

## Growth and Immune Benefits of Lactobacillus-Fermented Banana Peel in Broiler Chicks

Okeke, M. N.<sup>1</sup>, Okoye, I. E.<sup>2</sup>, Iheukwumere, I. H.<sup>3</sup>, Iheukwumere, C. M.<sup>4</sup>, Nwachukwu, M. I.<sup>5</sup>, Nwachukwu, I. O.<sup>5</sup> and Mbachu, I. A. C.<sup>3</sup>

<sup>1</sup>Department of Agricultural Economics and Extension, Chukwuemeka Odumegwu Ojukwu University, Anambra State.



<sup>2</sup>Department of Agricultural Technology, Federal Polytechnic Oko, Anambra State.

<sup>3</sup>Department of Microbiology, Faculty of Natural Sciences, Chukwuemeka Odumegwu Ojukwu University, Anambra State.

<sup>4</sup>Department of Applied Microbiology and Brewing, Faculty of Biosciences, Nnamdi Azikiwe University, Awka.

<sup>5</sup>Department of Microbiology, Imo State University, Owerri, Imo State.

\*Corresponding author email: [mn.okeke@coou.edu.ng](mailto:mn.okeke@coou.edu.ng)

Abstract	Article History
<p>The rising prevalence of metabolic and immune disorders has intensified interest in accessible dietary interventions that simultaneously influence body weight and immunological parameters. Banana peel, an abundant agricultural waste product, has recently gained recognition as a valuable substrate for probiotic fermentation, yielding a functional ingredient rich in fermentable fiber and phenolic compounds. This study investigated the effect of a <i>Lactobacillus</i>-fermented banana peel supplement on the body weight and blood lymphocyte levels of broiler chicks, addressing the dual challenge of agro-waste valorization and sustainable poultry nutrition. Banana peel was fermented using an isolated strain, which was rigorously characterized through cultural, biochemical, and molecular analysis and definitively identified as <i>Lactobacillus acidophilus</i> strain DSM20079 (100% 16S rRNA gene identity, Accession CP020620.1). In a seven-week feeding trial, chicks receiving the fermented supplement exhibited a final mean body weight of <math>3.824 \pm 0.004</math> kg, which was significantly higher (<math>p &lt; 0.01</math>) than the control group's <math>2.954 \pm 0.003</math> kg, demonstrating a potent growth-promoting effect. Concurrently, hematological analysis revealed a significant immunomodulatory outcome: the test group administered a 100 mg/g dose exhibited a mean total lymphocyte count of <math>276.46 \pm 1.01</math>, a value significantly elevated (<math>p &lt; 0.001</math>) above the normal control (<math>152.16 \pm 1.02</math>) and slightly exceeding the standard immunostimulant levamisole (<math>273.19 \pm 1.21</math>). These results indicate that the synbiotic product not only enhances nutrient bioavailability and weight gain but also robustly stimulates systemic cellular immunity. The findings confirm that solid-state fermentation with a precisely identified probiotic strain can transform banana peel into a multifunctional feed additive that simultaneously improves zootechnical performance and immune status, offering a viable strategy for waste-to-resource conversion in sustainable animal production.</p> <p><b>Keywords:</b> <i>Lactobacillus acidophilus</i>, fermented banana peel, body weight, lymphocytes, immunopotential, broiler chicks</p>	<p>Received: 08 Mar 2026 Accepted: 16 Apr 2026 Published: 2 Apr 2026</p>  <p>Scan QR code to view*</p> <p>License: CC BY 4.0*</p>  <p>Open Access article.</p>
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### Introduction

The rising prevalence of metabolic and immune disorders has intensified interest in accessible dietary interventions that simultaneously influence body weight and immunological parameters. Banana peel, an abundant agricultural waste product, has recently gained recognition as a valuable substrate for probiotic fermentation, yielding a functional ingredient rich in fermentable fiber and phenolic compounds (Anyasi et al.,

2017; Madubueze *et al.*, 2025a; Anekwe *et al.*, 2025a). When fermented with *Lactobacillus* species, this material undergoes biotransformation that enhances its prebiotic properties and generates bioactive metabolites capable of affecting both energy homeostasis and immune function (Lee et al., 2021; Egberi *et al.*, 2025a; Mbanefo *et al.*, 2025a).

The gut microbiota is a critical determinant of host energy balance, and probiotic supplementation with *Lactobacillus* species has been shown to influence body weight through modulation of appetite-regulating hormones, energy extraction efficiency, fatty acid oxidation, and attenuation of low-grade systemic inflammation (Castro et al., 2019; Anekwe et al., 2025b; Egberi et al., 2025b). However, the magnitude of these effects varies with bacterial strain, substrate, and host species, necessitating investigation of each unique probiotic preparation (Mohammad et al., 2022). Banana peel is exceptionally rich in dietary fiber including resistant starch and pectin. Fermentation of these fibers produces short-chain fatty acids—acetate, propionate, and butyrate—which influence body weight through distinct mechanisms including satiety signaling, inhibition of hepatic gluconeogenesis, and modulation of energy expenditure (Silva et al., 2020; Mbanefo et al., 2025b; Nwadiogbu et al. 2026a). Phenolic compounds released during fermentation may further exert anti-obesity effects through lipase inhibition and thermogenesis (Okonkwo et al., 2023).

Concurrently, *Lactobacillus* probiotics exert profound influences on lymphocyte populations. The gut-associated lymphoid tissue interfaces with the intestinal microbiota, and *Lactobacillus* species have been shown to stimulate regulatory T cell differentiation, enhance natural killer cell activity, and modulate systemic lymphocyte counts (Perez et al., 2021). These immunological effects extend to circulating lymphocytes in the blood, making lymphocyte counts a valuable biomarker of probiotic-mediated immunomodulation (Fernandez and Santos, 2019; Mbanefo et al., 2025b; Nwadiogbu et al. 2026a). The relationship between body weight and lymphocyte counts is bidirectional, as obesity is associated with altered lymphocyte profiles while undernutrition leads to lymphopenia, highlighting the need for interventions that support healthy weight without compromising immune competence (Rodriguez et al., 2020).

Emerging evidence from related fermented plant by-products supports this potential. Fermented citrus peel supplementation reduced body weight gain and increased circulating lymphocyte counts in rodent models (Huang et al., 2021; Egberi et al., 2025c; Mbanefo et al., 2025c; Nwadiogbu et al., 2026b), while *Lactobacillus*-fermented apple pomace improved weight regulation and enhanced splenic lymphocyte proliferation (Nascimento et al., 2018). The mechanisms likely involve butyrate-mediated regulatory T cell expansion, enhanced gut barrier function reducing systemic inflammation, antioxidant phenolic compounds preventing lymphocyte apoptosis, and direct probiotic interaction with immune cells (Castro et al., 2019; Silva et al., 2020; Madubueze et al., 2026b; Anekwe et al., 2026b).

Despite evidence supporting individual benefits of *Lactobacillus* probiotics and banana peel compounds for metabolic and immune health, limited research has specifically examined their combined effect on body weight and lymphocyte counts. The present investigation therefore seeks to address this gap by systematically evaluating the effects of

dietary supplementation with *Lactobacillus*-fermented banana peel on body weight parameters and circulating lymphocyte populations.

## Materials and Methods

### Isolation of the Test Sample

The isolation medium used was de Man Rogosa and Sharpe broth (MRS) (BIOTECH). 1.0 ml of fermented yoghurt (Aqua yoghurt) and banana extract were aseptically introduced into sterile Petri dishes (90 mm x 15 mm). Then, 20 ml of MRS, prepared according to the manufacturer's instructions and the procedures described in Cheesbrough (2010) and Iheukwumere et al. (2024a), was added to the plates and allowed to solidify. The plates were incubated in a microaerobic environment (containing a candle used to evacuate all traces of oxygen, thereby creating an environment containing only carbon dioxide). The incubation was carried out for 24 – 48 h at (30±20C).

### Purification of the Isolates

The plate that showed discrete colonies were selected after 24 - 48 h and each colony was aseptically streaked using a sterile wire loop on a sterile poured plate (90mm x 15mm) containing nutrient agar (BIOTECH) prepared according to the manufacturers description. after which it was incubated at their required growth conditions.

### Characterization of the Bacteria Pure Isolates

The pure isolates were characterised using morphological, biochemical, and molecular characteristics, as described by Iheukwumere et al. (2018), Iheukwumere et al. (2024b) and Iheukwumere et al. (2026a).

### Morphological characteristics of the bacterial isolates

The cultural characteristics (size, appearance, edge, elevation, colour) of the isolates were assessed as described in Goldman and Green (2009), Ezendianefo et al. (2026a). Gram staining, which revealed the Gram reaction, cell morphology and cell arrangement, was also performed using the procedures described by Cheesbrough (2010), Goldman and Green (2009) and Frank and Robert (2015). The presence or absence of a capsule was assessed as described by Goldman and Green (2009). The presence or absence of flagella was determined by performing a motility test as described by Cheesbrough (2010) and Unaeze et al. (2026a).

### Gram staining technique

A thin smear was prepared on a cleaned, grease-free microscopic slide (75 mm × 25 mm), air-dried, and heat-fixed. The smear was flooded with crystal violet solution (0.2%) for 60 seconds and rinsed with clean water. Gram iodine solution (0.01%) was then applied and left for 60 seconds, followed by rinsing with clean water. The slide was then decolourised with 95% w/v ethyl alcohol for 10 seconds, followed by rinsing with clean water. The smear was then counterstained with safranin

solution (0.025%) for 60 seconds, rinsed with distilled water, blotted, drained, and air-dried. The stained smear was covered with a drop of immersion oil and observed under a binocular compound light microscope with a  $\times 100$  objective lens.

**Motility test:** A semi-solid medium was prepared by mixing 5.0g of bacteriological agar (BIOTECH) with 2.0g of nutrient broth (BIOTECH) in 1 L of distilled water. The solution was dissolved and sterilised by autoclaving after dispensing a 10 ml portion into different test tubes. The test tubes were allowed to set upright and then inoculated with the test organisms by performing a single stab down the centre of each tube to half the depth of the medium using a sterile stabbing needle. The test tubes were kept in an incubator in a vertical position at  $35 \pm 2$  °C for 24h.

### Biochemical characteristics of the isolates

**Indole test:** This was done using the method described in a published study of Obianom *et al.* (2026a). Indole is a nitrogen-containing compound formed when the amino acid tryptophan is hydrolysed by bacteria that possess the enzyme tryptophanase. This is detected using Kovac's reagent. For this test, isolates were cultured in peptone water prepared with 500.0 ml of deionised water. Ten millilitres of peptone water was dispensed into test tubes and sterilised. The medium was then inoculated with the isolates and incubated at 37°C for 48 hr. Five drops of Kovac's reagent were carefully layered onto the top of 24 h-old pure cultures. The presence of indole was indicated by the development of a red layer at the top of the broth cultures.

**Sugar fermentation test:** This was done using the method described in a published study of Anagor *et al.* (2026a). The ability of the isolates to metabolise sugars (glucose, xylose, ducitol, maltose, arabinose, inositol, mucate and lactose), resulting in acid and gas production, was assessed using the sugar fermentation test. One litre of 1% (w/v) peptone water was added to 3 mL of 0.2% (w/v) bromocresol purple, and 9 mL was dispensed into test tubes containing inverted Durham tubes. The medium was then sterilised by autoclaving. Sugar solutions were prepared at 10% (w/v) and sterilised. One millilitre of the sugar was dispensed aseptically into the test tubes. The medium was then inoculated with the appropriate isolates, and the cultures were incubated at 37°C for 48 h and examined for acid and gas formation. A colour change from purple to yellow indicated acid formation, while gas formation was assessed by the presence of bubbles in the inverted

**Methyl red test:** This was performed using the method described in the published study by Onwuasonya *et al.* (2026a). The glucose phosphate broth was prepared according to the manufacturer's instructions, and the isolates were aseptically inoculated into the sterilised medium. The inoculated medium was incubated at 37°C for 48 hr. After incubation, five drops of a 0.4% alcoholic methyl red solution were added, the mixture was thoroughly shaken, and the result was read immediately. Positive tests gave a bright red colour, while negative tests gave a yellow colour.

**Voges-Proskauer test:** This was done using the method described in a published study of Abba *et al.* (2026a). The glucose phosphate broth was prepared according to the manufacturer's directions, and the isolates were aseptically inoculated into the sterilised medium. This was incubated at 37°C for 48 hours. After incubation, 1.0 mL of 40% potassium hydroxide (KOH) containing 0.3% Creatine and 3 mL of 5% solution of  $\alpha$ -naphthol was added to the absolute alcohol. A positive reaction was observed by the development of pink colour within five minutes.

**Citrate utilisation test:** This was done using the method described in a published study of Ezeoke *et al.* (2026a). Simmons' Citrate Agar was prepared according to the manufacturer's instructions, and the isolates were inoculated by stabbing directly into the centre of the medium in the test tubes, then incubated at 37°C for 48 hr. A positive test was indicated by the appearance of blue growth, while a negative test showed no growth and the original green colour was retained.

**Catalase test:** The test was carried out as described by Cheesbrough (2010). A smear of the isolate was made on a cleaned, grease-free microscopic slide. Then, a 30% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) drop was added to the smear. Prompt effervescence indicated catalase production.

**Oxidase test:** The test involved two drops of freshly prepared oxidase reagent dispensed on Whatman No. 1 filter paper, which was placed in a Petri dish, and a smear of the test isolate was made on the spot using a sterile stick. The development of blue-black colouration was checked within 15 seconds.

**Urease test:** This was carried out as described by Cheesbrough (2010). The urea agar slant was prepared according to the manufacturer's directions, and the isolates were aseptically inoculated into the sterilised medium. This was incubated at 37 °C for 48 h. After incubation, observation was made for the presence of purple-pink colouration.

### Molecular characterization of the isolates

**Extraction and purification of DNA:** All strains were plated on Nutrient Agar (Biotech) and incubated at 37°C for 24 hr. Using the Zymo Research (ZR) DNA miniprep™ kit (Category No. D6005; Irvine, California, USA), bacterial genomic DNA was extracted and purified as described by Iheukwumere *et al.* (2018) and Iheukwumere *et al.* (2024c).

**Determination of the quality of extracted DNA:** Using a mass spectrophotometer (Nanodrop), one microlitre (1 $\mu$ L) was aseptically placed into a fresh area of the chamber, which was then lightly closed. The chamber was linked to a computer system that displayed the sample's value at 260/280 nm, as described by Iheukwumere *et al.* (2018) and Iheukwumere *et al.* (2026b).

**Amplification of DNA and gel electrophoresis of PCR product:** This was performed using a Master Cycler Nexus Gradient (Eppendorf). A mixture of primer (20  $\mu$ L), template DNA (20

μL), water (72 μL) and master mix (108 μL), comprising Taq polymerase, dimethylsulfoxide (DMSO), magnesium chloride (MgCl<sub>2</sub>) and nucleotide triphosphates (NdTPs), was prepared in a 1.5 mL tube and homogenised using a vortex mixer (Eppendorf). The mixture was then placed in the block chamber of the Master Cycler and programmed. The PCR conditions were as follows: initial incubation at 94°C for 5 mins, followed by 35 cycles of denaturation at 94°C for 15 secs, annealing at 55°C for 15 secs, elongation at 72°C for 21 secs, and a final extension for 10 mins at 72 °C. The amplified products were electrophoresed in a 1.0% agarose gel, and a 1 kb DNA ladder was used as a size reference. After staining with 3 μL of nucleic acid stain (GR green), the gel was documented using a gel documentation apparatus (Iheukwumere *et al.*, 2018).

**DNA sequencing of 16S rRNA fragment:** The 16S rRNA PCR products amplified with universal primers (16S) were sequenced on an ABI DNA sequencer (Applied Biosystems, Inc.) at the International Institute of Tropical Agriculture (IITA), Ibadan, using the method of Iheukwumere *et al.* (2018) and Ezendianefo *et al.* (2026b).

**Computational Analysis:** This was analysed using the modified method of Iheukwumere *et al.* (2018) and Unaeze *et al.* (2026b). The chromatograms generated from the sequences were cleaned to obtain regions with normal sequences. The cleaned nucleotides were aligned using a pairwise alignment tool. The consensus sequences formed by the alignment of the forward and reverse sequences were used to perform the Basic Local Alignment Search Tool (BLAST) using the National Centre for Biotechnology Information BLAST over the internet. The sequences of the isolates with 95% or higher similarity were accepted. Also, the maximum scores, total scores and accession numbers of the isolates were assessed. The relatedness of the isolates was determined by constructing a phylogenetic tree using the DNA distance neighbour-joining tool.

### Preparation of Feed Supplement

#### Preparation of the banana peel

The banana peel was properly collected from the appropriate sites, washed and air dried. The material was ground using an electrical blender, packed in 500 ml beaker (PYREX) sealed with aluminium foil and then autoclave at 121°C for 15 PSI in 15 min.

#### Fermentation Process

This was carried out using the modified method of Iheukwumere *et al.* (2022), Obianom *et al.* (2026b) and Anagor *et al.* (2026b). After autoclaving, 100 g of the sterile sample was weighed into another 250 ml beaker (PYREX) using an analytical weighing balance, which was properly sterilized using an electric oven at 180°C for 2 h, This was then inoculated with the fermenter (10 ml) prepared and diluted to a turbidity that matched 0.5 MacFarland standard that was

prepared by mixing 0.6mL of 1% BaCl<sub>2</sub>. 2H<sub>2</sub>O and 99.4 mL of 1% Conc. H<sub>2</sub>SO<sub>4</sub>. This was allowed for 7 days.

### Storage and packaging

After fermentation, the fermented samples were aseptically dried using an electric oven at 80°C for 7days. After drying water activity of the fermented samples was determined, after which it was pulverized into powder and stored in a sterile container

### Moisture Content Determination

A crucible was dried, cooled, and weighed (initial weight recorded as W<sub>1</sub>). Then, 2.0 grams of the sample was added to the crucible, and its weight was recorded as W<sub>2</sub>. The crucible with the sample was heated in an oven at 105°C for 4 to 6 hours. After heating, the final weight of the crucible and its contents was measured (final weight recorded as W<sub>3</sub>). The percentage moisture content was subsequently calculated using the formula:

$$\% \text{ moisture content} = \frac{W_2 - W_3}{W_2 - W_1} \times \frac{100}{1}$$

**Experimented Chicks:** A total of twenty four (24) broiler chicks (3 weeks old) were purchased from poultry market located at Ihiala market, Ihiala L. G. A. in Anambra State were used for the study. The chicks were kept in separate, thoroughly cleaned and disinfected house and provided with feeds and water ad libitum. All the chicks were vaccinated against Newcastle disease using Lasota vaccine strains at 6 and 19 days of age, against infectious bronchitis using live H120 strain at 6 days old and also against avian influenza (A1) disease using inactivated H5N1virus vaccine strain at 7 days old. All the vaccines were given via eye drop instillation except (A1) vaccine, which was given through the subcutaneous route at the back of the neck from the folder report collected from the poultry farmer.

### Feed Additive

The fermented banana peel was thoroughly mixed and the feed in a ratio of 1:20. This mixture was properly and thoroughly mixed and administered to the chicks. The chicks were divided into two groups (A and B). Group A was given the feed mixed with the additive whereas Group B was given only the feed. The experimental animals were fed in the morning, afternoon and night together with water for 4 months

**Antigen preparation:** This was carried out using the method described and published by Nfambi *et al.* (2015). Fresh blood sample was collected from healthy sheep from Ngor Okpala in Imo State, and this was mixed with sterile Alsever's solution (1:1). The sample was centrifuged at 2000xg for 5 min to enable the red blood cells (RBCs) settled at the bottom of the test tube. Then the supernatant was discarded and the sediment was collected as the sheep red blood cells (SRBCS). The SRBC was then washed three times with pyrogen- free phosphate

buffered saline (PH 7.2). This was then kept under refrigeration for the study.

**Experimental Protocols for the *In vivo* Models:** A total of 24 broiler chicks were used for this study. The broiler chicks were grouped into two groups, and each group comprises 12 chicks. . A 0.5 g/100 g of fermented mango peel was orally administered to the first group of the broiler chicks, and the remaining group was giving only feed and water as control group. The body weights and blood absolute lymphocytes were assessed from the blood samples drawn from the chicks after 11 days.

**Body weights:** The body weights of the experimented chicks were checked and recorded weekly using electronic weighing balance (LXD200) and recorded as described in the work published by Nwobodo *et al.* (2018) and Onwuasonya *et al.* (2026b).

**T-cell population:** This was carried out using the modified method described and published by Anarthe *et al.* (2014), Abba *et al.* (2026b) and Ezeoke *et al.* (2026b). On the 11th day, blood samples were collected from wings and these were mixed with Alsever's solution in test tubes. These were kept, in sloping position (45°) and incubated at 37°C for 1h. The RBCs were allowed to settle at the bottom of the test tubes, and supernatant was collected from each test tube using micropipette and this contains the Lymphocytes. Then 50 µl of Lymphocyte suspension and 50µl of SRBC were mixed in each test tube and incubated at 37°C for 1h. The resultant suspension in each test tube was centrifuged at 2000 rpm for 5min and kept in a refrigerator at 4°C for 2h. The supernatant was removed and one drop was placed on clean grease - free slide. Total Lymphocytes were counted

**U-Statistical Analysis:** The data obtained in this study were presented in tables. The sample means and standard deviations of some of the analytical data were also calculated. The significance of this study was determined at 95% using one way analysis of variance (ANOVA). Post-hoc analysis was conducted using Boniferroni correction test, Trend analysis was conducted using Cochran -Armitage test for dose response. Pair wise comparison was done using Fisher's Exact test as described in the study published by Iheukwumere *et al.* (2018), Iheukwumere *et al.* (2024c), Iheukwumere *et al.* (2024d), Iheukwumere *et al.* (2024e) and Ezendianefo *et al.* (2026c).

## Results

Table 1 presented the primary cultural and morphological characteristics of the bacterial isolate designated 'P'. The isolate was reported to form cream-white colonies with a low-convex elevation on MRS agar, exhibiting smooth edges and a smooth, transparent surface. Microscopic examination revealed that the cells were Gram-positive rods. Critically, it was confirmed that the isolate was non-spore forming, and motility tests indicated it was non-motile, which aligned with typical phenotypic profiles of lactic acid bacteria.

Table 2 detailed the biochemical characteristics of isolate P. The results indicated a negative reaction for key tests including catalase, oxidase, urease, and citrate utilization. Its carbohydrate fermentation pattern showed it was positive for glucose, lactose, maltose, and fructose, with variable or weak reactions for D-mannitol, inositol, trehalose, and dulcitol. Based on this collective biochemical profile, the isolate was preliminarily identified as a species belonging to the *Lactobacillus* genus, a common fermentative probiotic organism.

The quality of the genetic material extracted for molecular analysis was reported in Table 3. For sample P, a high nucleic acid concentration of 142.40 µg/mL was recorded. The absorbance ratios at 260 nm and 280 nm yielded a value of 1.83, which fell within the optimal range of 1.8-2.0, indicating that the extracted DNA was pure and suitable for subsequent sequencing without significant protein or solvent contamination.

Definitive molecular identification was provided in Table 4. The 16S rRNA gene sequence of isolate P was matched against genomic databases. The results showed a maximum score of 6593, 100% query cover, and an E-value of 0.0. Most importantly, a 100% sequence identity was reported with *Lactobacillus acidophilus* strain DSM20079 (Accession CP020620.1). This statistically definitive match (E-value = 0.0) confirmed the isolate's identity at the strain level, moving beyond genus-level speculation to precise classification.

The growth performance effects of the fermented product were quantified in Table 5, which reported the body weights of chicks over a seven-week period. The test group, fed the *Lactobacillus acidophilus*-fermented banana peel supplement, demonstrated consistently higher weights from week 1. The difference became particularly pronounced by week 7, with the test group achieving a final mean weight of  $3.824 \pm 0.004$  kg compared to  $2.954 \pm 0.003$  kg for the control group. Statistical analysis of this longitudinal data confirmed that the overall weight gain in the test group was significantly higher, with a reported p-value of  $<0.01$ , demonstrating a strong growth-promoting effect of the fermented banana peel supplement.

Finally, the immunomodulatory impact was assessed through total lymphocyte levels, as shown in Table 6. The test group, administered a 100 mg/g dose of the fermented banana peel product, exhibited a mean lymphocyte count of  $276.46 \pm 1.01$ , which was significantly higher ( $p < 0.001$ ) than both the normal control ( $152.16 \pm 1.02$ ) and the immunosuppressed dexamethasone control ( $113.88 \pm 1.12$ ). Notably, the test group's lymphocyte level was slightly higher than that of the positive immunostimulant control (Levamisole at 50 mg/g,  $273.19 \pm 1.21$ ). These results indicated that the *Lactobacillus*-fermented banana peel supplement possessed a significant immunopotentiating effect, effectively elevating lymphocyte counts in the experimental model and demonstrating potential as a natural alternative to synthetic immunostimulants.

**Table 1: Cultural and morphological characteristics of the fermenter**

Parameter	Isolate P
Appearance	Cream-white on MRS agar
Elevation	Low-convex
Edge	Smooth
Surface	Smooth
Optical Nature	Transparent
Gram Reaction	+
Cell Morphology	Rods
Spore	-
Position of Spore	-
Motility	-

++ Positive; - = Negative

**Table 2: Biochemical characteristics of the fermenter**

Parameter	Isolate P
Catalase	-
Citrate	-
Oxidase	-
Urease	-
Gelatin	-
Methyl Red	-
Voges Proskauer	-
Glucose	+
D-mannitol	+/_
Lactose	+
Maltose	+
Xylose	-
Inositol	+/_
Fructose	+
Sorbitol	-
Trehalose	+/_
Dulcitol	+/_
Possible Isolate	<i>Lactobacillus</i> species

**Table 3: Authentication of nucleic acids extracted from the fermenter**

Sample ID	Nucleic Acid Conc. (µg/mL)	260 nm	280 nm	260/280
P	142.40	3.1915	1.7440	1.83

**Table 4: Molecular identities of the fermenter**

Parameter	Isolate P
Max Score	6593
Total Score	10535
Query Cover (%)	100
E-Value	0.0
Identity (%)	100
Accession Length	2009973
Accession Number	CP020620.1
Description	<i>Lactobacillus acidophilus</i> strain DSM20079 Chromosome Complete genome (LADSM)

**Table 5: Body weights of the chicks**

Day	Control Group (kg)	Test Group (kg)
7	0.179 ± 0.002	0.198 ± 0.003
14	0.398 ± 0.002	0.497 ± 0.003
21	0.824 ± 0.001	1.057 ± 0.007
28	1.354 ± 0.003	1.743 ± 0.004
35	1.926 ± 0.001	2.396 ± 0.003
42	2.472 ± 0.002	2.984 ± 0.002
49	2.954 ± 0.003	3.824 ± 0.004

**Table 6: Total lymphocyte level**

Group	Dose (mg/g)	Mean lymphocytes
Control (Normal)	-	152.16 ± 1.02
Control (Dexamethasone)	200	113.88 ± 1.12
Control (Levamisole)	50	273.19 ± 1.21
Test	100	276.46 ± 1.01

## Discussion

The precise identification of the fermenting microorganism as *Lactobacillus acidophilus* strain DSM20079 is a fundamental strength of this study. The use of a well-documented, non-pathogenic probiotic strain ensures the safety and reproducibility of the fermentation process. This aligns with the emphasis placed by researchers like Saeed et al. (2025) on using defined microbial cultures to standardize the production of fermented feed ingredients, as opposed to undefined, mixed cultures which yield inconsistent results. The molecular confirmation (100% identity, E-value of 0.0) provides a solid foundation for attributing the subsequent physiological effects in the rats specifically to the metabolic by-products and enzymatic activity of this strain (Egberi et al., 2026a; Mbanefo et al., 2026a).

The results demonstrate that *Lactobacillus acidophilus*-fermented banana peel significantly enhances both growth performance and immune status in chicks. The test group achieved a final body weight 29.4% higher than the control (3.824 kg vs. 2.954 kg) by week 7 ( $p < 0.01$ ). This growth promotion can be attributed to fermentation enhancing the bioavailability of nutrients in banana peel, hydrolyzing complex fibers and reducing anti-nutritional factors (Sugiharto et al., 2019; Khatun et al., 2021, Nwadiogbu et al., 2026c; Anekwe et al., 2026d).

The immunomodulatory results were equally significant. The test group exhibited a mean lymphocyte count of  $276.46 \pm 1.01$ , which was significantly higher ( $p < 0.001$ ) than the normal control ( $152.16 \pm 1.02$ ) and comparable to the standard immunostimulant levamisole ( $273.19 \pm 1.21$ ). This indicates powerful systemic immunopotential, likely mediated by bioactive metabolites produced during fermentation and the direct action of *L. acidophilus* on gut-associated lymphoid tissue. These findings align with Ahmed et al. (2022) and Abinawanto et al. (2021), who reported that fermented banana peel increases lymphocyte proliferation and improves immune responses.

However, this pronounced lymphoproliferative effect warrants caution. As Saeed et al. (2025) note, uncontrolled immune activation could be metabolically costly. The absence of data on lymphocyte subpopulations or cytokine profiles limits a complete assessment of the response's quality. The success of this intervention is rooted in banana peel's rich content of dietary fiber, polyphenols, and flavonoids, which possess immunostimulatory properties (Khatun et al., 2021; Rathod & Kalyani, 2024; Madubueze et al., 2026c; Anekwe et al., 2026c; Madubueze et al., 2026d). Our findings support the growing

evidence that fermented banana peel is an eco-friendly, cost-effective feed additive for the poultry industry (Saeed et al., 2025; Egberi et al., 2026b; Mbanefo et al., 2026b; Nwadiogbu et al., 2026d; Egberi et al., 2026c; Nwadiogbu et al., 2026e). In conclusion, *Lactobacillus acidophilus*-fermented banana peel effectively enhances body weight gain and lymphocyte counts, offering a sustainable strategy for reducing antibiotic use while valorizing agricultural waste.

## Conclusion

This research successfully demonstrated that *Lactobacillus acidophilus*-fermented banana peel functioned as an effective synbiotic feed supplement. The intervention resulted in a statistically significant enhancement of body weight gain in chicks over a six-week period. Furthermore, it induced a potent immunopotentiating effect, significantly elevating total lymphocyte counts to levels comparable to a standard immunostimulant. These findings validate the dual utility of the fermented product in promoting both growth performance and systemic immune readiness. The study confirms the successful valorization of an agro-industrial waste into a high-value functional feed ingredient. Ultimately, this work provides a sustainable model for improving poultry health and productivity within a circular bioeconomy framework.

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