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Original Research Article

Microencapsulated *Lactobacillus plantarum* promotes intestinal development through gut colonization of layer chicks

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ABSTRACT

The effects of *Lactobacillus plantarum* in microencapsulation (LPM) on intestinal development in layer chicks were investigated in this study, as well as the colonization of *L. plantarum* in the gut. A total of 480 healthy Hy-Line Brown layer chicks at 0 d old were randomly divided into 4 groups (8 replicates each treatment), and the diets of these birds were supplemented with nothing (control), *L. plantarum* (0.02 g/kg feed; 10^9 CFU/kg feed), LPM (1.0 g/kg feed; 10^9 CFU/kg feed) and wall material of LPM (WM; 0.98 g/kg feed), respectively. Compared to control, LPM improved growth performance and intestinal development of layer chicks, evidenced by significantly increased body weight, average daily gain, average daily feed intake, villus height, villus height/crypt depth, as well as weight and length of the duodenum, jejunum and ileum ($P < 0.05$). These results could be attributed to the increased colonization of *L. plantarum* in the gut, which was verified by significant increases in lactic acid content, viable counts in chyme and mucosa ($P < 0.05$), as well as a visible rise in number of strains labeled with fluorescein isothiocyanate. Meanwhile, the relative abundances of *Lactobacillus* and *Bifidobacterium* significantly increased in response to microencapsulated *L. plantarum* supplementation ($P < 0.05$), accompanied by the significant up-regulation of colonization related genes ($P < 0.05$), encoding solute carrier family, monocarboxylate transporter, activin A receptor, succinate receptor and secretogranin II. To sum up, microencapsulated *L. plantarum* supplementation promoted intestinal development, which could be attributed to the enhancement of *L. plantarum* colonization in the intestine through the mutual assistance of *Bifidobacterium* and interactions with colonization related transmembrane proteins.

1. Introduction

As intestinal maldevelopment in young animals can irreversibly impair their growth and production performance in subsequent developmental phases, the importance of intestinal development has garnered increasing attention. In reality, the positive benefits of *Lactobacillus plantarum* on intestinal development have been widely reported in various animal models, such as piglet, broiler and tilapia (Dawood et al., 2020; Humam et al., 2019; Wang et al., 2019). However, there are still several inconsistent reports that *L. plantarum* supplementation may not significantly improve the intestinal development of animals (Lee et al., 2017; Zheng et al., 2018). The foremost reason for these inconsistent results could be due to the discrepancy in viable counts of *L. plantarum* arriving in the intestine. Such situations may be attributed to the different degrees of decline in the survival rate of *Lactobacillus* during processing, storage and digestion (Lee et al., 2019). In fact, having established microencapsulation technology of *L. plantarum* M616, we found that *L. plantarum* in microencapsulation (LPM) could smoothly pass through the gastric juices and maintain a high survival rate upon arrival in the intestine (Song et al., 2022). Given the aforementioned inconsistent reports, it is necessary to explore the precise effects of *L. plantarum* on intestinal development in the

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form of microencapsulation, which can provide scientific guidance for production.

Colonization in the gut is an important prerequisite for *L. plantarum* to exert long-term and stable benefits on intestinal development (Tuomola et al., 1999; Zmora et al., 2018). Adhesion to mucosa was thought to provide *Lactobacillus* with a competitive advantage for intestinal colonization (Frese et al., 2013). The mucosa layer lines the gastrointestinal tract, serves as the first contact between the intestinal microbiota and host, and provides a habitat for these microbes (Atuma et al., 2001; Johansson et al., 2011, 2008). Although the intestinal mucosal tissue plays a prominent role in the colonization of probiotics, most of the current research concerning *L. plantarum* focuses only on its probiotic functions, with very limited investigation into the colonization of *L. plantarum* in the intestinal mucosa. Therefore, *L. plantarum* adhesion to and colonization in intestinal mucosal tissue need to be explored in further detail.

The achievement of *L. plantarum* colonization is closely related to the interaction between host and microbe, and many factors influence the colonization process. These factors can be divided into 2 main categories: microbial molecules (Buck et al., 2005; Jacobson et al., 2018; Kankainen et al., 2009) and substances derived from the host (Joglekar et al., 2019). Furthermore, host and microbe genotypes and their expression may vary (Crook et al., 2019; Song et al., 2018) during *Lactobacillus* colonization in the gut. Additionally, metabolic interactions among incoming strains and original residents have been purported to facilitate microbial colonization (Krumbeck et al., 2015; Turroni et al., 2016). Clarifying the mechanisms can enable probiotics to colonize the intestine more efficiently, thereby exerting their beneficial effects. However, due to complex interactions and a multitude of unknown factors, the gut colonization mechanisms of *L. plantarum* remain unclear. Therefore, more work is needed to explore the colonization mechanisms of *L. plantarum* in the intestine.

Poultry production has been an enormous industry in China, and more than 15.74 billion chickens were fed in 2021. In production, the incidence of diarrhea and intestinal injury in chicken flocks reached 54.5% and 53.0% (Lobani et al., 2016; ter Veen et al., 2017) respectively, which reflects the serious intestinal health challenges in the poultry industry. Besides the EU and US, China banned the addition of antibiotics in animal feed from 2020. The mortality of broilers increased from 2.8% to 4.2% after antibiotics were prohibited (Ritter et al., 2019), making intestinal health issues more serious. In fact, *Lactobacillus* colonization in the gut has been considered effective for improving intestinal development and gut health (Yan et al., 2017). Probiotic intervention during the early stages of life has been found to more easily colonize the bowel lumen (Gueimonde et al., 2006). Because layers have a long lifespan, early-phase intestinal maldevelopment means higher losses in later stages. Hence, in this study, we investigated the impact of microencapsulated *L. plantarum* on intestinal development, the colonization of *L. plantarum* in the intestinal mucosa and the underlying colonization mechanisms in layer chicks.

2. Materials and methods

2.1. Animal ethics statement

The animal protocols for this study were approved by the Animal Care and Use Committee of Henan University and Technology.

2.2. Birds and experimental diets

A total of 480 healthy Hy-Line Brown layer chicks at 0 d old were randomly divided into 4 groups, with 8 replicates per group and 15 birds per replicate. The diets for birds in these groups were basal diets supplemented with nothing, *L. plantarum*, LPM and wall material of LPM (WM), respectively. The LPM was prepared through *L. plantarum* M616 microencapsulation with WM, and the latter contained enzymatic hydrolysate of soybean protein isolate (4%; hydrolyzed by pepsin), modified phospholipid (10%), soybean oil (20%), sorbitol (60%) and glycerol (4%), according to a previous report (Song et al., 2022). The viable counts in LPM reached 10^9 CFU/g. Hence, the supplemental levels of *L. plantarum*, LPM and WM were 0.02 g (10^9 CFU), 1.0 g and 0.98 g, per kilogram feed, respectively. Feed and water could be accessed freely, and the experimental management was according to the feeding management manual of Hy-Line Brown layer chicks. Air quality was ensured by a programmed ventilation system and timely cleaning of litter. Experimental diets were formulated according to the Chinese Feeding Standard of Chicken (NY/T 33-2004) and NRC (1994). The feeding trial lasted for 3 weeks, and the nutrient values of the basal diets are shown in Table S1. Feed raw materials were dried to a constant weight in a forced-air oven at 55 °C for a minimum of 48 h, and finely ground to pass through a 2-mm screen. Analytical dry matter content of the samples was measured by drying at 105 °C for 6 h (AOAC, 2016; method 930.15). Crude protein was analyzed by the Kjeldahl method (AOAC, 2006; method 984.13). A spectrophotometer (UV-2700, Shimadzu, Japan) was adopted to analyze P content, while flame atomic absorption spectrophotometry (Zeenit700P, Analytik Jena, Germany) was used to measure the content of Ca. The calculated nutrition level (like AME) in Table S1 referred to the Tables of Feed Composition and Nutritive Values in China (2020). The calculation methods were as follows:

$$AME_{total} = \sum_{i=1}^{n} (AME_i \times C_i)$$

where AME_{total} was calculated AME value of feed, AME_i was the AME value of feedstuff *i* adopted in feed, and C_i were the percentage of feedstuff *i* in feed.

2.3. Growth performance and intestine collection

In this study, body weight (BW) was recorded at both the beginning and end of the formal experiment. Feed intake was recorded weekly. The performance indicators, including average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR; feed intake/BW gain, g/g), were carefully calculated. After the trial ended, 24 birds from each group were randomly selected (3 birds per replicate at average BW) and weighed prior to slaughter. The weight and length of the duodenum, jejunum, and ileum were measured to evaluate the impacts of LPM supplementation on intestinal development.

Segments, approximately 1.5 cm in length, in the middle portion of the duodenum, jejunum, ileum were obtained, washed with PBS and fixed in 10% neutral-buffered formalin for histology analysis. The remaining intestinal segments were opened longitudinally, and then chyme and mucosal samples were collected. Then ileal mucosal tissue samples were immersed in liquid nitrogen, and then stored at −80 °C. These samples were used for subsequent mRNA expression measurement of the investigated genes (Table 1), microflora and transcriptomic profile analyses.

2.4. Intestinal morphology

The intestinal samples underwent a series of procedures, including washing, dehydration, clarification, and embedding in paraffin. Subsequently, sections were cut into 5 μm thickness,

Table 1
Primer sequence of target and reference genes.

Gene	Forward primer (5′–3′)	Reverse primer (3′–5′)	GenBank number	Product length, bp
SLC15A1	TCTCTGTCCGTCCCTCGGTC	GGGGTAGCCAAAGCAGTTCG	NM_204365.2	116
LOC416086	AGTTTCTTATAAAGTGCTGGGACAC	ATACAAGCCAGTGGAAGGGC	XM_040646407.2	201
ACVR1C	TTCTGACGCACAAACAGGGA	TTGCACTGCTCAACACAAGC	XM_040703732.2	124
SUCNR1	GGCCAATAACTTCACGTGCC	GAACCCAAAGAGCGTCAGGA	XM_025153465.3	91
SCG2	GCAAGGTTGGCTTCGCTC	TACTCCAGCACCTTTGCCAG	XM_040706039.2	107
TMEM174	TGCTTTCTGTCGGCGTAACT	TTCCGGTGAACACGAAGGAC	NM_001282271.2	141
GDF8	AAACGGTCCCGCAGAGATTT	CAGGTGAGTGTGCGGGTATT	NM_001001461.2	195
β-Actin[1]	GAGAAATTGTGCGTGACATCA	CCTGAACCTCTCATTGCCA	L08165	

SLC15A1 = solute carrier family 15 member 1; *LOC416086* (*MCT2L*) = monocarboxylate transporter 2-like; *ACVR1C* = activin A receptor type 1C; *SUCNR1* = succinate receptor 1; *SCG2* = secretogranin II; *TMEM174* = transmembrane protein 174; *GDF8* (*MSTN*) = myostatin.
[1] Sequences refer to Feng et al. (2021).

deparaffinized in xylene, rehydrated, stained with hematoxylin and eosin, fixed with neutral balsam, and observed by light microscopy (BX51, Olympus Co., Tokyo, Japan). The intestinal morphometry was then evaluated by villus height (VH; from the tip of villus to the villus-crypt junction), crypt depth (CD; from the base up to the crypt-villus transition region) and the villus height to crypt depth ratio (VCR), as described by Forte et al. (2016).

2.5. Lactic acid content, pH and L. plantarum population analysis

The lactic acid content was measured using the method described by Borshchevskaya et al. (2016), and P-hydroxybiphenyl colorimetry was adopted. The value of intestinal pH was measured by a pH-meter (Testo 206 pH-meter, Germany). The amount of *L. plantarum* was determined using the method of plate counting. To solidify the counting media, agar powder was added at a rate of 18 g per liter. Following thorough dispersion, samples were serially diluted and plated in triple to obtain the viable count.

2.6. L. plantarum marked by fluorescein isothiocyanate (FITC)

The cultured *L. plantarum* M616 was centrifuged at 4612 × *g* for 10 min, and the supernatant was removed. Resuspension of bacteria was carried out using sterile PBS buffer 3 times. FITC dye solution was added into the bacterial solution, and the mixture was incubated for 2 h with shaking at 37 °C. After centrifuging the stained bacterial solution at 4612 × *g* for 10 min, the supernatant was discarded. The sterile PBS buffer was used to resuspend the mixture (4612 × *g* for 10 min), and this process was repeated several times until no fluorescence remained in the supernatant. The cultivation of *L. plantarum* and preparation of LPM were conducted according to the instructions in our former report (Song et al., 2022).

2.7. Quantification of mRNA with real-time PCR

Total RNA was extracted using the TRIzol reagent (Tiangen Biotech Co., Ltd., Beijing, China). The RNA yield was measured using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA), and the integrity was assessed using agarose-ethidium bromide electrophoresis. Quantification was performed through a 2-step reaction process involving reverse transcription and PCR, in accordance with the instructions of the FastQuant RT Kit (KR106, Tiangen, Beijing, China). Each reverse transcription reaction contained 1 μg RNA. Real-time PCR was carried out using a Light Cycler 480 Real-Time PCR Instrument (Roche Diagnostics, Basel, Switzerland) with a 20 μL PCR reaction mixture, which contained 2 μL cDNA, as per the instructions of the SuperReal PreMix Plus kit (SYBR Green; KR106, Tiangen, Beijing, China). Real-time quantitative PCR reactions were conducted in duplicate, using the Bio-Rad C1000 thermal cycler (CFX-96 real-time PCR detection systems; Bio-Rad, Hercules, CA, USA). The protocol involved an initial step at 95 °C for 15 min, followed by 40 cycles of amplification at 95 °C for 10 s and 60 °C for 30 s. The relative mRNA expression levels were normalized to avian β-actin according to the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). The primer sequences are listed in Table 1.

2.8. Analysis of microflora in ileal mucosal tissue

Microbial DNA was extracted from ileal mucosal tissue samples (approximately 0.3 g) obtained from layer chicks using a DNA Kit (Omega Bio-tek, Norcross, GA, USA). The quality and integrity of the DNA samples were assessed using 1% agarose gel electrophoresis and a Nanodrop D-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Microbial 16S rDNA sequences, spanning the hypervariable regions v3–v4, were amplified using forward primer 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and reverse primer 806R (5′-GGACTACHVGGGTWTCTAAT-3′). The PCR reaction conditions were as follows: a 2-min denaturation step at 95 °C, followed by 25 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 30 s; and a final extension step at 72 °C for 5 min. The AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) was used to purify the amplicons extracted from 2% agarose gel, with the purpose of eliminating any superabundant primer dimers and dNTPs. At Beijing Biomarker Biotechnology Co., Ltd. (Beijing, China), the purified amplicons underwent qualification and sequencing using the MiSeq platform. The raw reads were deposited into the database of NCBI Sequence Read Archive (SRA; accession number: PRJNA991241).

For the microbial community analysis, raw paired-end sequences were generated by the Illumina HiSeq 2500. After sequencing, the raw data underwent base calling and was converted to raw reads. The subsequent steps included filtration (Trimmomatic v0.33) and screen (cutadapt 1.9.1) to obtain high-quality reads. The high-quality reads were then pieced together through overlap (FLASH v1.2.7) to obtain clean reads. To obtain effective reads, the chimera sequences were first identified and removed using UCHIME (v4.2). The effective reads were then clustered into operational taxonomic units (OTUs) with 97% sequence identity using USEARCH (Edgar, 2013). The estimation of β-diversity was conducted through the computation of weighted UniFrac distance, followed by visualization using principal coordinate analysis (PCoA).

2.9. Transcriptomic profiling analysis

TRIzol reagent (Tiangen Biotech Co., Ltd., Beijing, China) was used to extract total RNA from the ileal mucosal tissue following the manufacturer's instructions. The RNA quality was evaluated using

the Agilent Bioanalyzer 2100 system (Agilent Technologies, CA, USA) prior to library preparation, and RNA samples (1 μg) were used for RNA library construction, which contained mRNA purification, fragmentation under elevated temperature, and the synthesis of double strand cDNA. Exonuclease/polymerase was used to transform the remaining overhangs into blunt ends. The cDNA library construction contained the adenylation of 3′ ends of DNA fragments, NEBNext Adaptor ligation and PCR. PCR products were purified using the AMPure XP system (Beckman Coulter, Beverly, USA), and the quality of the library was evaluated using the Agilent Bioanalyzer 2100 system (Agilent Technologies, CA, USA). After clustering, the library sequencing was conducted using an Illumina platform. The raw sequencing data have been deposited in the NCBI SRA (accession number: PRJNA993654).

Clean reads were obtained through low quality raw read elimination, or with adapter and ploy-N. The Q20, Q30, GC content and sequence duplication level of clean data were evaluated. Alignment of the clean reads to the reference genome (*Gallus gallus* 5.0) was conducted using the HISAT2 tool in soft mode. Function annotation was carried out on the basis of the databases below: Nt (NCBI non-redundant nucleotide sequences), COG (Clusters of Orthologous Groups) and GO (Gene Ontology). Gene expression levels were estimated using fragments per kilobase of transcript per million fragments mapped (FPKM). Differentially expressed genes (DEGs) analysis was conducted using DESeq2, and the obtained *P*-values were subjected to Benjamini and Hochberg's method for controlling the false discovery rate (FDR). GO analysis of DEGs (fold change >1.5, FDR <0.05) was performed using the GOseq R packages based Wallenius non-central hyper-geometric distribution and the KEGG pathway enrichment analysis of DEGs was carried out using KOBAS software (Mao et al., 2005).

2.10. Statistical analysis

SAS (version 9.2, SAS Institute Inc., Cary, NC, USA), was utilized for data analyses. The experimental unit for growth performance analysis was the replicate, with each replicate in 1 cage. As for the other parameter measurements, the experimental unit for statistical analysis was the mean of 3 birds. The homogeneity of variances and normality of the data were first tested, among them the normality analysis using the Shapiro–Wilk test. Then, a one-way ANOVA was conducted and the means were compared using Duncan's Multiple Range Test. Differences were considered statistically significant at $P < 0.05$, and the data were expressed as mean and pooled SEM.

3. Results

3.1. Growth performance

The effects of microencapsulated *L. plantarum* inclusion on growth performance of layer chicks are listed in Table 2. In this experiment, there was no significant difference in initial BW among all treatments ($P = 0.471$). Furthermore, no significant differences were observed in FCR among all the treatments during the whole experiment ($P > 0.05$). Compared with the control, the significantly higher values of BW (7 and 21 d) and ADG (0–7 and 0–21 d) and ADFI (0–7, 14–21 and 0–21 d) were observed in the treatment of LPM ($P < 0.05$). Significantly higher values of ADFI were also observed in free *L. plantarum* inclusion treatment (0–7 d) compared with the control ($P < 0.05$).

Table 2
Effect of microencapsulated *Lactobacillus plantarum* supplementation on the growth performance of layer chicks[1].

Items	Treatments				SEM	*P*-value
	Control	LP	WM	LPM		
BW, g						
0 d of age	42.5	42.6	42.5	41.2	0.37	0.471
7 d of age	76.6^{b}	78.9^{ab}	75.8^{b}	80.0^{a}	0.60	0.033
14 d of age	140	146	143	148	1.3	0.079
21 d of age	229^{b}	243^{ab}	234^{b}	250^{a}	2.8	0.020
0–7 d of age						
ADG, g	4.88^{b}	5.19^{ab}	4.76^{b}	5.55^{a}	0.105	0.022
ADFI, g	9.00^{b}	9.71^{a}	9.08^{b}	9.62^{a}	0.072	<0.001
FCR	1.85	1.89	1.91	1.75	0.033	0.364
7–14 d of age						
ADG, g	9.04	9.54	9.60	9.78	0.169	0.482
ADFI, g	21.0^{a}	20.2^{b}	21.0^{a}	19.9^{b}	0.14	0.002
FCR	2.34	2.13	2.20	2.05	0.041	0.080
14–21 d of age						
ADG, g	9.4	10.2	9.5	10.7	0.22	0.115
ADFI, g	24.2^{b}	25.4^{b}	24.5^{b}	28.0^{a}	0.40	<0.001
FCR	1.91	1.84	1.91	1.94	0.035	0.876
0–21 d of age						
ADG, g	8.89^{b}	9.53^{ab}	9.10^{b}	9.97^{a}	0.136	0.014
ADFI, g	18.1^{b}	18.4^{ab}	18.2^{b}	19.1^{a}	0.14	0.023
FCR	2.04	1.94	2.00	1.92	0.024	0.303

LP = *Lactobacillus plantarum*; LPM = *Lactobacillus plantarum* in microencapsulation; WM = wall material of LPM; BW = body weight; ADG = average daily gain; ADFI = average daily feed intake; FCR = feed conversion ratio (feed intake/body weight gain, g/g).
[a,b] Values within a row with no common superscripts differ significantly ($P < 0.05$).
[1] Data are the mean of 8 replicates.

3.2. Intestinal development

The development of the small intestine, including the duodenum, jejunum and ileum in response to microencapsulated *L. plantarum* inclusion, is shown in Table 3. No significant differences were observed in the indices of the duodenum (7, 14 and 21 d) among all groups ($P > 0.05$). Compared with the control, significantly higher values for duodenum weight (7 and 21 d), jejunum weight (7 and 21 d), ileum weight (7 and 21 d), duodenum length (7 d), jejunum length (14 and 21 d) and ileum length (14 and 21 d) were observed with LPM treatment ($P < 0.05$). Compared with the control, jejunum index (7 d) was significantly higher with LPM treatment ($P < 0.05$). Compared with LPM treatment, significantly lower values for duodenum weight (7 and 21 d) and ileum weight (21 d) occurred with free *L. plantarum* supplementation treatment ($P < 0.05$).

3.3. Intestinal morphology

The changes in intestinal morphology in response to microencapsulated *L. plantarum* supplementation are shown in Figs. 1 and 2. Compared with the control, significantly higher VH values of duodenum (7 and 21 d), jejunum (14 d) and ileum (7 and 21 d) were observed with LPM treatment ($P < 0.05$); meanwhile, significantly lower CD values for duodenum (21 d), jejunum (7 d) and ileum (21 d) occurred with LPM supplementation treatment ($P < 0.05$). In addition, a significantly higher VCR in the duodenum (7 and 21 d), jejunum (7, 14 and 21 d) and ileum (7, 14 and 21 d) was observed in the treatment of LPM ($P < 0.05$), compared with the control.

3.4. Intestinal pH and lactic acid content

The effects of microencapsulated *L. plantarum* inclusion on intestinal pH and lactic acid content are shown in Fig. 3. No significant

Table 3
Effect of microencapsulated *Lactobacillus plantarum* supplementation on the development of small intestine in layer chicks[1].

Items	Treatments				SEM	*P*-value
	Control	LP	WM	LPM		
7 d of age						
Duodenum						
Weight, g	1.48^{b}	1.46^{b}	1.54^{b}	1.80^{a}	0.039	0.002
Index, %	1.81	1.72	1.71	1.80	0.034	0.663
Length, cm	13.4^{b}	13.6^{b}	13.6^{b}	15.2^{a}	0.24	0.020
Jejunum						
Weight, g	1.47^{bc}	1.63^{ab}	1.36^{c}	1.74^{a}	0.042	0.002
Index, %	1.79^{a}	1.92^{a}	1.51^{b}	1.81^{a}	0.040	<0.001
Length, cm	26.5	27.7	27.0	27.9	0.35	0.454
Ileum						
Weight, g	0.86^{c}	1.02^{ab}	0.89^{bc}	1.11^{a}	0.030	0.006
Index, %	1.05^{ab}	1.20^{a}	0.99^{b}	1.15^{ab}	0.030	0.040
Length, cm	20.7^{b}	23.9^{ab}	20.9^{b}	24.4^{a}	0.48	0.002
14 d of age						
Duodenum						
Weight, g	2.46	2.43	2.41	2.61	0.033	0.115
Index, %	1.49	1.46	1.45	1.52	0.018	0.588
Length, cm	15.3	15.5	15.3	16.1	0.14	0.102
Jejunum						
Weight, g	2.32	2.39	2.44	2.71	0.059	0.094
Index, %	1.40	1.43	1.47	1.58	0.030	0.164
Length, cm	25.6^{b}	27.6^{ab}	26.6^{ab}	28.6^{a}	0.40	0.038
Ileum						
Weight, g	1.51	1.55	1.55	1.63	0.033	0.664
Index, %	0.906	0.926	0.929	0.946	0.0182	0.897
Length, cm	26.2^{b}	27.4^{ab}	27.4^{ab}	29.0^{a}	0.38	0.049
21 d of age						
Duodenum						
Weight, g	3.08^{b}	3.33^{b}	3.29^{b}	3.75^{a}	0.081	0.017
Index, %	1.31	1.29	1.37	1.32	0.028	0.832
Length, cm	15.7	16.2	16.0	16.8	0.21	0.312
Jejunum						
Weight, g	3.13^{b}	3.53^{ab}	3.21^{b}	3.90^{a}	0.091	0.003
Index, %	1.33	1.37	1.33	1.38	0.026	0.880
Length, cm	26.4^{b}	28.0^{ab}	27.0^{b}	29.2^{a}	0.35	0.015
Ileum						
Weight, g	2.14^{b}	2.29^{b}	2.24^{b}	2.70^{a}	0.072	0.017
Index, %	0.920	0.888	0.929	0.956	0.0220	0.759
Length, cm	27.6^{c}	29.6^{ab}	28.8^{bc}	30.5^{a}	0.32	0.004

LP = *Lactobacillus plantarum*; LPM = *Lactobacillus plantarum* in microencapsulation; WM = wall material of LPM.
[a-c] Values within a row with no common superscripts differ significantly ($P < 0.05$).
[1] Data are the mean of 8 replicates with 3 birds each.

differences were observed in the pH values of the duodenum at 14 and 21 d of age, the pH values of the jejunum at 7 and 14 d of age, and the pH value of the ileum at 14 d of age, between the control and LPM treatment ($P > 0.05$). Significantly lower pH values in the duodenum at 7 d, jejunum at 21 d, and ileum at 7 and 21 d were observed with LPM treatment ($P < 0.05$), compared with the control. The remarkable thing was that all the pH values with LPM treatment, including in the duodenum, jejunum, ileum (at 7, 14 and 21 d), were numerically lower than those of the control.

No significant differences were observed in lactic acid content of ileal chyme at 7 d of age between the control and LPM treatments ($P = 0.268$). Compared with the control, significantly higher values for lactic acid content in the duodenum (7, 14 and 21 d), jejunum (7, 14 and 21 d) and ileum (14 and 21 d) were observed with LPM treatment ($P < 0.05$).

3.5. Intestinal L. plantarum population

The effects of microencapsulated *L. plantarum* inclusion on intestinal *L. plantarum* viable counts are shown in Fig. 4. Compared with the control, significantly higher values of *L. plantarum* viable counts in both digesta and mucosal tissue of the duodenum, jejunum and ileum were simultaneously observed following LPM treatment at 7, 14 and 21 d ($P < 0.05$).

3.6. Colonization of L. plantarum labeled with FITC in intestinal mucosa

The colonization of *L. plantarum* in the small intestine is detailed in Fig. 5. Microencapsulation distinctly enhanced the colonization efficiency of *L. plantarum* (labeled with FITC) in small intestinal sections, including the duodenum, jejunum and ileum. Moreover, the highest colonization counts were observed in ileal mucosal tissue.

3.7. Ileal microbial diversity and community

After filtering, an average of 83,488 effective sequences per sample were acquired. The sequencing depths were evaluated by plotting rarefaction curves and examining the numbers of shared OTUs. The curves of all the samples reached plateaus, indicating that sampling depth was adequate. As shown in the heatmap of bacterial community composition of the 14 samples (Fig. 6A), the predominant strains clustered together in the control and LPM treatment respectively. As illustrated in Fig. 6B, β-diversity analysis was carried out to compare the microbial profiles in ileal mucosa between the control and LPM treatment. PCoA was performed to show a holistic perception of these microbes. Results visually showed that these groups were mainly scattered into 2 distinct clusters, which indicated the microbiota compositions were quite dissimilar to each other.

To investigate the effect of LPM supplementation on ileal mucosal microbiota, the taxonomic compositions were explored at the phylum and genus levels. Three major phyla (Fimicutes, Proteobacteria and Actinobacteria; relative abundance > 1%) dominated the bacterial community (Fig. 6C). Meanwhile, these phyla could be allocated into 18 major genera (Fig. 6D). Compared with the control, significantly higher values of Firmicutes and Actinobacteria phyla, and *Lactobacillus*, *Bifidobacterium* and *Lachnospiraceae_NK4A136_group* genera occurred with LPM treatment ($P < 0.05$; Table 4). Meanwhile, significantly lower values of the phylum Proteobacteria, and the genera *unclassified_f_Lachnospiraceae*, *Escherichia-Shigella*, *Blautia*, *Ruminococcus_torques_group*, *Eisenbergiella*, *Flavonifractor*, *Enterococcus* and *Klebsiella* occurred in this treatment ($P < 0.05$; Table 4).

Linear discriminant analysis (LDA) effect size (LEfSe) analysis was adopted to identify the significant differentially abundant OTUs for ileal mucosal microbiota at levels from phylum to genus (LDA >4.0). As shown in Fig. 6E and F, discrepant microbiota from ileal mucosal tissue in the control was mainly enriched in Proteobacteria phylum, and its downstream microbes (Gammaproteobacteria, Enterbacterrales, Enterobacteriaceae, *Escherichia-Shigella* and *Klebsiella*). The LPM group exhibited increased abundances of the following microbes: phylum Actinobacteriota (Actinobacteria, Bifidobacteriales, Bifidobacteriaceae and *Bifidobacterium*) and Firmicutes (Bacilli, Lactobacillales, Lactobacillaceae and *Lactobacillus*).

3.8. Ileal mucosa transcriptome analysis

A total of 150.96 Gb of clean data were obtained from 16 libraries divided into 2 groups, with more than 7.09 Gb clean data from each sample. Moreover, 91.11% to 92.98% of the total raw reads were uniquely mapped to *Gallus gallus* (GRCg6a). More than 92.58% bases had a quality score of ≥ Q30 and the GC content of the libraries ranged from 49.80% to 51.91%, indicating a reliable quality of RNA sequence results. The clustered heatmaps based on discrepant genes showed that samples in each treatment (the control or LPM)

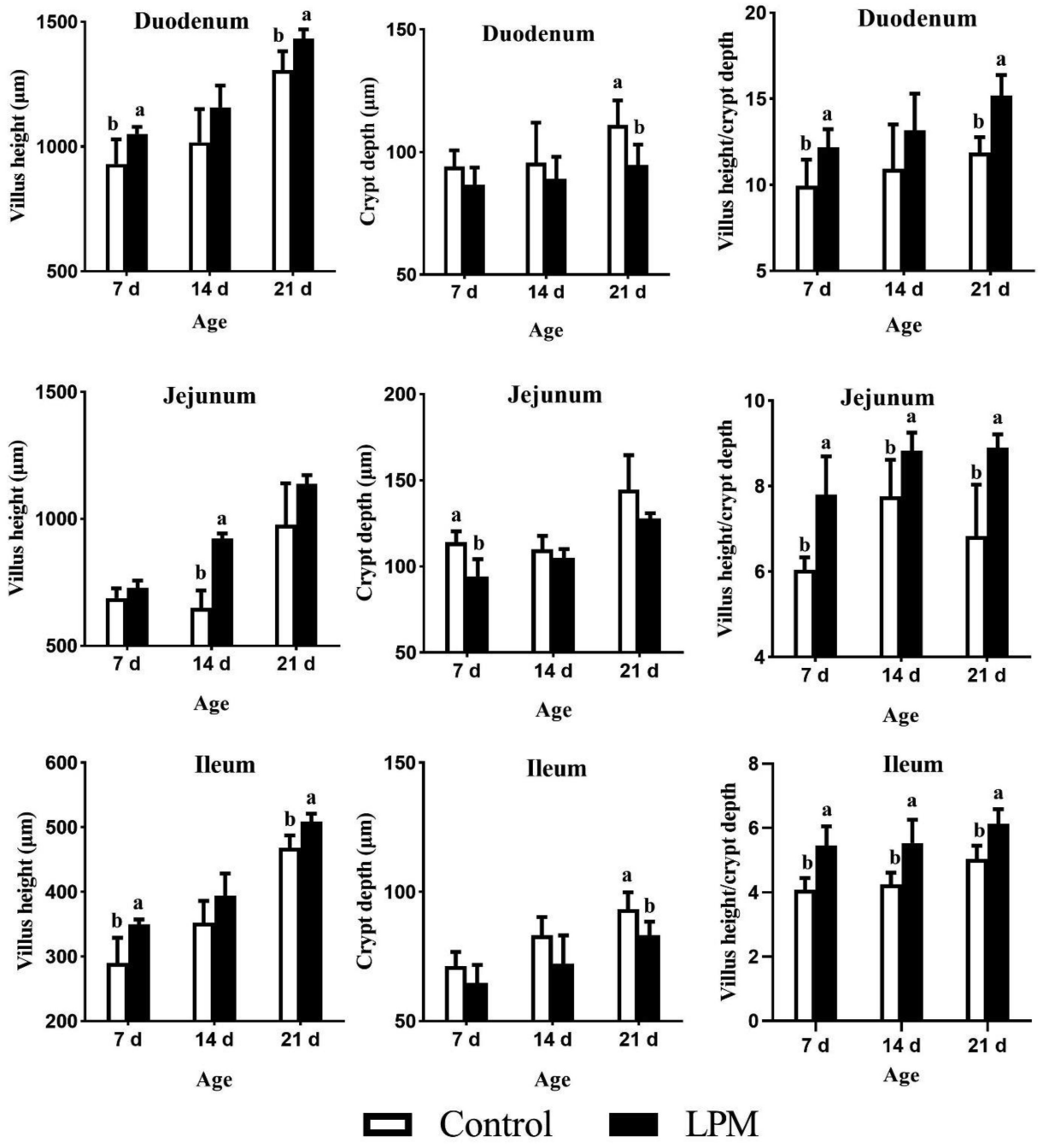

Fig. 1. Effect of microencapsulated *Lactobacillus plantarum* supplementation on small intestine morphology of layer chicks. LPM = *Lactobacillus plantarum* in microencapsulation. [a,b]Values within groups at the same day of age with no common superscripts differ significantly ($P < 0.05$). The error bars mean standard deviation (SD).

occurred in the same group through clustering. The transcripts of samples from the same treatment exhibited good similarity (Fig. 7A). A total of 101 DEGs were identified in the ileal mucosa between the control and LPM treatment. Among them, there were 30 significantly up-regulated and 71 significantly down-regulated genes in the LPM treatment relative to the control. The differences in the gene expression profile between these 2 groups were visualized in a volcano plot (Fig. 7B).

GO annotations and enrichment analysis were conducted to obtain valuable information for function prediction of DEGs. These DEGs were annotated into 3 major function categories: biological process, cellular component and molecular function (Fig. 7C). The most enriched terms in the category of biological process were cellular process, biological regulation and metabolic process. Cell part, organelle, membrane part, protein-containing complex, organelle part, membrane, extracellular region part and extracellular region were most enriched in the category of cellular component. Meanwhile, binding, catalytic activity and transporter activity were most enriched in the category of molecular function. Furthermore, the most DEGs in the LPM treatment relative to the control were enriched in the extracellular region (14 DEGs, $P < 0.05$, Fig. 7D).

Differentially expressed genes related to *L. plantarum* colonization are listed in Table 5. After screening (fold change >1.5 at a FDR

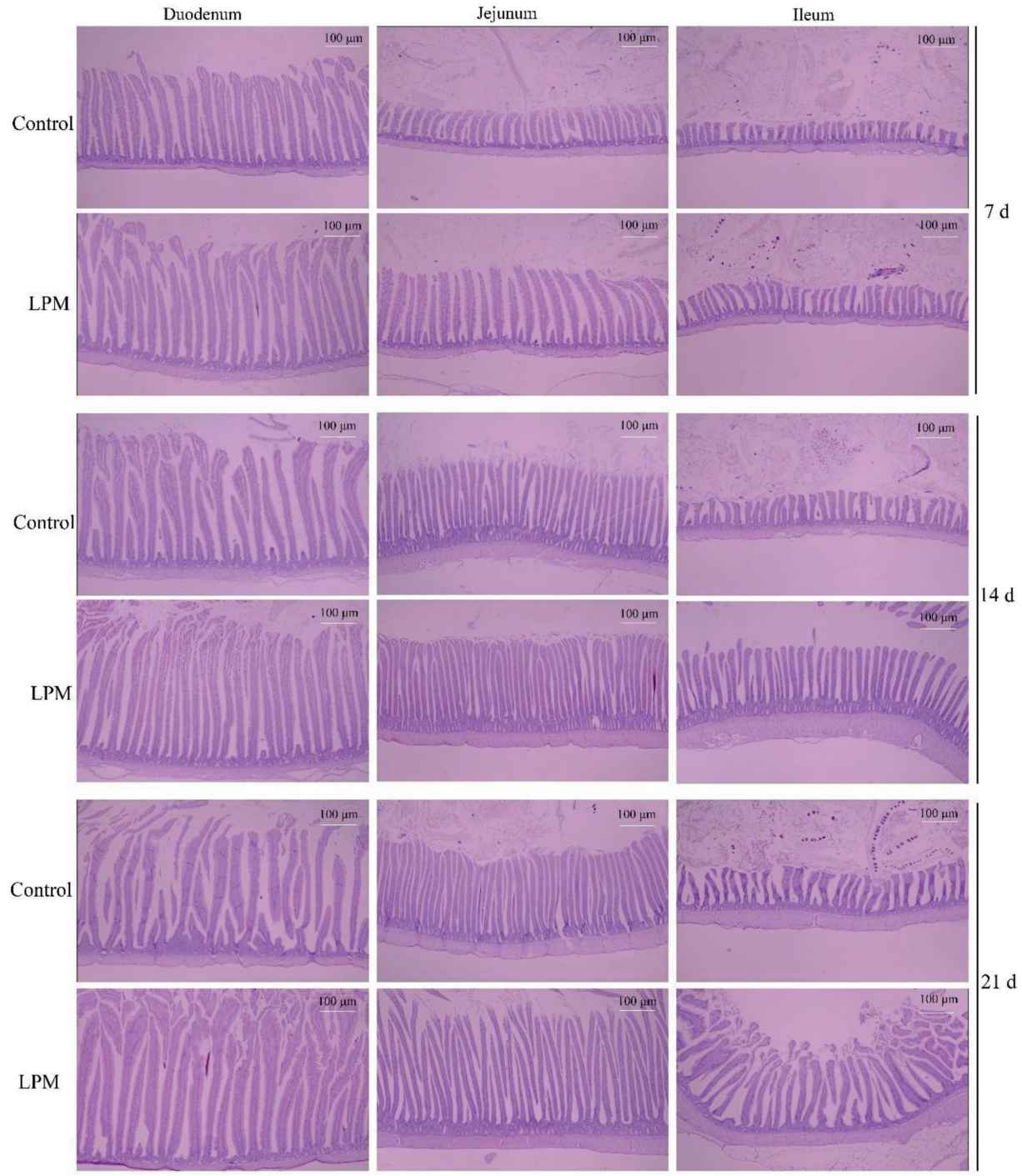

Fig. 2. Effect of microencapsulated *Lactobacillus plantarum* supplementation on small intestinal morphology. Magnification, 100×. Scale bar, 100 μm. LPM = *Lactobacillus plantarum* in microencapsulation.

<0.05), 7 probable key genes were obtained: solute carrier family 15 member 1 (*SLC15A1*), monocarboxylate transporter 2-like (*LOC416086*, *MCT2L*), activin A receptor type 1C (*ACVR1C*), succinate receptor 1 (*SUCNR1*), secretogranin II (*SCG2*), transmembrane protein 174 (*TMEM174*) and myostatin (*GDF8*, *MSTN*). The descriptions of these genes are detailed in Table 5. These 7 DEGs were verified by RT-PCR (Fig. 8A and B).

4. Discussion

The beneficial effects of *L. plantarum* on intestinal development and health have been widely reported in various animals, such as pigs, broilers, laying hens, etc. (Pupa et al., 2021; Xu et al., 2020; Yang et al., 2020). However, there are still some inconsistent results supporting the effectiveness of *L. plantarum* (Han et al., 2018;

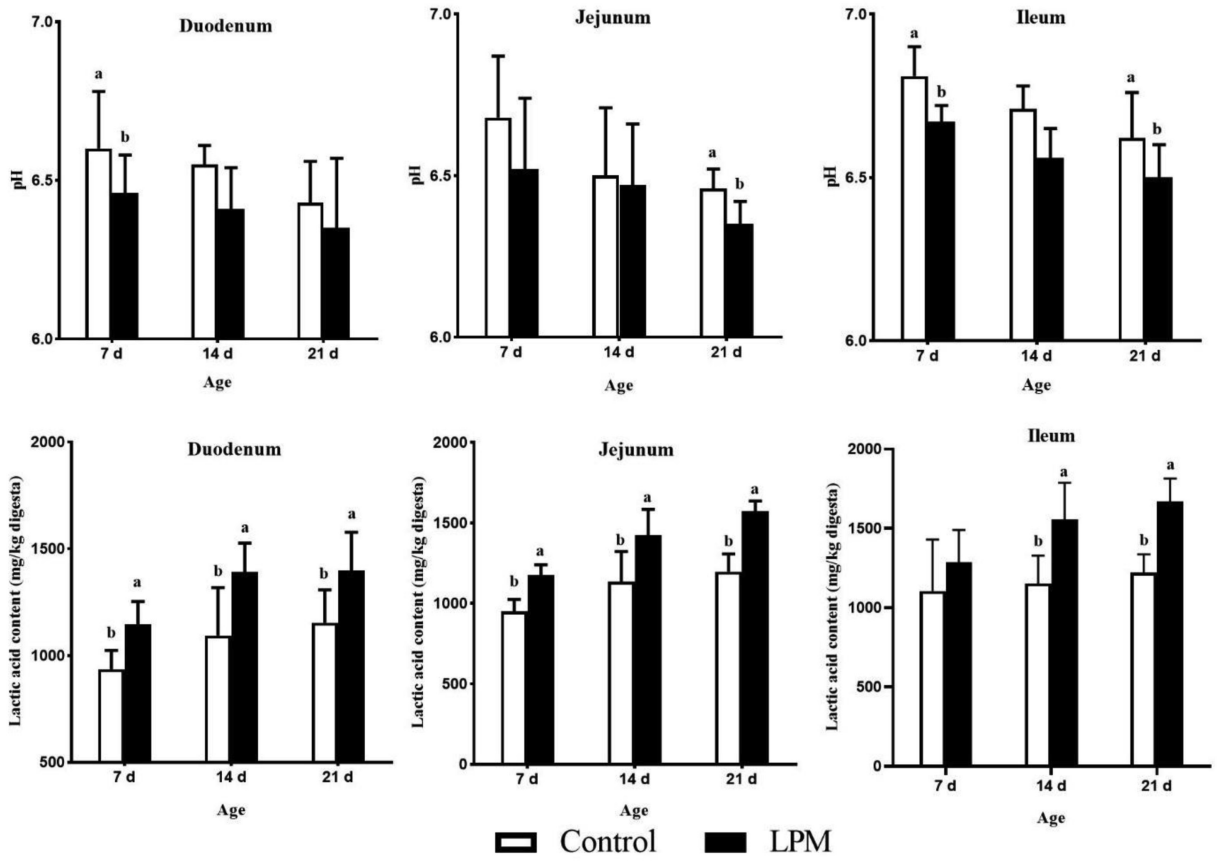

Fig. 3. Effect of microencapsulated *Lactobacillus plantarum* supplementation on small intestine pH and lactic acid content of layer chicks. LPM = *Lactobacillus plantarum* in microencapsulation. [a,b]Values within groups at the same day of age with no common superscripts differ significantly ($P < 0.05$). The error bars mean standard deviation (SD).

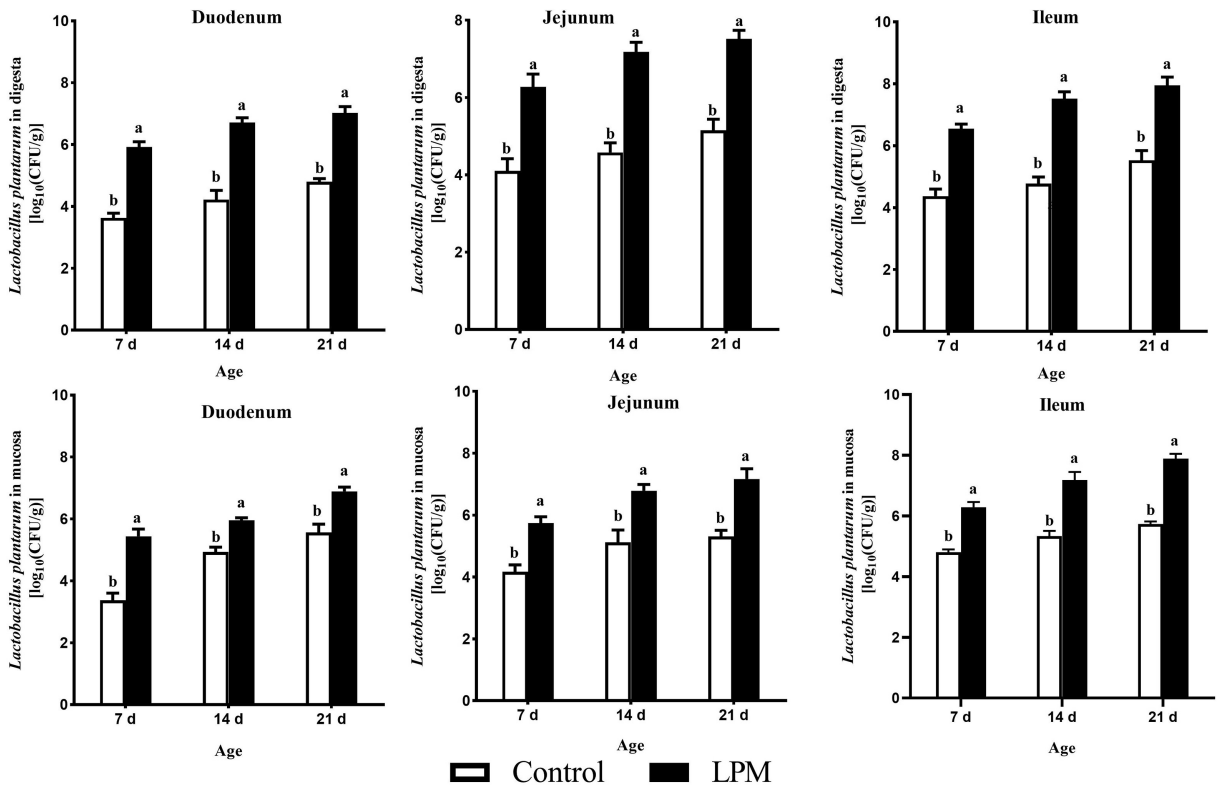

Fig. 4. Effect of microencapsulated *Lactobacillus plantarum* supplementation on *L. plantarum* count in digesta and mucosa of layer chicks. LPM = *Lactobacillus plantarum* in microencapsulation. [a,b]Values within groups at the same day of age with no common superscripts differ significantly ($P < 0.05$). The error bars mean standard deviation (SD).

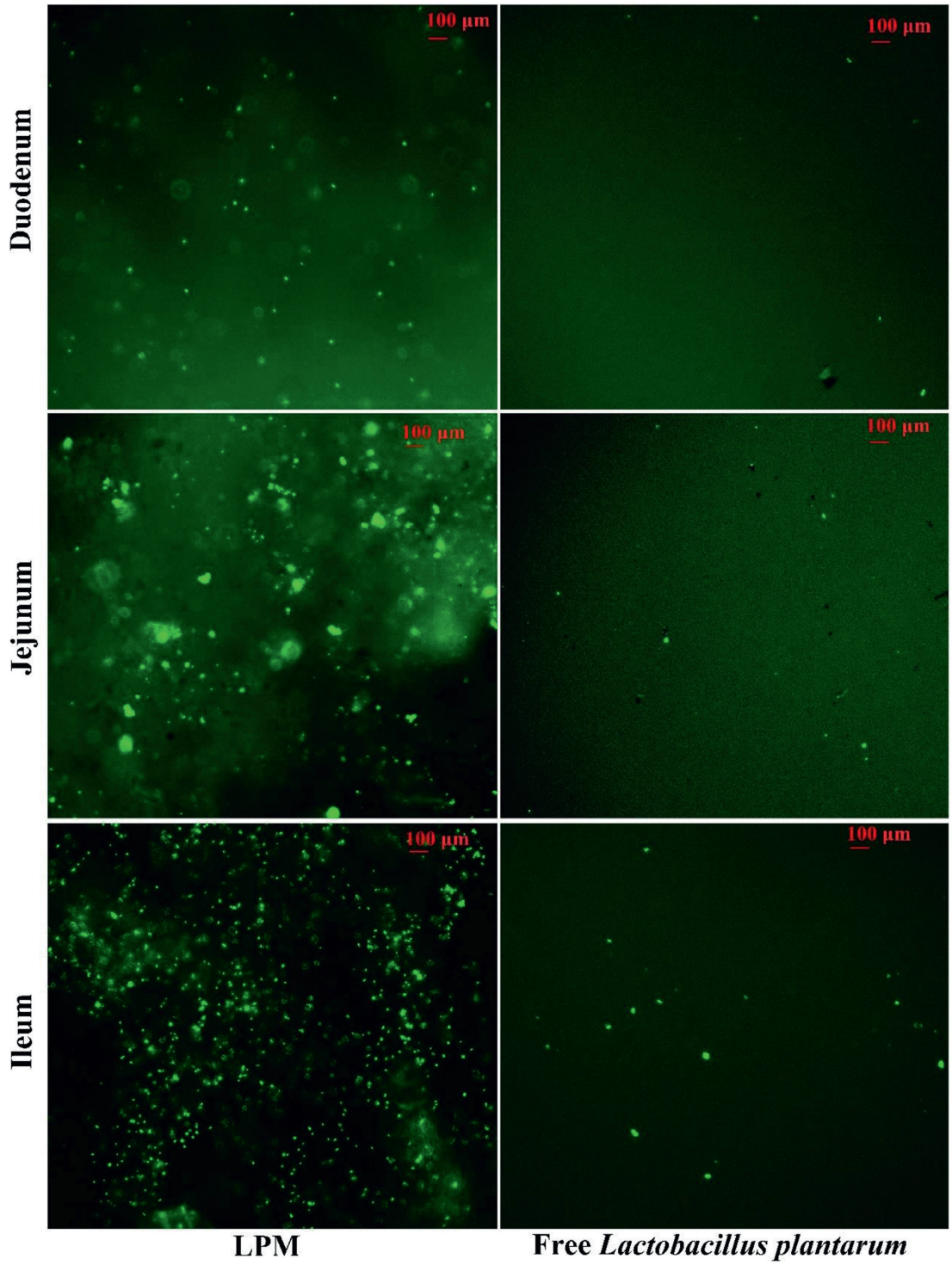

Fig. 5. *Lactobacillus plantarum* colonization in small intestinal mucosa of layer chicks in response to its supplemental form. Magnification, 40×. Scale bar, 100 μm. LPM = *Lactobacillus plantarum* in microencapsulation. *L. plantarum* was marked by fluorescein isothiocyanate (FITC).

Hashemi et al., 2018; Lee et al., 2017), which may be attributed to the different viable counts of *L. plantarum* when it reaches bowel lumen (Chan et al., 2010). In our previous research, we observed that microencapsulated *L. plantarum* exhibited a significantly enhanced survival rate in artificial intestinal juice following passage through gastric juice (Song et al., 2022). Therefore, more reliable effects of *L. plantarum* could be elucidated by examining the effects of LPM supplementation on the growth performance and intestinal development of layer chicks in vivo. Consistently, free *L. plantarum* supplementation has significantly increased ADFI only, while LPM supplementation significantly increased BW, ADG and ADFI concurrently, compared with the control. These findings were consistent with a previous report that free *L. plantarum* supplementation had limited benefits on growth performance (Lee et al., 2017). Meanwhile, these results also indicated *L. plantarum* supplementation in the form of microencapsulation exhibited

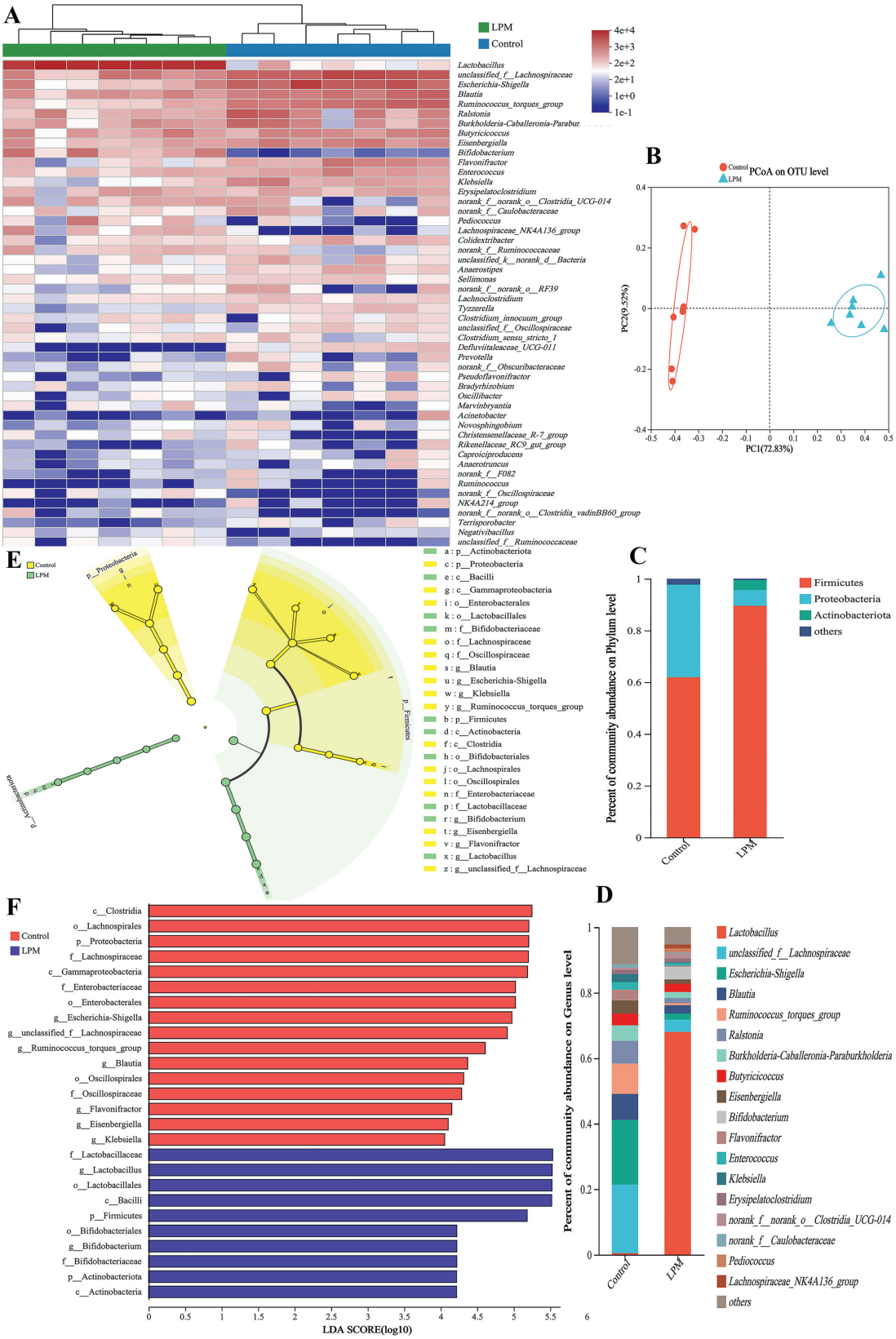

Fig. 6. Effect of microencapsulated *Lactobacillus plantarum* supplementation on the bacterial community in ileal mucosa of layer chicks (7 d of age). (A) Clustered heatmap. (B) Principal coordinate analysis (PCoA) of microbiota. (C) Composition of microbiota at phylum level (>1%). (D) Composition of microbiota at genus level (>1%). (E) Cladogram generated from linear discriminant analysis (LDA) effect size (LEfSe) analysis. The diameters of the circles are proportional to the taxon's abundance. (F) Histogram of the LDA scores computed for features differentially abundant among control and LPM groups. Species with significant difference that have an LDA score greater than 4.0 are presented. The length of the histogram represents the LDA score, which can be interpreted as the effect size of each differentially abundant feature. And p_, c_, o_, f_ and g_ represent the phylum, class, order, family and genus, respectively. Data are the mean of 7 replicates. LPM = *Lactobacillus plantarum* in microencapsulation.

Table 4
Effect of microencapsulated *Lactobacillus plantarum* supplementation on microbial relative abundance components in ileal mucosa of layer chicks (7 d of age) [1].

Items	Treatments		SEM	*P*-value
	Control	LPM		
Phylum, %				
Firmicutes	61.9[b]	89.5[a]	4.89	0.003
Proteobacteria	35.8[a]	6.1[b]	4.99	0.002
Actinobacteria	0.07[b]	3.87[a]	0.892	0.043
Genus, %				
Lactobacillus	0.5[b]	68.0[a]	9.62	<0.001
unclassified_f__Lachnospiraceae	21.0[a]	3.8[b]	2.89	0.001
Escherichia-Shigella	19.7[a]	1.8[b]	3.43	0.010
Blautia	7.94[a]	2.52[b]	1.029	0.006
Ruminococcus_torques_group	9.27[a]	0.67[b]	1.349	0.001
Ralstonia	6.89	1.56	1.466	0.084
Burkholderia-Caballeronia-Paraburkholderia	4.76	1.83	0.937	0.139
Butyricicoccus	3.63	2.55	0.538	0.336
Eisenbergiella	4.07[a]	1.32[b]	0.570	0.009
Bifidobacterium	<0.01[b]	3.84[a]	0.891	0.042
Flavonifractor	3.25[a]	0.33[b]	0.620	0.023
Enterococcus	2.27[a]	0.82[b]	0.344	0.041
Klebsiella	2.44[a]	0.24[b]	0.469	0.024
Erysipelatoclostridium	1.32	1.18	0.195	0.734
norank_f__norank_o__Clostridia_UCG-014	0.61	1.43	0.251	0.105
norank_f__Caulobacteraceae	1.11	0.34	0.221	0.099
Pediococcus	0.96	1.29	0.489	0.257
Lachnospiraceae_NK4A136_group	0.03[b]	1.15[a]	0.269	0.049

LPM = *Lactobacillus plantarum* in microencapsulation.
[a,b] Values within a row with no common superscripts differ significantly ($P < 0.05$).
[1] Data are the mean of 7 replicates (1 chick each replicate).

significantly better promotion effects on growth performance than free *L. plantarum*. These findings may be attributed to the fact that microencapsulation helped *L. plantarum* pass through adverse environments (such as gastric acid), thus improving its ability to exert beneficial effects. Hence, it can be supposed that microencapsulated *L. plantarum* could effectively improve the growth performance of layer chicks.

Growth performance is closely related to intestinal development. Hence, intestinal development was further investigated. In this research, variations in small intestine development were consistent with the changes in growth performance in response to *L. plantarum* supplementation. In particular, free *L. plantarum* supplementation significantly enhanced ileal weight and length, while microencapsulated *L. plantarum* significantly and comprehensively improved intestinal weight, index and length of the duodenum, jejunum and ileum at 7, 14 and 21 d of age. Such findings displayed excellent promotion effects of microencapsulated *L. plantarum* on intestinal development, which was consistent with former reports (Dawood et al., 2020; Humam et al., 2019; Wang et al., 2019). Optimal intestinal development is usually accompanied by good intestinal morphology. Therefore, intestinal morphology was further investigated. In this study, significantly improved VH, CD and VCR were simultaneously observed in the duodenum, jejunum and ileum (7, 14 and 21 d) with LPM treatment. Such findings were consistent with a previous report that *L. plantarum* significantly improved the morphology of the small intestine, including the duodenum, jejunum and ileum, in pigs (Pupa et al., 2021). These results could be attributed to the role of *Lactobacillus* on accelerating the proliferation and differentiation of intestinal epithelial stem cells (Xie et al., 2019). All of these findings indicated that microencapsulated *L. plantarum* could promote intestinal development in layers at an early age.

Colonization in the intestinal mucosa is a prerequisite for probiotics to effectively and durably exert their beneficial effects on the host (Zmora et al., 2018). Therefore, colonization of *L. plantarum* in the intestinal mucosa of layer chicks was further explored in the current research. Significant decreases were observed in the pH of the duodenum, jejunum and ileum in response to LPM supplementation at 7, 14 and 21 d of age, accompanied with significantly higher lactic acid content. The decrease in intestinal pH could be attributed to an increase in intestinal lactic acid content. Lactic acid is the representative metabolite of *L. plantarum* (Passos et al., 1994), and thus the enhanced lactic acid content indicated an increase in *L. plantarum* colonization in the gut. Consistently, in this research, significantly higher viable counts of *L. plantarum* were observed both in ileal chyme and mucosal tissue in response to LPM supplementation, accompanied by a visual increase in *L. plantarum* colonization through FITC labeling. All of these findings demonstrated that microencapsulation increased *L. plantarum* colonization in the intestinal mucosa.

The interaction between microorganisms plays a crucial role in determining the successful colonization of probiotics in the intestinal tract (Turroni et al., 2016); thus gut microflora analysis was conducted to further explore the underlying mechanism of *L. plantarum* colonization in the intestinal mucosa of layer chicks. Microencapsulated L. *plantarum* supplementation significantly increased the Firmicutes phylum percentage in the microbiota composition, which could be attributed to the increased counts in Bacilli class, Lactobacillales order, Lactobacillaceae family and *Lactobacillus* genus. These findings were consistent with the above results that the colonization of *L. plantarum* significantly increased in response to LPM supplementation. Such findings were similar to a previous report in which the fecal relative abundance of *Lactobacillus* significantly increased in response to *L. plantarum* supplementation in the diet of laying hens (Qiao et al., 2019). The increase in colonization of *L. plantarum* could be attributed to the interactions with original resident strains (Krumbeck et al., 2015). In this research, several original resident strains in the gut significantly increased with LPM supplementation, which could be mainly allocated into phylum Actinobacteriota, class Actinobacteria, order Bifidobacteriales, family Bifidobacteriaceae, genus *Bifidobacterium*. These findings could be ascribed to the reason that *Bifidobacterium* exhibited positive effects on the intestinal colonization of *L. plantarum* through creating more acidic conditions in the gut (Salminen et., 2016). Hence, the mutual assistance from *Bifidobacterium* could be proposed to facilitate the colonization of *L. plantarum* in the gut.

The successful colonization of *L. plantarum* is closely linked to the interactions between the host and microbes. In fact, numerous factors act in these interactions (Cervantes-Barragan et al., 2017; Ganesh et al., 2018; Tang et al., 2018), many of which are still unidentified. Therefore, ileal mucosal tissue was subjected to transcriptomic analysis in this research to identify key factors in the process of *L. plantarum* colonization in the gut of layer chicks. The clustered heatmap indicated that *L. plantarum* supplementation had distinct influences on the transcripts in ileal mucosal tissue. The GO annotations and enrichment analysis showed that the DEGs mainly focused on molecular binding function and several cellular components. The latter included membrane, membrane part, extracellular region, and extracellular region part. Such findings were similar to the previous report that 16 *L. plantarum* strains from human sources adhered to and colonized in extracellular matrix components, while 4 strains showed significant binding to both fibronectin and mucin (Yadav et al., 2015). In this research, DEGs were annotated in developmental process, which was consistent with the benefits of LPM supplementation on intestinal development. In addition, DEGs were also enriched in metabolic process, biological regulation and response to stimulus. Based on such findings, it could be speculated that after intestinal mucosal exposure to *L. plantarum*, responsive stimuli were induced and metabolism was changed in host through biological regulation to

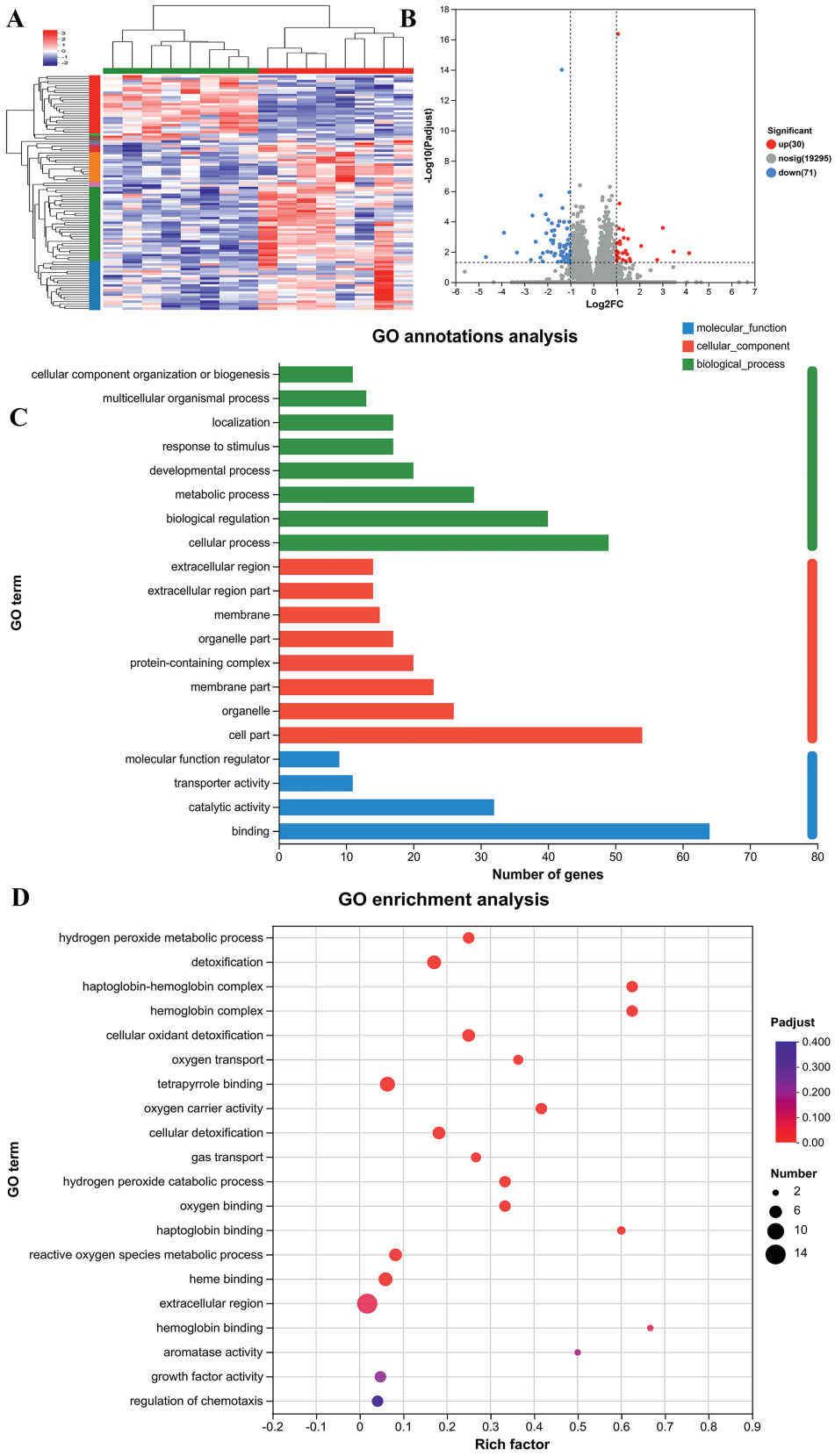

Fig. 7. Effect of microencapsulated *Lactobacillus plantarum* supplementation on transcripts of ileal mucosal tissue in layer chicks (7 d of age). (A) Clustered heatmap. (B) Volcano plot. (C) Gene ontology analysis of differentially expressed genes. (D) Pathway analysis of differentially expressed genes. LPM = *Lactobacillus plantarum* in microencapsulation. Data are the mean of 8 replicates.

Table 5
Analysis of differentially expressed genes (fold change >1.5 at a false discovery rate <0.05) related to microencapsulated *Lactobacillus plantarum* colonization (microencapsulated *L. plantarum* vs. control).

Gene	Fold change	*P*-adjust value	Gene description
SLC15A1	2.19	<0.001	Solute carrier family 15 member 1
LOC416086	2.16	<0.001	Monocarboxylate transporter 2-like (*MCT2L*)
ACVR1C	2.84	0.001	Activin A receptor type 1C
SUCNR1	2.64	0.008	Succinate receptor 1
SCG2	2.41	0.012	Secretogranin II
TMEM174	2.01	0.039	Transmembrane protein 174
GDF8	2.64	0.004	Myostatin (*MSTN*)

promote the colonization of *L. plantarum*. This hypothesis needs further verification.

In reality, through screening, 7 DEGs related to *L. plantarum* colonization stood out in this research, including *SLC15A1*, *LOC416086*, *ACVR1C*, *SUCNR1*, *SCG2*, *TMEM174* and *GDF8*. Consistently, Wang et al. (2020) found that significantly higher *SLC15A1* expression occurred accompanied by improved intestinal morphology and higher *Lactobacillus* percentage, which may be attributed to the fact that SLC15A1 (SLC PepT1) could deliver *L. plantarum* to intestinal mucosa (Kotka et al., 2008; Vavricka et al., 2004). Oral administration of live *L. plantarum* enhanced sodium-coupled monocarboxylate transporter 1 mRNA expression in the colonic and ileal tissues of C57BL/6 mice (Borthakur et al., 2010), which was consistent with the findings in this research. The

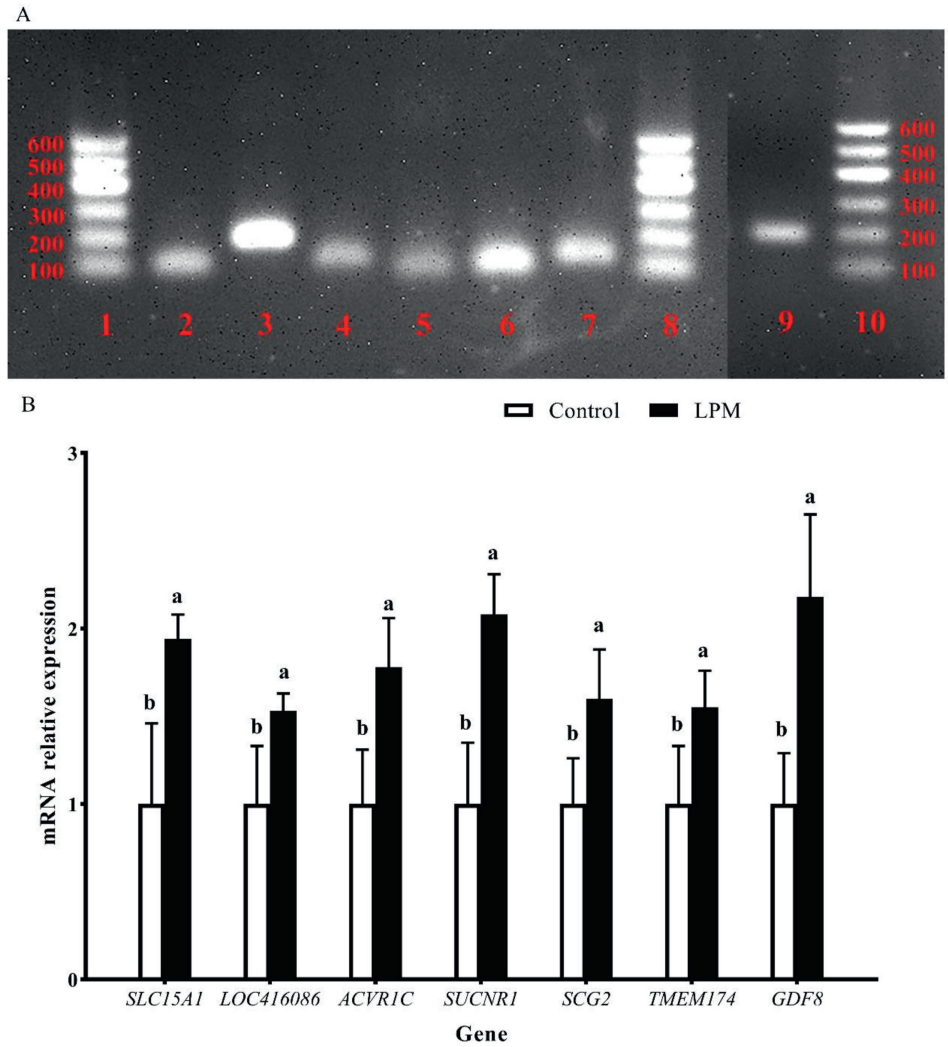

Fig. 8. RT-PCR confirms the differentially expressed genes and their primers. (A) A representative image of RT-PCR results from lane 1 to lane 10 are DNA ladder (100, 200, 300, 400, 500 and 600 bp), *SLC15A1* (116 bp), *LOC416086* (201 bp), *ACVR1C* (124 bp), *SUCNR1* (91 bp), *SCG2* (107 bp), *TMEM174* (141 bp), DNA ladder, *GDF8* (195 bp) and DNA ladder, respectively. (B) Relative mRNA expression of the differentially expressed genes by RT-PCR. LPM = *Lactobacillus plantarum* in microencapsulation. *SLC15A1* = solute carrier family 15 member 1; *LOC416086* (*MCT2L*) = monocarboxylate transporter 2-like; *ACVR1C* = activin A receptor type 1C; *SUCNR1* = succinate receptor 1; *SCG2* = secretogranin II; *TMEM174* = transmembrane protein 174; *GDF8* (*MSTN*) = myostatin. [a,b]Values within a group with no common superscripts differ significantly ($P < 0.05$). The error bars mean standard deviation (SD).

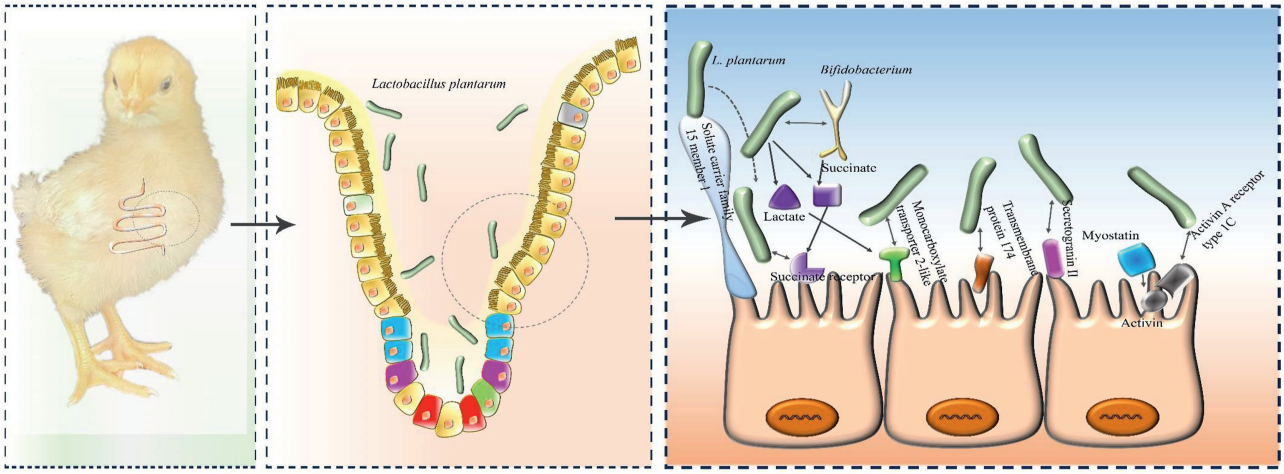

Fig. 9. A schematic model displaying the potential mechanism of *Lactobacillus plantarum* colonization in the ileum.

upregulation of monocarboxylate transporter (encoded by *LOC416086*) may be due to the induction of lactate produced by *L. plantarum*. In other research, extracellular succinate was found to facilitate intestinal microbiota colonization through interacting with its cognate receptor SUCNR1 (Serena et al., 2018), while succinic acid was an important by-product of *L. plantarum* (Tsuji et al., 2013) and *Bifidobacterium* (Jalili et al., 2009). Meanwhile, the significantly up-regulated SUCNR1 suggested that the colonization of *L. plantarum* likely occurred through its interaction with the succinic acid receptor SUCNR1, which was induced by increased levels of succinic acid. The increased succinic acid contents could be supported by the enhanced percentages of *Lactobacillus* and *Bifidobacterium*. Besides, dietary *Lactobacillus casei* supplementation significantly increased the activin gene expression in male zebrafish (Safari et al., 2022), and activin could be activated by GDF8 (Sako et al., 2010). Meanwhile, the up-regulated *GDF8* and activin A receptor expressions were observed in the present study, which indicated activin A was probably activated by GDF8 and then induced the expression of its receptor to promote *L. plantarum* colonization in gut. Host derived secretogranins were reported to influence gut microbial composition (Sundin et al., 2018). Meanwhile, significantly up-regulated SCG2 (encoding this transmembrane protein) was observed in this work, which indicated SCG2 could probably promote *L. plantarum* colonization through their interaction. Moreover, transmembrane protein LMxysn_1693 promoted Lm XYSN adhesion and invasion to intestinal epithelial cells in vitro, as well as colonization in the ileum of mice (Jin et al., 2022), indicating that the transmembrane protein was crucial in the colonization of probiotics. These results were consistent with the significantly up-regulated of TMEM174 in response to *L. plantarum* supplementation in this study. All these 7 DEGs encode transmembrane proteins, and the up-regulation of these genes could be attributed to the induction of *L. plantarum* on intestinal mucosal tissue, which was consistent with the published research (Son et al., 2020; Wei et al., 2022). These findings could provide evidence supporting the previous speculation that the host up-regulated the expression of genes encoding colonization-related transmembrane proteins in the intestinal mucosa in response to *L. plantarum* supplementation. Based on the above analysis, the colonization of *L. plantarum* in the gut could be attributed to its interactions with transmembrane proteins, including the solute carrier family, monocarboxylate transporter, activin A receptor, succinate receptor and SCG2. Therefore, mutual assistance from *Bifidobacterium* and interactions with crucial transmembrane proteins may facilitate the colonization of *L. plantarum* in the ileal mucosa of layer chicks (Fig. 9). Thus, the underlying mechanisms of *L. plantarum* colonization in different intestinal sections as well as strategies to enhance colonization efficiency are potential areas of future study.

5. Conclusion

In conclusion, microencapsulated *L. plantarum* supplementation improved growth performance and intestinal development of layer chicks, which could be attributed to the increase of *L. plantarum* colonization in intestinal mucosa. The colonization of *L. plantarum* was likely achieved through mutual assistance from *Bifidobacterium* and interactions with specific transmembrane proteins, including the solute carrier family, monocarboxylate transporter, activin A receptor, succinate receptor, and SCG2.

Author contributions

Yaoming Cui: Formal analysis, Data curation, Writing-original draft, Conceptualization. **Yanxia Liu:** Formal analysis, Investigation. **Jing Yang:** Formal analysis. **Haitao Duan**: Methodology, Conceptualization. **Yanjiao Guo** and **Suying Li:** Conceptualization. **Yating Zhao:** Conceptualization. **Peng Wang** and **Linna Guo:** Methodology, Resources. **Guanghai Qi:** Conceptualization, Supervision. **Junjun Guan** and **Jinrong Wang:** Supervision, Writing-review & editing.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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Appendix Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aninu.2024.03.016.

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