Animal Microbiome

RESEARCH

Open Access

Dynamic development of gut microbiota and metabolism during and after weaning of kittens

Hong Zhang[1†], Yang Ren[2†], Siyu Wei[1], Hongli Jin[1], Yizhen Wang[1] and Mingliang Jin[1*]

Abstract

Background As the pet population grows, there is increasing attention on the health and well-being of companion animals. Weaning, a common challenge for young mammals, often leads to issues such as diarrhea, growth retardation, and in severe cases, even mortality. However, the specific changes in gut microbiota and metabolites in kittens following weaning remain unclear. In this study, we conducted a comprehensive investigation of the dynamic changes in the gut microbiota, serum metabolism, antioxidant capacity, and immune function of kittens at various time points: days 0, 4, and 30 post-weaning.

Results Significant changes in the immune response and gut microbiota were observed in kittens following weaning. Specifically, IgM levels increased significantly ($P < 0.01$, n = 20), while IgA and IgG levels showed a sustained elevation. Weaning also disrupted the intestinal microbiota, leading to notable changes in serum metabolism. On day 4 post-weaning, there was a decrease in beneficial bacteria such as *Bacteroides vulgatus*, *Fusobacterium nucleatum*, *Anaerostipes caccae*, and *Butyricico-ccaceae*. However, by day 30, beneficial bacteria including *Candidatus Arthromitus*, *Holdemanella*, and *Bifidobacterium* had increased ($P < 0.05$, n = 20). Serum metabolites showed clear separation across time points, with day 0 and day 4 exhibiting similar patterns. A total of 45 significantly altered metabolites ($P < 0.05$, n = 20) were identified, primarily related to vitamins, steroids, peptides, organic acids, lipids, and carbohydrates. Pathway analysis revealed significant enrichment in eight metabolic pathways, with key changes in arginine metabolism and biosynthesis. Additionally, bacteria such as *Bacteroides fragilis*, *Bacteroides stercoris*, *Leuconostoc citreum*, and *Bifidobacterium adolescentis* were positively correlated with serum metabolic changes, emphasizing the link between gut microbiota and systemic metabolism ($P < 0.05$, n = 20).

Conclusion Our study demonstrated that the composition and function of intestinal microorganisms as well as serum metabolic profiles of weaned kittens presented dynamic changes. These findings not only deepen our understanding of the effects of weaning on kitten health, but also provide valuable insights into post-weaning nutritional regulation strategies for kittens.

Keywords Kitten, Weaning, Microbiota, Metabolomics

†Hong Zhang and Yang Ren have contribute equally.

*Correspondence:
Mingliang Jin
mljin@zju.edu.cn
Full list of author information is available at the end of the article

Introduction

The 2023 China Pet Industry Development Report, published at the 12th IPIS Asian CEO Summit, highlights the continued growth of the pet population in China, with cats remaining the most popular pet. In 2023, there were nearly 70 million cats in urban households [1, 2], reflecting the increasing importance of understanding the health of cats, particularly kittens [3–6]. Kittens are especially vulnerable during their early developmental stages, particularly during the critical weaning period, which can lead to significant physiological changes, including shifts in gut microbiota [7–13]. Understanding these changes is crucial to improving their overall health and well-being.

Weaning represents a major health challenge for kittens. Unlike adult cats, kittens undergo a dramatic transition from milk to solid food, which places considerable stress on their developing gastrointestinal and immune systems [14–19]. Studies have shown that this transition can result in metabolic disturbances, gastrointestinal dysfunction, and impaired mucosal barrier function, all of which can negatively impact growth and development [20–27]. Additionally, kittens' immune systems are particularly vulnerable during this period, making them more susceptible to infections and diseases. Given these challenges, identifying strategies to mitigate the adverse effects of weaning is essential. Recent research underscores the pivotal role of the gut microbiota in maintaining gastrointestinal health and immune function during this vulnerable period [28]. Thus, regulating the gut microbiota has emerged as a promising approach to improve health outcomes for kittens during and after weaning.

While substantial research has focused on gut microbiota dynamics during weaning in animals like piglets and lambs, studies on kittens remain limited. This gap is significant because kittens, as obligate carnivores undergoing a unique dietary transition, may experience distinct microbiota shifts compared to omnivorous species [29–31].

By elucidating the dynamic changes in the gut microbiota and metabolism of kittens during weaning, this study aims to contribute to a better understanding of how early-life microbiota regulation can support gut health, enhance immune function, and reduce disease susceptibility in kittens. The findings may offer valuable strategies for improving the health and well-being of kittens during this critical transition.

Materials and methods

Animal ethics

All methods in this study were in accordance with Guidelines for the Management and Use of Laboratory Animals prepared by the Animal Management and Use Institution, and all animals utilized in this experiment received approval from Zhejiang University Animal Protection and Use Committee (ZJU20240985).

Animals and experimental design

Twenty healthy 2-month-old kittens, half male and half female, were selected, including 4 British short blue cats, 4 British short gradient cats, 6 Siamese cats and 6 American shorthair cats (each breed of cat comes from two litters), with a weight difference of 0.90 ± 0.10 kg (Table S1). Blood and fecal samples from the kittens were collected on days 0, 4, and 30 (Fig. 1). Under the same feeding management conditions, all cats had free access to water and cat food, and were fed the diets with similar nutritional composition after weaning. Litter boxes were cleaned twice daily, in the morning and evening, and the cat litter was changed once a week. The cat enclosures were cleaned and disinfected daily to maintain hygiene, and each cat was played with for at least 30 min every day. At 9:00 every day, a measured amount of feed was weighed and provided to each cat. The food bowls were cleaned after weighing in the morning. The daily feed intake and the amount of leftover feed the following day were accurately recorded for each cat. During the afternoon litter cleaning, the fecal condition was observed and documented. The basal diet met the nutritional requirements

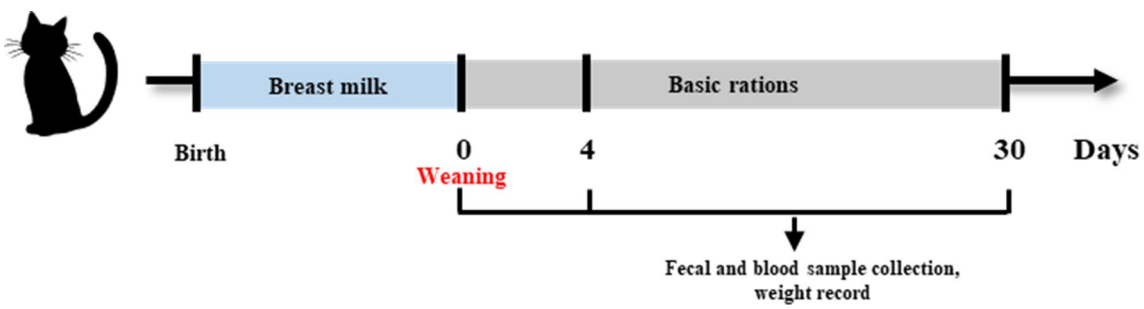

Fig. 1 Study design for the whole experiment

of AAFCO (2017) and its composition and nutrient levels were shown in Table 1. The environmental temperature was controlled at 20–22 °C and humidity at 45–60%.

Sample collection

Blood samples were collected from the forelimb vein after an overnight fast, approximately at 9:00, before feeding on days 0, 4, and 30. After collection, the blood was left to stand until serum separation occurred, then centrifuged at 4000 r/min for 10 min. The supernatant was carefully extracted, aliquoted into 200 µL centrifuge tubes, and stored at −80 °C. Fresh fecal samples (within 15 min of defecation) were collected using clean, sterile cotton swabs, placed into sterile collection bags, and then aliquoted into 2 mL cryotubes for storage at −80 °C.

Assessment of serum biochemical parameters

Serum biochemical parameters were analyzed using an automated biochemical analyzer (Hitachi, Japan). The levels of Immunoglobulin A (IgA), Immunoglobulin G (IgG) and Immunoglobulin M (IgM) in serum were detected by Enzyme Linked Immunosorbent Assay (ELISA) kit (Jingmei Biotechnology, Jiangsu, China). The total antioxidant capacity (T-AOC), and concentrations of glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and malondialdehyde (MDA) in serum were determined using commercial assay kits (Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China).

Table 1 Ingredients and nutrient contents of the basal diet

Ingredients	Content, %	Nutrient[b]	Content, %
Chicken meal	30.00	Crude protein	36.00
Sweet potato starch	10.00	Crude fat	15.00
Pea	14.00	Calcium	1.20
Potato starch	5.00	Phosphorus	1.00
Fish meal	15.00	Ash	8.00
Beer yeast	2.00		
Cheese powder	2.00		
Beet powder	2.00		
Cat premix[a]	4.00		
Chicken fat	13.00		
Chicken liver enzymatic hydrolysate	3.00		
Total	100.00		

[a] The premix provided the following per kg of the diet: mineral and vitamin premix (VA, 22 565.85 IU; VD3, 484.50 IU; VE, 53.92 IU; VK3, 5.73 mg; VB1, 17.68 mg; VB2, 7.33 mg; VB6, 8.31 mg; VB12, 30 IU; biotin, 330 IU; pantothenic acid, 8.75 mg; nicotinamide, 97.10 mg; choline chloride, 2 641.16 mg; Fe, 72.00 mg; Cu, 13.50 mg; Mn, 70.00 mg; Zn, 70.00 mg), 1%; cellulose, 0.5%; dicalcium phosphate, 1%; others (calcium carbonate, potassium chloride, choline chloride, sodium chloride, taurine, selenium yeast), 1.5%

[b] Nutrient levels were measured values

16S rRNA sequencing and data analysis

Fecal microbial genomic DNA was extracted using the QIAamp® Fecal DNA Extraction Kit (QIAGEN N.V., USA), combined with the bead-beating method. Integrity of the genomic DNA was verified by 2% agarose gel electrophoresis. The V5-V6 region of the 16S rRNA gene was amplified by Polymerase Chain Reaction (PCR) using the following primers: 784F (5′-RGG ATTAGATACCC-3′) and 1064R (5′-CGACRRRCC ATGCAN CACCT-3′). Three biological replicates were used for each sample. The PCR products from the same sample were pooled, and amplification success was confirmed via 2% agarose gel electrophoresis. Target bands were excised and purified using the DNA Gel Recovery Kit (Axygen, USA). The PCR product library was quantified based on the preliminary electrophoresis results, and further fluorescence quantification was performed using the PicoGreen dsDNA Quantification Kit (Bioo Scientific, USA). Equal quantities of samples were mixed, and a genomic sequencing library was constructed for paired-end sequencing on the Illumina MiSeq platform.

Sequencing data were quality controlled and low-quality sequences were discarded (< 150 base pairs). The quality-controlled sequences were assembled using Illumina-utils, and chimeric sequences resulting from PCR amplification were removed using UCHIME. QIIME analysis software, in conjunction with USEARCH, was employed to group sequences into Operational Taxonomic Units (OTUs) based on a 97% pairwise sequence identity threshold, and representative sequences for each OTU were identified. α-diversity of the gut microbiota was assessed using the Chao1, Shannon, and Simpson indices, while β-diversity was evaluated using Principal Coordinates Analysis (PCoA) and Non-metric Multidimensional Scaling (NMDS), based on Bray–Curtis, Weighted Unifrac, and Unweighted Unifrac distance metrics. Species annotation was performed by comparing the representative sequences to the Greengenes database using the RDP Classifier (https://sourceforge.net/projects/rdp-classifier/). The microbial community composition was visualized as a heatmap. Statistical analysis of species differences was conducted using the Wilcoxon rank-sum test, two-tailed test, and False Discovery Rate (FDR) correction for multiple testing. Linear Discriminant Analysis (LDA) Effect Size (LEfSe) and Statistical Analysis of Metagenomic Profiles (STAMP) software were used to identify microbial taxa that were significantly influenced by weaning. Microbiota analyses were performed on an online cloud platform (https://cloud.majorbio.com/).

Metabolomic profiling by LC–MS

A total of 100 µL of serum was added to a 1.5 mL centrifuge tube, followed by the addition of 400 µL of a 1:1 (v:v) acetonitrile: methanol mixture containing 0.02 mg/mL of 1–2-chlorophenylalanine as an internal standard for metabolite extraction. The mixture was subjected to vortex agitation for 30 s, and low-temperature sonicated for 30 min (5 °C, 40 kHz). Subsequently, the samples were incubated at −20 °C for 30 min to facilitate protein precipitation. After centrifugation at 4 °C, 13,000 × g for 15 min, the supernatant was carefully decanted, and the residue was dried under a nitrogen stream. The residue was then reconstituted in 100 µL of an acetonitrile: water mixture (1:1, v:v) and further extracted using low-temperature ultrasonication at 5 °C and 40 kHz for 5 min. Following centrifugation at 4 °C, 13,000 × g for 10 min, the supernatant was transferred to sample vials for subsequent LC–MS analysis.

The raw data were processed to remove noise by filtering with a single peak, followed by simulation of missing values and normalization. Soft Independent Modeling of Class Analogy (SIMCA) software was used for logarithmic transformation and data centering, and principal component analysis (PCA) was performed to gain an initial understanding of the overall metabolic differences between sample groups and the variation within groups. PCA, including quality control (QC) samples, was used to assess the metabolic profiles. Unrelated signals were filtered using SIMCA, followed by mean centering for data transformation to construct a partial least squares discriminant analysis (PLS-DA) model. Model reliability was validated using 200 permutations. The quality of the model was assessed using cross-validation, and its validity was tested through permutation experiments. The criterion for evaluating the permutation test is the intercept of the Q^2 regression line with the Y-axis; an intercept less than 0.05 indicates that the model is robust and reliable, with no overfitting.

Differential metabolites were identified based on P-value, fold change (FC) from Student's t-test, and the variable importance in projection (VIP) of the first principal component in the PLS-DA model. The Euclidian distance matrix was calculated for differential metabolite quantities, and a heatmap was generated by hierarchical clustering of the differential metabolites using the complete linkage method. Screening criteria for differential metabolites included FC > 1.5, *P*-value < 0.01, and VIP > 1. Differential metabolites across the three groups were mapped to biochemical pathways using the KEGG database (http://www.genome.jp/kegg/). These metabolites were classified based on their involvement in specific pathways and functions. Pathway enrichment and topological analyses were then performed to identify key pathways most closely associated with the differential metabolites.

Statistical analyses

The data for serum biochemical parameters were analyzed using SPSS 26.0 (SPSS Inc., Chicago, IL, USA) with one-way ANOVA followed by Duncan's multiple range test to identify significant differences. Data visualization was performed using GraphPad Prism version 8.0.2 (GraphPad Software, San Diego, CA, USA). The methods for microbiome data analysis are outlined in the '16S rRNA sequencing and data analysis' section, while the metabolomics data analysis methods are detailed in the 'Metabolomic profiling by LC–MS' section. Pearson correlation analysis was conducted using the Corrplot package in R software to examine the relationship between gut microbiota significantly influenced by weaning stress and differential metabolites. Data are presented as mean ± standard deviation (SD), and $P < 0.05$ was considered statistically significant.

Result

Serum biochemical parameters and antioxidant capacity of kittens at different stages

The biochemical parameters of the serum in kittens at different weaning stages are summarized in Table 2. Serum triglycerides (TG) increased significantly on day 4 ($P < 0.05$), but returned to baseline levels by day 30. Total bile acid (TBA) levels were significantly higher on day 4 compared to day 0 ($P < 0.05$), with a trend toward a decrease observed by day 30. The levels of aspartate aminotransferase (ALT), aspartate aminotransferase (AST), albumin (ALB), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and total cholesterol (TC) in serum did not show significant changes ($P > 0.05$).

Table 2 Changes of serum biochemical parameters in kittens at different stages

Item	Day 0	Day 4	Day 30	*P*-value
ALT(U/L)	40.95 ± 3.07	41.19 ± 2.83	37.76 ± 2.76	0.453
AST(U/L)	24.77 ± 1.45	25.15 ± 1.55	19.5 ± 1.47	0.284
ALB(g/L)	24.47 ± 0.43	25.81 ± 0.63	26.75 ± 0.75	0.063
HDL-C(mmol/L)	2.06 ± 0.10	1.94 ± 0.07	1.89 ± 0.09	0.150
LDL-C(mmol/L)	1.29 ± 0.10	1.17 ± 0.12	1.11 ± 0.08	0.311
TG(mmol/L)	0.45 ± 0.03^{b}	0.56 ± 0.05^{a}	0.44 ± 0.02^{b}	0.046
TBA(umol/L)	6.24 ± 0.22^{b}	7.73 ± 0.59^{a}	6.61 ± 0.28^{ab}	0.033
TC(mmol/L)	3.00 ± 0.15	3.05 ± 0.18	2.93 ± 0.15	0.495

Results are expressed as mean ± SD (n = 20), and different shoulder labels (lowercase English letters) for data in the same row indicate significant differences ($P < 0.05$)

With the extension of the weaning period, the levels of immune factors in serum changed. On day 30, the serum IgM level was significantly higher than on day 0 and day 4 ($P<0.01$), while the levels of IgA and IgG followed a similar trend, but did not show significant differences ($P>0.05$). No significant differences were observed in serum antioxidant parameters; however, antioxidant activity decreased on day 4, followed by a trend of recovery by day 30 (Fig. 2).

Gut microbial diversity of weaned kittens at different stages

The Rarefaction curve based on the Shannon diversity index, flattens as sequencing depth increases, suggesting that the data adequately capture the microbial diversity of the samples (Fig. S1A). It shows that the characteristic OTU numbers for kittens on days 0, 4, and 30 of weaning are 24, 6, and 7, respectively, indicating a decrease followed by a slight increase, with an overall decline in OTU numbers from day 0 to day 30 (Fig. S1B). There was no significant difference in α diversity between the different stages of the kittens (Fig. 3A). The results of PCoA based on the weighted unifrac algorithm showed significant differences in microbiota structure among the three groups at both the genus level ($R^2=0.0645$, $P=0.02$) and the OTU level ($R^2=0.0555$, $P=0.041$) (Fig. 3B).

Changes of gut microbial communities at different stages after weaning

The bacteria with relative abundance exceeding 0.5% included Firmicutes, Actinobacteriota, Bacteroidota and Proteobacteria and Fusobacteriota (Fig. 4A). The abundance of Bacteroidetes, Proteobacteria, and Fusobacteria increased continuously with the extension of the weaning period, whereas the abundance of Proteobacteria and Fusobacteria decreased steadily, though no significant differences were observed ($P>0.05$, Fig. 4B). At the genus level, the top 10 dominant bacteria in each group in terms of relative abundance included *Peptoclostridium, Collinsella, Olsenella, Blautia, Prevotella, Megasphaera, Bacteroides, Bifidobacterium, Dialister* and *Aloprevotella* (Fig. 4C). Compared to day 0, the abundance of *Ruminococcus*, *Libanicoccus*, and *Marvinbryanlia* increased significantly on day 4 after weaning.

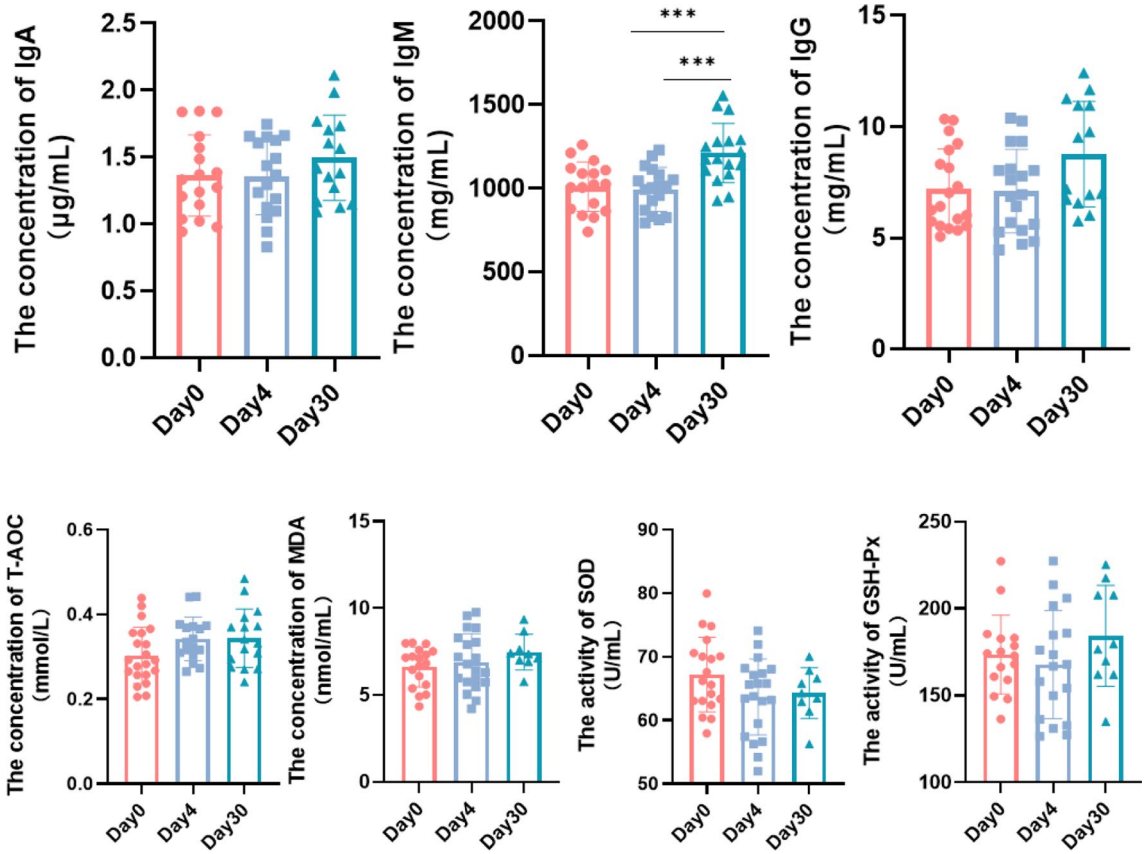

Fig. 2 Effect of weaning on serum immunoglobulin and antioxidant capacity in kitten (n = 20). Data are expressed as mean ± SD, * means *p* value < 0.05, ** means *p* value < 0.01, *** means *p* value < 0.001

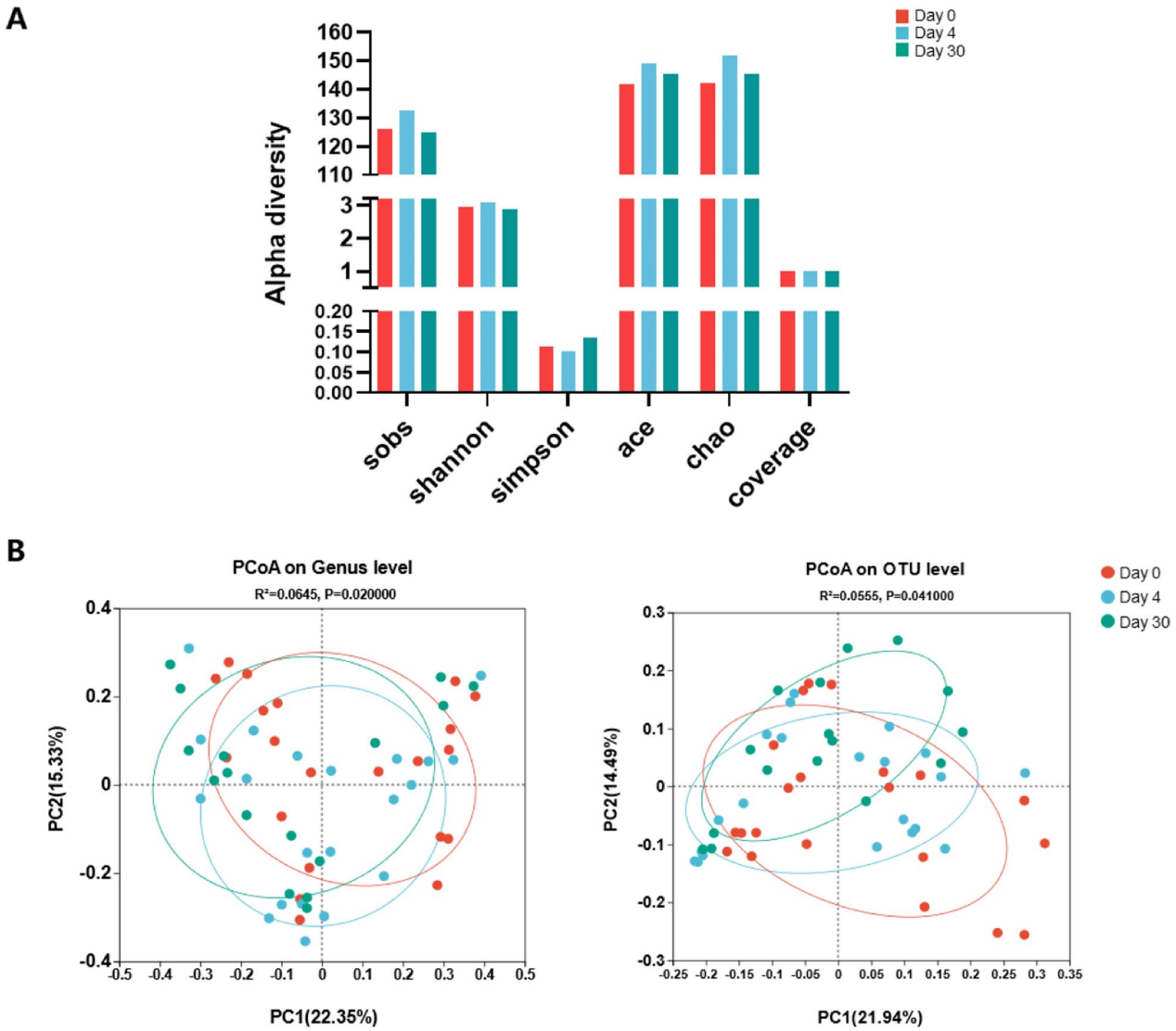

Fig. 3 The diversity and overall structure of gut microbes at three different stages (n = 20). (**A**) α diversity based on OTU levels; (**B**) β diversity based on genus and OTU levels

The abundance of *Bifidobacterium, Holdemanella,* and *Solobacterium* increased significantly on day 30 after weaning. *Bacteroides, Parabacteroides, Fusobacterium* and *Odoribacter* were significantly lower on day 4 post-weaning compared to day 30 post-weaning ($P < 0.05$) (Fig. 4D). Moreover, differences in relative abundance of bacteria at OTU level between groups were detected by using the non-parametric Kruskal–Wallis (KW) sum-rank test (Fig. 4E). LDA linear discriminant analysis was used to estimate the influence of these different bacteria on the difference between groups. According to Fig. 4F, it could be concluded that on day 4 post-weaning, beneficial bacteria such as *Bacteroides fragilis, Bacteroides vulgatus, Bacteroides stercoris, Fusobacterium nucleatum* and *Anaerostipes caccae* were reduced, while on day 30 post-weaning, bacteria such as *Candidatus Arthromitus, Holdemanella, Clostridium spiroformes* and *Bifidobacterium* increased ($P < 0.05$).

Metabolic differences of gut microbiota at different stages after weaning

Serum metabolic profile by LC–MS indicated that the three sets of sample points were clearly separated in the negative, positive and negative–positive mode were significantly distinguished by both PCA (negative: PC1 = 20.80%, PC2 = 9.37%; positive: PC1 = 43.90%, PC2 = 9.71%; MIX: PC1 = 27.70%, PC2 = 12.60%) and PLS-DA (negative: $R^2Y = 0.982$, $Q^2 = 0.844$, $R^2X = 0.482$;

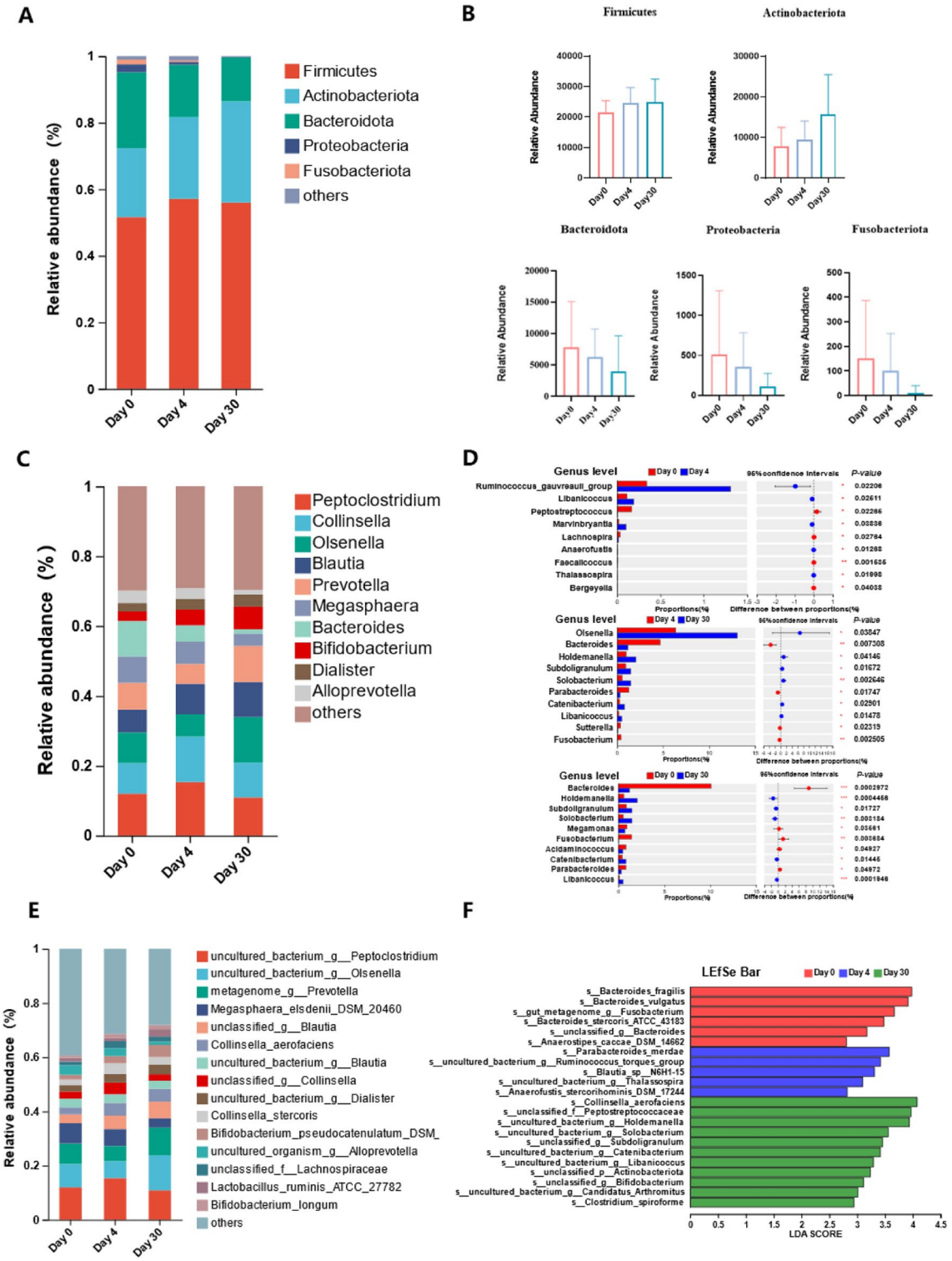

Fig. 4 Effect of weaning on intestinal microbiota at different level in kittens (n = 20). (**A**) Relative abundance of the bacteria at the phylum level; (**B**) comparison of species with differences in phylum levels; (**C**) relative abundance of the bacteria at the genus level; (**D**) comparison of species with differences in genus levels; (**E**) relative abundance of the bacteria at the species level; (**F**) microbial communities that were significantly different in the three groups by linear discriminant analysis plus effect sizes using default parameters (LEfSe)

positive: $R^2Y = 0.989$, $Q^2 = 0.83$, $R^2X = 0.627$; MIX: $R^2Y = 0.976$, $Q^2 = 0.781$, $R^2X = 0.446$) analysis methods (Figs. 5A and B, S2A). Further, the differential metabolites were compared and analyzed through pair comparison, and the volcanic map of differential metabolites was showed with *P*-value < 0.05, VIP > 1 and Fold change ≥ 1 as screening conditions (Fig. 5C). The results indicated that a total of 794 metabolites were detected. Compared with day 0, 10 metabolites were significantly up-regulated and 49 metabolites were significantly down-regulated in the day 4 group, while 100 metabolites were significantly up-regulated and 123 metabolites were significantly down-regulated in the day 30 group. Compared with day 4, 101 metabolites were significantly up-regulated and 83 metabolites were significantly down-regulated in the day 30 group.

There were significant differences in the concentration of metabolites, and the composition of metabolites on day 0 and day 4 was more similar (Fig. 6A). Different metabolites were involved in different biological metabolic processes. Therefore, we performed KEGG pathway enrichment analysis of differential metabolites, among which lipid metabolism and amino acid metabolism pathways were more related to differential metabolism (Fig. 6B). Furthermore, phenylalanine metabolism, serotonergic synapse, cAMP

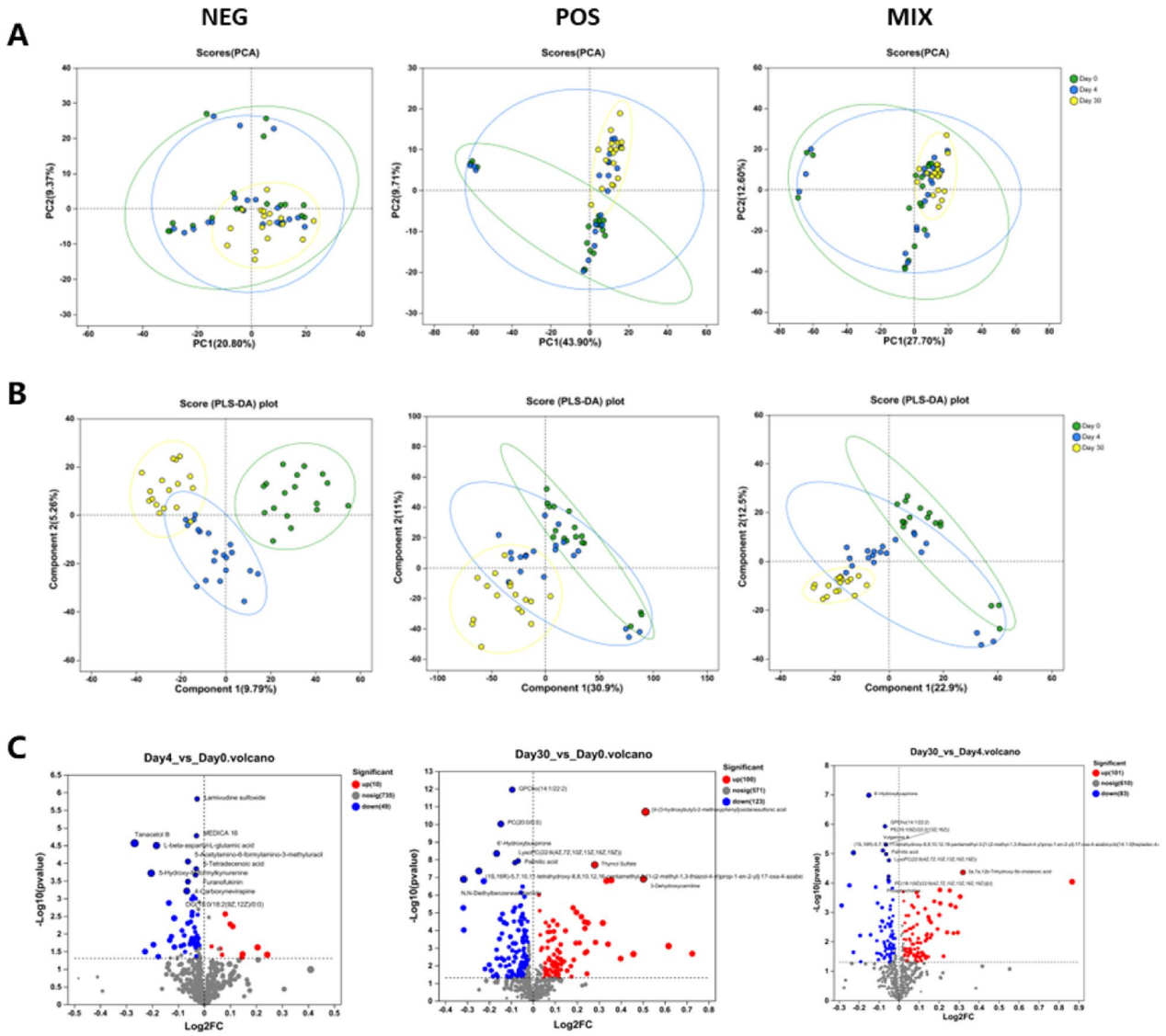

Fig. 5 Comparative analysis of serum metabolites (n = 20). (**A**) PCA plot (from left to right, negative, positive and mix); (**B**) PLA-DA plot (from left to right, negative, positive and mix); (**C**) volcano plots of differential metabolites in both positive and negative modes

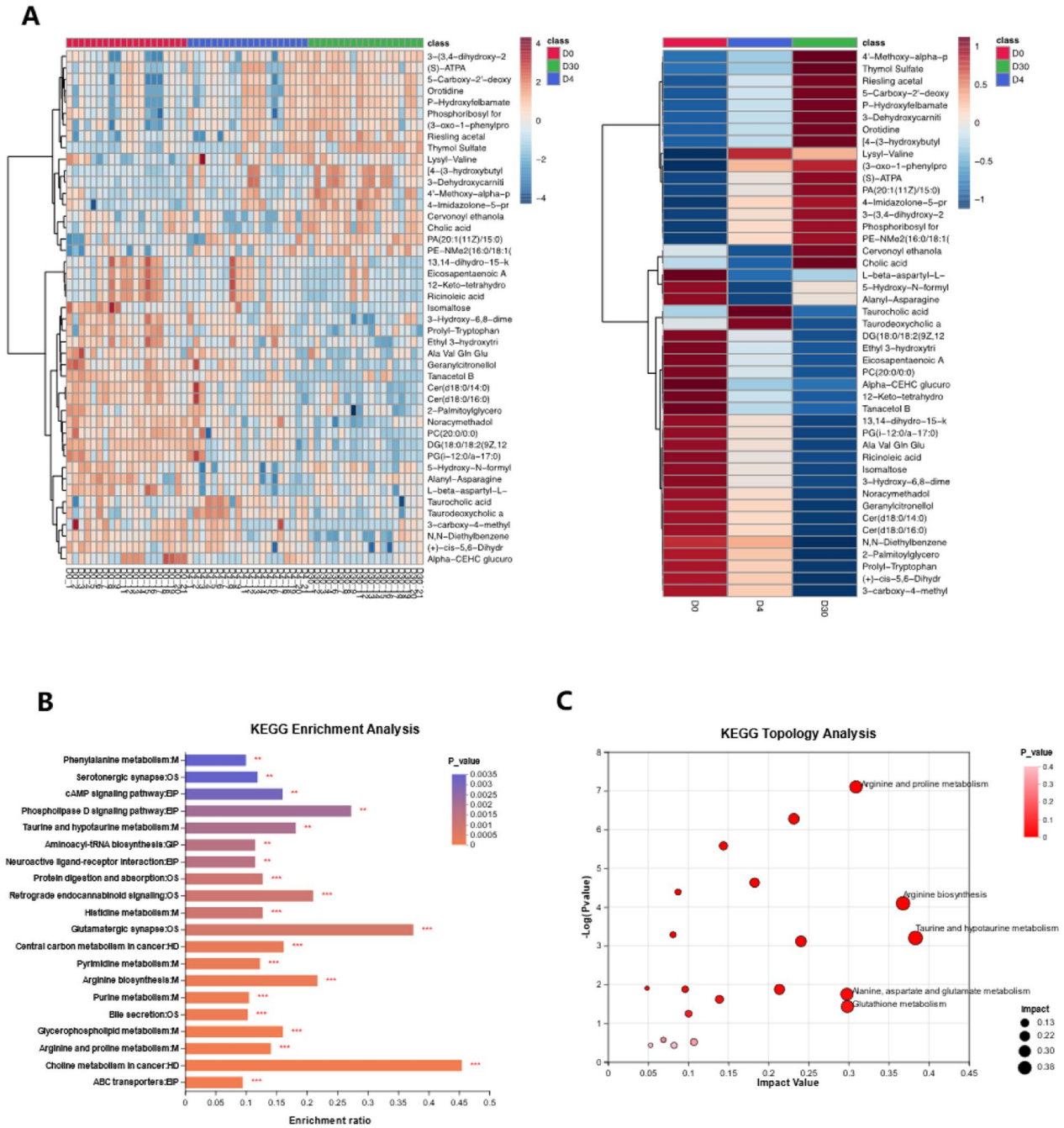

Fig. 6 Changes of metabolic pathways and metabolites in kittens at different stages after weaning. (**A**) The heatmap shows the differences of metabolites of the different stage; (**B**) enrichment analysis of metabolic pathways among the three groups; (**C**) the important metabolism pathway among three groups by topological analysis

signaling pathway, phospholipase d signaling pathway and taurine and hypotaurine metabolism were the top five metabolic pathways. In view of the high impact value and P-value of the bubble plot, five significantly changed metabolic pathways included arginine and proline metabolism, arginine biosynthesis, taurine and hypotaurine metabolism, alanine, aspartate and glutamate metabolism and glutathione metabolism ($P < 0.01$) (Fig. 6C).

Relationship between serum metabolites and gut microbiota at different stage after weaning by integrated correlation analysis

By correlating the different microorganisms with metabolites, we initially screened and obtained four strains that differed significantly before and after weaning that were significantly associated with nutrient metabolism, namely *Bacteroides fragilis, Bacteroides stercoris, Leuconostoc citreum* and *Bifidobacterium adolescentis*. The differential microorganisms and metabolites among the three groups were selected for association analysis. The association analysis showed that *Bacteroides fragilis, Bacteroides stercoris* and *Bacteroides vulgatus* enriched in day 0 group were positively correlated with (S)-ATPA, orotidine,5-Carboxy-2'-deoxyuridine and p-hydroxyfelbamate ($P < 0.05$) and were negatively correlated with cer(d18:0/14:0), geranylcitronellol and ethyl 3-hydroxytridecanoate. *Collinsella aerofaciens, Solobacterium, Coriobacteriale* and *Bifidobacterium* which enriched in day 30 group, were associated with these metabolites, but showed an opposite trend. *Bifidobacterium* was significantly positively correlated with 3-hydroxy-6,8-dimethoxy-7(11)-eremophilen-12,8-olide,3-carboxy-4-methyl-5-pentyl-2-furanpropanoic acid, noracymethadol and cer(d18:0/16:0), but was negatively correlated with 4-Methoxy-alpha-pyrrolidinopropiophenone, 4-imidazolone-5-propionic acid, riesling acetal, [4-(3-hydroxybutyl)-2-methoxyphenyl] oxidanesulfonic acid, 3-dehydroxycarnitine and (3-oxo-1-phenylpropoxy) sulfonic acid (Fig. 7).

Discussion

The weaning process represents a significant and intricate transition for young animals, encompassing changes in gut morphology, physiological functions, and gut microbes due to alterations in their diet, social interactions, and living environment [20]. Weaning stress is a substantial factor influencing the growth of young animals. As a late-maturing breed, the weaning stress degree of kittens is slightly higher compared to other animals [32, 33]. Studies have indicated that there is an abrupt shift in the classification and function of the gut microbiota after weaning, yet the precise mechanisms influencing the gut microbiota of kittens and the corresponding interventions to alleviate the weaning stress remain unclear. We conducted this study to better understand the serum biochemical parameters and the structure and function of intestinal microorganisms during the weaning period of kittens, so that we can target the nutritional means to alleviate the weaning stress of kittens in the future.

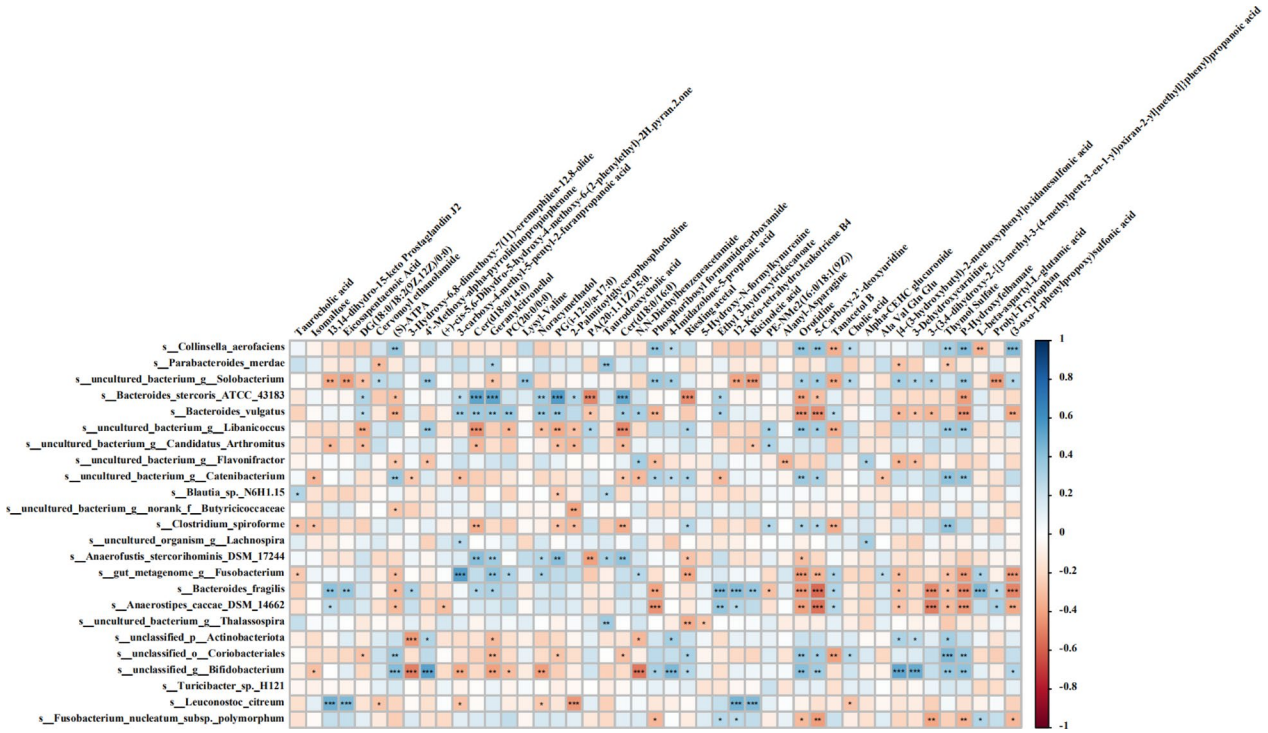

Fig. 7 Correlation analysis of differential metabolites with differential microorganisms. * means *p* value < 0.05, ** means *p* value < 0.01, *** means *p* value < 0.001

During the weaning process of kittens, their intestinal development remains incomplete, which coincides with a sudden dietary transition. This abrupt change leaves their immune and defense systems compromised, making their bodies more susceptible to stress. The resulting stress triggers an excessive production of free radicals, which in turn disrupts the equilibrium between the oxidation and antioxidant systems, ultimately leading to oxidative stress [34–36]. The body's defense against oxidative stress involves both enzymatic and non-enzymatic antioxidants, with the enzymatic antioxidants playing a pivotal role [37, 38]. Oxidative stress can be manifested as increased lipid peroxidation, altered glutathione (GSH) redox status, decreased levels of antioxidant enzymes, and decreased activity of these enzymes and serum MDA levels, all of which are hallmarks of oxidative damage [34, 39, 40]. Oxidative stress is a known factor in the development of postweaning intestinal diseases in kittens. The research revealed that the levels of SOD, CAT, and GSH were significantly lower in cats with periodontal disease compared to recovered cats, suggesting that external stimuli or diseases may disrupt the oxidant/antioxidant balance in the body [40–42]. Similarly, our findings suggest that the levels of antioxidant enzymes decrease in kittens after weaning, possibly due to the stress of weaning, which disrupts the antioxidant enzyme system, leading to decreased GSH-Px and SOD activities and increased MDA levels [43]. Nonetheless, kittens may be able to mitigate this oxidative burden by enhancing the activity of endogenous antioxidant enzymes, such as GSH-Px.

In contrast, IgA and IgG showed similar trends over time, but their concentrations did not significantly change during the weaning period. IgA, which is primarily involved in mucosal immunity, and IgG, associated with systemic immunity and immune memory, did not exhibit significant alterations during this early phase of immune development. This may suggest that the maturation of both mucosal and systemic immunity is a gradual process, potentially taking longer than the study period to become evident [44–46]. Previous studies have shown that IgM levels are relatively high in neonatal kittens and play a key role in their initial immune responses. Our findings align with these studies, as we observed elevated IgM levels by day 30, consistent with its role in responding to external stimuli early in life. However, IgA and IgG were less responsive, possibly due to the kittens' continued dependence on passive immunity from maternal antibodies during this early stage, as reported in other studies [47, 48]. At days 0 and 4, immunoglobulin levels were lower, indicating that the kittens' immune systems were still developing, making them more vulnerable to infections. By day 30, all three immunoglobulins had increased, indicating the maturation of their immune systems. These results suggest that the kittens may be gradually transitioning from passive immunity provided by maternal antibodies to active immunity, beginning to produce their own immunoglobulins in response to environmental challenges.

Our results confirmed significant differences in the gut microbiota composition of kittens across different developmental stages at the OTU level. Post-weaning, alterations in the intestinal nutrient source and a transient insufficiency in supply result in structural and functional modifications of the gastrointestinal tract in young animals. These alterations are primarily manifested as damage to intestinal morphology and structure, reduced digestion and absorption capacity, decreased microbial diversity, and an imbalance of intestinal immune homeostasis. This aligns with our observations of shifts in the gut microbial structure and diversity following weaning. Following weaning, the dominant phyla observed were Firmicutes, Actinobacteria, Bacteroides, Proteobacteria, and Fusobacteriota, which is consistent with previous studies [49, 50]. The abundance of Actinobacteria phylum gradually increases, and at the genus level, we observed that *Bifidobacterium* is the dominant genus, suggesting that *Bifidobacterium* may be one of the key probiotics for protecting the health of young kittens post-weaning. In addition to *Bifidobacterium*, we also noted changes in the abundance of the *Prevotella*, belonging to the Actinobacteria phylum, which was higher after weaning. *Prevotella* have been reported to be associated with the fermentation of plant non-starch polysaccharides into short-chain fatty acids [51, 52]. Additionally, in humans, it has been documented that *Prevotella* species are capable of producing enzymes, including β-glucanase, mannase, and xylanase, which are involved in the degradation of polysaccharides within the plant cell wall [53].

It was posited that structural changes to the fecal microbiome were largely driven by five genera: *Lactobacillus*, *Bifidobacterium*, *Bacteroides*, *Prevotella*, and *Megasphaera* [54]. In our study, *Bacteroides fragilis, Bacteroides vulgatus, Bacteroides stercoris* were reduced. Previous studies have shown that *Bacteroides fragilis* can prevent *Clostridium difficile* infection, possibly by hindering pathogen colonization, promoting probiotic colonization, and improving intestinal barrier integrity [55]. Bacteroides is rich in genes encoding carbohydrate active enzymes and is engaged in the metabolism of carbohydrates. Additionally, *Bacteroides* species are known for their carbohydrate-active enzymes, playing a significant role in carbohydrate metabolism. Among them, *B. stercoris* is considered a core strain and has been shown to influence the microbial community structure, potentially by promoting the colonization of beneficial bacteria. As

the diet of young kittens shifts from the highly liquid mother's milk to solid commercial food, the intestinal microbiota involved in carbohydrate metabolism should increase to adapt to this change. However, this was not the case in our results. On one hand, this may be due to the influence of weaning, which disrupts the balance of the original intestinal microbiota. On the other hand, it may be related to the change in the gut microbiota composition structure caused by the cats' high-protein diet.

The gut microbiota plays a pivotal role in a wide range of metabolic and physiological processes in animals. By engaging in diverse metabolic pathways, the microbiota produces and regulates a wide range of bioactive compounds that influence nutrient digestion, lipid metabolism, and hormone biosynthesis [4, 47, 56, 57]. Our study identifies that the differential metabolites observed were predominantly enriched in pathways such as taurine and hypotaurine metabolism, primary bile acid biosynthesis, histidine metabolism, and starch and sucrose metabolism, consistent with previous reports of alterations in serum biochemical parameters. KEGG pathway analysis revealed that lipid and amino acid metabolism pathways were frequently represented among the metabolism-related pathways. This suggests that glucose, lipid, and amino acid metabolism play key roles in maintaining homeostasis in weaned kittens. Nutritional interventions targeting these metabolic pathways could potentially help regulate metabolic balance and support kittens during the weaning process [4]. Additionally, studies have indicated that *Bacteroides* and *Prevotella* species are associated with 3-dehydroxycarnitine, a carnitine derivative involved in fatty acid metabolism [58, 59]. In our study, *Bacteroides* was predominantly enriched on day 0 but significantly decreased by day 30, which could impact fatty acid absorption in kittens. This suggests that *Bacteroides* supplementation post-weaning may alleviate weaning stress by enhancing fatty acid metabolism. Probiotics have also shown promise in improving gut health. For example, multi-strain probiotics have been shown to enhance gut health in domestic cats by modulating gut microbes, increasing short-chain fatty acid (SCFA) production, reducing inflammation, and boosting antioxidant status [3]. We hypothesize that supplementing *Bacteroides* may improve immunity in kittens by modulating fatty acid metabolism and mitigating metabolic disorders in serum. In addition to *Bacteroides*, other strains like *Bacillus* species have been considered as dietary supplements to alleviate diarrhea in pet cats [60], and *Enterococcus hirae* may counteract *aEPEC*-induced gut dysfunction in kittens [61]. Furthermore, *Leuconostoc citricum* plays a role in carbohydrate metabolism and exhibits strong antibacterial effects against pathogens like *Staphylococcus aureus*, suggesting its probiotic potential [62, 63]. *Bifidobacterium adolescentis* has also been linked to enhanced health span and lifespan through modulation of catalase activity and host metabolism [64]. These findings support the potential for probiotics to alleviate weaning stress and promote disease prevention in kittens by modulating host-microbe interactions. Future research should explore the application of these probiotics, including the four strains identified in our study, as novel nutritional interventions for weaned kittens.

Conclusion

This study demonstrates that post-weaning kittens experience a decline in immune function, disruption of the gut microbiota, and some degree of impaired nutrient metabolism and absorption. As the weaning period progresses, the abundance of Actinobacteria in the kitten gut microbiota increases. Notably, the prevalence of commensal bacteria, such as *Bacteroides vulgatus*, decreases after weaning. However, *Bifidobacterium* and *Enterococcus* are consistently present at all stages and play a critical role in maintaining gut microbiota balance and supporting the health of the kittens. Furthermore, weaning significantly impacts five metabolic pathways, including arginine and proline metabolism. These findings offer new insights into the nutritional regulation of weaned kittens and suggest that manipulating host-microbiome metabolic interactions could support disease prevention during weaning. Probiotics identified through comparative analysis may serve as potential candidates for further investigation.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42523-024-00373-w.

Additional file1 (PDF 170 KB)

Additional file2 (XLSX 11 KB)

Acknowledgements

None.

Author contributions

MLJ, Supervision, Project administration, Funding acquisition; YZW: Visualization, Supervision; HZ, Conceptualization, Methodology, Investigation, Formal analysis, Writing original draft; YR, Validation, Methodology, Resources, Formal analysis; SYW: Formal analysis, Data curation; HLJ: Conceptualization, Methodology. The authors read and approved the final manuscript.

Funding

This research was supported by the fund from National Key Research and Development Program of China (2023YFD1301101, 2022YFD1300505), National Natural Science Foundation of China (32372889).

Availability of data and materials

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

All the experimental procedures and animal care in this study were performed in accordance with guidelines from the Institutional Animal Care and Use Committee at Zhejiang University, and met the requirements of experimental animal ethics.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]Present Address: National Engineering Research Center for Green Feed and Healthy Breeding, Key Laboratory of Molecular Animal Nutrition, Ministry of Education, Key Laboratory of Animal Nutrition and Feed Science (Eastern of China), Ministry of Agriculture and Rural Affairs, Key Laboratory of Animal Feed and Nutrition of Zhejiang ProvinceInstitute of Feed Science, Zhejiang University, Hangzhou 310058, People's Republic of China. [2]Present Address: Fubei Pet Food, Shanghai 201600, People's Republic of China.

Received: 27 February 2024 Accepted: 28 December 2024
Published online: 18 January 2025

References

1. Li Y, Ali I, Lei Z, Li Y, Yang M, Yang C, et al. Effect of a multistrain probiotic on feline gut health through the fecal microbiota and its metabolite SCFAs. Metabolites. 2023;13:228.
2. China: number of cat 2023 [Internet]. Statista. [cited 2024 Sep 26]. Available from: https://www.statista.com/statistics/992420/china-number-of-cats/
3. Sonnenburg JL, Bäckhed F. Diet-microbiota interactions as moderators of human metabolism. Nature. 2016;535:56–64.
4. Mondo E, Marliani G, Accorsi PA, Cocchi M, Di Leone A. Role of gut microbiota in dog and cat's health and diseases. Open Vet J. 2019;9:253–8.
5. Schoeler M, Caesar R. Dietary lipids, gut microbiota and lipid metabolism. Rev Endocr Metab Disord. 2019;20:461–72.
6. Fischer MM, Kessler AM, Kieffer DA, Knotts TA, Kim K, Wei A, et al. Effects of obesity, energy restriction and neutering on the faecal microbiota of cats. Br J Nutr. 2017;118:513–24.
7. Kałużna-Czaplińska J, Gątarek P, Chartrand MS, Dadar M, Bjørklund G. Is there a relationship between intestinal microbiota, dietary compounds, and obesity? Trends Food Sci Technol. 2017;70:105–13.
8. Kawase T, Nagasawa M, Ikeda H, Yasuo S, Koga Y, Furuse M. Gut microbiota of mice putatively modifies amino acid metabolism in the host brain. Br J Nutr. 2017;117:775–83.
9. Han H, Wang M, Zhong R, Yi B, Schroyen M, Zhang H. Depletion of gut microbiota inhibits hepatic lipid accumulation in high-fat diet-fed mice. Int J Mol Sci. 2022;23:9350.
10. Yin J, Li Y, Han H, Chen S, Gao J, Liu G, et al. Melatonin reprogramming of gut microbiota improves lipid dysmetabolism in high-fat diet-fed mice. J Pineal Res. 2018;65: e12524.
11. Swartz TD, Duca FA, de Wouters T, Sakar Y, Covasa M. Up-regulation of intestinal type 1 taste receptor 3 and sodium glucose luminal transporter-1 expression and increased sucrose intake in mice lacking gut microbiota. Br J Nutr. 2012;107:621–30.
12. Tremaroli V, Bäckhed F. Functional interactions between the gut microbiota and host metabolism. Nature. 2012;489:242–9.
13. Kumari M, Kozyrskyj AL. Gut microbial metabolism defines host metabolism: an emerging perspective in obesity and allergic inflammation. Obes Rev. 2017;18:18–31.
14. Wang C, Wei S, Liu B, Wang F, Lu Z, Jin M, et al. Maternal consumption of a fermented diet protects offspring against intestinal inflammation by regulating the gut microbiota. Gut Microbes. 2022;14:2057779.
15. Young W, Moon CD, Thomas DG, Cave NJ, Bermingham EN. Pre- and post-weaning diet alters the faecal metagenome in the cat with differences in vitamin and carbohydrate metabolism gene abundances. Sci Rep. 2016;6:34668.
16. Frese SA, Parker K, Calvert CC, Mills DA. Diet shapes the gut microbiome of pigs during nursing and weaning. Microbiome. 2015;3:28.
17. Isaacson R, Kim HB. The intestinal microbiome of the pig. Anim Health Res Rev. 2012;13:100–9.
18. García-Mantrana I, Selma-Royo M, González S, Parra-Llorca A, Martínez-Costa C, Collado MC. Distinct maternal microbiota clusters are associated with diet during pregnancy: impact on neonatal microbiota and infant growth during the first 18 months of life. Gut Microbes. 2020;11:962–78.
19. Kieler IN, Mølbak L, Hansen LL, Hermann-Bank ML, Bjornvad CR. Overweight and the feline gut microbiome—a pilot study. J Anim Physiol Anim Nutr (Berl). 2016;100:478–84.
20. Jia J, Frantz N, Khoo C, Gibson GR, Rastall RA, McCartney AL. Investigation of the faecal microbiota of kittens: monitoring bacterial succession and effect of diet. FEMS Microbiol Ecol. 2011;78:395–404.
21. Hooda S, Vester Boler BM, Kerr KR, Dowd SE, Swanson KS. The gut microbiome of kittens is affected by dietary protein:carbohydrate ratio and associated with blood metabolite and hormone concentrations. Br J Nutr. 2013;109:1637–46.
22. Yang H, Xiong X, Wang X, Tan B, Li T, Yin Y. Effects of weaning on intestinal upper villus epithelial cells of piglets. PLoS ONE. 2016;11: e0150216.
23. Gaillard V, Chastant S, England G, Forman O, German AJ, Suchodolski JS, et al. Environmental risk factors in puppies and kittens for developing chronic disorders in adulthood: a call for research on developmental programming. Front Vet Sci. 2022;9: 944821.
24. Gresse R, Chaucheyras-Durand F, Fleury MA, Van de Wiele T, Forano E, Blanquet-Diot S. Gut microbiota dysbiosis in postweaning piglets: understanding the keys to health. Trends Microbiol. 2017;25:851–73.
25. Pilla R, Suchodolski JS. The gut microbiome of dogs and cats, and the influence of diet. Vet Clin North Am Small Anim Pract. 2021;51:605–21.
26. Hayes JD, McMahon M. NRF2 and KEAP1 mutations: permanent activation of an adaptive response in cancer. Trends Biochem Sci. 2009;34:176–88.
27. Suchodolski JS, Foster ML, Sohail MU, Leutenegger C, Queen EV, Steiner JM, et al. The fecal microbiome in cats with diarrhea. PLoS ONE. 2015;10: e0127378.
28. Carding SR, Davis N, Hoyles L. Review article: the human intestinal virome in health and disease. Aliment Pharmacol Ther. 2017;46:800–15.
29. Bosch G, Hagen-Plantinga EA, Hendriks WH. Dietary nutrient profiles of wild wolves: insights for optimal dog nutrition? Br J Nutr. 2015;113(Suppl):S40-54.
30. Rochus K, Janssens GPJ, Hesta M. Dietary fibre and the importance of the gut microbiota in feline nutrition: a review. Nutr Res Rev. 2014;27:295–307.
31. Brosey BP, Hill RC, Scott KC. Gastrointestinal volatile fatty acid concentrations and pH in cats. Am J Vet Res. 2000;61:359–61.
32. Veronesi MC, Fusi J. Feline neonatology: from birth to commencement of weaning—what to know for successful management. J Feline Med Surg. 2022;24:232–42.
33. Fournier A, Masson M, Corbière F, Mila H, Mariani C, Grellet A, et al. Epidemiological analysis of reproductive performances and kitten mortality rates in 5,303 purebred queens of 45 different breeds and 28,065 kittens in France. Reprod Domest Anim. 2017;52(Suppl 2):153–7.
34. Cao S, Wu H, Wang C, Zhang Q, Jiao L, Lin F, et al. Diquat-induced oxidative stress increases intestinal permeability, impairs mitochondrial function, and triggers mitophagy in piglets. J Anim Sci. 2018;96:1795–805.
35. Sies H. Oxidative stress: a concept in redox biology and medicine. Redox Biol. 2015;4:180–3.
36. van der Pol A, van Gilst WH, Voors AA, van der Meer P. Treating oxidative stress in heart failure: past, present and future. Eur J Heart Fail. 2019;21:425–35.
37. Mishra V, Shah C, Mokashe N, Chavan R, Yadav H, Prajapati J. Probiotics as potential antioxidants: a systematic review. J Agric Food Chem. 2015;63:3615–26.
38. Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 2007;39:44–84.
39. Zhang H, Chen Y, Chen Y, Ji S, Jia P, Li Y, et al. Comparison of the protective effects of resveratrol and pterostilbene against intestinal damage and redox imbalance in weanling piglets. J Anim Sci Biotechnol. 2020;11:52.

40. Webb CB, Falkowski L. Oxidative stress and innate immunity in feline patients with diabetes mellitus: the role of nutrition. J Feline Med Surg. 2009;11:271–6.
41. Yurdakul İ, Çatak MA.: Evaluation of the relationship between periodontal diseases and oxidative stress parameters in cats. RC FCV-LUZ. (2024) XXXIV:1–6.
42. Polat E, Acisu TC, Sönmez M, Ünsaldi E, Otlu Ö, Tanrisever M, et al. Investigation of changes in Hematological parameters and levels of Oxidative stress factors in castrated Cats. RC FCV-LUZ. (2023) XXXIII: 1–7.
43. Wang C, Zhu Q, Li Y, Guo J, Li L. Alterations in the intestinal microbiome and metabolic profile of british shorthair kittens fed with milk replacer. Animals. 2024;14:2346.
44. Bakar E, Ulucam E, Cerkezkayabekir A, Sanal F, Inan M. Investigation of the effects of naringin on intestinal ischemia reperfusion model at the ultrastructural and biochemical level. Biomed Pharmacother. 2019;109:345–50.
45. Fadlallah J, El Kafsi H, Sterlin D, Juste C, Parizot C, Dorgham K, et al. Microbial ecology perturbation in human IgA deficiency. Sci Transl Med. 2018;10:eaan1217.
46. Macpherson AJ, Yilmaz B, Limenitakis JP, Ganal-Vonarburg SC. IgA function in relation to the intestinal microbiota. Annu Rev Immunol. 2018;36:359–81.
47. Sanidad KZ, Amir M, Ananthanarayanan A, Singaraju A, Shiland NB, Hong HS, et al. Maternal gut microbiome-induced IgG regulates neonatal gut microbiome and immunity. Sci Immunol. 2022;7:eabh3816.
48. Claus MA, Levy JK, MacDonald K, Tucker SJ, Crawford PC. Immunoglobulin concentrations in feline colostrum and milk, and the requirement of colostrum for passive transfer of immunity to neonatal kittens. J Feline Med Surg. 2006;8:184–91.
49. Desai AR, Musil KM, Carr AP, Hill JE. Characterization and quantification of feline fecal microbiota using cpn60 sequence-based methods and investigation of animal-to-animal variation in microbial population structure. Vet Microbiol. 2009;137:120–8.
50. Handl S, Dowd SE, Garcia-Mazcorro JF, Steiner JM, Suchodolski JS. Massive parallel 16S rRNA gene pyrosequencing reveals highly diverse fecal bacterial and fungal communities in healthy dogs and cats. FEMS Microbiol Ecol. 2011;76:301–10.
51. De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, et al. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proc Natl Acad Sci USA. 2010;107:14691–6.
52. Ivarsson E, Roos S, Liu HY, Lindberg JE. Fermentable non-starch polysaccharides increases the abundance of Bacteroides–Prevotella–Porphyromonas in ileal microbial community of growing pigs. Animal. 2014;8:1777–87.
53. Flint HJ, Bayer EA. Plant cell wall breakdown by anaerobic microorganisms from the mammalian digestive tract. Ann N Y Acad Sci. 2008;1125:280–8.
54. Deusch O, O'Flynn C, Colyer A, Swanson KS, Allaway D, Morris P. A longitudinal study of the feline faecal microbiome identifies changes into early adulthood irrespective of sexual development. PLoS ONE. 2015;10:0144881.
55. Zhang H, Duan Y, Cai F, Cao D, Wang L, Qiao Z, et al. Next-generation probiotics: microflora intervention to human diseases. Biomed Res Int. 2022;2022:5633403.
56. Wernimont SM, Radosevich J, Jackson MI, Ephraim E, Badri DV, MacLeay JM, et al. The effects of nutrition on the gastrointestinal microbiome of cats and dogs: impact on health and disease. Front Microbiol. 2020;11:1266.
57. Clarke G, Stilling RM, Kennedy PJ, Stanton C, Cryan JF, Dinan TG. Minireview: gut microbiota: the neglected endocrine organ. Mol Endocrinol. 2014;28:1221–38.
58. Falony G, Vieira-Silva S, Raes J. Microbiology meets big data: the case of gut microbiota-derived trimethylamine. Annu Rev Microbiol. 2015;69:305–21.
59. Rombouts C, Hemeryck LY, Van Hecke T, De Smet S, De Vos WH, Vanhaecke L. Untargeted metabolomics of colonic digests reveals kynurenine pathway metabolites, dityrosine and 3-dehydroxycarnitine as red versus white meat discriminating metabolites. Sci Rep. 2017;7:42514.
60. Wang F, Mei X, Wang Q, Zhao P, Zhou Y, Tang L, et al. Compound bacillus alleviates diarrhea by regulating gut microbes, metabolites, and inflammatory responses in pet cats. Anim Microbiome. 2023;5:49.
61. Watson VE, Jacob ME, Bruno-Bárcena JM, Amirsultan S, Stauffer SH, Píqueras VO, et al. Influence of the intestinal microbiota on disease susceptibility in kittens with experimentally-induced carriage of atypical enteropathogenic Escherichia coli. Vet Microbiol. 2019;231:197–206.
62. Han H-S, Soundharrajan I, Valan Arasu M, Kim D, Choi K-C. Leuconostoc citreum inhibits adipogenesis and lipogenesis by inhibiting p38 MAPK/Erk 44/42 and stimulating AMPKα signaling pathways. Int J Mol Sci. 2023;24:7367.
63. Wang X, Zhao L, Wang Y, Xu Z, Wu X, Liao X. A new Leuconostoc citreum strain discovered in the traditional sweet potato sour liquid fermentation as a novel bioflocculant for highly efficient starch production. Food Res Int. 2021;144: 110327.
64. Chen S, Chen L, Qi Y, Xu J, Ge Q, Fan Y, et al. Bifidobacterium adolescentis regulates catalase activity and host metabolism and improves healthspan and lifespan in multiple species. Nat Aging. 2021;1:991–1001.

Publisher's Note