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RESEARCH ARTICLE

Could *Paenibacillus xylanexedens* MS58 be an Ecofriendly Antibiotic in Poultry Production? Impacts on Performance, Blood Biochemistry, Gut Microbiota and Meat Quality

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ABSTRACT

The search for novel natural antibiotic alternatives is continuous and crucial to overcome the antibiotic-resistant bacteria. In this study, the isolation and identification of *Paenibacillus xylanexedens* MS58 (PX) on the gene level as a new probiotic, antibiotic alternative, and feed additive has been assessed. The effects of PX on growth performance, blood biochemical parameters, immune response, gut microbiota and meat quality of broilers were also evaluated. The selected isolate, *Paenibacillus xylanexedens* MS58, inhibited the growth of pathogenic bacteria with MIC of 5-15 %. One hundred sixty chicks were casually divided into four equal experimental groups: the control group (CON) delivered the basal diet, group 1 received a basal diet supplemented with 0.3 mg/kg PX, and groups 2 and 3 provided a basal diet fortified with PX at 0.9 and 1.5 mg/kg, respectively. Adding PX at 1.5 mg/kg to the diet of chicks significantly enhanced the highest body weight influenced all growth parameters during 35 days (2.304 kg) with a relative increase of 10 % compared to control. Additionally, the dietary PX enhanced the liver and kidney enzymes; ALT activity decreased by 25% and AST by 67% compared to control. The uric acid was lowered by dietary PX with a relative decrease of 18 %. In response to adding dietary PX, the abdominal fat reduced from 1.1 to 0.8 in the PX 1.5 treatment with a relative reduction of 25 %, and total cholesterol decreased by 33 %; however, HDL increased. Additionally, the immune response and beneficial gut microbiota of chicks significantly improved with the addition of the dietary PX compared to the control group. The chicken meat's moisture and protein content were enhanced, influenced by the juiciness and tenderness properties compared to control; also, the lightness of meat was enhanced with PX addition. Generally, adding dietary PX to broilers could enhance growth, blood biochemical properties, and immune response, modulate the gut microflora structure, and improve meat quality.

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INTRODUCTION

Extensive use of antibiotics in the veterinary sector has developed antibiotic-resistant microorganisms (Yaqoob *et al.,* 2021; Abd El-Hack *et al.,* 2022a). Although these pathogens can induce diseases in animals and humans, these pathogens can be transmitted to humans directly and indirectly. Direct contact with infected animals or contaminated food items poses a risk, as well as indirect interaction with the environment where non-food-producing animals reside (El-Saadony *et al.,* 2021a,b; Salem *et al.,* 2022; Umair *et al.,* 2022). The residues of antibiotics in poultry products such as eggs or meats harm human health. Such substances make pathogenic microbes of human flora resistant to those groups of antibiotics (Mancuso *et al.,* 2021). The current training includes using probiotics as antibiotic alternatives and productive agents to promote growth (Rahman *et al.,* 2022).

The probiotics are defined as the feed components that stimulate the growth properties and activate the beneficial microorganisms in the gastrointestinal tract, resulting in valuable health (Spacova *et al.,* 2023; Zhou *et al.,* 2023). They are expected to be the component that is resistant to digestive enzymes of humans or non-ruminants; it is the substrate related to helpful microbes in the large intestine because it is wholly fermented by beneficial microorganisms such as *Lactobacillus, Bacillus, Peanibacillus, Bifidobacteria,* and *Bacteroides,* thereby having the likely effect of modifying the arrangement of bacterial collections in the digestive gut (Béghin *et al.,* 2021).

Generally, there are two kinds of microorganisms in the chicken gastrointestinal tract. The first type is allochthonous bacteria or transitory microflora, which is exogenous and is added complementarily in feed or drinking water as a probiotic nutritional supplement (György *et al.,* 2021). The second one is autochthonous or established bacteria found in the gut by inoculation resulting from normal feeding behavior and the surrounding environment of the bird (Terzić-Vidojević *et al.,* 2020). Some researches explored that allochthonous bacteria introduced by probiotics may inhibit the colonization and infection of pathogen microbes in the gastrointestinal tract (Yousaf *et al.,* 2022).

The probiotics may benefit animal performance by regulating gastrointestinal tract microflora and inhibiting the population of pathogens, so they are a potential alternatives to antibiotics enteric conditioners for performance enhancement (Aghamohammad *et al*., 2023). Adding probiotics and their metabolites as antibiotic alternatives to chicks' diet considerably enhanced the production (Swelum *et al.,* 2021; Abd El-Hack *et al.,* 2022b; El-Saadony *et al.,* 2022a). Additionally, they improve growth rates, feed conversion ratios, body weight, and carcass output and boost the bioavailability of amino acids (Sharma *et al.,* 2023).

Previous studies reported that probiotics helped in the production of H_2O_2 that damaged many harmful bacteria. Therefore the oxidation stress in the gastrointestinal tract was released, impeding the growth of aerobic pathogens and toxic amines, boosting the appetite and feed intake, and producing (B) group vitamins (Khromova *et al.,* 2022).

The *Bacillus* species are found in soils, air, and sea sediments (Stenfors Arnesen *et al.,* 2008). *Bacillus* and *Paenibacillus* have been identified or isolated in several habitats; nevertheless, most of their occurrences are seen in soils of plant roots (Grady *et al.,* 2016). Contrary to well-recognized probiotics, research on the impact of *Paenibacillus* species on animal health and performance is quite limited. A prior research has shown that *Paenibacillus xylanexedens* ysm1 adhered to enterocytes and inhibited *E. coli* growth under *in-vitro* conditions (Calik *et al.,* 2017).

The probiotic effects depend upon many factors like strain, dose, age of the bird, administration method, ability of the selected strain to survive at environmental temperature, viability and term storage, which may be responsible for the differences in results (Aluwong *et al.,* 2013; Elshaghabee *et al*., 2017).

Further, previous studies did not find changes in antioxidant status; hence, in this study, *Paenibacillus xylanexedens* MS58 was introduced as ecofriendly antibiotics alternative in chicken diet and investigated their beneficial effects on performance, immunity, blood biochemistry, intestinal bacterial counts, and meat quality enhancers of chickens.

MATERIALS AND METHODS

***Paenibacillus* isolation, screening and identification:** *Paenibacillus xylanexedens* MS58 strain was used as an antibiotic alternative and growth promoter in the broilers. This strain was isolated from soil. Representative soil samples (25g) were stirred in peptone buffer (225mL) for thirty minutes to prepare dilutions from 10^{-1} to 10^{-7}. The prepared dilutions were inoculated on count agar plates. 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 PCA 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, 6mm disks previously suspended in bacterial isolates were put on both sides of LB plates and then incubated at 37°C for 24h. The inhibition zone diameters around the disks were measured, indicating the antibacterial activity of selected isolates (Saad *et al.,* 2022). In comparison to the Bergy's handbook, the salt bile-tolerant bacteria were identified predominantly by the use of biochemical assays and light microscopy. Furthermore, identification was conducted by MALDI-TOF spectroscopy.

Antibacterial activity of the selected isolate: The disc assay estimated antibacterial activity, and discs (7mm) were soaked in *Paenibacillus* concentrations. The antibacterial effects of the chosen *Paenibacillus* isolates were tested against the following pathogens*: Klebsiella pneumonia, Staphylococcus aureus, Salmonella typhi*, *Listeria monocytogenes,* and *Escherichia coli*. The saturated discs were put on the LB plates after

inculcating 0.1ml of pathogenic bacteria, then after incubation, the zones of inhibtion (mm) measured by a ruler (Zhou *et al.,* 2023).

Experimental layout: One hundred sixty broiler chicks were randomly allocated into four equal groups with triplicates, each of ten at equal body weights. Four experimental groups: the control group delivered the typical diet, group 1 received a basal diet fortified with 0.3 mg/kg PX, and groups 2 and 3 provided a basal diet fortified with PX (0.9 and 1.5 mg/kg), respectively. (Zhou *et al.,* 2023). Batteries were utilized to shelter the birds; the batteries consisted of two parts cages and three decks, which were provided with automated watering and were freely fed and hydrated.

Growth and carcass parameters: The assessment of body weight (LBW), feed intake, and weight growth (BWG) was performed on the chicks. Furthermore, FCR was estimated by dividing the feed weight by the feed consumption (Saad *et al.,* 2022). The PI (performance index) was calculated by dividing (Zhou *et al.,* 2023).

$$Performance\ index\ (PI) = BWG/FCR$$

$$Growth\ rate\ (GR) = \frac{(BW\ 35 - BW1)}{0.5 \times (BW1 + BW35)}$$

$$\text{Weight gain (WG)} = \text{FBW} - \text{IBW}$$

Blood parameters: At 35 days of age, twenty-four chicks were weighed accurately and then dispatched. The serum was isolated from the collected blood samples by centrifugation at 3000rpm for 15min, and kept in a freezer until further use in blood tests. The following enzymes were evaluated in the blood serum of chicks: liver enzymes, aspartate aminotransferase (AST), alanine aminotransferase (ALT); lipid profile, total cholesterol (Chol), low-density lipoproteins (LDL), and high-density lipoproteins (HDL) as defined by James (2001); The intestinal enzymes; lipase and amylase and trypsin enzyme were analyzed using ELISA Kit (Cat. No. CH 203) as Tan *et al*. (2017). The quantitative detection of Triiodothyronine (T3) and thyroxine (T4) was achieved using an ELISA kit using an ELISA reader (Biochrom G 020151, USA). IgA and IgG immunoglobulin isotypes were determined by ELISA kit (NA.46) (Gao *et al.,* 2023). The blood biochemical parameters were estimated using commercial diagnostic kits (Biodiagnostic Company, Giza, Egypt).

Intestinal microbial counts: The intestinal digesta was collected after slaughter, emptied, and mixed in sterile glass bottles. The containers were stored at 4°C till the enumeration of the microbial population. The microbial population was examined for the total bacterial count, *E. coli*, TYMC and *Lactobacilli* spp., on specific media, according to Abd El-Wahab *et al*. (2022). The counts were calculated as log CFU/g of digesta.

Meat quality: The chicken breasts (*n*=3) were collected and cut into pieces (3cm); the color properties of meat samples were calculated by Hunter Lab spectrophotometer (Vista, Reston, VA, USA) following the method of Sayed-Ahmed *et al*. (2022). The oxidation of lipids was evaluated using a 2-thiobarbituric acid assay (TBA) (Sayed-Ahmed *et al*., 2022). Total Volatile Basic Nitrogen (TVBN) is a method to assess the amount of nitrogenous molecules (ammonia, dimethyl, and trimethyl amine) in meat, showing the degree of freshness; the technique was evaluated following Moosavi-Nasab *et al*. (2021). The pH value of breast samples was measured with a HANNA pH meter (Woonsocket, USA). The approx. Analysis of chicken breast was estimated according to AOAC, (2012) as follows: moisture content was tested by oven technique, crude protein was identified by Kjeldahl method, crude fat was calculated by the Soxhlet apparatus, and a muffin evaluated ash at 500°C (El-Saadony *et al*., 2022b). Sensory evaluation: Meat cubes (3cm) were evaluated by ten experienced panelists; breast samples were coded with three random digits and introduced to the panelists. The panelists rated the sensory characteristics (color, flavor, appearance, and juiciness) on a nine-point hedonic scale (Saad *et al*., 2021). Tap water was administered between sessions to change the mouthfeel (Saad *et al*., 2015; Abd El-Hack *et al*., 2021).

Statistical analysis: The statistical analysis was performed utilizing Excel software and one-way ANOVA (Microsoft Excel, 2021). The means of replicates were compared using the LSD test, ensuring significance at a probability of $p<0.05$.

RESULTS

Isolation, screening, and identification of selected *Paenibacillus* isolates: Seventeen *Paenibacillus* isolates were isolated from soil samples, coded as MS1, MS2, MS4, and MS54. The tested isolates were screened depending on their antimicrobial activity against the pathogenic bacteria *Bacillus cereus* and *Klebsiella pneumonia*. The results showed that one isolate (MS58) recorded the highest inhibtion zones against *Bacillus cereus* and *Klebsiella pneumonia* and was selected for the identification tests. The chosen isolate was identified under the microscope as a gram-positive, motile, long rod, spore-forming bacteria, further confirming and its classification as a *Paenibacillus* species organism. The biochemical assays conducted by the Beregy's handbook identified this isolate as *Paenibacillus xylanexedens* MS58, which exhibited a 99 % identity to *Paenibacillus xylanexedens* DSM 2048T.

Antibacterial activity of *Paenibacillus xylanexedens* MS58: The concentration of IZDs of *Paenibacillus xylanexedens* MS58 dose independently against the tested bacteria, is shown in Table 1. The IZDs were wide for the bacterial suspension with the highest concentration (60 %); the highest IZD (37 mm) was noticed against *S. aureus,* followed by *B. cereus* at 31mm; however, the lowest IZD recorded against *K. pneumonia* and *S. typhi* with 25 and 24mm, respectively. *S. aureus* was vulnerable to MS58, recording the lowest MIC of 5 µg/mL, and *S. typhi* was the most resistant, which inhibited at 15 µg/mL

Growth performance: Table 2 shows the effect of the inclusion of *Paenibacillus xylanexedens* MS58 at three levels (0.3, 0.9, and 1.5) mg/kg feed in broilers' diet on

Table 1: Antibacterial activity of *Paenibacillus xylanexedens* MS58 against pathogenic bacteria presented as IZDs (mm)

Bacterial strain	Bacterial supernatant concentration (%)				
	15	30	45	60	MIC
BC	21±0.0	25±0.2	28±0.1	31±0.1	10
LM	19±0.3	22±0.1	26±0.4	29±0.7	10
SA	25±0.8	28±0.6	33±0.3	37±0.1	5
EC	16±0.1	19±0.0	22±0.5	27±0.3	10
ST	14±0.2	17±0.2	20±0.8	24±0.5	15
KP	15±0.7	18±0.1	21±0.1	25±0.3	15

Values are presented as mean ±SD, n=3, *Bacillus cereus* (BC), *Listeria monocytogenes* (LM), *Staphylococcus aureus* (SA), *Escherichia coli* (EC), *Salmonella typhi* (ST), and *Klebsiella pneumonia* (KP).

Table 2: Effect of dietary treatments of *Paenibacillus xylanexedens* MS58 at three levels on broiler growth performance.

Treatments (mg/kg)	LBW (g)		BWG (g)	FI (g)	FCR	GR	PI
	1d	35d	1-35d	1-35d	1-35d	1-35d	1-35d
Control	45.01	2200.11^{b}	2154.53^{b}	3755.00^{c}	1.77a	192.61b	120.61c
PX0.3	45.44	2290.35^{ab}	2244.56^{ab}	3772.14^{b}	1.62b	194.18a	132.22b
PX0.9	45.30	2310.69^{a}	2264.53^{ab}	3741.43^{c}	1.65b	195.56a	139.64ab
PX1.5	45.11	2350.63^{a}	2304.85^{a}	3811.18^{a}	1.64b	195.99a	145.55a
p-value	0.91	0.01	0.02	0.001	0.02	0.001	0.01

a-c Means within the same column with different superscripts differ significantly ($p \leq 0.05$) SEM[1]: Pooled standard error, LBW: Live body weight BWG: body weight gain, FCR: feed conversion ratio, PI: performance index, GR: growth rate, PX0.3= basal diet + 0.3 mg PX/kg, PX0.9 = basal diet + 0.9 mg PX/kg, PX1.5 = basal diet + 1.5 mg PX/kg.

body structure parameters (GR, BW, WG, PI, and FCR) during the growth period or experimental period of 35d age. Adding PX to a 1.5 mg/kg diet of chicks significantly enhanced all growth parameters; the chicks offered with a diet containing PX 1.5 gained the highest body weight during 35 days (2.304 kg) with a relative increase of 10 % compared to control. These increased because the appetite of these chicks enhanced, recording the highest feed intake (3811g) compared to 3755g in the control group, followed by an increase in the growth rate of 195.99 and performance index of 145 with a relative increase of 21% than control; however, the FCR value decreased. All performance indicators of the broilers that received diets containing PX were determined to be superior to those of broilers fed the control diet ($p < 0.001$). This enhancement in the performance of broilers can be attributable to PX, which might increase the digestibility of fiber and protein.

Liver and kidney enzyme parameters: The impact of supplementing broiler chicks with varying concentrations of PX (0.3–1.5 mg/kg food) on their renal and liver status is illustrated in Table 3. The addition of PX to chicks' diet at three levels gradually regulated the activity of liver and kidney enzymes. The liver enzymes, ALT and AST, were significantly lowered in the PX 1.5 treatment. The values of ALT activity were 219, 205, and 194 u/g in PX treatments, while in the control group, the activity of ALT was 250; similarly, the activity of AST in control was 6.5 U/g that gradually lowered by PX treatments as 5.1, 4.3, 3.9. The ALT activity decreased by 25% and AST by 67% compared to control. Regarding the kidney parameters, uric acid recorded various values between control and treatments, where in control was 5.31 and PX treatments were 4.8, 4.1, and 4.5; the uric acid lowered by dietary PX with a relative decrease of 18 %; the creatinine level slightly reduced from 0.32 to 0.28. Fortifying the broiler diet with PX 1.5 mg/kg considerably benefits the broiler health, including preserving standard liver size and function.

Table 3: Effect of *Paenibacillus xylanexedens* MS58 dietary treatments on broilers' serum biochemical parameters.

Serum biochemical parameters	Treatments (mg/kg)				*P* value
	Control	PX0.3	PX0.9	PX1.5	
Liver functions (U/L)					
AST	250^{a}	219^{b}	205^{c}	194^{d}	0.001
ALT	6.5^{a}	5.1^{b}	4.3^{c}	3.9^{d}	0.001
Kidney functions (mg/dl)					
Uric acid	5.31^{a}	4.8^{b}	4.1^{c}	4.5^{bc}	0.0101
Creatinine	0.32	0.30	0.29	0.28	0.045
Lipid profile					
Abdominal fat	1.1^{a}	0.91^{ab}	0.88^{b}	0.81^{c}	0.025
HDL (mg/dl)	91.9^{bc}	93.4^{b}	94.5^{ab}	95.3^{a}	0.011
LDL (mg/dl)	43.2^{a}	31.2^{b}	28.6^{c}	21.7^{d}	0.001
Total cholesterol (mg/dl)	136.1^{a}	118.4^{b}	110.3^{c}	102.8^{d}	0.001
Immune response					
IgG (mg/dl)	950.0^{c}	1061.2^{b}	1075.9^{b}	1190.3^{a}	0.012
IgA (mg/dl)	180.2^{c}	198.5^{b}	195.6^{b}	220.9^{a}	0.015

n=3 a-d Means within the same row with different superscripts differ significantly ($P \leq 0.05$). ALT Alanine aminotransferase: AST Aspartate aminotransferase, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, T_3: Triiodothyronine T_4: Thyroxine IgG, IgA Immunoglobulins Isotypes G, and A. PX0.3= basal diet + 0.3 mg PX/kg, PX0.9 = basal diet + 0.9 mg PX/kg, PX1.5 = basal diet + 1.5 mg PX/kg.

Lipid profile: The effects of feeding broiler chicks with varying concentrations of PX on total cholesterol, its components, and % belly fat are illustrated in Table 3. The addition of dietary PX significantly regulated the lipid profile in chicks; the abdominal fat reduced from 1.1 to 0.8% in the PX 1.5 treatment with a relative decrease of 25%. The total cholesterol recorded was 136 mg/dl in control, which gradually decreased in PX (118.4, 110.3, and 102.8 mg/dl); the total cholesterol decreased by 33 %; consequently, the LDL levels decreased by 50 % from 43.2 mg/dL in control to 21.7 mg/dl in PX 1.5 treatment. However, HDL increased (95.3 mg/dl) compared to control (91.9 mg/dl). Lowering the bad lipids in chicks considerably enhances their health.

Immune response: The findings given in Table 3 presented the influence of PX on immunoglobulins levels

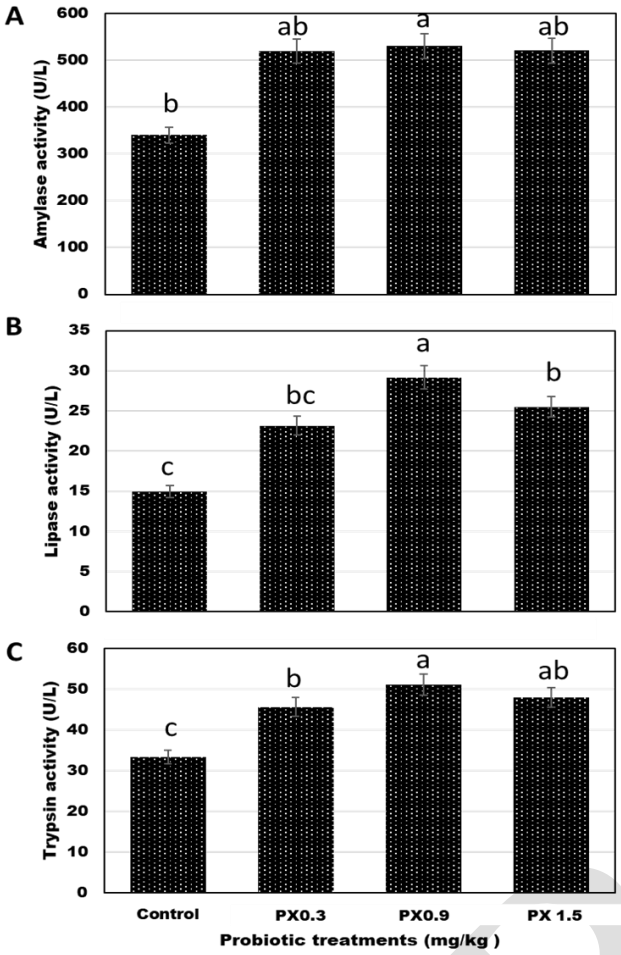

Fig. 1: Effect of dietary treatments *Paenibacillus xylanexedens* MS58 on serum digestive enzymes of broilers. [a-b:] Means within the same column with different superscripts differ significantly (P≤0.05). PX0.3= basal diet + 0.3 mg PX/kg, PX0.9 = basal diet + 0.9 mg PX/kg, PX1.5 = basal diet + 1.5 mg PX/kg.

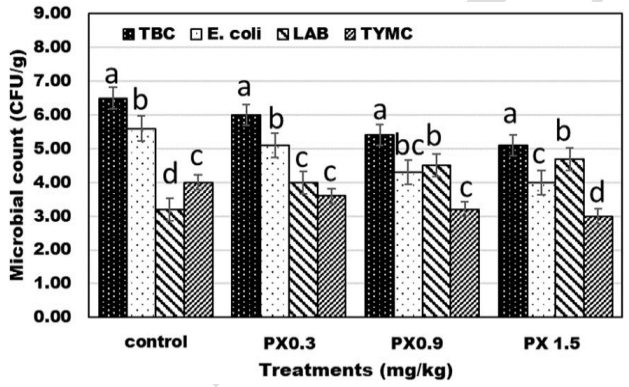

Fig. 2: Effect of dietary treatments *Paenibacillus xylanexedens* MS58 on the intestinal microbial count of broilers. TBC, total bacterial count, TYMC, total yeast and mold count, *E. coli: Escherichia coli* CFU/g: logarithm of colony forming unit per gram of digesta LAB: *Lactobacillus* spp. PX0.3= basal diet + 0.3 mg PX/kg, PX0.9 = basal diet + 0.9 mg PX/kg, PX1.5 = basal diet + 1.5 mg PX/kg

in broiler chicks. The immune response of chicks significantly improved with adding the dietary PX at three levels. The immunoglobulin G increased with adding PX, recording 1061.2, 1075.9, and 1190.3 for PX 0.3, 0.9, and 1.5 mg/kg, respectively; these values increased by 15-25 % compared to control. On the other hand, immunoglobulin A also increased with the addition of PX, where the PX 1.5 increased the immune response by 23 % compared to control from 180 to 220 mg/dl.

Digestive enzymes: The Fig. 1 illustrates the effect of increasing PX doses on digestive enzyme levels (amylase, lipase, and trypsin) in blood serum. The enzyme values increased steadily from control group (lowest values) to the 1.5 mg/kg PX dose (29.55, 51.89, and 533.11, U/L for lipase, trypsin, and amylase, respectively). However, adding PX at 1.5 mg/kg to the broiler diet reduced intestinal enzyme activity, i.e., 24.89, 47.56, and 519.17 U/L respected enzymes. This suggests that other factors, such as gut microbiota, may influence the relationship between PX and digestive enzymes.

Intestinal microbial count: The Fig. 2 shows the number of microorganisms in the gut. The total bacteria, yeast and molds, and *E. coli* are highest in the control group (6.5, 4, and 5.6 Log_{10} CFU/g, respectively). Adding PX to the diet reduces the number of these microorganisms, with the lowest significant values occurring at the highest PX level (BT 1.5; 5.1, 3, and 4 Log $_{10}$ CFU/g, respectively). In contrast, the number of lactic acid bacteria is lowest in the control group (3.2) and increases with the addition of PX to the diet, reaching a peak at the highest PX level (BT 1.5; 4.7 Log $_{10}$ CFU/g).

Meat quality: The Table 4 shows that the chemical composition of chicken breast fluctuated withadding PX levels to the broiler diet; the BT 0.9 group recorded the best value among the other groups. The moisture content increased by 20% and protein content by 10% compared to the control; the enhancement in moisture and protein in the breast significantly affected the sensory parameters (juiciness and tenderness), which they scored the highest values (9 and 8.8) in PX 0.9 group by panelists. The enhanced test was found in the control and PX 0.9 groups (Table 5). The fat and ash content decreased by adding PX levels. As for the quality parameters of meat, pH increased to 6.7, and the nitrogen compounds and TBA decreased to 4.7 and 0.2, respectively (Table 4). The color parameters were significantly affected by PX addition, where the lightness of the meat was enhanced, while *a* and *b* were not affected (Table 5).

Table 4: Effect of *Paenibacillus xylanexedens* MS58 dietary treatments on meat quality of broiler parameters.

Parameters	Treatments (mg/kg)				*P* value
	Control	PX0.3	PX0.9	PX1.5	
Chemical composition					
Moisture	63.3^{c}	66.3^{b}	69.1^{a}	65.2^{b}	0.01
Protein	20.36^{c}	21.6^{b}	22.5^{a}	21.1^{b}	0.015
Fat	14.2^{a}	11.3^{b}	8.1^{c}	10.3^{b}	0.01
Ash	0.9^{a}	0.8^{b}	0.31^{d}	0.6^{c}	0.001
pH	5.8^{c}	5.9^{c}	6.7^{a}	6.0^{b}	0.01
TVBN	6.5^{a}	5.4^{b}	4.7^{c}	5.1^{bc}	0.01
TBA	0.6^{a}	0.4^{b}	0.2^{d}	0.38^{c}	0.0011

n=3 a-d Means within the same row with different superscripts differ significantly (P≤0.05). Total Volatile Basic Nitrogen (TVBN), Thiobarbituric acid (TBA). PX0.3= basal diet + 0.3 mg PX/kg, PX0.9 = basal diet + 0.9 mg PX/kg, PX1.5 = basal diet + 1.5 mg PX/kg.

Table 5: Effect of *Paenibacillus xylanexedens* MS58 dietary treatments on meat color and sensory properties of broiler.

Color parameters	Treatments (mg/kg)				*p*-value
	Control	PX0.3	PX0.9	PX1.5	
L	60^{ab}	60^{ab}	61^{a}	58^{b}	0.045
a	6^{a}	5.8^{a}	5.9^{a}	5.4^{b}	0.049
b	14^{a}	14.1^{a}	14.3^{a}	14.5^{a}	0.12
	Sensory properties				
Jiuicness	8.9^{b}	8.9^{b}	9^{a}	8.6^{c}	0.01
Tenderness	8.7^{a}	8.5^{b}	8.8^{a}	8.4^{b}	0.03
Aroma	8.5^{a}	8.0^{b}	8.1^{b}	8.0^{b}	0.041
Taste	8.8^{a}	8.3^{c}	8.7^{a}	8.6^{b}	0.023

n=3 a-d Means within the same row with different superscripts differ significantly ($p \leq 0.05$). PX0.3= basal diet + 0.3 mg PX/kg, PX0.9 = basal diet + 0.9 mg PX/kg, PX1.5 = basal diet + 1.5 mg PX/kg. L, lightness; a, Redness; b, blueness.

DISCUSSION

Antimicrobial resistance is characterized by bacterial resistance to the antimicrobial medications that are employed to treat their associated diseases (Osei Sekyere and Mensah, 2020; Samad *et al.,* 2022). Nevertheless, the popular viewpoint is mostly linked to the improper use or overutilization of antibiotics in both human and animal populations (Chiș *et al.,* 2022). The most urgent challenge in treating infectious illnesses is the dissemination of related genes and antibiotic resistance among populations of aggressive pathogens (Alenazy, 2022). During the initial stages of antibiotic utilization, drugs targeted genes alterations, the principal source of antibiotic resistance. Horizontal gene transfer, which refers to the capacity of bacteria to exchange genes, plays a pivotal role in developing and disseminating the antibiotic resistance among dangerous bacteria (Xavier *et al*., 2019; Wainstein *et al*., 2022).

Those responsible for the veterinary sector play a critical role in managing antimicrobial resistance through the regulation and supervision of the antimicrobial usage in animals, the provision of guidance to farmers and animal owners regarding responsible antimicrobial usage, and the strategy formulation and data sharing facilitation with the human health field (Palma-Lara *et al.,* 2020).

Understanding the mechanisms of resilience is crucial in order to develop novel approaches to counteract this menace. Hence, we conducted this investigation by extracting *Bacillus* isolates from marine sediments, screening for the most NaCl-tolerant bacteria, and classifying them using biochemical assays and MALDI-TOF analysis. Instead of antibiotics, *Paenibacillus xylanexedens* MS58 was included in the broiler's food. The inclusion of PX in the diet significantly improved the growth performance metrics; The impact of our isolate is similar when applying the microorganisms as feed additives as in the study of Hatab *et al*. (2016) on broilers, (Czech *et al.,* 2020) on turkey, (Soomro *et al.,* 2019) on quail, and Ye *et al.* (2021) on partridge. Furthermore, Zhang *et al*. (2021) recently reported that the addition of microorganisms significantly increased the liveweight (LB), FCR, and mean daily gain of female chickens aged 42 days.

However, other studies Karimi Torshizi *et al*. (2010) stated that adding microorganisms in poultry diet decreased average daily intake and average daily gain in male chickens, they concluded that the addition of microbial supplements to feeding diets or drinking water did not have a significant impact on the growth performance of broilers.

Multiple studies demonstrate the function and significance of EM in enhancing feed consumption efficiency (Kulkarni *et al.,* 2022). Supplementation with efficacious microorganisms raised digestibility, decreased the amount of feed eaten and improved animal growth performance (Ye *et al*., 2021).

Enhancing the growth performance of animals by supplementing diets with PX may occur through the reduction of detrimental bacteria (*E. coli*) and the proliferation of *Lactobacillus* in their gastrointestinal tract (Xu *et al*., 2019). Adding microorganisms to broiler feeds improves intestinal pH, digestive enzyme activity, and the structure of intestinal bacteria (Liu *et al.,* 2018). And that may explain the enhancement in the performance of broilers can be attributable to PX, which might increase the digestibility of fiber and protein.

Moreover, the inclusion of PX in the feed of broilers demonstrated an improvement in liver enzymes, which is consistent with Kakhki *et al*. (2016), who reported that probiotics did not affect the amounts of ALT and AST in the serum. Although liver cells are vital for detoxification, they are susceptible to harm caused by toxins in meals. Liver health may be evaluated by measuring AST and ALT levels in the blood; ALT is a more specific sign of damage to liver cells than AST (Wang *et al.,* 2020). Elevated concentrations of serum liver enzymes indicate liver damage; conversely, decreased levels indicate enzyme leakage. There were no statistically significant impacts of dietary probiotics on blood creatinine levels. In contrast, the amounts of cholesterol, uric acid, ALT, triglycerides, serum AST and (TG) were all considerably lower in all experimental groups than in the control group (Hatab *et al*., 2016).

PX influences the diet as follows, in accordance with the principle that it preserves the typical proportions of organs, i.e., the liver. The therapies did not affect the liver weight (Musazadeh *et al.,* 2022). Also, a probiotic or probiotic mixture increased the weight of the thymus, bursa of fabricius, and spleen of broilers. Based on prior research, various PPs had the most significant impact on the broiler spleen index in our investigation (Tarradas *et al.,* 2020).

Probiotics are living microorganisms that influence serum triglycerides and total cholesterol when introduced to meals. The present findings are in accordance with Wajizah (2021). Various studies stated that chicken feed supplemented with probiotics could exhibit certain beneficial properties through the inhibition of blood triglyceride and cholesterol levels. Also, Shah *et al.* (2022) demonstrate that supplementing avian species with probiotics can lower their blood total cholesterol levels. This might result from several processes, including increased bile acid excretion: Probiotics can facilitate the elimination of bile acids via the feces after cholesterol has been transformed into bile acids.

Consequently, the quantity of cholesterol in the bloodstream is decreased. Probiotics can also inhibit the activity of hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, an enzyme that participates in

the cholesterol production process. Bile salt recycling can be diminished, and probiotics can inhibit this process in the gastrointestinal tract. As a result, bile acids are produced with more cholesterol, which reduces serum cholesterol levels.

Inclusion of PX in the feed of IR, broilers improves immunity, consistent with Elbaz *et al.* (2021), which showed that probiotic-containing meals enhanced the blood triiodothyronine level of broiler chickens. Hatab *et al*. (2016) identified a potential causal relationship between thyroid serum hormone and electromagnetic interference's growth-promoting effects in layer hens. Probiotics can enhance immune cell proliferation and secretion and stimulate nonspecific immunological responses by inducing macrophage phagocytic activity.

On the other hand, Fazelnia *et al*. (2021) cited as the reason why probiotic supplementation in avian diets increases blood antibody production, namely Immunoglubins (A and G) and interferon γ. In contrast to the results of the current inquiry, previous research documented a substantial elevation in the Immunoglubins (A and G) levels in male and female broilers by the administration of probiotics. An increased mass of the follicular fluid, spleen, and thymus in chickens indicates a more robust immune system (Slawinska *et al.*, 2014). A study by Zhang *et al*. (2021) also found that supplementing broiler chickens with probiotics reduces harmful *E. coli* and *Salmonella* in their stomachs while boosting digestive enzyme production.

In a recent study, Ye *et al*. (2021) discovered that adding probiotics increased amylase and protease activity. *Salmonella* and *E. coli* are harmful bacteria seldom observed in chicken husbandry (Vieco-Saiz *et al.*, 2019). Supplementation with PX lowered *E. coli* and increased *Lactobacillus* numbers in this research. The findings are consistent with those documented by Zhang *et al*. (2021), who observed a comparable pattern in the intestinal microbial community of broilers that were supplemented with probiotics. In general, introducing PX supplementation into animal feed may enhance competitiveness in the intestinal microbiota in favor of beneficial bacteria. The current results indicate that the inclusion of probiotics in the diets of broiler chickens may increase their economic efficiency, which is consistent with the findings of Zaghari *et al*. (2020).

Conclusion: The inclusion of probiotics in the feed of broiler chicks improved not only growth performance but also carcass quality, certain blood metabolites, and enzymes, hence enhancing their economic efficiency. The gut health improvements in broiler chicks supplemented with probiotics were marked by a rise in lactobacillus populations and a decline in *E. coli* levels. These positive gut changes ultimately resulted in enhanced meat quality.

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