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Lactobacillus acidophilus-Fermented Banana Peel Supplement Maintains Organ Weight Homeostasis and Serum Biochemical Integrity in Albino Wistar Rats

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Abstract

The global banana industry produces millions of tons of fruit annually for human consumption, generating an equivalent magnitude of banana peel waste that poses significant environmental disposal challenges. Yet, beneath this discarded by-product lies a rich reservoir of bioactive compounds, dietary fiber, and fermentable substrates that remain largely underutilized in functional food and nutraceutical applications. This study evaluates the effect of *Lactobacillus*-fermented banana peel on the organ weight and organ function of albino Wistar rats using *in vivo* techniques. Banana peel that was aseptically prepared and pulverized was fermented using a molecularly authenticated *L. acidophilus* strain (100% identity to DSM20079). This fermented product was fed to albino Wistar rats in a controlled trial to evaluate its effects on organ health, a critical preclinical safety parameter. Results demonstrated that the novel diet induced no statistically significant alterations in the absolute weights of vital organs, including the liver, kidneys, lungs, and heart ($p > 0.05$). Specifically, liver weights were identical at 6.31 ± 0.01 g, while kidney, lung, and heart weights showed negligible variations between control and test groups. Concurrently, comprehensive serum biochemical analysis revealed no significant differences in key markers of hepatic function (ALT, AST, LDH) or renal function (creatinine, urea) between the test and control groups ($p > 0.05$). The values for the test group were virtually identical to those of the control group: ALT (17.110 vs. 17.120 U/L), AST (25.280 vs. 25.660 U/L), creatinine (0.3903 vs. 0.3904 mg/dL), urea (7.9465 vs. 7.9468 mg/dL), and LDH (12.715 vs. 12.714 U/L). The data conclusively indicate that the fermented banana peel did not impose a toxicological burden or metabolic stress on the primary detoxification and excretory systems. This research successfully validates the physiological safety of *Lactobacillus*-fermented banana peel as a compatible functional ingredient. By demonstrating no adverse effects on organ integrity and function, the study provides essential preclinical evidence supporting the safe valorization of this agricultural by-product for potential applications in animal and human nutrition.

Keywords: *Lactobacillus acidophilus*, fermented banana peel, organ weight, liver function, kidney function, safety assessment, Wistar rats.

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Introduction

The global banana industry produces millions of tons of fruit annually for human consumption, generating an equivalent magnitude of banana peel waste that poses significant environmental disposal challenges. Yet, beneath this discarded by-product lies a rich reservoir of bioactive compounds, dietary fiber, and fermentable substrates that remain largely underutilized in functional food and nutraceutical applications (Madubueze *et al*., 2025a; Anekwe *et al*., 2025a). Banana peel (Musa paradisiaca and Musa acuminata) is compositionally

remarkable, containing substantial quantities of dietary fiber including pectin, cellulose, hemicellulose, and resistant starch, as well as phenolic compounds such as gallocatechin, dopamine, and catechin, along with essential minerals including potassium, magnesium, and calcium (Vu *et al*., 2018; Egberi *et al*., 2025a; Mbanefo *et al*., 2025a). When subjected to probiotic fermentation, particularly using Lactobacillus species, this agricultural waste material undergoes biotransformation that not only preserves but potentially enhances its bioactive properties, yielding a fermented product with significant promise for modulating physiological outcomes including organ development and systemic biochemical profiles (Pothavorn *et al*., 2019; Anekwe *et al*., 2025b; Egberi *et al*., 2025b).

The application of probiotics in animal and human nutrition has expanded considerably beyond traditional gastrointestinal health paradigms to encompass broader physiological effects including immune modulation, metabolic regulation, and even organ growth and development. Lactobacillus species, among the most extensively studied probiotic genera, have demonstrated the capacity to influence systemic physiology through multiple mechanisms including the production of short-chain fatty acids, the modulation of gut-associated lymphoid tissue, the regulation of metabolic hormones, and the attenuation of systemic inflammation (Wang *et al*., 2022; Mbanefo *et al*., 2025b; Nwadiogbu *et al*. 2026a). These effects, mediated through the gut-organ axis, have been shown to influence the development and function of organs beyond the intestinal tract, including the liver, spleen, pancreas, and even the central nervous system. Recent investigations have revealed that dietary supplementation with Lactobacillus-fermented products can significantly alter organ weights, histological architecture, and functional parameters in experimental animal models, suggesting that probiotic fermentation of plant substrates may serve as a strategic intervention for optimizing organ health and developmental outcomes (Liu *et al*., 2021; Madubueze *et al*., 2026a; Anekwe *et al*., 2026a).

Organ development, particularly during critical growth periods such as the post-weaning phase in mammals or the early growth phase in production animals, is highly sensitive to nutritional and microbial influences. The liver, as the primary metabolic organ responsible for nutrient processing, detoxification, and protein synthesis, responds dynamically to dietary interventions including probiotic supplementation. Studies have demonstrated that Lactobacillus-fermented products can enhance liver antioxidant capacity, reduce hepatic steatosis, and modulate the expression of genes involved in lipid and glucose metabolism (Kim *et al*., 2020; Egberi *et al*., 2025c; Mbanefo *et al*., 2025c; Nwadiogbu *et al*., 2026b). Similarly, the spleen, as a key secondary lymphoid organ, reflects the status of systemic immune function and has been shown to exhibit altered mass and cellular composition following probiotic administration, indicating enhanced immune surveillance and responsiveness. Furthermore, the kidneys, heart, and lungs may also be influenced indirectly through the attenuation of systemic inflammation and oxidative stress, highlighting the pleiotropic effects of probiotic-fermented dietary interventions on organ systems throughout the body (Cho *et al*., 2019; Madubueze *et al*., 2026b; Anekwe *et al*., 2026b).

The biochemical profile of an organism represents a comprehensive readout of metabolic health, nutritional status, and organ function. Key biochemical parameters including serum proteins, liver enzymes, lipid profiles, glucose homeostasis markers, and kidney function indicators collectively provide insight into the systemic effects of any dietary intervention. Probiotic supplementation has been consistently associated with favorable alterations in biochemical profiles, including reductions in serum cholesterol and triglycerides, improvements in glucose tolerance, attenuation of liver enzyme elevations, and optimization of protein metabolism (Ran *et al*., 2020). Specifically, Lactobacillus species have been shown to reduce circulating low-density lipoprotein cholesterol through mechanisms involving bile salt deconjugation, cholesterol assimilation into bacterial cell membranes, and the promotion of cholesterol conversion to coprostanol in the gut lumen. Additionally, the production of short-chain fatty acids, particularly propionate and butyrate, influences hepatic gluconeogenesis and lipid metabolism, contributing to improved glycemic control and reduced hepatic fat accumulation (Koh *et al*., 2016).

The integration of banana peel as a fermentation substrate for probiotic cultivation presents a particularly compelling strategy for producing a cost-effective, sustainable, and functionally potent supplement. Unlike refined commercial prebiotics and probiotics that require extensive processing, banana peel is abundantly available as a waste product, requiring only minimal processing before fermentation. The inherent fiber-rich matrix of banana peel provides an ideal growth substrate for Lactobacillus species, which readily ferment the available carbohydrates to produce organic acids, bacteriocins, and exopolysaccharides that may contribute additional health benefits beyond those conferred by the probiotic organisms alone (Yang *et al*., 2020). Moreover, fermentation has been demonstrated to release bound phenolic compounds from the banana peel matrix, enhancing their bioavailability and antioxidant capacity. This is particularly significant given that oxidative stress is a common underlying factor in impaired organ development and dysfunctional biochemical profiles across a range of pathological and physiological conditions (Han *et al*., 2021).

The relationship between probiotic-fermented banana peel consumption and organ development has received limited but encouraging research attention. Studies examining related fermented plant by-products have reported increases in relative organ weights, particularly of immune organs such as the spleen and thymus, following probiotic supplementation, suggesting enhanced immune maturation and function. Similarly, improvements in liver histology and reductions in hepatic inflammatory infiltrates have been documented in animals receiving Lactobacillus-fermented plant materials (Wang *et al*., 2022). Concurrently, favorable alterations in biochemical profiles have been observed, including reductions in serum alanine aminotransferase and aspartate aminotransferase activities, decreases in total cholesterol and triglyceride concentrations, improvements in the high-density

lipoprotein to low-density lipoprotein ratio, and enhanced antioxidant enzyme activities including superoxide dismutase and glutathione peroxidase (Liu *et al*., 2021).

Despite the growing body of evidence supporting the health benefits of both Lactobacillus probiotics and banana peel-derived bioactive compounds, limited research has specifically examined the combined effect of Lactobacillus-fermented banana peel on organ development and biochemical profiles.

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 ×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 ± 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 α-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 (H2O2) 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 miniprepTM 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 (1µL) 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 µL), template DNA (20 µL), water (72 µL) and master mix (108 µL), comprising Taq polymerase, dimethylsulfoxide (DMSO), magnesium chloride (MgCl2) 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 121^0C 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 180^0C 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% $BaCl_2$. $2H_2O$ and 99.4 mL of 1% Conc. H_2SO_4. This was allowed for 7 days.

Storage and packaging

After fermentation, the fermented samples were aseptically dried using an electric oven at 80^0C 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 W1). Then, 2.0 grams of the sample was added to the crucible, and its weight was recorded as W2. 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 W3). The percentage moisture content was subsequently calculated using the formula:

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

Feed Additive and experimental protocols
The fermented banana peel was mixed thoroughly and the feed in a ratio of 1:20. This mixture was properly and thoroughly mixed and administered to the rats. The rats 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

Organ 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).

Liver function test
Aspartate aminotransferase (AST) activity: This was carried out as described by Nwobodo *et al*. (2018) and Abba *et al*. (2026b). The blood sample was centrifuged and the serum was collected and dispensed 0.1 ml into test tube (pyrex), 0.5 ml of phosphate was added and mixed thoroughly. This was incubated at 37^0C for 30 min. Then 2,4 – dinitrophenylhydrazine was added to the mixture, mixed thoroughly and allowed to stand for 20min. Sodium hydroxide was added to the solution, mixed and allowed to stand for 5 min after which the absorbance was read at 546n m. The procedure was repeated for the blank without the sample and that of the standard. The AST activity was determined by the calibration curve provided in the kit.

Alanine aminotransferase (ALT) activity: This was carried out as described by Nwobodo *et al*. (2018) and Ezeoke *et al*. (2026b). The clotted blood sample was centrifuged and the serum was collected and dispensed 0.1ml into the test tube and this was followed by the addition of 0.5 ml of phosphate buffer. This was mixed thoroughly and incubated at 37^0C for 30min. Then 2,4 – dinitrophenylhydrazine was added into the test tube, and this was mixed thoroughly and allowed to stand for 20min. Sodium hydroxide was then added into the solution and mixed thoroughly and allowed for 5 min after which the absorbance was read at 546nm. The procedure was repeated for the blank without the addition of the sample and that of standard by adding the standard in place of the sample. The ALT activity was determined using the standard curve provided in the kit.

Heart function test
Lactate dehydrogenase (LDH) activity: This was carried out using the method published by Nwobodo *et al*. (2018). One vial of NADH (Rib) was reconstituted with 3 ml of buffer/substrate. Two milliliters of this mixture was added to 0.06 ml of serum. The absorbance was read after 0.5, 1.0. 2.0 and 3.0 min against the reagent blank at 340nm.
LDH Activity = 4921 x ΔA340nm/min (u/l)

Kidney function test
Blood Urea: This was determined by adopting the method published by cheesebrough (2010). The blood samples were allowed to settle and then centrifuged to collect the serum. Ten microliter (10µl) of the sample and standard were pipetted into different test tubes, this was followed by the addition of 100µl of Nitroprusside, and urease. The mixture was shaked and incubated at 37^0C for10 min. Then phenol concentrate and hypochloride concentrate were added, mixed and incubated at 37^0C for 10min. Then the absorbances were read at 546nm against the sample blank.
Urea concentration (mg/dl) = Asample x Conc standard/Astandard

Creatinine: This was determined using the method described in Cheesbrough (2010). Two milliliters (2 ml) of creatinine reagents (prepared by mixing equal volumes of sodium dodecyl sulphate, phosphate borate buffer and picric acid) was added in different test tubes for the blank, test and standard. This was followed by the addition of 0.2ml distilled water to the blank, 0.2 ml serum to the test and 0.2ml standard (0.362g anhydrous creatinine in 100ml HCL) to the standard test tubes. The contents of the test tubes were thoroughly mixed and allowed for 90 min at room temperature. Then the absorbances were checked at 490nm. The other sets of test tubes containing the blank test and standard test reagents were then added 0.05 ml of 60% (v/v) Acetic acid and allowed for 6min. The absorbances were also checked at 490nm. The differences in the absorbances of the former and the later were determined after the instrument has been zero with the blank solution.
Creatinine (mg/dl) = AT X CS / AS

AT = Absorbance of the test
AS = Absorbance of the standard
CS = Concentration of the standard

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

The fermenting organism, designated Isolate P, was first characterized phenotypically. On MRS agar, it presented as cream-white colonies with a low-convex elevation, smooth edges, and a smooth, transparent surface. Microscopic examination revealed it to be Gram-positive rods. Further tests confirmed it was non-motile and did not produce spores. This initial cultural and morphological profile was highly suggestive of a bacterium belonging to the *Lactobacillus* genus, which is commonly employed in the fermentation of food and feed due to its safety and probiotic properties.

A comprehensive biochemical characterization was performed. Isolate P tested negative for key diagnostic enzymes including catalase, citrate utilization, oxidase, urease, and gelatin hydrolysis. The isolate also yielded negative results for the Methyl Red and Voges-Proskauer tests, which are associated with specific metabolic end-products. In contrast, it demonstrated a positive fermentation profile for several carbohydrates, including glucose, lactose, maltose, and

fructose, with variable reactions observed for others like D-mannitol and inositol. This specific biochemical signature catalase-negative, non-proteolytic, and fermentative provided strong corroborative evidence that Isolate P was a *Lactobacillus* species.

Definitive identification was achieved through molecular genetic analysis. High-quality genomic DNA was successfully extracted from Isolate P, as evidenced by a concentration of 142.40 µg/mL and a 260/280 absorbance ratio of 1.83, which is indicative of pure nucleic acid free from significant protein contamination. Subsequent sequencing and BLAST analysis against the GenBank database yielded unambiguous results. The analysis returned a maximum score of 6593 with 100% query cover and 100% identity. The E-value of 0.0 confirmed an extremely high statistical significance for this match, which was to *Lactobacillus acidophilus* strain DSM20079 (Accession CP020620.1). This conclusively identified the fermenting microorganism.

The physiological impact of the dietary treatment, consisting of *Lactobacillus*-fermented banana peel, was assessed through the absolute weights of vital organs in the rats. The mean weights of the liver, kidneys, lungs, and heart were measured and compared between the control and test groups. The results showed no statistically significant differences ($p > 0.05$) for any organ. Specifically, liver weights were identical at 6.31 ± 0.01 g, while kidney, lung, and heart weights showed negligible variations (kidneys: 0.46 ± 0.00 g vs. 0.48 ± 0.00 g; lungs: 1.04 ± 0.00 g for both groups; heart: 0.43 ± 0.00 g vs. 0.42 ± 0.00 g). This indicated that the novel feed formulation did not induce organ hypertrophy, atrophy, or gross pathological changes, supporting its safety at the dietary level administered as shown in Table 5.

To evaluate the functional integrity of key metabolic and excretory organs, a panel of serum biochemical markers was analyzed. Hepatic function was assessed via alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) activities. Renal function was evaluated through serum creatinine and urea concentrations. The values for the test group were virtually identical to those of the control group: ALT (17.110 vs. 17.120 U/L), AST (25.280 vs. 25.660 U/L), creatinine (0.3903 vs. 0.3904 mg/dL), urea (7.9465 vs. 7.9468 mg/dL), and LDH (12.715 vs. 12.714 U/L). Statistical analysis confirmed that there were no significant differences ($p > 0.05$) for any of these parameters, demonstrating that the fermented diet did not impair liver or kidney function as shown in Table 6.

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 fermenters

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	*Lactobacillus* species

Table 3: Authentication of nucleic acids extracted from the fermenters

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 fermenters

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	*Lactobacillus acidophilus* strain DSM20079 Chromosome Complete genome (LADSM)

Table 5: Organ weight of the rats

Organ	Control group	Test group
Liver (g)	6.31 ± 0.01	6.31 ± 0.01
Kidney (g)	0.46 ± 0.00	0.48 ± 0.00
Lungs (g)	1.04 ± 0.00	1.04 ± 0.00
Heart (g)	0.43 ± 0.00	0.42 ± 0.00

Table 6: Organ functions of the rats

Parameter	Control	Test
ALT (U/L)	17.120	17.110
AST (U/L)	25.660	25.280
Creatinine (mg/dL)	0.3904	0.3903
Urea (mg/dL)	7.9468	7.9465
LDH (U/L)	12.714	12.715

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 most significant finding of this study was the demonstration of physiological safety of *Lactobacillus*-fermented banana peel. The absence of statistically significant differences in the absolute weights of the liver, kidneys, lungs, and heart ($p > 0.05$) between the control and test groups indicates that the fermented banana peel did not induce organomegaly, hypertrophy, or atrophy. Specifically, liver weights were identical at 6.31 ± 0.01 g, while kidney, lung, and heart weights showed negligible variations (kidneys: 0.46 ± 0.00 g vs. 0.48 ± 0.00 g; lungs: 1.04 ± 0.00 g for both groups; heart: 0.43 ± 0.00 g vs. 0.42 ± 0.00 g). This result strongly agrees with earlier findings from Pahm and Dante (2000), who reported that feeding protein-enriched banana peelings to mice at 50% substitution of soybean meal produced no adverse effects on growth performance. The maintenance of normal organosomatic indices is a primary, non-invasive indicator of dietary safety and suggests excellent biocompatibility of the fermented banana peel (Nwadiogbu *et al*., 2026c; Anekwe *et al*., 2026d).

Complementing the organ weight data, the serum biochemical analysis provided direct evidence of unimpaired organ function following banana peel probiotic feeding. The virtually identical levels of hepatic enzymes (ALT, AST), renal markers (creatinine, urea), and LDH between groups ($p > 0.05$) demonstrate that the diet did not cause hepatocellular damage, cholestasis, or compromised renal filtration. The values for the test group were nearly identical to those of the control group: ALT (17.110 vs. 17.120 U/L), AST (25.280 vs. 25.660 U/L), creatinine (0.3903 vs. 0.3904 mg/dL), urea (7.9465 vs. 7.9468 mg/dL), and LDH (12.715 vs. 12.714 U/L). This finding is particularly critical, as the liver and kidneys are the primary sites for the metabolism and excretion of both nutrients and potential toxins (Madubueze *et al*., 2026c; Anekwe *et al*., 2026c; Madubueze *et al*., 2026d). Our results disagree with some earlier studies on poorly processed plant proteins, which have occasionally shown elevated liver enzymes due to anti-nutritional factors such as tannins and phytates commonly found in raw banana peel. The successful fermentation process, as indicated here, likely degraded such compounds, a benefit underscored by Abdel-Latif *et al*. (2023), who demonstrated that microbial conversion of banana peels using *Saccharomyces cerevisiae* produced sophorolipids that improved liver function tests and enhanced antioxidant pathways in rats. This confirms that the bioprocessing was effective in producing a non-toxic, hepatorenal-safe final product from banana peel.

The use of banana peel as a fermentation substrate is particularly advantageous given its rich nutritional profile. Saeed *et al*. (2025) reported that banana peel contains 6-9% crude protein, 43.2-49.7% total nutritional fiber, and 3.8-11% crude fat, making it an excellent source of nutrition for animals. Additionally, banana peels contain flavonoids, tannins, alkaloids, glycosides, and terpenoids, which possess antibacterial, antioxidant, anti-inflammatory, and immunostimulant properties. Fermentation improves the

nutritional quality of banana peel by increasing digestibility, reducing anti-nutritional factors, and enriching it with probiotics and bioactive compounds (Saeed *et al*., 2025; Egberi *et al*., 2026b; Mbanefo *et al*., 2026b; Nwadiogbu *et al*., 2026d). These properties support our finding that the fermented banana peel did not induce any adverse physiological effects.

Furthermore, recent research by the IOP Conference Series (2025) demonstrated that banana peel powder inclusion in broiler diets up to 15% did not significantly alter body weight, weight gain, or feed intake compared to control groups, confirming its safety for growth performance (Egberi *et al*., 2026c; Nwadiogbu *et al*., 2026e). The study also suggested that the phenolic compounds and dietary fibers in banana peel provide antioxidant and detoxifying functions, which aligns with our observation of normal serum biochemical markers in the test group. Similarly, Louhasakul *et al*. (2024) reported that fermented banana peel treated with microorganisms contained high carbohydrate and fiber contents with no detectable mycotoxin fragments, indicating its potential as a safe roughage source in animal feed.

Conclusion

Lactobacillus acidophilus-fermented groundnut chaff blended with fish meal was shown to be physiologically safe for Albino Wistar rats. The dietary intervention did not significantly alter vital organ weights or impair hepatic and renal function. These findings validate the safety of this novel, valorized feed ingredient, supporting the principles of circular economy and sustainable nutrition. The study provides a crucial preclinical foundation for its potential application in livestock diets.

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