

# Comparison of gut microbiome and plasma metabolome profiles between domesticated and wild Eurasian perch (*Perca fluviatilis*)

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## Abstract

Domestication has strongly influenced the physiological characteristics of fish due to selection pressure from human beings and local environments. Here, we comprehensively analysed the gut microbiome and plasma metabolome through 16S rRNA sequencing and untargeted metabolomic detection using liquid chromatography–mass spectrometry. We found significant divergences in the gut microbiota and plasma metabolome between domesticated and wild *Perca fluviatilis*. The reduced abundance of Proteobacteria in the gut and lowered abundance of L-lysine, L-valine, piperidine, beta-alanine, L-glutamate and L-histidine in the plasma metabolome of domesticated *P. fluviatilis* indicated potential reduced protein digestion and absorption capability in domesticated *P. fluviatilis*. By contrast, the increased abundance of Bacteroidetes in the gut might imply the improved digestive capability for plant polysaccharides in domesticated *P. fluviatilis*. Digestive system alterations may be due to changed selection pressures on diet during long-term domestication. Our study provided preliminary results on the physiological changes in domesticated and wild *P. fluviatilis* which will affect the digestive system and growth performance of *P. fluviatilis*. The data obtained in this study will benefit germplasm improvement programmes for *P. fluviatilis* in the future.

## KEY WORDS

digestion, domestication, gut microbiome, immune, metabolome

## 1 | INTRODUCTION

Domestication is a great innovation that has dramatically changed human life, helped human ancestors build farming societies and promoted human civilization (Wang et al., 2014). It is a sustained process of utilizing and enhancing the specific attributes of organisms to provide agricultural resources and satisfy the specific requirements of human beings (Zeder, 2012). Long-term domestication causes a series of phenotypic and genetic changes that may be evident in the morphology, physiology, behaviour and reproduction of a species (Bélteky et al., 2016; Larson et al., 2014). Domestication

has increased the seed oil content of cultivated soybean and peanut compared with that of their wild accessions (Zhang et al., 2019; Zhuang et al., 2019). Domestication caused modern dogs to thrive on a starch-rich diet instead of the carnivorous diet of their wolf ancestors (Axelsson et al., 2013) and increased the meat-production and egg-laying capacity of chickens (Rubin et al., 2010). Aquatic species play essential roles in providing high-quality protein resources and promoting economic development worldwide (Houston et al., 2020). Compared with agricultural plants, livestock and poultry species, the domestication process of aquatic species is in its early stages. However, aquatic species has more advantages than

agricultural species, such as high genetic diversity, high fertility and external fertilization, indicating the great potential for genetic improvement programmes in the future (Houston et al., 2020; Teletchea & Fontaine, 2014). Therefore, understanding the underpinnings of the domestication is critical for future genetic improvement programmes.

Eurasian perch (*Perca fluviatilis*) is a common predatory freshwater fish, that is naturally distributed in Europe and northern Asia (Ben Khadher et al., 2016). It is also found in the Irtysh (Eltrixhe) River in the northern part of Xinjiang Province, China (Ren et al., 2002). High-quality fish fillets are warmly welcomed in the local market, and *P. fluviatilis* is considered to be a promising candidate species for inland aquaculture (Stejskal et al., 2011). The domestication of *P. fluviatilis* started in the early 1990s, and long-term domestication has significantly shaped the phenotype, physiology and genetic characteristics of this species (Ben Khadher et al., 2016). Different digestion abilities were presented between wild and domesticated *P. fluviatilis* (Palińska-Żarska et al., 2020). Wild *P. fluviatilis* presented higher arachidonic acid (ARA, 20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3) and linoleic acid (LA, 18:2n-6) than that in domesticated *P. fluviatilis* (Mairesse et al., 2006). Wild and domesticated *P. fluviatilis* presented different stress response and affected their growth performance (Douxfil, Mandiki, et al., 2011a; Douxfils, Mathieu, et al., 2011b). It is reported that higher HSP70 protein and cortisol level, along with various changes in the abundance of serum proteins related to immunity and acute phase response were observed in domesticated *P. fluviatilis* (Douxfil et al., 2012; Douxfils, Mandiki, et al., 2011a; Douxfils, Mathieu, et al., 2011b; Mairesse et al., 2006; Stejskal et al., 2011). Domesticated *P. fluviatilis* can acclimate to the rearing systems (extensive, semi-extensive and intensive system) and presented normal growth performance (Mairesse et al., 2005). Moreover, our previous study also indicated that domestication may have altered the digestive and immune system of *P. fluviatilis* through comparative transcriptome analysis (Chen, Chen, et al., 2017a; Chen, Wang, et al., 2017b).

It is well known that long-term domestication could at some content change the digestive system, immune system, endocrine system of domesticated species due to human selection (Fabrice, 2019; Wang et al., 2014). Recently, the gut microbiota has been extensively researched and played fundamental roles in many aspects of organisms, such as growth, development and immunology (Alberdi et al., 2016; Chen, Chen, et al., 2017a; Chen, Wang, et al., 2017b; Chevalier et al., 2015; Chuang et al., 2020; Jarak et al., 2018; Singh et al., 2015; Sommer et al., 2016). Moreover, the metabolomic analysis provides valuable tools to illustrate the physiological changes in organisms and have been widely utilized for detection and identification of key metabolites in the organisms in different conditions (Johnson et al., 2016). Although many related functional studies were conducted on the physiological changes in domesticated *P. fluviatilis*, the impacts of long-term domestication on the *P. fluviatilis* gut microbiome and metabolome profiles were still limited and not in-depth studied. We have conducted domestication of *P. fluviatilis* in Xinjiang Province of China in early 2000s. The founding population

was sampled from Irtysh (Eltrixhe) River (47°25'N, 87°35'E). During the domestication process, we provided artificial formula feed for daily diet and parental fish with fast growth rate and five clear strips on the skin were selected for the next generation breeding. After successive generations of selective breeding, the growth rate of the domesticated *P. fluviatilis* was 15–20% faster than that of wild *P. fluviatilis* (Data unpublished). In the present study, gut microbiome and plasma metabolome sequencing were conducted and microbiome and metabolome profiles between wild and domesticated *P. fluviatilis* were compared and finally the obtained results were linked to the physiological variation of the studied fish.

## 2 | MATERIALS AND METHODS

### 2.1 | Sample collection and ethics statement

In this study, wild adult *P. fluviatilis* (WP) individuals were collected from Irtysh (Eltrixhe) River (Xinjiang, China) (47°25'N, 87°35'E). Domesticated *P. fluviatilis* (DP) individuals from the sixth generation (F6 progenies) were collected from the Fisheries Technology Extension Station of Xinjiang Production and Construction Corps (Urumqi, Xinjiang). All the *P. fluviatilis* were cultured in artificial ponds and raised with *P. fluviatilis* formula feed twice a day, which was bought from the Wanghai Feed Industry Company Limited (Guangdong, China) from May to September 2019. To keep consistency, all the selected wild and domesticated *P. fluviatilis* for the experiment (10 biological replicates for each group, 2-year-old) were with nearly the same body weight and body length ( $650.04 \pm 2.94$  g,  $25.13 \pm 0.27$  cm for DP,  $649.45 \pm 2.54$  g,  $24.94 \pm 0.28$  for WP,  $p > 0.05$ ). Before dissection, all the collected individuals were temporarily reared and fasted in two identical plastic containers for 24 h. After 24 h, the collected *P. fluviatilis* were quickly dissected after being washed with phosphate buffer. After dissection, the midgut tissues from the WP and DP groups were collected, snap-frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  before the experiment. A total of 500  $\mu\text{l}$  whole blood was extracted from *P. fluviatilis* individuals using a sterile injection syringe with anticoagulant. The plasma was then extracted after the centrifugation of whole blood at 10,000g for 10 min at  $4^{\circ}\text{C}$ . This study was approved by the Institutional Animal Care and Use Committee of Shanghai Ocean University (Shanghai, China). Sampling procedures complied with the guidelines of the Institutional Animal Care and Use Committee on the care and use of animals for scientific purposes.

### 2.2 | 16s RNA sequencing and data analysis

DNA was extracted from the midguts of collected samples ( $n = 10$  for each group) by using FastDNA SPIN Kit for Faeces (MP Biomedical) in accordance with the manufacturer's protocols. The concentration and purity of extracted DNA were evaluated with a NanoDrop 2000 platform (Shanghai, China). We amplified the V3–V4 region of

16s RNA by using 338F (5'-ACTCCTACGGAGGCAGCAG-3') and 806R (5'-GGACTTACHVGGGTWTCTAA-3') primers. The PCR was performed in a 20 $\mu$ l PCR reaction mixture, including 10 ng DNA, 4  $\mu$ l FastPfu Buffer, 2  $\mu$ l 2.5 mM dNTPs, 0.8  $\mu$ l of 338F and 806R primers, 0.4  $\mu$ l FastPfu Polymerase, 0.2  $\mu$ l BSA and ddH<sub>2</sub>O. The PCR procedure used was: 95°C for 3 min, followed by 28 cycles of 95°C for 30 s, 55°C for 30 s and 72°C for 45 s. Paired-end sequencing libraries (PE300) were constructed by using a TruSeqTM DNA Sample Prep Kit (Illumina, San Diego, USA). Constructed sequencing libraries were sequenced on an Illumina MiSeq platform (Illumina, San Diego, CA, USA).

Raw sequencing reads were first quality-filtered before data processing by using fastp v0.19.6 software. Reads with an average quality score below 20 in a 50 bp sliding window were trimmed, reads with quality below 20 at the end were removed and reads shorter than 50 bp were removed for further analysis (Chen et al., 2018). After filtering, the paired-end reads were merged into a consensus sequence with overlaps longer than 10 bp between sequencing reads and mismatch error rates <2% by utilizing FLASH v1.2.11 (Magoč & Salzberg, 2011). Sequences with  $\geq 97\%$  similarity were clustered into representative operational taxonomic units (OTUs) with UPARSE (7.0.1090) (Edgar, 2013). For each individual in the WP and DP groups, the numbers of OTUs were recorded and summarized with USEARCH 7.0 (Edgar, 2013). We conducted subsampling for each sample by using the minimum sequencing depth to reduce sequence bias. The representative sequences of each OTU were selected to annotate taxonomic information by using the Ribosomal Database Project classifier with an identity threshold of 0.7 based on Silva database (version 138) (Wang et al., 2007). OTU level alpha diversity was calculated using Sobs, Chao and Shannon indexes by applying Mothur 1.30.2 software (Schloss et al., 2009). The abundance weighted jaccard distance matrixes were used to calculate beta diversity and were visualized via principal coordinate analyses (PCoA). Analysis of similarities (ANOSIM) was conducted to detect differences between the WP and DP groups by using QIIME software (Caporaso et al., 2010) with abundance weighted jaccard distance.

## 2.3 | Plasma liquid chromatography-mass spectrometry metabolomic processing and data analysis

Untargeted metabolomic analysis was conducted using liquid chromatography-mass spectrometry (LC-MS) technology with extracted 10 plasma samples from each group. A total of 100  $\mu$ l of extracted plasma was mixed with 400  $\mu$ l of biochemical solution (methanol: acetonitrile = 1:1) and 20  $\mu$ l of the internal standard. Then, each sample was vortexed, ultrasonically extracted (5°C, 40 KHz), incubated at -20°C for 30 min and centrifuged at 11,000 g for 15 min at 4°C. Next, the supernatant was extracted and dried. A total of 120  $\mu$ l of 50% acetonitrile solution was used to redissolve dried samples for LC-MS analysis. The LC-MS experiment was performed using a Thermo UHPLC system equipped with a binary solvent delivery

manager and a sample manager coupled with a Thermo Q Exactive HF-X Mass Spectrometer equipped with an electrospray interface. Quality control (QC) samples were measured during the entire experiment to assess the reproducibility of our dataset. Equal volumes of QC samples from each sample were mixed to assess the reproducibility and reliability of the LC-MS system. Mass spectrometric data were collected by using a Thermo UHPLC-Q Exactive HF-X Mass Spectrometer equipped with an electrospray ionization source operating in either positive or negative ion mode.

Generated raw data were processed by using Progenesis QI (Waters Corporation, Milford, CT, USA) for peak picking, peak alignment and peak filtering. Then, the data matrixes for retention time, M/Z and peak intensity were normalized under the following parameters: (a) only the metabolites present in >80% of all the samples within a group were retained; (b) missing values were replaced with 1/2 of the minimum value; (c) peak intensities were normalized to the total spectral intensity; and (d) raw data were log10 transformed to obtain the final data set. Then, the normalized data were used to obtain accurate qualitative results for each metabolite by matching against HMDB (<http://www.hmdb.ca/>) and the Metlin (<https://metlin.scripps.edu>) public database (Wishart et al., 2017).

Principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) were conducted to visualize metabolic differences between the WP and DP groups using the ROPLS v1.6.2 software package with positive and negative data (Thévenot et al., 2015). Differential metabolites (DMs) between groups were identified with variable importance in the projection (VIP)>1 and  $p<0.05$ . DMs were subjected to KEGG enrichment analysis by using the software MetaboAnalyst 4.0. We determined the impact value and -log(P) and performed metabolic pathway analysis by using MetaboAnalyst 4.0 to gain further insight into the underlying biological mechanisms associated with domestication (Chong et al., 2019).

## 3 | RESULTS

### 3.1 | Differences in gut microbial communities between the WP and DP groups

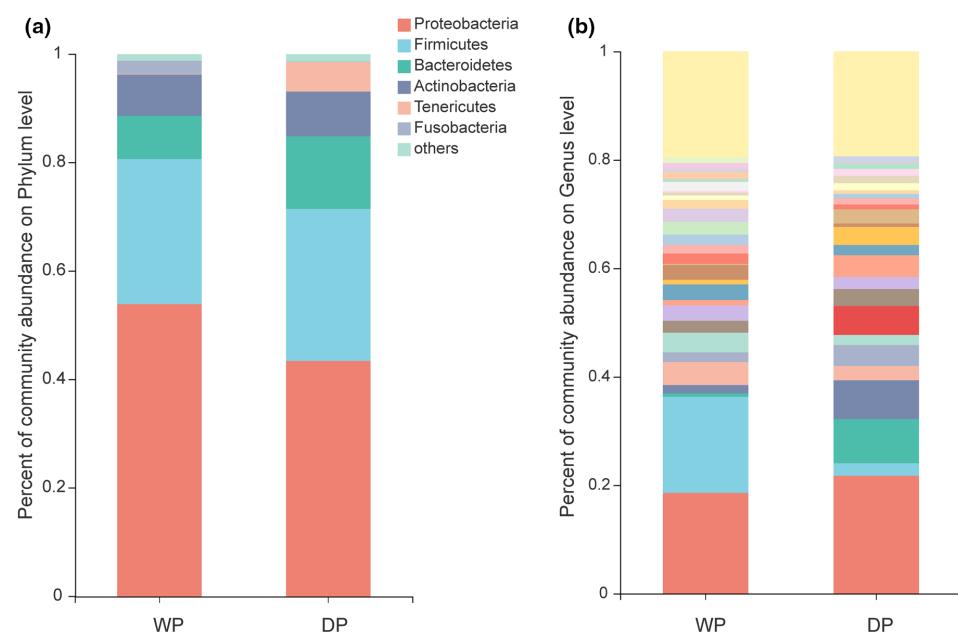
On average  $45,050 \pm 10,161$  and  $45,294 \pm 5905$  sequences with average read length  $420.08 \pm 4.64$  bp and  $419.98 \pm 4.26$  bp were obtained after sequencing for DP and WP groups, respectively. After sequence clustering and annotation, a total of 1998 different OTUs, representing 28 bacterial phyla and 636 bacterial genera were identified. The rarefaction curves of the number of reads sampled and Sobs index on the OTU level indicated the nearly saturated sampling of the microbial communities (Figure S1).

Venn diagram analysis indicated that the WP and DP groups shared 1041 OTUs, whereas 578 and 379 OTUs were identified only in the DP and WP groups, respectively (Figure S2). The WP and DP groups presented various gut microbiome compositions and abundances, the dominant bacterial phyla in *P. fluvialis*

included Proteobacteria, Firmicutes, Bacteroides, Actinobacteria, Tenericutes and Fusobacteria (Figure 1a). Compared with the DP group, the WP group harboured a higher proportion of Proteobacteria and Fusobacteria. Compared with the WP group, the DP group harboured a higher proportion of Bacteroidetes and Tenericutes (Figure 1a,b). The top three bacterial genera in the WP group were *Burkholderia-Caballeronia-Paraburkholderia* (18.56%), *Aeromonas* (17.73%) and *Rhodococcus* (4.23%), whereas those in the DP group were *Burkholderia-Caballeronia-Paraburkholderia* (21.73%), *Muribaculaceae* (8.16%) and *Klebsiella* (7.16%) (Figure 1b). At the phylum level, the proportion of Proteobacteria, Bacteroidetes, and Tenericutes was slightly different between the DP and the WP groups (Student's t-test,  $p > 0.05$ , Figure 2a). Meanwhile, at the genus level, the proportion of *Klebsiella* and *Lactobacillus* were significantly different between the DP and WP groups (Student's t-test,  $p < 0.05$ , Figure 2b).

### 3.2 | Variations in diversity indexes between the WP and DP groups

The alpha diversity of Sobs (the number of observed OTUs) and Shannon index in the DP group were slightly higher than those in the WP groups, although no significant difference was observed ( $p > 0.05$ ) (Figure 3a,b). The ANOSIM analysis using abundance weighted jaccard distance based on the total samples showed that significant different gut microbiome communities between the WP and the DP groups ( $R = 0.4927$ ,  $p < 0.01$ ), and beta diversity analysis using PCoA clustering revealed a clear separation between the DP and WP groups (Figure 3c,d).



**FIGURE 1** Composition and proportion of the gut microbiome of the wild (WP) and domesticated *P. fluviatilis* (DP). (a) Compositions and proportion of the gut microbiome at phylum level of the wild and domesticated *P. fluviatilis*. (b) Compositions and proportion of the gut microbiome at genus level of the wild and domesticated *P. fluviatilis*.

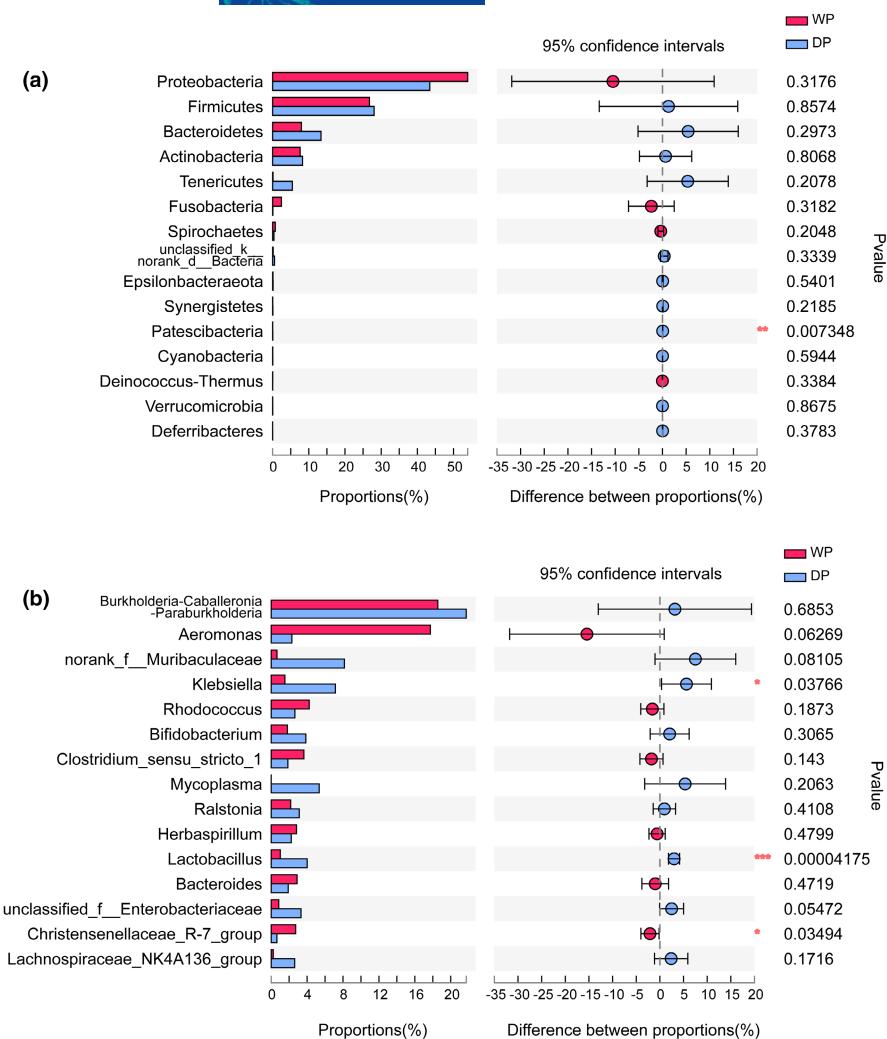
### 3.3 | Plasma metabolomic profiles of the WP and the DP groups

A total of 591 annotated metabolites (442 in positive ionized mode and 149 in negative ionized mode) were identified in *P. fluviatilis*. PCA and PLS-DA were performed to obtain a global overview of the metabolites identified in the groups. The positive and negative data revealed clear separation and discrimination between the two groups, indicating significantly divergent metabolomic profiles between the WP and DP groups (Figure S3).

A total of 194 DMs with  $VIP > 1$  and  $p < 0.05$  were identified, among which 63 were upregulated and 131 were downregulated in the DP group relative to the WP group. OPLS-DA score plots were generated on the basis of DMs, and the results presented significant discrimination between the two groups (Figure 4a,b). The OPLS-DA model was validated via the 200 permutation test, and the  $R^2$  value (0.6807 for positive mode and 0.7108 for negative mode) and Q2 value (-0.423 for positive mode and -0.2457 for negative mode) indicated the satisfactory effectiveness of the model (Figure 4c,d).

Among the DMs, 88 were classified as lipids and lipid-like molecules, 19 as organic acids and derivatives, 16 as organoheterocyclic compounds, 7 as organic oxygen compounds and 6 as phenylpropanoids and polyketides (Figure S4). Furthermore, KEGG enrichment analysis indicated that upregulated metabolites in the DP group were enriched in the glycerophospholipid metabolism, purine metabolism, cholinergic synapse, arginine and proline metabolic pathways (Figure 5a). Downregulated metabolites in the DP groups were enriched in protein digestion and absorption, taurine and hypotaurine metabolism, linoleic acid metabolism and steroid hormone biosynthesis (Figure 5a). KEGG topology analysis also indicated that

<i>Burkholderia-Caballeronia-Paraburkholderia</i>
<i>Aeromonas</i>
<i>norank_f_Muribaculaceae</i>
<i>Klebsiella</i>
<i>Rhodococcus</i>
<i>Bifidobacterium</i>
<i>Clostridium_sensu_stricto_1</i>
<i>Mycoplasma</i>
<i>Ralstonia</i>
<i>Herbaspirillum</i>
<i>Lactobacillus</i>
<i>Bacteroides</i>
<i>unclassified_f_Enterobacteriaceae</i>
<i>Christensenellaceae_R-7_group</i>
<i>Lachnospiraceae_NK4A136_group</i>
<i>Faecalibacterium</i>
<i>Blautia</i>
<i>Terrisporobacter</i>
<i>Acinetobacter</i>
<i>Cetobacterium</i>
<i>Escherichia-Shigella</i>
<i>Subdoligranulum</i>
<i>unclassified_f_Lachnospiraceae</i>
<i>Ruminococcaceae_UCG-014</i>
<i>Chryseobacterium</i>
<i>Collinsella</i>
<i>Shewanella</i>
<i>Streptococcus</i>
<i>Flavobacterium</i>
<i>Sphingomonas</i>
others



**FIGURE 2** Comparison of the gut microbiome communities between wild (WP) and domesticated *P. fluviatilis* (DP). (a) Pairwise comparison of the microbiome communities between the WP and DP groups at the phylum level. (b) Pairwise comparison of the gut microbiome communities between the WP and DP groups at the genus level.

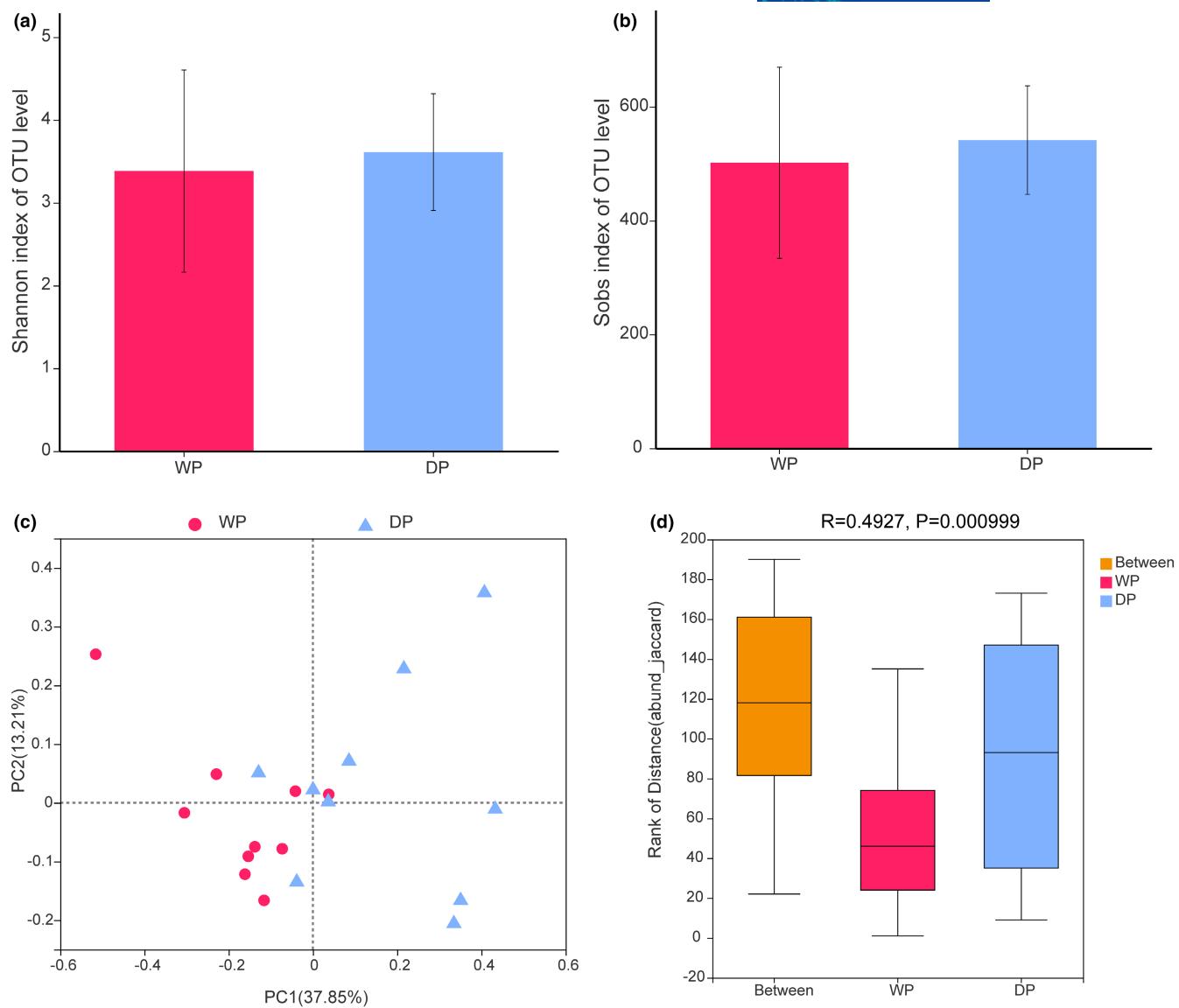
the glycerophospholipid metabolism, histidine metabolism, beta-alanine metabolism, taurine and hypotaurine metabolism and citrate cycle (TCA cycle) metabolic pathways showed reduced *p* values and increased pathway impact (>0.1) between the DP and the WP groups (Figure 5b). Meanwhile, the abundance of L-lysine, L-valine, piperidine, beta-alanine, L-glutamate, L-histidine in the protein digestion and absorption pathway and the abundance of 9-OxoODE, 9,10,13-TriHOME, 9,12,13-TriHOME, and PC (22:6/22:6) in linoleic acid metabolism in the DP group were all significantly lower than those in the WP group (Figures 6a,b and S5). The abundance of inosine, hypoxanthine, guanine and guanosine involved in purine metabolism in the DP group were higher than those in the WP group. The abundance of cortisol in the WP group was higher than that in the DP group (Figure S5).

## 4 | DISCUSSION

Domestication is the purposeful culture of organisms in a given environment; it involves changing the living space, dietary resources, and behaviour of an organism to satisfy the special needs of human beings (Zeder, 2012). This process has exerted strong selection

pressures that have shaped the phenotypic, physiological and even genetic characteristics of organisms (Bélteky et al., 2016; Palińska-Żarska et al., 2020; Teletchea & Fontaine, 2014). In this study, the domesticated *P. fluviatilis* has been domesticated for up to six generations with strong selection on growth rate. They presented a fast growth rate and are able to live on formula feed compared with wild *P. fluviatilis*. The significant divergence in the gut microbiome and plasma metabolome between the DP and WP groups may indicate that domestication has effected the gut microbiome and plasma metabolome profiles of *P. fluviatilis*. This change might have contributed to the varied physiological characteristics of domesticated *P. fluviatilis*.

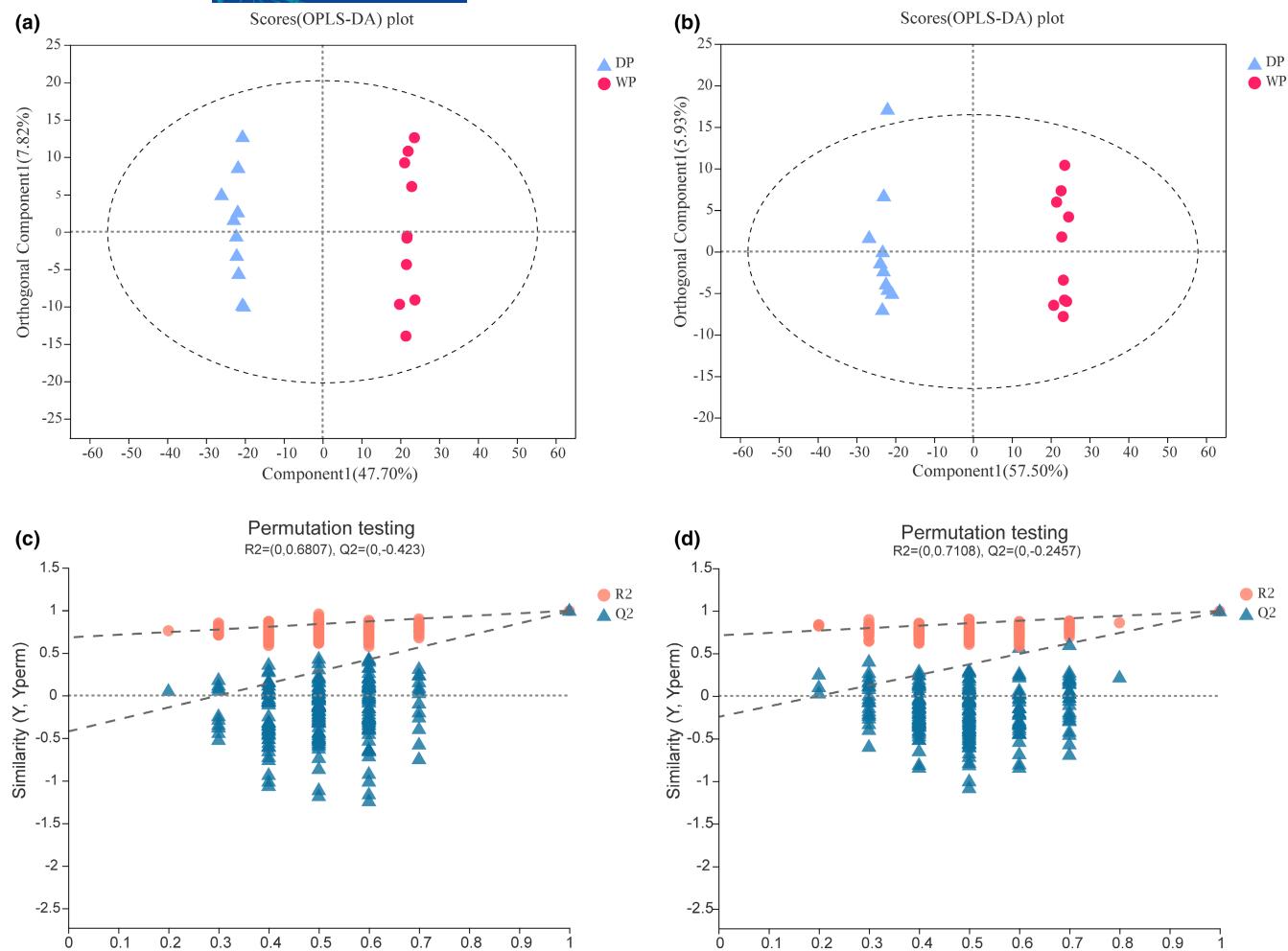
Domestication has altered the digestive system of many domesticated organisms. Through domestication, dogs adapted to a starch-rich diet instead of the carnivorous diet of wolves (Zeder, 2012), whereas cats developed a hypercarnivorous diet due to the positive selection of genes that are enriched in lipid metabolism pathways (Chuang et al., 2020). Among the physiological characteristics that varied between wild and domesticated *P. fluviatilis*, the most interesting is the change in the original dietary habits of domesticated *P. fluviatilis*, which accept formulated feed diet as its food source; by contrast, wild *P. fluviatilis* is a piscivorous predator



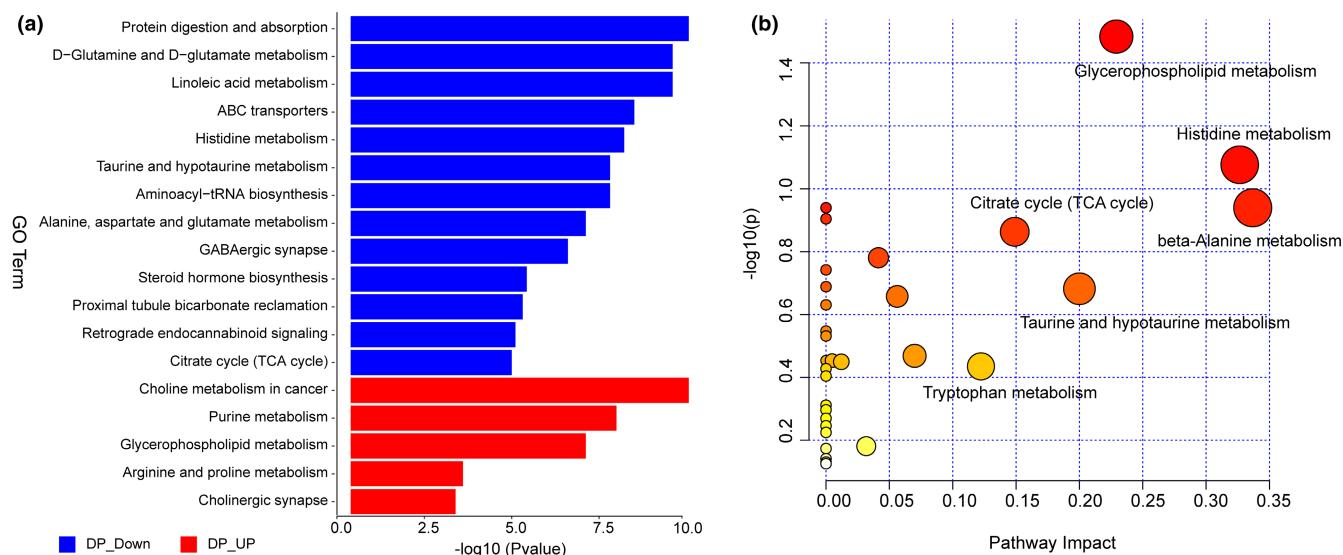
**FIGURE 3** Alpha and beta diversity analyses of the gut microbiota between wild (WP) and domesticated *P. fluviatilis* (DP). (a) Alpha diversity (Shannon index) estimate at the OTU level between the WP and DP groups. (b) Alpha diversity (sobs index) estimate at the OTU level between the WP and DP groups. (c) Beta diversity (PCoA) estimates for the gut bacterial communities of the WP and DP groups. (d) ANOSIM estimate for the WP and DP groups with abundance weighted jaccard distance.

that feeds on live macroinvertebrates and fish (Ceccuzzi et al., 2011; Mustamäki et al., 2014). In this study, we found that the abundance of Proteobacteria in the WP group was higher than that in the DP group (Figure 1). Proteobacteria is significantly elevated in the guts of carnivorous fish, dogs and cats when fed with high-protein diets (Gao et al., 2020; Moon et al., 2018). Moreover, the relative abundances of L-lysine, L-valine, piperidine, beta-alanine, L-glutamate and L-histidine involved in the protein digestion and absorption pathway in the plasma metabolome of the WP group were all higher than those in the plasma metabolome of the DP group. We previously pointed out that digestion-related genes, such as trypsin-1 (*try1*), chymotrypsin-like protease *CTRL-1* (*ctrl*) and chymotrypsin B (*ctrb*), in the protein digestion and absorption pathway are expressed at low levels in the domesticated group, indicating that this molecular

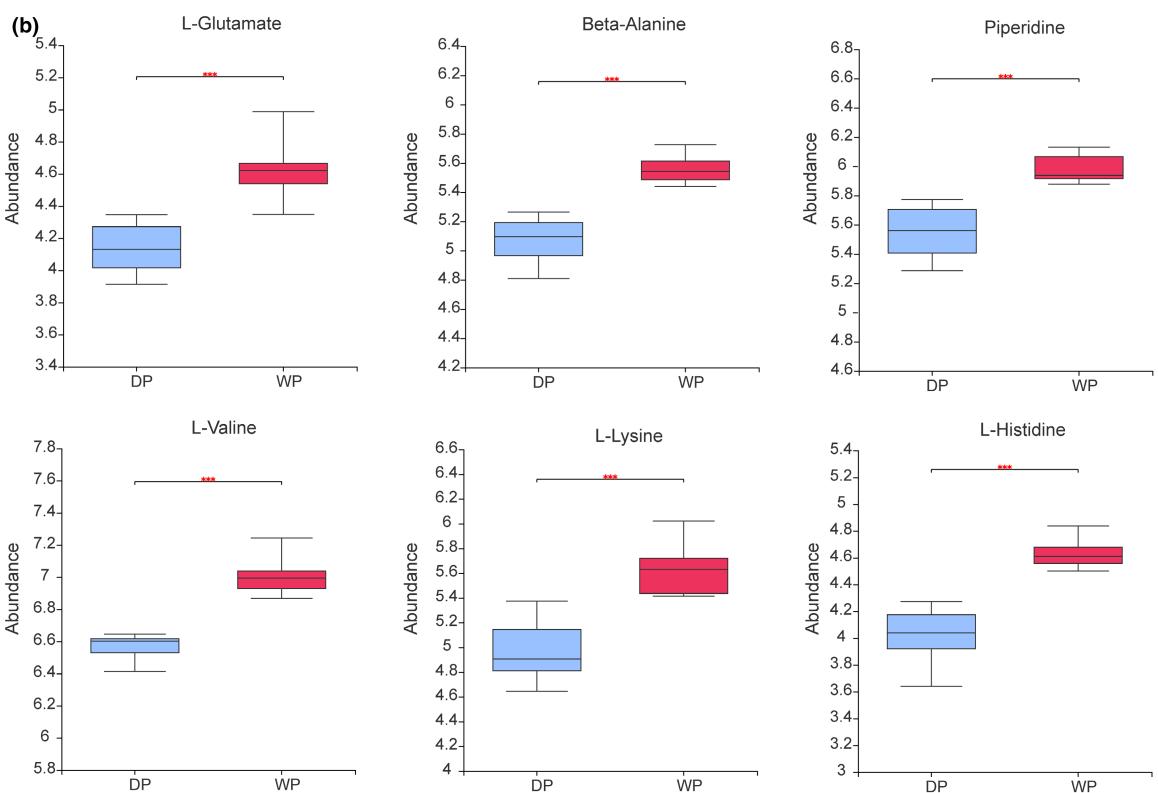
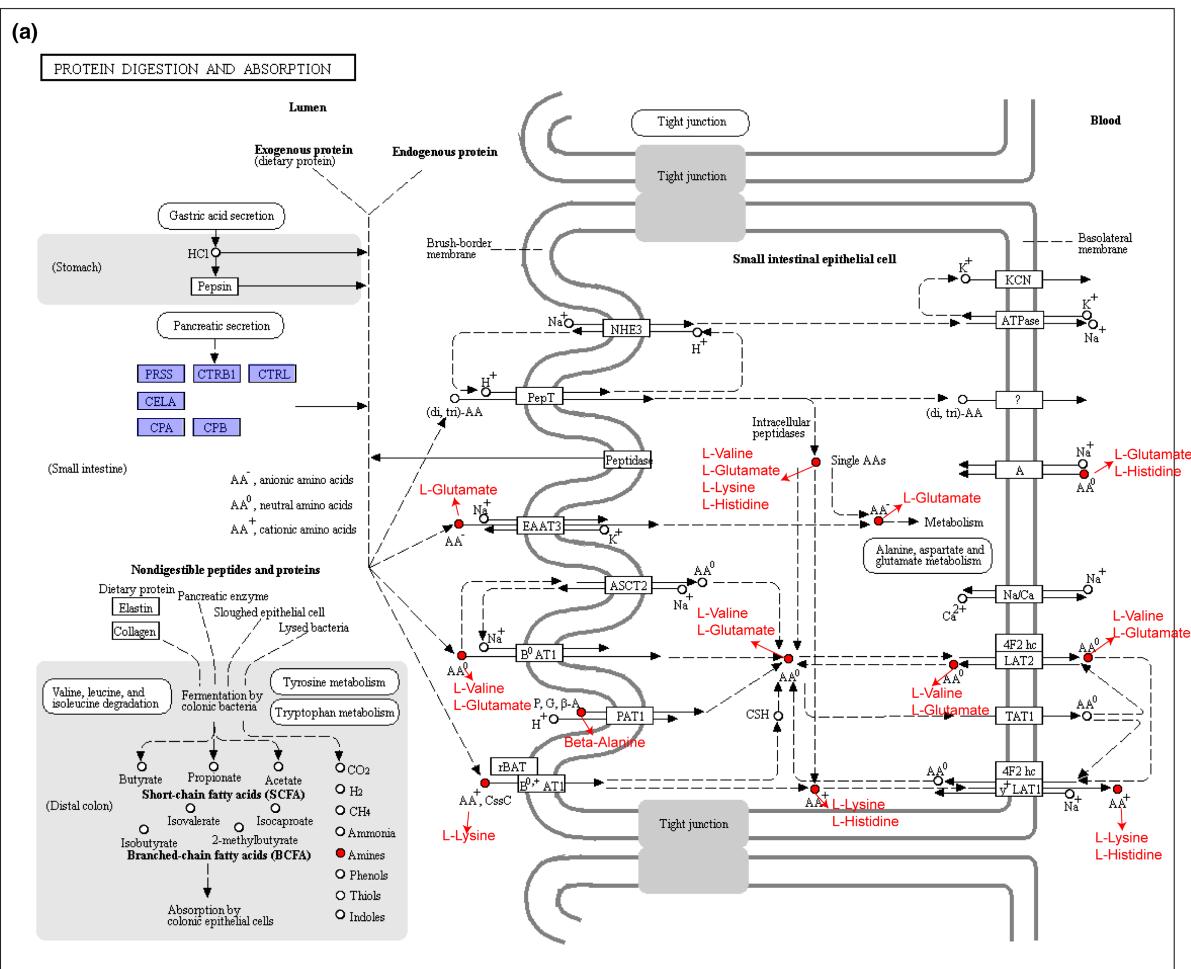
pathway is downregulated in domesticated *P. fluviatilis* (Chen, Chen, et al., 2017a; Chen, Wang, et al., 2017b). *P. fluviatilis* living in the wild environment requires superior protein digestion and absorption capability to extract the required energy from a limited carnivorous diet supply. By contrast, the provision of an abundant formula feed to domesticated *P. fluviatilis* reduces the selection pressure exerted by diet resources and abundance. Thus, after generations of domestication, the domesticated *P. fluviatilis* may have shaped the digestive capability to adapt to artificial rearing systems with formula feed. Meanwhile, a recent research found that the activity of the enzymes and the expression levels of genes encoding for digestive enzymes in domesticated larvae are lower than those in the wild larvae of *P. fluviatilis*, which also support our results (Palińska-Żarska et al., 2020). Collectively, our results suggested potential reductions



**FIGURE 4** Orthogonal partial least squares discriminant analysis (OPLS-DA) plot of plasma metabolites in comparisons of the wild (WP) and domesticated *P. fluviatilis* groups (DP) following (a) positive and (b) negative mode ionization. Permutation test result of the OPLS-DA models of differential plasma metabolites between the WP and DP groups in the positive (c) and negative (d) modes.



**FIGURE 5** KEGG pathway analysis on the differential metabolites between the wild (WP) and domesticated *P. fluviatilis* groups (DP). (a) KEGG enrichment analysis on the identified differential metabolites. (b) KEGG topology analysis on the identified differential metabolites.



**FIGURE 6** Altered protein digestion and absorption metabolic pathway in domesticated *P. fluviatilis*. (a) Downregulated metabolites (labelled with red) involved in protein digestion and absorption metabolic pathway of domesticated *P. fluviatilis*. Note: Genes in the blue box were identified as downregulated in domesticated *P. fluviatilis* in our previous study (Chen, Wang, et al., 2017b). (b) The relative abundance of identified differential metabolites between wild (WP) and domesticated *P. fluviatilis* groups (DP) in protein digestion and absorption metabolic pathway.

in the protein digestion and absorption capability of *P. fluviatilis* during domestication.

Formulated feed diets are provided to domesticated *P. fluviatilis* rearing in artificial conditions. In aquaculture, plant protein sources are widely used to replace animal protein resources in formula feed diets to save cost (Huang et al., 2018). Therefore, the plant polysaccharides content of the formula feed diet for domesticated *P. fluviatilis* is higher than that for wild *P. fluviatilis*, which is mainly fed on animal-type diets. Bacteroidetes, which is believed to play essential roles in the degradation of complex plant polysaccharides, such as starch and cellulose, dominates the gut microbiome of herbivorous fish (Naas et al., 2014). In this study, the high abundance of Bacteroidetes in domesticated *P. fluviatilis* may suggest improved digestive capability for plant polysaccharides during domestication. However, the varied gut microbiotas may be also attributed to different feed types of wild and domesticated *P. fluviatilis*, although they were cultured in the same environment. Together, domestication might alter the composition of the gut microbiome to improve the digestive capability for plant polysaccharides for adaptation to changes in feed diet habit.

We also discovered that the abundance of cortisol in the plasma metabolome of the domesticated *P. fluviatilis* were significantly lower than those in the plasma metabolome of wild *P. fluviatilis* (Figure S5). As reported, cortisol is a biomarker that reflects of stress levels in organisms, and its high levels are usually associated with increased stress response (Douxifils, Mandiki, et al., 2011a; Douxifils, Mathieu, et al., 2011b). The varied cortisol level between the DP and WP groups may be due to the varied environmental stress or may imply that long-term domestication impacts the physiological stress response of *P. fluviatilis* to a certain extent. In this study, domesticated *P. fluviatilis* has been cultured in controlled ponds for generations. Sensitivity to environmental stress is a vital breeding character that is selected by breeders during selective breeding practices (Lepage et al., 2000). Fish individuals with high sentensity to environment stress usually cause injuries and lead to inferior growth performance. They will not be selected for parental fish for subsequent selective breeding and after generations of selective breeding, the domesticated fish may be less sententive to environmental stress (Lepage et al., 2000). However, cortisol only can not truly reflect the sentensity to environmental stress between domesticated and wild *P. fluviatilis*, further detailed experiments are needed to verify this hypothesis.

In conclusion, through the analysis on gut microbiome and plasma metabolome, we demonstrated that long-term domestication may alter the physiological characteristics of *P. fluviatilis*, although we could not neglect the effect of environmental stress and/or other effects. Reduced protein digestion and absorption capability and

improved plant polysaccharide digestion capability could be attributed to altered selection pressure on diet resources and abundance during domestication. However, more comprehensive genetic work needs to be conducted in future to support the hypothesis.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## AUTHOR CONTRIBUTIONS

Xiaowen Chen performed experiments, statistical analysis, and drafted paper; Long Qian, Tao Ai and Wei Xiang curated data and performed experiments; Xin Hou, Qinyu Xu and Bolin Hu collected samples and performed experiments; Jun Wang conceptualized study, performed statistical analysis, drafted paper and revised paper; Chenghui Wang conceptualized study, supervised experiments, acquired funding, edited paper. All authors have read and approved the final manuscript.

## DATA AVAILABILITY STATEMENT

All sequences have been uploaded to the NCBI sequences read archive under BioProject accession numbers PRJNA674947.

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