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Article

Laminaria Japonica Polysaccharide Improved the Productivities and Systemic Health of Ducks by Mediating the Gut Microbiota and Metabolome

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ABSTRACT: This study investigated the beneficial effects of a *Laminaria japonica* polysaccharide (LJPS) on the systemic health of ducks by modulating the gut microbiome and metabolome. Our findings demonstrated that the LJPS supplementation enhanced the overall growth performance and physiological immune and antioxidant index of ducks. In addition, the LJPS-fed group significantly increased abundances of intestinal *Bacteroides* and *Prevotellaceae* with decreased α-diversity than that in the control group. Among the total of 1840 intestinal metabolites, 186 metabolites were identified to be differentially regulated by LJPS feeding (upregulated 143 metabolites and downregulated 43 metabolites), which is closely associated with some of the growth-related metabolic pathways. Lastly, the correlation analysis recapitulates that the beneficial effects of LJPS underlie the alterations in intestinal microbiota and metabolites. Taken together, LJPS supplementation improved the physiological parameters and richness of some beneficial microbes and upregulated certain metabolic pathways, which facilitated better productivities and systemic health of ducks.

KEYWORDS: *Laminaria japonica polysaccharide, health parameters, intestinal microbiome, metabolome, ducks*

INTRODUCTION

Currently, intensively raised ducks have become an important contributor of meat production worldwide, with the number of ducks raised in China accounting for more than 60% of the production worldwide.[1,2] However, due to limitations in the farming environment and rearing technologies, duck production faces some challenges including low feed efficiency, poor immunity, and susceptibility to various diseases, which lead to decreased production efficiency and poor meat quality.[2] Therefore, improving production performance and systemic health of ducks is a concern in the duck industry.

Previous studies revealed that the natural plant-derived polysaccharides (PSs) exerted beneficial effects on the immunity, gut integrity, and production performance of broilers[3] and piglets.[4] A *Laminaria japonica* polysaccharide (LJPS) is a biomacromolecule with a complex molecular structure[5] and some health-beneficial bioactivities referring to the growth-promoting,[6] antioxidant, anti-inflammatory,[5] antiviral,[7] lipid-lowering, and antiobesity properties.[8,9] A recent study on postweaned pigs demonstrated that the polysaccharides from the macroalgal ameliorated postweaning intestinal dysfunction by favorably regulating the microbiota.[10] This observation provides new perspectives to treat human metabolic diseases and animal health as well as food security and quality.

Kim et al.[11] documented that dietary supplementation with seaweed PSs alleviated diarrhea and improved gut barrier function by inhibiting pathogens and increasing the proliferation of beneficial bacteria. Similarly, Zuo et al.[12] reported that LJPS reduced the stress caused by high concentrations of ammonia in aquatic animals. Furthermore, Vigors et al.[10] observed that dietary laminarin-rich microalgal extract supplementation enhanced the abundance of *Prevotella* and concentrations of short-chain fatty acids (SCFAs) in the gut, and the increased beneficial bacteria level exerted the positive relationship with the enhanced performance involving average daily gain (ADG), nutrient digestibility, and feed efficiency. Thus, regulating the intestinal microbiota and metabolome could be one of the vital ways by which LJPS regulates the performance and systemic health of animals.[13,14] Emerging studies have revealed that the structural characteristics of the intestinal microbiota, including the components and abundance of bacteria, significantly influenced the productivity,[15] health status, and product quality of animals.[16] Additionally, the composition and metabolism of the gastrointestinal microbiota are affected by dietary intake, particularly bioactive components, which creates a connection between microbes, diet, and the physical condition and characteristics of the host.[17] Moreover, the increased digestibility of dietary nutrients is tightly associated with a greater abundance of gut microbes and upregulated metabolic pathways,[13,18] rather than increased overall diversity.[19]

However, few studies focused on the modulatory effects of LJPS supplementation on intestinal microbiota, metabolites, and relative metabolic pathways of ducks. Furthermore, the correlation among the systematic health, microbiota, and

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metabolome of ducks fed with an LJPS-supplemented diet remains unclear. We hypothesized that dietary LJPS supplementation would affect growth performance by modulating gut microbiota and their metabolites, as well as health-related metabolic pathways. This study aimed to investigate the effects of dietary LJPS supplementation on productivities, health status, intestinal microbiota, and metabolites of ducks, as well as the potential association of these parameters with one another.

MATERIALS AND METHODS

The current experimental procedures were approved by the Department of Animal Nutrition of Shandong Agricultural University following the guidelines for animal research (Protocol No. S20210072).

Experimental Animals, Design, and Feeding Management. The LJPS was obtained using the water extraction method, and the monosaccharides and their molecular and structural characteristics were elucidated in our previous study.[5] The experimental diets were prepared according to the duck industry standard in China (NY/T 2122-2012). The information on the dietary component and nutrient levels is presented in Supporting Table 1. Overall, 120 healthy Cherry Valley ducks (mixed sex) (21-day-old) with similar body weights were randomly allocated to 20 cages for two treatment types (6 ducks/cage and 10 cages/treatment). One cage was considered a replicate unit for monitoring body weight (BW) and average daily feed intake (ADFI). Each cage was randomly allocated to either one of the two dietary treatments with 10 replicates per treatment. One treatment group was fed a basal diet denoted as the Con group, and the other was fed a basal diet supplemented with 400 mg LJPS/kg diet representing the LJPS group. The LJPS was first added into a premix and then mixed with other feedstuffs well, thus forming a nutritionally balanced diet for ducks. During the experimental period, ducks had free access to feed and fresh water through automatic feeding and drinking equipment in the experiment. An 18-day duck feeding experiment was carried out, with the first 4 days as the acclimation period and the last 14 days as the sample collection stage. ADFI and ADG were measured by determining the BW (on days 21 and 39) of the ducks and their feed consumption per cage. Feed efficiency was estimated as the ADG divided by the corresponding ADFI (ADG/ADFI was denoted as G:F).

Sample Collection and Determination. After an 18-feeding experiment, when the ducks were at 39 days of age, 10 healthy ducks from each treatment (1 duck/cage) were randomly taken and sacrificed for collecting samples. Before slaughter, blood samples were collected from the wing veins of the ducks to determine serum biochemical and immune indices primarily regarding high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglyceride (TG), total cholesterol (TCHO), and serum IgA and IgG, according to the methods depicted by Li et al.[20] and Yan et al.[21] After neck bloodletting and slaughter, the blood and liver samples were collected to determine the antioxidant status (T-AOC and CAT) and physiological health indicators of ducks. Abdominal fat was collected and weighed. A 3 cm intestinal segment was cut off from the middle of the ileum and the jejunum, respectively, with surgical scissors for making histologic slices to determine the intestine development. These collected intestinal samples were rinsed with a salt solution (9 g/L, w/v) and fixed in formaldehyde−phosphate buffer (100 g/L, w/v) for no less than 24 h before being paraffin sections. For each intestinal sample, 5−10 observation samples (slices with 5 μm thickness) were cut continuously using a microtome and stained with hematoxylin and eosin (H&E). The intestinal morphology was evaluated by determining the villus height (V), crypt depth (C), and the villus-to-crypt ratio (*V*:C) using a light microscope (BX-51, Olympus, Tokyo, Japan) equipped with Image-Pro Plus software (version 6.0, Motic Images software, Motic China Group Co., Ltd., Xiamen, China).[20] Measurements were repeated 10 times for each sample.

Digestibility of Feed Nutrients. During the last 3 days of the feeding experiment, fecal and feed samples were collected from the two groups for determining the digestibility of nutrients of the two groups of ducks using the internal indicator method (hydrochloric acid-insoluble ash).[22] The contents of dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and hydrochloric acid-insoluble ash in feed and feces were determined according to the methods described by Prawirodigdo et al.,[22] and the digestibility of feed nutrients was calculated following the formula:

$$\text{digestibility} = \left(1 - \frac{A \times B}{C \times D}\right) \times 100\%$$

In the formula, *A* and *C* represent the content of acid-insoluble ash in the feed or chyme, respectively, and *B* and *D* indicate the content of certain nutrients in the chyme or feed, respectively. The digestibility of DM, CP, NDF, and ADF was represented as DDM, DCP, DNDF, and DADF.

Cecal Microbiota Determination Using 16S rDNA Amplicon Sequencing. At the end of the experiment, after the sacrifice of the duck, 10 cecum digesta samples were immediately collected from each group (1 duck/cage) to determine the microbiome and metabolome. The determination of the microbiome was conducted following our previous procedure[13,20] using the IonS5TMXL sequencing platform (Novogene, Beijing, China). In brief, the first step was extracting DNA from cecum digesta samples using QIAamp DNA fecal mini kits (Qiagen Inc., Hilden, Germany); then, the V3−V4 hypervariable region of the 16S rRNA gene was amplified. Amplicon libraries were sequenced on an IonS5TMXL sequencing platform (Novogene, Beijing, China) for single-end reads of 400 and 600 bp (SE400 and SE600). The detailed procedure is listed in the Supporting Information.

Determination of the Metabolome of the Cecal Digesta. The cecal metabolome was quantitatively measured following the procedure described in our previous paper[13] using liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Novogene Co., Ltd., Beijing, China). In brief, the measurement process contained three main steps: (1) metabolite extraction, (2) metabolites in the chyme were quantified by ultrahigh-performance liquid chromatography-tandem mass spectrometry (UHPLC−MS/MS) analysis, and (3) data processing and metabolite identification.[13,23]

Calculations and Statistical Analysis. Data for growth performance including the BW, ADFI, ADG, and G:F were analyzed by one-way ANOVA according to Dunn's multiple comparisons using the IBM SPSS Statistics 23. Discrepant microbes were filtrated using the linear discriminant analysis (LDA) effect size analysis by the online procedure of BIC (http://www.ehbio.com/Cloud_Platform/front/#/). The correlation between the differentiated microbes, metabolites, and healthy parameters was conducted using Spearman analysis and presented in a heatmap using the heatmap illustrator program (version 1.0.3.7). The analysis of metabolic pathways was conducted referring to the *Gallus gallus* KEGG pathway database using MetaboAnalyst 4.0 online software (https://www.metaboanalyst.ca/).

RESULTS

Dietary Supplementation with LJPS Improved Productivities of Ducks. Effects of dietary supplementation with LJPS on the BW and growth performance (ADG, ADFI, and G:F) of ducks are presented in Table 1. Compared to the con group, dietary supplementation with LJPS improved the BW ($P = 0.02$) and ADG ($P = 0.04$), while decreasing ADFI ($P < 0.01$), and thus enhanced the feed efficiency (G:F) ($P = 0.01$). Thus, LJPS supplementation exhibited a beneficial effect on the productivities of ducks.

Effects of LJPS on the AD of Nutrients, Serum Immune and Physiological Indices, Abdominal Fat, and Liver Antioxidant Properties of Ducks. As shown in

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Table 1. Effects of LJPS Supplementation on Productivities of Ducks

items	treatment		SEM	*P*-values
	Con	LJPS[a]		
body weight, kg				
day 21	1.23	1.24	0.02	>0.05
day 39	3.09	3.18	0.59	0.02
ADFI[b], g	218.50	214.00	8.25	<0.01
ADG, g	109.35	114.76	5.65	0.04
G:F	0.50	0.53	0.00	0.01

[a]LJPS, *L. japonica* polysaccharide (LJPS)-supplemented group. [b]Average daily feed intake (ADFI), average daily gain (ADG), and feed efficiency was calculated by dividing ADG by ADFI (G:F).

Figure 1A, dietary supplements with LJPS enhanced the digestibility of DM, (DDM $P < 0.01$) and NDF (DNDF, $P < 0.01$), compared with those of the con group. The LJPS-supplemented group had lower serum TG, TCHO, and LDL levels ($P < 0.05$) than those of the con group, while serum HDL levels between the two groups were similar (Figure 1B). Additionally, higher serum IgA and IgG levels were observed in the LJPS-supplemented group ($P < 0.01$) compared to those of the con group (Figure 1C). In addition, the LJPS-supplemented group had significantly higher ($P < 0.01$) antioxidant enzyme activities regarding T-AOC and CAT in the liver than those of the con group (Figure 1E), while LJPS-supplemented group ducks exhibited decreased abdominal fat percentage ($P < 0.05$) compared to that of the con group ducks (Figure 1D). Dietary LJPS supplementation ameliorated the intestinal morphology of ducks (Figure 1F–K). The significantly increased villus height (V) ($P < 0.05$) and the value of V/C ($P < 0.05$), whereas a decreased crypt depth (C) of the ileum and jejunum were observed in the LJPS-supplemented group compared to those in the con group.

Profile and Characteristics of Intestinal Microbial Communities in the Ducks Fed with the LJPS-Supplemented or Normal Diet. A total of 86,918 and 81,867 raw reads were determined from the LJPS and con groups, respectively. Of them, 53,064 and 48,372 effective tags were identified for the LJPS and con groups by eliminating low-quality sequences. All effective tags were analyzed according to the standard of 97% sequence similarity, and 663 and 972 operational taxonomic units (OTUs) were

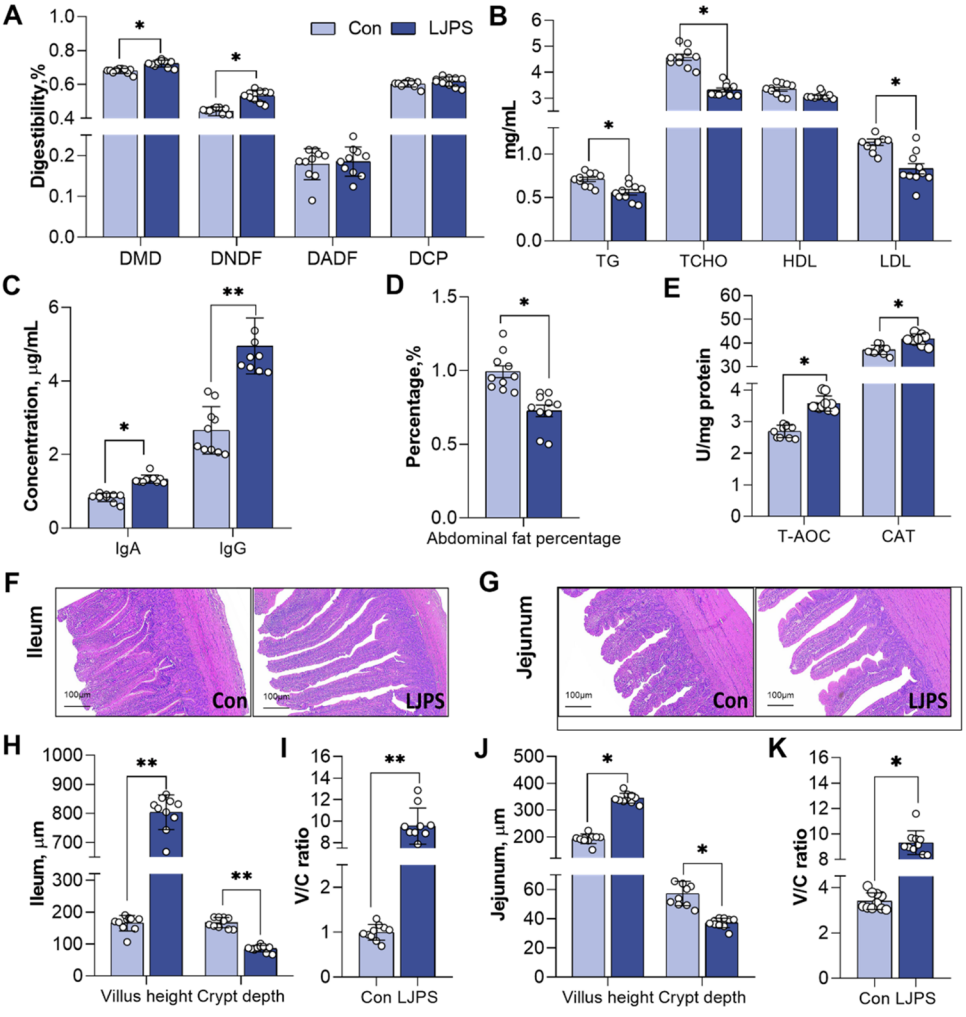

Figure 1. (A) Digestibility of nutrients in ducks (DMD, DNDF, DADF, and DCP); (B) serum biochemical indices of the lipid metabolism intermediate; (C) level of IgA and IgG in serum; (D) abdominal fat percentage; (E) activities of antioxidant enzymes in the liver; and (F–K) comparison of intestinal morphology of the villus height, crypt depth, and the V/C values of the ileum and the jejunum of ducks. *$P < 0.05$ and **$P < 0.01$ mean significant differences.

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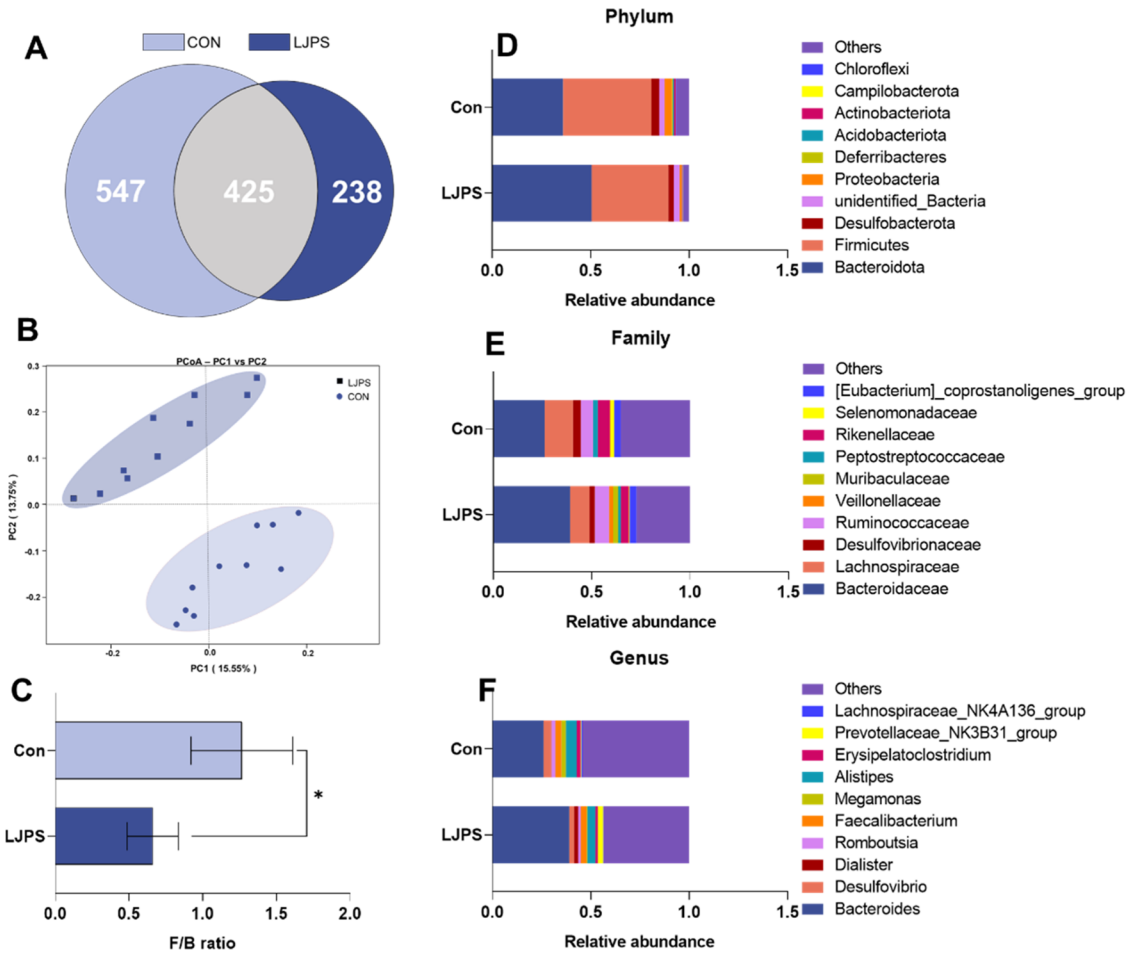

Figure 2. Profile and characteristics of the intestinal microbiota of ducks between two groups. (A) Operational taxonomic unit (OTU) numbers in the cecal microbiota represented in the Venn diagram; (B) β-diversity that was indicated by principal coordinate analysis (PCoA); and (C) ratio of the relative abundance of *Firmicutes* to *Bacteroidetes* (F/B). The relative abundances of bacteria at the (D) phylum, (E) family, and (F) genus levels of the cecal microbiota of ducks are shown. *$P < 0.05$ suggested the significant difference.

Table 2. Analysis of α-Diversity Indices

		richness estimators			α-diversity		
items	coverage, %	OS	Chao 1	ACE	PD_whole_tree	Shannon	Simpson
con	0.995	891.88	980.36	1023.57	76.21	6.22	0.95
LJPS	0.997	593.00	677.88	698.04	57.72	5.59	0.92
P-value	0.016	<0.01	<0.01	<0.01	0.04	0.03	0.05

identified from the LJPS group and con group, respectively. In the LJPS group, all OTUs were then allocated to 43 phyla, 97 classes, 196 orders, 254 families, 341 genera, and 183 species. Similarly, in the con group, 972 OTUs were categorized into 54 phyla, 120 classes, 259 orders, 349 families, 486 genera, and 192 species. Notably, 425 OTUs were shared between the two groups (Figure 2A), 247 exclusive OTUs to the LJPS group, and 547 to the con group.

The analysis of gut microbial characteristics and diversity shows that the sequencing depth has superior coverage to all microbial species (good coverage >99%), while the majority of OTUs present a low richness (Supporting Table 2). As shown in Table 2, the con group has greater richness and diversity of gut microbiota than that in the LJPS group, as demonstrated by the observed species, Chao 1, ACE, PD_whole_tree, Shannon, and Simpson indices ($P < 0.05$) than those in the LJPS group (Table 2). This resulted in decreased α-diversity and richness of the intestinal microbial community in the LJPS group. β-diversity analysis revealed a clear clustering between the con and LJPS groups, suggesting that LJPS supplementation reshaped the intestinal microbiota components. Principal component analysis (PCA) demonstrated that the gut microbial community was nonoverlapping between two groups due to LJPS supplementation (Figure 2B), suggesting that LJPS played an important role in shaping the composition and abundance of intestinal microbiota.

The relative abundances (the value was more than 0.50%) at the phylum, family, and genus levels of gut microbiota are presented in Figure 2D–F (Supporting Table S2). At the phylum level, the dominant phyla were *Bacteroidota* and *Firmicutes* in both groups, and the proportions of *Bacteroidota* were 50.49 and 35.85%, and those of the *Firmicutes* (regardless of treatment) account for 39.06 and 45.00% of the total abundance in the LJPS and con groups, respectively. In

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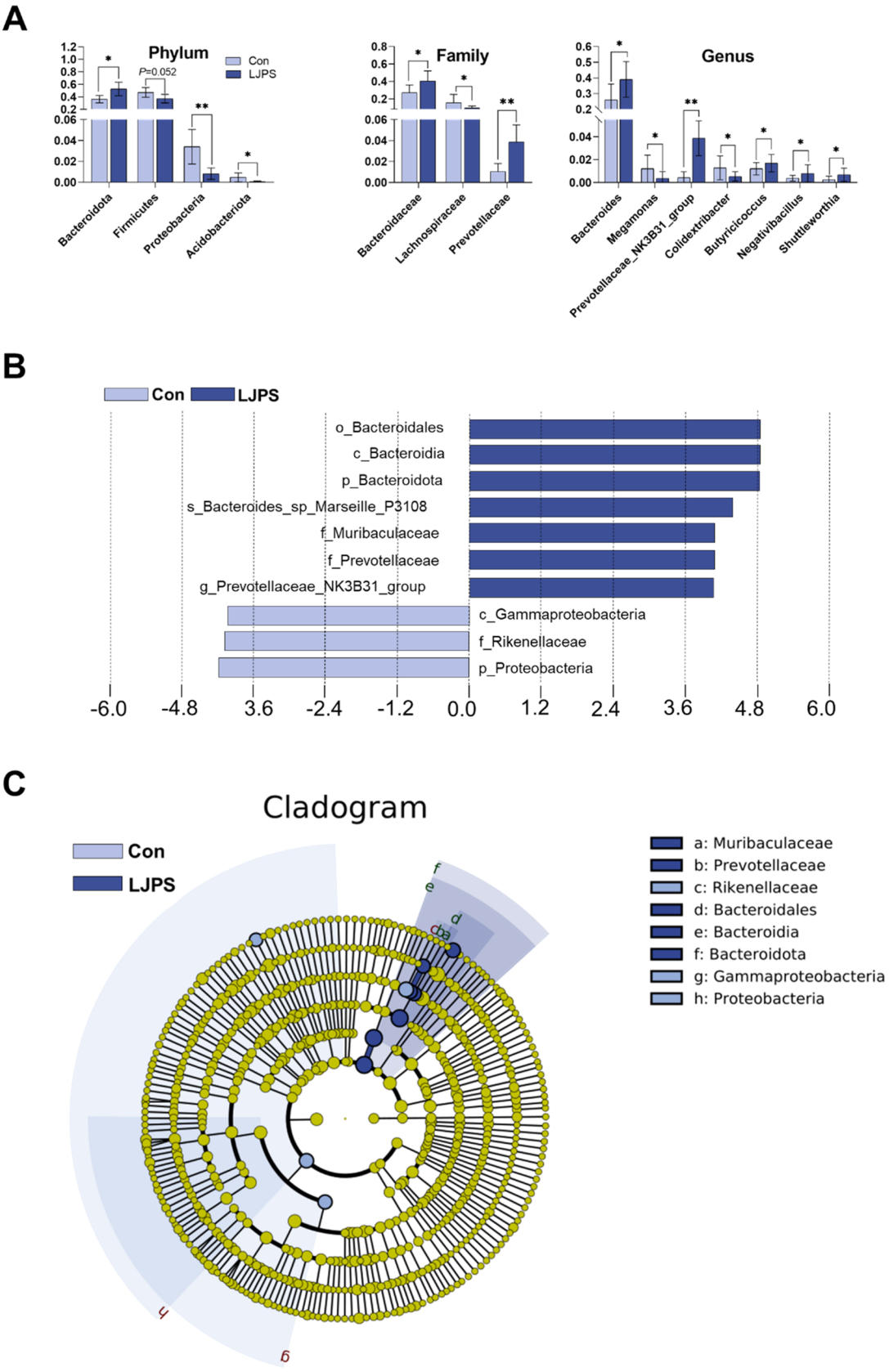

Figure 3. Discrepant microbes between the LJPS and con groups at the (A) histogram show the differentiated microbes at phylum, family, and genus levels. Herein, only the microbes with more than 0.5% relative abundance were compared. Linear discriminant analysis (LDA) values of different microbes were displayed in the histogram and the cladogram (LDA score >3.5). (B) LDA values are expressed in the histogram. (C) Cladogram showing the taxonomic level and phylogenetic relationships of the identified differentiated microbes of the two treatments. *$P < 0.05$ and **$P < 0.01$ mean the significant difference between groups.

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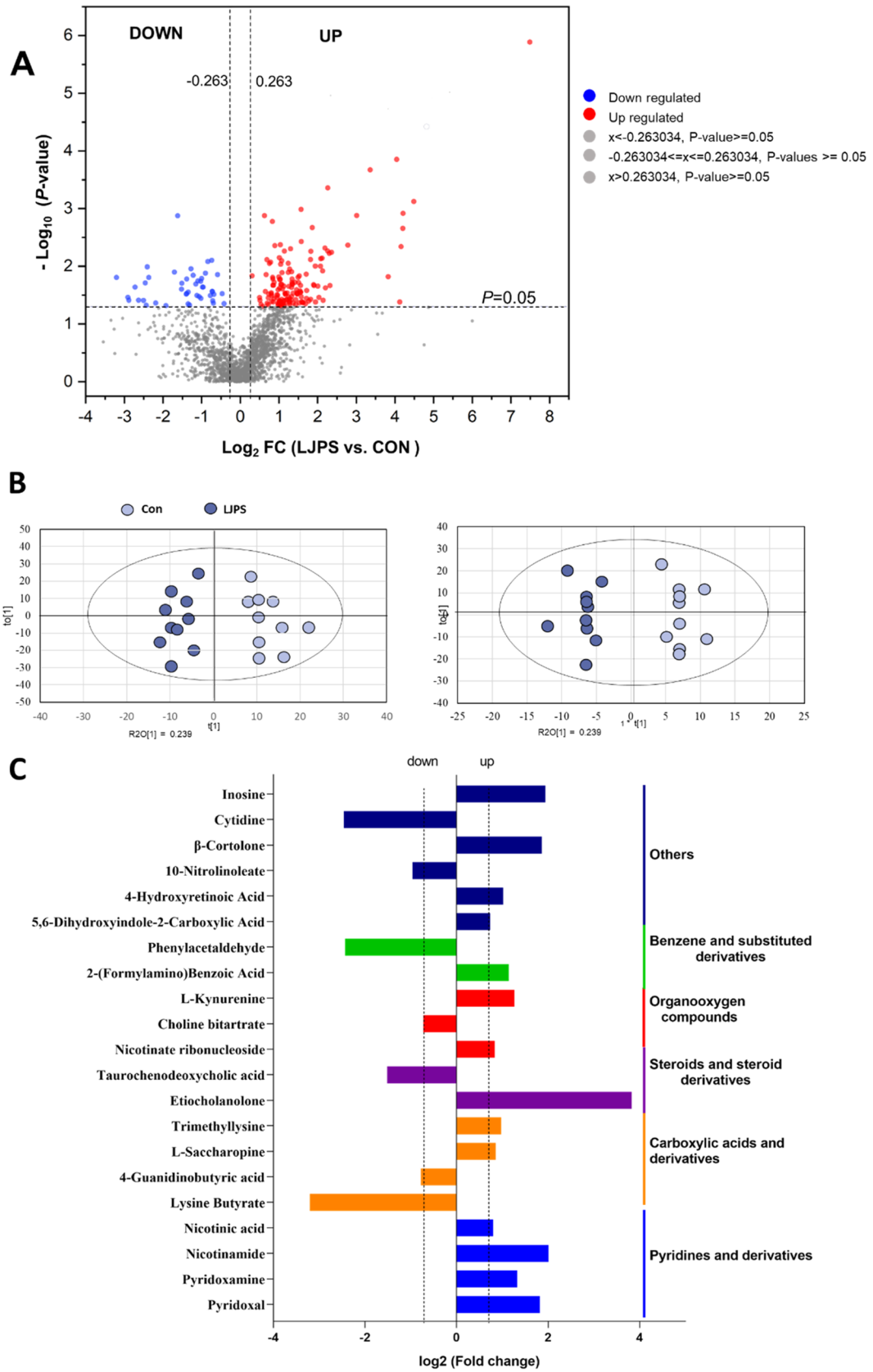

Figure 4. Differential metabolites and their classification. (A) Volcano plot displaying the discrepant metabolites between the LJPS group and the con group. Taking fold change (FC, LJPS vs) and variable importance in the projection (VIP) of metabolite contents in LJPS or con, 186 differentiated metabolites were identified. Of them, 143 are observed to be upregulated, indicated by red dots, and 43 are downregulated, displayed by blue dots. (B) Distribution and discreteness of intestinal metabolites from the con and/or LJPS group are presented by the orthogonal partial least squares-discriminant analysis (OPLS-DA). (C) Ascertained classifications of discrepant metabolites ($P < 0.05$) that were upregulated or downregulated in the LJPS group than those in the con group by matching the *G. gallus* (chicken) KEGG database.

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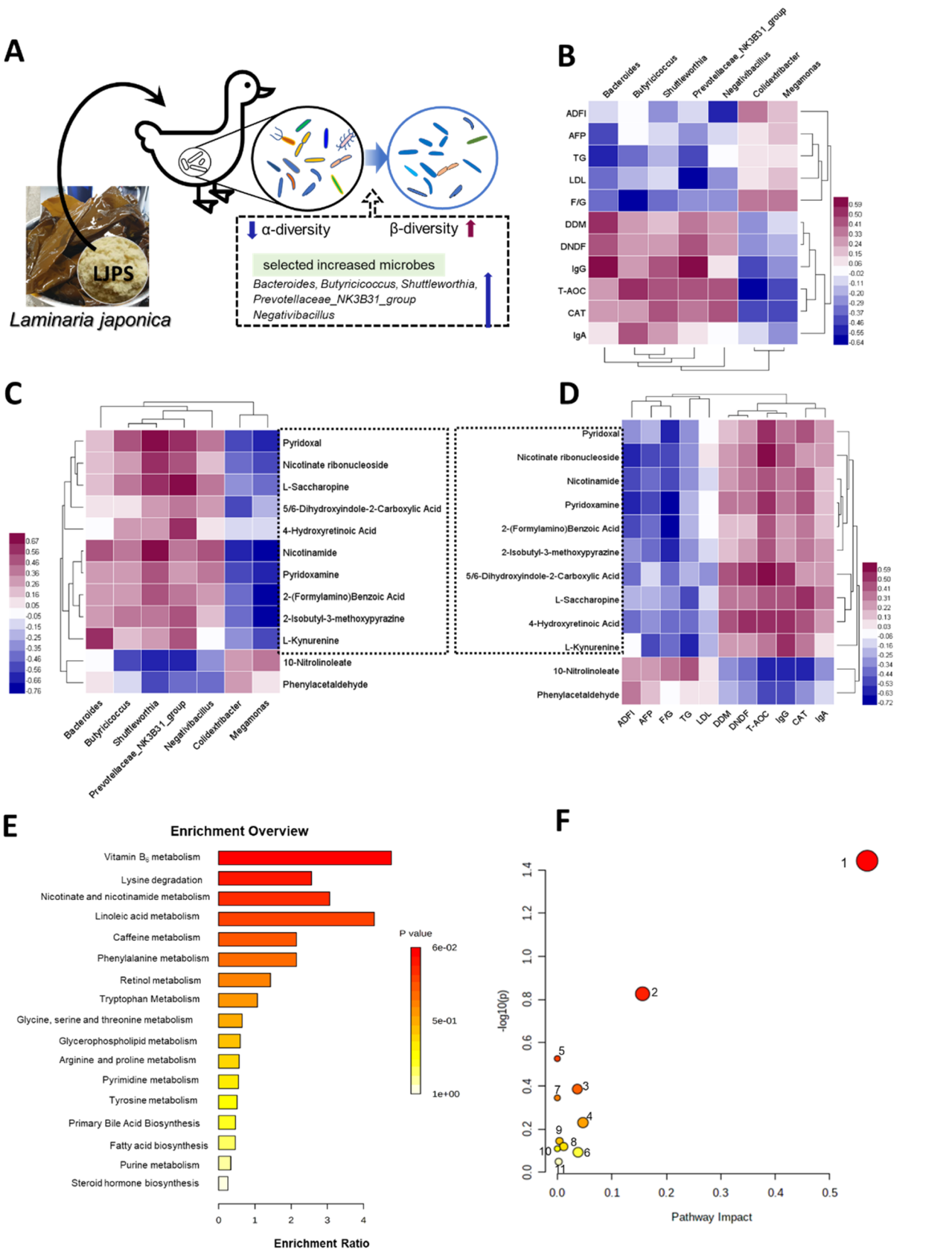

Figure 5. Cause-and-effect process of *L. japonica* polysaccharide (LJPS) supplementation that affects the growth performance, microbiome, and metabolome of ducks. (A) LJPS supplementation especially impacted the diversity of the cecum microbial community and the abundance of some special bacteria. (B) Correlation between the health parameters [average daily feed intake (ADFI), abdominal fat percentage (AFP), feed to gain (F/G), total glucose (TG), low-density lipid protein (LDL), digestibility of dry matter (DDM), digestibility of neutral detergent fiber (DNDF), total antioxidant capacity (T-AOC), catalase (CAT), immunoglobulin A (IgA), immunoglobulin G (IgG)] and differentiated microbes. (C, D) Exerting the correlation between the differentiated microbiota and metabolites and (D) growth performance and differential metabolites.

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Figure 5. continued

Metabolites circled in the black dashed box indicated that these specific metabolites showed a positive correlation both to the productivities and microbes that increased due to the dietary LJPS supplementation. (E) Metabolic pathways between the LJPS and con groups, which were predicated by matching the differentiated metabolites in the pathway-associated metabolite set library (KEGG). (F) Significantly modulated metabolic pathways following the analysis of differentiated metabolites between two groups following the *G. gallus* (chicken) KEGG pathway database. The node colors presented the difference in metabolites. (1) Vitamin B_6 metabolism, (2) tryptophan metabolism, (3) ascorbate and aldarate metabolism, (4) nicotinate and nicotinamide metabolism, (5) lysine degradation, (6) primary bile acid biosynthesis, (7) retinol metabolism, (8) pyrimidine metabolism, (9) steroid hormone biosynthesis, (10) steroid biosynthesis, and (11) purine metabolism.

addition, the abundance of *Proteobacteria* was 3.63% in the con group, which was higher ($P < 0.05$) than that in the LJPS group (0.75%). Additionally, LJPS supplementation decreased the ratio of relative abundance of *Firmicutes* to *Bacteroidota* (F/B) of intestinal microbiota compared to that of the ducks fed with a normal diet (Figure 2C). At the family level, the families *Bacteroidaceae*, *Lachnospiraceae*, and *Desulfovibrionaceae* dominated the cecum microbiota, with their proportions at 39.00, 14.40, and 2.60% in the LJPS group and 26.10, 9.80, and 4.00% in the con group, respectively. At the genus level, the predominant genera were *Bacteroides*, *Desulfovibrio*, and *Dialister*.

Discrepant Bacterial Communities in Cecum Microbiota of Ducks Fed with the LJPS-Supplemented Diet. Discrepant microbes between the LJPS and con groups at different phylogenetic levels (phylum, family, and genus) are shown in Figures 2 and 3. Dietary LJPS supplementation increased the abundance of phylum *Bacteroidota* ($P = 0.008$), while it decreased that of *Firmicutes* ($P = 0.052$), *Proteobacteria* ($P < 0.01$), and *Acidobacteria* ($P = 0.044$), with the relative proportions of 50.49, 39.06, 0.76, and 0.07% in the LJPS group and 35.86, 45.01, 3.69, and 0.58% in the con group, respectively. At the family level, compared to that of the con group, dietary supplementation with LJPS significantly increased the abundance of *Bacteroidaceae* ($P = 0.030$) and *Prevotellaceae* ($P < 0.01$) but decreased the richness of *Lachnospiraceae* ($P = 0.026$) in the intestinal microbiota. Similarly, at the genus level, the LJPS group had increased ($P < 0.05$) the abundance of *Bacteroides*, *Prevotellaceae_NK3B31_group*, *Butyricicoccus*, *Negativibacillus*, and *Shuttleworthia* (39.05, 2.80, 1.69, 0.79, and 0.68%) but decreased ($P < 0.05$) the abundance of *Megamonas* (0.33%) and *Colidextribacter* (0.54%) compared to that of the con group.

The results of the linear discriminant analysis effect size (LefSe) also displayed the typical effects of LJPS supplementation on the component and richness of the intestinal microbiota in ducks (Figure 3B,C). Dietary LJPS supplementation significantly increased the relative abundance of *Bacteroidales*, *Bacteroidia*, *Bacteroidota*, *Muribaculaceae*, *Prevotellaceae*, and *Prevotellaceae_NK3B31_group* and decreased the richness of *Gammaproteobacteria*, *Rikenellaceae*, and *Proteobacteria* in the gut compared to that of the con group (Figure 3B, LDA score >3.5). Furthermore, the cladograms revealed the phylogenetic distribution of discrepant microbes in the LJPS and con groups (Figure 3C).

Profile of the Intestinal Metabolome and Discrepant Metabolites. The metabolome of intestinal content samples was determined using LC-MS, and 1840 metabolites were detected in the two groups. Regarding the fold change (FC) and variable importance in the projection (VIP) of metabolite contents in LJPS or con groups, of which 186 differentiated metabolites were identified (positive and negative ions, Supporting Table S3, the FC values are more than 1.2 or less than 0.833, i.e., $\log_2$ FC (LJPS to con) > 0.263 or $\log_2$ FC (LJPS to con) < −0.263, $P < 0.05$, and VIP > 1). Among the 186 metabolites, 143 metabolites were classified to be upregulated ($\log_2$ FC (LJPS to con) > 1, VIP > 1, and $P < 0.05$; represented in red dots in Figure 4A) and 43 downregulated ($\log_2$ FC (LJPS to Con) < −1, VIP > 1, and $P < 0.05$; indicated by blue dots in Figure 4A) in the LJPS group compared to those in the con group (Figure 4A). To further investigate the similarity and dispersion of the intestinal metabolites between the two groups, orthogonal projections to latent structure discrimination analysis (OPLS-DA) was performed. The findings indicated that the metabolites of the LJPS group could be completely separated from those of the con group, indicating that dietary LJPS supplementation altered the intestinal metabolites (Figure 4B). These findings indicated a completely separated clustering in the determined metabolites between the con and LJPS groups, suggesting that dietary LJPS supplementation shaped a completely different metabolite community in ducks.

For further identifying the target metabolites modulated by LJPS supplementation, the differentiated metabolites were matched with the database of *G. gallus* (chicken) KEGG (https://www.metaboanalyst.ca/MetaboAnalyst/home.xhtm), and 21 effective metabolites were obtained (Figure 4C), which were attributed to benzene and substituted derivatives, organooxygen compounds, steroids and steroid derivatives, carboxylic acids and derivatives, pyridines and derivatives, and others, respectively.

Correlation among the Performance, Differentiated Microbes, Metabolites. The correlation among health parameters, intestinal microbiota, and metabolites was assessed by Spearman's rank analysis, and the results were presented in a heatmap (Figure 5A−D). Additionally, referring to the pathway-associated metabolite set (KEGG) database, taking 186 different metabolites between the two groups to enrich the modulated metabolic pathways, the results showed that 17 metabolic pathways were identified, primarily including growth-related steroid biosynthesis and fatty acid and amino acid metabolism (Figure 5E). These changed metabolic pathways further implied the potential mechanism of dietary LJPS supplementation impacting performance, feed efficiency, and systemic health condition. As shown in Figure 5, the LJPS supplementation decreased the α-diversity of the intestinal community and increased the abundance of some special microbes (Figure 5A). Especially, the genera *Bacteroides*, *Butyricicoccus*, *Shuttleworthia*, *Prevotellaceae_NK3B31_group*, and *Negativibacillus*, with increased ($P < 0.05$) relative abundance in the LJPS-supplemented group, positively linked with DDM, DNDF, T-AOC, CAT, IgA, and IgG, whereas they negatively ($P < 0.05$) correlated with ADFI, AFP, TG, F/G, and LDL levels of the ducks (Figure 5B). In contrast, the genera *Colldextribacter* and *Megamonas* with greater richness in the Con group negatively correlated with alterations in DDM,

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DNDF, T-AOC, CAT, IgA, and IgG ($P < 0.05$; Figure 5B). In addition, the genera *Bacteroides*, *Butyricicoccus*, *Shuttleworthia*, *Prevotellaceae_NK3B31_group*, and *Negativibacillus*, with increased abundance in the LJPS group, exhibited a positive correlation with specific metabolites, including pyridoxal, nicotinate ribonucleoside, nicotinamide, pyridoxamine, 2-isobutyl-3-methoxypyrazine, 2-(formylamino)benzoic acid, L-saccharopine, 4-hydroxyretinoic acid, 5,6-dihydroxyindole-2-carboxylic acid, and L-kynurenine, and negatively correlated with 10-nitrolinoleate and phenylacetaldehyde ($P < 0.05$; Figure 5C). Moreover, these metabolites that were significantly modified due to LJPS supplementation positively correlated with DDM, DNDF, T-AOC, CAT, IgA, and IgG in the ducks ($P < 0.01$; Figure 5D) and were also classified into 11 health-linked metabolic pathways by matching the *G. gallus* (chicken) KEGG pathway database (Figure 5E,F). Of these metabolic pathways, four differential pathways namely, vitamin B_6, tryptophan, nicotinate/nicotinamide metabolism, and lysine degradation were upregulated and had an impact (impact value > 0) on the health of ducks, and the metabolic pathway of primary bile acid biosynthesis and pyrimidine metabolism were downregulated in the LJPS group compared to those in the con group (Figure 5F, Table 3). Additionally, the analysis of the metabolic pathways modulated by dietary LJPS supplementation indicated that these pathways were interrelated and tightly correlated with the energy metabolism of the hosts (Figure 6). Especially, the improved metabolism of vitamin B_6, tryptophan, nicotinate/nicotinamide, and lysine degradation in the LJPS-fed ducks contributed to the increase of the three central energy metabolic biochemical process of glycolysis, pentose phosphate pathway, and tricarboxylic acid cycle (Figure 6 and Table 3). Thus, this might be one of the vital regulating factors to increase energy efficiency through dietary LJPS supplementation.

Table 3. Profile of Metabolic Pathways Modulated by *L. japonica* Polysaccharide (LJPS) Supplementation[a]

names of pathway	total	hits	raw P	$-\log 10(P)$	impact
vitamin B_6 metabolism	9	2	0.036	1.443	0.57
tryptophan metabolism	39	3	0.1489	0.827	0.16
ascorbate and aldarate metabolism	10	1	0.2972	0.527	0
nicotinate and nicotinamide metabolism	15	1	0.4114	0.386	0.04
retinol metabolism	17	1	0.4518	0.345	0
lysine degradation	25	1	0.5879	0.231	0.05
steroid hormone biosynthesis	71	2	0.7159	0.145	0
pyrimidine metabolism	40	1	0.7598	0.119	0.01
steroid biosynthesis	42	1	0.7765	0.109	0
primary bile acid biosynthesis	46	1	0.8067	0.093	0.04
purine metabolism	62	1	0.8923	0.049	0

[a]Total is the total number of compounds in the pathway; hits is the matched compound amounts; raw P is the P-value from the enrichment analysis; and impact is the impact from pathway topology analysis.

These findings suggested that changes in the structure and abundance of intestinal microbiota induced by dietary LJPS supplementation underlay the alterations of metabolites and metabolic pathways, which facilitated the productivities and systemic health of ducks.

DISCUSSION

A *L. japonica* polysaccharide is a long-chain biological macromolecule with highly branched and helical domains.[5] It exerts a variety of bioactivities, including immunomodulatory effects,[5,25] antiviral activity, vascular calcification preventive effects,[24,25] bone health,[26] intestinal microbiota regulatory effects of treating metabolic and cardiovascular disease, and chelating effects (i.e., iron, selenium, and zinc, and bile acids).[27] In this study, a notable finding was that the LJPS-fed ducks presented the ameliorated final production performance (BW, ADG, ADFI, and G:F) compared to those fed with a normal diet. This was consistent with the findings of the study of Venardou et al.,[6] where they addressed that adding laminarin (LJPS) to the diet increased the BW, ADG, and ADFI of broilers after a 35-day feeding experiment. In addition, our findings indicated decreased serum TG, TCHO, and LDL levels and AFP in the LJPS-supplemented group compared to those in the con group. Similarly, our previous study showed that dietary alfalfa PS supplementation promoted the production performance and systemic immunity of broilers.[20] Moreover, Wassie et al.[3] observed that *Enteromorpha* polysaccharides improved antioxidant conditions and ameliorated lipid metabolism in broilers. Thus, dietary LJPS supplementation not only improved the growth performance and feed efficiency of ducks but also ameliorated the lipid profile in serum and deposition.

We also observed that LJPS supplementation increased IgA and IgG levels in serum and the activities of liver antioxidases including CAT and T-AOC. This was well-reconciled with our previous in vitro study where LJPS treatment appeared to have immune-promoting and anti-inflammatory properties in stimulated macrophage cells.[5] Similarly, Luan et al.[9] also documented that antioxidant and immunomodulatory activities are among the primary bioactivities of LJPS.

The intestine's health plays an important role in nutrient absorption.[21] The intestinal morphological development was generally evaluated by determining the villus height, crypt depth, and V/C.[28] The crypt is the place where intestinal cells proliferate and differentiate, and it differentiates continuously from the base to the villi, promoting villi growth. Studies have shown that the length and width of the intestinal villi determine the longitudinal cross-sectional area of the villi, and the width can improve the villus chylous duct reflux and the exchange and absorption of substances.[16,29] The other main function of intestinal villi and crypt cells is to promote the absorption of nutrients in the gut, thus contributing to greater growth performance.[30] Our results indicated that dietary supplementation with LJPS significantly improved the villus height and V/C while decreasing the crypt depth of the ileum and the jejunum. Therefore, dietary LJPS supplementation can ameliorate the intestinal structure and promote intestinal development in ducks.

In this study, another noteworthy finding was that dietary supplementation decreased the α-diversity of the gut microbiota compared to that of the con group while shaping a different gut microbiota between the two groups following the OPLS-DA plot. Thus, feeding LJPS to ducks decreased the diversity of the intestinal microbial community relative to normal diet-fed ducks; therefore, the two different diets shaped two varied microbiota communities with differential composition and abundance, which might be the vital factor for the improved performance. At the phylum level, LJPS supple-

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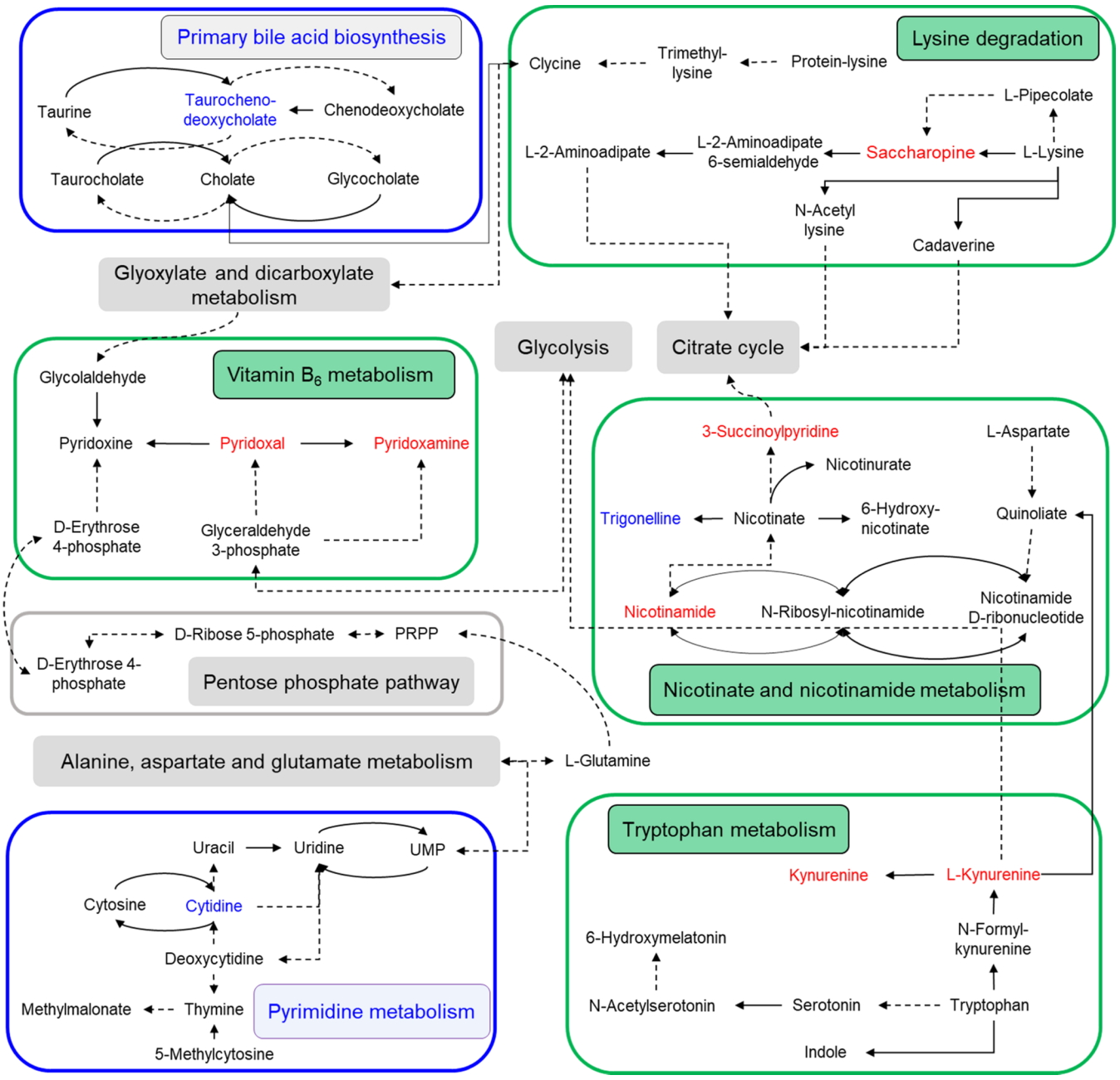

Figure 6. Correlation among the significantly modified metabolic pathways by LJPS supplementation based on the specific metabolites and KEGG pathways. The metabolites written in red font indicate an increase in the concentration, whereas those written in blue font indicate a decrease in the concentration. The rounded rectangles in different colors represent the pathways enriched by KEGG analysis. The metabolic pathways marked with green boxes are upregulated metabolic pathways and those marked with blue boxes are downregulated pathways. The gray rectangles represent the physiological and/or biochemical processes affected by the LJPS-modulated metabolic pathways.

mentation significantly increased the abundance of *Bacteroides* and decreased the richness of *Firmicutes*, *Proteobacteria*, and F/B ratio. In particular, *Bacteroides* are known to promote carbohydrate degradation and utilization,[31] which might play a partial role in the enhanced DDM and DNDF observed in this study. The phylum *Proteobacteria* includes some pathogenic bacteria, such as *Salmonella* and *Escherichia coli*, and their outer membrane is mainly composed of lipopolysaccharide, a major endotoxin. The structure of the microbial community is a pivotal factor that affects nutrient digestibility, energy-harvesting efficiency, and intestinal health.[19,32] The changes in the microbial community were also consistent with the augmented intestinal morphology in terms of an increased villus height and V/C ratio and a decreased crypt height. Additionally, the gut of the LJPS-fed group ducks had significantly increased abundance of *Bacteroides*, *Butyricicoccus*, *Shuttleworthia*, *Prevotellaceae_NK3B31_group*, and *Negativibacillus* compared to that in the gut of the con group ducks, and these microbes positively correlated with the altered health parameters, such as DDM, DNDF, T-AOC, CAT, IgA, and IgG. In contrast, the LJPS-fed group had a decreased abundance of the genera *Colldextribacter* and *Megamonas* compared to that of the con group, and these microbes negatively correlated with the altered parameters, such as DDM, DNDF, T-AOC, CAT, IgA,

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and IgG. Thus, feeding the LJPS-supplemented diet increased the abundance of certain specific beneficial bacteria, such as *Bacteroides*, *Butyricicoccus*, *Shuttleworthia*, *Prevotellaceae_NK3B31_group*, and *Negativibacillus*, which positively linked with the improved growth performance and systematic health of ducks.

In the present study, the OPLS-DA analysis displayed two completely nonoverlapping metabolites community, and 186 discrepant metabolites were distinguished owing to dietary LJPS supplementation. Additionally, Spearman's rank correlation analysis demonstrated that the increased metabolites in the LJPS-supplemented group exhibited a positive linkage with health parameters and modulated beneficial microbes (*Bacteroides*, *Butyricicoccus*, *Shuttleworthia*, *Prevotellaceae_NK3B31_group*, and *Negativibacillus*), whereas they exerted the negative correlation with the increased abundance of *Colidextribacter* and *Megamonas* in the con group. This certified that the increased abundance of the microbes in the LJPS-fed group enhanced the level of pyridoxal, nicotinate ribonucleoside, nicotinamide, pyridoxamine, 2-isobutyl-3-methoxypyrazine, and 2-(formylamino)benzoic acid in intestine content, which further improved the productivities, feed efficiency, and health parameters of the ducks. Similarly, the component and abundance of intestinal microbiota confer the constituents and contents of metabolites in the gut content.[33] The predicted differential metabolic pathways by comparing differentiated metabolites in the KEGG database primarily referred to the vitamin and amino acid metabolism (vitamin B_6, tryptophan, nicotinate, and nicotinamide metabolism and lysine degradation) and bile acid biosynthesis in the LJPS group compared to those in the con group. Of these, vitamin B_6, tryptophan, nicotinate, and nicotinamide metabolism and lysine degradation were upregulated, whereas bile acid biosynthesis was downregulated in the LJPS group compared to those in the con group.

As the most important coenzyme factor of transaminase and decarboxylase, vitamin B_6 is involved in the metabolism of carbohydrates, amino acids, and lipids. Emerging studies have identified more than 50 enzymes in the human active enzyme system are pyridoxal phosphate-dependent enzymes.[34] These enzymes play important roles in reducing skin inflammation, regulating the nervous system, promoting hormone secretion, enhancing immunity, and improving nutrient and energy metabolism.[35] Nicotinamide is involved in energy metabolism as a coenzyme component of NAD and NADP.[36] Thus, the increased nicotinamide metabolism improves energy utilization.

Tryptophan is an essential amino acid for animals' growth and production and plays vital biological roles in maintaining health, nutrient metabolism, and promoting animal growth.[16] Thus, increased tryptophan metabolism might be one of the vital contributors to improved production performance. In addition, tryptophan is the precursor of nicotinamide and is metabolized in the body to produce 5-hydroxytryptophane and serotonin, which improve stress resistance and performance in animals. As a functional amino acid, tryptophan is closely associated with protein metabolism in animals and plays an important role in modulating physiological metabolism, reducing oxidative stress, and improving intestinal morphology.[37] Lastly, lysine is an essential amino acid for ducks and plays an important role in protein and energy metabolism, mineral absorption, and immunity promotion.[38] Thus, dietary LJPS supplementation contributed to improved physiological health, primarily by enhancing the metabolism of vitamin B_6, tryptophan, lysine, and nicotinamide. Consequently, nutrient metabolism, particularly the central energy metabolic pathways, namely, glycolysis, pentose phosphate pathway, and TCA cycle, were enhanced, which are responsible for the superior productivities and systemic health of ducks. In contrast, the metabolic pathway for bile acid biosynthesis was downregulated in the LJPS-supplemented group. This finding was inferred from the decreased bile acid concentration in the intestinal content. In this study, six secondary bile acids, including tauroursodeoxycholic acid, dihydrate, deoxycholic acid, taurochenodeoxycholic acid, sodium dehydrocholate, and glycolithocholic acid, were determined with the lowered concentrations in the gut of LJPS-fed ducks compared with those in the con group. It has been documented that polysaccharides can bind with bile acids to promote excretion via feces, thereby reducing the bile acid circulating back into the liver through enterohepatic circulation.[27] Consequently, it promotes cholesterol conversion into bile acids in the liver, thus decreasing the cholesterol concentrations in the liver or blood.[39] This finding agreed well with the decrease in serum cholesterol concentration observed in our study.

In this study, the results indicated that the LJPS supplementation increased the abundance of some specific bacteria, including *Bacteroides*, *Butyricicoccus*, *Shuttleworthia*, *Prevotellaceae_NK3B31_group*, and *Negativibacillus*, which aroused the upregulation of some pivotal metabolic pathways involving the vitamin B_6 metabolism (proportion of impact, 57%), tryptophan metabolism (impact, 16%), lysine degradation (impact, 5%), and nicotinate and nicotinamide metabolism (impact, 4%). These modulated metabolic pathways are closely associated with protein, lipid, and energy metabolism in ducks. Further exploration revealed that the gut microbiota of LJPS-fed ducks was characterized by a lower α-diversity, while an increased abundance of certain beneficial bacteria relative to the con group. It has been documented that there are quantitative associations between the gut microbes, metabolites, and healthy parameters of the host,[29,40] and especially promoted microbes, rather than greater diversity, improved the energy-harvesting efficiency of the host.[19,41] Thus, in this study, the selectively promoted gut microbes by LJPS supplementation contributed to the increased nutrient availability and productivity of ducks.

Taken together, the current findings confirmed our hypothesis that dietary LJPS supplementation reshaped the exclusive intestinal microbial community and their metabolites, as well as selected upregulated certain growth/health-linked metabolic pathways, which facilitated the ameliorated productivities and systemic health. Additionally, this current study put forwards an innovative perspective in deciphering the intrinsic mechanisms that dietary LJPS supplementation modulated the productivities and systemic health of ducks by interfering with the "diet-gut microbiota/metabolome-physiological health" axis. These findings also implied that these growth-linked microbes/metabolites might be preferred targets for performing nutritional regulations for productivities of animals by supplementing specific plant-derived polysaccharides.

In conclusion, dietary LJPS supplementation enhanced growth performance, nutrient digestibility, systemic antioxidant status, immunity, and intestinal development of ducks. Additionally, compared with feeding a normal diet to ducks, feeding an LJPS-supplemented diet characterized the intestinal

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microbiota with decreased α-diversity, while it did increase the abundance of specific bacteria, including *Bacteroides*, *Butyricicoccus*, *Shuttleworthia*, *Prevotellaceae_NK3B31_group*, *Fournierella*, and *Negativibacillus*. Also, LJPS upregulated the energy utilization and health-promoting pathways that are primarily associated with the metabolism of vitamin B_6, tryptophan, nicotinamide, and lysine and decreased the bile acid concentration in the gut. Taken together, it can be inferred that dietary supplementation with LJPS can selectively augment the abundance of intestinal beneficial bacteria and their metabolites, and thereby facilitate the superior performance and systemic health of ducks. In summary, supplementation with LJPS in the duck diet would be a recommended nutritional strategy to achieve better productivities in the current duck farming industry.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.2c08731.

Composition and nutrient contents of the experimental diet (DM basis); relative abundance (%) of the cecal microbiota in the phylum, family, and genus levels of ducks receiving LJPS and con; different metabolites between LJPS and con groups with LC-MS/MS; and the determination of the gut microbiome (PDF)

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Author Contributions

G.Z. and Y.L. designed the experiments. A.L., E.K., J.C., and J.L. conducted the experiments and analyzed the data. A.L. and J.L. performed the determination of samples. G.Z. and Y.L. wrote and revised the manuscript.

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Notes

The authors declare no competing financial interest.

The microbiome data sets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/sra/PRJNA871872.

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■ ABBREVIATIONS USED

LJPS, *Laminaria japonica* polysaccharide; DMD, digestibility of dry matter; DNDF, digestibility of neutral detergent fiber; DADF, digestibility of acid detergent fiber; DCP, digestibility of crude protein; TG, total glucose; TCHO, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PRPP, phosphoribosyl diphosphate; UMP, uridine monophosphate

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