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Effects of *Lactobacillus plantarum* on Chicks' Intestinal and Immune Indexes

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ABSTRACT

Background: In the context of a complete prohibition on antibiotic utilization, This research sought to investigate the effect of *Lactobacillus plantarum* supplementation on slaughter performance, serum antioxidant capacity, intestinal morphology and structure, the expression of relevant genes and serum immune function in chickens.

Methods: A total of 120 healthy one-day-old chicks were randomly divided into two groups as control group and treatment group, each with 6 replicates of 10 chicks. The control group was maintained on a standard feeding regimen, whereas the experimental group was fed with the same feed but supplemented with *L. plantarum*. Samples were collected and tested after a feeding period of 30 days.

Result: The *L. plantarum* supplemented group demonstrated a notable elevation ($p<0.05$) in thigh muscle percentage, spleen index, as well as an enhanced thymus index when compared to the control group. Furthermore, intestinal villus height exhibited a marked increase ($p<0.05$). The levels of gene expression were substantially upregulated. particularly for *TLR-21*, *AVBD-2*, *AVBD-9*, *IFN-*α and *IL-10*. Conversely, the expression of *TNF-*α was markedly suppressed. The antioxidant capacity and level of immunoglobulin A (IgA) were markedly elevated ($p<0.05$). To summarize, the inclusion of *L. plantarum* in the diet notably improved chickens' slaughter outcomes, serum antioxidant potential and intestinal anatomical structure. These findings strongly support the potential use of *L. plantarum* as an antibiotic substitute in poultry production.

Key words: Chicks, Immune index, Intestine, *Lactobacillus plantarum.*

INTRODUCTION

The broiler chicken is a crucial source of animal protein for human consumption and the commercial poultry industry employs intensive indoor farming techniques extensively to increase production (Robins and Phillips, 2011). However, intensive feeding practices subject chickens to various environmental stresses, rendering them more vulnerable to common infectious and metabolic diseases, which can lead to death (Bellet and Rushton, 2019). Traditionally, poultry farms have employed antibiotics to promote chicken growth and prevent diseases; however, this practice has contributed to the emergence of antibiotic resistance. The development and spread of antibiotic resistance have posed a serious threat to animal and human health and aroused global concern (Aidara-Kane *et al.,* 2018). Consequently, many nations are actively engaged in efforts to restrict the use of antibiotics in animal feed. Antibiotics as growth promoters in feed have been completely banned in China, where the feed industry entered an antibiotics prohibition era in 2020 (Wang *et al.,* 2020). The excessive utilization of antibiotics can result in drug residues in meat and the development of antibiotic resistance. Therefore, the search for a safe, environmentally friendly, non-polluting feed additive is of utmost importance. *Lactobacillus plantarum*, a naturally occurring feed additive bacterium, offers significant advantages over antibiotics in terms of food safety and animal welfare. The discovery of new strains with better commercial applications can greatly benefit the local poultry industry, particularly farmers in China (Geeta *et al.,* 2021). *L. plantarum* supplementation has been documented to improve broiler intestinal health and growth performance by boosting immunity and promoting balanced intestinal microbiota (Chen *et al.,* 2023). The utilization of microbial feed has been reported to significantly improve broilers' weight gain, food intake and feed efficiency (Gao *et al.,* 2016). In addition, microbial feed additives can facilitate optimal growth in animals, maintain the intestinal microbiome's balance, improve feed conversion efficiency, mitigate animal morbidity rates and, overall, have emerged as a viable alternative to antibiotics (Abbad *et al.,* 2024). Lactic acid bacteria, one of the earliest discovered probiotics, has gained widespread popularity in the industry since its discovery and subsequent use as

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a feed additive in animal farming (Patterson and Burkholder, 2003). Lactic acid bacteria's probiotic properties have been extensively investigated in China since the 1980s. Among these bacteria, *L. plantarum* has garnered significant attention as a feed additive due to its capacity for vitamin synthesis, pH modulation, bacteriocin and analog release, micro-ecological flora balance adjustment, immune system support and promotion of nutrient digestion and absorption (Rolfe, 2000). Reportedly, dietary *lactobacillus* supplementation can significantly increase the ratio of jejunum villus height to crypt depth and improve the digestibility of ileal dry matter and protein. The findings of this study align with previous research, demonstrating that dietary lactobacillus supplementation can significantly increase jejunal villi height in broilers. This suggests that the inclusion of lactobacillus promotes jejunal villi growth in broilers, improving their digestive and nutrient-absorptive functions (Vimon *et al.,* 2020). Because of its good gastrointestinal environment tolerance, high adhesion, broad-spectrum bacteriostasis and other probiotic properties, *L. plantarum* can be applied to poultry breeding. A study conducted by Song *et al.* (2022) demonstrated that inclusion of *L. plantarum* in broiler diets significantly improves serum immune performance and, to a certain extent, improves antioxidant capacity (Song *et al.,* 2022). Probiotics affect poultry through various mechanisms, including their ability to withstand acidic environments and bile, as well as their capacity to adhere to the host's intestinal epithelial cells, where they exhibit antagonistic properties against pathogenic bacteria. The results of several studies affirm that using probiotics in poultry farming can significantly increase chickens' weight, optimize feed conversion and increase egg weight. Specifically, probiotic supplementation results in significant increases in body weight and feed utilization efficiency and decreases mortality (Mountzouris *et al.,* 2007). Therefore, the utilization of *L. plantarum* as a probiotic in animal feed holds significant implications. Sugandhi (2018) have demonstrated that probiotics can effectively increase the levels of natural antibodies in chickens' serum and gut against diverse foreign antigens. In addition, various studies have demonstrated that probiotics can elicit a favorable early immune response, facilitating local immune function. *L. plantarum* is a strain of gram-positive bacteria that can be anaerobic or facultative anaerobic and is primarily derived from plants. It exhibits notable resistance to high temperatures, acidity and bile salts. As one of the feed additives approved by the Ministry of Agriculture, it has garnered significant attention and wide-spread application in production. This study sought to investigate whether the inclusion of *L. plantarum* in daily feed improves slaughter performance, antioxidant capacity, intestinal morphology, gene expression levels and immune function in chickens.

MATERIALS AND METHODS

Animals and experimental design

The experimental procedures and animal-related protocols implemented in this investigation were authorized by the Institutional Animal Care and Use Committee at the College of Life Sciences, Leshan Normal University (778 Binhe Road, Leshan City, Sichuan Province, China), where all laboratory work was performed. A total of 120 one-day-old yellow-feathered broilers (purchased from Jiayyuan Breeder Farm, Leshan City, Sichuan Province, China) were randomly divided into control and experimental groups with 6 replicates of each group and 10 chickens in each replicate.

The comparison group (NC) was given the standard feed. The basal diet formulation (Table 1) adheres to the agricultural industry standard set by the People's Republic of China (NY/T3645-2020). In the experimental group (For short: *L. plantarum*), *L. plantarum* solution strains obtained from the laboratory and cultured for volume, (Table 2) was added to the same amount of basal diet given to the control group. During the feeding period, cage disinfection and routine immunization of the chicks were performed according to standard chicken farm management procedures. After ensuring that the treatment group consumed their daily allowance of *L. plantarum*, ample drinking water and feed were provided for ad libitum feeding. The chicks were maintained at a consistent temperature and humidity level (60%~70%) and their nutritional needs were met for 30 days. The feeding cycle was 30 days and the subsequent result data analysis was carried out after slaughter.

Detection index and method

Slaughter performance and immune organ index

On the 30th day of the feeding experiment, 6 chickens from each replicate were selected for slaughter *via* bloodletting from the jugular vein. Blood samples were collected and the chest muscles, leg muscles, abdominal fat, spleen and the bursa of Fabricius and thymus were separated, weighed and recorded. The poultry slaughtering performance calculation method of the National Poultry Breeder Committee was adopted.

The formulas are:

$$\text{Full evisceration rate (\%)} = \frac{\text{Full evisceration weight}}{\text{Live body weight}} \times 100$$

$$\text{Leg muscle rate (\%)} = \frac{\text{Leg muscle weight}}{\text{Full evisceration weight}} \times 100$$

$$\text{Pectoral muscle rate (\%)} = \frac{\text{Pectoral muscle weight}}{\text{Full evisceration weight}} \times 100$$

$$\text{Abdominal fat rate (\%)} = \frac{\text{Abdominal fat weight}}{\text{Full evisceration weight}} \times 100$$

$$\text{Organ index} = \frac{\text{Organ weight (g)}}{\text{Live body weight (kg)}}$$

Premortem weight

Body weight after a 12-hour fasting period before slaughter.

Morphological structure and gene expression level

Intestinal segments including the duodenum, jejunum and ileum were carefully excised and subsequently preserved through immersion in 4% paraformaldehyde solution for histological processing. These tissue samples were then embedded in paraffin blocks for sectioning and microscopic analysis. Each intestinal segment was flash-frozen in an enzyme-free storage tube with liquid nitrogen and subsequently preserved at -80°C for total RNA extraction. Paraffin sections were prepared from each fixed intestinal segment, the height of the intestinal villi and crypt depth were measured under a microscope and the ratio of villi height to crypt depth was calculated. The duodenum, jejunum and ileum were subjected to total RNA extraction using TRIzol@Reagent (Ambion,15596018). The total RNA was then subjected to reverse transcription to generate cDNA. The mRNA expression levels of *TLR-21*, *AVBD-2*, *AVBD-9*, *TNF-α*, *IFN-α* and *IL-10* were quantified with real-time PCR. The reference gene was glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*). The primers were designed based on the conserved regions of chicken *TLR-21*, *AVBD-2*, *AVBD-9*, *TNF-α*, *IFN-α* and *IL-10* genes as documented in GenBank.

Serum antioxidant index

Six broilers were randomly selected from each group and blood samples were collected from their wing veins after weighing. The blood samples were incubated for 20 minutes and then centrifuged at 3,500 revolutions per minute for 15 minutes. The resulting supernatant was extracted and stored at-80°C. A catalase (CAT) detection kit, malondialdehyde (MDA) content detection kit, superoxide dismutase (SOD) activity detection kit and total antioxidant capacity (T-AOC) detection kit were used to analyze the samples according to the kit instructions. All kits were purchased from Sangon Biotech (Shanghai) Co., Ltd.

Serum immune index

Six chickens were randomly selected from each group and blood samples were collected from the wing veins after weighing. The blood samples were incubated for 20 minutes and then centrifuged at 3,500 revolutions per minute for 15 minutes. The resulting supernatant was collected and stored at 80°C. Detection kits for immunoglobulin G (IgG), immunoglobulin A (IgA) and immunoglobulin M (IgM) were purchased from Shaanxi Yuanjia Biotechnology Company and used to analyze the samples according to the kit instructions.

Statistical analysis

Statistical analyses were conducted using SPSS version 16.0, employing single-factor analysis of variance (ANOVA) to evaluate the experimental data. Quantitative results are expressed as mean values accompanied by their standard errors. The threshold for statistical significance was established at $p<0.05$, with more stringent significance denoted at $p<0.01$.

RESULTS AND DISCUSSION

Effects of *L. plantarum* on chickens slaughter performance and immune organ index

In terms of slaughter indicators, relative to the control group (Table 3), the percentage of leg muscle in the *L. plantarum* group was signally greater ($p<0.05$) and the percentages of full evisceration, half evisceration and chest muscle all increased, although they failed to attain the significance level ($p>0.05$). The spleen index and thymus index in the *L. plantarum* group exhibited significantly elevated levels ($p<0.05$) in comparison to the control group, as indicated by the immune organ index, whereas the bursa of Fabricius index, cecum development and small intestine length did not significantly increase ($p>0.05$).

Effects of *L. plantarum* on the morphology and structure of chickens' duodenum, ileum and jejunum

The crypt depth of the duodenum, as well as the ileum and jejunum, in the *L. plantarum* cohort (Table 4) did not vary notably from that observed in the control cohort ($p>0.05$). In comparison to the control group, the *L. plantarum* cohort exhibited a notably increased villus height in both the

Table 1: Basic diet composition and nutritional components.

Dietary raw material composition	(%)	Nutrient composition	(%)
Corn	63.85	Moisture	14
Refined soybean oil	1.8	Crude protein	20
Soybean meal	26.3	Ca	0.6-1.2
Corn gluten meal	4	Total phosphorus	0.5
Mineral meal	1ÿ25	NaCl	0.2-0.8
$CaHPO_4$	1.7	Crude fibre	6
1% premix	1	Crude ash	8
Preservatives	0.1	Methionine + Cystine	0.74
Total	100		

Note: Premix offers the following nutrient content per kilogram of diet: VA, 17500IU; VD3, 3850 IU; VE, 26 mg; VK_3, 7 mg; VB_1, 3.5 mg; VB_2, 10. 5 mg; VB_6, 7 mg; VB_{12}, 35 μg; Nicotinic acid: 70 mg; Pantothenic acid, 21 mg; Folic acid, 1.75 mg; Biotin, 175 μg. It also provides Cu, 6.4 mg; Fe, 80 mg; Zn, 80 mg; I, 0.56 mg; Mn, 96 mg; Se, 0.24 mg; Co, 0.32 mg; $NaHCO_3$, 1500 mg; Choline chloride, 500 mg; Salt, 3000 mg; Lysine, 1220 mg; Methionine, 850 mg; *Lactobacillus plantarum*, 1000 mg or 2000 mg.

duodenum and jejunum ($p<0.05$). The villus height of the ileum was notably higher than of the control cohort at a greater significance level ($p<0.01$). These findings demonstrate that *L. plantarum* increased the height of the intestinal villi of the duodenum, ileum and jejunum (Fig 1).

Effects of *L. plantarum* on expression levels of related genes in chickens' intestinal tissues

As Fig 2 shows, the expression levels of *TLR-21*, *AVBD-2*, *AVBD-9* and *IFN-α* within the duodenum, ileum and jejunum were significantly elevated in the *L. plantarum* group as opposed to the control group; the expression level of *IL-10* was significantly higher in both the duodenum and jejunum. The level of *IL-10* expression in the ileum, however, showed no significant change. The level of *TNF-α* expression did not show a significant increase and was suppressed in both the jejunum and ileum.

Table 2: Medium formulation.

Component	Amt
Yeast powder	0.5 g
Beef extract	0.5 g
Peptone	1.0 g
D-glucose	2.0 g
K_2HPO_4	0.2 g
CH_3COONa	0.5 g
Ammonium citrate	0.2 g
$MgSO_4$	0.02 g
$MnSO_4$	0.01 g
$CaCO_3$	0.5 g
Tween-80	0.1 mL
L-cysteine hydrochloride	0.05 g

Effects of *L. plantarum* on chickens' antioxidant capacity and serum immune indexes

The T-AOC, MDA, SOD, along with catalase (CAT) levels in the *L. plantarum* group (Table 5) were substantially greater than those in the control cohort ($p<0.05$). The findings suggest a notable difference between the *L. plantarum* group and the control group. The ability to resist oxidation in the *L. plantarum* cohort was substantially higher than of the control cohort. The degree of IgA in the *L. plantarum* cohort was also markedly greater than of the control cohort ($p<0.05$). However, the *L. plantarum* cohort did not exhibit a substantial growth in IgG and IgM levels compared to the control cohort.

The impact of *L. plantarum* supplementation on chickens' slaughter performance and immune organ index

As probiotic bacteria, *L. plantarum* has demonstrated its distinctive usefulness in chicken breeding. Including *L. plantarum* in chicken feed improved meat tenderness and significantly increased post-slaughter net meat yield compared to chickens fed unsupplemented basal diets. Compared with the control group, the slaughter performance of experimental group was improved by adding proper dosage of lactic acid bacteria. Furthermore, the experimental group exhibited a significant increase in weight gain compared to the control group. This finding

Table 3: Effects of *Lactobacillus plantarum* on slaughter performance and immune organ index of chickens.

Item	NC	*L. plantarum*	P-value
Full evisceration rate	38.17±1.13	38.26±1.19	0.896
Half bore rate	58.67±1.71	60.52±1.76	0.094
Pectoral muscle rate	71.73±6.16	76.13±5.18	0.21
Leg muscle rate	36.15±4.78	43.09±5.67	0.044
Spleen index	6.09±0.68	7.04±0.75	0.043
Thymus index	5.21±1.98	8.33±1.76	0.016
Bursae index of Fabricius	3.57±1.74	5.04±2.61	0.277
Cecal development	8.77±0.74	9.35±0.90	0.25
Small intestine length	90.83±5.61	91.50±4.38	0.823

Table 4: Effects of *Lactobacillus plantarum* supplementation on intestinal development of chicks.

Items	Segment	NC	*L. plantarum*	P-value
Villus height/μm	Duodenum	90.22±13.67	105.38±4.34	0.041
	Ileum	66.34±4.32	78.19±5.05	0.001
	Jejunum	63.82±7.56	85.94±5.53	0.01
Crypt depth/μm	Duodenum	15.80±2.26	18.12±2.42	0.117
	Ileum	17.20±2.55	18.54±3.50	0.465
	Jejunum	14.94±3.18	18.33±2.10	0.054
Villus height/crypt depth	Duodenum	5.90±1.71	5.90±0.86	0.993
	Ileum	3.94±0.74	4.32±0.72	0.394
	Jejunum	4.42±0.93	4.74±0.57	0.492

aligns with Xiao *et al.* (2024) findings. In addition, adding *L. plantarum* to feed can significantly improve chickens' immune organ index. The developmental status of a*via*n species can be evaluated *via* immune organ indices, such as the bursa of Fabricius, thymus, spleen and other relevant parameters. Bursae of Fabria is a unique central immune organ of birds, which has a strong ability of immune regulation (Wang *et al.,* 2023). The experimental data demonstrated that chickens that ingest *L. plantarum* long-term exhibited greater weight and improved immune organ development compared to control groups. Chen *et al.* (2020) have shown that lactic acid bacteria can improve thymus index and spleen index. Thus, the experiment proved that compared with the control group, dietary *L. plantarum* improved the slaughter index and immune organ index of the experimental group.

Table 5: Effects of *Lactobacillus plantarum* on antioxidant capacity and serum immune indexes of chickens.

Item	NC	*L. plantarum*	P-value
T-AOC	1.68±0.24	2.08±0.23	0.014
MDA	2.10±0.95	3.43±0.26	0.017
SOD	79.20±7.38	107.35±30.05	0.05
CAT	1.84±1.12	3.14±0.89	0.05
IgG	15.43±2.65	17.78±3.04	0.185
IgM	16.19±2.15	17.72±2.30	0.261
IgA	9.06±0.82	11.02±1.72	0.03

Effects of *L. plantarum* on the morphology and structure of chickens' duodenum, ileum and jejunum

The development of intestinal villi is closely related to animals' intestinal absorption capacity. Villi height directly determines the effective area of nutrient absorption. Taller villi result in more efficient nutrient absorption; meanwhile, crypt depth reflects the rate at which intestinal epithelial cells are renewed and greater depth may indicate a decreased rate of epithelial cell formation. The ratio of villus height to crypt depth is an important index to evaluate the digestive and absorption function of animals, directly reflecting their bodies' digestive and absorptive capacity (Munoz *et al.,* 2023). *L. plantarum* can improve chickens' health. Cui *et al.* (2024) demonstrated that the addition of an appropriate quantity of *L. plantarum* to chickens' feed significantly improved the morphological structure of their intestinal tracts. *L. plantarum* can promote villi growth and increase villi height in chickens' duodenum, ileum and jejunum, improving the nutrient absorption capacity of the intestine. In addition, *L. plantarum* can reduce the depth of intestinal crypts, promote the growth of intestinal epithelial cells and optimize intestinal structure (Vineetha *et al.,* 2012). This study's results affirmed that dietary *L. plantarum* has a favorable effect on the morphological and structural integrity of chickens' duodenum, ileum and jejunum and promotes optimal intestinal health.

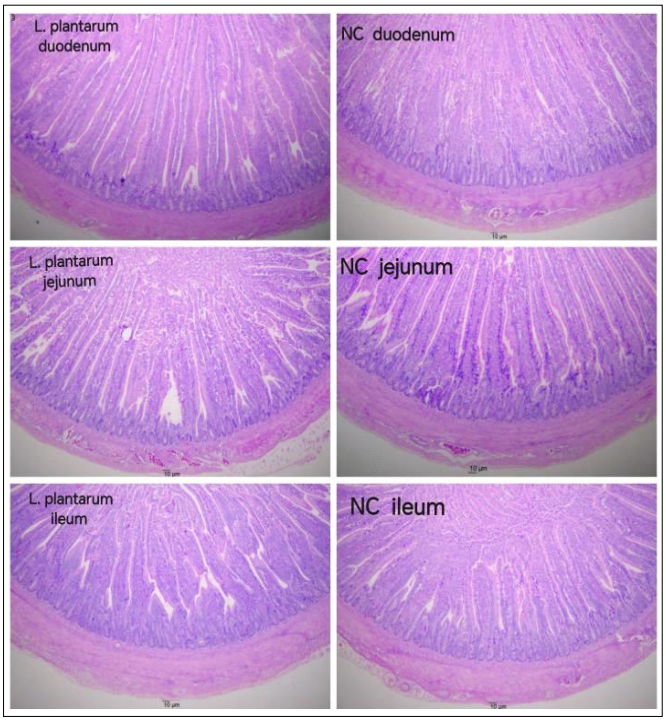


Fig 1: The effect of *Lactobacillus plantarum* on the morphology and structure of chickens' duodenum, ileum and jejunum.

Effects of feeding *L. plantarum* on chickens' serum immune indexes

Serum immune indicators encompass a set of crucial parameters employed to assess the body's immune status, comprising immunoglobulins, complements and interleukins. Immunoglobulin, a crucial component of the humoral immune system, plays an indispensable role in maintaining overall bodily health. Immunoglobulin A, specifically, plays a crucial role in local mucosal immunity and is indispensable for safeguarding the mucosa against pathogens. Immunoglobulin G constitutes the predominant fraction of immunoglobulins in the body, accounting for approximately 75% of the total. It can not only activate complement but also exhibits toxin-neutralizing properties, serving as a direct reflection of the body's immune status. Immunoglobulin M also includes the activation of complement that plays a crucial role in immune defense mechanisms (Dong *et al.,* 2024). The elevation of serum proinflammatory immune factor levels will increase the nutrient metabolic rate in livestock and poultry, increasing the protein degradation rate and decreasing protein synthesis. These changes are expected to reduce livestock's and poultry's production performance, potentially compromising farming efficiency overall (Klasing, 2007). The IgA level in *Lactobacillus plantarum* group was significantly higher than that in control group ($p<0.05$). In addition, the IgG and IgM levels in the *L. plantarum* group were higher than those of the control group, indicating that *L. plantarum* plays a crucial role in increasing serum immune indexes and promoting chickens' immune ability.

The impact of *L. plantarum* supplementation on chickens' serum antioxidant markers

Glutathione peroxidase, superoxide dismutase and catalase are indispensable as key enzymes in the body's antioxidant defense system. These enzymes effectively eliminate harmful reactive oxygen species, such as peroxides and hydrogen peroxide, safeguarding cell membranes against damage and ensuring their structural and functional integrity. Besides the role of maintaining a balanced redox metabolism in livestock and poultry, these enzymes are crucial for normal physiological metabolic activities. The serum malondialdehyde level can also serve as a crucial indicator of the extent of lipid peroxidation and cellular membrane impairment (Surai *et al.,* 2019). Tang *et al.* (2017) and Lin *et al.* (2020) found that *L. plantarum* carries genes encoding antioxidant enzymes, including key ones like glutathione peroxidase and catalase. *L. plantarum* can significantly upregulate the expression of these genes, exerting a positive effect on antioxidant function, efficiently scavenging free radicals and promoting overall body health. In this experiment, compared with the control group, the levels of T-AOC, MDA, SOD and CAT in *L. plantarum* group were significantly increased ($p<0.05$). The conclusion can be drawn, the antioxidant capacity of the *L. plantarum* group was significantly superior to that of the control group.

The effects of feeding *L. plantarum* on expression levels of intestinal tissue-related genes in chickens

As pattern-recognition receptors located on the surface of cells, toll-like receptors can recognize specific, relevant molecules under specific stimuli, trigger the body's immune response and act like bridges to communicate

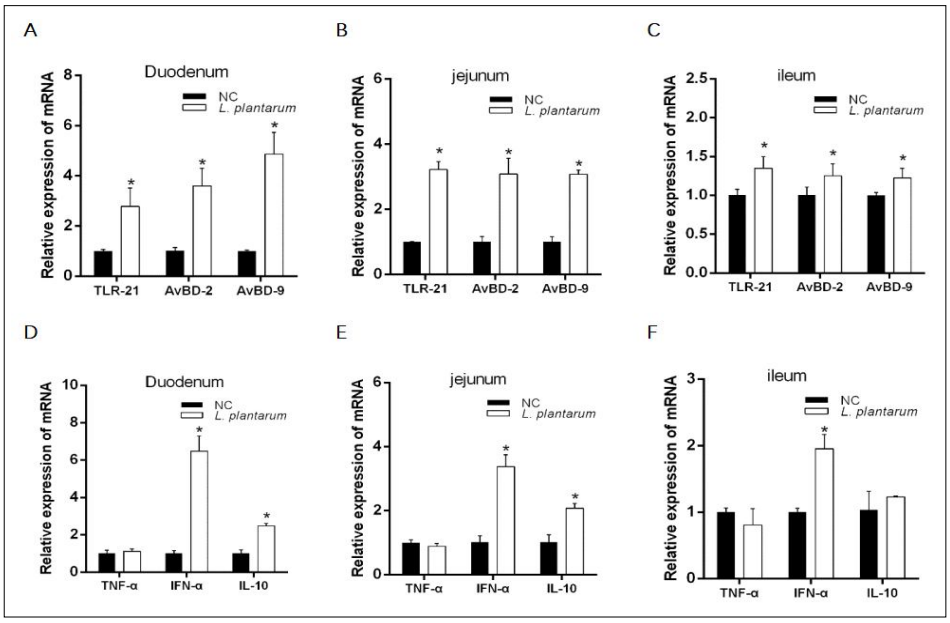


Fig 2: The effect of *L. plantarum* on the expression level of related genes in chickens' intestinal tissue.

and link innate and adaptive immunity and maintain the stability of the internal environment (Fitzgerald and Kagan, 2020). *TLR-21* is a member of the Toll-like receptor family that plays a crucial role in various disease processes. Antimicrobial peptides are a novel class of cationic active peptides that are widely distributed in various organisms and exhibit diverse antimicrobial activities and immunomodulatory functions, acting as crucial natural immune barriers for the host (Hancock *et al.,* 2016). Currently, the predominant antimicrobial peptide found in chickens' digestive tract tissues is known as chicken β-defensin. Researchers have identified 14 types of avian β-defensin from chicken tissues, from *AvBD1* to *AvBD14* (Cuperus *et al.,* 2013; Zhang and Sunkara, 2014). Studies have demonstrated that *TNF*-α can effectively stimulate antigen-presenting cells in living organisms, promoting the proliferation and activation of immune cells, it can also impair the function of effector T cells (Pandurangan *et al.,* 2022). Controlling the activity of *TFN*-α can mitigate inflammatory responses and tissue damage. IFN is divided into three types based on gene characteristics, receptor specificity and chromosomal localization. Of these, type I interferons encompass more than 20 subtypes, including *IFN*-α, *IFN*-α, *etc.* and their main biological functions are antiviral activity and tumor growth inhibition (Wang and Fish, 2019). Cytokines belonging to the *IL-10* family are essential for preserving tissue homeostasis amid infections and inflammatory conditions. They achieve this by modulating excessive inflammatory reactions, enhancing innate immune mechanisms and facilitating tissue regeneration processes, as demonstrated in recent studies (Li *et al.,* 2021). *IL*-10 is a multi-cell derived, multifunctional cytokine secreted by a variety of immune cells; It plays a vital role in inflammation, immunosuppression and anti-tumor processes. The production of pro-inflammatory cytokines can be inhibited by *IL*-10, attenuating the body's exaggerated immune response (Sabat *et al.,* 2010). The results of this experiment demonstrated that, compared to the control group, the *L. plantarum* group exhibited significant upregulation of *TLR-21*, *AVBD-2*, *AVBD-9* and *IFN*-α expression within the duodenum, ileum and jejunum. Additionally, a notable increase in the expression levels of *IL-10* within the duodenum and jejunum was observed. The expression level of *TNF*-α in jejunum and ileum was not significantly increased and was inhibited. The results affirmed that feeding *L. plantarum* could effectively increase chickens' immune response and ability to resist pathogen infection.

CONCLUSION

Compared to the control group, the *L. plantarum* group exhibited a significantly increased percentage of leg muscle, full evisceration rate, half evisceration rate (Half eviscopation rate refers to the percentage of half eviscopation weight to live weight and half eviscopation refers to the state after the body weight has removed the trachea, esophagus, crop, intestine, spleen, pancreas, gallbladder and reproductive organs) and pectoral muscle rate. In addition, the treatment group exhibited an increased immune organ index with a significantly increased spleen index and thymus index compared to the control group. These findings demonstrated that feeding *L. plantarum* can effectively improve chickens' slaughter performance and immune organ index.

L. plantarum can also improve intestinal villi growth, increase villus height, reduce crypt depth and promote epithelial cell proliferation, increasing chickens' capacity to absorb nutrients intestinally and promoting overall intestinal health.

The expression levels of *TLR-21*, *AVBD-2*, *AVBD-9* and *IFN*-α in chickens' duodenum, ileum and jejunum were significantly increased by feeding *L. plantarum*. Furthermore, the expression levels of *IL-10* in chickens' duodenum and jejunum were also significantly higher. This improved the chickens' immune response and strengthened their resistance to pathogen infection.

Compared with the control group, the *L. plantarum* group demonstrated a significantly greater T-AOC and MDA, SOD and CAT levels, indicating remarkable growth in their antioxidant capacity.

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Not applicable.

Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

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Data availability statement

Data is available upon request.

Conflict of interest

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