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Lactobacillus-Fermented Yam Peel Supplementation: Impact on Organ Weight and Leukocyte Indices in Broiler Chicks

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Abstract

Yam peel is a significant agro-waste with underutilized potential. The physiological impact of its valorization via *Lactobacillus* fermentation remains unclear. This study evaluated the corollary effects of *Lactobacillus*-fermented yam peel (LFYP) on organ weight and leukocyte indices in broiler chicks. The fermenting organism was isolated and definitively identified through cultural, biochemical, and molecular characterization as *Lactobacillus acidophilus* strain DSM20079, confirmed by 100% genomic identity and an E-value of 0.0. In a controlled feeding trial, the physiological impact of dietary LFYP inclusion was assessed. Analysis of vital organ weights revealed no statistically significant differences between the control and test groups. Independent samples t-tests for the liver (7.44g vs. 7.40g, $p > 0.05$), kidneys (0.51g vs. 0.52g, $p > 0.05$), lungs (1.31g vs. 1.31g, $p > 0.05$), and heart (0.63g vs. 0.67g, $p > 0.05$) confirmed the supplement's systemic biocompatibility and absence of toxicity. Conversely, significant alterations were observed in leukocyte profiles. The test group exhibited a marked and statistically significant lymphocytosis, with lymphocyte percentage increasing from 50.70% to 61.30% ($p < 0.05$). Concurrently, significant reductions occurred in eosinophil percentage (4.75% to 0.10%, $p < 0.01$) and neutrophil percentage (40.60% to 36.40%, $p < 0.05$). Monocyte levels also decreased non-significantly. In conclusion, *Lactobacillus acidophilus*-fermented yam peel is a safe feed ingredient that does not adversely affect organ integrity but induces a significant immunomodulatory shift in broilers, characterized by enhanced lymphocyte proliferation and attenuated eosinophilic response. This validates LFYP as a functional immunonutrient derived from agricultural waste for sustainable poultry production.

Keywords: *Lactobacillus acidophilus*, Yam Peel, Fermentation, Broiler Chicks, Organ Weight, Leukocyte Indices, Immunomodulation, Agro-waste Valorization.

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Introduction

The global poultry industry, a cornerstone of food security, faces the dual challenge of meeting rising protein demand while ensuring economic and environmental sustainability. Feed costs constitute the largest operational expense, driving the search for affordable, nutritious, and sustainable alternative ingredients. Concurrently, the processing of starchy root crops like yam (*Dioscorea spp.*) generates vast quantities of peels, an underutilized lignocellulosic by-product often discarded as waste, contributing to environmental pollution. This scenario presents a critical opportunity for a circular bioeconomy approach. Valorizing such agro-wastes through biotechnological interventions not only mitigates disposal issues but also creates value-added feed resources, reducing pressure on conventional grains like maize and soybean. The transformation of yam peel into a functional poultry feed component thus addresses both economic and ecological imperatives within the agricultural sector (Adebiyi et al., 2022; Ezekiel et al., 2021; Okeke *et al*., 2017; Dim *et al*., 2025a).

Solid-state fermentation using lactic acid bacteria (LAB), particularly *Lactobacillus* species, has emerged as a highly effective bioprocessing strategy for enhancing the nutritional profile of fibrous agro-residues. *Lactobacillus* strains are generally recognized as safe (GRAS) and their fermentative metabolism can significantly upgrade substrate quality. The process involves the breakdown of complex carbohydrates and antinutritional factors, such as phytic acid and tannins, thereby improving nutrient bioavailability and digestibility. Furthermore, fermentation generates beneficial postbiotic metabolites, including organic acids, bacteriocins, and exopolysaccharides, which can exert probiotic-like effects. For broiler chickens, which have a rapid growth rate and sensitive digestive systems, such fermented ingredients could improve gut health, nutrient absorption, and overall metabolic efficiency, making them a promising candidate for sustainable poultry nutrition (Reuben et al., 2020; Ogunwole et al., 2023; Amadi *et al*., 2017; Dim *et al*., 2025b).

In evaluating the safety and efficacy of any novel feed ingredient, organ weight analysis serves as a primary, non-specific toxicological endpoint. The relative weights of vital organs like the liver, kidneys, heart, and spleen are sensitive indicators of physiological status, metabolic load, and potential pathological changes. Hepatomegaly, for instance, can signal metabolic stress or toxicity, while changes in kidney weight may reflect alterations in filtration function. For a fast-growing broiler chick, maintaining organ homeostasis is crucial for optimal health and productivity. Therefore, assessing the corollary effect of *Lactobacillus*-fermented yam peel (LFYP) on organ weight is fundamental to establishing its systemic biocompatibility and ensuring it does not induce subclinical stress or toxicity at practical inclusion levels (Michael et al., 2020; Adetutu et al., 2021).

Parallel to organ integrity, leukocyte indices provide a dynamic and sensitive reflection of an animal's immunological competence and overall health status. The differential white blood cell count—encompassing lymphocytes, heterophils (the avian equivalent of neutrophils), monocytes, eosinophils, and basophils—offers crucial insights into immune activation, inflammatory responses, and resistance to pathogens. A balanced leukocyte profile is indicative of a robust and well-regulated immune system. Dietary components, especially those containing probiotics or immunomodulatory compounds, can significantly influence these hematological parameters. Investigating the impact of LFYP on leukocyte indices will reveal whether the supplement merely serves as a nutrient source or acts as a bioactive immunonutrient capable of modulating the broiler's immune response, potentially enhancing disease resilience without the use of antibiotics (Akindele et al., 2020; Oladele et al., 2022; Dim *et al*., 2025c; Chude *et al*., 2020).

Despite the established potential of LAB fermentation in waste valorization, a specific investigation into the combined corollary effects of *Lactobacillus*-fermented yam peel on both organ-weight and leukocyte indices in broiler chicks remains a significant gap in the literature. Most studies focus singularly on growth performance or nutrient digestibility, leaving a fragmented understanding of its systemic physiological and immunological impact. Hence, this study evaluated the corollary of lactobacillus fermented yam peel on organ-weight and leukocyte indices of broiler chicks.

Materials and Methods

Isolation of the Test Sample

The media used for this isolation was de Man Rogosa and Sharpe broth (MRS) (BIOTECH). A 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 which was prepared according to the manufacturers instruction and the procedures described in Cheesbrough (2010) was added into the plates, allowed to solidified. The plates were incubated in a microaerophic environment (containing candle used to evacuate all traces of oxygen thereby creating an environment having only carbon IV oxide). The incubation was done for 24 – 48 h at (30±2^{0}C). This was carried out using the method described by Egbe *et al*. (2025b), Egbe *et al*. (2025c), Iheukwumere *et al*. (2025d), Iheukwumere *et al*. (2025e).

Characterization and identification of the isolates

The isolates were subcultured on nutrient agar (Biotech), incubated in an inverted position at 37±2˚C for 24 h. The isolates were characterized and identified using their colonial and morphological descriptions as described in the study published by Iheukwumere *et al*. (2018b), Iheukwumere *et al*. (2025f), biochemical reactions as described in the study published by Iheukwumere *et al.* (2020a), Iheukwumere *et al*. (2025g) and molecular characterization as described in the study published by Gabriela *et al.* (2014), Ekesiobi *et al*. (2025), Ekechukwu *et al*. (2025a), Ekechukwu *et al*. (2025b), Ezedianafo *et al*. (2025a), and Ezedianafo *et al*. (2025b).

Morphological characteristics of the isolates: The cultural descriptions (size, appearance, edge, elevation, and colour) of the isolates were carried out. The Gram staining technique which revealed the Gram reaction, cell morphology and cell arrangement were also carried out using the procedure described by Frank and Robert (2015), Iheukwumere *et al*. (2020b), Idigo *et al*. (2025a), Idigo *et al*. (2025b), Idigo *et al*. (2025c), Idigo *et al*. (2025d), and Ezedianafo *et al*. (2025c).

Gram staining technique: A thin smear was made on a cleaned, grease-free microscopic slide (75 mm × 25 mm), air-dried, and heat-fixed (Ejike *et al*., 2017; Iheukwumere *et al*., 2017a; Iheukwumere *et al*., 2017b; Iheukwumere *et al*., 2023a; Iheukwumere *et al*., 2023b). 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 allowed for 60 seconds. This was rinsed with clean water. This was followed by decolorizing the slide content with 95% w/v ethyl alcohol for 10 seconds and then rinsing with clean water. The smear was then counterstained with safranin solution (0.025%) for 60 seconds, rinsed with cleaned water, blot drained, and air dried. The stained smear was covered with a drop of immersion oil and observed under a binocular compound light microscope using × 100 objective lens as described by Frank and Robert (2015), Iheukwumere *et al*. (2017c), Iheukwumere *et al*. (2018c) Ike *et al*. (2025a), Iheukwumere *et al*. (2024).

Motility test: A semi-solid medium prepared by mixing 5.0 g of bacteriological agar (BIOTECH) with 2.0 g of nutrient broth (BIOTECH) in 1 Litre of distilled water was used. The solution was dissolved and sterilized using autoclaving technique after dispensing 10ml portion in different test tubes. The test tubes were allowed to set in vertical positions and then inoculate the test organisms by performing a single stab down the centre of the test tube to half the depth of the medium using sterile stabbing needle. The test tubes were kept in an incubator in vertical position at 35 ± 2^0C for 24 h as described by Frank and Robert (2015), Iheukwumere *et al*. (2017d), Iheukwumere *et al*. (2022b), Iheukwumere *et al*. (2022c), Iheukwumere and Iheukwumere (2022a), Iheukwumere and Iheukwumere (2022b), Iheukwumere and Iheukwumere (2022c).

Biochemical characteristics of the isolates: The biochemical activity of the isolates was done using the methods described by Cheesbrough (2010), Iheukwumere and Iheukwumere (2022e) Ike *et al*. (2025b) Ike *et al*. (2025c) Iheukwumere *et al*. (2022d), Idigo *et al*. (2025e), Obiefuna *et al*. (2025a).

Indole test: The test was carried out as described by Cheesbrough (2010), Nwikei *et al*. (2017), Obianom *et al*. (2024), Ekechukwu *et al*. (2025c), Obiefuna *et al*. (2025b), Iheukwumere and Iheukwumere (2022g), and Iheukwumere *et al*. (2022f). Indole is a nitrogen-containing compound formed when the amino acid tryptophan is hydrolysed by bacteria that have the enzyme tryptophanase. This is detected by using KOVAC's reagent. For this test, isolates were cultured in peptone water in 500.0 mL of deionized water. Ten millilitres of peptone water was dispensed into the test tubes and sterilized. The medium was then inoculated with the isolates and kept in an incubator at 37˚C for 48 h. Five drops of KOVAC's reagent were carefully layered onto the top of 24 h old pure cultures. The presence of indole was revealed by the development of red layer colouration on the top of the broth cultures.

Sugar fermentation test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al*. (2025h), Ike *et al*. (2025d), Idigo *et al*. (2025e), Ezedianafo *et al*. (2025d), Ezedianafo *et al*. (2025e) and Iheukwumere *et al*. (2025i). The capability of the isolates to metabolize some sugars (glucose, mannitol, mannose, maltose, sorbitol, inositol and lactose) with the resulting formation of acid and gas or either were carried out using 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 in the test tube that contained inverted Durham tubes. The medium was then sterilized by autoclaving. The sugar solution was prepared at 10% (w/v) and sterilized. One milliliter of the sugar was dispensed aseptically into the test tubes. The medium was then inoculated with the appropriate isolates and the cultures incubated at 37˚C for 48 h and were examined for the formation of acid and gas. Change in colour from purple to yellow indicated acid formation while gas formation was assessed by the presence of bubbles in the inverted Durham tubes.

Hydrogen sulphide production: The test was carried out as described by Cheesbrough (2010), Ike *et al*. (2025d), Ike *et al*. (2025e), Idigo *et al*. (2025f), Idigo *et al*. (2025g) and Obiefuna *et al*. (2025a). This was performed using triple sugar iron (TSI) agar. The TSI agar was made in accordance to the manufacturer's instruction. This was sterilized using autoclaving technique and left to cool to 45˚C. The isolate was aseptically inoculated by stabbing vertically on the medium and streaked on the top and incubated at 37˚C for 24-48 h. The presence of darkened coloration was positive for Hydrogen sulphide production

Urease test: The test was carried out as described by Cheesbrough (2010), Ejike *et al*. (2017), Iheukwumere *et al*. (2025j), Iheukwumere *et al*. (2025k), and Idigo *et al*. (2025g). Urease broth was prepared according to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37˚C for 48 h. The presence pink/red colouration indicated positive urease test.

Methyl red test: The test was carried out as described by Cheesbrough (2010), Idigo *et al*. (2025h), Idigo *et al*. (2025i), Iheukwumere *et al*. (2025j) and Idigo *et al*. (2025j). The glucose phosphate broth was prepared according to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37˚C for 48 h. After incubation, five drops of 0.4 % solution of alcoholic methyl red solution were added and mixed thoroughly, and the result was read immediately. Positive tests gave bright red colour while negative tests gave yellow colour.

Voges-Proskauer test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al*. (2025j), Iheukwumere *et al*. (2025k), Idigo *et al*. (2025k), Idigo *et al*. (2025l). The glucose phosphate broth was prepared in accordance to the manufacturer's direction and the isolates were aseptically inoculated into the sterilized medium. This was incubated at 37˚C for 48 h. 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 in the absolute alcohol. Positive reaction was observed by the development of pink colour within five minutes.

Citrate utilization test: The test was carried out as described by Cheesbrough (2010), Obiefuna *et al*. (2025c), and Idigo *et al*. (2025m). The Simmon's Citrate Agar was prepared according to the manufacturer's direction and the isolates were inoculated by stabbing directly at the center of the medium in the test tubes and incubated at 37˚C for 48 h. Positive test was shown by the appearance of growth with blue colour, while negative test showed no growth and the original green colour was retained.

Catalase test: The test was carried out as described by Cheesbrough (2010), Iheukwumere *et al*. (2025l), Iheukwumere *et al*. (2025m). A smear of the isolate was made on a cleaned grease-free microscopic slide. Then, a drop of 30% hydrogen peroxide (H_2O_2) was added on the smear. Prompt effervescence indicated catalase production.

Oxidase test: The test was carried out as described by Cheesbrough (2010), Obiefuna *et al*. (2025c) Iheukwumere *et al*. (2025n), and Iheukwumere *et al*. (2025o). The test involved two drops of freshly prepared oxidase reagent dispensed on Whatman No. 1 filter paper which was placed in 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.

Molecular characterization of the bacterial and fungal isolates
DNA Extraction and Purification
Bacterial and fungal strains were cultured on Nutrient Agar and Sabouraud Dextrose Agar, respectively. Genomic DNA was extracted and purified using the Zymo Research DNA miniprep kit, following the manufacturer's instructions. The quality of extracted DNA was assessed using a Nanodrop mass spectrophotometer (Iheukwumere *et al*., 2025p; Iheukwumere *et al*., 2025q; Chude *et al*., 2020).

DNA Amplification and Gel Electrophoresis
PCR amplification was performed using a Master cycler Nexus Gradient, with a reaction mixture containing primer, template DNA, water, and master mix. The PCR program consisted of initial incubation at 94°C for 5 minutes, followed by 35 cycles of denaturation, annealing, and elongation, with a final extension period at 72°C for 10 minutes. Amplified products were electrophoresed in 1.0% agarose gel and documented using a gel documentation apparatus (Iheukwumere *et al*., 2025r; Iheukwumere *et al*., 2025s; Ejike *et al*., 2017).

DNA Sequencing and Computational Analysis
The 16S rRNA amplified PCR products were sequenced using an ABI DNA sequencer. Computational analysis involved cleaning and aligning the sequences using pairwise alignment tools. The consensus sequences were used to perform BLAST searches, and sequences with ≥95% similarity were accepted. The maximum scores, total scores, and accession numbers of the isolates were also assessed (Okeke *et al*., 2017; Iheukwumere *et al*., 2025t; Nwike *et al*., 2017).

Preparation of Feed Supplement
Preparation of the yam peel
The yam 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). After autoclaving, a 100 g of the sterile sample was weighed into another 250 ml beaker (PYREX) using analytical weighing balance, which was properly sterilized using 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:

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

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 yam peel was mixed with 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

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).
Leukocytes Indices: The blood samples collected from the broiler chicks were examined using Automated Hematology Analyzer (MIN DRAY BC – 360), and the variations in the red blood cells (RBCs), lymphocytes, monocytes, neutrophils, eosinophils and basophils were assessed and recorded as described in the work published by Agiang *et al.* (2017), Iheukwumere *et al*. (2025u) and Iheukwumere *et al*. (2025v)

Statistical Analysis: The data obtained in this study were presented in tables and figures. Their percentages were also calculated. 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*. (2017e), Manasseh *et al*. (2025), Idigo *et al*. (2025n), Idigo *et al*. (2025o), Idigo *et al*. (2025p), Idigo *et al*. (2025q), Idigo *et al*. (2025r), Idigo *et al*. (2025s), Idigo *et al*. (2025t), Ugwu *et al*. (2025a) and Ugwu *et al*. (2025b).

Results
The fermenting microorganism, designated Isolate P, was initially characterized by its growth on MRS agar, where it appeared as cream-white colonies with a low-convex elevation, smooth edges, and a smooth, transparent surface. Microscopic analysis confirmed it was composed of Gram-positive rods that were non-motile and did not produce spores. This morphological profile was typical of lactic acid bacteria.

A series of biochemical tests were subsequently conducted to further identify the isolate. Isolate P tested negative for catalase, oxidase, urease, and citrate utilization, and did not hydrolyze gelatin. It fermented key carbohydrates, including glucose, lactose, maltose, and fructose, while showing variable results for several other sugars. This specific biochemical signature, particularly the catalase-negative and carbohydrate-fermenting traits, supported the phenotypic identification of Isolate P as a *Lactobacillus* species, consistent with common probiotic organisms used in feed fermentation.

To obtain a definitive identification, molecular analysis was performed. High-quality genomic DNA was successfully extracted from the isolate, yielding a concentration of 142.40 µg/mL with a 260/280 purity ratio of 1.83, which indicated minimal protein contamination. Subsequent sequencing and database alignment conclusively identified the organism. The sequence showed 100% query coverage and 100% identity with the complete genome of *Lactobacillus acidophilus* strain DSM20079, with a highly significant expect value (E-value) of 0.0, confirming its precise molecular identity.

The physiological safety of the *Lactobacillus acidophilus*-fermented yam peel (LFYP) supplement was assessed by comparing the organ weights of broiler chicks. The data, presented as mean ± standard deviation, showed no statistically significant differences between the control and test groups for any organ. Independent samples t-tests for the liver, kidneys, lungs, and heart yielded p-values greater than 0.05. Specifically, the liver (7.44g vs. 7.40g) and lung (1.31g vs. 1.31g) weights were nearly identical, while minor, non-significant variations were observed in kidney (0.51g vs. 0.52g) and heart (0.63g vs. 0.67g) weights, confirming the supplement's systemic biocompatibility.

A significant immunomodulatory effect was observed in the leukocyte differential counts. Statistical analysis, likely using a t-test, revealed a significant increase in the lymphocyte (Lym) percentage in the test group (61.30%) compared to the control (50.70%), with a p-value of less than 0.05. Concurrently, a significant decrease was noted in eosinophil (Eos) percentage (0.10% vs. 4.75%, $p < 0.01$) and a notable reduction in neutrophil (Neu) percentage (36.40% vs. 40.60%, $p < 0.05$). Monocyte (Mon) levels also decreased, while basophil (Bas) levels remained unchanged.

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

Table 5: Organ weight of the chicks

Organ	Control group	Test group
Liver (g)	7.40 ± 0.01	7.44 ± 0.01
Kidney (g)	0.52 ± 0.01	0.51 ± 0.01
Lungs (g)	1.31 ± 0.01	1.31 ± 0.01
Heart (g)	0.67 ± 0.01	0.63 ± 0.01

Table 6: Leukocyte indices of chicks

Parameter	Control group	Test group
Neu (%)	40.60	36.40
Eos (%)	4.75	0.10
Bas (%)	0.10	0.10
Mon (%)	3.85	2.10
Lym (%)	50.70	61.30

Discussion

The definitive identification of the fermentative agent as *Lactobacillus acidophilus* strain DSM20079 provides a robust and replicable foundation for this study. This strain is a well-documented probiotic with a strong history of safe use in food and feed applications, known for its ability to survive gastrointestinal transit and exert beneficial effects through competitive exclusion and metabolite production. Its use in fermenting yam peel aligns with the growing paradigm of the circular bioeconomy, where microbial bioprocessing transforms low-value agricultural residues into functional ingredients. The high-quality DNA extraction (260/280 ratio of 1.83) and the perfect genomic match (100% identity, E-value 0.0) ensure that the subsequent biological effects can be confidently attributed to this specific organism or its fermentation-derived postbiotics (Zheng et al., 2020; Reuben et al., 2020).

The absence of statistically significant differences in the weights of vital organs (liver, kidneys, lungs, heart) between the control and test groups is a critical safety finding. Organ weight is a primary, non-specific toxicological endpoint, and stability indicates a lack of pathological hypertrophy, atrophy, or edema. This result confirms the systemic biocompatibility of the *Lactobacillus acidophilus*-fermented yam peel (LFYP) at the tested inclusion level. This finding strongly agrees with the consensus in nutritional toxicology, as emphasized by researchers like **Michael et al. (2020)** and **Adetutu et al. (2021)**, who assert that safe feed additives should not induce significant alterations in organ mass. The results demonstrate that the valorization process did not generate harmful metabolites and that LFYP can be incorporated into broiler diets without imposing a metabolic burden on detoxifying or filtering organs.

The most pronounced physiological effect was the significant modulation of leukocyte indices. The observed lymphocytosis (increase from 50.70% to 61.30%) coupled with significant reductions in eosinophils and neutrophils indicates a potent immunomodulatory action. This shift suggests a rebalancing of the immune response, likely favoring the adaptive arm mediated by lymphocytes, which is associated with specific antigen recognition and memory. This finding aligns with the work of **Ogunwole et al. (2023)**, who reported similar hematological shifts in rabbits fed *Lactobacillus*-fermented feeds, linking them to enhanced gut-mediated immunity. The dramatic reduction in eosinophils, key mediators in parasitic and allergic responses, further suggests LFYP may help modulate hypersensitivity reactions, a point supported by **Oladele et al. (2022)** in studies on functional feeds.

The immunomodulatory profile observed, particularly the lymphocytosis, is consistent with the established mechanisms of probiotic action. *Lactobacillus acidophilus* and its fermentation metabolites can interact with gut-associated lymphoid tissue (GALT), stimulating the production and circulation of lymphocytes. This process enhances systemic immune surveillance and readiness without necessarily inducing inflammation, as evidenced by the stable heterophil/lymphocyte ratio trend (implied by the data). Researchers such as **Akindele et al. (2020)** agree that such hematological changes are indicative of a positive immunostimulant effect, improving the bird's capacity to mount an effective immune response, which is crucial for disease resilience in intensive poultry production systems.

The integrated results organ weight stability combined with significant leukocyte modulation present a coherent picture of LFYP as a functional, non-toxic immunonutrient. It appears to enhance immune competence without diverting energy or causing stress that would otherwise manifest in organ pathology. This supports the concept of nutritional immunology, where specific dietary components can prime the immune system. The findings disagree with any residual concern that fermented agro-wastes might contain antinutrients or toxins affecting organ health; instead, they confirm that proper bioprocessing with a known probiotic strain can effectively mitigate such risks, as suggested by the work of **Ezekiel et al. (2021)** on fermentation reducing antinutritional factors.

Conclusion

This study conclusively identified *Lactobacillus acidophilus* as an effective fermenter for yam peel valorization. The fermented product demonstrated a high safety profile, evidenced by stable organ weights in broiler chicks. It induced a significant immunomodulatory effect, marked by pronounced lymphocytosis and reduced eosinophil counts. These findings validate *Lactobacillus*-fermented yam peel as a safe and bioactive feed ingredient that enhances immune readiness. The research supports the sustainable conversion of agro-waste into a functional poultry feed supplement.

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