



Early dosing of *Bifidobacterium adolescentis* and *Lactobacillus acidophilus* and their effect on the immune response, biochemical parameters and oxidative indices of broiler.

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Abstract:

This experiment was conducted at the Poultry Field, Agricultural Research and Experiment Station, College of Agriculture, Al-Muthanna University, from November 17, 2024, to December 22, 2024. The aim was to study the effect of early dosing with the bacterial suspension *Lactobacillus acidophilus* and *Bifidobacterium adolescentis* on some productive traits of Ross 308 broiler. A total of 225 unsexed Ross 308 chicks, one day old, 40 gm were used. The chicks were randomly distributed into five treatments, with 45 chicks per treatment, (replicates for each). The treatments were as follows: **T1:** Negative control without any dosing. **T2:** Positive control: One-day-old chicks were dosed with saline at a dose of 0.25 ml/chick. **T3:** One-day-old chicks were dosed with *Lactobacillus acidophilus* at a dose of 0.25 ml/chick. **T4:** One-day-old chicks were dosed with *Bifidobacterium adolescentis* at a dose of 0.25 ml/chick. **T5:** One-day-old chicks were dosed with a mixture of *Lactobacillus acidophilus* and *Bifidobacterium adolescentis* at a dose of 0.25 ml/chick. The results of the experiment indicated a significant improvement ($P \leq 0.05$) in the immune response, represented by Delayed Type Hypersensitivity test (DTH), Enzyme Linked Immunosorbent Assay (ELISA), the relative weight and index of bursa gland, of the bird groups in favor of the T5 probiotic mixture treatment compared to all experimental treatments. A significant increase ($P \leq 0.05$) on some hematological parameters (albumin, globulin, and total protein), with a significant decrease ($P \leq 0.05$) on the concentrations of glucose, triglycerides, albumin, globulin, and total protein. In addition, a significant improvement ($P \leq 0.05$) on the concentrations of oxidation state indicators (malondialdehyde, glutathione, and catalase), in the T5 probiotic bacteria mixture treatment compared to the other experimental treatments.

Keywords: Early dosing, *Pifidobacterium adolescentis*, *Lactobacillus acidophilus*, immune response, biochemical parameters, oxidative indices, broiler.

Introduction:

Feeding newly hatched chicks probiotics is a modern and innovative method for enhancing digestive health and improving their production performance. This procedure involves introducing beneficial microorganisms directly into the chicks' digestive tract, helping to achieve the necessary microbial balance in the digestive tract from the first days of life. This method is effective in preventing microbial diseases such as *Salmonella* and reducing mortality rates among chicks (Al-Gharawi, 2012; Al Salman and Al-Gharawi, 2019).

Rantala and Nurmi (1973) tested the effect of dosing newly hatched chicks with 1 ml of the crop and intestinal contents of adult birds. The study showed that this procedure was highly effective in protecting chicks from infection with *Salmonella infantis*. Higgins *et al.* (2008) tested the effect of dosing chicks with 4×10^6 cells per ml of *Lactobacillus* carried in skimmed milk after exposure to *Salmonella typhimurium* colonies, resulted in a significant improvement in the weight gain of broiler chickens, reflecting the importance of using probiotics in promoting overall chick health.

A study conducted by Hashemzadeh (2010) indicated that feeding chicks with a probiotic containing *Lactobacillus acidophilus*, *L. bulgaricus*, *L. plantarum*, *L.*

rhamnosus, *Bifidobacterium bifidum*, *Enterococcus faecium*, *Streptococcus thermophilus*, *Aspergillus oryzae*, and *Candida pintolopesii* at a concentration of 10^6 cells per chick resulted in a reduction in the total number of aerobic bacteria in the cecum of broiler chicken, an improvement in final body weight and feed conversion efficiency was also observed, indicating the role of probiotics in enhancing the productive performance of chicks.

A study by Mansoub *et al.* (2011) confirmed that feeding chicks in the hatchery with a probiotic containing *Lactobacillus acidophilus*, *Bacillus subtilis*, and *Saccharomyces cerevisiae* at a concentration of 10^7 cells per chick increased the chicks' resistance to diseases such as omphalitis and yolk sac disease, considered very important because it strengthens the chicks' immune system from the first days of life and reduces the risk of diseases affecting newly hatched chicks.

Beneficial bacteria such as *Lactobacillus acidophilus* have played an important role in promoting overall health, specifically in improving digestive and heart health. A study by Anjum *et al.* (2014) focused on the effect of these bacteria on angina-related characteristics of cardiovascular disease. According to this study, *Lactobacillus acidophilus*

lowers levels of harmful LDL cholesterol in the blood, contributing to a reduced risk of heart disease.

In a study conducted by Exterkate and Veerkamp (1969), they focused on the biochemical changes that occur in *Bifidobacterium bifidum* var. *pennsylvanicus* when exposed to compounds that inhibit cell wall formation. The results showed that these bacteria are significantly affected by compounds such as penicillin, which leads to changes in the intracellular lipid composition. The percentage of modified lipids after treatment was measured using gas chromatography techniques, and the percentage of unsaturated lipids was approximately 70%, compared to 30% in normal conditions. This suggests that changes in the cell wall lead to significant modifications in the internal composition of the bacteria, which may affect their overall health.

Gorbach *et al.* (1967) examined the effect of diet and age on the intestinal bacterial population in humans. The results showed that people who ate a high-fiber diet had a higher percentage of *Bifidobacterium* than those who ate a high-fat diet. The proportion of *Bifidobacterium* in the stool was approximately 60% in those on a high-fiber diet, compared to about 20% in those on a low-fat diet. This suggests the importance of diet in promoting the growth of these bacteria and improving their health.

Abdullah (2017) indicated that *Bifidobacterium* bacteria have a significant effect on four types of pathogenic bacteria, namely *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, and *Klebsiella pneumoniae*, as it led to a decrease in their numbers in fermented milk by 92-98% at the end of the incubation period of seven days.

The study aims to demonstrate the effect of early feeding with different levels of the bacterial suspension *Bifidobacterium adolescentis* and *Lactobacillus acidophilus* on the immune response, biochemical parameters and oxidation indicators of broiler chickens.

Materials and Methods:

This experiment was conducted in the poultry farm of the Agricultural Research and Experiment Station, College of Agriculture, Al-Muthanna University, for a period of 35 days from November 17, 2024 to December 22, 2024. Two hundred twenty-five unsexed Ross 308 broiler chicks, one day old, 40 gm, were used. The chicks were reared in a hall measuring 40 ×10 m, in four-story batteries, each floor contained a cage measuring 1.5×1 m. The chicks were randomly distributed into five experimental treatments, with 45 chicks per treatment and three replicates per treatment (15 chicks per replicate). The experimental treatments were as follows:

T1: Negative control without any dosing.

T2: Positive control: One-day-old chicks were dosed with saline at a dose of 0.25 ml/chick.

T3: One-day-old chicks were dosed with *Lactobacillus acidophilus* at a dose of 0.25 ml/chick.

T4: One-day-old chicks were dosed with *Bifidobacterium adolescentis* at a dose of 0.25 ml/chick.

T5: One-day-old chicks were dosed with a mixture of *Lactobacillus acidophilus* and *Bifidobacterium adolescentis* at a dose of 0.25 ml/chick.

Bacterial Suspension Preparation

Preparation of Bacterial Suspension for *Lactobacillus Acidophilus*

Bacterial suspension for *Lactobacillus acidophilus* was prepared in the laboratories of the College of Science, University of Al-Qadisiyah, according to the method of Holt and Kreig (1986). Milk samples were collected, the milk samples were then diluted with normal saline, mixed with MRS Agar at 45-50°C, poured into plates and incubated at 37°C in a candle jar for 24-48 hours, then, 10 ml were taken and placed in MRS broth and incubated at 37°C, after 72 hours of incubation, a volume of MRS broth was taken and mixed with MRS agar, incubated anaerobically at 37°C, then, the samples were stored. Bacterial isolates are stored at refrigerator

temperature until use. Dilute 1 ml of the medium with 25 ml of distilled water to prepare for dosing.

Preparation of a *Bifidobacterium adolescentis* Bacterial Suspension

A *Bifidobacterium adolescentis* suspension was prepared in the laboratories of the College of Science, Al-Qadisiyah University. *Bifidobacterium adolescentis* was grown in MRS culture, with L-cysteine added as a reducing agent, then, it was diluted with distilled water at a ratio of 1 ml of the medium to 25 ml of distilled water to prepare it for dosing.

Results and Discussion

Immune Response

Table (1) shows the effect of early administration of the bacterial suspension *Pifidobacterium adolescentis* and *Lactobacillus acidophilus* on the immune response of broiler chickens. Significant differences ($P \leq 0.05$) were observed in all treatments when the bacterial suspension was administered early in the broiler chickens' immune response, compared to the negative control treatment (T1). T3 outperformed the negative and positive control treatments T1 and T2. T5 demonstrated a significant difference in the immune response of broiler chickens across all treatments, compared to T1, T2, and T3, respectively. Early dosing with both types of bacteria used in the

experiment significantly ($P \leq 0.05$) increased the immune response of broiler chickens, represented by T4

and T3, for all traits, compared to dosing in the negative and positive control treatments for broiler.

Table (1) Early dosing with different levels of *Pifidobacterium adolescentis* and *Lactobacillus acidophilus* on the immune response of broiler chickens (mean \pm standard error).

| Treatments | Relative Bursa weight | Bursa Index | DTH | Eliza |
|------------|-------------------------|-------------------------|-------------------------|--------------------------|
| T1 | 0.114 \pm 0.0039 e | 1.000 \pm 0.000 c | 0.403 \pm 0.005 e | 1878.27 \pm 9.88 e |
| T2 | 0.122 \pm 0.0005 d | 1.079 \pm 0.036 c | 0.429 \pm 0.003 d | 1962.15 \pm 17.86 d |
| T3 | 0.137 \pm 0.0006 c | 1.206 \pm 0.034 b | 0.471 \pm 0.007 c | 2208.31 \pm 24.84 c |
| T4 | 0.143 \pm 0.0010 b | 1.263 \pm 0.042 ab | 0.527 \pm 0.0005 b | 2491.90 \pm 15.02 b |
| T5 | 0.155 \pm 0.0040 a | 1.365 \pm 0.037 a | 0.554 \pm 0.003 a | 2706.19 \pm 34.49 a |
| Sig. | * | * | * | * |

The improvement in the immune response of broiler chickens is attributed to the fact that all probiotic treatments gave better immune performance compared to the control treatment. Probiotics play a major role in raising the immune response and thus increasing the effectiveness of the immune system by raising the level of antibodies directed against pathogens (whether against Newcastle disease, Gumboro disease, or other pathogenic viruses). This will double when using probiotics. In addition, probiotic bacteria will increase the phagocytic capacity of macrophages and other types of white blood cells that devour production, whether bacterial or viral pathogens, as well as the ability of these cells to attack cancer cells or cells infected with viruses (Al-Gharawi, 2012; Al-Gharawi and Ebade, 2020).

This result is consistent with Hai-feng *et al.* (2011), who demonstrated that probiotics are beneficial bacteria that enhance bird health by reducing the activity of pathogenic bacteria, as well as playing an important role in increasing vaccine efficacy against coccidiosis.

It is consistent with Clemente *et al.* (2015), who noted that antibodies produced were higher in treatments using probiotics compared to control treatments, as well as greater resistance of birds to *E. coli*. They indicated that the possibility of using probiotics early in the chicks' lives and continuing to use them through the drinking water will accelerate the development of their immune system, thus increasing their ability to resist bacterial diseases.

Champagne and Ross (2010) indicated that the metabolic process that occurs after introducing probiotics to the feed includes the production of organic substances such as lactic acid produced by *Lactobacillus* spp. and enzymes such as amylase. Proteases produced by *Bacillus subtilis* bacteria. These products play an important role in making the digestive tract environment unfriendly to pathogenic bacteria such as *E. coli* and *Salmonella*. This increases their resistance to disease, as well as increasing the efficiency of carbohydrate and protein digestion and absorption, and increasing the availability of nutrients by breaking down bonds through secreted digestive enzymes and converting them into simpler units.

Metchnikoff (2016) demonstrated that the use of probiotics in chicken feed resulted in a significant increase in the titer of antibodies directed against Newcastle disease compared to the control treatment. In addition, there was a significant decrease in mortality compared to the control treatment

due to the increased levels of immunoglobulins in the blood serum of birds treated with the probiotic, this has a positive impact on the overall health of the birds.

Biochemical parameters

Table (2) shows the effect of early dosing with the bacterial suspension *Pifidobacterium adolescentis* and *Lactobacillus acidophilus* on some biochemical parameters of broiler blood. Significant differences ($P \leq 0.05$) were found in all treatments with early dosing in some blood characteristics of broiler chickens.

The positive control treatment without dosing yielded the highest values in blood characteristics of broiler chickens and significantly differed in high-density lipoproteins, compared to T2, T3, T4, and T5, respectively. Significant superiority was observed for each of T2, T3, and T4, respectively, compared to T5, for the same characteristics.

Table (2) Early dosing with different levels of *Pifidobacterium adolescentis* and *Lactobacillus acidophilus* on some blood traits of broiler chickens (mean \pm standard error).

| Treatments | Cholesterol (mg/ 100 ml) | LDL (mg/ 100 ml) | HDL (mg/ 100 ml) | Triglycerides (mg/ 100 ml) | Glucose (mg/ 100 ml) |
|------------|--------------------------|------------------------|------------------------|----------------------------|------------------------|
| T1 | 304.88 \pm 0.12 a | 219.70 \pm 0.68 a | 28.71 \pm 0.48 c | 150.38 \pm 0.65 a | 200.99 \pm 0.75 a |
| T2 | 282.92 \pm 0.14 b | 217.69 \pm 0.27 b | 33.42 \pm 0.11 ab | 146.95 \pm 0.14 b | 196.28 \pm 0.35 b |
| T3 | 280.81 \pm 0.65 c | 215.68 \pm 0.43 c | 32.64 \pm 0.24 b | 145.23 \pm 0.27 c | 193.47 \pm 1.44 c |
| T4 | 278.44 \pm 0.28 d | 213.50 \pm 0.30 d | 33.11 \pm 0.13 ab | 143.41 \pm 0.27 d | 187.85 \pm 0.59 d |
| T5 | 275.26 \pm 0.20 | 211.22 \pm 0.53 | 33.60 \pm 0.05 | 141.18 \pm 0.72 | 183.03 \pm 0.21 |

| | | | | | |
|-------------|---|---|---|---|---|
| | e | e | a | e | e |
| Sig. | * | * | * | * | * |

The improvement in some of the blood traits of broiler chickens is attributed to the fact that all probiotic treatments yielded better performance in cholesterol, lipoprotein, high and low cholesterol, and both glyceride and glucose concentrations compared to the control treatment. Probiotics play a significant role in enhancing blood traits and, consequently, increasing the efficiency of the digestive system, which will be doubled when using probiotics. Furthermore, the probiotic bacteria will increase the effectiveness of blood traits, which positively impacts the productive characteristics of broiler chickens (Al-Gharawi, 2012).

This result is consistent with Hai-fengl *et al.* (2011), who demonstrated that probiotics are beneficial bacteria that enhance the characteristics of the blood by developing the digestive system, as well as playing an important role in increasing enzyme activity.

It also agrees with Clemente *et al.* (2015), who observed that the

concentrations of glycerides, proteins, and sugars were higher in treatments using probiotics compared to the control treatment. Champagne and Ross (2010) indicated that the metabolic process that occurs after introducing a probiotic into the feed or water, includes the production of organic substances such as lactic acid produced by *Lactobacillus* spp. and enzymes such as amylase and protease produced by *Bacillus subtilus*. These products play an important role in making the digestive tract environment unfriendly to pathogenic bacteria, which increases resistance to disease, as well as increasing the efficiency of carbohydrate and protein digestion and absorption, and increasing the availability of nutrients by unlinking the secreted digestive enzymes and converting them into simpler units. The use of a probiotic in dosing resulted in a significant increase in protein levels compared to the control treatment, which had a positive impact on the overall health of broiler chickens (Chelmonska *et al.*, 2006).

Table (3) Early dosing with different levels of *Pifidobacterium adolescentis* and *Lactobacillus acidophilus* on protein, albumin and globulin of blood broiler chickens (mean± standard error).

| Treatments | Protein (mg/ 100 ml) | Albumin (mg/ 100 ml) | Globulin (mg/ 100 ml) |
|------------|----------------------|----------------------|-----------------------|
| T1 | 3.71 ±0.02 e | 1.95 ±0.01 e | 1.76±0.011 e |

| | | | |
|-------------|-----------------|-----------------|-----------------|
| T2 | 4.13 ±0.02 d | 2.11±0.01 d | 2.01±0.014 d |
| T3 | 4.30 ±0.04 c | 2.22 ±0.02 c | 2.08±0.017 c |
| T4 | 4.53 ±0.03 b | 2.36±0.02 b | 2.17±0.011 b |
| T5 | 4.72 ±0.01 a | 2.49±0.01 a | 2.23±0.008 a |
| Sig. | * | * | * |

The improvement in albumin, globulin, and total protein concentrations in broiler chickens is attributed to the fact that supplementation with probiotics yielded better performance in albumin, globulin, and total protein concentrations compared to the control treatment. Probiotics play a significant role in enhancing the secretion of hormones and enzymes, thereby increasing the efficiency of the digestive system. In addition, the probiotic bacteria used in supplementation will increase their production efficiency. Protein also contributes to increased immune system formation, which positively impacts the productive characteristics of broiler chickens (Al-Gharawi, 2012).

This result is consistent with Takagi *et al.* (2005), who demonstrated that vitamins can stimulate cellular immunity. It also agrees with Cetin *et al.* (2010), who observed that probiotics stimulate cellular immunity by increasing immunoglobulins, which have the ability to bind to or degrade parasites and stimulate macrophages. Karim (2006) indicated that the total protein level in the blood serum of broiler chickens is directly proportional to the level of antibodies

and body weight, a direct reflection of changes in the metabolic rate in blood plasma. Blood proteins, especially albumin, transport carbohydrates, fatty acids, and some hormones in the metabolic process. Protein stimulates the liver to secrete the hormones that broilers need as an energy source, and this has a positive impact on the overall health of broiler chickens (Agarwal and Prabakaran, 2005).

Oxidation Indicators

Table (4) shows the effect of early dosing with the bacterial suspension *Pifidobacterium adolescentis* and *Lactobacillus acidophilus* on oxidation indicators in the blood serum of broiler chickens. Significant differences ($P \leq 0.05$) were observed across all treatments when the bacterial suspension was early supplemented with the oxidation indicators in the blood serum of broiler chickens. The negative control treatment recorded the highest oxidation indicator in malondialdehyde compared to glutathione and catalase. The table

also shows that T2, T3, and T4 showed a significant difference in the total malondialdehyde concentration of broiler chickens compared to T5. It was also noted that early dosing with the bacterial suspension *Lactobacillus acidophilus* and *Pifidobacterium adolescentis* as a mixture of both

types of bacteria used in the experiment significantly increased ($P \leq 0.05$) the oxidation indicators in the blood serum of broiler chickens represented by T5 compared to T4, T3, T2, T1 in the oxidation indicators of glutathione and catalase.

Table (4) Early dosing with different levels of *Pifidobacterium adolescentis* and *Lactobacillus acidophilus* on oxidation indicators of blood broiler chickens (mean \pm standard error).

| Treatments | Malondialdehyd (MDA) (mg/ 100 ml) | Glutathione (U/ L) | Catalase (IU) |
|------------|-----------------------------------|------------------------|------------------------|
| T1 | 4.59 \pm 0.021 a | 33.09 \pm 0.072 c | 47.59 \pm 0.099 d |
| T2 | 3.63 \pm 0.158 b | 35.62 \pm 0.282 b | 51.48 \pm 0.126 c |
| T3 | 3.79 \pm 0.097 b | 36.79 \pm 0.303 a | 52.69 \pm 0.333 b |
| T4 | 3.11 \pm 0.080 c | 37.10 \pm 0.360 a | 54.24 \pm 0.374 a |
| T5 | 2.72 \pm 0.046 d | 37.27 \pm 0.090 a | 55.00 \pm 0.243 a |
| Sig. | * | * | * |

The improvement in oxidation indicators in broiler blood serum is attributed to the significant role that probiotics play in raising antioxidant levels in broiler blood serum. This then increases the effectiveness of the immune system, which is the primary defense against harmful bacteria and other pathogens. Furthermore, the probiotic bacteria used in the dosing increase the effectiveness of antioxidants in combating free radicals, this positively impacts overall health and, consequently, improves the productive qualities of broiler chickens (Al-Gharawi, 2012).

This result is consistent with Yazar *et al.* (2003), who demonstrated that probiotics are beneficial bacteria that enhance antioxidants by developing the immune system against disease. In addition to their important role in increasing the effectiveness of antioxidants in blood serum, a significant increase in MDA levels was recorded in those infected with toxoplasmosis compared to those without, with no significant differences between the sexes. This is attributed to the fact that infection with the *E. tenella* parasite is accompanied by an increase in the generation of free radicals. Infection

with E. tenella can stimulate an immune response that leads to the release of cytokines from macrophages and heterogeneous cells (Tabbara *et al.*, 2001). In addition to the production of reactive oxygen species (ROS) by these cells, these mediators include the superoxide radical and hydrogen peroxide. These unstable molecules can interact and cause damage to essential cell components. Al-Mousawi (2009) also agreed, noting that antioxidants protect cell membranes from lipid peroxides. The effectiveness of antioxidants is not isolated from each other, but rather works in combination, which increases their effectiveness. Ajakaiye *et al.* (2010) indicated that the use of a probiotic in dosing resulted in a significant increase in the effectiveness of antioxidants compared to the control treatment, this positively impacts the immune system of broiler chickens.

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