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Dietary *Paenibacillus polymyxa* AM20 as a new probiotic: Improving effects on IR broiler growth performance, hepatosomatic index, thyroid hormones, lipid profile, immune response, antioxidant parameters, and caecal microorganisms

Luli Zhou,[*] Khaled Abouelezz,[*,†] Maha Abdullah Momenah,[‡] Majed A. Bajaber,[§] Narjes Baazaoui,[#] Taha F. Taha,[‖] Ahmed E. Awad,[‖] Soha A. Alamoudi,[¶] Eman A. Beyari,[**] Yasmene F. Alanazi,[††] Aminah Allohibi,[¶] and Ahmed M. Saad [‖,1]

[*]*Tropical Crops Genetic Resources Institute, Chinese Academy of Tropical Agricultural Sciences, Haikou 571101, China;* [†]*Department of Poultry Production, Faculty of Agriculture, Assiut University, Assiut 71526, Egypt;* [‡]*Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, Riyadh 11671, Saudi Arabia;* [§]*Chemistry Department, Faculty of Science, King Khalid University, Abha 61413, Saudi Arabia;* [#]*Biology Department, College of Sciences and Arts Muhayil Assir, King Khalid University, Abha 61421, Saudi Arabia;* [‖]*Biochemistry Department, Faculty of Agriculture, Zagazig University, Zagazig 44511, Egypt;* [¶]*Biological Sciences Department, College of Science & Arts, King Abdulaziz University, Rabigh 21911, Saudi Arabia;* [**]*Department of Biological Sciences, Microbiology, Faculty of Science, King Abdulaziz University, Jeddah, 21589, Saudi Arabia; and* [††]*Department of Biochemistry, Faculty of Science, University of Tabuk, Tabuk 71491, Saudi Arabia*

ABSTRACT The search for a natural antimicrobial agent is ongoing and critical because of the rise and rapid proliferation of antibiotic-resistant pathogenic bacteria. The current study aims to examine the effect of *Paenibacillus polymyxa* AM20 as an alternative antibiotic and feed additive on Indian river broiler performance, digestive enzymes, thyroid hormones, lipid profile, hepatosomatic index, immunological response, gut bacteria, and antioxidant parameters. The bacterial isolate AM20 was identified at the gene level by isolating DNA and using PCR to detect genes. Based on 16S rRNA gene sequence analysis, the bacterial isolate was identified as *Paenibacillus polymyxa*. One hundred twenty Indian river broilers (1-day old) were randomly divided into 4 groups of 10 chicks each, with 3 replicates. The control group was fed a basal diet only, while the other 3 were administered control diets supplemented with *P. polymyxa* at 3 concentrations: 0.5, 1, and 1.5 mg/kg. The findings revealed that all groups that received graded amounts of *P. polymyxa* increased all growth parameters throughout the study. *P. polymyxa* treatment at 1.5 mg/kg increased body gain by 9% compared to the control due to increased feed intake ($P = 0.0001$), growth rate ($P = 0.0001$), and decreased feed conversion ratio. Compared to the control group, *P. polymyxa* (1.5 mg/kg) enhanced kidney functions in chickens by reducing uric acid and creatinine levels ($P = 0.0451$). Compared to the control group, alanine aminotransferase and aspartate transaminase levels in the liver were significantly reduced at all *P. polymyxa* doses. Liver function values were highest for *P. polymyxa* at 1.5 mg/kg. Compared to the control group, those whose diets included P. polymyxa had significantly better blood cholesterol levels, high-density lipoprotein, low-density lipoprotein, immunological response, thyroid function, and gut microbiota. In general, broiler chickens' economic efficiency was improved by including P. polymyxa in their diet, which also improved their growth performance, carcass dressing, specific blood biochemical levels and enzymes, and the composition of the gut microbiota.

Key words: antibiotics alternatives, cecal microbiota, organic poultry, *Paenibacillus polymyxa*, probiotic

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INTRODUCTION

Antibiotics are essential for most therapeutic procedures in both human and veterinary medicine. As a result, developing resistance against such substances is a critical concern (Swelum et al., 2021; Abd El-Hack et al., 2022a,b). For many years, veterinarians and

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[1]Corresponding author. ahmedm4187@gmail.com

farmers have relied on antibiotics to treat and improve animal health, growth rate, and feed conversion ratio (**FCR**) (Landers et al., 2012; Alagawany et al., 2021a). According to conservative estimates, at least 23,000 people die each year in the United States because of illnesses with organisms resistant to antibiotics (Hao et al., 2014). A new study estimates that antibiotic resistance will be responsible for around 300 million premature deaths worldwide by the yr 2050 and will place a burden of up to $100 trillion on the economy of the entire world (Hao et al., 2014).

The European Union (2005) warned that the use of antibiotics as growth promoters in animal feed posed a serious threat due to the constant discovery of new antibiotic resistance mechanisms in various bacteria. Antibiotic overuse in livestock causes selective pressure that favors the development of microorganisms resistant to these drugs (Weinstein, 2001; El-Tarabily et al., 2021; Abd El-Hack et al., 2021a). These antibiotic-resistant bacteria can transmit to humans through direct contact with infected animals or food products and by indirect environmental interaction with non−food-producing animals, rendering human pathogenic bacteria resistant to certain antibiotic classes (Mancuso et al., 2021; El-Saadony et al., 2023a,b).

Prebiotics (natural or herbal feed additives), probiotics, and symbiotics are all being used in modern feeding regimens as growth promoters and antibiotic replacements (Abd El-Hack et al., 2021b; Samad, 2022). Probiotics are a type of feed additive that has been shown to increase the population of beneficial bacteria in the gut and improve overall health (Abd El-Hack et al., 2020, 2021c). They might improve digestibility and performance by making conditions more favorable for beneficial bacteria and lowering the number of harmful bacteria (Alavi et al., 2012; De Oliveira et al., 2019; Shini et al., 2020). In addition, mannan oligosaccharides and other macromolecules are increasingly being investigated for their prebiotic properties (Craig et al., 2020).

In addition to preventing the proliferation of potentially dangerous microorganisms probiotics can also regulate the microbiota of the gastrointestinal tract, making them a promising alternative to antibiotics for boosting animal performance (Kulkarni et al., 2022). Probiotic bacteria and their metabolites supplements in poultry production may have positive effects both as feed additives and as antibiotic replacements (Ayalew et al., 2022) and have many beneficial effects, including the improvement of general health, FCR, growth rates, body resistance, body weight, carcass yield, digestibility of amino acids such as lysine, valine, and cysteine, and thus increase production (Sharma et al., 2023).

Synbiotics are a mixture of live microorganisms and substrate(s) specifically utilized by host microorganisms that provide a health benefit to the chickens (Swanson et al., 2020). Pourrajab et al. (2021) shown that synbiotics derived from the combination of pre- and probiotics are more effective in broiler diets than either prebiotic or probiotic alone. Similarly, Nuzhat et al. (2023) reported that a synbiotic product improved broiler performance more than a probiotic product.

Probiotics are resistant to digestion by humans and ruminant animals, but they can be digested by beneficial bacteria in the chicken gut, such as *Lactobacillus, Bacillus, Paenibacillus*, bifidobacteria, and *Bacteroides*. This fermentation process can assist to change the composition of the bacterial community in the gut, making it more resistant to colonization by harmful bacteria (Xu et al., 2019). Probiotics have been shown to have a number of beneficial effects, including the induction of H_2O_2 that kills off harmful bacteria, the reduction of oxidative stress in the gut, the inhibition of aerobic pathogens and toxic amines, the production of essential digestive enzymes, the generation of vitamin B, and the stimulation of appetite and feed intake (Xu et al., 2019).

Many recent researches have centered on the role of probiotics and prebiotics as effective additives in shaping the microflora and microbial count in the gastrointestinal tracts of animals (Abdelnour et al., 2020a,b; Alagawany et al., 2021b, 2022; El-Saadony et al., 2021; Wang et al., 2021). In recent years, several researchers have concentrated on the role that probiotics and prebiotics play as effective additives that can alter the microflora and microbial count in the guts of broiler chicks via 2 points (Wang et al., 2021). The first probiotic benefit is that it improves the gut bacterial equilibrium. Second, the prebiotic advantage related to the host is demanding, encouraging a limited number of bacteria in the hindgut (Wang et al., 2021). According to Wang et al. (2021), adding supplements like these to the food of broilers has been shown to have a beneficial effect on the microbiota of the intestines, as well as increase metabolic function and nutrient absorption.

Paenibacillus polymyxa is a gram-positive spore-forming rhizobacterium that generates extracellular polymeric substances, enzymes, phytohormones, and antibiotics (Rybakova et al., 2016). The whole genome sequences have been reported for several strains of *P. polymyxa* strains, and these sequences code for the production of various physiologically active compounds (Liu et al., 2017b). These chemicals have extremely diverse physiological and biotechnological activities and minimal toxicity (Liang and Wang, 2015).

In the current investigation, we isolated different isolates of *P. polymyxa* from corn roots, tested them for their antibacterial effectiveness against multidrug-resistant bacteria, and finally identified them using biochemical and matrix-assisted laser desorption/ionization-time of flight (**MALDI-TOF**) mass spectrometry (**MS**) techniques. *P. polymyxa* AM20 was given to the broiler diet as an alternative to antibiotics. They were then evaluated as growth boosters for their effects on broiler performance, carcass yield, digestibility, blood biochemistry, intestine bacterial counts, and net income.

MATERIALS AND METHODS

Isolation of Paenibacillus

Corn roots were used to isolate *Paenibacillus* isolates. The roots were rinsed with tap water, then submerged

in ethanol (80%), sodium hypochlorite (2.5%), and sterilized water 3 times. Surface sterilized roots were chopped into pieces; 10 g were chosen and homogenized in 90 mL peptone water for 30 min to generate a 10^{-1} dilution; repeated dilutions up to 10^{-7} were prepared.

Tryptic soy agar (**TSA**, Lab M Limited, Lancashire, UK) plates were inoculated with each dilution, and then incubated for 2 d at 28°C. Streaking on TSA plates allowed for selecting and purifying colonies of varying morphologies (Saad et al., 2022).

Screening Paenibacillus *Isolates Against Pathogenic Bacteria*

The *Paenibacillus* isolates were screened based on the antibacterial activity of selected isolates against the pathogenic bacteria *Bacillus cereus* and *Klebsiella pneumonia*. The pathogenic bacteria were cultivated into TSB for 3 d at 28°C, then centrifuged to suspend the bacterial cells. Fifty microliters of each suspension of selected bacterial isolate (1.5×10^8 CFU/mL) were spread on the LB (10 g of Bacto-tryptone and 10 g of NaCl per liter, pH 7.5.) plates' surface; after 20 min, 6 mm disks previously suspended in bacterial isolates were put on both sides of LB plates and then incubated at 37°C for 24 h. The inhibition zone diameters around the disks were measured, indicating the antibacterial activity of selected isolates (Adeoyo et al., 2019).

The second screening was conducted against 6 pathogenic bacteria: *Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus, Escherichia coli, Salmonella typhi*, and *Klebsiella pneumonia*.

Identifying the Selected Isolate

The chosen isolate was identified by morphological, biochemical, and physiological examinations following Bergey's manual (Logan and De Vos, 2009), The bacteria were treated with mutanolysin twice, followed by treatment with mutanolysin, chromopeptidase, and lysostaphin. The phenol-chloroform was used to extract the DNA from bacterial cells (Mannerová et al., 2003). The DNA was characterized by agarose gel electrophoresis (1.5%), with TBE buffer used. Ethidium bromide was used for the gel staining, and the developed bands were shown under ultraviolet light. The size of the DNA fragments was measured by comparing them with a known set of DNA fragments of different sizes. The DNA fragments were amplified using PCR, which is a technique that uses short pieces of DNA called primers to copy specific DNA sequences are PFf 5′ AGGGATGTATTTATTAGATAAAAAATCAA3′ and PFr 5′ AGTAGTTTCTTCAGTAAATC 3′. RNAmmer version 1.2 is a software tool used to piece together gene sequences from short DNA fragments obtained from whole-genome sequencing (**WGS**) (Yoon et al., 2017) compared to other *Paenibacillus* species.

Antibacterial Activity of Paenibacillus *Selected Isolate*

Different concentrations of bacterial suspension were prepared (20, 40, 60, and 80%). Six millimeter disks were immersed in each level for 30 min. The *Paenibacillus* selected isolate concentrations were tested against *Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus, Escherichia coli, Salmonella typhi*, and *Klebsiella pneumonia*. The pathogenic bacteria were inoculated on LB plates, and the *Paenibacillus*-saturated disks were placed on the plates. The LB plates were incubated, and the inhibition zones were measured (mm) (Saad et al., 2021).

Experimental Birds Design and Diets

At 1-day old, 120 broilers (Indian river) were purchased from Elwattania company and were randomly divided at equal body weights into 4 groups, with 3 replicates of 10 chicks each. The G1 served as control and fed the basal diet, while the G2, G3, and G4 fed the basal diet supplemented with *Paenibacillus* powder at 0.5, 1, and 1.5 mg/kg diet, respectively. Birds were housed in batteries consisting of 3 decks and 2 sections of cages with automatic watering and were fed and watered ad libitum. The ingredients and chemical composition of basal diets are displayed in Table 1.

Growth Performance and Carcass Traits Measured-

Chicks' live body weights (**LBW**) and feed consumption (per pen) were recorded, and then body weight gain (**BWG**) was calculated by the difference between the final live body weight (35 d of age) and initial live body weight (1-day old). Also, the FCR was calculated by BWG divided by feed consumption, and according to Saad et al. (2022), the performance index (**PI**) was computed through PI = BWkg/FCR. The growth rate (**GR**) was calculated as follows (Brody and Lardy, 1945).

$$\text{Body weight gain(BWG)} = \text{Final live body weight} - \text{Initial live body weight} \quad (1)$$

$$\text{GR} = (\text{LBW35} - -\text{LBW1})/0.5 \times (\text{LBW1} + \text{LBW35}) \quad (2)$$

$$\text{PI} = \text{BWG/FCR} \quad (3)$$

At 35 d of age, 24 chickens (4 treatments × 3 samples × 2 sex) were reweighed and slaughtered by cutting the Jugular vein, then de-feathered and eviscerated. Carcass yield and giblets weight were measured, and the dressing % and giblets were computed. The blood samples were taken, then the serum was separated (through

Table 1. Composition of the basal diet at different ages of Indian river (IR) broiler chickens.

Ingredient (%)	Prestarter 1−7 d	Starter 8−20 d	Grower 21−28 d	Finisher 29−35 d
Yellow corn	55.80	57.17	61.50	68.70
Soybean meal (44%)	28.20	28.97	25.00	19.31
Corn gluten meal (60%)	10.17	7.78	7.46	5.82
Soybean oil	1.20	1.40	2.00	2.50
L-Lysine HCL	0.50	0.50	0.50	0.50
DL methionine	0.13	0.18	0.14	0.16
Calcium carbonate	1.60	1.60	1.33	1.22
Calcium phosphate, mono	1.73	1.73	1.40	1.12
Salt, NaCl	0.37	0.37	0.37	0.37
Vitamin and mineral mix[1]	0.30	0.30	0.30	0.30
Total	100.00	100.00	100.00	100.00
Calculated analysis[2]				
Dry matter (%)	87.73	87.53	86.95	86.46
Crude protein (%)	23.8	22.85	21.26	18.40
ME kcal/kg	3000	2990	3084	3181
Ether extract (%)	2.77	2.78	2.88	3.03
Crude fiber (%)	3.55	3.58	3.39	3.11
Lysine (%)	1.46	1.46	1.35	1.18
Methionine (%)	0.60	0.61	0.55	0.52
Methionine + cysteine (%)	0.98	0.98	0.90	0.82
Calcium (%)	0.97	0.97	0.90	0.85
Available phosphorus (%)	0.51	0.51	0.44	0.40
Determined				
Dry matter (%)	88.5	88.7	86.8	85.6
Crude protein (%)	23.89	22.77	20.85	18.29
Ether extract (%)	3.00	3.00	3.00	2.95
Ash (%)	5.22	4.9	4.6	5.1

[1]Each 3.0 kg of mineral and vitamin mix contain vitamin A, 12,000,000 IU; vitamin E, 10 g; vitamin D_3, 2,500,000 IU; vitamin K_3, 2.5 g; vitamin B_1, 1 g; vitamin B_2, 5 g; vitamin B_6, 1.5 g; vitamin B_{12}, 10g; biotin 50 mg; folic acid, 1 g; nicotinic acid, 30 g; pantothenic acid, 10 g; choline 250,000 mg, Zn, 55 g; Cu, 10 g; Fe, 35 g; Co, 250 mg; Se, 150 mg; I, 1 g; Mn, 60 g; and antioxidant, 10 g.
[2]According to NRC (1994). ME, metabolizable energy.

centrifugation at 3,000 rpm for 15 min) and stored till use for blood analyses. The following equation calculated the Hepatosomatic Index according to Yesuf et al. (2023).

$$\text{Hepatosomatic Index(HSI)} = \text{weight of the liver(g)/final body weight(g)} \times 100 \quad (4)$$

Blood Biochemical, Antioxidant, and Immunity All following determinations were done in serum using kits of Biodiagnostic Company, Egypt; total cholesterol (**Chol**, CAT No. CH 12 20), low-density lipoproteins (**LDL**), high-density lipoproteins (**HDL**) according to Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (2001), aspartate aminotransferase (**AST**), alanine aminotransferase (**ALT**), lipase and amylase enzymes were measured (Wainstein et al., 2022).

Trypsin enzyme was determined (Bovine Trypsin ELISA Kit MBS706461). Triiodothyronine (T_3) and thyroxine (T_4) were quantitatively detected as an immunoassay by ELISA technique using the automated ELISA reader Expert Plus UV, Biochrom., G 020151. All blood biochemical parameters are determined through commercial diagnosing kits (manufactured by Spectrum Diagnostics Company, Egypt). Immunoglobulin isotypes (IgA and IgG) were assayed in ELISA (Gao et al., 2023).

Cecal Microbial Count Intestinal content from each treatment's cecum was collected separately in sterile glass flasks after slaughter. Digesta were evacuated and mixed. Flasks were kept at 4°C till the determination of microbial counts. Ten-fold serial dilutions of up to 10^7 of each sample were prepared. Total bacterial counts, coliform count, *E. coli*, and lactobacilli count were estimated. A nutrient agar medium (Lab M Limited, Lancashire, UK) was used to enumerate aerobic bacteria (Amadi and Wami, 2023). MacConkey agar medium (Kabary et al., 2021) was used for counting coliform bacteria (forming red color colonies).

The eosin methylene blue (**EMB**) agar medium (Oxoid, UK) was used for *E. coli* counts. Casein Soy Bean Digest medium was used to count the TYMC. The deMan, Rogosaand Sharpe (**MRS**) agar medium was used for lactobacilli. Three dilutions of each treatment were plated for each medium (10^2, 10^3, and 10^5 for counting *E. coli* and coliform, whereas 10^3, 10^5, and 10^7 were used for total aerobic bacteria and lactobacilli). After incubation, colonies were counted. Numbers of colony-forming units (**CFU**) are expressed as log colony-forming units per gram of digest content.

Economic Efficiency The economic efficiency was calculated according to Kalia et al. (2018). The extra price of the *P. polymyxa* preparation was added to the feed price, whereas other productive costs were disregarded since they were constant; the following equations declared the calculations

$$\text{Feed cost} = \text{Feed intake}\left(\text{kg}\frac{\text{feed}}{\text{kg}}\text{meat}\right) \times \text{price of kg feed} \quad (5)$$

$$\text{Net revenue} = \text{Price of kg live body weight} - \text{Feed cost} \quad (6)$$

$$\text{Economic efficiency} = \frac{\text{Net revenue}}{\text{Feed cost}} \quad (7)$$

Statistical Analysis

Statistical analysis was conducted using 1-way ANOVA of SPSS software (2021). All tested means (treatment) were compared by LSD test at a probability of $P < 0.05$, which was required for significance. The sample size was calculated from the following equation.

$$n = \left(\frac{ZSD}{E}\right)^2$$

RESULTS

Isolation, Screening, and Identification of Selected Paenibacillus Isolate

Thirty-three *Paenibacillus* isolates isolated from corn roots samples, coded as AM1, AM2, AM3, …. AM33. The isolates were screened based on their antibacterial activity against multidrug-resistant bacteria; the first screening was against 2 pathogens, *Bacillus cereus*, and *Klebsiella pneumonia*, and showed that isolates AM5, AM11, AM15, AM20, AM26, and AM31 have considerable antibacterial activity against the tested pathogens with inhibition zones in the range of 18 to 29 mm; however, other isolates have lower inhibition zones. The second screening was against *Bacillus cereus* (**BC**), *Listeria monocytogenes* (**LM**), *Staphylococcus aureus* (**SA**), *Escherichia coli* (**EC**), *Salmonella typhi* (**ST**), and *Klebsiella pneumonia* (**KP**) showed that *Paenibacillus* isolate AM20 had the highest inhibition zones diameters against the tested pathogens compared to other isolates, the IZDs were 33, 30, 39, 28, 27, and 29 mm against *B. cereus, L. monocytogenes, S. aureus, E. coli, S. typhi*, and *K. pneumonia*, respectively (Table 2).

Under the microscopic examination, the selected isolate was gram-positive, motile, long rod, and spore-forming, revealing that this bacterium belongs to the *Paenibacillus* species. The biochemical tests based on the Beregy manual revealed that this isolate was nominated as *Paenibacillus polymyxa* AM20. The PCR results showed a single band, meaning the PCR test successfully amplified the 360-base-pair 16S rRNA gene sequence. The 16S ribosomal DNA sequencing test suggested that pathogenic bacteria-tolerant isolate AM20 (band 5) is closely related to *Paenibacillus* strains (bands 2−4). In addition, quantitative real-time PCR (**RT-PCR**) showed that AM20 expresses all pathogenic bacteria tolerance genes. The findings of this study add a new member to the *Paenibacillus* genus, which was previously thought to be the only genus of bacteria that can tolerate pathogenic and multidrug-resistant bacteria (Figure 1).

Antibacterial Activity of Paenibacillus Polymyxa AM20

Table 3 shows that IZDs of *Paenibacillus polymyxa* AM20 increased concentration independently ($P < 0.05$) against the tested bacteria. The highest concentration of bacterial suspension (80%) had the highest IZDs; *S. aureus* was the most sensitive bacteria to AM20 concentration, 41 mm, followed by *B. cereus*, 33 mm; however, the most resistant bacteria to AM20 was *S. typhi*, 27 mm followed by *E. coli*, 29 mm.

Growth Performance

Table 4 shows the effect of feeding IR broilers on graded levels of *Paenibacillus polymyxa* AM20 (0.5, 1, and 1.5 mg/kg diet) on different growth promoters (LBW, BWG, FCR, GR, and PI) during the overall period (1−35 d of age). The findings showed that all addition levels of *P. polymyxa* addition were significantly superior ($P < 0.05$) to the control group. The results were in a concentration-dependent manner between *P. polymyxa* levels and control or among *P. polymyxa* concentrations, where the highest addition level (1.5 mg/kg diet) was the best value for the studied

Table 2. Inhibition zone diameters (mm) of *Paenibacillus* isolates against multidrug-resistant bacteria.

Isolates	BC	LM	SA	EC	ST	KP
AM5	22 ± 0.3^{d}	20 ± 0.2^{e}	25 ± 0.6^{e}	23 ± 0.7^{c}	21 ± 0.0^{c}	24 ± 0.3^{cd}
AM11	30 ± 0.6^{b}	28 ± 0.6^{b}	36 ± 0.5^{b}	25 ± 0.6^{b}	24 ± 0.5^{b}	27 ± 0.5^{b}
AM15	25 ± 0.7^{cd}	24 ± 0.2^{d}	29 ± 0.7^{d}	22 ± 0.3^{c}	23 ± 0.4^{bc}	23 ± 0.1^{d}
AM20	33 ± 0.5^{a}	30 ± 0.7^{a}	39 ± 0.8^{a}	28 ± 0.4^{a}	27 ± 0.8^{a}	29 ± 0.5^{a}
AM26	27 ± 0.9^{c}	26 ± 0.9^{c}	31 ± 0.9^{cd}	23 ± 0.5^{c}	22 ± 0.2^{c}	25 ± 0.6^{c}
AM31	29 ± 0.7^{b}	27 ± 0.8^{b}	32 ± 0.0^{c}	25 ± 0.9^{b}	24 ± 0.6^{b}	26 ± 0.9^{b}
P value	<0.0001	<0.0001	<0.0001	0.001	0.0103	0.002

Values are presented as mean ± SD, $n = 3$. BC, *Bacillus cereus*; LM, *Listeria monocytogenes*; SA, *Staphylococcus aureus*; EC, *Escherichia coli*; ST, *Salmonella typhi*; and KP, *Klebsiella pneumonia*. Lowercase letters (a−e) in the same column indicate significant differences ($P < 0.05$)

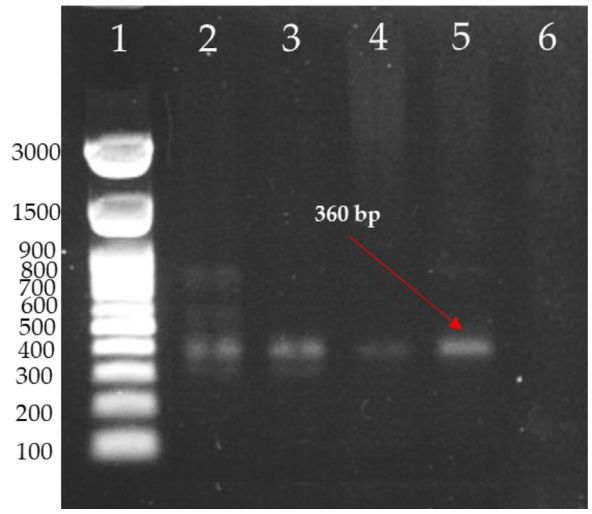

Figure 1. 16S rRNA genes of zinc-tolerant isolate, Lane 1, Ladder [(L) 100−1,500 bp], Lane 2 to 4 positive controls (P, *Paenibacillus polymyxa*), Lane 5, identified isolate at 360 bp, Lane 6 negative control (N, *Listeria monocytogenes*).

Table 3. Antibacterial activity of *Paenibacillus polymyxa* AM20 supernatant at different concentrations (20−80%) against pathogenic bacteria presented as diameters of inhibition zones in mm.

Bacterial strain	20%	40%	60%	80%	*P* value
BC	23 ± 0.1^{d}	27 ± 0.3^{c}	30 ± 0.2^{b}	33 ± 0.2^{a}	<0.0001
LM	22 ± 0.5^{d}	24 ± 0.5^{c}	27 ± 0.3^{b}	31 ± 0.5^{a}	<0.0001
SA	28 ± 0.3^{d}	33 ± 0.3^{c}	38 ± 0.3^{b}	41 ± 0.2^{a}	<0.0001
EC	20 ± 0.2^{d}	23 ± 0.0^{c}	25 ± 0.6^{b}	29 ± 0.6^{a}	<0.0001
ST	18 ± 0.9^{d}	21 ± 0.1^{c}	25 ± 0.7^{b}	27 ± 0.9^{a}	<0.0001
KP	22 ± 0.5^{d}	25 ± 0.9^{c}	27 ± 0.8^{b}	30 ± 0.5^{a}	<0.0001

Values are presented as mean ± SD, $n = 3$. BC, *Bacillus cereus*; LM, *Listeria monocytogenes*; SA, *Staphylococcus aureus*; EC, *Escherichia coli*; ST, *Salmonella typhi*; and KP, *Klebsiella pneumonia*. Lowercase letters (a−e) in the same row indicate significant differences ($P < 0.05$).

growth parameters LBW (2,350.33 g), BWG (2,310.03 g), FCR (1.64), GR (192.75), and PI (147.72).

The lowest values for the control group were 2,196.87, 2,141.54, 3,735.57, 1.75, 190.61, and 125.68 for the same previous parameters, respectively. From the statistical view, the *P. polymyxa* administration at 1.5 mg/kg significantly ($P = 0.0011$) enhanced the body gain by 9% compared to the control because of increasing the feed intake ($P = 0.0001$) and growth rate ($P = 0.0001$) and lowering FCR. It was found that all performance parameters were better in broilers fed in *P. polymyxa*-supplemented diets than those provided in the basal diet ($P < 0.05$). This improvement in broiler performance may be related to *P. polymyxa*, which could be caused by enhancing crude protein and fiber digestibility.

Blood Parameters

Table 5 shows how feeding broiler chicks different levels of *P. polymyxa* (0.5, 1, and 1.5 mg/ kg diet) affect serum liver and kidney function. The results showed that regarding the kidney functions, creatinine level was not significantly affected by any treatments, and the numerical improvement in creatinine values was achieved for the group of *P. polymyxa* 1.5 mg/kg, which recorded 0.31 mg/dL compared with the control group, while the uric acid positively affected by *P. polymyxa* supplementation only with *P. polymyxa* 1.5 mg/kg (5.39 mg/dL) which superior control group and the rest 2 levels *P. polymyxa* 0.5 mg/kg and *P. polymyxa* 1.0 mg/kg diet (5.11, 4.13, and 4.55 mg/dL), respectively.

Concerning liver functions, the findings displayed a significant improvement in ALT and AST with each level of *P. polymyxa* compared to the control group, and the best values of liver functions were with the highest levels of *P. polymyxa*. Concerning sex effect, there was no significant difference between males and females, whether in liver or kidney functions. Regarding liver enzymes (in serum), the current results detected a significant reduction in liver enzymes ALT and AST compared with the control.

Hepatosomatic (**HIS**) is an indicator utilized to test liver weight relative to final body weight. In this study, the *P. polymyxa* 0.5 mg/kg group recorded the highest ($P = 0.003$) HSI value; the other groups (1 and 1.5 mg/kg) recorded higher HIS values than the control. The hepatosomatic index is a trusty hepatic growth and development parameter related to age and the liver's physiochemical or physiological status. The hepatosomatic index is crucial because it describes the changes caused by feed supplementation, so it is a suitable parameter for animal feeding activity. Also, the current study displays that the broilers had a higher dose of *P. polymyxa* in the feed, positively impacting health.

Table 4. Effect of dietary treatments of *Paenibacillus polymyxa* AM20 at 3 levels on broilers growth performance.

Treatments (mg/kg)	LBW 1d	LBW 35d	BWG 1−35 d	FI 1−35 d	FCR 1−35 d	GR 1−35 d	PI 1−35 d
Control	45.37^{a}	2196.87^{d}	2141.54^{d}	3735.57^{d}	1.75^{a}	190.61^{b}	125.68^{d}
P. polymyxa 0.5	45.48^{a}	2289.18^{c}	2235.69^{c}	3746.43^{c}	1.65^{b}	191.88^{ab}	136.26^{c}
P. polymyxa 1.0	45.35^{a}	2311.76^{b}	2266.50^{b}	3788.77^{b}	1.67^{b}	192.36^{a}	141.64^{b}
P. polymyxa 1.5	45.32^{a}	2350.33^{a}	2310.03^{a}	3801.98^{a}	1.64^{b}	192.75^{a}	147.72^{a}
SEM	0.26	27.07	28.39	4.04	0.01	0.12	3.52
P value	0.96	0.0012	0.0011	0.0001	0.003	0.0001	0.0101

Within columns, values followed by the same letter are not significantly ($P > 0.05$) different. SEM, pooled standard error. LBW, live body weight; BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio; GR, growth rate, PI, performance index. *P. polymyxa* 0.5 = basal diet + 0.5 mg *P. polymyxa*/kg; *P. polymyxa* 1.0 = basal diet + 1.0 mg *P. polymyxa*/kg; *P. polymyxa* 1.5 = basal diet + 1.5 mg *P. polymyxa*/kg.

Table 5. Effect of *Paenibacillus polymyxa* AM20 dietary treatments on broilers' serum kidney and liver functions.

	Kidney functions		Liver functions		
Treatments (mg/kg)	Creatinine (mg/dL)	Uric acid (mg/dL)	ALT (U/L)	AST (U/L)	Hepatosomatic index (HSI)
Control	0.34[a]	5.11[ab]	6.32[a]	261.15[a]	1.29[d]
P. polymyxa 0.5	0.31[a]	4.13[c]	5.78[b]	221.17[b]	1.65[a]
P. polymyxa 1.0	0.33[a]	4.55[b]	5.69[bc]	212.63[c]	1.4[c]
P. polymyxa 1.5	0.32[a]	5.39[a]	5.58[c]	210.13[c]	1.50[b]
SEM	0.015	0.90	0.20	7.83	0.075
P value	0.87	0.041	0.022	0.008	0.003

Within columns, values followed by the same letter are not significantly ($P > 0.05$) different. SEM, pooled standard error. ALT, alanine aminotransferase; AST, aspartate aminotransferase. *P. polymyxa* 0.5 = basal diet + 0.5 mg *P. polymyxa*/kg; *P. polymyxa* 1.0 = basal diet + 1.0 mg *P. polymyxa*/kg; *P. polymyxa* 1.5 = basal diet + 1.5 mg *P. polymyxa*/kg.

This denotes that the liver maintains a standard size without reverse influencing its function.

Lipid Profile

Table 6 represents the influence of feeding broiler chicks on different levels of *P. polymyxa* on total cholesterol, its fractions, and abdominal fat percentage. Total cholesterol and LDL had the highest significant value in control and then declined by increasing the level of *P. polymyxa*. The *P. polymyxa* 1.5 mg/kg group recorded the highest HDL value without significant differences to control. However, the other groups of 0.5 and 1 mg/kg recorded significantly lower HDL values than the control group. All tested groups achieved significantly lower abdominal fat percentage rather than control. In addition, the abdominal fat is lowered by *P. polymyxa* treatments.

Thyroid Hormones and Immune Response

The data in Table 7 show the impact of feeding broiler chick diets supplemented with graded *P. polymyxa* levels on blood thyroid hormones (ng/dL), immune globulin (mg/dL), and spleen index (g/100g BW). It is clear from the data that there is a significant effect ($P = 0.0451$) among the different treatments on the thyroid (T3) hormone; regarding the T4 hormone, the highest-level detected value was recorded for the *P. polymyxa* 1.5 group followed by the control group and *P. polymyxa* 1 group without significant differences between them but the lowest value was recorded for *P. polymyxa* 0.5 group, to be 129.51, 126.06, 129.71 and 133.25 ng/dL, respectively.

As for the immunoglobulins (IgG and IgA), the control treatment showed the lowest level of immune response compared to the rest of the treatments, while the rest of the treatments added with *P. polymyxa* had a significantly high immune response and the highest were *P. polymyxa* 1.5 mg/kg in IgG (1,126.09 mg/dL) and in IgA (215.72 mg/dL). When tracking the percentage of the spleen to LBW, it is clear that the lowest rate appears with the control treatment, while the highest percentage is with the treatments of *P. polymyxa* 0.5 and 1 mg/kg.

There is a significant difference between treatments of T3 ($P = 0.0451$), while T4 significantly increased ($P = 0.07$) by *P. polymyxa* addition to 133 ng/dL compared to 129 ng/dL; additionally, adding *P. polymyxa* 1.5 mg/kg significantly boosted $P < 0.001$ the immunity response of broiler by a relative increase of 9 and 7% for IgG and IgA, respectively.

Digestive Enzymes

The data shown in Table 8 show the impact of adding graded levels of *P. polymyxa* on the digestive enzymes in the blood serum (amylase, lipase, and trypsin), where the values of the 3 previous enzymes showed an upward trend starting from the control group, which included the lowest values of enzymes and the more the addition of *P. polymyxa* increased the content of the 3 enzymes to achieve the peak with treatment *P. polymyxa* 1.5 mg/kg to be (530.50, 29.17, and 51.13 for amylase, lipase, and trypsin, respectively). The enzymatic activity decreased with the increase of the addition of microbes in the *P. polymyxa* greater than 1.5 mg/kg treatment.

Table 6. Effect of dietary treatments of *Paenibacillus polymyxa* AM20 on serum cholesterol profile of broilers.

Treatments (mg/kg)	TC (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	Abdominal fat (g)
Control	136.10[a]	92.07[ab]	41.06[a]	1.00[a]
P. polymyxa 0.5	120.03[b]	89.29[c]	30.35[b]	0.92[b]
P. polymyxa 1.0	115.16[c]	91.72[b]	26.31[c]	0.88[c]
P. polymyxa 1.5	105.91[d]	96.70[a]	23.65[d]	0.82[c]
SEM	1.41	0.89	0.55	0.01
P value	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$

Within columns, values followed by the same letter are not significantly ($P > 0.05$) different. SEM, pooled standard error. HDL, high-density lipoprotein; LDL, low-density lipoprotein. *P. polymyxa* 0.5 = basal diet + 0.5 mg *P. polymyxa*/kg; *P. polymyxa* 1.0 = basal diet + 1.0 mg *P. polymyxa*/kg; *P. polymyxa* 1.5 = basal diet + 1.5 mg *P. polymyxa*/kg.

Table 7. Effect of dietary treatments of *Paenibacillus polymyxa* AM20 on serum thyroid functions and immune response kidney and liver functions of broilers.

	Thyroid hormones		Immune response		
Treatments (mg/kg)	T3 (ng/dL)	T4 (ng/dL)	IgG (mg/dL)	IgA (mg/dL)	Spleen % to LBW
Control	2.68^{a}	129.51^{a}	931.15^{d}	179.52^{c}	0.11^{c}
P. polymyxa 0.5	2.25^{d}	126.06^{b}	1016.24^{c}	192.33^{b}	0.17^{a}
P. polymyxa 1.0	2.33^{c}	129.71^{ab}	1056.91^{b}	197.83^{b}	0.15^{ab}
P. polymyxa 1.5	2.41^{b}	133.25^{a}	1126.09^{a}	215.72^{a}	0.14^{bc}
SEM	0.22	1.62	16.23	2.89	0.01
P value	0.00451	0.070	$P < 0.001$	$P < 0.001$	$P < 0.001$

Within columns, values followed by the same letter are not significantly ($P > 0.05$) different. SEM, pooled standard error. T_3, triiodothyronine; T_4, thyroxine IgG; IgA, immunoglobulins isotypes G and A; LBW, live body weights of chicks. *P. polymyxa* 0.5 = basal diet + 0.5 mg *P. polymyxa*/kg; *P. polymyxa* 1.0 = basal diet + 1.0 mg *P. polymyxa*/kg; *P. polymyxa* 1.5 = basal diet + 1.5 mg *P. polymyxa*/kg.

Intestinal Bacterial Count

Figure 2 shows the gut microorganism's count. Total bacterial count, yeast and molds, and *E. coli* show the highest values with the control coefficient of 8.89, 5.11, and 7.85 Log10 CFU/g, respectively. It decreases with the augment in the dietary *P. polymyxa* level until it reaches the lowest significant value with the treatment *P. polymyxa* 1.5 (6.9, 3.9, and 6.68 Log 10 CFU/g). As for lactic acid bacteria count, the lowest value appears with the control 6.89; the values increase with adding *P. polymyxa* in the diet to achieve the peak height with treatment *P. polymyxa* 1.5 (7.87 Log 10 CFU/g).

Economic Efficiency

Data presented in Table 9 show the effect of adding different levels of *P. polymyxa* to broiler diets on economic efficiency, indicating that all levels of *P. polymyxa* had the highest economic efficiency than the control group, and the treatment *P. polymyxa* 1.5 mg/kg was the highest value in economic efficiency and relative economic efficiency. This may be due to the highest final body weight of those treatments compared to the control group.

DISCUSSION

Antimicrobial resistance (**AMR**) occurs when bacteria, parasites, viruses, and fungi become resistant to the antimicrobial drugs used for treating their related infections. However, the common perception is exclusively associated with the overuse or misuse of antibiotics in humans and animals (Osei Sekyere and Mensah, 2020; El-Saadony et al., 2022; El-Shall et al., 2022).

Antibiotic resistance and circulation of the related genes in virulent bacterial populations is the most critical issue in infectious disease treatment (Alenazy, 2022). In the early era of antibiotic use, there was an extensive use of antibiotics; the mutations in the bacterial genes that were the target of antibiotics were the primary cause of antibiotic resistance. However, it soon became evident that acquiring antibiotic resistance-related genes through horizontal gene transfer has a significant role in developing and spreading pathogenic bacteria (Alenazy, 2022).

Therefore, veterinarians and veterinary paraprofessionals are crucial in fighting against antimicrobial resistance by regulating and supervising antibiotic use, offering professional advice to farmers and animal owners, and collaborating with the human healthcare sector (Xavier et al., 2019). Understanding the resistance mechanisms is paramount for developing novel strategies to cope with this threat. Therefore, in this study, we isolated the *Paenibacillus* isolates from corn roots, screening for the best antibacterial activity against multi-drug-resistant bacteria and then identifying them by biochemical tests and PCR analysis. The *Paenibacillus polymyxa* AM20 was added as an alternative to antibiotics to the broiler diet.

The dietary addition of *P. polymyxa* considerably enhanced the growth performance parameters, and that agrees with those of Hatab et al. (2016) on the broiler, Czech et al. (2020) on turkey, Soomro et al. (2019) on quail, and Ye et al. (2021) on partridge when using microorganisms as feed additives. Recently, Zhang et al. (2021) revealed that microbial additives from 0 to 42 d of age markedly augmented LBW, average daily gain, and average daily consumption in female chickens and significantly ameliorated LBW and feed conversion ratio in male chickens. In contrast to the previous authors, Nuengjamnong and Luangtongkum (2014) and Jayathilaka et al. (2017) reported that broiler growth performance was not affected significantly by feeding diets or drinking water added with microbial additives. Various studies point to the role and importance of probiotics in better feed utilization efficacy. The probiotics

Table 8. Effect of dietary treatments of *Paenibacillus polymyxa* AM20 on serum digestive enzymes and intestinal microbial count of broilers.

	Digestive enzymes (U/L)		
Treatments (mg/kg)	Amylase	Lipase	Trypsin
Control	340.00^{c}	15.00^{c}	33.33^{c}
P. polymyxa 0.5	519.33^{b}	23.17^{b}	45.62^{b}
P. polymyxa 1.0	520.17^{b}	25.50^{b}	48.00^{ab}
P. polymyxa 1.5	530.50^{a}	29.17^{a}	51.13^{a}
SEM	13.59	0.71	2.58
P value	$P < 0.001$	$P < 0.001$	0.006

Within columns, values followed by the same letter are not significantly ($P > 0.05$) different. SEM, pooled standard error. *P. polymyxa* 0.5 = basal diet + 0.5 mg *P. polymyxa*/kg; *P. polymyxa* 1.0 = basal diet + 1.0 mg *P. polymyxa*/kg; *P. polymyxa* 1.5 = basal diet + 1.5 mg *P. polymyxa*/kg.

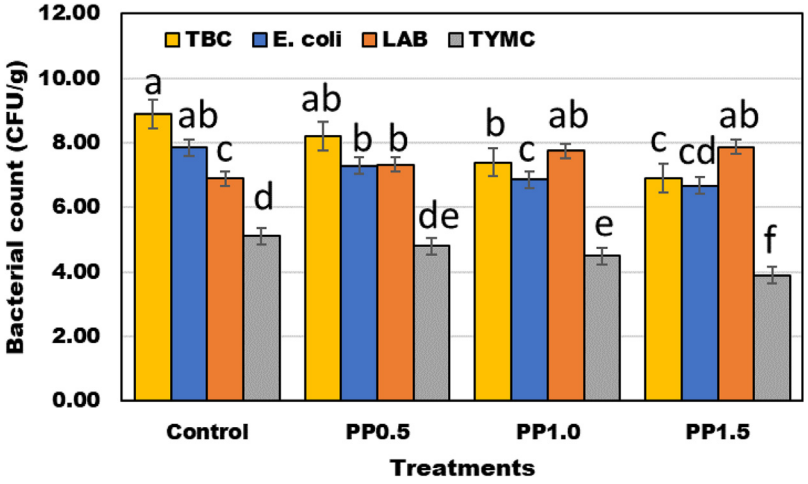

Figure 2. Effect of dietary treatments of *Paenibacillus polymyxa* AM20 on the intestinal microbial count of broilers. TBC, total bacterial count; *E coli, Escherichia coli*; LAB, *Lactobacillus* spp.; and TYMC, total yeast and mold count. CFU/g: $\log_{10}$ of colony forming units per gram of cecum. Bars represent standard error. Mean values followed by different letters are significantly ($P < 0.05$) different from each other. *P. polymyxa* 0.5 = basal diet + 0.5 mg *P. polymyxa*/kg; *P. polymyxa* 1.0 = basal diet + 1.0 mg *P. polymyxa*/kg; *P. polymyxa* 1.5 = basal diet + 1.5 mg *P. polymyxa*/kg. Different lowercase letters over columns indicate significant differences.

supplementation improved digestibility, reduced the feed consumed quantity, and enhanced animal growth performance (Biswas et al., 2023).

This enhancement in growth performance due to feeding diets enriched with *P. polymyxa* may be related to decreasing the counts of pathogenic bacteria (*E. coli*) and increasing counts of beneficial bacteria (*Lactobacillus* spp.). The same conclusion was reported by Liu et al. (2018), who showed that supplementing broiler diets with microorganisms affects the gut system by improving digestive enzyme activity, intestinal bacteria composition, and intestinal pH.

Furthermore, Adding *P. polymyxa* to the diet in our study improved the liver enzymes in broilers, agreeing with Rashidi et al. (2020) and Çelik et al. (2016), who indicated that probiotics had no effects on the serum ALT and AST concentrations. Hepatocytes are essential in absorbing and metabolizing various poisonous chemicals, so they are susceptible to damage by many chemicals in the food. Liver function and health can be detected by aspartate aminotransferase. and Alanine aminotransferase activities in serum, so liver cellular damage may augment the AST and ALT levels in serum. Alanine aminotransferase chiefly exists in the liver and is considered more specific than Aspartate aminotransferase for revealing injury of liver cells (Wang et al., 2020). The high levels of hepatic enzymes serum (ALT and AST) reflect the hepatic damage and enzyme leakage in the bloodstream and vice versa. Furthermore, Hatab et al. (2016) found no significant effects of microbial additives to diets on serum creatinine concentrations, while uric acid, serum AST, ALT, cholesterol, and triglycerides concentrations in all tested groups were lower significant than in the control group.

Regarding the effect of adding *P. polymyxa* to the diet, it maintains the standard size of organs, including the liver, agreeing with Musazadeh et al. (2022) reported that the treatments did not influence the weight of the liver. Also, Tarradas et al. (2020) showed that treating a single probiotic or combination can improve broilers' spleen, bursa of Fabricius, and thymus weight. In our study, different *P. polymyxa* expended the best effect on the broiler spleen index, which agreed with the previous findings.

Probiotics are live microbes that, when added to feed, affect serum triglycerides and total cholesterol. The obtained results align with those of Imran et al. (2021), who point out that feed containing probiotics may have

Table 9. Effect of dietary treatments of *Paenibacillus polymyxa* AM20 on broiler diets economic efficiency.

Item	Control	*P. polymyxa* 0.5	*P. polymyxa* 1.0	*P. polymyxa* 1.5	*P* value
Average feed intake, kg feed/kg meat (A)	1.75^{a}	1.69^{b}	1.65^{c}	1.63^{c}	0.002
Price kg feed (LE)[1] (B)	8.31^{c}	8.34^{b}	8.37^{ab}	8.39^{a}	0.0021
Total feed cost C = (A × B)	14.54^{a}	14.09^{b}	13.81^{c}	13.67^{d}	<0.0001
Price/1 kg gain[2](D)	24.00	24.00	24.00	24.00	0.9
Net revenue (L.E.) = E = D − C	9.45^{d}	9.90^{c}	10.18^{b}	10.32^{a}	<0.0001
Economic efficiency[3] (E/C)	0.65^{c}	0.70^{b}	0.73^{ab}	0.75^{a}	0.003
Relative efficiency[4]	100.00^{c}	107.69^{b}	112.30^{ab}	115.38^{a}	0.0025
Viability rate (%)	100.00	100.00	100.00	100.00	0.98

[1]Price of kg feed according to local market in December 2020.
[2]Price of kg live body weight according to the local market in December 2020.
[3]Net revenue per unit cost.
[4]Compared to the economic efficiency of the control group. *P. polymyxa* 0.5 = basal diet + 0.5 mg *P. polymyxa*/kg; *P. polymyxa* 1.0 = basal diet + 1.0 mg *P. polymyxa*/kg; *P. polymyxa* 1.5 = basal diet + 1.5 mg *P. polymyxa*/kg. Similar lowercase letters within the row indicate no significant difference.

some beneficial impacts by decreasing the concentration of serum triglycerides and cholesterol in chickens. Also, Shah et al. (2021) showed that the serum total cholesterol levels decreased by adding probiotics to a bird's diet. The reduction in total cholesterol in serum may be due to the synthesis of hepatocyte cholesterol, which is regulated and converted to bile acids; the adherence of Effective Microorganisms promotes the excretion of these acids. So, it may have a role in diminishing serum cholesterol levels.

Probiotics improve pH, color, fatty acid profile, chemical composition, water retention capacity and oxidation stability (Popova, 2017). The probiotics affect the fat contents. The inclusion of *Aspergillus awamori* and *Saccharomyces cerevisiae* in chicken feed reduced blood-saturated fatty acids and increased the polyunsaturated (Saleh et al., 2012); through inhibiting hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase activity and related to a lowered recycling of bile salts in the gut (Liu et al., 2017a).

The dosage of probiotic treatment is an essential factor influencing the effects of probiotics on lipid abnormalities. Our results reveal that a high dose of probiotics led to a more significant reduction in LDL levels when compared to a lower dose. A similar result was observed in a previous *meta*-analysis, where a more significant blood pressure reduction was observed after administering a high dose of probiotics than a lower dose. Similarly, the results of some dose−response trials suggest that a high dose of probiotics more effectively alleviates a range of dysfunctions, such as obesity (Szulińska et al., 2018; Shehata et al., 2022; Chen,et al., 2023) and constipation (Waller et al., 2011), compared to lower doses.

P. polymyxa addition in the IR broiler diet enhances immunity, which agrees with Elbaz et al. (2021), who reported that broiler chickens fed diets containing probiotics increased their serum triiodothyronine level. Hatab et al. (2016) provided a potential causative relation between thyroid serum hormone and the EM effects as a growth-promoting of layer chickens. EM can boost the secretion of immunoglobulins and immune cell proliferation and promote nonspecific immunity stimulation, such as the induction of the phagocytic activity of macrophages (Tarradas et al., 2020).

Fazelnia et al. (2021) observed that birds fed with probiotics in their diets have increased serum antibody production levels, including IgM, interferon γ, and IgG. Zhang et al. (2021) reported that probiotics and feeding with EM highly promoted the IgG and IgA concentration in female and male broilers, contradicting the current study. Poultry lymphatic organs are the bursa of Fabricius, spleen, and thymus, and their weight directly explains the internal immune function strength (Slawinska et al., 2014).

Regarding gut microbiota, Zhang et al. (2021) showed that probiotics supplementation for broiler chickens (male and female) decreased the number of harmful *E. coli* and *Salmonella* in the gut and augmented the digestive enzymes. Also, recent investigations by Ye et al., (2021) found that probiotic addition enhanced the amylase and protease activity. The spared encountered pathogenic microorganisms in poultry farming are *Salmonella E. coli* enterica (Vieco-Saiz et al., 2019). In this study, *P. polymyxa* supplementation decreased *E. coli* and improved *Lactobacillus* counts. The findings were consistent with the results obtained by Zhang et al. (2021), who noticed the same trend in the gut microbial population in broilers fed with probiotics. Generally, supplementation of *P. polymyxa* in feed could improve competition in favor of beneficial bacteria in the gut's internal environment.

From the economic view, Our findings are in the same trend as mentioned by Zaghari et al. (2020) on broiler, who reported that the supplementation of probiotics improved economic efficiency compared to the control.

CONCLUSIONS

The addition of probiotics to the feed of broiler chickens positively impacted the growth performance, carcass dressing, certain blood metabolites, and enzymes, as well as increased the economic efficiency of broiler chicks. In addition, the improved gut health of these supplemented broiler chicks resulted in an increase in total count and lactobacilli and a reduction in *E. coli*.

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DISCLOSURES

The authors declare no conflicts of interest.

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