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Prevotella and succinate treatments altered gut microbiota, increased laying performance, and suppressed hepatic lipid accumulation in laying hens

Min Liu^{1†}, Zeyue Kang^{1†}, Xikang Cao¹, Hongchao Jiao¹, Xiaojuan Wang¹, Jingpeng Zhao¹ and Hai Lin^{1*} 

Abstract

Background This work aimed to investigate the potential benefits of administering *Prevotella* and its primary metabolite succinate on performance, hepatic lipid accumulation and gut microbiota in laying hens.

Results One hundred and fifty 58-week-old Hyline Brown laying hens, with laying rate below 80% and plasma triglyceride (TG) exceeding 5 mmol/L, were used in this study. The hens were randomly allocated into 5 groups and subjected to one of the following treatments: fed with a basal diet (negative control, NC), oral gavage of 3 mL/hen saline every other day (positive control, PC), gavage of 3 mL/hen *Prevotella melaninogenica* (10^7 CFU/mL, PM) or 3 mL/hen *Prevotella copri* (10^7 CFU/mL, *P. copri*) every other day, and basal diet supplemented with 0.25% sodium succinate (Succinate). The results showed that PM and *P. copri* treatments significantly improved laying rate compared to the PC ($P < 0.05$). The amount of lipid droplet was notably decreased by PM, *P. copri*, and Succinate treatments at week 4 and decreased by *P. copri* at week 8 ($P < 0.05$). Correspondingly, the plasma TG level in Succinate group was lower than that of PC ($P < 0.05$). Hepatic TG content, however, was not significantly influenced at week 4 and 8 ($P > 0.05$). PM treatment increased ($P < 0.05$) the mRNA levels of genes *PGC-1 β* and *APB-5B* at week 4, and *ACC* and *CPT-1* at week 8. The results indicated enhanced antioxidant activities at week 8, as evidenced by reduced hepatic malondialdehyde (MDA) level and improved antioxidant enzymes activities in PM and Succinate groups ($P < 0.05$). Supplementing with *Prevotella* or succinate can alter the cecal microbiota. Specifically, the abundance of *Prevotella* in the Succinate group was significantly higher than that in the other 4 groups at the family and genus levels ($P < 0.05$).

Conclusions Oral intake of *Prevotella* and dietary supplementation of succinate can ameliorate lipid metabolism of laying hens. The beneficial effect of *Prevotella* is consistent across different species. The finding highlights that succinate, the primary metabolite of *Prevotella*, represents a more feasible feed additive for alleviating fatty liver in laying hens.

Keywords Fatty liver, Gut microbiota, Laying hen, *Prevotella*, Succinate

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Background

The disorder of lipid metabolism is a prevalent symptom observed in the late stage of laying hens, which results in excessive lipid accumulation in the liver (fatty liver disease, FLD) and in turn decreased laying performance and increased mortality [1–5]. The fatty liver of laying hens is characterized by higher rates of hepatic lipogenesis and overaccumulation of triglycerides (TG) [6, 7].

Previous researches have provided evidence indicating that gut microbiota play a crucial role in the development of FLD [8, 9]. Recently, emerging evidence has highlighted the significant association between the gut-liver axis and the pathogenesis of liver injury in laying hens with fatty liver [10]. Dietary modulation has emerged as a potential therapeutic approach to address excess fat deposition in the liver, as it has the ability to modify the composition and metabolic activity of the gut microbiota [2, 11]. For example, Lee et al. [12] found that *Lactobacillus* and *Pediococcus* were a promising therapeutic approach in non-alcoholic fatty liver disease (NAFLD) by modulating gut-microbiome and inflammatory pathway in mammals. In recent years, there are increasing reports on the positive regulatory effects of bacteria and probiotics on fatty liver in monogastric animals [13, 14]. The intestinal dysbiosis is associated with the activation of the inflammatory cascade in the development of NAFLD [15]. The application of prebiotics and probiotics in laying hens could reshape the gut microbiota. For example, utilization of *Clostridium butyricum* and *Bacillus subtilis* enhanced the abundance of Firmicutes phylum in laying hens [16]. The supplementation of *C. butyricum* accelerated hepatic fatty acid oxidation, and shaped gut microbiota and bile acid (BA) profile, thus reducing fat deposition in the liver of aged laying hens [17]. Furthermore, mounting evidences support that gut microbiomes are involved in the regulation of hepatic metabolism of host via their metabolites short-chain fatty acids (SCFAs) [18, 19]. For example, gut *Akkermansia muciniphila* could ameliorate metabolic dysfunction-associated FLD by regulating lipid oxidation and improving gut-liver interactions through the modulation of L-aspartate metabolism [20]. *Lactobacillus salivarius* SNK-6 shown efficacy in inhibiting fat deposition and decreasing serum TG levels in a NAFLD model of laying hens, in which, SCFAs (acetate, butyrate, and propionate), the microbial metabolites, play an important role [21]. The positive effects of probiotics (*L. salivarius* and *B. subtilis*) on the host metabolism could improve the production performance and egg quality [22]. Dietary supplementation with *C. butyricum* showed beneficial with respect to hen performance and egg quality coordinate improving the gut health [23].

Dietary interventions are the potential effective approach for regulating the composition of gut microbiome and their associated metabolites. High dietary fiber intake leads to an increase in *Prevotella*, a genus of Gram-negative anaerobic bacteria [24, 25]. In addition, multiple studies have shown the association of *Prevotella* with glucose homeostasis [26–28]. However, there is limited knowledge about the role of *Prevotella* in the regulation of lipid metabolism. Recent studies indicated that bacteria regulate host metabolism through producing microbial metabolites [19, 29]. *Prevotella* has been reported to produce succinic acid, which has the potential to exert metabolic effects and overcome the challenges of anaerobic fermentation [27]. However, little is known about the function of *Prevotella* in the regulatory of lipid metabolism.

Recent evidences in mammals commended that the NAFLD development could be closely associated with mitochondrial dysfunction in the liver [30–32]. Mitochondrial function is related to the β -oxidation of fatty acids, TG synthesis, and the reactive oxygen species (ROS) accumulation. However, further elucidation is required to understand the mitochondrial function in the liver of FLD hens.

Therefore, we hypothesized that *Prevotella* and its metabolite, succinate, could alter lipid accumulation in the liver and improve laying performance of laying hens. The incidence of fatty liver adversely affects both laying performance and egg quality [10, 27, 28]. In the present study, the laying hens with a laying rate less than 80% and plasma TG levels exceeding 5 mmol/L were selected according to our preliminary clinical investigation. The effects of oral intake of *Prevotella* and dietary supplementation of succinate on laying performance and liver lipid metabolism of laying hens were measured.

Methods

Microorganisms culture

This work employed two distinct strains belonging to the *Prevotella* genus: *Prevotella melaninogenica* (ATCC 43982, ATCC, MD, USA) and *Prevotella copri* (BNCC 337399, BeNa Culture Collection, Beijing, China). The strains were precultured in complex KVLB broth medium (HB8817, Qingdao Hope Bio-Technology Co., Ltd., Qingdao, China) containing defibrated sheep blood (5%) (1001339-1, Qingdao Hope Bio-Technology Co., Ltd.), vitamin K₁ (1%) (2100501, Qingdao Hope Bio-Technology Co., Ltd.), and kanamycin-vancomycin complex (2%) (HB8817a, Qingdao Hope Bio-Technology Co., Ltd.). The medium was cultured under the condition of 37 °C in an anaerobic environment.

Birds and experimental design

A total of 150 healthy 58-week-old Hyline brown laying hens with similar laying rate below 80% ($75.89\% \pm 1.47\%$) and similar plasma TG levels (Additional file 1) were randomly divided into 5 groups with 5 replicates per group and 6 hens per replicate and subjected to one of the following treatments: fed with the basal diet (negative control, NC), fed with the basal diet and oral gavage of 3 mL/hen saline every other day (positive control, PC), fed the basal diet and oral gavage of 3 mL/hen *Prevotella melaninogenica* (10^7 CFU/mL, PM) or 3 mL/hen *Prevotella copri* (10^7 CFU/mL, *P. copri*) every other day, and fed with the basal diet supplemented with 0.25% sodium succinate (S112882, Aladdin, Shanghai, China) and oral gavage of 3 mL saline/hen every other day (Succinate). The composition and nutrient levels of basal diet were presented in Table 1. The experiment lasted for 8 weeks and the hens had free access to feed and water during the whole experimental period. The lighting program was 16 h light period and 8 h dark period. Egg numbers and egg weight were recorded daily, laying rate and feed efficiency were calculated weekly.

Sample collection

Blood samples were collected every two weeks. One hen was randomly selected from each replicate in every treatment, a blood sample was collected from the brachial vein of each bird and centrifuged at 4 °C to get plasma and stored at -80 °C for further analysis. Five eggs were respectively obtained from each replicate at week 4 and week 8 and used for the measurement of egg quality. At the end of week 4 and 8, eight hens were randomly obtained from each replicate. The hens were weighted and sacrificed by exsanguination after cervical dislocation [33, 34]. The liver was excised and weighted, and then the morphological observation was conducted. After removed blood with PBS, tissue sample was obtained from the middle part of the liver, part of the sample was fixed in 4% formaldehyde for morphology observation, and another part of sample was snap frozen in liquid nitrogen for further analysis. The abdominal fat pad was weight to calculated organ index. The cecal content were obtained and was immediately snap frozen in liquid nitrogen and stored at -80 °C for further analysis.

Physical egg parameters

Eggshell breaking strength was evaluated using the Egg Force Reader (EFG-0503, Robotmation Co., Ltd., Tokyo, Japan), and sharp end, equator, blunt end was measured to obtain the value of eggshell thickness with

the Egg Shell Thickness Gauge (ESTG-1, ORKA Food Technology LLC, West Bountiful, UT, USA). Albumen height, Haugh units, and yolk color were tested by the Egg Multi Tester EMT-500 (EMT-5200, Robotmation Co., Ltd., Tokyo, Japan).

Plasma parameters

The plasma TG, total cholesterol (TCH), glucose (GLU), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) levels were analyzed using the Hitachi L-7020 fully automatic biochemical analyzer (7020, HITAGHUI, Tokyo, Japan).

Hepatic TG and TCH content measurements

TG and TCH contents in the liver were measured using commercial kits (A110-1-1 and A111-1-1, GPO-PAP and CHOD-PAP, Nanjing Jiancheng Biotechnology Institute, Nanjing, China).

Liver histological observation

The liver samples were cut into 5 μm serial sections sliced, Hematoxylin and eosin (H&E) stained, and sheet sealed, each slice was chosen with 10 visions for observing the liver morphology using a light microscope (CK-40, Olympus, Tokyo, Japan).

Oil Red O staining

Liver sample was put in sucrose solution (15%) for 6 h and then shifted in sucrose solution (30%) overnight at 4 °C. The tissue was embedded in OTC and immediately put on the -20 °C quick-freezing machine, and then cut into 8 μm slide by the CryoStar NX50 HOVPD freezing slicer (CryoStar NX50 HOVPD, Thermo, MA, USA). These sliced stored at -20 °C. The room temperature was restored for 20 min before staining. It was stained for 30 min in a fresh diluted Oil Red O (ORO) solution (G1262, Solarbio, Beijing, China). After color separation of 60% isopropanol (20 s), softly flow flush of H₂O (3 min), lining dyeing of hematoxylin (20 s), rinsing with distilled water, the slide was mounted in glycerine jelly. The image of each group was photographed with a light microscope (CK-40, Olympus, Tokyo, Japan). The lipid droplets in ORO staining was measured by Image J software and the ratio of lipid droplets in ORO staining was calculated.

Lipid metabolism associated enzyme activities and free fat acid content

The hepatic fatty acid synthase (FAS) (H231-1-1, Nanjing Jiancheng Biotechnology Institute, Nanjing, China), lipoprotein lipase (LPL), hepatic lipase (HL) activities and free fat acid (FFA) contents (ml060836, ml036976, and

ml556655, Shanghai Enzyme-linked Biotechnology Co., Ltd., Shanghai, China) and malic enzyme (ME) (BC1120, Solarbio, Beijing, China) were analyzed using commercial kits.

Hepatic antioxidant enzyme activities

The activities of glutathione (GSH), glutathione peroxidase (GSH-Px), catalase (CAT), superoxide dismutase (SOD), and contents of malondialdehyde (MDA) and total antioxidant capacity (T-AOC) were performed by commercial kits (A006-2-1, A005-1-2, A007-1-1, A001-3-2, A003-1-2, A015-2-1, Nanjing Jiancheng Biotechnology Institute, Nanjing, China).

RNA extraction, cDNA synthesis, and quantitative real-time PCR

The method of RNA isolation and reverse transcription and qRT-PCR was followed. Total RNA of the liver was isolated using Trizol reagent (M5100, New Cell & Molecular Biotech Co., Ltd., Suzhou, China). The concentration of RNA was tested by Nanodrop 2000c spectrophotometer (Thermo, MA, USA). The mRNA was reversed transcribed into cDNA by Primer Script RT reagent kit (CW2569, Jiangsu CoWin Biotech, Taizhou, China). Gene expression of the liver was measured by quantitative real-time PCR (7500, ABI, CA, USA), including acetyl-CoA carboxylase (*ACC*), adenosine monophosphate-5B (*ATP-5B*), cytochrome IV (*COX IV*), carnitine palmitoyl-transferase 1 (*CPT-1*), *FAS*, isocitrate dehydrogenase 3 α (*IDH3 α*), nuclear respiratory factor 1 (*NRF1*), peroxisome proliferator-activated receptor γ -1 α (*PGC-1 α*), *PGC-1 β* , peroxisome proliferator-activated receptor γ (*PPAR γ*), sterol regulatory element binding proteins-1c (*SREBP-1c*), mitochondrial transcription factor A (*TFAM*). The primer was synthesized by Sangon Biotech (Shanghai) Co., Ltd. (Table 2), and *GAPDH* was as the housekeeping gene and calculated using the $2^{-\Delta\Delta Ct}$ method.

16S rRNA sequencing analysis

DNA was extracted from cecal content using the Magnetic Soil and Stool DNA kit (DP336-02, TianGen, Beijing, China) and its integrity was tested through electrophoresis on a 1% agarose gel, and then its amplification was performed with the TransStart[®] FastPfu DNA Polymerase (AP221-01, TransGen Biotech, Beijing, China). The PCR production as the templates was amplified using the universal primer (338F: 5'-ACTCCTACGGGAGGCAGCAG-3', 806R: 5'-GGACTACHVGGGTWTCTAAT-3') targeting the V3-V4 hyper variable region. The PCR reaction conditions consisted of an initial denaturation step at 95 °C for 3 min, followed by 30 cycles of denaturation at 95 °C 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s, with a final extension of 10 min at

Table 1 Composition and nutrient levels of experimental diets of laying hens

| Ingredient, % | Content | Calculated nutrition levels, % | Content |
|-----------------------------|---------|--------------------------------|---------|
| Corn (8.5%) | 58.41 | Crude protein | 16.50 |
| Wheat bran | 5.91 | Metabolizable energy, kcal/kg | 2,700 |
| Soya-bean oil | 1 | Calcium | 3.50 |
| Soybean meal (46% CP) | 22.68 | Available phosphorus | 0.404 |
| Salt | 0.35 | Lysine | 0.78 |
| Limestone | 9.61 | Methionine | 0.40 |
| CaHPO ₄ | 1.44 | Threonine | 0.505 |
| Choline chloride, 50% | 0.09 | Tryptophan | 0.161 |
| DL-Methionine, 99% | 0.16 | Methionine + cystine | 0.619 |
| Lysine, 99% | 0.10 | | |
| Vitamin premix ^a | 0.05 | | |
| Mineral premix ^b | 0.20 | | |

^a Vitamin premix provides the following per kg of diet: VA, 8,800 IU; VD₃, 3,300 IU; VE, 16.5 IU; VK, 2.2 mg; VB₁, 1.7 mg; VB₂, 5.5 mg; VB₃, 6.6 mg; VB₅, 28 mg; VB₆, 3.3 mg; VB₇, 0.1 mg; VB₉, 0.6 mg; VB₁₂, 0.05 mg

^b Mineral premix provides the following per kg of diet: Fe (as ferrous sulfate), 55 mg; Zn (as zinc sulfate), 88 mg; Mn (as manganese sulfate), 88 mg; Cu (as copper sulfate) 5.5 mg; I (as potassium iodide), 1.7 mg; Se (as sodium selenite), 0.3 mg

Table 2 Primers used in the work

| Gene | Primer sequence (5'→3') | Accession No. |
|---------------------------------|-----------------------------------------------------------|---------------|
| <i>GAPDH</i> | F: ACATGGCATCCAAGGAGTGAG R: GGGGAGACAGAAGGGAACAGA | NM-204305.2 |
| <i>ACC</i> | F: AATTGGCAGCTTTGGAGGTGT R: TCTGTTTGGGTGGGAGGTG | NM-205505 |
| <i>FAS</i> | F: CTATCGACACAGCCTGCTCCT R: CAGAATGTTGACCCCTCTACC | J03860 |
| <i>PPARγ</i> | F: CCAGCGACATCGACCAGTT R: GGTGATTTGTCTGCTGTTTCCC | AF163811 |
| <i>CPT1</i> | F: GGGACCTGAAACCAGAGAAGC R: ACAGAGGAGGGCATAGAGGATG | AY675193 |
| <i>SREBP1c</i> | F: GCCCTCTGTGCCTTTGTCTTC R: ACTCAGCCATGATGCTTCTCC | NM_204126.2 |
| <i>PGC-1α</i> | F: GACTCAGGTGTCATGGAAGTG R: ATCAGAACAAGCCCTGTGGT | NC_006091 |
| <i>PGC-1β</i> | F: AGGGTCTCGACACGCACTA R: CTTTGTAACTGGCTCTGATCTCC | NC_006100.5 |
| <i>ATP-5B</i> | F: AGAGATGAGCGTCGAGCAGGAG R: ACACCAGCGAACACCGAATAACC | NC_003283.11 |
| <i>NRF1</i> | F: GGCCAACGTCCGAAGTGAT R: CCATGACACCCGCTGCTT | NC_052532.1 |
| <i>TFAM</i> | F: AGCAGAACCAGAACTGAAC R: CAAGCACAGTCAATTCTCTC | NC_0.2537.1 |
| <i>COX IV</i> | F: CAACCACCCAACACTCTGG R: CTCTTGCTCTCTCTCTCA | NM_001030577 |
| <i>IDH3α</i> | F: TGCTGGATTGATTGGAGGTCCTGG R: AGGTGCTGTTCCATGAACCGATT | NC_000015.10 |

72 °C. The integrity was assessed by electrophoresis on a 2% agarose gel, and then purified by the AxyPrep DNA Gel extraction kit (AP-GX-50, Axygen Scientific, Inc., CA, USA). The purification was qualified and sequenced by the Quantus™ Fluorometer (E6150, Promega, Madison, WI, USA). Purified amplicons were pooled in equimolar and paired-end sequenced on an Illumina Novaseq 6000 PE250 platform (Illumina, San Diego, CA, USA). Raw data FASTQ files were imported by QIIME2 system. In our work, the average count of raw sequences and sample depth amounts to 86,561 bp. Demultiplexed sequences from each sample were quality filtered and trimmed, de-noised, and merged. Subsequently, chimeric sequences were identified and removed to obtain the feature table of amplicon sequence variant (ASV) and further generate the taxonomy table. The average sequence counts, post-quality control, is 67,199 bp. Subsequently, the QIIME2 feature-classifier plugin was used to align the ASV sequences with a pre-trained GREENGENES 13_8 99% database for the purpose of generating the taxonomy table. The linear discriminant analysis effect size (LEfSe) tool was utilized for detecting bacterial exhibiting differential abundance within the sample and groups at phylum level ($LDA \geq 4.0$). Diversity metrics were calculated using the core-diversity plugin within QIIME2. Alpha diversity indices at the feature level, including observed operational taxonomic unit (OTU), the Chao1 estimator, Shannon index, Simpson index and Faith PD index, were computed to estimate microbial diversity within individual samples. Beta diversity distance measurements, Bray–Curtis, was employed to explore structural variations in microbial communities across the samples. The results were visualized using principal coordinate analysis (PCoA). Partial least squares discriminant analysis (PLS-DA) was employed as a supervised model for discerning microbiota variations among the groups. This analysis was conducted using the "plsda" function within the R package "mixOmics". Redundancy analysis (RDA) was conducted to elucidate the connections between microbial communities and environmental factors, utilizing relative abundances of microbial species at various taxonomic levels. This analysis was performed using the "vegan" package in R. Co-occurrence analysis was

conducted by calculating Spearman's rank correlations among the dominant taxonomic groups, and the results were visualized using a network plot to represent the relationships among these taxa. The 16S rRNA sequencing of the gut microbiome was performed in Wekemo Tech Group Co., Ltd. (Shenzhen, China).

Statistical analysis

All the data were presented as means \pm SEM. For variables laying performance, plasma parameters, hepatic enzyme activity and redox parameters, and hepatic gene expression, the main effect of treatment was estimated with one-way ANOVA (SAS software, version 9.4). Means were compared using Tukey post-tests where significant ($P < 0.05$). Statistically significant was determined at a P value threshold of < 0.05 .

For microbial community analysis, appropriate methods include ANCOM, ANOVA, Kruskal Wallis, and LEfSe were used to identify the bacteria with different abundance among samples and groups.

Results

Prevotella improved the laying performance in laying hens

The laying rate in the PM and *P. copri* groups was significantly higher than that in the PC group ($P < 0.05$, Table 3). *P. copri* treatment significantly increased ($P < 0.05$) egg weight in comparison to NC group. Moreover, *P. copri* treatment notably elevated egg mass when compared to PC and NC groups ($P < 0.05$). The ADFI and FCR were not altered ($P > 0.05$) by dietary treatment. For the egg quality, there was no difference ($P > 0.05$) in egg-shaped index, eggshell thickness, eggshell strength, albumen height, yolk color, Haugh units, eggshell proportion and yolk proportion among treatments at the fourth and eighth weeks (Additional file 2).

Prevotella and succinate decreases plasma TG levels

The initial TG, TCH, GLU, LDL-C and HDL-C content in plasma of laying hens among all the groups showed no remarkable difference ($P > 0.05$, Additional file 1). At the fourth week, plasma TG content was notable lowered ($P < 0.05$) in *P. copri* compared with NC and PC groups and was decreased by Succinate treatment compared to

Table 3 Effect of *Prevotella* and sodium succinate on the laying performance in the laying hens

| Item | NC | PC | PM | <i>P. copri</i> | Succinate | P-value |
|-------------------|--------------------------------|--------------------------------|--------------------------------|-------------------------------|--------------------------------|---------|
| Laying rate, % | 73.45 \pm 1.25 ^{ab} | 71.33 \pm 1.08 ^b | 76.66 \pm 1.83 ^a | 78.25 \pm 1.94 ^a | 75.31 \pm 1.91 ^{ab} | 0.045 |
| Egg weight, g | 58.81 \pm 0.93 ^{bc} | 60.37 \pm 0.36 ^{ab} | 60.57 \pm 0.64 ^{ab} | 61.26 \pm 0.33 ^a | 58.33 \pm 0.73 ^c | 0.019 |
| Egg mass, g/hen/d | 43.45 \pm 0.75 ^{bc} | 42.28 \pm 0.75 ^c | 46.23 \pm 0.92 ^{ab} | 48.31 \pm 1.57 ^a | 44.37 \pm 1.37 ^{bc} | 0.005 |
| ADFI, g/d/h | 104.17 \pm 2.91 | 107.47 \pm 2.91 | 108.21 \pm 2.83 | 105.86 \pm 3.39 | 105.93 \pm 3.82 | 0.951 |
| FCR, g/g | 2.42 \pm 0.08 | 2.54 \pm 0.06 | 2.34 \pm 0.03 | 2.29 \pm 0.05 | 2.40 \pm 0.09 | 0.106 |

The assessment measures were conducted at week 8. All data are presented as mean \pm SEM ($n = 5$). ^{a-c}Means with different superscripts within a column differ significantly ($P < 0.05$)

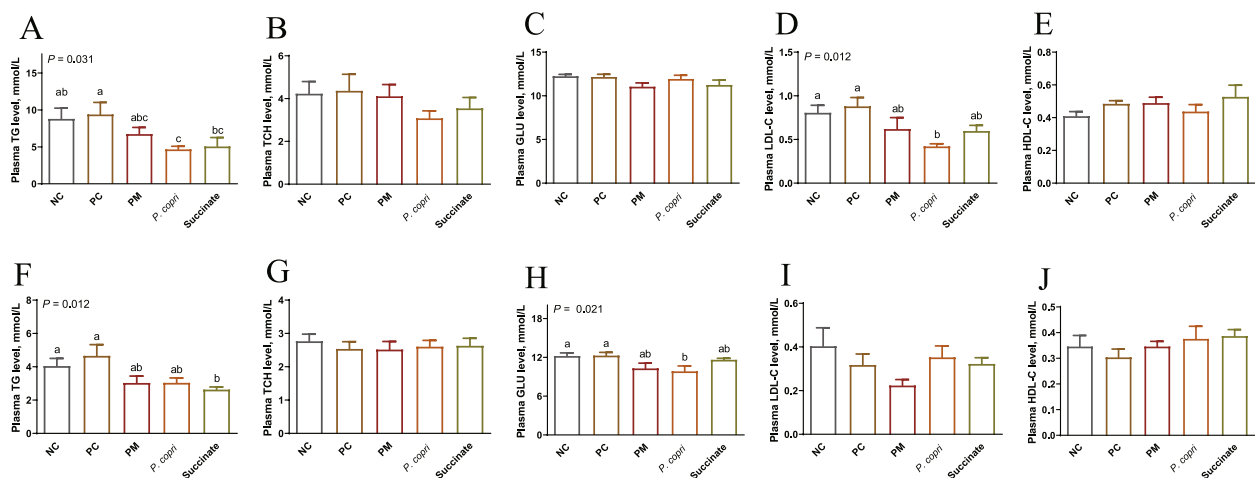


Fig. 1 Effect of *Prevotella* (3×10^7 CFU) and sodium succinate (0.25%) on plasma parameters of hens. **A** and **F** Triglyceride (TG). **B** and **G** Total cholesterol (TCH). **C** and **H** Glucose (GLU). **D** and **I** Low-density lipoprotein-cholesterol (LDL-C). **E** and **J** High-density lipoprotein-cholesterol (HDL-C). The assessment measures were conducted at two different time points: at week 4 (**A–E**) and week 8 (**F–J**). ^{a–c}Means with different letter differ significantly ($P < 0.05$)

PC ($P < 0.05$, Fig. 1A). Plasma TCH, GLU, and HDL-C levels were not influenced by treatment ($P > 0.05$, Fig. 1B, C and E). In contrast, plasma LDL-C level was decreased by *P. copri* treatment ($P < 0.05$) compared with NC and PC groups (Fig. 1D). At week 8, however, plasma TG was decreased ($P < 0.05$) by Succinate treatment compared to NC and PC groups and there was no detectable difference among PM, Succinate, and *P. copri* treatments ($P < 0.05$, Fig. 1F). The GLU level in *P. copri* group was significantly lower than those in the NC and PC groups ($P < 0.05$, Fig. 1H). Dietary treatments had no significant effect on TCH, LDL-C, and HDL-C levels ($P > 0.05$, Fig. 1G, I, J).

Prevotella and succinate suppress the hepatic TG accumulation

At week 4, *P. copri* treatment decreased liver index compared to NC group ($P < 0.05$, Fig. 2A). All the treatments had no significant influence ($P > 0.05$) on hepatic TG and TCH contents (Fig. 2B and C). The abdominal fat pad index was decreased by *P. copri* compared to NC and PC groups, and decreased in PM group compared to NC group ($P < 0.05$, Fig. 2D). At week 8, PM and Succinate treatments notably decreased the abdominal fat pad index compared to NC group ($P < 0.05$, Fig. 3D). The liver of NC group and PC group appeared yellow color, while the liver of hens in PM, *P. copri*, and Succinate groups appeared normal red color (Fig. 2E and 3E). The ORO staining and H&E staining showed that there was less amount of lipid droplets in PM, *P. copri*, and Succinate treatments, compared with PC or NC group (Fig. 2E and F and 3E and F). Figure 2G revealed that the lipid

droplets in the PM, *P. copri* and Succinate groups exhibited a significant decrease compared to both the NC and PC groups at the fourth week ($P < 0.01$). At week 8, liver index and hepatic TG and TCH contents were not changed ($P > 0.05$) by dietary treatment (Fig. 3A–C). The abdominal fat pad index was decreased ($P = 0.05$) by PM and Succinate treatments compared with NC (Fig. 3D). The *P. copri* treatment had less lipid droplets in comparison to the NC group ($P < 0.05$, Fig. 3G).

The effect of Prevotella and succinate on lipid metabolism associated enzyme activities and FFA content

At week 4, FAS, ME, LPL, and HL activities and FFA content were not significant influenced by treatment ($P > 0.05$, Fig. 4A–E). At week 8, PM treatment increased ($P < 0.05$) ME activity compared with NC, while Succinate notably increased ($P < 0.01$) the activity of ME compared to NC, PC, and *P. copri* groups (Fig. 4G). The HL activity in the PM and Succinate groups exhibited a significant increase compared to NC and PC groups ($P < 0.01$, Fig. 4I). The FAS and LPL activities and FFA level were not altered ($P > 0.05$) by treatment (Fig. 4E, H and J).

Prevotella and succinate regulate CPT-1 and mitochondrial function related mRNA expression

At week 4, for the key genes related to lipid metabolism, the mRNA expression of *FAS*, *ACC*, *CPT-1*, *SREBP-1c*, and *PPAR γ* displayed no obvious difference among these five groups ($P > 0.05$, Fig. 5A). The genes expression associated with mitochondrial function was then evaluated. The mRNA expression of *PGC-1 β* was increased ($P < 0.05$) by PM compared with PC and *P. copri* (Fig. 5B).

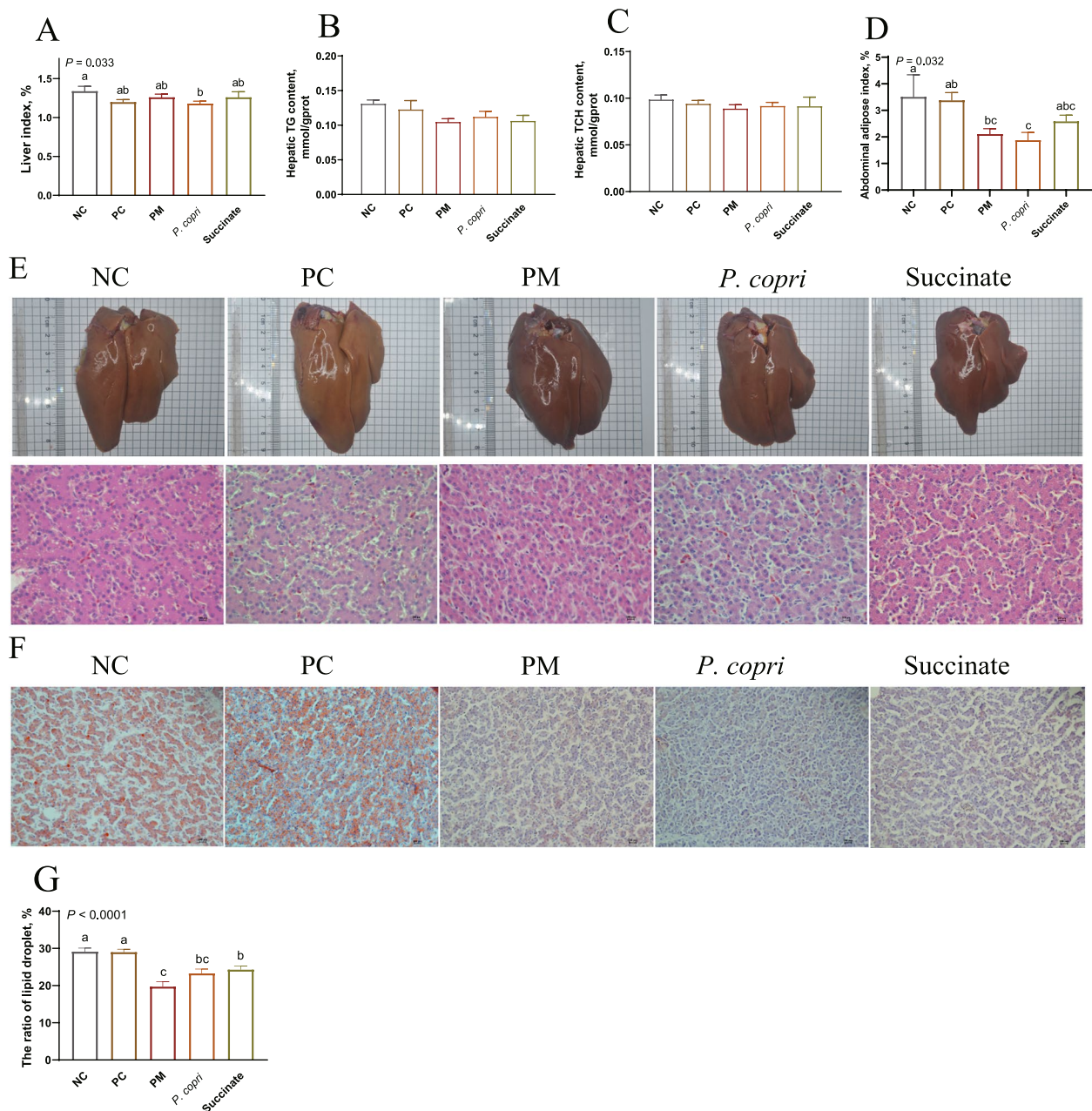


Fig. 2 *Prevotella* (3×10^7 CFU) and sodium succinate (0.25%) suppressed the hepatic lipid accumulation at week 4. **A** Liver index. **B** Hepatic triglyceride (TG) content. **C** Hepatic total cholesterol (TCH) content. **D** Abdominal fat pad index. **E** Liver morphology (upper), and H&E staining (lower). **F** Hepatic ORO staining. **G** The ratio of lipid droplets in ORO staining. ^{a-c}Means with different letters differ significantly ($P < 0.05$)

In contrast, the expression of *ATP-5B* was significantly increased in PM group compared with PC, NC, and Succinate ($P < 0.05$, Fig. 5B). The expression of *PGC-1 α* , *NRF1*, *TFAM*, *COX IV*, and *IDH3 α* was not changed by all the treatment ($P > 0.05$, Fig. 5B).

At week 8, the expression level of *ACC* was significantly reduced in Succinate group when compared with PM group ($P < 0.05$, Fig. 5C). The *CPT-1* expression level

was increased by PM compared to the other four groups ($P < 0.01$, Fig. 5C). By contrary, the expression of *FAS*, *SREBP-1c*, and *PPAR γ* was not changed by dietary treatment ($P > 0.05$, Fig. 5C). The expression of genes *PGC-1 α* , *PGC-1 β* , *NRF1*, *ATP-5B*, *TFAM*, *COX IV*, and *IDH3 α* was not changed by treatment ($P > 0.05$, Fig. 5D).

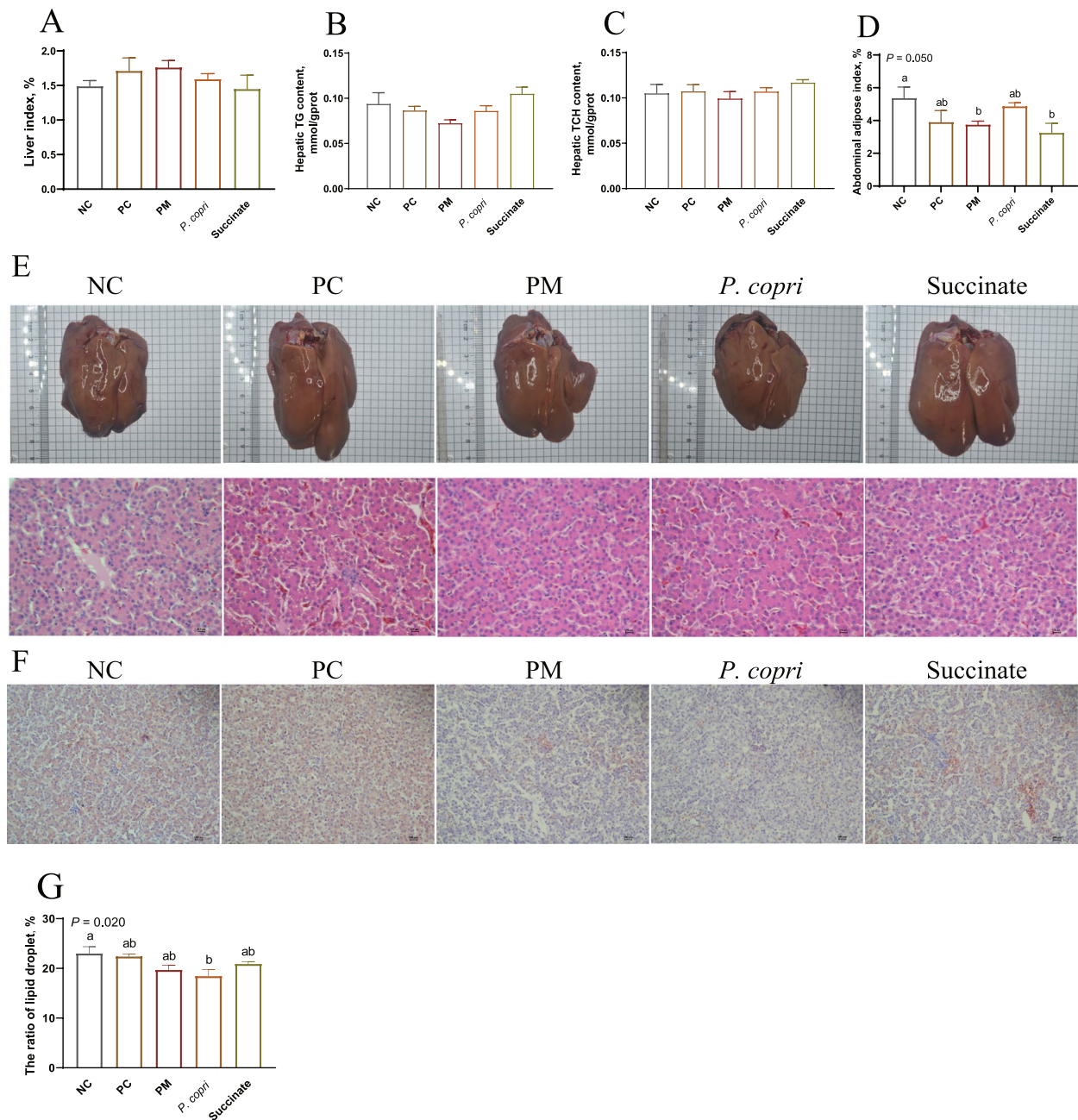


Fig. 3 *Prevotella* (3×10^7 CFU) and sodium succinate (0.25%) suppressed the hepatic lipid accumulation at week 8. **A** Liver index. **B** Hepatic triglyceride (TG) content. **C** Hepatic total cholesterol (TCH) content. **D** Abdominal fat pad index. **E** Liver morphology (upper) and H&E staining (lower). **F** Hepatic ORO staining. **G** The ratio of lipid droplets in ORO staining. ^{a,b}Means with different letters differ significantly ($P < 0.05$)

Prevotella and succinate could improve the hepatic antioxidant activities

At week 4, all the measured hepatic oxidative parameters MDA, GSH, T-AOC, GSH-Px, SOD, and CAT were not significantly ($P > 0.05$) changed by treatment (Fig. 6A–F). At week 8, MDA levels in the Succinate group were significantly lower than those in the NC, PC, and *P. copri*

groups ($P < 0.05$, Fig. 6G). PM treatment significantly increased the levels of GSH compared to PC group ($P < 0.05$, Fig. 6H). The T-AOC content was significantly ($P < 0.05$) increased by Succinate treatment, compared with PC, NC, and PM (Fig. 6I). Compared with PC and NC groups, Succinate had higher ($P < 0.05$) SOD activity, while PM and Succinate treatments had elevated

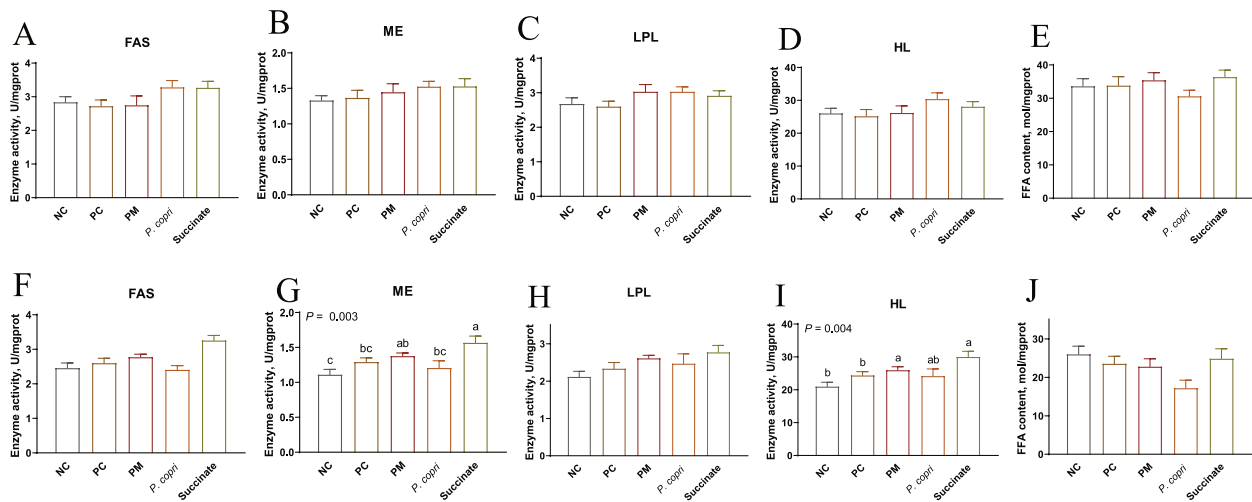


Fig. 4 Effect of *Prevotella* and sodium succinate on enzymes activities related to hepatic lipid metabolism. **A** and **F** Fatty acid synthase (FAS) activity. **B** and **G** Malic enzyme (ME) activity. **C** and **H** Lipoprotein lipase (LPL) activity. **D** and **I** Hepatic lipase (HL) activity. **E** and **J** Free fat acid (FFA) content. The assessment measures were conducted at two different time points: at week 4 (**A–E**) and at week 8 (**F–J**). ^{a–c}Means with different letters differ significantly ($P < 0.05$)

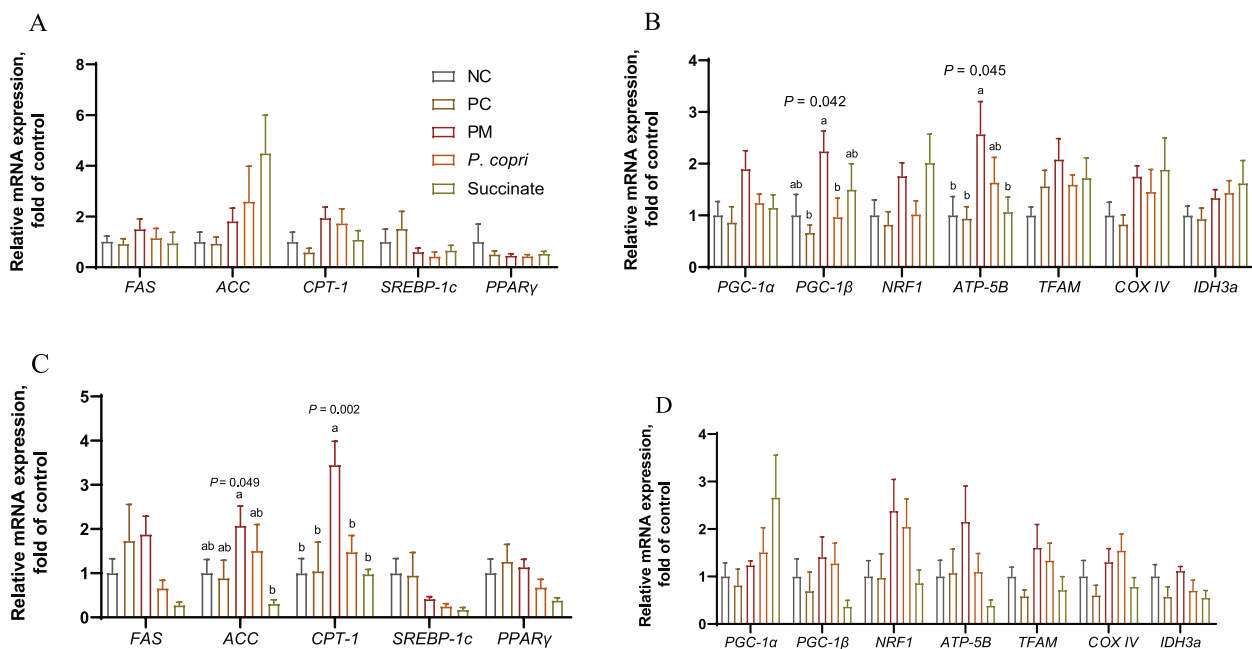


Fig. 5 *Prevotella* and sodium succinate regulated hepatic lipid metabolism and mitochondrial function related mRNA expression. The assessment measures were conducted at two different time points: at week 4 (**A** and **B**) and at week 8 (**C** and **D**). ^{a–b}Means with different letters differ significantly ($P < 0.05$)

($P < 0.01$) CAT activity (Fig. 6K and L). The GSH-Px activity was not changed by treatment ($P > 0.05$, Fig. 6J).

Intestinal microbial diversity and community

As shown in Fig. 7A, the *Prevotella* and succinate could alter the abundance of microbiome, particularly by

enriching the abundance of microbiota diversity through the presence of succinate. Phylum distributions and heatmap analysis revealed changes in composition and clustering due to the treatment of *Prevotella* and succinate, particularly in the Succinate group (Fig. 7B and C). The LEfSe analysis ($LDA > 4.0$) indicated significant

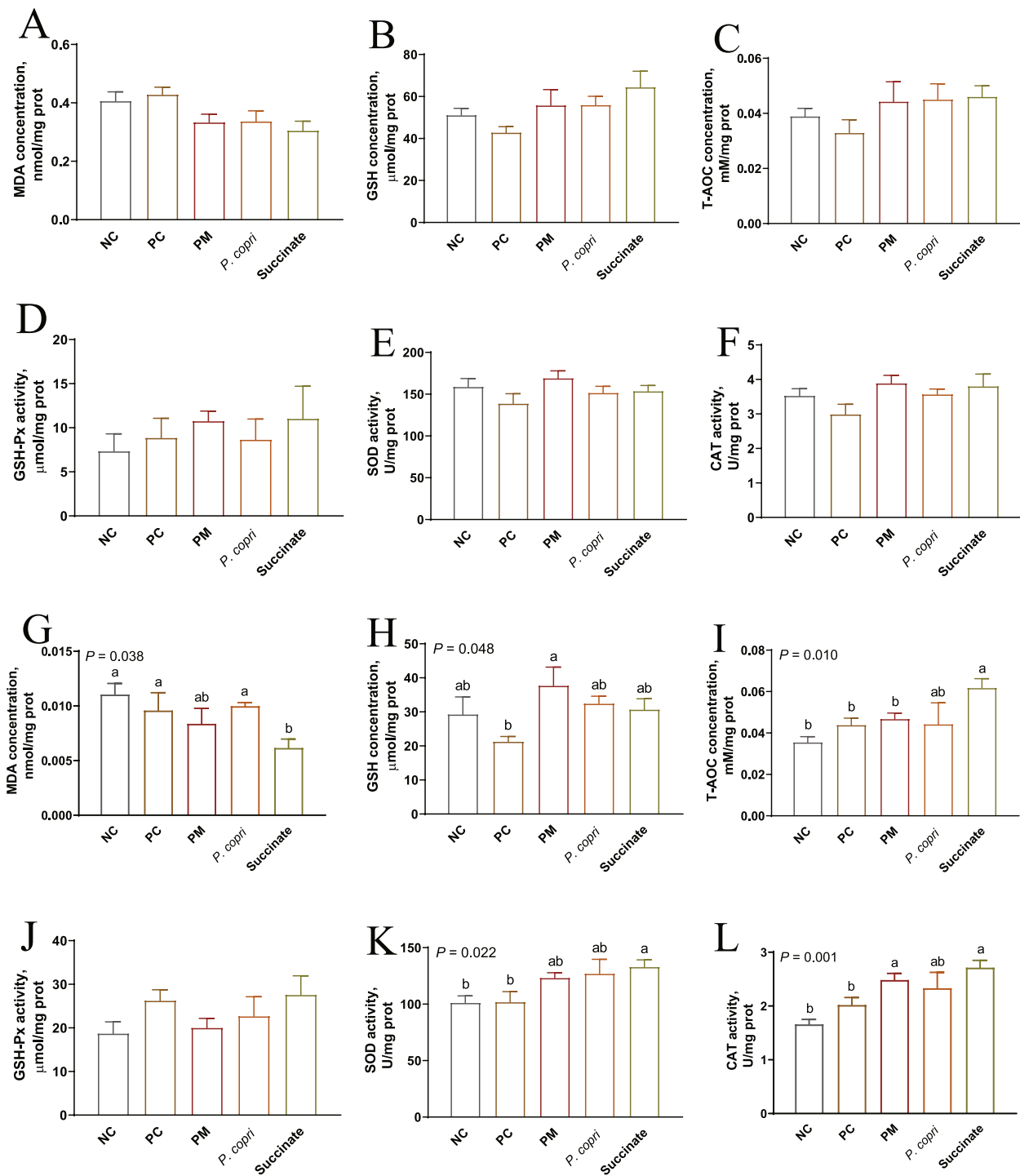


Fig. 6 *Prevotella* and sodium succinate improved the hepatic antioxidant activities. **A** and **G** Malondialdehyde (MDA) content. **B** and **H** Glutathione (GSH) concentration. **C** and **I** Total antioxidant capacity (T-AOC) concentration. **D** and **J** Glutathione peroxidase (GSH-Px) activity. **E** and **K** Superoxide dismutase (SOD) activity. **F** and **L** Catalase (CAT) activity. The assessment measures were conducted at two different time points: at week 4 (**A-F**) and at week 8 (**G-L**). ^{a,b}Means with different letters differ significantly (*P* < 0.05)

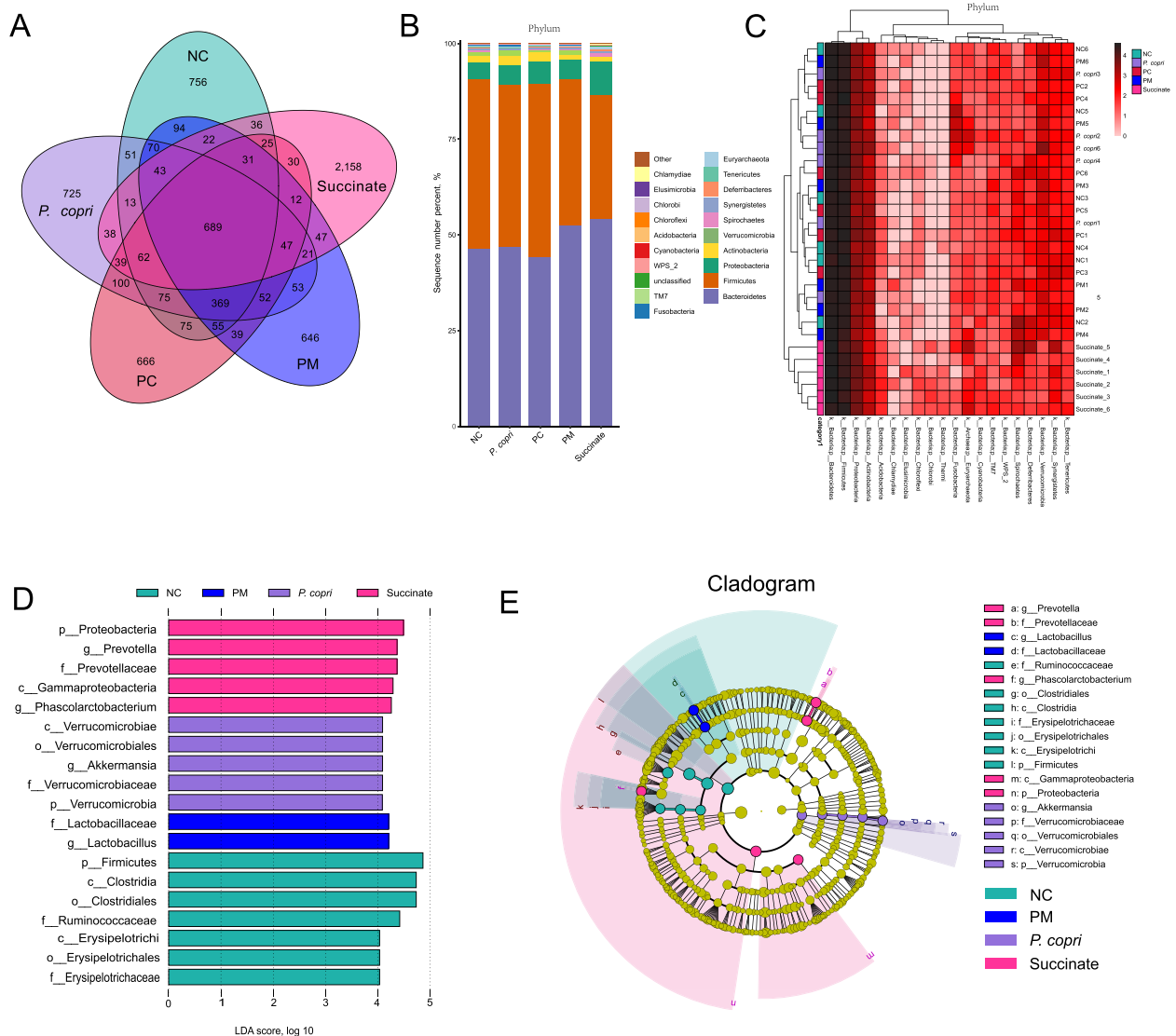


Fig. 7 Effect of *Prevotella* and sodium succinate on the gut microbiota. **A** Venn diagram illustrated the overlap of operational taxonomic unit (OTU) in all tested chicken. **B** The composition of gut microbiota of community bar plot analysis on phylum level. **C** Heatmap hierarchical cluster analysis based on the 20 most abundant phylum among groups. **D** Linear discriminant analysis effect size (LEfSe) analyses (LDA score ≥ 4.0) at the phylum level to the genus level. **E** Cladogram generated from LEfSe analysis (LDA score ≥ 4.0). Different color nodes represent the significant enriched microbial groups in the corresponding treatment groups which have a significant impact on the differences among groups, and the diameters of the circles are proportional to the axon's abundance

differences in the abundance of OTU at the phylum levels within the entire microbiota (Fig. 7D and E). In the *P. copri* group, LEfSe highlighted the greater differential abundance of Verrucomicrobia (phylum level) and its derivatives (Verrucomicrobiae, Verrucomicrobiales, and Verrucomicrobiaceae), and the genus *Akkermansia*. The marker microorganism in the Succinate group belonged to the phylum Proteobacteria, class Gammaproteobacteria, genus *Phascolarctobacterium*, family Prevotellaceae, and its derivative genus *Prevotella*.

Faith PD index was increased significantly by the supplementation of succinate compared with NC, PC, PM, and *P. copri* groups ($P < 0.01$, Fig. 8A), indicating a notable increase in evolutionary divergence and microbiome diversity. There were no significant differences in the alpha diversity of the gut microbiome between the *Prevotella* (PM or *P. copri*) group and control (NC or PC) group ($P > 0.05$, Fig. 8A–D). However, the Succinate group exhibited a decreased Shannon index compared to NC, PC, PM, and *P. copri* groups ($P < 0.05$, Fig. 8C).

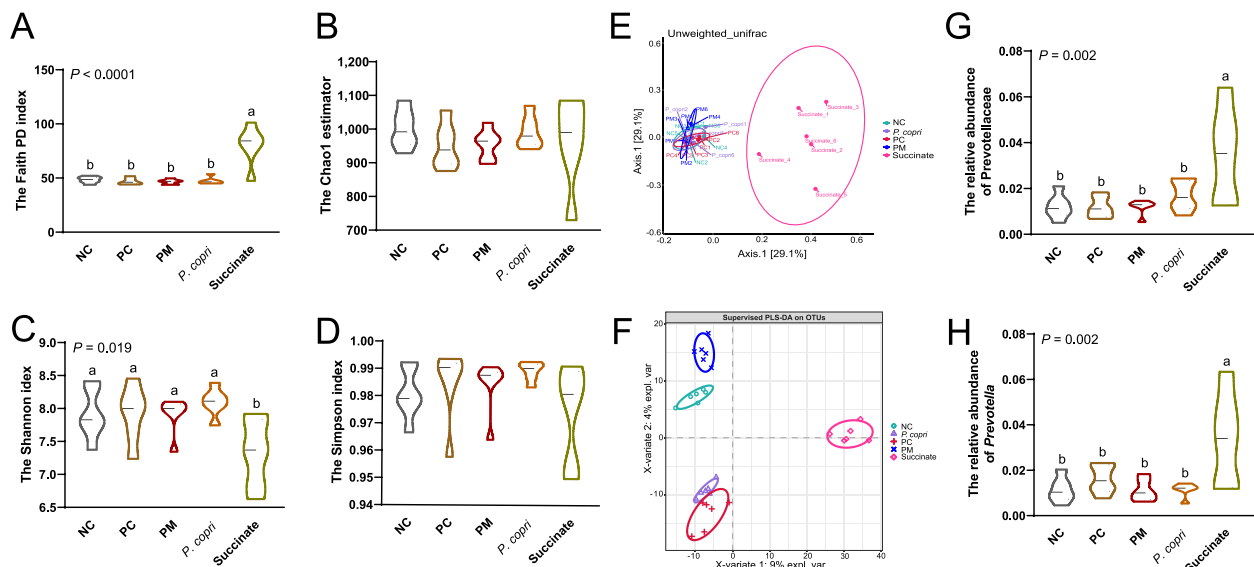


Fig. 8 Effect of *Prevotella* and sodium succinate on the gut microbiota. **A–D** Alpha diversity among groups (Faith PD, Chao1, Shannon, and Simpson index). **E** Principal coordinate analysis (PCoA) based on Bray–Curtis dissimilarity. **F** Partial least squares discriminant analysis (PLS-DA). **G** The relative abundance of Prevotellaceae. **H** The relative abundance of *Prevotella*. ^{a,b}Means with different letters differ significantly ($P < 0.05$)

The PCoA using the Bray distance metric demonstrated distinct separation between the succinate dietary group and other four groups, including NC, PC, PM and *P. copri* groups (Fig. 8E). Additionally, PLS-DA clearly demonstrated distinct classification separation among the NC, PC, PM, *P. copri* and Succinate groups (Fig. 8F). The relative abundance of Prevotellaceae and *Prevotella* remarkably increased in the Succinate group compared to NC, PC, PM, and *P. copri* groups ($P < 0.01$, Fig. 8G and H).

Association analysis and phylogenetics analysis

RDA revealed that hepatic TG levels were among the most influential parameters in explaining the majority of variations in the community compositions of the samples (Fig. 9A). It indicated a negative correlation between the relative abundance of *Prevotella* and plasma TG levels, while a positive correlation was observed between the relative abundance of *Prevotella* and T-AOC levels. The correlation heatmap revealed a significant negative correlation between plasma TG levels and the abundance of *Prevotella* ($P < 0.01$). Additionally, a noteworthy positive correlation was observed between T-AOC levels and the relative abundance of *Prevotella* ($P < 0.05$) (Fig. 9B). The genus-level microbiome Spearman's correlation network, as depicted in Fig. 9C, revealed intricate interactions among the microbial species present in the samples. In terms of the relative abundance of *Prevotella*, it exhibited a direct positive correlation with *Dorea*, *Akkermansia*, *Phascolarctobacterium*, *Blautia*, *Sutterella*,

Eubacterium, and a direct negative correlation with *Mucispirillum* and *Clostridium*. The phylogenetic tree of intestinal microbes showed that *Prevotella*, *YRC22*, *Barnesiella*, *Odoribacter*, *Parabacteroides*, and *Rikenella* clustered together in the same phylogenetic clades, and they belonged to the phylum Bacteroidetes, and *Prevotella* was particularly enriched in the Succinate group.

Discussion

The incidence of fatty liver increases with laying hens adversely affects both laying performance and egg quality, resulting in economic losses [10, 27, 28]. In this study, the laying hens with relative lower laying rate ($< 80\%$) and higher plasma TG level (> 5 mmol/L) were selected. The result indicated that *Prevotella*, along with its associated metabolite succinate, effectively improved the laying performance of laying hens by alleviating hepatic lipid accumulation. Oral administration of *Prevotella* significantly reduce the plasma TG level. Succinate, the main metabolite from *Prevotella* [35], can play the alternative role in reducing the hepatic lipid droplet by improving mitochondrial function. Additionally, succinate eliminated the challenges with anaerobic culture. This work demonstrated that succinate could be considered as a potential feed additive for enhancing the laying performance and alleviating fatty liver and of laying hens.

Gut microbiome is associated with laying performance of hens [10, 36, 37]. Zhang et al. [22] demonstrated that the application of probiotics, specifically a combination

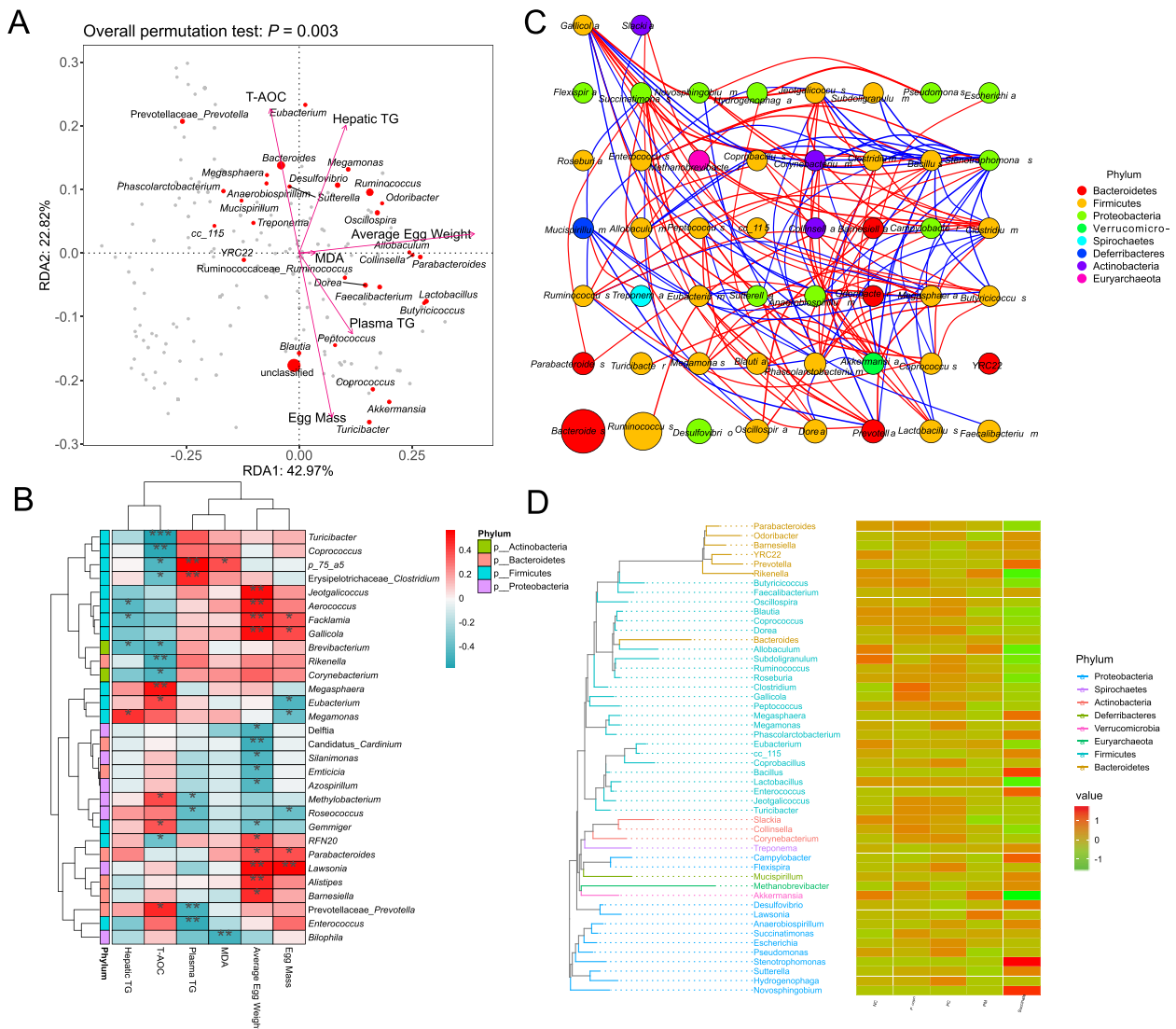


Fig. 9 *Prevotella* and sodium succinate influenced the correlations between intestinal flora and physicochemical parameters. **A** Redundancy analysis (RDA), parameters are represented by arrows. The longer the arrows are, the greater the correlation coefficients are. The Angle between the arrow line and the ordering axis represents the correlation between an environmental factor and the ordering axis. The smaller the Angle is, the higher the correlation will be. The larger the dot, the higher the corresponding species abundance. **B** Spearman's correlation analysis between serum biochemical indexes and cecal microbiota at the phylum level, R-values are shown in different colors in the heatmap, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. **C** The genus-level microbiome Spearman's correlation network, the circles represent a species, the size represents its relative abundance, and different colors represent different phylum species classification. The lines between the circles represent significant correlation between the two species ($P < 0.05$), and the lines color red represents positive correlation and blue represents negative correlation. The thicker the lines, the greater the absolute value of the correlation coefficient. **D** Phylogenetic tree and heatmap, on the left, different colored branches of the evolutionary tree represent different phyla, and each end branch represents an OTU, annotated with the genus classification to which the corresponding OTU belongs, the heat map on the right represents the normalized abundance, with larger values representing higher relative abundance

of heat-inactivated *L. salivarius*^{CB} and *B. subtilis*, led to significant increases in egg production and daily egg yield, as well as significant reductions in feed conversion. In this study, the increased egg mass by *P. copri* treatment compared with PC and NC indicated the beneficial effect of *P. copri* treatment, in line with findings in previous

probiotic research [22, 38]. The improved laying rate and egg mass by PM compared to PC indicating the favorable effect of PM treatment on laying performance. Moreover, the comparable effect of PM with *P. copri* suggests that the positive effect of *Prevotella* on laying performance is consistent across different species. Moreover, the laying

performance was not significantly altered in Succinate group, suggesting the metabolites of PM and *P. copri* play a minor role in the supplemental effect of *Prevotella* on laying performance. Feed intake was not changed by dietary treatment, indicating that *Prevotella* or succinate supplementation has no unfavorable influence on the appetite. In the present study, the supplemental effect of succinate on laying performance was less obvious compared with PM or *P. copri*. The adequate supplemental dose needs further determined.

In previous studies, it is observed that the introduction of probiotic strains like *C. butyricum* could increase eggshell strength [23], and *B. subtilis* was associated with improvements in albumen height and Haugh unit [39]. In this study, however, all the measured parameters related egg quality were not influenced by dietary treatments. If the effect of probiotic strain and their metabolites on egg quality needs to be investigated further.

Liver is the main site for lipid synthesis in poultry, and the occurrence of fatty liver is strongly associated with blood lipid disorders [40–42]. Our work found that none of the three treatments PM, *P. copri*, or Succinate had any detectable influence on the HDL-C level, which aligns with the findings of Wu et al. [43]. Wu et al. [43] reported that the lack of significant changes in HDL-C may be attributed to liver damage without hepatic lipodosis, and the limited diagnostic value of liver function and blood lipid metabolism biomarkers for NAFLD. In the present work, the supplement of *Prevotella* and succinate decrease of plasma TG levels and plasma LDL-C levels. The findings are consistent with a previous study by Zhu et al. [44], which demonstrated that dietary supplementation with an herbaceous mixture could effectively lower serum TG and LDL-C levels. *Prevotella* was shown to effectively reduce TG levels in patients with hyperlipidemia or cardiovascular disease and prevent liver disorders [45]. Elevated TG levels are widely established as the primary characteristic of NAFLD, leading to poor egg production, high mortality and lower production performance in layer chickens [46]. And in our work, we observed a significant improvement in the production performance of laying hens with reduced levels of TG. However, another study by Yao et al. [47] demonstrated that dimethylglycine supplementation could improve the laying rate by reducing of the abdominal fat rate, without affecting plasma TG levels.

Excessive accumulation of TGs can be observed through anatomical and pathological features, including liver enlargement, brittleness, and a tawny color [48]. TG is the main lipid deposited in the liver [49]. The histological changes observed in the livers of these hens provide evidence for anatomical pathological features. The hepatocyte exhibited similarities to those previously described

for NAFLD [50]. Administration of PM or succinate can induce alterations in the pathological changes, thereby indicating a potential alleviation of the TGs accumulation in the liver of laying hens. The decreased abdominal fat accumulation by Succinate was in line with the reduced plasma TG level. The decreased plasma TG level, lipid droplets in hepatocytes, and abdominal fat pad index in PM, *P. copri* or Succinate treatment indicated the arrested development of fatty liver, align with previous works [40, 51], in which the increased lipid droplets and fat vacuoles accumulation were observed in the livers of fatty liver-afflicted laying hens. And these syndromes could be alleviated by dietary probiotics, such as *Bacillus* [38], *L. salivarius* [21], *A. muciniphila* [52], and *C. butyricum* [53].

The de novo hepatic lipogenesis is strongly associated with the functioning of hepatic mitochondria, which is the important contributing factor to NAFLD development [54]. The ACC is the rate-limiting enzyme in fatty acid synthesis and is believed to be responsible for inhibiting fat deposition [55]. Another fatty acid de novo synthesis enzyme, FAS, resides predominantly in the liver and fat tissues, where it facilitates the conversion of acetyl-CoA and malonyl-CoA into long-chain fatty acids [56]. Moreover, down-regulation of the transcription factor SREBP-1c, which binds to the DNA sequence involved in sterol biosynthesis, has been suggested as the cause for the decreased synthesis of sterols and TC. We found that PM or Succinate increased ME and HL activities, while had no obvious influence on the enzyme activities and gene expression related to lipid biosynthesis, suggesting the lipid synthesis play a minor role in the reduced hepatocytic lipid droplet by PM and succinate. The elevated expression of *CPT-1*, a rate-limiting enzyme in mitochondrial β -oxidation, indicated that PM promoted the process of fatty acid β -oxidation. This speculation was supported by the elevated expression of genes related to mitochondrial function. These results are in accordance with previous studies that have reported the prevention of downregulation of fatty acid β -oxidation genes by betaine further, thereby alleviating hepatic lipid accumulation [57]. This result suggested that PM or succinate has a beneficial effect on mitochondrial function.

Mitochondrial dysfunction can lead to electron leakage at respiratory complexes, resulting in the excess accumulation of ROS. And excessive ROS can disrupt the balance between oxidation and antioxidation, inducing lipid oxidation, DNA damage and ultimately resulting in poor production performance in aged hens [50, 58]. MDA is a significant oxidation product derived from peroxidized polyunsaturated fatty acids and serves as a crucial marker for assessing lipid peroxidation [59]. The findings of our study demonstrated that the incorporation of dietary

succinate supplementation led to a significant reduction in MDA and elevated antioxidative enzyme activities, suggesting the suppressed lipid peroxidation. Numerous studies have reported that the antioxidant defense system, which includes T-AOC, CAT and GSH-Px, plays a crucial role in maintaining the balance. T-AOC represented the overall antioxidant effects, CAT converts hydrogen peroxide into water and oxygen [60], and GSH-Px scavenges excessive hydroperoxides [61]. A recent study by Dai et al. [62] found that reducing the level of MDA and increasing the activities of T-AOC and GSH-Px could enhance the capacity to scavenge free radicals in ovary, consequently alleviating the laying performance. In this work, the scavenging of oxygen free radicals was investigated to determine its impact on reducing the attack on polyunsaturated fatty acids in biofilms and lipid peroxidation. Moreover, the study aimed to assess whether this scavenging activity could alleviate liver damage. Similarly, in a previous study, Jian et al. [63] confirmed that fatty liver syndrome (FLS), when combined with oxidative stress, inhibited anti-oxidase production and increased ROS levels. Furthermore, the inclusion of feed additives possessing antioxidative properties, such as pyrroloquinoline quinone, demonstrated a hindrance in the prevention of metabolic dysfunction-associated FLD [64].

The gut microbiota plays an essential role in regulating lipid metabolism of the host [65]. It has been proved that the imbalance of microbial community is associated with hepatic lipid metabolism of laying hens, and laying hens with fatty liver exhibited alterations in both microbial abundance and the composition of their gut microbiota [17, 66]. Hamid et al. [10] discovered that aged laying hens with non-alcoholic steatohepatitis (NASH) exhibited changes in the composition of their intestinal microbiota. Specifically, there was an increased abundance of *Bacteroidetes* and *Proteobacteria*, but a significant decrease in the abundance of *Proteobacteria*. It was commonly known that *Bacteroides* was associated with NASH [67]. In our study, the gut microbiota of laying hens was altered by treatment with *Prevotella* and succinate. Particularly, the succinate treatment demonstrated a notable increase in microbial diversity in the cecal contents, significantly influencing the beta diversity and resulting in a pronounced clustering effect on the gut microbes. It indicated that the alleviation of fatty liver in laying hens is associated with the diversity of intestinal microbiota. The finding, consistent with previous research on fatty liver in laying hens, demonstrated a reduction in gut microbiota diversity, while also suggesting that the condition of fatty liver could be mitigated by enhancing gut microbial diversity through the administration of probiotics or

prebiotics [53, 68]. For example, Xu et al. [68] reported that *L. salivarius* CML352 led to an increase in the beta diversity of cecal microbiota, a reduction in abdominal fat deposition, and an improvement in egg quality among laying hens. Another relevant study indicated that the inclusion of dietary choline could potentially decrease egg production by promoting hepatic lipid deposition and reducing abundances of beneficial intestinal bacteria and microfloral biodiversity in laying hens [66].

More interesting, succinate administration could significantly augment the abundance of *Prevotella* at the genus level, whereas the two strains of *Prevotella* failed to achieve a similar effect. This may be attributed to the relatively mature colonization of intestinal microorganisms in laying hens, and *Prevotella* employed both its own components and metabolites to act on the intestinal flora after entering the body. Upon succinate entering the body, succinate not only exerted direct effect but also significantly promoted the growth of *Prevotella* in the intestinal tract, while synergistically regulating the fat metabolism of laying hens. Similarly, we observed a positive correlation between plasma TG levels and cecal *Prevotella* abundance in this work, providing further evidence for its potential role in alleviating fatty liver in laying hens.

Conclusions

In summary, this study showed that dietary supplementation of PM and *P. copri* both has a favorable effect on laying performance of hens by reducing hepatic lipid accumulation, suggesting that different species of *Prevotella* has comparable beneficial effect on laying performance and prevention of fatty liver development. The result suggests that the beneficial effect is associated with the manipulation of gut microbiota composition. Dietary supplementation of succinate, the primary metabolite of *Prevotella*, induces a moderate suppression of lipid droplets accumulation in hepatocytes, compared with *Prevotella* treatment. The result highlights that succinate presents a more feasible feed additive for alleviating fatty liver in laying hens. The adequate dietary supplemental level of succinate is warranted to further evaluate the effect of succinate on laying performance and the incidence of fatty liver in laying hen.

Abbreviations

| | |
|--------|----------------------------------|
| ACC | Acetyl-CoA carboxylase |
| ASV | Amplicon sequence variant |
| ATP-5B | Adenosine monophosphate-5B |
| BA | Bile acid |
| CAT | Catalase |
| COX IV | Cytochrome IV |
| CPT-1 | Carnitine palmitoyltransferase 1 |
| FAS | Fatty acid synthase |

| | |
|-----------------|-----------------------------------------------------------------|
| FFA | Free fat acid |
| FLD | Fatty liver disease |
| FLS | Fatty liver syndrome |
| GLU | Glucose |
| GSH | Glutathione |
| GSH-Px | Glutathione peroxidase |
| H&E | Hematoxylin and eosin |
| HDL-C | High density lipoprotein cholesterol |
| HL | Hepatic lipase |
| IDH3a | Isocitrate dehydrogenase 3a |
| LDL-C | Low density lipoprotein cholesterol |
| LEFSe | Linear discriminant analysis effect size |
| LPL | Lipoprotein lipase |
| MDA | Malondialdehyde |
| ME | Malic enzyme |
| NAFLD | Non-alcoholic fatty liver disease |
| NASH | Non-alcoholic steatohepatitis |
| NC | Negative control |
| NRF1 | Nuclear respiratory factor 1 |
| ORO | Oil Red O |
| OTU | Operational taxonomic unit |
| <i>P. Copri</i> | <i>Prevotella copri</i> |
| PC | Positive control |
| PCoA | Principal coordinate analysis |
| PGC-1 α | Peroxisome proliferator-activated receptor γ -1 α |
| PLS-DA | Partial least squares discriminant analysis |
| PM | <i>Prevotella melaninogenica</i> group |
| PPAR γ | Peroxisome proliferator-activated receptor γ |
| RDA | Redundancy analysis |
| ROS | Reactive oxygen species |
| SCFAs | Short-chain fatty acids |
| SOD | Superoxide dismutase |
| SREBP-1c | Sterol regulatory element binding proteins-1c |
| Succinate | Succinate group |
| T-AOC | Total antioxidant capacity |
| TCH | Total cholesterol |
| TFAM | Mitochondrial transcription factor A |
| TG | Triglyceride |

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s40104-023-00975-5>.

Additional file 1: Fig. S1. The initial plasma parameters during grouping of hens.

Additional file 2: Table S1. Effect of *Prevotella* and sodium succinate on the egg quality at week 4. **Table S2.** Effect of *Prevotella* and sodium succinate on the egg quality at week 8.

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Not applicable.

Authors' contributions

ML and ZYK conducted the experiments; ML, ZYK and XKC processed the experimental data, prepared the paper and designed the figures; H CJ, XJW, and JPZ were involved in planning and work supervision; HL and ML contributed to the research design, funding acquirement, project supervision, and paper reviewing and editing. The results were discussed, and the paper was commented on by all authors.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Declarations

Ethics approval and consent to participate

All the experimental protocol in this work were approved by the Ethics Committee of the Shandong Agricultural University and conducted in accordance with the "Guidelines for Experimental Animals" of the Ministry of Science and Technology (Beijing, China).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflicts of interest.

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