Microbiome

RESEARCH

Open Access

Early fecal microbiota transplantation continuously improves chicken growth performance by inhibiting age-related *Lactobacillus* decline in jejunum

Qiyao Liu[1†], Muhammad Akhtar[1†], Na Kong[1†], Rumeng Zhang[1], Yue Liang[1], Yaqian Gu[1], Danyi Yang[1], Abdallah A. Nafady[1], Deshi Shi[2], Abdur Rahman Ansari[3], El-Sayed M. Abdel-Kafy[4], Syed Umair-Ali-Shah Naqvi[5] and Huazhen Liu[1*]

Abstract

Background At an early age, chickens commonly exhibit a rise in the average daily gain, which declines as they age. Further studies indicated that the decrease in chicken growth performance at a later age is closely associated with an age-related decline in *Lactobacillus* abundance in the small intestines. Whether inhibiting the age-related decline in *Lactobacillus* in the small intestine by early fecal microbiota transplantation (FMT) could improve chicken growth performance is an interesting question.

Results 16S rRNA gene sequencing revealed a higher jejunal *Lactobacillus* abundance in high body weight chickens in both two different chicken breeds (yellow feather chickens, H vs L, 85.96% vs 55.58%; white feather chickens, H vs L, 76.21% vs 31.47%), which is significantly and positively associated with body and breast/leg muscle weights ($P < 0.05$). Moreover, the jejunal *Lactobacillus* abundance declined with age (30 days, 74.04%; 60 days, 50.80%; 120 days, 34.03%) and the average daily gain rose in early age and declined in later age (1 to 30 days, 5.78 g; 30 to 60 days, 9.86 g; 60 to 90 days, 7.70 g; 90 to 120 days, 3.20 g), indicating the age-related decline in jejunal *Lactobacillus* abundance is closely related to chicken growth performance. Transplanting fecal microbiota from healthy donor chickens with better growth performance and higher *Lactobacillus* abundance to 1-day-old chicks continuously improved chicken growth performance (Con vs FMT; 30 days, 288.45 g vs 314.15 g, $P < 0.05$; 60 days, 672.77 g vs 758.15 g, $P < 0.01$; 90 days, 1146.08 g vs 1404.43 g, $P < 0.0001$) even after stopping fecal microbiota transplantation at 4th week. Four-week FMT significantly inhibited age-related decline in jejunal *Lactobacillus* abundance (Con vs FMT, 30 days, 65.07% vs 85.68%, $P < 0.01$; 60 days, 38.87% vs 82.71%, $P < 0.0001$ and 90 days, 34.23% vs 60.86%, $P < 0.01$). Moreover, the numbers of goblet and Paneth cells were also found significantly higher in FMT groups at three time points ($P < 0.05$). Besides, FMT triggered GH/IGF-1 underlying signaling by significantly increasing the expressions of GH, GHR, and IGF-1 in the liver and IGF-1 and IGF-1R in muscles along age ($P < 0.05$).

[†]Qiyao Liu, Muhammad Akhtar and Na Kong contributed equally as the first co-authors.

*Correspondence:
Huazhen Liu
lhz219@mail.hzau.edu.cn
Full list of author information is available at the end of the article

Conclusion These findings revealed that age-related decline in jejunal *Lactobacillus* abundance compromised chicken growth performance, while early fecal microbiota transplantation continuously improved chicken growth performance by inhibiting age-related jejunal *Lactobacillus* decline, promoting the integrity of jejunal mucosal barrier and up-regulating the expression level of genes related to growth axis.

Keywords Chicken, Jejunum, Fecal microbiota transplantation, *Lactobacillus*, Growth performance

Introduction

The chicken growth rate is a critical determinant of economic benefits, and considerable attention has been paid to its optimization. The growth rate is regulated by multiple factors, including inheritance, nutrition, environment, and infectious diseases [1–3]. With the improvements in genetic and production management levels, the growth rate has been significantly accelerated. However, it is common that the average daily gain of healthy chickens increases at an earlier age but declines at a later age during the growth periods, especially in chickens with longer growth periods, which significantly reduces production efficiency [4–6]. When the genetic background is the same, maintaining intestinal health and nutrient digestion and absorption are very important for chicken growth [7, 8], which crucially depends on the balance between gut eubiosis and gut dysbiosis in the chicken gut [9]. Gut dysbiosis decreases the beneficial bacteria, particularly *Lactobacillus*, favoring the proliferation of pathogenic microbes, i.e., *Salmonella* and *Escherichia coli*, which become an extra burden on chickens, slowing meat production [10, 11]. As *Lactobacillus* regulates inflammatory response in the chicken gut, a decline in *Lactobacillus* abundance could cause low-grade chronic intestinal inflammation [12–14]. Chronic intestinal inflammation could lead to mucosal lining erosion and ulceration, compromised nutrient digestion and absorption, and dysregulation in barrier permeability and immune responses, resulting in a decrease in chicken growth performance [7, 8, 15, 16]. Thus, finding an instant strategy to improve animal growth performance by mitigating intestinal inflammation through balancing gut eubiosis and gut dysbiosis is an urgent need in the current decade.

Lactobacillus is a principal bacterium which is predominantly found in the gastrointestinal tracts of humans and animals [17]. *Lactobacillus* has been documented as a potential probiotic that can enhance chicken growth performance [15, 18]. For instance, a recent study reported that *Lactobacillus acidophilus* (*L. acidophilus*) promoted growth in *Clostridium perfringens*-exposed broilers [19]. Likewise, *L. rhamnosus* enhanced body weight gain in dual-purpose chickens [20], *L. johnsonii* BS15 increased chicken meat quality and growth [21], and *L. reuteri* promotes growth and helps conserve immune homeostasis in chickens [22]. *Lactobacillus* also limits pathogens colonization and promotes other gut commensals, i.e., *Bacillus subtilis* in the chicken gut [23, 24]. The above findings indicated that *Lactobacillus* could increase chicken growth. However, some scientists revealed that the abundance of *Lactobacillus* decreased with age in chickens, which might impair their body growth [25, 26]. For example, the relative proportion of *Lactobacillus* in the intestinal tract of 53-day-old chickens is lower than 27-day-old chickens [27], and as the birds got older, the relative abundance of *Lactobacillus* decreased throughout their growth cycle, which could influence chicken growth performance [28]. So, it is hypothesized that inhibiting the age-related decline in *Lactobacillus* in the small intestine would promote growth performance during the growth and development stage. Recently, the fecal microbiota transplantation (FMT) technique in reshaping chicken gut microbiota has become a useful tool and could be implemented to improve animal growth [15]. Other studies also reported that FMT could reshape early microbial colonization and boost growth performance in chickens and other animals, i.e., calves [29, 30]. The chicken jejunum has the utmost significance as it can provide enough surface area for proficient nutrient absorption, and if inflammation damages its epithelial structure, the chicken growth could be compromised [15, 31]. Therefore, whether *Lactobacillus* abundance in chicken jejunum could be altered by using fecal microbiota transplantation and maintained at a higher level with age are some critical questions, which are still under investigation.

To answer these questions, firstly, the chickens with significantly different growth performances from two different chicken breeds (yellow feather chickens and white feather chickens) were used to confirm whether jejunal *Lactobacillus* abundance is the key factor affecting chicken growth performance. Secondly, the differences in *Lactobacillus* abundance in jejunum at different time points were compared and the correlation with growth performance was analyzed to elucidate whether the age-related decrease in jejunal *Lactobacillus* could significantly affect chicken growth performance. Thirdly, to verify whether increasing jejunal *Lactobacillus* abundance and inhibiting

the age-related decrease of *Lactobacillus* could continuously improve chicken growth performance, transplanting fecal microbiota from healthy donor chickens with better growth performance and higher *Lactobacillus* abundance to 1-day-old chicks was accomplished. In addition, the interaction between fecal microbiota transplantation-mediated increased *Lactobacillus* and chicken growth performance was also investigated.

Results

The abundance of *Lactobacillus* in jejunum was positively related to chicken growth performance

In order to explore the correlation between the abundance of *Lactobacillus* in the jejunum and chicken growth performance, chickens from two different breeds (yellow feather chickens and white feather chickens) with significantly different growth performances were used in the present study. The volumes of breast and leg muscles (Fig. 1A) and the weights of body, breast, and leg muscles (Fig. 1B) were significantly higher in high body weight chickens (H) compared with low body weight chickens (L) of both breeds, indicating a significantly different growth performance. Further, 16S rRNA gene sequencing results indicated that *Lactobacillus* was the dominant genus of high and low-body-weight chickens. Interestingly, the relative abundance of *Lactobacillus* was much higher in high-body weight chickens in both breeds (yellow feather chickens, H vs L, 85.96% vs 55.58%; white feather chickens, H vs L, 76.21% vs 31.47%) (Fig. 1C). Furthermore, Spearman correlation analysis indicated that the relative abundance of jejunal *Lactobacillus* was significantly and positively correlated with the growth performance (Fig. 1D) ($P < 0.05$). The above results indicated that the abundance of *Lactobacillus* in the jejunum was closely related to chicken growth performance.

The chicken daily gain was significantly associated with age-related decline in *Lactobacillus* abundance in jejunum

To elucidate whether age-related decline in jejunal *Lactobacillus* abundance was related to chicken daily gain, the bacterial community composition in the jejunal contents of Turpan cockfighting × white Leghorn chickens at three different ages (30 days, 60 days, 120 days) and average daily gain were compared. The results showed that the *Lactobacillus* abundance in the jejunum decreased with age (30 days, 74.04%; 60 days, 50.80%; 120 days, 34.03%) (Fig. 2A). The average daily gain (ADG) of chickens accelerated initially until reaching 60 days, after which it gradually slowed down (1 to 30 days, 5.78 g; 30 to 60 days, 9.86 g; 60 to 90 days, 7.70 g; 90 to 120 days, 3.20 g) (Fig. 2B). Further, Spearman correlation analysis results indicated that *Lactobacillus* abundance was significantly and positively correlated with ADG (Fig. 2C) ($P < 0.05$). These results suggested that the declining abundance of *Lactobacillus* in the jejunum with age was closely associated with the chicken growth rate during the growth period.

Early fecal microbiota transplantation improved chicken growth performance by increasing jejunal *Lactobacillus* abundance

To investigate whether increased jejunal *Lactobacillus* abundance could improve chicken growth performance, transplanting fecal microbiota from healthy donor chickens with better growth performance and higher *Lactobacillus* abundance to 1-day-old chicks in two different chicken breeds (Turpan cockfighting × white Leghorn chickens and yellow feather chickens) was performed. The results showed that the body weight, breast muscle weight, and leg muscle weight were significantly higher in FMT groups (Fig. 3A) ($P < 0.05$). Further, hematoxylin and eosin (HE) staining results exhibited that the single breast ($P < 0.01$) and leg ($P < 0.0001$) muscle cell's cross-sectional area was significantly larger in FMT groups (Fig. 3B). Moreover, 16S rRNA gene sequencing results revealed that the abundance of *Lactobacillus* was significantly higher in the FMT groups (Turpan cockfighting × white Leghorn chickens, Con vs FMT, 72.52% vs 94.36%, Fig. 3C; and yellow feather chickens, Con vs FMT, 63.24% vs 88.70%, Fig. 3D). The above results indicated that early FMT significantly improves growth performance by increasing the abundance of *Lactobacillus* in the jejunum.

(See figure on next page.)

Fig. 1 Comparison of growth performance and jejunal microbiota between high and low body weight chickens in two different breeds (yellow feather chickens and white feather chickens). The comparison of **A** the volume of breast muscles and leg muscles, **B** body weight, breast muscle weight, and leg muscle weight, and **C** microbial communities in the jejunum in high vs low body weight chickens. **D** Heatmap of Spearman's correlations between differential jejunal microbiota and chicken growth performance, red color; positive correlation, blue color; negative correlation. Statistically significant differences between high vs low groups were determined using unpaired Student's *t* tests. Data are presented as mean± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$. H, high body weight group ($n = 15$); L, low body weight group ($n = 15$)

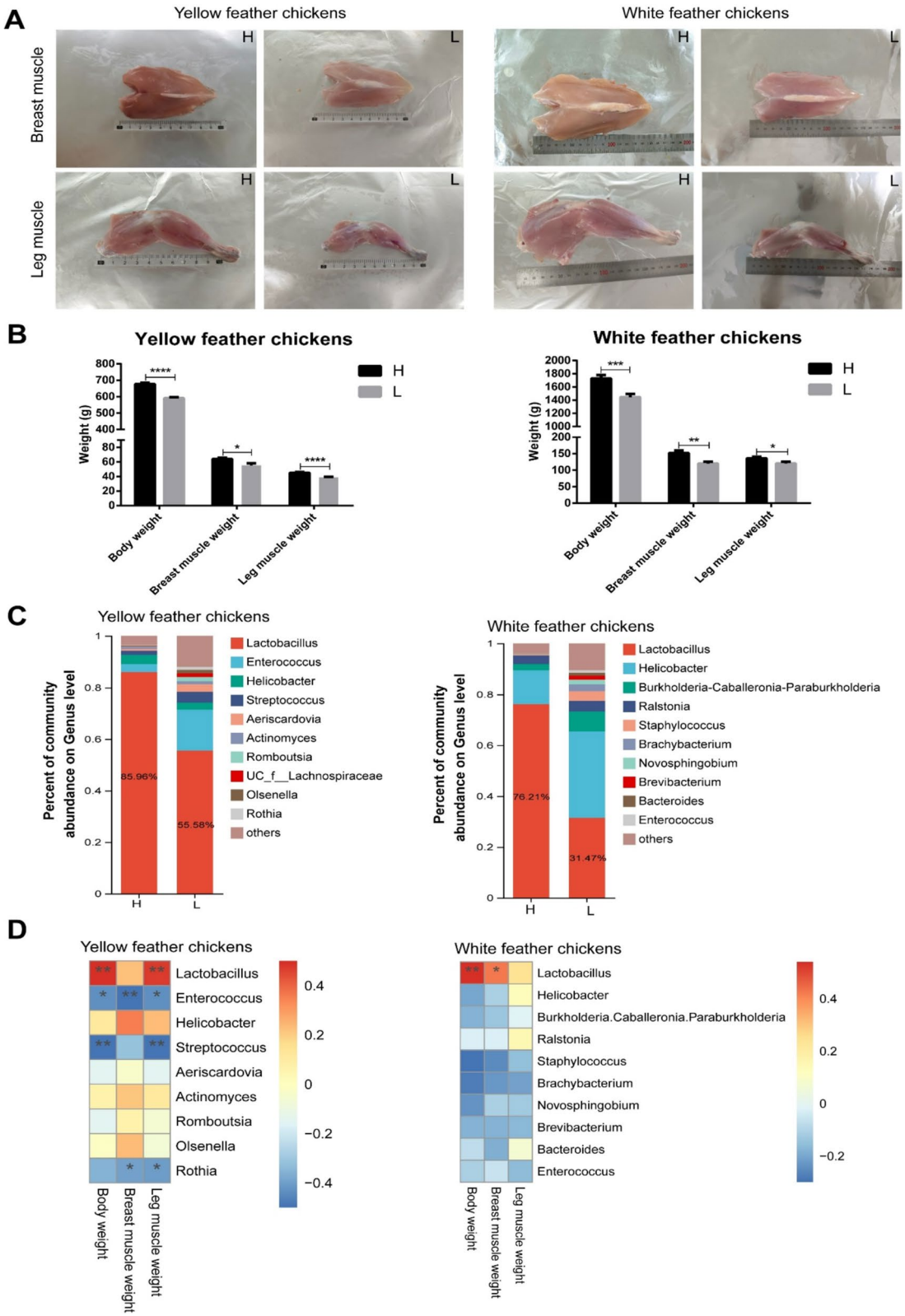

Fig. 1 (See legend on previous page.)

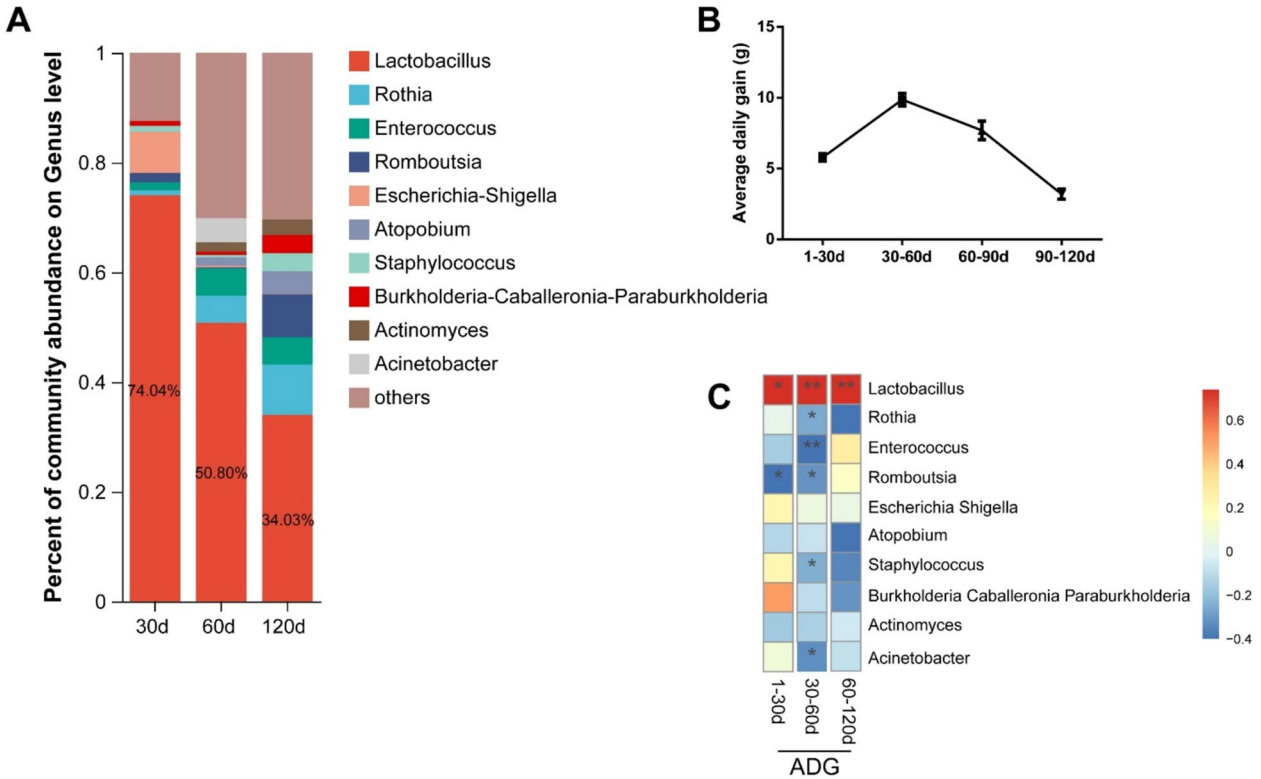

Fig. 2 The changes of jejunal *Lactobacillus* abundance and average daily gain of chickens with age. **A** Genus level community composition of jejunal contents of chickens at 30, 60, and 120 days of age. **B** Average daily gain (ADG) of chickens at different stages (1 to 30, 30 to 60, 60 to 90, and 90 to 120 days). **C** Heatmap of Spearman's correlations between ADG and jejunal microbiota on 30, 60, and 120 days, red color; positive correlation, blue color; negative correlation. Data are presented as mean ± SEM. **P* < 0.05, ***P* < 0.01. d, age in days (*n* = 20)

Early fecal microbiota transplantation continuously improved chicken growth performance by mitigating the age-related decrease of *Lactobacillus* abundance in jejunum

Whether FMT could continuously improve chicken growth performance even after stopping FMT is an interesting question. Therefore, Guishanhuang chickens with longer growth periods were selected for 4-week FMT and continued until 90 days. The results showed that the body size (Fig. 4A), body weight (Con vs FMT; 30 days, 288.45 g vs 314.15 g, $P<0.05$; 60 days, 672.77 g vs 758.15 g, $P<0.01$; 90 days, 1146.08 g vs 1404.43 g, $P<0.0001$, Fig. 4B), average daily gain (Fig. 4C), breast and leg muscle weight (Fig. 4D) and breast and leg muscle index (Fig. 4E) in FMT group at different time points were significantly higher compared with the control group ($P<0.05$). Further investigation indicated that the *Lactobacillus* abundances in the FMT group at three-time points were significantly higher (30 days, Con vs FMT, 65.07% vs 85.68%, $P<0.01$; 60 days, Con vs FMT, 38.87% vs 82.71%, $P<0.0001$ and 90 days, Con vs FMT, 34.23% vs 60.86%, $P<0.01$) (Fig. 4F). Spearman correlation analysis indicated that the relative abundance of *Lactobacillus* was significantly and positively correlated with body weight, breast muscle weight, leg muscle weight and breast muscle index, and leg muscle index at three time points (Fig. 4G) ($P<0.05$). These results revealed that an early FMT could continuously improve chicken growth performance by mitigating the age-related decrease of *Lactobacillus* abundance in the jejunum.

Early fecal microbiota transplantation improved jejunal development and health

Intestinal health is essential for chicken growth. To further understand whether FMT-mediated elevated jejunal *Lactobacillus* abundance improved jejunal development and health, the jejunal length, villus height, crypt depth, and numbers of goblet and Paneth cells were compared. The results showed that the jejunal length was significantly longer in the FMT group at all time points (Fig. 5A) ($P<0.05$). In addition, HE staining revealed that the jejunum villus length was significantly longer and the depth of the jejunal crypts was significantly higher in FMT group (Fig. 5B) ($P<0.05$). The Periodic Acid-Schiff (PAS) staining and Lendrum

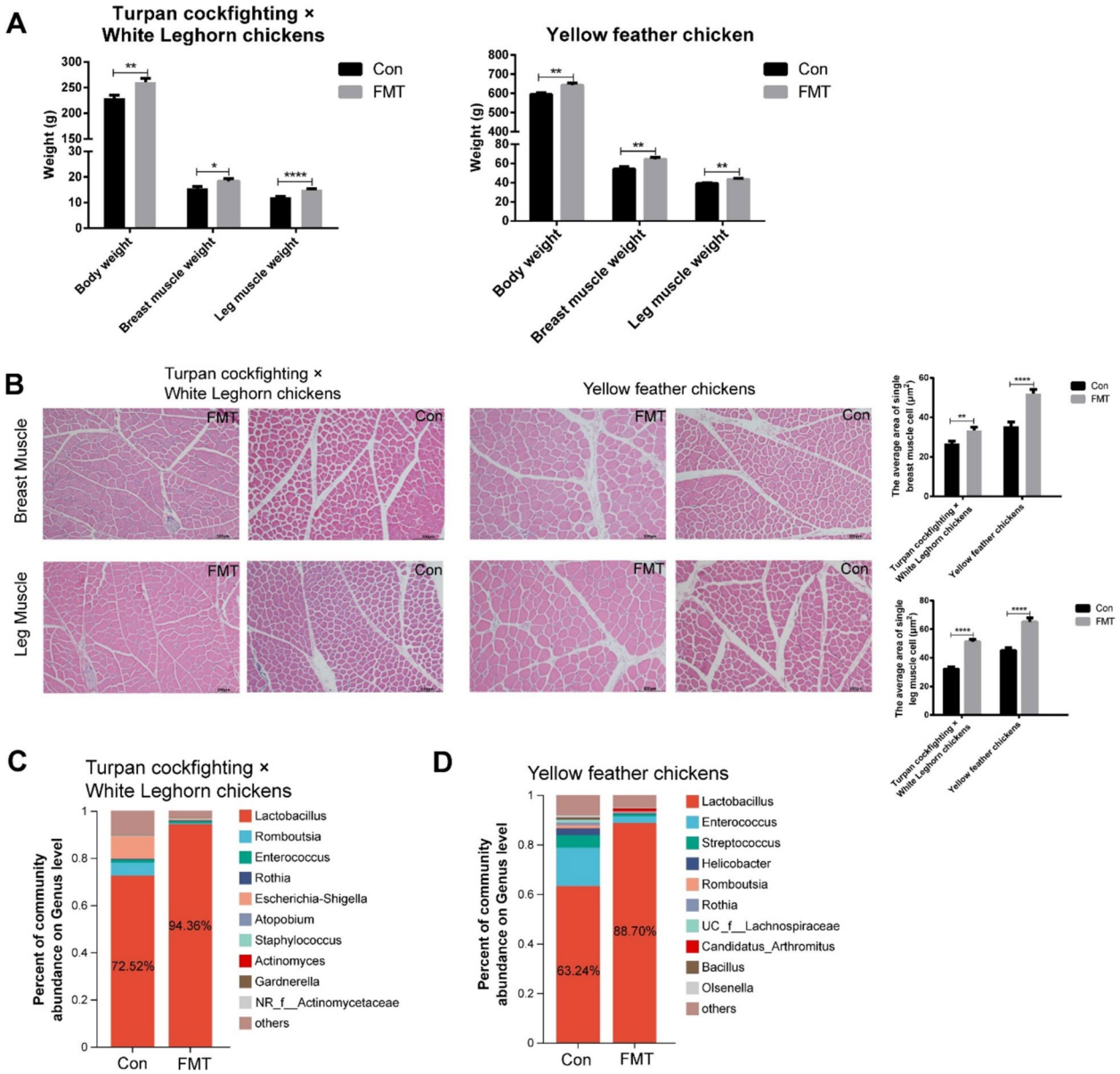

Fig. 3 The effects of early fecal microbiota transplantation (FMT) on chicken growth performance and jejunal microbiome in two different breeds (Turpan cockfighting × white Leghorn chickens and yellow feather chickens). **A** Comparison of body weight, breast muscle weight, and leg muscle weight in FMT vs control groups. **B** Comparison of the cross-sectional area of single muscle fibers of breast and leg muscles in FMT vs control groups, HE staining, magnification; 10 × 20, scale bar; 100 μm. **C** Genus level community composition of jejunal contents in Turpan cockfighting × white Leghorn chickensin FMT vs control groups. **D** Genus level community composition of jejunal contents in yellow feather chickens in FMT vs control groups. Statistically significant differences between FMT vs Con groups were determined using unpaired Student's *t* tests. Data are presented as mean± SEM. Con, control group ($n = 15$); FMT, fecal microbiota transplantation group ($n = 15$). $^{*}P < 0.05$, $^{**}P < 0.01$,$^{****}P < 0.0001$

staining results showed that the numbers of goblet cells (Fig. 5C) and Paneth cells (Fig. 5D) were significantly higher in the FMT groups at three time points ($P < 0.05$). These results indicated that early FMT promotes chicken growth performance by improving jejunal development and health.

Early fecal microbiota transplantation promotes growth and GH/IGF-1-associated gene expression

Growth hormone (GH) facilitates the secretion of insulin-like growth factor 1 (IGF-1) in the liver, which promotes the growth and development of the body through blood circulation to other organs, i.e., muscles. Whether

FMT could regulate the GH/IGF-1 pathway, the expressions of related genes were compared in the liver, blood, and muscles of Guishanhuang (GSH) chickens at different time points. The qPCR results indicated significantly higher mRNA expressions of GH, GHR, and IGF-1 at 60th day and GHR and IGF-1 at 90th day in the liver of FMT groups (Fig. 6A) ($P < 0.05$). The immunohistochemical (IHC) and enzyme-linked immunosorbent assay (ELISA) results showed that the liver IGF-1 protein expression (Fig. 6B) and the serum IGF-1 concentration (Fig. 6C) in FMT groups was significantly higher at 60th day and 90th day ($P < 0.05$). The qPCR results indicated significantly higher mRNA expressions of IGF-1 and IGF-1R in the breast (Fig. 6D) and leg (Fig. 6E) muscles of FMT groups at different time points ($P < 0.05$). These results suggested that early FMT promotes the expression of GH/IGF-1-related genes, which subsequently promotes the growth and development of chickens.

Discussion

Lactobacillus, as a commensal inhabitant both in human and animal small intestine, boosts intestinal health, strengthens intestinal barrier functions and intestinal ecosystem, regulates the immune system, facilitates the restoration of the normal microflora in the intestine, and promotes daily weight gain and chicken growth [18, 20, 32, 33]. In the present study, we found a decreasing trend of *Lactobacillus* abundance in the jejunal contents with increasing age (30 days, 74.04%; 60 days, 50.80%; 120 days, 34.03%), which is significantly associated with lower body weight and decreased breast and leg muscle weight in low body weight chickens. Likewise, the average daily gain (ADG) of chickens accelerated initially until reaching 60 days, after which it gradually slowed down. Consistent with our findings, recent studies also demonstrated that a decrease in *Lactobacillus* abundance caused a reduction in body weight gain and chicken growth because optimal levels of stable *Lactobacillus* are the indicator of balanced gut microbiome-mediated higher growth and vice versa [25, 34]. Other studies indicated that the *Lactobacillus* abundance in the jejunum also decreased with age [35, 36], which supported the point that age-related decline in *Lactobacillus* could cause gastrointestinal health and immunity issues and challenge chicken growth [37]. Some studies have shown that an increase in *Lactobacillus* abundance in the small intestine has a substantial contribution to chicken growth performance, while a decline in *Lactobacillus* abundance flourishes the harmful bacteria that affect chicken growth performance [18, 38]. In agreement with these reports, the current study also found a higher abundance of *Lactobacillus* in the FMT group compared with the control group, and *Corynebacterium* and *Streptococcus*, negatively associated with chicken growth performance, could be the potential pathogens, are found higher in the control group at 30 days, 60 days, and 90 days. These reports indicated that the *Lactobacillus* abundance in the small intestine is a key factor affecting chicken growth performance, and successfully maintaining a higher level of *Lactobacillus* abundance in the jejunum as the chicken ages could enhance their growth performance.

It has been established that fecal microbiota transplantation (FMT) is an emerging technique to substantially reshape the recipient gut microbiome, which facilitates *Lactobacillus* colonization in chickens [15, 29, 39]. Several studies demonstrated that FMT could manipulate the chicken gut microbiome by reconstituting their intestinal microecology and altering host phenotypic traits, i.e., body and carcass weight through modulation of nutrient metabolism, which increased feed intake and body weight, thus positively affecting chicken growth performance [40, 41]. Another study described that FMT is associated with the increased *Lactobacillus* abundance in broilers, which is also consistent with our findings [42]. In the present study, it is amazing that the growth performance and *Lactobacillus* abundance in the jejunum of the FMT group were still higher even after FMT cessation. We hypothesized that an increase in *Lactobacillus* could be inhibited through various mechanisms, such as competitive exclusion and pathogen antagonism [43]. The intestine of newly hatched chicks is virtually non-colonized and a variety of bacteria start colonizing later as the chicks grow [44]. Pathogenesis and colonization of the harmful bacteria initiate after binding to the host gut epithelium. However, due to competitive advantages over other bacteria, if *Lactobacilli* succeeds in colonizing the

(See figure on next page.)

Fig. 4 The effects of early 4-week fecal microbiota transplantation (FMT) on chicken growth performance and jejunal *Lactobacillus* abundance of Guishanhuang chickens throughout their whole growth period. Comparison of **A** chicken body size, **B** body weight, **C** average daily gain, **D** breast muscle weight and leg muscle weight, and **E** breast muscle index and leg muscle index in FMT vs control groups at 30th, 60th, and 90th days of age. **F** Comparison of genus level community composition and relative abundance of *Lactobacillus* in jejunal contents. **G**, Heatmaps of Spearman's correlation between jejunal microbiota and body weight, breast muscle weight, leg muscle weight, breast muscle index, and leg muscle index, red color; positive correlation, blue color; negative correlation. Statistically significant differences between FMT vs Con groups were determined using unpaired Student's *t*tests. Data are presented as mean ± SEM. d, age in days; Con, control group ($n = 15$); FMT, fecal microbiota transplantation group ($n = 15$).*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$

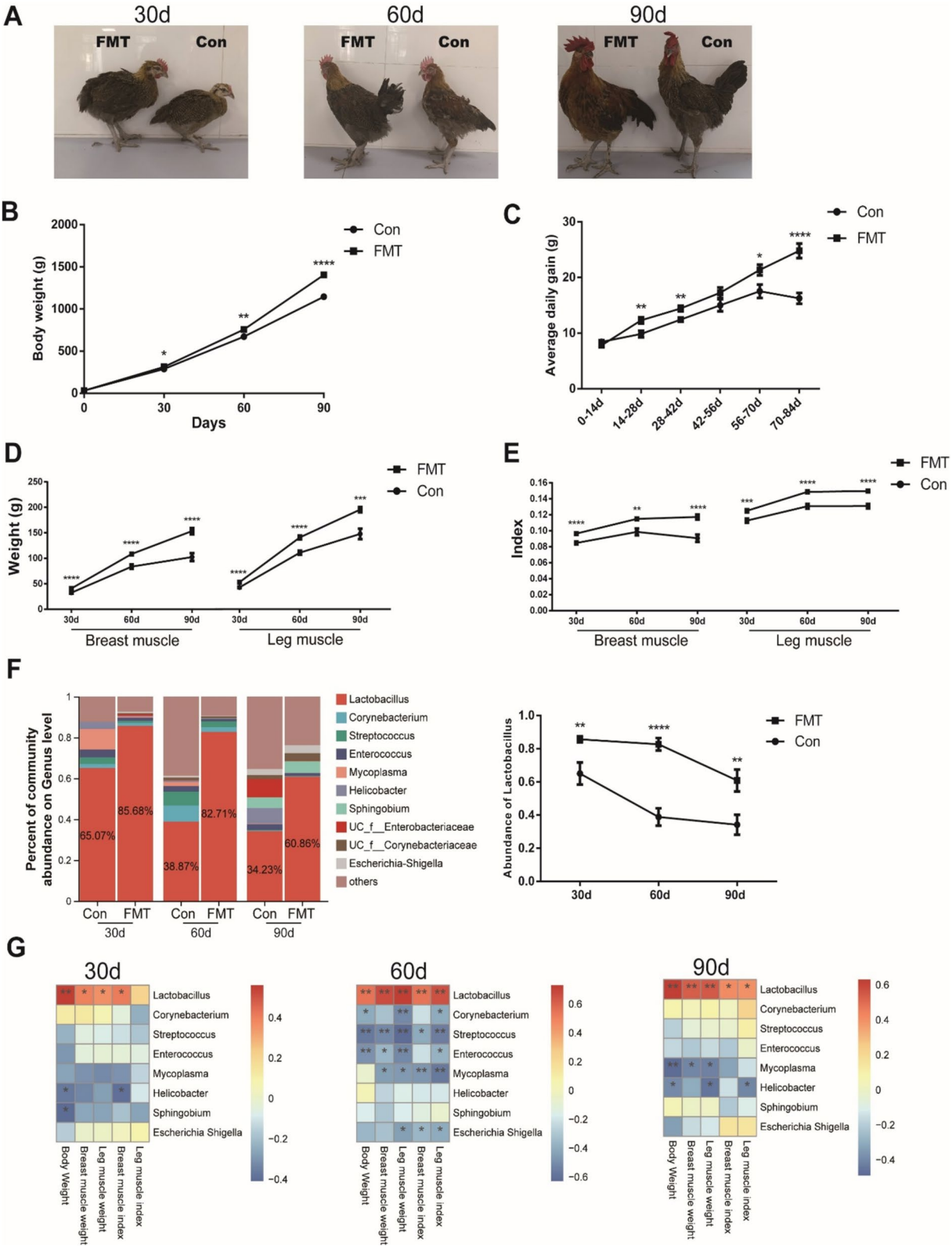

Fig. 4 (See legend on previous page.)

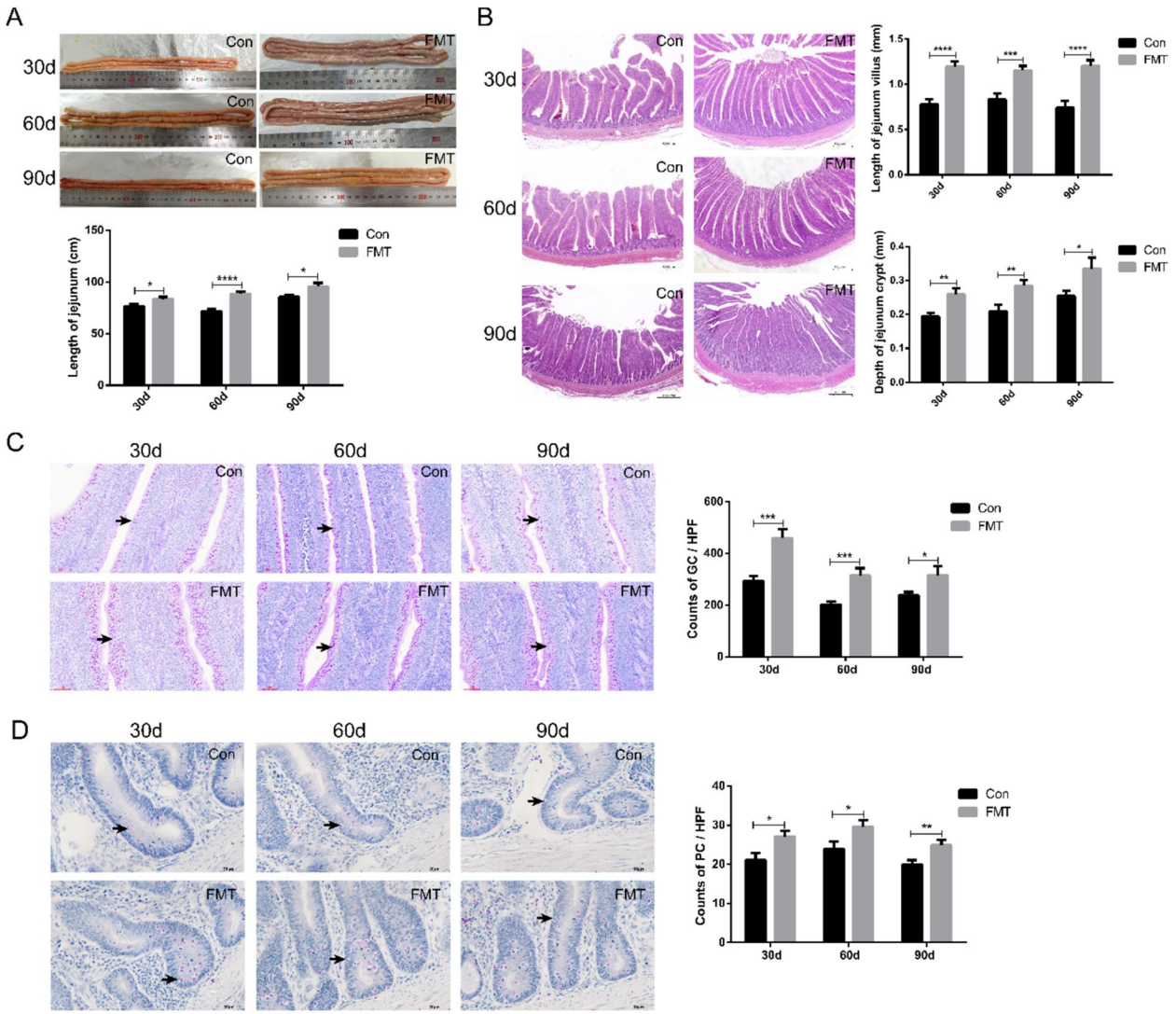

Fig. 5 The effects of early 4-week fecal microbiota transplantation (FMT) on jejunal health and development of Guishanhuang chickens throughout their whole growth period. FMT vs control groups at 30th, 60th, and 90th days of age. The comparison of **A** jejunal length, **B** the jejunum villus length and jejunal crypts, HE staining, magnification; 10 × 4, scale bar; 500 μm; **C,** goblet cell counts in the jejunum, PAS staining, magnification; 10 × 20, scale bar; 60 μm; and **D,** Paneth cells count in the jejunum, Lendrum staining, magnification; 10 × 40, scale bar; 50 μm. Statistically significant differences between FMT vs Con groups were determined using unpaired Student's *t* tests. Data are presented as mean ± SEM. d, age in days; Con, control group ($n = 15$); FMT, fecal microbiota transplantation group ($n = 15$), HPF, high power field, GC, goblet cells, PC, Paneth cells. *$P < 0.05$, **$P < 0.01$,***$P < 0.001$, ****$P < 0.0001$

chicken gut, they prevent the colonization of such pathogenic bacteria by avoiding their binding to the adhesion sites of the gut epithelium [43, 45]. Besides, the colonized *Lactobacilli* could also produce many molecules, including short-chain fatty acids (SCFAs), bacteriocins, nisin, soluble peptides, amino acids, and anti-bacterial compounds, i.e., H_2O_2, which inhibit other harmful bacteria and maintain a relative stable microbial population [43, 46]. *Lactobacillus*-released bactericidal molecules challenge pathogen growth by damaging their cellular integrity and inhibiting their cell division [47]. Therefore, early fecal microbiota transplantation is an effective way to keep higher *Lactobacillus* abundance in the jejunum.

How increased *Lactobacillus* abundance in the jejunum improves chicken growth performance is very interesting. Chicken growth performance is influenced by many factors including healthy intestine, hormone, and growth-related genes [48]. Paneth cells in the crypt's base secrete α-defensin, an anti-microbial peptide, which impedes microbial entry into the intestine lumen.

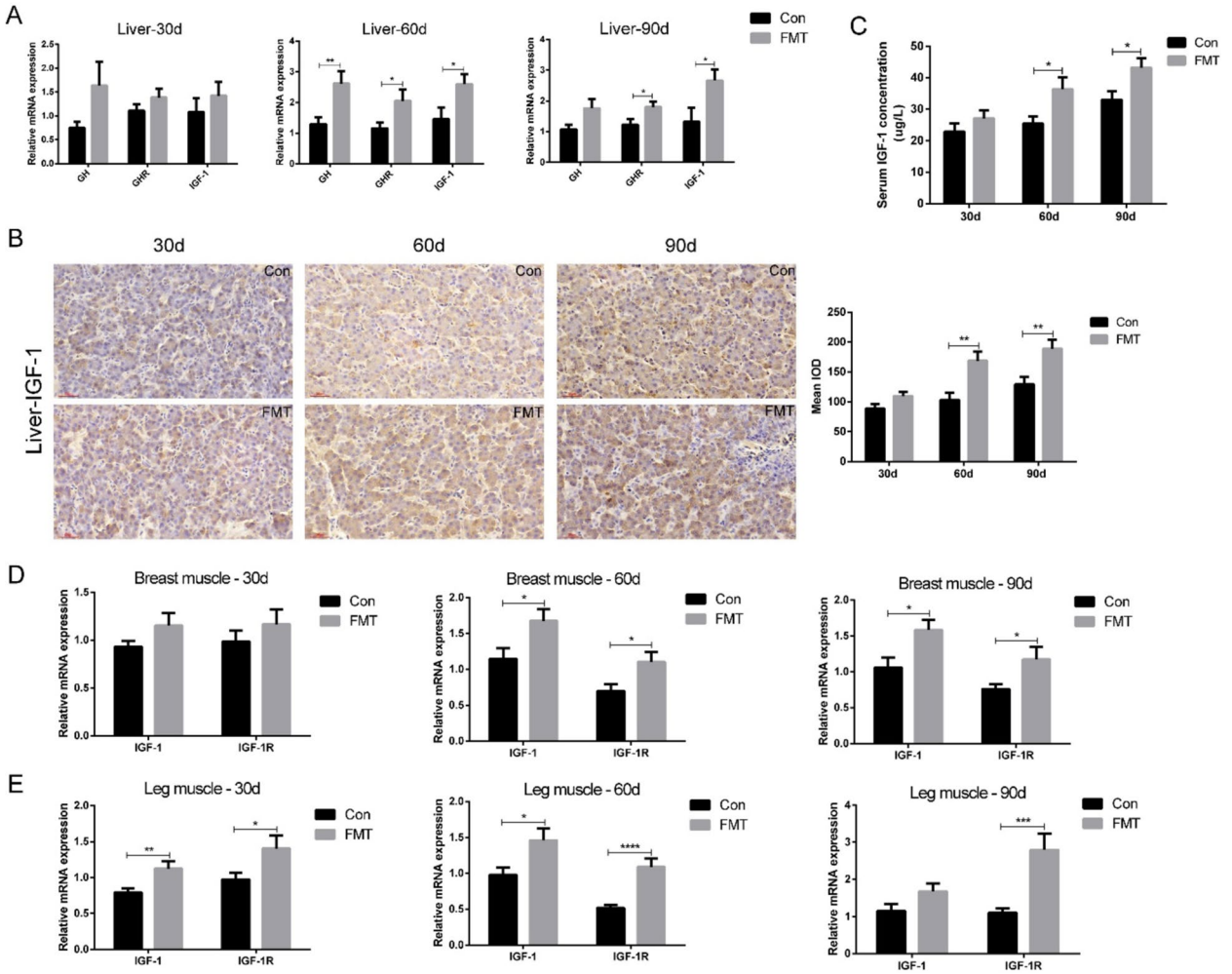

Fig. 6 The Effects of early 4-week fecal microbiota transplantation (FMT) on the expression of GH/IGF-1 related genes in the liver, blood, and muscles of Guishanhuang chickens throughout their whole growth period. FMT vs control groups at 30th, 60th, and 90th days of age. **A** The comparison of relative mRNA expression of GH/IGF-1 related genes in the liver, qPCR. **B** The comparison of protein expression of IGF-1 in the liver, immunohistochemistry staining, magnification; 10 × 40, scale bar; 30 μm. **C** The comparison of serum IGF-1 concentrations, ELISA.In **D** and **E**,relative mRNA expression levels of IGF-1 and IGF-1R in chicken breast muscles and leg muscles, qPCR. Statistically significant differences between FMT vs Con groups were determined using unpaired Student's *t* tests. Data are presented as mean ± SEM. d, age in days; Con, control group ($n = 15$); FMT, fecal microbiota transplantation group ($n = 15$). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$

Besides, goblet cells release the resistin-like molecule-β and trefoil peptide along with mucus, which promotes intestinal defense and epithelial layer repairing, maintaining epithelial homeostasis [49]. It has been reported that oral transplantation of *Lactobacillus,* typically *Lactobacillus* reuteri 22, *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Lactobacillus plantarum* regulate the differentiation of intestinal stem cells into goblet cells, which promotes intestinal mucosal immunity and barrier integrity in the broiler jejunum [18, 50–53]. Other studies revealed that *Lactobacillus agilis* and *Lactobacillus salivarius*-released lactic acid metabolite enhances the Paneth cell's proliferation [54]. These studies indicated that an increased *Lactobacillus* abundance in the jejunum could keep the gut healthy by promoting Paneth and goblet cells, which is consistent with our findings. Growth hormone (GH), together with insulin-like growth factor 1 (IGF-1), is very important for the growth of chicken skeletal muscles. A coordinated response to the growth signals is mediated through the GH receptor (GHR) and IGF-1 receptor (IGF-1R) on the target cells, which is called the GH/IGF-1 axis [55, 56]. A recent study revealed that higher serum levels of GH/IGF-1 are positively associated with chicken growth performance [57].

It is anticipated that *Lactobacillus*-boosted IGF-1/IGF-1R expression enhances anabolism in muscle tissues by increasing amino acids' uptake and their incorporation into proteins, cell reproduction, glucose uptake, and thymidine and uridine synthesis in chickens [58]. Particularly, *Lactobacillus plantarum*-produced SCFAs induce IGF-1 levels in circulation by directly or indirectly affecting liver tissues and promoting skeletal muscle development in broilers [59]. Consequently, other studies in pigs, mice, and Drosophila also revealed that *Lactobacillus* could positively influence GH function by enhancing IGF-1 levels and promoting host growth by interacting with the other distinct gut microbes for a longer period [60]. The present study also found an up-regulation of GH, GHR, and IGF-1 in the liver and IGF-1 and IGF-1R in the breast and leg muscles of FMT chickens, indicating that the binding of GH and IGF-1 to their relevant receptors stimulated the target cells and mediated their biological effects. These findings suggest that the GH/IGF-1 signaling pathway ensures a particular mechanism over chicken growth performance, influencing breast/leg muscle growth and development and overall chicken growth performance in a coordinated manner. However, which *Lactobacillus* strains are particularly involved in improving chicken growth performance still needs to be investigated. Additionally, although we observed that the GH/IGF-1 signaling pathway helps develop chicken breast/leg muscles, the exact mechanism is still warranted.

Conclusion

To sum up, age-related decline in *Lactobacillus* abundance is significantly associated with a decrease in chicken growth performance. An early fecal microbiota transplantation (FMT) could persistently improve chicken growth performance by maintaining *Lactobacillus* abundance at a higher level in the jejunum, which could keep the jejunum healthy and up-regulate the gene expression level of the GH/IGF-1 pathway. These results contribute to an understanding that inhibiting the age-related decline of *Lactobacillus* in the jejunum by early microbiota transplantation is an effective way to improve chicken growth performance.

Materials and methods

Animals

All relevant procedures associated with animal experiments were approved by the Institutional Animal Care and Use Committee, Huazhong Agricultural University No. HZAUCH-2018–008. All applied methods were completed following the Committee's regulations/guidelines.

To explore the correlation between jejunal *Lactobacillus* abundance and growth performance cocks with significantly different growth performance were selected from two different chicken breeds (4 weeks old yellow feather chickens (Lingnan yellow chickens) and (6 weeks old white feather chickens (Arbor Acres broiler chickens) ($n=15$). To investigate whether the abundance of *Lactobacillus* in the jejunum decreases with age, cocks with similar body weight were selected from Turpan cockfighting × white Leghorn chickens at three different ages (30 days, 60 days, and 120 days) ($n=20$). Further, to understand whether fecal microbiota transplantation (FMT) could maintain *Lactobacillus* abundance in the jejunum of recipient chickens and continuously affect their growth performance, 1-day-old male chicks from three different breeds of yellow feather chickens, Turpan cockfighting × white Leghorn chickens (hybrid chickens) and Guishanhuang chickens were selected as recipients. All recipient chicks from each chicken breed were randomly divided into FMT and control groups ($n=15$). All types of chickens were reared without medications and/or vaccinations at the chicken farm of Huazhong Agricultural University following the standard rearing conditions described in our previous study [15]. All management conditions for all experimental phases, including the testing environment, were the same, i.e., the chicken coop was ventilated, the ground remained dry and clean, and the rooms were equipped with air conditioning and ventilation equipment and temperature and humidity detectors. Each cage had access to clean drinking water systems and feed buckets. *Ad libitum* feed and water were provided. Daily feed intake was calculated by weighing the feed before feeding and the remaining feed at the end of the day. The commercial broiler feed (Supplementary Table S1) (Charoen Pokphand Group, Wuhan, Hubei, China) was used throughout the growth periods, and fresh feed was provided every day. Different feed formulations, i.e., (Broiler Chick Compound Feed 810, Broiler Medium Chicken Compound Feed 811, and Broiler Large Chicken Compound Feed 813) were selected according to the chicken's growth stages. Fast-growing chickens (yellow and white feathered breeds) received Broiler Chick Compound Feed until 21 days of age, Broiler Medium Chicken Compound Feed from 22 to 35 days, and Broiler Large Chicken Compound Feed thereafter. Slow-growing breeds (Turpan cockfighting × white Leghorn and Guishanhuang) were fed Broiler Chick Compound Feed until 45 days, Broiler Medium Chicken Compound Feed from 46 to 60 days, and Broiler Large Chicken Compound Feed after 61 days.

Fecal microbiota transplantation donor's selection

The donor chickens with better body weight and higher abundance of *Lactobacillus* in feces for fecal microbiota transplantation (FMT) experiment were obtained. The feces from chickens with higher body weight were collected, and screening of *Lactobacillus* in all donor

chickens was performed using 16S rRNA gene sequencing. According to the sequencing results, 3-month-old three donor chickens (two Turpan cockfighting × white Leghorn chickens and one white-feathered laying hen) with a higher abundance of *Lactobacillus* in feces (Donor1, 81.44%; Donor2, 91.75%; Donor3, 94.77%) and body weights (796.7 g, 802.4 g, and 678.5 g) were selected as the FMT donors (Supplementary Fig. S1).

Fecal suspension preparation

Fresh feces without white urate fraction were aseptically collected in a sterile sealed centrifuge tube (50 mL) every morning and transported to the laboratory for processing on ice. Ten grams of feces were diluted with 60 mL of 0.75% sterile normal saline and filtered with germ-free gauze to obtain fecal bacteria solution in accordance with our previous work [15].

Animal treatments

On a daily afternoon, 1 mL oral fecal suspension was given to each chick in the FMT group and 1 mL oral saline (0.75%) to each chick in the control group for 4 weeks. The chickens of yellow feather and Turpan cockfighting × white Leghorn breeds were killed on the 30th day of their age. To investigate whether an early FMT can persistently improve chicken growth performance, Guishanghuang chickens were killed at 30, 60, and 90 days of age.

Samples collection

Before sacrificing, the chickens were fasted for 12 h, and the body weights of all chickens were measured. The chickens ($n=15$) were killed through puncturing jugular veins, followed by sample collection for later analysis [39]. Some parts of the jejunum, breast muscles, leg muscles, and liver were harvested and fixed using 4% paraformaldehyde solution for morphological study. Whereas some parts of these tissues were harvested and frozen using liquid nitrogen immediately and stored in a − 80 °C refrigerator for molecular study. Jejunal content samples (1 to 1.5 g from each bird) were obtained, instantly frozen using liquid nitrogen, and stored in a − 80 °C refrigerator for microbiota analysis. About 3 mL blood sample from every chicken was collected and centrifuged at the speed of 3000 × *g* for 15 min at 4 °C to get the serum, which was stored in a − 80 °C refrigerator for IGF-1 concentration analysis.

Muscle-index calculation

A formula for calculating the index of breast and leg muscles is given below:

$$\text{Muscle index} = \text{muscle weight}\ (\text{g})/\text{body weight}\ (\text{g}).$$

16S ribosomal RNA and sequencing data analysis

For 16S ribosomal RNA analysis, total DNA from the bacterial genome was extracted with a Fast-DNA-SPIN extraction kit from MP Biomedicals, Santa Ana, CA, USA, following guidelines given in the company's instructional manual [39]. Both quality and quantity of obtained DNA were estimated using the spectrophotometer (NanoDrop ND-1000) from Thermo Fisher Scientific, Waltham, MA, USA, and evaluated its integrity using 0.8% (w/v) agarose gel. A (V3–V4) region of bacterial 16S ribosomal RNA gene was amplified with the PCR using 338F-(5′-ACTCCTACGGGAGGCAGCA-3′) and 806R-(5′-GGACTACHV GGGTWTCTAAT-3′) as the forward primer and the reverse primer, respectively. All other steps for 16S rRNA gene sequencing were performed as demonstrated in our previously published study [29].

For sequencing data analysis, de-multiplexing of raw FASTQ files of 16S ribosomal-RNA gene sequencing was performed with an in-house perl-script followed by the quality filtering with fastp version (0.19.6) and merging with FLASH version (1.2.7) (https://ccb.jhu.edu/software/FLASH/index.shtml) using the steps described in our previous study [15].

Hematoxylin and eosin, periodic acid-Schif (PAS) and Lendrum staining

In the hematoxylin and eosin (H&E) staining technique, the tissue samples from the jejunum, leg, and breast muscles were embedded in the paraffin and sliced into thin sections (3 μm) using the rotary slicer of LEICARM2245, Leica, Germany. Then, the H&E staining technique was applied to staining these slices following the steps, which have been reported by our earlier study [15]. Periodic acid-Schif (PAS) staining technique was used for the observation of goblet cells in the jejunum. Firstly, the routine deparaffinization of all sections was performed twice in the xylene, followed by its rehydration in the alcohol (graded series). 1% periodate acid was dropped onto the slides and allowed to stay on the slides for 10 min. The Schiff reagent was then added to slides, placed in the dark for 15 min, and rinsed using running water. Lastly, these slides were then counterstained with hematoxylin, followed by mounting these slides with the coverslip. Lendrum staining was used to observe jejunal Paneth cells. Firstly, the sections were routinely deparaffinized. Then, the staining of these slides was performed using hematoxylin for 4 min, followed by phloxine for 20 min, rinsed with running water, and the tartrazine stock solution was

separated until the cells were red, rinsed with running water for 3 min, washed with 2-ethoxyethanol, and then washed with methanol for 30 min. Finally, the slides were sealed with routine dehydration and transparency.

Immunohistochemical staining

The distribution and protein expression levels of insulin-like growth factor 1 (IGF-1) (1:200), (20,215–1-AP, Proteintech, China) in the liver were performed with immunohistochemical staining technique following steps in our previous study [15].

Enzyme-linked immunosorbent assay (ELISA)

To calculate the serum concentrations of IGF-1, the Chicken-IGF-1 ELISA-Kit (Jiyinmei, China) was used following an instruction manual of the manufacturer. We measured the absorbance at 450 nm and calculated an average absorbance value (A450) for every sample following standard curve.

Quantitative real-time polymerase chain reaction PCR (qPCR)

To compare differential expressions of all genes among all different groups, isolation of the total RNA was performed with Trizol reagent from Takara, Japan, following the given instructional manual of the manufacturer. After getting the DNA of the genome, reverse transcription of the isolated RNA (1 μg) to complementary DNA (cDNA) was performed with gDNA Eraser and PrimeScript™ RT kit from Takara, Japan. The qPCR was completed with a Bio-Rad CFX Connect real-time qPCR Detection system from Bio-Rad, Hercules, CA, USA, following the steps described in our previously published study [15]. To compare the levels of expression differences of all genes, a reference gene (β-actin) was used. The quantification of differential expressions of all genes was performed following the $2^{-\Delta\Delta CT}$ method. The relevant primer sequences of all described genes are presented in Table 1.

Statistical analysis

The experimental unit in this study was the individual chicken. All digital images used in this study were obtained with an optical microscope from BH-2; Olympus, Japan, through the digital camera from DP72; Olympus Mons. A total of 15 sections were carefully chosen from every experimental group, and five field images were randomly attained from every section of each type of tissue. To determine and calculate the immunohistochemical positive signals, jejunal villus length, crypt depth, goblet cell counts, Paneth cell counts, and an average cross-sectional area of individual leg or breast muscle cells in every section, an Image-Pro Plus (IPP) 6.0 software from Media Cybernetics, USA was applied. To analyze the data and to obtain data graphs, Prism software 8 (GraphPad Software, Inc., San Diego, USA) was applied. The data are shown here as "mean" ± "standard error of the mean" (SEM). The mean differences between the two groups were compared by using a Student's t-test following the $P < 0.05$ value as the statistically significant.

Table 1 The relevant primer sequences of all described genes (qPCR)

Gene	Primer sequences (5′ to 3′)	Accession no.
β-actin	F-GTGGATCAGCAAGCAGGAGT	NM_205518.1
	R-ATCCTGAGTCAAGCGCCAAA	
IGF-1	F-TACCTTGGCCTGTGTTTGCT	NM_001004384.3
	R-CCCTTGTGGTGTAAGCGTCT	
GH	F-ACATGGAGCTGCTTCGGTTT	NM_204359.2
	R-CAGCAGGCCGTAGTTCTTCA	
GHR	F-GAAGGCCGCATTTTGCAGTT	NM_001001293.2
	R-GAAGCATCATCCACCCCCTG	
IGF-1R	F-CTGTGTCCGACAAATGGGGA	NM_205032.3
	R-TGACGGTCAGTTTCGGGAAG	

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40168-024-02021-6.

Supplementary Material 1: Figure S1. The fecal microbial composition of three candidate donor chickens at the genus level.

Supplementary Material 2: Table S1. Commercial feed composition (Charoen Pokphand Group, Wuhan, Hubei, China)

Acknowledgements

Not applicable.

Authors' contributions

All authors, QYL, MA, NK, RMZ, YL, YQG, DYY, AAN, DSS, ARA, E-SMA-K, SU-A-SN and HZL contributed to the conceptualizations and experimentations of the manuscript. QYL, MA, NK, DSS and HZL: contributed to the design of the work, writing the original draft, sample collection and analysis, DSS, MA, RMZ, YL, YQG, DYY, AAN, ARA, E-SMA-K, SU-A-SN: animal handling, sample collection, analysis, and interpretation of data. DSS, MA, AAN, ARA, E-SMA-K, SU-A-SN: software, validation, analysis, and interpretation of data, reviewing and editing the manuscript. MA, QYL, NK, DSS and HZL: writing, reviewing and editing. HZL: supervision, funding acquisition, and project administration. All authors read and approved the final manuscript.

Funding

The present study was financially supported by the National Key Research and Development Program of China (2017YFE0113700) and the Supporting High Quality Development of Seed Industry Fund Project of Hubei Province (HBZY2023B007).

Data availability

The raw 16S rRNA gene sequencing data are available at the NCBI Sequence Read Archive (SRA), under BioProject PRJNA1114918.

Declarations

Ethics approval and consent to participate
All relevant procedures associated with animal experiments were approved by the Institutional Animal Care and Use Committee, Huazhong Agricultural University No. HZAUCH-2018-008. All applied methods were completed following the Committee's regulations/guidelines.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Key Laboratory of Agricultural Animal Genetics, Breeding and Reproduction, Ministry of Education, Huazhong Agricultural University, Wuhan 430070, People's Republic of China. [2]Department of Preventive Veterinary Medicine, College of Animal Science and Veterinary Medicine, Huazhong Agricultural University, Wuhan 430070, People's Republic of China. [3]Section of Anatomy and Histology, Department of Basic Sciences, College of Veterinary and Animal Sciences (CVAS) Jhang, University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan. [4]Animal Production Research Institute (APRI), Agricultural Research Center (ARC), Ministry of Agriculture, Giza, Egypt. [5]Department of Zoology, Govt. Islamia Graduate College, Civil Lines, Lahore 54000, Pakistan.

Received: 30 May 2024 Accepted: 19 December 2024
Published online: 10 February 2025

References

1. Podisi BK, Knott SA, Burt DW, Hocking PM. Comparative analysis of quantitative trait loci for body weight, growth rate and growth curve parameters from 3 to 72 weeks of age in female chickens of a broiler-layer cross. BMC Genet. 2013;14:22.
2. Yegani M, Korver DR. Factors affecting intestinal health in poultry. Poult Sci. 2008;87(10):2052–63.
3. Maulidi IS, Puspita UE, Mahardhika I, Daryono BS. The inheritance of phenotype character of feather color and growth of hybrid chicken (Gallus gallus gallus, Linnaeus 1758) derived from crossing of F1♀ Kamper and♂ Kambro. In: AIP Conf Proc., vol. 2260. Melville: AIP Publishing; 2020. p. 060010.
4. Korver DR. Review: current challenges in poultry nutrition, health, and welfare. Animal. 2023;17:100755.
5. Kamel E. Comparative study of growth and economic performance of Fayoumi, Rhode Island Red and their reciprocal crossbred chickens. Int J Curr Res. 2016;8(05):30613–9.
6. Mueller S, Taddei L, Albiker D, Kreuzer M, Siegrist M, Messikommer RE, et al. Growth, carcass, and meat quality of 2 dual-purpose chickens and a layer hybrid grown for 67 or 84 D compared with slow-growing broilers. J Appl Poult Res. 2020;29(1):185–96.
7. Pastorelli L, De Salvo C, Mercado J, Vecchi M, Pizarro T. Central role of the gut epithelial barrier in the pathogenesis of chronic intestinal inflammation: lessons learned from animal models and human genetics. Front Immunol. 2013;4:280.
8. Rubin DC, Shaker A, Levin MS. Chronic intestinal inflammation: inflammatory bowel disease and colitis-associated colon cancer. Front Immunol. 2012;3:107.
9. Ducatelle R, Goossens E, Eeckhaut V, Van Immerseel F. Poultry gut health and beyond. Anim Nutr. 2023;13:240–8.
10. Mon KK, Saelao P, Halstead MM, Chanthavixay G, Chang HC, Garas L, et al. Salmonella enterica serovars Enteritidis infection alters the indigenous microbiota diversity in young layer chicks. Front Vet Sci. 2015;2:61.
11. Ringseis R, Eder K. Heat stress in pigs and broilers: role of gut dysbiosis in the impairment of the gut-liver axis and restoration of these effects by probiotics, prebiotics and synbiotics. J Anim Sci Biotechnol. 2022;13(1):126.
12. Mukhopadhya I, Hansen R, El-Omar EM, Hold GL. IBD—what role do Proteobacteria play? Nat Rev Gastroenterol Hepatol. 2012;9(4):219–30.
13. Cardoso Dal Pont G, Farnell M, Farnell Y, Kogut MH. Dietary factors as triggers of low-grade chronic intestinal inflammation in poultry. Microorganisms. 2020;8(1):139.
14. Soares I, Belote BL, Santin E, Dal Pont GC, Kogut MH. Morphological assessment and biomarkers of low-grade, chronic intestinal inflammation in production animals. Animals. 2022;12:3036.
15. Ma Z, Akhtar M, Pan H, Liu Q, Chen Y, Zhou X, et al. Fecal microbiota transplantation improves chicken growth performance by balancing jejunal Th17/Treg cells. Microbiome. 2023;11(1):137.
16. Zou X, Ji J, Wang J, Qu H, Shu DM, Guo FY, et al. Dextran sulphate sodium (DSS) causes intestinal histopathology and inflammatory changes consistent with increased gut leakiness in chickens. Br Poult Sci. 2018;59(2):166–72.
17. Dewi G, Kollanoor JA. Lactobacillus in food animal production—a forerunner for clean label prospects in animal-derived products. Front Sustain Food Syst. 2022;6:831195.
18. Forte C, Manuali E, Abbate Y, Papa P, Vieceli L, Tentellini M, et al. Dietary Lactobacillus acidophilus positively influences growth performance, gut morphology, and gut microbiology in rurally reared chickens. Poult Sci. 2018;97(3):930–6.
19. Li Z, Wang W, Liu D, Guo Y. Effects of Lactobacillus acidophilus on the growth performance and intestinal health of broilers challenged with Clostridium perfringens. J Anim Sci Biotechnol. 2018;9(1):25.
20. Fesseha H, Demlie T, Mathewos M, Eshetu E. Effect of lactobacillus species probiotics on growth performance of dual-purpose chicken. Vet Med (Auckl). 2021;12:75–83.
21. Liu L, Ni X, Zeng D, Wang H, Jing B, Yin Z, et al. Effect of a dietary probiotic, Lactobacillus johnsonii BS15, on growth performance, quality traits, antioxidant ability, and nutritional and flavour substances of chicken meat. Anim Prod Sci. 2016;57:920–6.
22. Sureshkumar S, Jung SK, Kim D, Oh KB, Yang H, Lee HC, et al. Oral administration of Lactobacillus reuteri expressing a 3D8 single-chain variable fragment (scFv) enhances chicken growth and conserves immune homeostasis. 3 Biotech. 2019;9(7):282.
23. Al-Khalaifa H, Al-Nasser A, Al-Surayee T, Al-Kandari S, Al-Enzi N, Al-Sharrah T, et al. Effect of dietary probiotics and prebiotics on the performance of broiler chickens. Poult Sci. 2019;98(10):4465–79.
24. Abdelqader A, Abuajamieh M, Hayajneh F, Al-Fataftah AR. Probiotic bacteria maintain normal growth mechanisms of heat stressed broiler chickens. J Therm Biol. 2020;92:102654.
25. Xi Y, Shuling N, Kunyuan T, Qiuyang Z, Hewen D, ChenCheng G, et al. Characteristics of the intestinal flora of specific pathogen free chickens with age. Microb Pathog. 2019;132:325–34.
26. Wang Y, Xu L, Sun X, Wan X, Sun G, Jiang R, et al. Characteristics of the fecal microbiota of high- and low-yield hens and effects of fecal microbiota transplantation on egg production performance. Res Vet Sci. 2020;129:164–73.
27. He Y, Li J, Wang F, Na W, Tan Z. Dynamic changes in the gut microbiota and metabolites during the growth of Hainan Wenchang chickens. Animals. 2023;13:348.
28. Shang Y, Kumar S, Oakley B, Kim WK. Chicken gut microbiota: importance and detection technology. Front Vet Sci. 2018;5: 254.
29. Zhang X, Akhtar M, Chen Y, Ma Z, Liang Y, Shi D, et al. Chicken jejunal microbiota improves growth performance by mitigating intestinal inflammation. Microbiome. 2022;10(1):107.
30. Kim HS, Whon TW, Sung H, Jeong Y-S, Jung ES, Shin N-R, et al. Longitudinal evaluation of fecal microbiota transplantation for ameliorating calf diarrhea and improving growth performance. Nat Commun. 2021;12(1):161.
31. Kim JE, Tun HM, Bennett DC, Leung FC, Cheng KM. Microbial diversity and metabolic function in duodenum, jejunum and ileum of emu (Dromaius novaehollandiae). Sci Rep. 2023;13(1):4488.
32. Djukovic A, Garzón MJ, Canlet C, Cabral V, Lalaoui R, García-Garcerá M, et al. Lactobacillus supports Clostridiales to restrict gut colonization by multidrug-resistant Enterobacteriaceae. Nat Commun. 2022;13(1):5617.
33. Drissi F, Raoult D, Merhej V. Metabolic role of lactobacilli in weight modification in humans and animals. Microb Pathog. 2017;106:182–94.
34. Chen CY, Chen SW, Wang HT. Effect of supplementation of yeast with bacteriocin and Lactobacillus culture on growth performance, cecal fermentation, microbiota composition, and blood characteristics in broiler chickens. Asian-Australas J Anim Sci. 2017;30(2):211–20.

35. Gautam H, Ayalew LE, Shaik NA, Subhasinghe I, Popowich S, Chow-Lockerbie B, et al. Exploring the predictive power of jejunal microbiome composition in clinical and subclinical necrotic enteritis caused by Clostridium perfringens: insights from a broiler chicken model. J Transl Med. 2024;22(1):80.
36. Lan PTN, Sakamoto M, Benno Y. Effects of two probiotic Lactobacillus strains on jejunal and cecal microbiota of broiler chicken under acute heat stress condition as revealed by molecular analysis of 16S rRNA genes. Microbiol Immunol. 2004;48(12):917–29.
37. Nouri A. Age-dependent development trends (models) of intestinal significant microbiota species and Eimeria oocysts in coccidia-challenged broiler chickens as affected by dietary encapsulated organic acids and anticoccidial drugs. Avian Pathol. 2024;53(4):264–84. https://doi.org/10.1080/03079457.2024.2319284.
38. Shokryazdan P, Faseleh Jahromi M, Liang JB, Ramasamy K, Sieo CC, Ho YW. Effects of a Lactobacillus salivarius mixture on performance, intestinal health and serum lipids of broiler chickens. PLoS One. 2017;12(5):e0175959.
39. Chen Y, Akhtar M, Ma Z, Hu T, Liu Q, Pan H, et al. Chicken cecal microbiota reduces abdominal fat deposition by regulating fat metabolism. NPJ Biofilms Microbi. 2023;9(1):28.
40. Zhang M, Li D, Yang X, Wei F, Wen Q, Feng Y, et al. Integrated multi-omics reveals the roles of cecal microbiota and its derived bacterial consortium in promoting chicken growth. mSystems. 2023;8(6):e00844-00823.
41. Song J, Luo C, Liu Z, Liu J, Xie L, Zhang X, et al. Early fecal microbiota transplantation from high abdominal fat chickens affects recipient cecal microbiome and metabolism. Front Microbiol. 2023;14:1332230.
42. Gilroy R, Chaloner G, Wedley A, Lacharme-Lora L, Jopson S, Wigley P. *Campylobacter jejuni* transmission and colonisation in broiler chickens is inhibited by faecal microbiota transplantation. BioRxiv. 2018:476119. https://doi.org/10.1101/476119.
43. Huang R, Wu F, Zhou Q, Wei W, Yue J, Xiao B, et al. Lactobacillus and intestinal diseases: mechanisms of action and clinical applications. Microbiol Res. 2022;260:127019.
44. Varmuzova K, Kubasova T, Davidova-Gerzova L, Sisak F, Havlickova H, Sebkova A, et al. Composition of gut microbiota influences resistance of newly hatched chickens to Salmonella Enteritidis infection. Front Microbiol. 2016;7:957.
45. Zeise KD, Woods RJ, Huffnagle GB. Interplay between Candida albicans and lactic acid bacteria in the gastrointestinal tract: impact on colonization resistance, microbial carriage, opportunistic infection, and host immunity. Clin Microbiol Rev. 2021;34(4):e00323-e320.
46. Anjana, Tiwari SK. Bacteriocin-producing probiotic lactic acid bacteria in controlling dysbiosis of the gut microbiota. Front Cell Infect Microbiol. 2022;12:851140.
47. Lee MD, Pedroso AA, Maurer JJ. Bacterial composition of a competitive exclusion product and its correlation with product efficacy at reducing Salmonella in poultry. Front Physiol. 2022;13:1043383.
48. Ibitoye EB, Lokman IH, Hezmee MNM, Goh YM, Zuki ABZ, Jimoh AA, et al. Gut health and serum growth hormone levels of broiler chickens fed dietary chitin and chitosan from cricket and shrimp. Poult Sci. 2019;98(2):745–52.
49. Chelakkot C, Ghim J, Ryu SH. Mechanisms regulating intestinal barrier integrity and its pathological implications. Exp Mol Med. 2018;50(8):1–9.
50. Zhang H, Pertiwi H, Hou Y, Majdeddin M, Michiels J. Protective effects of Lactobacillus on heat stress-induced intestinal injury in finisher broilers by regulating gut microbiota and stimulating epithelial development. Sci Total Environ. 2024;918:170410.
51. Xie S, Zhao S, Jiang L, Lu L, Yang Q, Yu Q. Lactobacillus reuteri stimulates intestinal epithelial proliferation and induces differentiation into goblet cells in young chickens. J Agric Food Chem. 2019;67(49):13758–66.
52. Cui Y, Huang P, Duan H, Song S, Gan L, Liu Z, et al. Role of microencapsulated Lactobacillus plantarum in alleviating intestinal inflammatory damage through promoting epithelial proliferation and differentiation in layer chicks. Front Microbiol. 2023;14:1287899.
53. Deng Z, Han D, Wang Y, Wang Q, Yan X, Wang S, et al. *Lactobacillus casei* protects intestinal mucosa from damage in chicks caused by *Salmonella pullorum* via regulating immunity and the Wnt signaling pathway and maintaining the abundance of gut microbiota. Poult Sci. 2021;100(8):101283.
54. Hong Y, Zhou Z, Yu L, Jiang K, Xia J, Mi Y, et al. *Lactobacillus salivarius* and Lactobacillus agilis feeding regulates intestinal stem cells activity by modulating crypt niche in hens. Appl Microbiol Biotechnol. 2021;105(23):8823–35.
55. Jia J, Ahmed I, Liu L, Liu Y, Xu Z, Duan X, et al. Selection for growth rate and body size have altered the expression profiles of somatotropic axis genes in chickens. PLoS One. 2018;13(4):e0195378.
56. Hu B, Hu S, Yang M, Liao Z, Zhang D, Luo Q, et al. Growth hormone receptor gene is essential for chicken mitochondrial function in vivo and in vitro. Int J Mol Sci. 2019;20(7): 1608.
57. Ibrahim D, Al-Khalaifah HS, Abdelfattah-Hassan A, Eldoumani H, Khater SI, Arisha AH, et al. Promising role of growth hormone-boosting peptide in regulating the expression of muscle-specific genes and related MicroRNAs in broiler chickens. Animals. 2021;11:1906.
58. Saleh AA, Amber K, Mohammed AA. Dietary supplementation with avilamycin and *Lactobacillus acidophilus* effects growth performance and the expression of growth-related genes in broilers. Anim Prod Sci. 2020;60(14):1704–10.
59. Salehizadeh M, Modarressi MH, Mousavi SN, Ebrahimi MT. Effects of probiotic lactic acid bacteria on growth performance, carcass characteristics, hematological indices, humoral immunity, and IGF-I gene expression in broiler chicken. Trop Anim Health Prod. 2019;51(8):2279–86.
60. Jensen EA, Young JA, Jackson Z, Busken J, Kuhn J, Onusko M, et al. Excess growth hormone alters the male mouse gut microbiome in an age-dependent manner. Endocrinology. 2022;163(7):bqac074.

Publisher's Note