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Effect of inulin supplementation in maternal fecal microbiota transplantation on the early growth of chicks

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Abstract

Background Fecal microbial transplantation (FMT) is an important technology for treating diarrhea and enteritis. Additionally, FMT has been applied to improve productivity, alter abnormal behavior, relieve stress, and reduce burdens. However, some previous studies have reported that FMT may cause stress in acceptor animals. Inulin, a prebiotic, can promote growth, enhance immunity, and balance the gut microbiota. Currently, there are limited reports on the effects of combining FMT with inulin on early growth performance in chicks.

Results In this study, a total of 90 1-day-old chicks were randomly divided into the control group (CON), FMT group, and inulin group (INU). The CON group was fed a basic diet, whereas the FMT and INU groups received fecal microbiota transplantation and FMT with inulin treatment, respectively. Compared with the FMT and CON groups, the INU group presented significantly greater average daily gain (ADG) and average daily feed intake (ADFI) values ($P < 0.05$). However, the organ indices did not significantly change ($P > 0.05$). The ratio of the villi to crypts in the ileum significantly differed at 21 and 35 days ($P < 0.05$). In addition, the cecum concentrations of acetic acid and butyric acid significantly increased in the INU group ($P < 0.05$). In addition, gut inflammation and serum inflammation decreased in the INU group, and immune factors increased after inulin supplementation. ($P < 0.05$). *Firmicutes* and *Bacteroidetes* were the dominant phyla, with more than 90% of all sequences being identified as originating from these two phyla. Inulin supplementation during mother-sourced microbial transplantation significantly increased the abundance of *Rikenella*, *Butyricicoccus*, and *[Ruminococcus]*, which contributed positively to the promotion of early intestinal health and facilitated the early growth of chicks.

Conclusion The results of this study suggest that inulin supplementation in maternal fecal microbiota transplantation can effectively promote early growth and probiotic colonization, which favors the health of chicks.

Keywords Fecal microbial transplantation, Inulin, Growth performance, Immune factors, Short chain fatty acids, Maternal microbiota, Metabolic pathway

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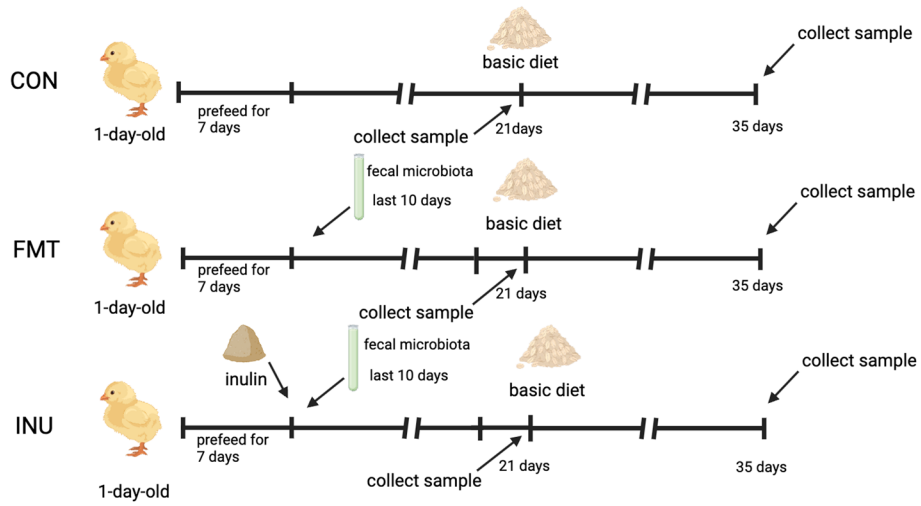
Graphical Abstract

Experimental design process diagram



Background

Maternal fecal microbiota plays a key role in disrupting the colonization and development of exogenous microbiota in early newborn to offspring [1]. The microbiota plays a critical role in regulating the chick weight, which colonizes the host gut, affects energy harvest from the diet, regulates host health, and promotes innate and acquired immunity [2]. Wang et al. [3] reported that fecal transplantation significantly increased the abundance of the intestinal flora, reduced the expression of proinflammatory factors, and increased the expression of anti-inflammatory factors within the intestine. A number of studies have shown that supplementation with fecal microbiota can significantly increase body weight during the early growth stages of livestock and poultry. Furthermore, the colonization of beneficial bacteria has been shown to exert long-term effects on health and development. These effects include the regulation of intestinal metabolites, enhancement of immune system function, and improved fermentation and absorption of dietary nutrients [4, 5]. However, in modern intensive culture, avian embryos grow in an incubator, limiting the delivery of gut bacteria from hens to offspring. Fecal microbiota transplantation (FMT) is an important technology for treating diarrhea and enteritis [6, 7]. Additionally, FMT has been applied to improve productivity; correct abnormal behavior, relieve stress, and reduce disease burdens [8]. Reports indicate that FMT improves weight gain by reshaping the gut microbiota in early life in laying chickens [9] and that FMT improves the gut morphology and microbial composition and increases nutrient absorption from food [10]. The gut function and composition are critical for body growth and development, and the intestinal tract of newborns is not fully developed and has an imperfect immune system. Therefore, the gut microbial composition is very important for gut growth and physiological plasticity in newborn chicks [11, 12].

FMT has a long history as a treatment for diarrhea and enteritis, and it has been increasingly used in medicine in recent years [13–16]. Brunse et al. [17] reported that oral FMT could improve bacterial adhesion, reduce mortality, and promote the colonization of pathogens in the internal organs, whereas rectal FMT could reduce the stimulation of the upper digestive tract and improve the effectiveness of FMT. These findings suggest that FMT benefits livestock growth and improves immunity and intestinal health. However, some reports indicate that FMT may damage the intestinal barrier and induce inflammation, which is a potentially dangerous health risk for animals [18]. Thus, a method is needed to fully harness the beneficial effects of FMT avoiding adverse impacts.

Inulin, a prebiotic, is a plant polysaccharide and a type of natural functional dietary fiber found in various plants [19, 20]. In animal husbandry, inulin can improve the growth performance of growing-finishing pigs; improving the immune system and promoting beneficial bacterial proliferation [21, 22]. Additionally, inulin can positively influence the gut microbiota by enhancing the balance of beneficial bacteria while reducing the presence of potential pathogens [21]. Inulin has also been confirmed to have anti-oxidative, anti-informatory, and anticancer activities, promote the colonic absorption

of minerals, and stimulate the immune system [23–25]. Awad et al. [26] reported that inulin promotes glucose absorption, increases glucose transport by increasing jejunal mucosa permeability, and increases the intestinal permeability barrier in weaning piglets. The above evidence supports the use of inulin as a functional food ingredient for reducing diarrhea and inflammation [27].

In this study, to increase the practical applicability of FMT in production, we also aimed to demonstrate that inulin plays a role in enhancing the beneficial effects of FMT. Therefore, we added inulin and maternal source fecal bacterial mixture to the basal diet and observed the growth performance and immunoglobulin, intestinal, and bacterial abundances of chicks to investigate whether maternal microbial transplantation supplemented with inulin could favor FMT during the early growth of offspring.

Materials and methods

Fecal microbiota and basic diet preparation

Twenty healthy (Hy-Line Brown) hens at 6 months of age, with no history of gastrointestinal diseases and no prior vaccinations, were used as donors for FMT. Wing venous blood was collected for blood agglutination tests (*Salmonella* and *Mycoplasma Galliscepticum*). Fresh fecal samples were collected, with the white portion removed, followed by intestinal microbiota testing to confirm the absence of pathogenic contamination in the transplanted fecal bacteria. Then, the samples were immediately processed under anaerobic conditions to prepare an FMT stock solution for use in recipient laying hens and stored on ice. Feces were treated according to the methods of Siegerstetter, and the samples were mixed and homogenized with sterile saline (1:2) and filtered through sterile gauze and a 0.25-mm filter [10]. After that, the filtered mixture was centrifuged at 800 rpm for 3 min, the supernatant was removed, 10% bacterial freezing solution was added, and the mixture was stored in a − 80 °C freezer. Notably, the stored bacterial mixture could not be frozen and thawed repeatedly. The basal diet was purchased from Hefeng, Changchun, China. The food-grade inulin was purchased from ShanYou Biotechnology, Shanghai, China. The composition and nutrition levels of the basal diets are shown in Table 1.

Table 1 Composition and nutrition levels of basal diets

Items	Content (%)	Nutrient level	Content (%)
Corn	56.09	DE, MJ/kg	14.17
Subflour	8.00	CP	20.16
Soybean meal	19.51	AP	0.53
Extruded soybean	8.00	Ca	1.05
Corn gluten meal	2.00	Sodium	0.16
Fish meal	2.00	Lysine	1.13
Stone powder	1.53	Methionine	0.49
Calcium hydrogen phosphate	1.10	Threonine	0.75
Premix	1.00		
Salt	0.25		
Lysine	0.15		
Threonine	0.10		
Choline chloride	0.06		
Actinic acid	0.20		
Methionine	0.01		
Total	100		

*1. The premix provided the following per kilogram of diet: Retinol 2.752 mg, cholecalciferol 93.75 μg, α-tocopherol 100 mg, menadione 3 mg, riboflavin 12.5 mg, pyridoxine 9 mg, cyanocobalamin 0.03 mg, pantothenic acid 18 mg, niacin 60 mg, folic acid 1.5 mg, biotin 0.225 mg, Fe 80 mg, Cu 9 mg, I 0.9 mg, Se 0.3 mg, Mn 12.55 mg, and Zn 25.2 mg. 2. Values in the table are calculated based on data provided by China Feed Database (2013)

Animal management

Chicks were provided by Changchun Agricultural Science and Technology College. This experiment was approved by the Animal Ethics Committee of Jilin Agricultural University (NO. 201,705,001). One-day-old chicks (initial body weight 39.23 ± 0.21 g) were considered recipients and randomly divided into three groups, the CON, FMT, and INU groups with 30 chicks in each group. Each group was subdivided into three cages (three replicates). The chicks were kept in cages (120 × 60 × 60 cm^3) equipped with two nipple drinkers and one feeder, which are named in each group CON, FMT, and INU, respectively. Before the actual experiment began, all chicks were fed. Before the actual experiment began, all chicks were fed a basal diet and allowed access to water freely for 7 days. The CON group was fed a basal diet, the FMT group received a basal diet supplemented with 40 mL of fecal microbiota, and the INU group was fed a basal diet containing 1.5% inulin combined with 40 mL of fecal microbiota. We noted that 40 mL of fecal microbiota was added to the diet in two doses (20 mL each). The management regulations were free based on the Hy-line Brown Layer Breeder's Manual.

Calculations of growth and development indicators

The chicks of the three groups were weighed each week. Feed intake was recorded every day, and the average daily gain (ADG) and conversion ratio (ADFI) were calculated. The immune organs were collected and their indices were calculated, while tibial length was measured. ADG is the (final body weight—initial body weight) number of days, whereas average daily feed intake (ADFI) was determined by dividing total feed consumption by the number of days and animals. The organ index was expressed as (organ weight/body weight) × 100.

Table 2 The sequences of primers

Gene name	Forward primer(5′−3′)	Reverse primer(5′−3′)
GAPDH	CAGAACATCATCCCAGCGTCCAC	CGGCAGGTCAGGTCAACAACAG
Muc2	TCACCCTGCATGGATACTTGCTCA	TGTCCATCTGCCTGAATCACAGGT
ZO-1	TAAAGCCATTCCTGTAAGCC	GTTTCACCTTTCTCTTTGTCC
Occludin	CGCAGATGTCCAGCGGTTACT	CAGAGCAGGATGACGATGAGGAA
IL-6	CTCCTCGCCAATCTGAAGTC	AGGCACTGAAACTCCTGGTCT
IL-17A	TTGACATTCGCATTGGCAGC	AGTTCAAGCAGCCCAAGAGG
TGF-β	ATGTGTTCCGCTTTAACGTGTC	GCTGCTTTGCTATATGCTCATC
Foxp3	AACGGCGAGACACCTTC	TTCGGAGACTTTAATCCACTA
IL-1β	ACCTACAAGCTAAGTGGGCG	ATACCTCCACCCCGACAAGG
Wnt3	TCCACAGCAAGGACAACGTA	ACGAGGGGTCTTTCACCCAT

Immune substance assay

The blood was collected ($n=5$ for each group) and left at 4 °C for 30 min before serum was obtained by centrifugation (3500 × g, 15 min). Serum levels of lysozyme (LZM), immunoglobulin G (IgG), immunoglobulin A (IgA), immunoglobulin M (IgM), and interleukin 10 (IL-10) were estimated according to the instructions of the manufacturer of the ELISA kits (Bioimmune Biotechnology, Shanghai, China), and the process was repeated three times for each chick.

Short-chain fatty acid (SCFA) assay

On day 21, 0.1 g of caecum content ($n=5$ for each group) was mixed with 1.5 mL of DEPC water and then centrifuged for 15 min (10 000 × g). One milliliter of the supernatant was collected and mixed with 0.2 mL of metaphosphoric acid (25%). Detection was performed via an Agilent 7980A gas chromatograph with three replicates.

Intestinal histologic observation

The ileum and jejunum ($n=5$ for each group) of chicks were treated with 4% paraformaldehyde, dehydrated embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin (Solberg Biotechnology, Beijing, China). Photographs of the ileal and jejunal sections were obtained using an Olympus light microscope, after which the villus height and crypt depth were measured via ImageJ software. Intestinal Paneth cells were colored red by fluorescent pink, and the background was recolored to yellow using tartar. Paneth cell stain was purchased from Leagene Biological, Beijing, China.

RNA extraction and qPCR analysis

RNA was extracted from the jejunum at 28 and day 35 days ($n=5$ for each group). The RNA was quantified using a NanoDrop 2000 spectrophotometer and reverse-transcribed to cDNA (Thermo Fisher Scientific Inc.). Then, qPCR analyses were carried out on a CFX Reali-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). In the present study, GAPDH was used as the housekeeping gene, and MUC2, ZO1, Occludin, IL-6, IL-17A, TGF-β, Foxp3, IL-1β, and Wnt3 were the target genes. The relative expression levels of the target genes in the jejunum were calculated via the $2^{-\triangle\triangle Ct}$, with three replicates per chick. The primers were designed and synthesized by Shenggong Biotechnology, Shanghai, China, and the sequences are shown in Table 2.

Sequencing analysis of bacterial colonies and statistical analysis

Total microbial RNA was extracted from the caecum contents of 21-day-old chicks ($n=5$ for each group), and the microbiota of the feces was analyzed ($n=3$). Sequencing analysis of bacterial colonies was performed by Personal Biotechnology, Shanghai, China. Classification of vital metabolic pathways was performed by analyzing the KEGG database and MetaCyc database. Protein function was predicted using the COG database. Finally, the metagenomeSeq method was combined with calling the fitFeatureModel function to use a zero-included log-normal model to fit the distribution of each pathway/group, and the results of this model were used to determine the significance of the differences. Group distributions use the results of this model to discriminate the significance of the differences.

The data were analyzed for significance using SPSS, and the mean ± standard errors were plotted using Prism software. *P*-values less than 0.05 were considered statistically significant, and *P*-values less than 0.01 were considered highly statistically significant [28, 29], * in the figure and lower case letters in the table are mean *p* values less than 0.05, ** in the figure and upper case letters are mean *p* values less than 0.01.

Table 3 Effects of FMT combined with inulin on the ADG and ADFI of the three groups per week

Growth performance			
Items	CON	FMT	INU
14 days			
ADG	4.72 ± 0.39[a]	4.17 ± 0.23[b]	5.26 ± 0.18[a]
ADFI	10.23 ± 0.57[a]	8.62 ± 0.62[b]	10.52 ± 0.53[a]
FCR	2.16 ± 0.17	2.07 ± 0.11	2.00 ± 0.13
21 days			
ADG	18.66 ± 0.31[ab]	17.14 ± 0.30[b]	21.29 ± 0.29[a]
ADFI	31.12 ± 2.12[ab]	30.23 ± 2.00[b]	32.23 ± 2.43[a]
FCR	1.67 ± 0.12[ab]	1.76 ± 0.14[a]	1.51 ± 0.10[b]
28 days			
ADG	17.10 ± 0.87	16.91 ± 0.71	18.80 ± 0.55
ADFI	41.37 ± 2.37	37.23 ± 2.94	42.32 ± 3.21
FCR	2.42 ± 0.21	2.20 ± 0.19	2.25 ± 0.22
35 days			
ADG	20.07 ± 0.86	20.85 ± 0.99	22.71 ± 0.76
ADFI	43.84 ± 2.12	42.76 ± 2.23	44.72 ± 1.93
FCR	2.18 ± 0.15	2.05 ± 0.10	1.97 ± 0.13

a and b represent statistically significant differences $p < 0.05$, with a being significantly higher than b, whereas ab is not significantly different from either a or b

Results

Effects of FMT combined with inulin on growth performance and organ index development

Effects of FMT combined with inulin on ADG and ADFI

As shown in Table 3, the ADG was significantly greater in the INU group than in the FMT group during the first 2 weeks (*P < 0.05)*. Additionally, the CON group also had a significantly higher ADG compared to the FMT group (*P < 0.05*). However, there was no significant difference between the CON and INU groups or among the three groups during the last 2 weeks (*P > 0.05*). Significant differences were detected among the three groups at 21 and 35 days, but not at 7 and 28 days. At 14 days, the INU and CON groups presented significantly greater ADFI than did the FMT group (*P < 0.05*), with no difference between the INU and CON groups (*P > 0.05*). At 21 days, the ADFI in the INU group was significantly greater than that in the FMT group (*P < 0.05*), but no differences were found between the INU and CON groups, or between the CON and FMT groups (*P > 0.05*). During the first 2 weeks of the experiment, no significant differences in the feed conversion ratio (FCR) were observed among the groups. However, in the second week, the FMT group presented a significantly greater FCR than the INU group.

Effects of FMT combined with inulin on tibial length

As shown in Table 4, tibial length differed significantly between 21 and 35 days; however, no significant differences were observed among the three groups at 1, 21, and 35 days. At 7 days, the tibial length in the INU group was significantly greater than that in the CON group (*P < 0.05*) and also significantly greater than that in the FMT group (*P < 0.05*). However, no significant difference was observed between the CON and FMT groups (*P > 0.05*). At 21 days, the INU group presented a significantly greater tibial length than did the CON group (*P < 0.05*), and the FMT group also presented a significant increase in tibial length compared with the CON group (*P < 0.05*). Nevertheless, no significant difference was detected between the INU and FMT groups (*P > 0.05*).

Effects of FMT combined with inulin on immune organ indices

As shown in Table 5, no significant differences in organ indices were observed among the CON, FMT, and INU groups at 21 and 35 days (*P < 0.05*).

Effects of FMT combined with inulin on immunological indices in serum

Histological examination of the ileum is presented in Fig. 1. As shown in Table 6, compared with those in the CON group, the levels of IgA, IgG, IgM, and LZM in the FMT group were significantly greater at 21 days (*P < 0.05*). The levels of IgA, IgM, and LZM in the INU group were also significantly greater than those in the CON group at 21 days *(P < 0.05*). In addition, the levels of IgG, IgM, and LZM in the FMT group were significantly greater than those in the INU group at 21 days (*P < 0.05*). Compared with those in the CON group, the levels of IgA in both the FMT and INU groups were significantly greater (*P < 0.05*). However, there were no significant differences in the levels of IgG, IgM, IL-10, or LZM among the three groups at 35 days (*P > 0.05*).

Table 4 Effects of FMT combined with inulin on tibial length at five different times

Tibial length (mm)					
Items	7 days	14 days	21 days	28 days	35 days
CON	26.68 ± 0.25	33.05 ± 0.26[b]	48.89 ± 0.50	57.62 ± 0.69[b]	66.64 ± 0.49
FMT	27.66 ± 0.40	33.02 ± 0.33[b]	48.71 ± 0.44	58.71 ± 0.69[ab]	64.92 ± 0.90
INU	27.33 ± 0.26	34.39 ± 0.32[a]	49.82 ± 0.24	60.61 ± 0.60[a]	66.10 ± 0.95

a and b represent statistically significant differences $p < 0.05$, a significantly higher than b, whereas ab is not significantly different from either a or b

Table 5 Effects of FMT on immune organ indices in the three groups at 21 and 35 days

Immune organ index			
Items	CON	FMT	INU
21 days			
Bursa of Fabricius Index (g/kg)	4.78 ± 0.50	4.30 ± 0.48	4.69 ± 0.48
Thymus Index (g/kg)	6.55 ± 0.31	6.57 ± 0.34	6.34 ± 0.31
Spleen Index (g/kg)	2.05 ± 0.10	1.96 ± 0.16	2.00 ± 0.14
35 days			
Bursa of Fabricius Index (g/kg)	5.88 ± 0.23	5.91 ± 0.30	5.06 ± 0.31
Thymus Index (g/kg)	5.55 ± 0.43	5.14 ± 0.31	5.73 ± 0.46
Spleen Index (g/kg)	2.39 ± 0.15	2.12 ± 0.06 2	2.11 ± 0.12

Effects of FMT combined with inulin on short-chain fatty acid levels in caecum

The effects of FMT combined with inulin on short-chain fatty acids in caecum as shown in Fig. 2. The concentrations of acetic acid and butyric acid were significantly greater in the INU group than in the CON and FMT groups ($P < 0.05$). The concentration of acetic acid in the FMT group was significantly greater than in the CON group ($P < 0.05$); however, butyric acid was significantly lower than that in the CON group ($P < 0.05$). Interestingly, the propionic acid content did not significantly differ among the three groups ($P > 0.05$).

Effects of FMT combined with inulin on intestinal morphology

Effects of FMT combined with inulin on ileum villi and crypts

The effects of FMT combined with inulin on ileal morphology are shown in Table 7. Compared with those in the INU group, the depths of the crypts in the CON and FMT groups were significantly greater ($P = 0.001$). The ratio of the villus height to the crypt depth in the INU group was significantly greater ($P < 0.001$), whereas no significant difference was detected in the villus height. At 35 days of age, the VH in the INU group was significantly greater than that in the FMT group ($P = 0.001$). However, no significant differences were detected in the depth of the crypts or the ratio of the villus height to the crypt depth among the three groups of chicks ($P > 0.05$).

Effects of FMT combined with inulin on paneth cells in jejunum

The histological staining of duodenal Paneth cells was shown in Fig. 3. In Table 8, the number of Paneth cells in the jejunum of chicks was greater in the FMT and INU groups than in the CON group ($P < 0.05$). Additionally, the expression of the Wnt3 gene was characterized. The expression of Wnt3 in the INU group was significantly greater than that in the FMT and CON groups ($P < 0.05$). Additionally, the expression of Wnt3 in the FMT group was significantly greater than that in the CON group ($P < 0.05$), as shown in Fig. 4.

FMT combined promotes the jejunal barrier and alleviates inflammation

As shown in Fig. 4, the expression levels of Muc2, ZO-1, and Occludin were significantly greater in the INU group than in the CON group ($P < 0.05$). Similarly, Muc2 and Occludin levels were significantly elevated in the FMT group ($P < 0.05$), although the expression levels of all three genes were markedly greater in the INU group than in the FMT group ($P < 0.05$). Additionally, Foxp3 expression was significantly increased in both the FMT and INU groups ($P < 0.05$), with higher levels observed in the INU group than in the FMT group ($P < 0.05$).

As for inflammatory markers, IL-6 expression was significantly lower in the FMT and INU groups than in the CON group ($P < 0.05$), whereas TGF-β levels were significantly higher in the INU group than in both the CON and FMT groups ($P < 0.05$). Furthermore, the IL-17A and IL-1β levels in the FMT group were significantly greater than those in the CON and INU groups ($P < 0.05$), with the IL-1β levels in the INU group being even lower than those in the CON group ($P < 0.05$).

Analysis of microbial composition differences in chicks

Analysis of microbial composition differences in the caecum

As shown in Fig. 5(a), there were a total of 488 microbial species in all the treatment groups, but there were 848 unique OTUs in the CON group, and 896 and 1252 unique OTUs in the FMT and INU groups, respectively. As shown in Fig. 5(b), PCoA analysis also revealed that the microbial community composition in the different treatment groups was different from that of the CON group at 21 days. However, the microbial community compositions of the FMT and INU groups were more similar at 21 days.

The top 20 microbial compositions of chicks at the phylum and genus levels

As shown in Fig. 6(a), at the phylum level, the top 20 most common microbiota sequences identified in chick guts were sequential, *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Actinobacteria*, *Tenericutes*, *Cyanobacteria*, *TM7*, *Verrucomicrobia*, *Lentisphaerae*, *Spirochaetes*, *NKB19*, *Deferribacteres*, *Synergistetes*, *Gemmatimonadetes*, *WS3*, *WWE1*, and *Fusobacteria* and *Chloroflexi*. Notably, the abundance of *Firmicutes* in the FMT group was lower than that in the INU group, whereas the abundance of *Bacteroidetes* and *Proteobacteria* was greater

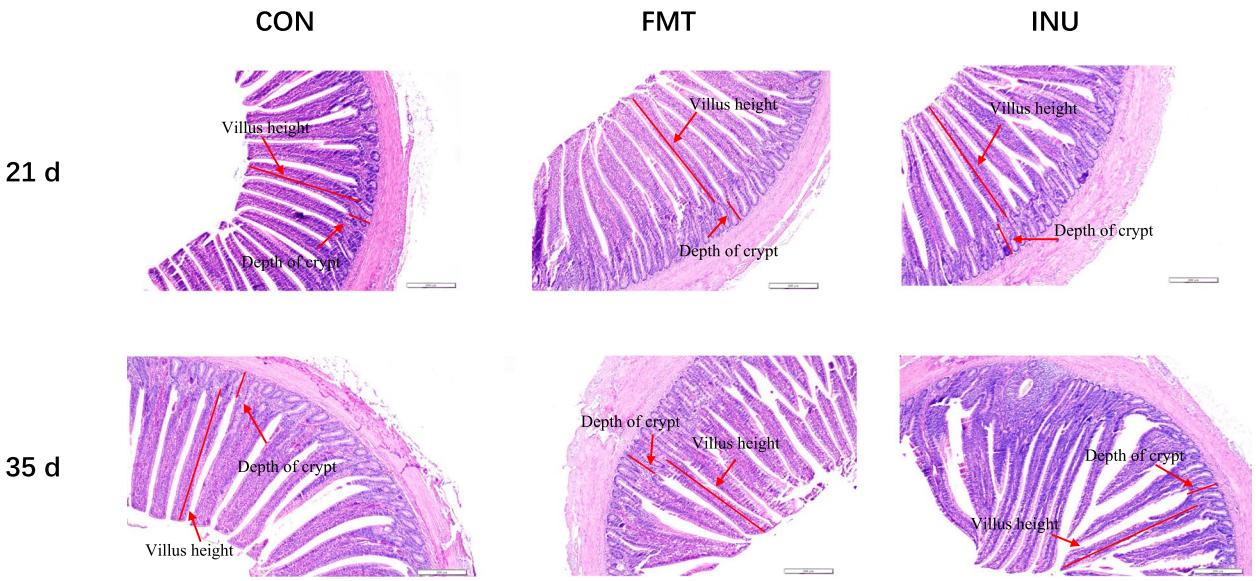


Fig. 1 Effects of FMT combined with inulin on Paneth cell numbers in jejunum. Zoom in with Topaz photo AI. Bar = 50 μm

in the FMT group than in the CON and INU groups. Compared with those in the CON and FMT groups, *Actinobacteria* and *Tenericutes* abundances were clearly increased in the INU group. As shown in Fig. 6b, at the genus level, the top 20 microbiota sequential proportions were *Faecalibacterium*, *Oscillospira*, *[Ruminococcus]*, *Phascolarctobacterium*, *Bacteroides*, *Ruminococcus*, *Butyricicoccus*, *Streptococcus*, *Lactobacillus*, *Campylobacter*, *Parabacteroides*, *Coprococcus*, *Rikenella*, *Blautia*, *Dorea*, *Barnesiella*, *Enterococcus*, *cc_115*, *Butyricimonas*, and *AF12*. Notably, *Oscillospira*, *[Ruminococcus]*, *Bacteroides*, *Lactobacillus*, *Campylobacter*, and *Parabacteroides* abundance were increased in the FMT group compared with the CON and INU groups, whereas *Faecalibacterium*, *Ruminococcus*, and *Streptococcus* abundance were decreased in the FMT group compared with the CON and INU groups. Additionally, *Oscillospira*, *[Ruminococcus]*, *Phascolarctobacterium*, *Bacteroides*, *Butyricicoccus*, *Streptococcus*, and *Parabacteroides* were more abundant in the INU group than in the CON group.

FMT promoted microbial diversity in the caecum

As shown in Fig. 7, the cecal microbial community was analyzed via alpha diversity analysis at 21 days. Compared with those in the CON group, the Chao1 index and observed species index tended to be greater in the INU group, whereas the Shannon index, Simpson index,

Table 6 Effect of FMT combined with inulin on immune indices in the serum at 21 and 35 days

Serum index			
Items	CON	FMT	INU
21 days			
IgA (ng·mL^{-1})	5491.08 ± 564.69^{b}	5917.07 ± 807.30^{a}	6132.01 ± 413.55^{a}
IgG (ng·mL^{-1})	78.64 ± 3.20^{a}	80.13 ± 3.90^{a}	60.81 ± 4.01^{b}
IgM (ng·mL^{-1})	1323.98 ± 98.02^{b}	2583.83 ± 197.26^{a}	1955.05 ± 209.28^{ab}
IL-10 (μg·L^{-1})	38.85 ± 5.31	59.90 ± 3.29	52.30 ± 5.06
LZM (μg·L^{-1})	1.01 ± 0.18^{b}	1.68 ± 0.13^{a}	1.11 ± 0.12^{ab}
35 days			
IgA (ng·mL^{-1})	5284.88 ± 520.49^{b}	7414.88 ± 451.28^{a}	7562.45 ± 357.62^{a}
IgG (ng·mL^{-1})	53.04 ± 3.18	50.02 ± 3.33	52.15 ± 4.97
IgM (ng·mL^{-1})	1670.53 ± 179.97	1731.78 ± 178.94	1670.27 ± 151.17
IL-10 (μg·L^{-1})	48.05 ± 4.77	53.29 ± 3.85	53.91 ± 5.90
LZM (μg·L^{-1})	1.46 ± 0.13	1.11 ± 0.17	1.25 ± 0.15

a and b represent statistically significant differences $p < 0.05$, a significantly higher than b, whereas ab is not significantly different from either a or b

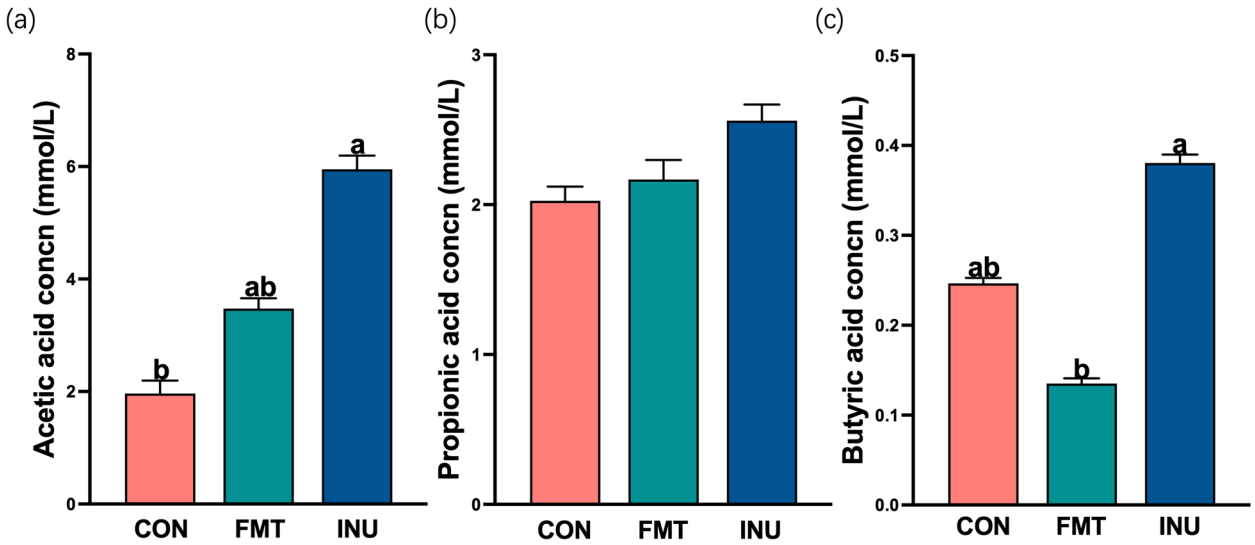


Fig. 2 Concentration of acetic acid (**a**), propionic acid (**b**), and butyric acid (**c**) in the cecal contents. Measurements were made at 21 days of age

Pielou's evenness index, and Faith's PD index were significantly greater in the INU group at 21 days ($P < 0.05$). However, the Good's coverage index was not significantly different between the CON group and the INU group ($P > 0.05$). Compared with those in the CON group, the PD indices in the FMT group were significantly greater ($P < 0.05$), but the other indices were not significantly different between the CON and FMT groups ($P > 0.05$). Compared with the FMT group, the INU group presented significantly higher Shannon, Simpson, and Pielou's evenness indices ($P < 0.05$), but other indices did not significantly differ between the INU group and FMT group ($P > 0.05$).

Table 7 Effects of FMT combined with inulin on ileum morphology

Ileum			
21 d			
Item	CON	FMT	INU
Villus height (*μm*)	783.53±30.66	739.02±16.85	751.14±12.80
Depth of crypt (*μm*)	165.40±8.40^{A}	169.50±8.07^{A}	131.92±6.22^{B}
The ratio from the villus to crypt	5.10±0.18^{B}	4.63±0.15^{B}	6.05±0.28^{A}
35 d			
Villus height (*μm*)	816.92±5.08^{AB}	755.41±20.64^{B}	823.54±17.50^{A}
Depth of crypt (*μm*)	121.10±6.43	114.99±6.33	117.64±5.42
The ratio from the villus to crypt	7.05±0.40	6.78±0.26	7.30±0. 24

A and B represent statistically significant differences $p < 0.01$, A significantly higher than B, whereas AB is not significantly different from either A or B

Assessment of the effects of FMT combined with inulin on the microbiome

LefSe analysis was used to determine significant microbiota enrichment at the phylum and genus levels in the three chick groups after 2 weeks. Figure 8(a) and (b) show that 49 significant taxa differed among the groups ($P < 0.05$). At the phylum level, *Actinobacteria* and *Tenericutes* were enriched in the INU group, whereas *Cyanobacteria* were enriched in the FMT group. At the genus level, seven genera (*Barnesiella*, *Parabacteroides*, *Oscillospira*, *Bilophila*, *Helicobacter*, *Tatumella*, and *Mycoplasma*) were enriched in the FMT group; nine genera (*Collinsella*, *Slackia*, *Butyricimonas*, *AF12*, *Rikenella*, *Streptococcus*, *Peptococcus*, *Phascolarctobacterium*, and *Sutterella*) were enriched in the INU group, and five genera (*Odoribacter*, *Fructobacillus*, *Anaerostipes*, *Lachnospira*, *Faecalibacterium*, *Phascolarctobacterium*, *cc_115*, and *Pseudomonas*) were enriched in the CON group. Heatmap analysis further revealed changes in the microbial composition of the three groups. Based on changes in ASV abundance, the top 20 genera were selected. Among them, *Ruminococcus* was changed; *Phascolarctobacterium*, *Rikenella*, and *Butyricimonas* were altered in the FMT and INU groups. *Faecalibacterium* and *cc_115* were enriched in the CON and INU groups. The *Bacteroides*, *Barnesiella*, *Lactobacillus*, *Campylobacter*, *Oscillospira*, *Enterococcus*, *Parabacteroides*, *Butyricicoccus*, and *Dorea* genera were enriched in the FMT group; *Blautia* was enriched in the CON group; and *Streptococcus*, *Coprococcus*, and *AF12* were enriched in the INU group.

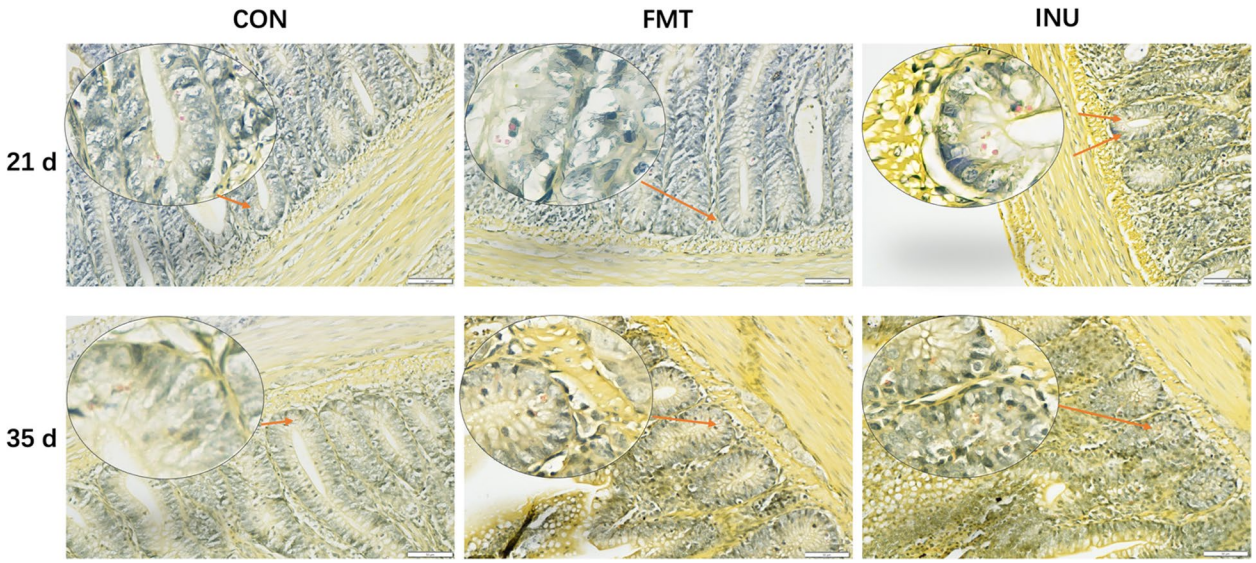


Fig. 3 Effects of FMT combined with inulin on the ileum, as shown by observation of the paraffin-embedded section. Bar = 200 μm

Relationships between microbiota communities and jejunal barrier function

The relationships between the microbiota community and barrier function and inflammation in the jejunum at 21 days were analyzed. As shown in Fig. 9, *Blautia* was positively correlated with IL-17A gene expression ($P < 0.01$). *Butyricimonas* and *Anaerofilum* were positively correlated with ZO-1 gene expression ($P < 0.05$). *Odoribacter*, *Lysinibacillus*, and *Allobaculum* were positively correlated with IL-6, Wnt3, and Muc2, respectively.

Effects of FMT combined with inulin on metabolic pathways

The metabolic pathway statistics, differential analysis of metabolic pathways, and species composition of metabolic pathways are shown in Fig. 10. As shown in Fig. 10 (a), improvements were observed in the following pathways: *amino acid biosynthesis*, *carbohydrate biosynthesis*, *cell structure biosynthesis*, *cofactor, prosthetic group, electron carrier*, and *vitamin biosynthesis*; *fatty acid and lipid biosynthesis, nucleoside and nucleotide biosynthesis*; and *secondary metabolite biosynthesis under the biosynthesis category*, as well as *carbohydrate degradation*, *carboxylate degradation*, and *nucleoside and nucleotide degradation under the generation of precursor metabolites* and *energy category*. The top 20 most common bacterial genera include 11 identified and 9 unidentified, as shown in Fig. 10 (b). Compared with the CON and INU groups, the FMT group presented highly significant increases in metabolic pathways, as shown in Fig. 10 (c) and (e). Figure 10 (d) shows significantly improved metabolic pathways in the INU group compared with those in the CON group.

Compared with the INU group, the FMT group presented highly significant differences in the following pathways ($P < 0.001$): *aerobactin biosynthesis*, *norspermidine biosynthesis*, *superpathway of L-arginine, putrescine*, and *4-aminobutanoate degradation*, and *superpathway of L-arginine* and *L-ornithine degradation*. Additionally, the FMT group presented significant improvements

Table 8 The number of Paneth cells in the jejunum

Number of Paneth cells			
Item	CON	FMT	INU
21 d			
Paneth cells	3.21±0.13^{b}	4.56±0.15^{ab}	5.02±0.21^{a}
35 d			
Paneth cells	3.56±0.12	3.89±0.23	3.72±0.17

a and b represent statistically significant differences $p < 0.05$, a significantly higher than b, whereas ab is not significantly different from either a or b

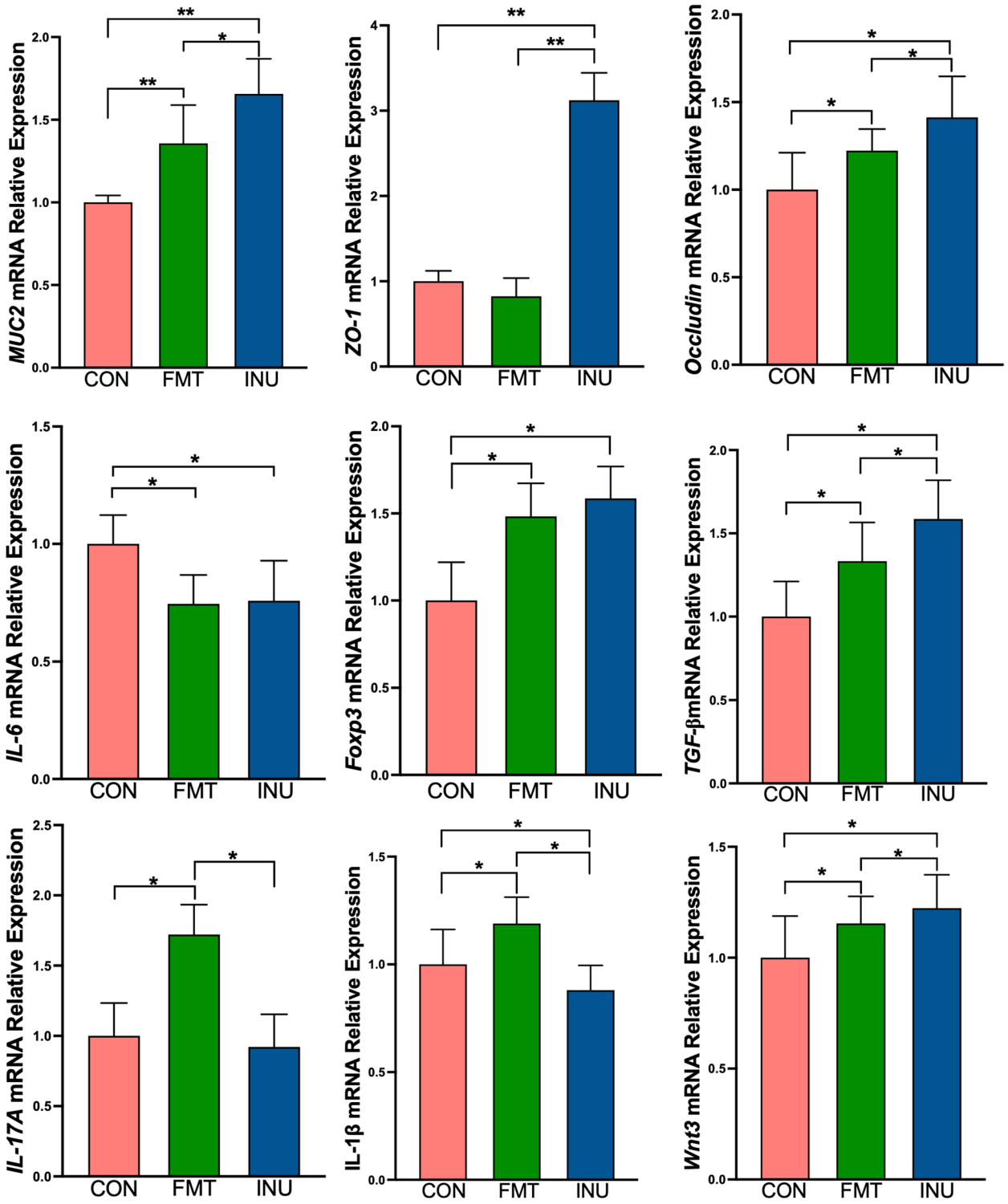


Fig. 4 Effects of FMT combined with inulin on the jejunum barrier and inflammation at 21 days

in the following pathways compared to the CON group ($P < 0.001$): *biotin biosynthesis II*, *chondroitin sulfate degradation I* (bacterial), *superpathway of taurine degradation*, and *superpathway of sulfolactate degradation*. Compared with those in the CON group, the following pathways in the INU group were highly significantly

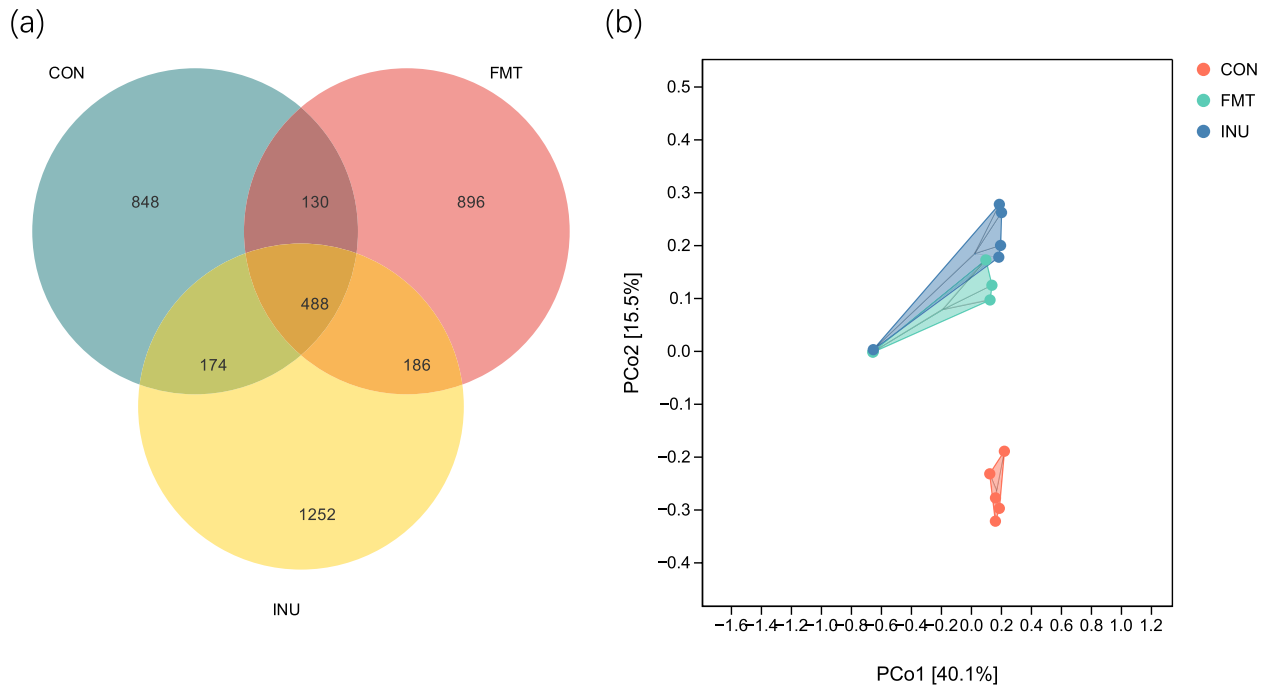


Fig. 5 The different microbial compositions of the three groups were analyzed by Venn and PCoA analysis, as shown in **a** and **b** in Fig. 5, respectively

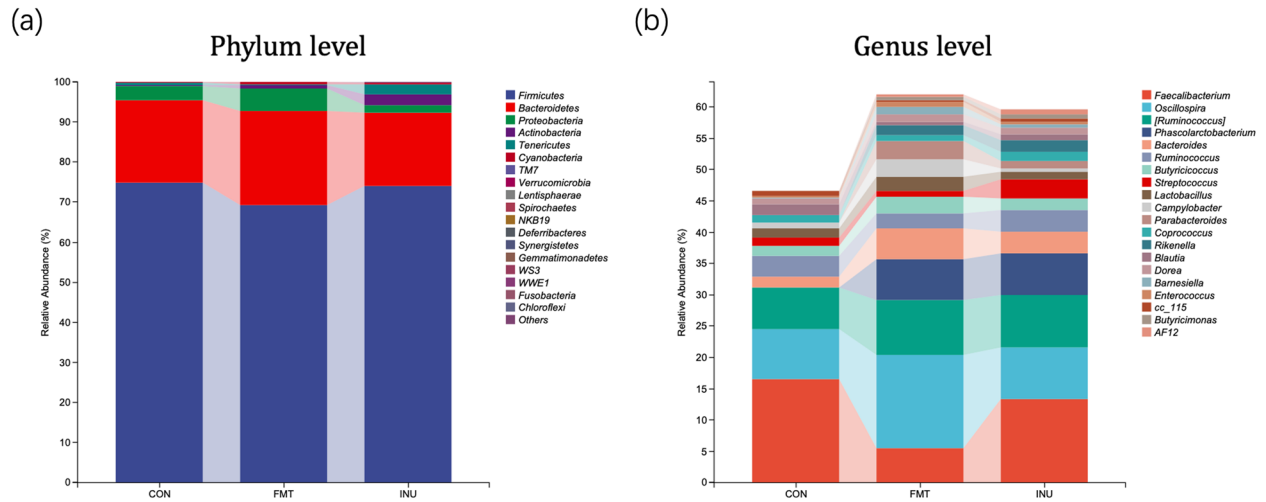


Fig. 6 The top 20 microbial compositions of three groups in the caecum at the phylum and genus level are shown in **a** and **b**, respectively

enriched ($P < 0.001$): *biotin biosynthesis II*, *L-leucine degradation I*, *reductive acetyl coenzyme A pathway*, and *chondroitin sulfate degradation I* (bacterial). The *photorespiration* and superpathway of sulfolactate degradation pathways were also significantly increased compared to the CON group ($P < 0.05$). However, the CON group presented significant improvements in the following pathways compared with the INU group ($P < 0.001$): *aerobactin biosynthesis, the superpathway of L-arginine, putrescine,* and *4-aminobutanoate degradation*, and the *superpathway of L-arginine* and *L-ornithine degradation*.

Analysis of the associations between hen fecal microbiota and chick intestinal microbiota

Analysis of microbial composition differences between hen feces and chick intestines

As shown in Fig. 11(a), there were a total of 127 microbial species in all the treatment groups, but there were 2087

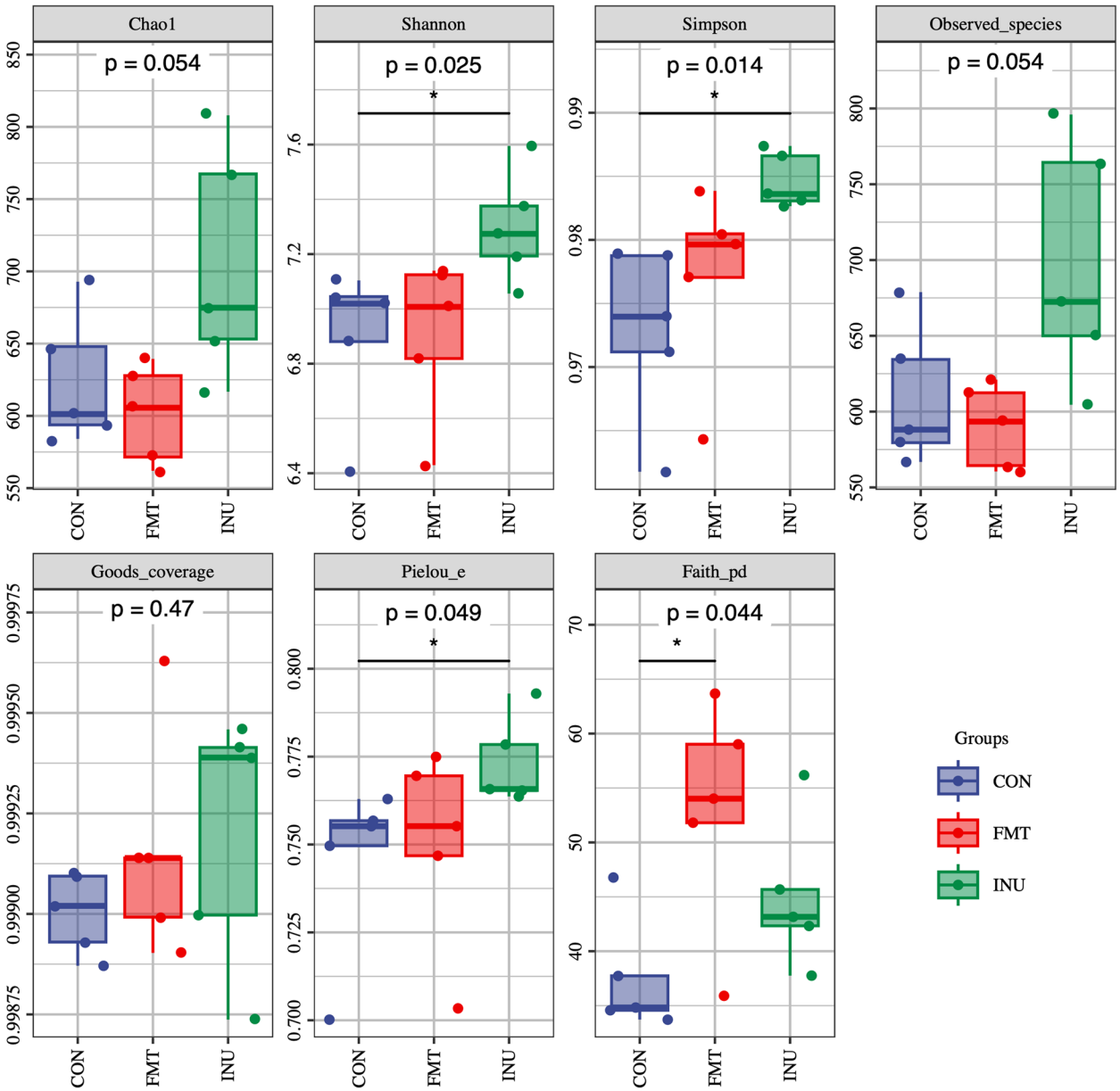


Fig. 7 *Alpha-diversity* analysis of the cecal microbiota at 21 days. *Alpha-diversity* analysis, including *Chao 1*, *Shannon*, *Simpson*, *observed* species, *Goods_coverage*, *Pielou_e*, and *Faith_pd* indices, was performed

unique OTUs in the Hen group, and 1090, 1323, and 1930 unique OTUs in the CON, FMT, and INU groups, respectively. As shown in Fig. 11(b), PCoA analysis also revealed that the microbial community composition in the Hen, FMT, and INU groups was different from that in the CON group. According to the PCoA results, the microbiota of the Hen group was more similar to that of the FMT and INU groups than of the CON group.

Comparison of fecal microbial richness in hens and intestinal microbial richness in chicks

As shown in Fig. 12, the richness of fecal microbiota in hens and intestinal microbiota was analyzed via Alpha diversity analysis. Compared with those in the CON group, the Chao 1 index and observed species index significantly increased in the Hen group ($P < 0.05$), but the values in the Hen group were not significantly different from those in the FMT and INU groups. The Simpson index, Pielou-E index, and Shannon index revealed that

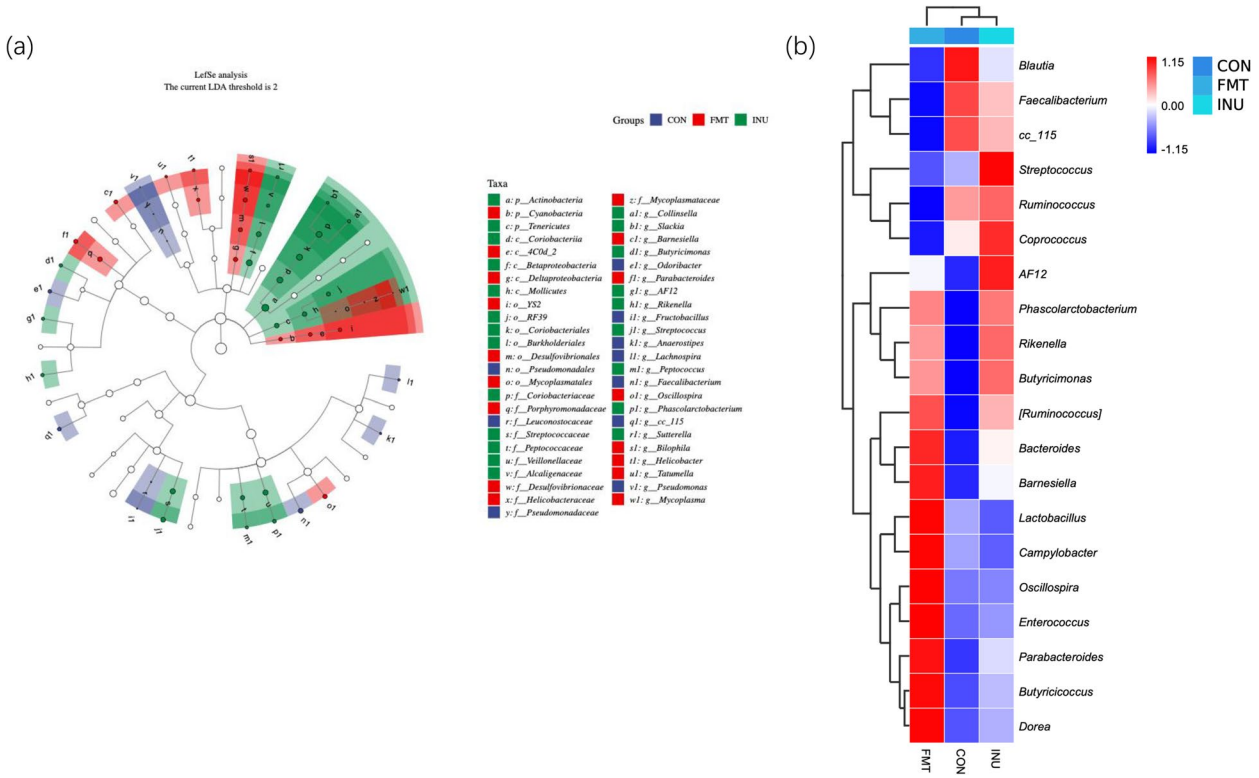


Fig. 8 Microbiota with significant differences from the phylum level to the genus level in the cecal microbiota of the three groups

the values in the FMT and INU groups were significantly greater than those in the Hen group ($P < 0.05$).

Comparison of hen feces and chick intestinal microbial species

The microbial taxa with differences in abundance are displayed by LEfSe from the phylum to genus level in Fig. 13 (a), and 100 taxa were significantly different among the groups. Three phyla (*Actinobacteria*, *Bacteroidetes*, and *Proteobacteria*) were enriched in the INU, FMT, and Hen groups, respectively. At the genus level, 24 genera were significantly enriched in the Hen group (*Corynebacterium*, *Leucobacter*, *Aeriscardovia*, *Wautersiella*, *Sphingobacterium*, *Bacillus*, *Solibacillus*, *Sporosarcina*, *Facklamia*, *Trichococcus*, *Enterococcus*, *GW_34*, *Epulopiscium*, *Erysipelothrix*, *Alcaligenes*, *Comamonas*, *Citrobacter*, *Escherichia*, *Proteus*, *Serratia*, *Shigella*, *Acinetobacter*, *Psychrobacter*, *Pseudomonas*); 8 genera were significantly enriched in the FMT group (*Bacteroides*, *Parabacteroides*, *[Ruminococcus]*, *Dorea*, *Butyricicoccus*, *Oscillospira*, *Clostridium*, *Bilophila*); and 9 genera were significantly enriched in the INU group (*Butyricimonas*, *AF12*, *Rikenella*, *Streptococcus*, *Coprococcus*, *Peptococcus*, *Ruminococcus*, *Phascolarctobacterium*, *Sutterella*). The top 20 microorganisms with changes in ASV abundance were analyzed using a heatmap to further observe their composition. Among them, the abundance of *Butyricimonas* and *Phascolarctobacterium* changed in the Hen, FMT, and INU groups; that of *Enterococcus* changed in the Hen and FMT groups; and that of *Rikenella*, *Butyricicoccus*, and *[Ruminococcus]* changed in the Hen and INU groups.

Discussion

Following China's comprehensive ban on antibiotics, except for limited therapeutic use, the search for green, safe, and effective alternatives has gained significant attention [30]. Fecal microbiota transplantation, initially recognized for its efficacy in treating diarrhea and colitis, has been increasingly adopted in research and clinical practice since 2013. Currently, FMT is widely utilized in clinical settings, particularly for treating *Clostridioides difficile* infection [31]. Studies have demonstrated that combining probiotics with FMT can effectively alleviate diarrhea and enteritis [32–34]. However, there are few reports on the effects of maternal microorganisms transplanted to offspring on their growth and even fewer studies on the effects of FMT combined with probiotics on the early growth of livestock and poultry. Inulin is extracted primarily from plant sources, with industrial synthesis and commercial procurement serving as

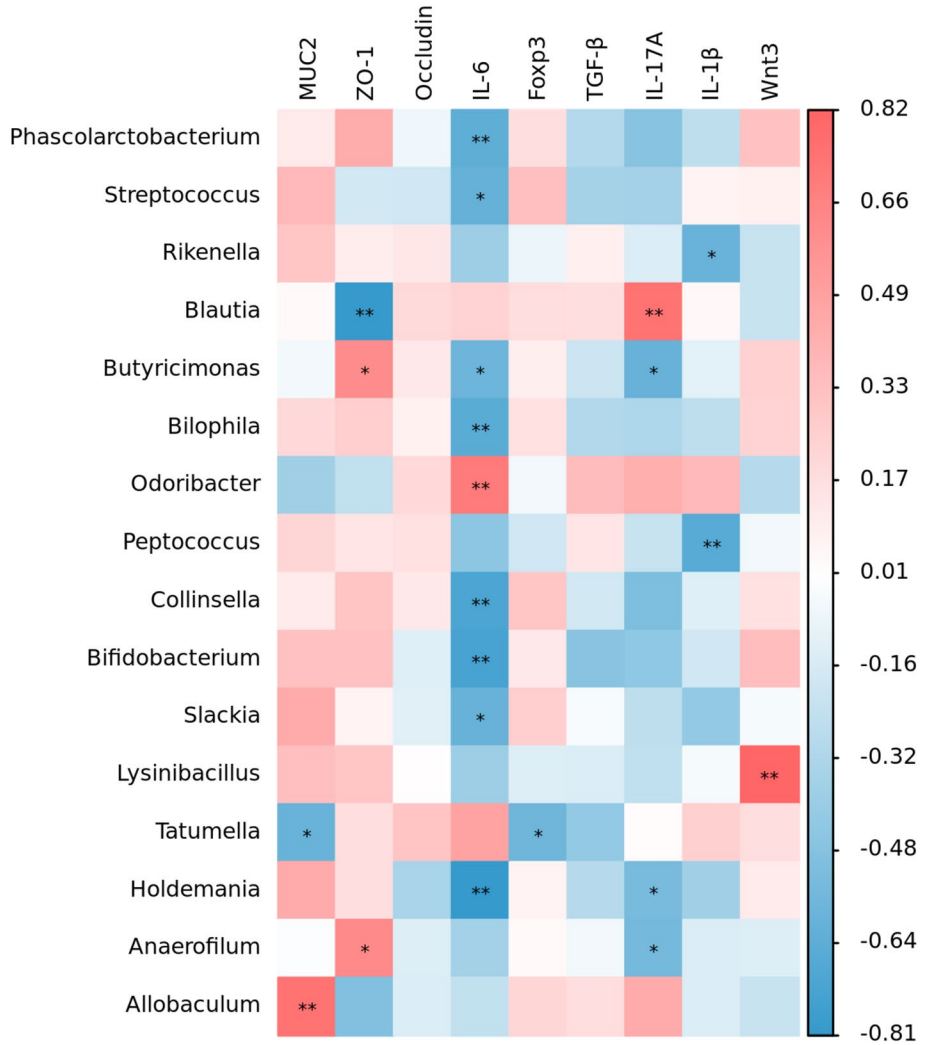


Fig. 9 Spearman's correlation between the genera level of the fecal bacterial communities and 21-day barrier function. Red indicates a positive correlation and blue indicates a strong negative correlation

alternative approaches. Currently, plant extraction currently represents the most widely adopted method for commercial production. Inulin has significant therapeutic potential in various clinical applications, particularly in the management of digestive health, metabolic regulation, and cardiovascular diseases. Although the initial production costs are relatively high, the optimization of large-scale production processes has improved cost-effectiveness, indicating substantial market potential in both the pharmaceutical and functional food industries [20, 35]. In this study, we combined FMT with inulin to investigate its effects on the early growth of chicks and to investigate the changes in the gut microbiota of offspring after the transplantation of maternal microorganisms.

The ADG and ADFI can reflect the growth status of organisms and feed utilization [36]. Interestingly, the INU group ADG increased at 7 and 14 days, whereas there was no significant change at 21 and 35 days. However, the results revealed a significant increase in the ADFI at 14 and 21 days in the INU group. In addition, the ADG and ADFI did not increase significantly in the FMT group and were even lower than those in the CON group. Previous studies have shown that not all the benefits of FMT treatment are positive, and some have noted other negative effects of FMT. Mccormack et al. [37] reported that feeding 8 mL of a donor fecal bacteria suspension to newborn piglets could seriously damage the intestinal morphology of piglets and lead to weight loss, and the negative effect was significantly greater after four feeds than after only one. The reason is that the complete fecal flora contains numerous pathogenic bacteria, such as *Streptococcus* and *Campylobacter*. Deng et al. [38] reported that some patients receiving FMT may have mild adverse reactions, mainly diarrhea, abdominal pain, vomiting, constipation, and other gastrointestinal symptoms, some patients have fever and a few have lung infections. The tibial length can

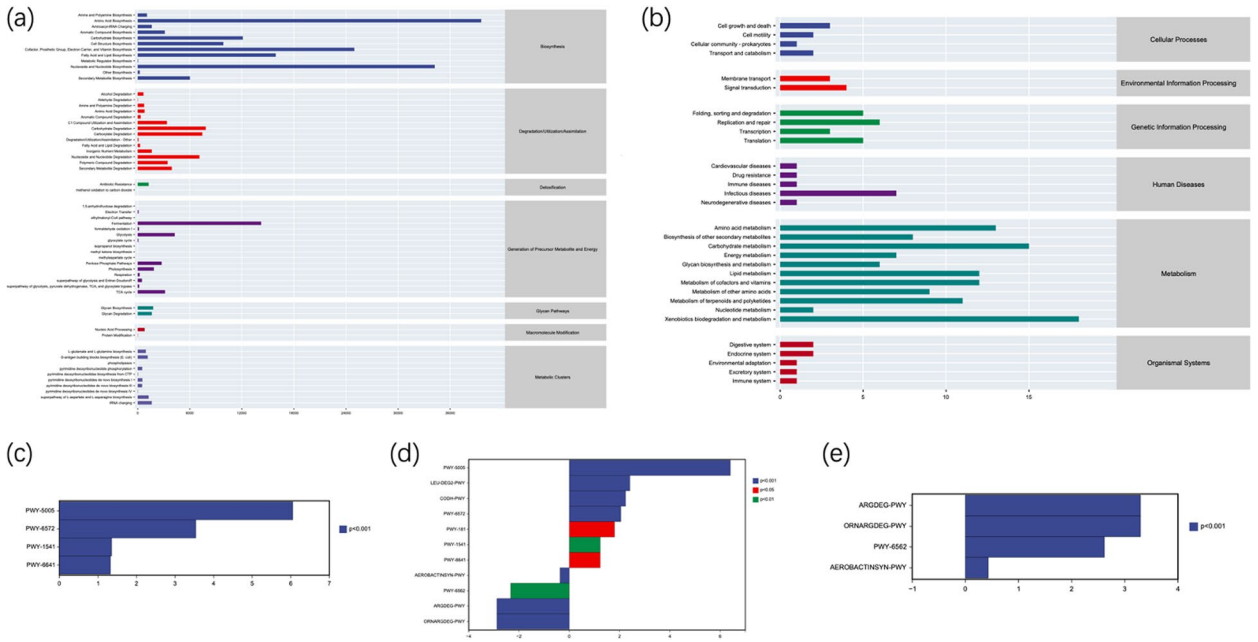


Fig. 10 Effects of FMT combined with inulin on metabolic pathways. **a** Metabolic statistic, **b** species composition of the metabolic pathway, and **c**–**e** differential analysis of the metabolic pathway

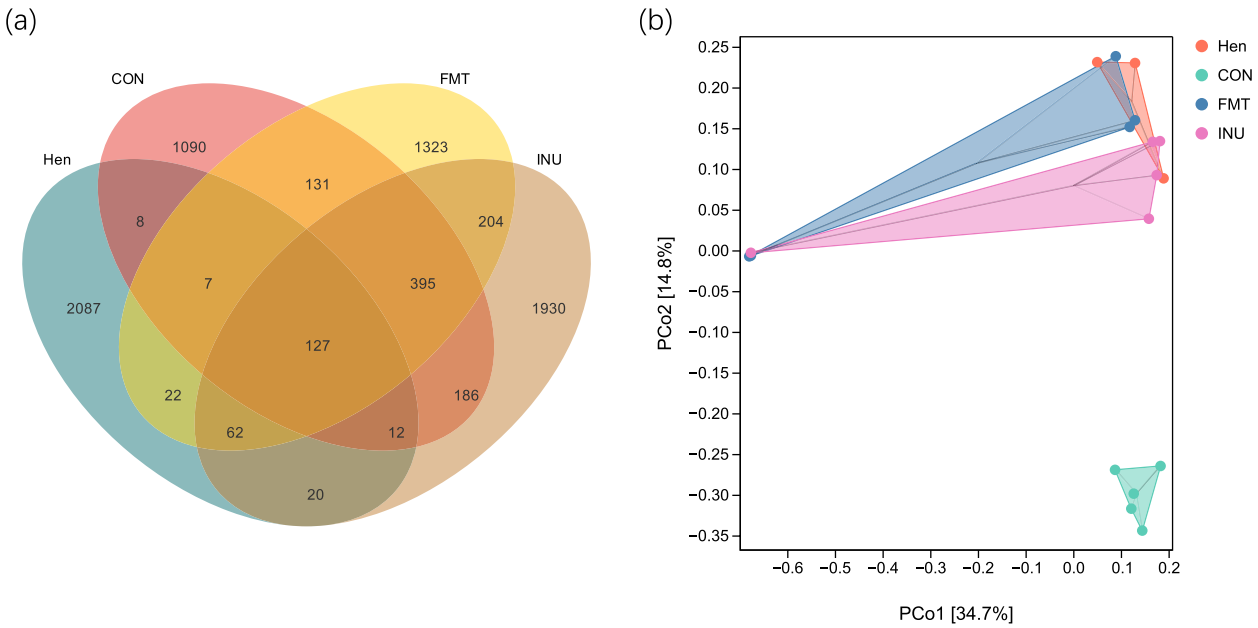


Fig. 11 The different microbial compositions of the four groups were analyzed by Venn and PCoA, as shown in **a** and **b**, respectively

reflect the growth conditions and growth performance of poultry. Another interesting phenomenon is that tibia length in the INU group increased significantly at 7 and 21 days, but did not change markedly at 14 days; it remained the greatest among the three groups. A possible reason may be errors in the measurement process. In this study, there was no significant difference in organ indices among the three groups, which was different from our team's previous studies. In addition, reports indicate that a single feeding of a fecal bacterial suspension with a bacterial volume of 1.2×10^9 CFU/mL (8 mL) may damage the intestinal environment homeostasis of newborn offspring, and

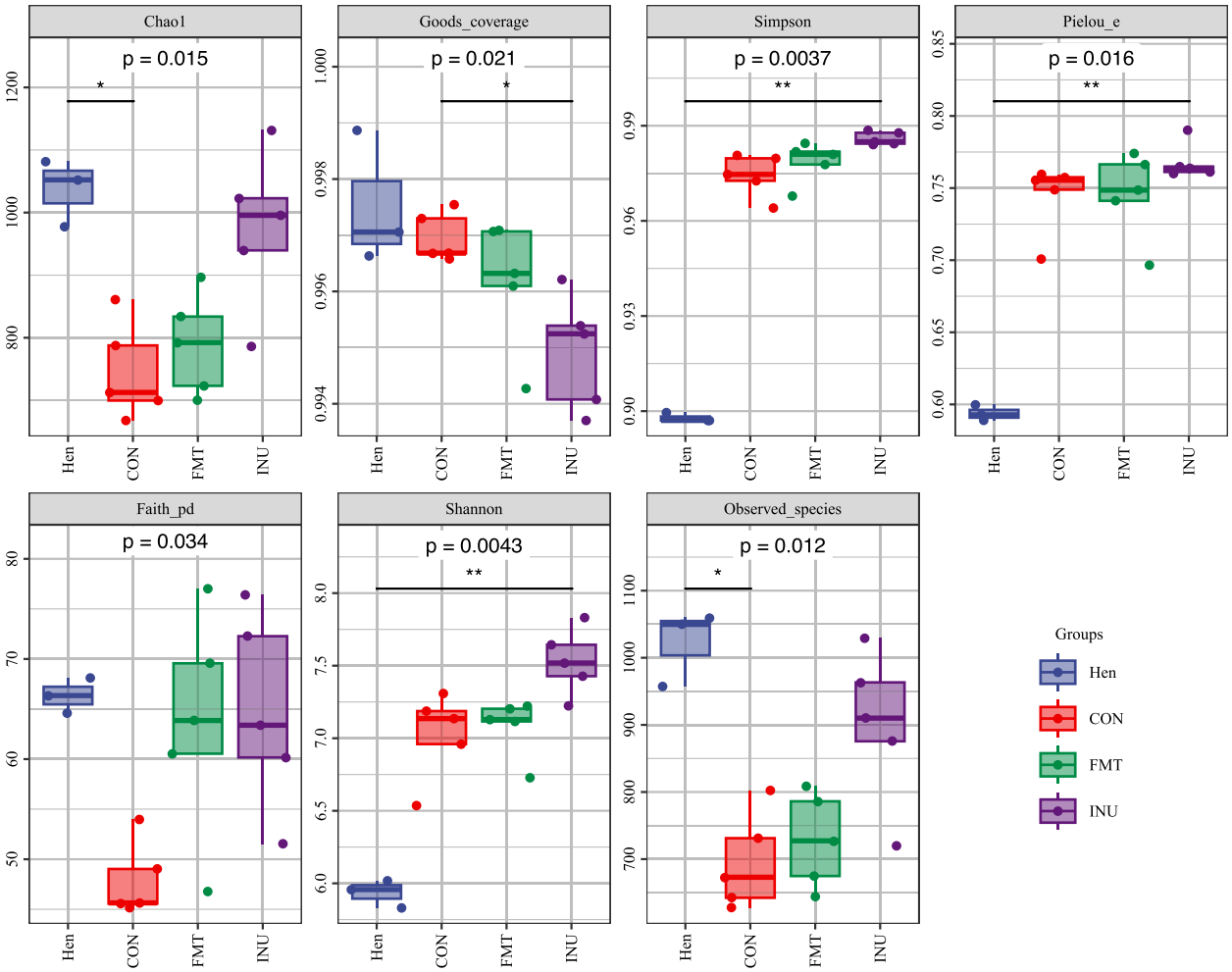


Fig. 12 *Alpha-diversity* indices, including the *Chao 1*, *Shannon* diversity index, *Simpson* index, *observed* species index, *Goods_coverage* index, *Pielou_e* index, and *Faith_pd* index, were analyzed

cause receptor diarrhea, growth and development retraction, inflammation, and so on [18, 39, 40]. A thorough comparison of the previous experiments revealed the possible reasons for the different results: first, the volume of bacterial suspension added was significantly higher than in the previous protocol; second, the fecal bacteria were added directly to the diet; and third, the type of fecal bacteria was added after 7 days instead of within 1 days. The above findings suggest that FMT may have adverse effects, but the present study revealed that both ADG and ADFI increased in the INU group compared with those in the CON group or FMT group, suggesting that inulin can promote the beneficial effects of FMT.

Surprisingly, the villus height and crypt depth at 21 and 35 days were greater in the INU group than in the FMT group. We speculated that the FMT might cause intestinal inflammation. The result showed that supplementation with inulin can promote intestinal development. Next, the serum levels of inflammatory factors and immunoglobulins were determined. Interestingly, the results revealed that the level of IL-10 did not significantly differ among the three groups, whereas the FMT group presented significantly higher levels of the IgG, IgM, and LZM immunokines than did the INU group. To explore this phenomenon, jejunal barrier function and inflammatory and immune gene expression were characterized. Interestingly, Muc2, ZO-1, and Occludin gene expression were significantly greater in the INU group than in the CON and FMT groups, and the levels of IL-17A and IL-1β were significantly lower in the INU group than in the FMT group. In addition, the gene expression levels of Foxp3 and TGF-β were markedly greater in the INU group than in the FMT group. These findings provide evidence for our speculation. To further prove our hypothesis, Paneth cells were counted, which revealed more increased Paneth cells in the jejunum in the INU and FMT groups than in those of the CON group. We also measured Wnt3 gene expression, which was increased expression in the INU group compared with the CON and

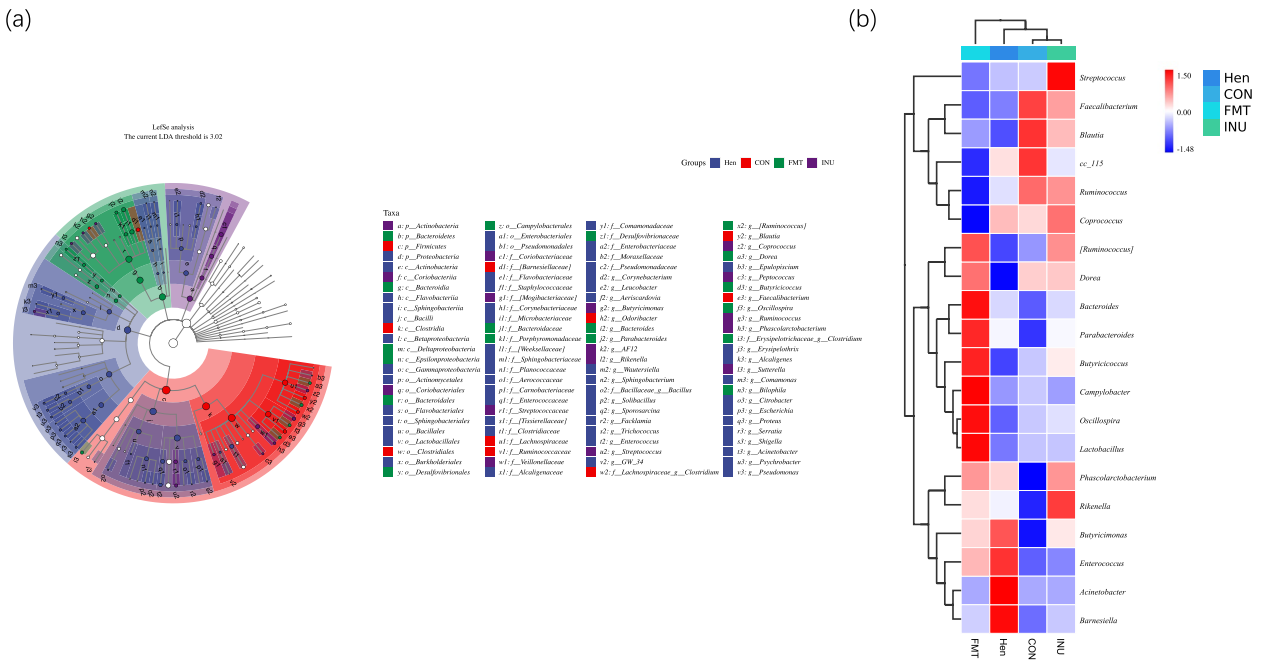


Fig. 13 Microbiota with significant differences from the phylum level to the genus level. Heatmap analysis of species composition at the genus level

FMT groups. In summary, FMT supplemented with inulin had a positive effect on chicks.

The intestinal microbiota is a source of immunity, and adhering to the intestinal mucosa can enhance the intestinal mucosal barrier function, preventing the entry of some pathogenic bacteria and preventing the invasion of harmful bacteria, generating inflammation and strengthening the host immune response [41, 42]. Our study analyzed the composition of the caecum microbiota. The results of the intestinal biodiversity of chicks in the FMT and INU groups revealed an increase in the richness of the intestinal microbiota relative to that in the CON group. Among the three groups, *Firmicutes* accounted for the largest proportion, followed by *Bacteroidetes*. *Faecalibacterium* is the most important butyrate bacteria in the intestine and plays an important role in intestinal health, and its abundance is significantly reduced in the intestines of patients with inflammatory bowel disease and various other diseases according to data from numerous previous reports [43–45]. At the phylum level, the fecal microbiota was dominated by the *Firmicutes* and *Bacteroidetes* phyla, with a total abundance greater than 90%. Similar to previous studies, Zhang et al. [46] reported that adding inulin to the diet of mice increased the abundance of *Firmicutes* and *Bacteroidetes*. In addition, Zhu et al. [47] reported that *Firmicutes* accounted for the largest percentage of the gut microbiota of rats subjected to FMT. Furthermore, reports have shown that *Faecalibacterium*, *[Ruminococcus]*, and *Phascolarctobacterium* can promote the production of short-chain fatty acids (SCFAs), mostly acetic, propionic, and butyric acids, which play an important role in maintaining intestinal health [48, 49]. We also tested the SCFAs in the cecum and found that acetic acid and butyric acid significantly increased in the INU group, whereas only acetic acid significantly increased in the FMT group. In addition, the relationship between bacterial symbiosis and gene expression was found to be positive for ZO-1 by *Butyricimonas* and *Allobaculum* and beneficial for Muc2 by *Allobaculum*. These findings suggest that FMT can increase gut biodiversity, that the addition of inulin can promote the colonization of probiotics, and that the supplementing of inulin can effectively promote chick growth in early life. Combining the analysis of hen fecal microbiota with chick gut microbiota, we found that the inulin supplementation favored the colonization of intestinal probiotics (*Coprococcus*, *Phascolarctobacterium*, *Butyricimonas*); *Coprococcus* is a genus of anaerobic cocci that actively ferment carbohydrates to produce butyric and acetic acids as well as formic or propionic and/or lactic acids, among others [50], *Butyricimonas*, a beneficial bacterium that produces short-chain fatty acids to reduce inflammation, was found in a report by Han et al. [51]. *Phascolarctobacterium*, a specialized anaerobic and gram-negative bacterium that produces acetate and propionate [52], and the increase in short-chain fatty acid-producing microbiota in the INU group is consistent with the results of our SCFA content assay.

In conclusion, FMT supplemented with inulin is beneficial for the colonization of beneficial intestinal bacteria and for the early growth of chicks.

Surprisingly, differences in metabolic pathways were observed. Compared with those in the CON group, PWY-5005 (biotin biosynthesis II) and PWY-6572 (chondroitin sulfate degradation I) were significantly increased in the FMT group and INU group. Additionally, LEUDEG2-PWY (L-leucine degradation I) and CODH-PWY (reductive acetyl coenzyme A pathway) were improved in the INU group compared with the CON group. Previous studies have demonstrated that L-leucine metabolism promotes growth, muscle tissue protein synthesis, and intestinal immune function [53, 54]. Moreover, L-leucine releases energy for ATP synthesis via the acetyl coenzyme A reduction pathway. Interestingly, however, the AEROBACTINSYN-PWY (aerobactin biosynthesis) pathway was significantly increased in the FMT group compared with the INU group. In combination with the supplementary figures, which show the microbiota composition of metabolic pathways at the phylum and genus levels, we suspect that aerobactin from probiotics constitutes a significant proportion of the gut microbiota. Therefore, we believe that the addition of inulin can promote chick growth and immunity. Although growth performance was lower in the FMT group than in the INU group, chick growth should be observed over a longer period. Therefore, in future experiments, we will extend the observation period to assess the long-term effects of the treatments.

Conclusions

In conclusion, our data demonstrate that dietary inulin supplementation significantly enhances growth performance in chicks through maternal fecal microbiota transplantation. This effect is attributed to the modulation of early intestinal microbiota, which promotes the colonization of beneficial bacteria and enhances immune function. Specifically, the combined intervention of inulin and maternal fecal microbiota improved gut barrier integrity, upregulated immune-related gene expression, and reduced the expression of proinflammatory factors. These findings highlight the potential of inulin supplementation as a strategy to optimize early growth and intestinal health in poultry.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40168-025-02084-z.

Supplementary Material 1.

Supplementary Material 2.

Supplementary Material 3.

Supplementary Material 4.

Supplementary Material 5.

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Authors' contributions

Conceptualization, M.C., J.P., S.L., S.Y., and X.Z.; methodology, M.C., J.P., Y.S., P.S. and X.Z.; formal analysis, M.C., J.P. and X.Z.; re-source, M.C., J.P.; data curation, M.C., J.P and P.S.; writing——original draft preparation, M.C.; writing ——review and editing, M.C., J.P., Y.S., P.S. and X.Z.; project administration, M.C., J.P., S.L., Y.S. and X.Z.; funding acquisition, X.Z. All authors have read agreed to the published version of the manuscript.

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Data availability

The sequences of 3 cases of fecal microbiota sequencing in hens and 15 cases of microbiota sequencing in the cecum of chicks improved in the manuscript have been shared on the NCBI platform under the project number of hen flora: PRJNA 1181668 (https://www.ncbi.nlm.nih.gov/sra/PRJNA1181668); and under the project number of chick flora: PRJNA 1181746 (https://www.ncbi.nlm.nih.gov/sra/PRJNA1181746).

Declarations

Ethics approval and consent to participate

This study was approved by the Experimental Animal Ethical Committee of Jilin Agriculture University. The chicks came from a local family farm, and the farm owner was informed and agreed to the implementation of this experimental protocol.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

1. Korpela K, Helve O, Kolho KL, Saisto T, Skogberg K, Dikareva E, Stefanovic V, Salonen A, Andersson S, de Vos WM. Maternal fecal microbiota

transplantation in cesarean-born infants rapidly restores normal gut microbial development: a proof-of-concept study. Cell. 2020;183:324-334.e325.
2. Song Y, Cui Y, Wang Y, Yu J, Wang B, Wen Q, Zheng X. Donor selection for fecal bacterial transplantation and its combined effects with inulin on early growth and ileal development in chicks. J Appl Microbiol. 2023;134:lxad099.
3. Wang X, Wu X, Cong X, Ren J, Li J, Zhu J, Dai M, Hrabchenko N, Du Y, Qi J. The functional role of fecal microbiota transplantation on Salmonella Enteritidis infection in chicks. Vet Microbiol. 2022;269: 109449.
4. Sonnenburg JL, Bäckhed F. Diet-microbiota interactions as moderators of human metabolism. Nature. 2016;535(7610):56–64.
5. Zhong Y, Cao J, Ma Y, Zhang Y, Liu J, Wang H. Fecal microbiota transplantation donor and dietary fiber intervention collectively contribute to gut health in a mouse model. Front Immunol. 2022;13: 842669.
6. Zhou H, Yu B, Sun J, et al. Gut microbiota absence and transplantation affect diarrhea: an investigation in the germ-free piglet model. Anim Biotechnol. 2023;34(8):3971–7.
7. Zhong S, Zeng J, Deng Z, Jiang L, Zhang B, Yang K, Wang W, Zhang T. Fecal microbiota transplantation for refractory diarrhea in immunocompromised diseases: a pediatric case report. Ital J Pediatr. 2019;45:116.
8. Chen YR, Jing QL, Chen FL, Zheng H, Chen LD, Yang ZC. Desulfovibrio is not always associated with adverse health effects in the Guangdong Gut Microbiome Project. PeerJ. 2021;9:e12033.
9. Elokil AA, Chen W, Mahrose K, Elattrouny MM, Abouelezz KFM, Ahmad HI, Liu HZ, Elolimy AA, Mandouh MI, Abdelatty AM, Li S. Early life microbiota transplantation from highly feed-efficient broiler improved weight gain by reshaping the gut microbiota in laying chicken. Front Microbiol. 2022;13: 1022783.
10. Siegerstetter SC, Petri RM, Magowan E, et al. Fecal Microbiota Transplant from Highly Feed-Efficient Donors Shows Little Effect on Age-Related Changes in Feed-Efficiency-Associated Fecal Microbiota from Chickens. Appl Environ Microbiol. 2018;84(2):e02330–17.
11. Rychlik I. Composition and function of chicken gut microbiota. Animals (Basel). 2020;10:103.
12. Kubasova T, Kollarcikova M, Crhanova M, Karasova D, Cejkova D, Sebkova A, Matiasovicova J, Faldynova M, Pokorna A, Cizek A, Rychlik I. Contact with adult hen affects development of caecal microbiota in newly hatched chicks. PLoS One. 2019;14: e0212446.
13. Zhu L, Fu J, Xiao X, Wang F, Jin M, Fang W, Wang Y, Zong X. Faecal microbiota transplantation-mediated jejunal microbiota changes halt high-fat diet-induced obesity in mice via retarding intestinal fat absorption. Microb Biotechnol. 2022;15:337–52.
14. Zou M, Jie Z, Cui B, Wang H, Feng Q, Zou Y, Zhang X, Yang H, Wang J, Zhang F, Jia H. Fecal microbiota transplantation results in bacterial strain displacement in patients with inflammatory bowel diseases. FEBS Open Bio. 2020;10:41–55.
15. Lin H, Wang Q, Yuan M, Liu L, Chen Z, Zhao Y, Das R, Duan Y, Xu X, Xue Y, et al. The prolonged disruption of a single-course amoxicillin on mice gut microbiota and resistome, and recovery by inulin, Bifidobacterium longum and fecal microbiota transplantation. Environ Pollut. 2020;265: 114651.
16. Pang J, Beyi AF, Looft T, Zhang Q, Sahin O. Fecal microbiota transplantation reduces Campylobacter jejuni colonization in young broiler chickens challenged by oral gavage but not by seeder birds. Antibiotics (Basel). 2023;12:1503.
17. Brunse A, Martin L, Rasmussen TS, Christensen L, Skovsted Cilieborg M, Wiese M, Khakimov B, Pieper R, Nielsen DS, Sangild PT, Thymann T. Effect of fecal microbiota transplantation route of administration on gut colonization and host response in preterm pigs. ISME J. 2019;13:720–33.
18. Diao H, Yan HL, Xiao Y, Yu B, Zheng P, He J, Yu J, Mao XB, Chen DW. Modulation of intestine development by fecal microbiota transplantation in suckling pigs. RSC Adv. 2018;8:8709–20.
19. Wang XJ. Omics Mechanism of inulin regulation of glycolipid metabolism and its application in fattening pigs. Master, Shandong Agricultural University; CNKI. 2023.
20. Shoaib M, Shehzad A, Omar M, Rakha A, Raza H, Sharif HR, Shakeel A, Ansari A, Niazi S. Inulin: properties, health benefits and food applications. Carbohydr Polym. 2016;147:444–54.
21. Gamboa RG, Basurto RIO, Santoyo MC, Madrigal JB, Álvarez BER, Avila MG. In vitro evaluation of prebiotic activity, pathogen inhibition and enzymatic metabolism of intestinal bacteria in the presence of fructans extracted from agave: A comparison based on polymerization degree[J]. LWT, 2018, 92.
22. Wang W, Chen D, Yu B, Huang Z, Luo Y, Zheng P, Mao X, Yu J, Luo J, He J. Effect of dietary inulin supplementation on growth performance, carcass traits, and meat quality in growing-finishing pigs. Animals (Basel). 2019;9:840.
23. Ahmed W, Rashid S. Functional and therapeutic potential of inulin: a comprehensive review. Crit Rev Food Sci Nutr. 2019;59:1–13.
24. Shang HM, Zhou HZ, Yang JY, Li R, Song H, Wu HX. In vitro and in vivo antioxidant activities of inulin. PLoS One. 2018;13: e0192273.
25. Mazraeh R, Azizi-Soleiman F, Jazayeri S, Noori SMA. Effect of inulin-type fructans in patients undergoing cancer treatments: a systematic review. Pak J Med Sci. 2019;35:575–80.
26. Awad WA, Ghareeb K, Paßlack N, Zentek J. Dietary inulin alters the intestinal absorptive and barrier function of piglet intestine after weaning. Res Vet Sci. 2013;95:249–54.
27. Zou YF, Li CY, Fu YP, Feng X, Peng X, Feng B, Li LX, Jia RY, Huang C, Song X, et al. Restorative effects of inulin from Codonopsis pilosula on intestinal mucosal immunity, anti-inflammatory activity and gut microbiota of immunosuppressed mice. Front Pharmacol. 2022;13: 786141.
28. Zhang F, Zheng W, Guo R, Yao W. Effect of dietary copper level on the gut microbiota and its correlation with serum inflammatory cytokines in Sprague-Dawley rats. J Microbiol. 2017;55:694–702.
29. Zhang F, Zheng W, Xue Y, Yao W. Suhuai suckling piglet hindgut microbiome-metabolome responses to different dietary copper levels. Appl Microbiol Biotechnol. 2019;103:853–68.
30. Baran A, Kwiatkowska A, Potocki L. Antibiotics and bacterial resistance-a short story of an endless arms race. Int J Mol Sci. 2023;24:5777.
31. Wang JW, Kuo CH, Kuo FC, Wang YK, Hsu WH, Yu FJ, Hu HM, Hsu PI, Wang JY, Wu DC. Fecal microbiota transplantation: review and update. J Formos Med Assoc. 2019;118(Suppl 1):S23-s31.
32. Seekatz AM, Safdar N, Khanna S. The role of the gut microbiome in colonization resistance and recurrent Clostridioides difficile infection. Therap Adv Gastroenterol. 2022;15: 17562848221134396.
33. Li Y, Li X, Wu Y, Zhang W. Effects of fecal microbiota transplantation from yaks on weaning diarrhea, fecal microbiota composition, microbial network structure and functional pathways in Chinese Holstein calves. Front Microbiol. 2022;13: 898505.
34. Wang B, Zhou Y, Mao Y, Gong L, Li X, Xu S, Wang F, Guo Q, Zhang H, Li W. Dietary supplementation with lactobacillus plantarum ameliorates compromise of growth performance by modulating short-chain fatty acids and intestinal dysbiosis in broilers under Clostridium perfringens challenge. Front Nutr. 2021;8: 706148.
35. Tawfick MM, Xie H, Zhao C, Shao P, Farag MA. Inulin fructans in diet: role in gut homeostasis, immunity, health outcomes and potential therapeutics. Int J Biol Macromol. 2022;208:948–61.
36. Sun WL, Chen LH, Liu KY, Fu XY, Li GY. Effects of different dietary metabolisable energy levels on growth performance, slaughter performance and meat quality of male pheasants aged 20 to 22 weeks. Chinese Journal of Animal Science and Veterinary Medicine. 2023;50(10):3959–66.
37. McCormack UM, Curião T, Wilkinson T, Metzler-Zebeli BU, Reyer H, Ryan T, Calderon-Diaz JA, Crispie F, Cotter PD, Creevey CJ, et al. Fecal microbiota transplantation in gestating sows and neonatal offspring alters lifetime intestinal microbiota and growth in offspring. mSystems. 2018;3:10.
38. Deng K, Zhou YP, Ye G. Key technical issues and development trend of clinical application of fecal bacteria transplantation. Modern Practical Medicine. 2021;33(03):284–5+88.
39. Zhang Z, Jiang Q, Liu ZH, Qi RL. Advances in fecal bacteria transplantation and its application in pigs. Chinese Journal of Animal Nutrition. 2022;34(08):4793–801.
40. Qi R, Zhang Z, Wang J, Qiu X, Wang Q, Yang F, Huang J, Liu Z. Introduction of colonic and fecal microbiota from an adult pig differently affects the growth, gut health, intestinal microbiota and blood metabolome of newborn piglets. Front Microbiol. 2021;12: 623673.
41. Yuan L, Li X, Yin Q, Xu M, Han J, Zhang Y, Zhuang P. Research progress of Chinese medicine intervention in intestinal flora to improve intestinal mucosal barrier function. Chin Herb Med. 2018;49(08):1932–8.
42. Xu P, Liu P, Zhou C, Shi Y, Wu Q, Yang Y, Li G, Hu G, Guo X. A multi-omics study of chicken infected by nephropathogenic infectious bronchitis virus. Viruses. 2019;11:1070.

43. Yang YJ, Chen PC, Lai FP, Tsai PJ, Sheu BS. Probiotics-containing yogurt ingestion and H. pylori eradication can restore fecal Faecalibacterium prausnitzii dysbiosis in H. pylori-infected children. Biomedicines. 2020;8:146.
44. Zhang M, Zhou L, Wang Y, Dorfman RG, Tang D, Xu L, Pan Y, Zhou Q, Li Y, Yin Y, et al. Faecalibacterium prausnitzii produces butyrate to decrease c-Myc-related metabolism and Th17 differentiation by inhibiting histone deacetylase 3. Int Immunol. 2019;31:499–514.
45. Auger S, Kropp C, Borras-Nogues E, Chanput W, Andre-Leroux G, Gitton-Quent O, Benevides L, Breyner N, Azevedo V, Langella P, Chatel JM. Intraspecific diversity of microbial anti-inflammatory molecule (MAM) from Faecalibacterium prausnitzii. Int J Mol Sci. 2022;23:1705.
46. Zhang Y, Zhang J, Wu J, Zhu Q, Chen C, Li Y. Implications of gut microbiota dysbiosis and fecal metabolite changes in psychologically stressed mice. Front Microbiol. 2023;14: 1124454.
47. Zhu F, Ke Y, Luo Y, Wu J, Wu P, Ma F, Liu Y. Effects of different treatment of fecal microbiota transplantation techniques on treatment of ulcerative colitis in rats. Front Microbiol. 2021;12: 683234.
48. Zhang HY, Tian JX, Lian FM, Li M, Liu WK, Zhen Z, Liao JQ, Tong XL. Therapeutic mechanisms of traditional Chinese medicine to improve metabolic diseases via the gut microbiota. Biomed Pharmacother. 2021;133: 110857.
49. Kelly CJ, Zheng L, Campbell EL, Saeedi B, Scholz CC, Bayless AJ, Wilson KE, Glover LE, Kominsky DJ, Magnuson A, et al. Crosstalk between microbiota-derived short-chain fatty acids and intestinal epithelial HIF augments tissue barrier function. Cell Host Microbe. 2015;17:662–71.
50. Valles-Colomer M, Falony G, Darzi Y, Tigchelaar EF, Wang J, Tito RY, Schiweck C, Kurilshikov A, Joossens M, Wijmenga C, et al. The neuroactive potential of the human gut microbiota in quality of life and depression. Nat Microbiol. 2019;4:623–32.
51. Lu H, You Y, Zhou X, He Q, Wang M, Chen L, Zhou L, Sun X, Liu Y, Jiang P, et al. Citrus reticulatae pericarpium extract decreases the susceptibility to HFD-induced glycolipid metabolism disorder in mice exposed to azithromycin in early life. Front Immunol. 2021;12: 774433.
52. Wu F, Guo X, Zhang J, Zhang M, Ou Z, Peng Y. Phascolarctobacterium faecium abundant colonization in human gastrointestinal tract. Exp Ther Med. 2017;14:3122–6.
53. Zhang J, Xu W, Yang Y, Zhang L, Wang T. Leucine alters blood parameters and regulates hepatic protein synthesis via mammalian/mechanistic target of rapamycin activation in intrauterine growth-restricted piglets. J Anim Sci. 2022;100:skac109.
54. Zhao Y, Niu Y, He J, Gan Z, Ji S, Zhang L, Wang C, Wang T. Effects of dietary dihydroartemisinin supplementation on growth performance, hepatic inflammation, and lipid metabolism in weaned piglets with intrauterine growth retardation. Anim Sci J. 2020;91: e13363.

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