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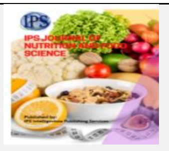

# Assessment of the Prebiotic Potential of Fermented Groundnut Chaff and Fish Meal Blend on the Performance of Broiler Chicks

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**Abstract**

The use of antibiotics in poultry production has led to the development of antibiotic-resistant bacteria, posing a significant threat to human health. The need for alternative feed additives to promote growth and health in broiler chicks is urgent. Fermented groundnut chaff and fish meal blend has shown potential as a prebiotic, but its effects on broiler chicks' performance are unknown.. This study was undertaken to evaluate the effect of fermented groundnut chaff mixed with fish meal as a chicken additive for healthy broiler chicks. The effects of the fermented feeds on the broiler chicks were determined using *in vivo* techniques. The isolate P was identified as *Lactobacillus acidophilus* strain DSN20079 (LADSM) based on cultural, morphological, biochemical, and molecular characteristics. The mixture of groundnut chaff and fish meal, fermented by LADSM was incorporated into the diet of broiler chicks, and its effects on growth performance, organ weights, feed intake, and hematological indices were evaluated. The results showed that the body weights of the chicks in the test group were significantly higher ($p<0.05$) than those in the control group from week 2 to week 6. The feed conversion ratio of the test group was significantly lower ($p<0.05$) than that of the control group from week 3 to week 6. The organ weights of the chicks were not affected by the inclusion of the feed additive in the diet. The white blood cell count and lymphocyte percentage were significantly higher ($p<0.05$) in the test group compared to the control group. The study suggest that the mixture of groundnut chaff and fish meal, fermented by LADSM has potential as a probiotic feed additive for broiler chicks, improving growth performance and blood indices without any adverse effects on organ weights.

**Keywords:** *Lactobacillus acidophilus*, Probiotic, Broiler chicks, Growth performance.

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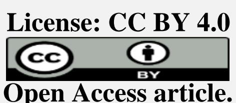

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## 1. Introduction

The use of antibiotics in poultry production has led to the development of antibiotic-resistant bacteria, posing a significant threat to human health (WHO, 2018; Iheukwumere *et al*., 2025a; Dim *et al*., 2025a; Ihekwumere *et al*., 2022a; Nwike *et al*., 2017). The need for alternative feed additives to promote growth and health in broiler chicks is urgent. Fermented feed additives have been shown to have potential as prebiotics, promoting the growth of beneficial microorganisms in the gut and improving the overall health of the birds (Gaggìa et al., 2010; Ekechukwu *et al.,* 2025a; Obianom *et al*., 2024; Dim *et al*., 2025b).

Groundnut chaff and fish meal are two common ingredients used in poultry feed, but their use as a fermented feed additive is not well documented. However, studies have shown that fermentation can improve the nutritional value of groundnut chaff and fish meal, making them more digestible and

increasing their protein content (Adeyemo *et al*., 2018; Ojha *et al.*, 2018; Iheukwumere *et al*., 2025b; Dim *et al*., 2025c).

*Lactobacillus acidophilus* is a commonly used probiotic bacterium that has been shown to have beneficial effects on the growth performance and immune response of broiler chicks (Kabir, 2009; Mountzouris *et al.*, 2010; Amadi *et al*., 2017; Ejike *et al*., 2017). However, there is a research gap in the use of *Lactobacillus acidophilus* isolated from fermented groundnut chaff and fish meal blend as a probiotic feed additive for broiler chicks.

The current study aims to evaluate the effect of fermented groundnut chaff mixed with fish meal as a chicken additive for healthy broiler chicks. The effects of the fermented feeds on the broiler chicks were determined using in vivo techniques.
The results of this study will contribute to the existing knowledge on the use of fermented feed additives in poultry production and provide insights into the potential use of *Lactobacillus acidophilus* isolated from fermented groundnut chaff and fish meal blend as a probiotic feed additive for 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), Ekechukwu *et al*. (2025b), Ekesiobi *et al*., (2025), Ezedianafo *et al*., (2025a) 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).

### 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 as described by Iheukwumere *et al*. (2020a), Ezedianafo *et al*. (2025b); Idigo *et al*. (2025a), Iheukwumere *et al*. (2025c).

### Characterization of the Bacteria Pure Isolates

The pure isolates were characterized using the morphological, biochemical and molecular characteristics as described by Iheukwumere *et al.* (2017a); Iheukwumere *et al.* (2018a), Ike *et al*. (2025a), Iheukwumere *et al*. (2025d).

### Morphological characteristics of the Bacteria isolates

The cultural descriptions (size, appearance, edge, elevation, colour) of the isolates were carried out as described in Goldman and Green (2009); Iheukwumere *et al*. (2017b), Iheukwumere *et al*. (2018b), Iheukwumere *et al*. (2020b). The Gram staining technique which revealed the Gram reaction, cell morphology and cell arrangement were also carried out using the procedure described by Cheesbrough (2010), Goldman and Green (2009) Frank and Robert (2015), Iheukwumere *et al.* (2022b), Iheukwumere *et al.* (2023a). The presence or absence of capsule was also carried out as described by Goldman and Green (2009), Ike *et al*. (2025b), Obiefuna *et al*. (2025a). The presence or absence of flagellum was determined by carrying out motility test as described by Cheesbrough (2010), Iheukwumere *et al*., (2017c), Iheukwumere *et al.* (2018c), Iheukwumere and Iheukwumere (2022a).

### Gram staining technique

A thin smear was made in a cleaned grease free microscopic slide (75mm×25mm), air dried heat fixed. The smear was flooded with crystal violet solution (0.2%) for 60 seconds and rinsed with cleaned water. Gram iodine solution (0.01%) was then applied and allowed for 60 seconds. This was rinsed with cleaned water. This was followed by decolourizing the slide content with 95%w/v ethyl alcohol for 10seconds and then rinsed with cleaned water. The smear was then counter stained 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 Iheukwumere *et al.* (2017d); Iheukwumere *et al.* (2020c), Chude *et al.* (2020), Iheukwumere and Iheukwumere (2022b), Iheukwumere *et al*. (2022c).

**Motility test:** A semi-solid medium prepared by mixing 5.0g of bacteriological agar (BIOTECH) with 2.0g of nutrient broth (BIOTECH) in 1 Litre of distilled water was used. The solution was dissolved and sterilized using autoclaving technique after dispensing 10 ml 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$^{0}$C for 24h (Iheukwumere *et al*., 2017e; Iheukwumere and Iheukwumere, 2022c; Iheukwumere *et al*., 2022d; Idigo *et al*., 2025b).

### Biochemical characteristics of the isolates

**Indole test:** Indole is a nitrogen containing compound formed when the amino acid tryptophan is hydrolyzed 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 hr. 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 as described by Iheukwumere *et al.* (2022e), Iheukwumere and Iheukwumere (2022d), Iheukwumere *et al*. (2023b), Egbe *et al*. (2025a), Ike *et al*. (2025c).

**Sugar fermentation test:** The capability of the isolates to metabolize some sugars (glucose, xylose, ducitol, maltose, arabinose, inositol, mucate 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 were 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 (Iheukwumere *et al*., 2022f; Iheukwumere and Iheukwumere, 2022e; Egbe *et al.,* 2025b; Idigo *et al*., 2025c)

**Methyl red test:** 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 hr. After incubation, five drops of 0.4 % solution of alcoholic methyl red solution was added and mixed thoroughly, and the result was read immediately. Positive tests gave bright red colour while negative tests gave yellow colour (Ezedianafo *et al*., 2025c; Ike *et al*., 2025c).

**Voges-Proskauer test:** 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 48hr. After incubation, 1.0 mL of 40% potassium hydroxide (KOH) containing 0.3% Creatine and 3 ml of 5% solution of *α*-naphthol was add ed in the absolute alcohol. Positive reaction was observed by the development of pink colour within five minutes (Egbe *et al*., 2025b; Ekechukwu *et al*., 2025c).

**Citrate utilization test:** The Simmon's Citrate Agar was prepare 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 hr. 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 (Idigo *et al*., 2025d; Ezedianafo *et al*. 2025d).

**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 drop of 30% hydrogen peroxide ($H_2O_2$) was added on the smear. Prompt effervescence indicated catalase production (Idigo *et al*., 2025e; Idigo *et al*., 2025f).

**Oxidase test:** 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.

**Urease test:** This was carried out as described by Cheesbrough (2010), Idigo *et al*. (2025g) and Idigo *et al*. (2025h). The urea agar slant was prepared in accordance to the manufacturer's direction and the isolates were aseptically inoculated into sterilized medium. This was incubated at $37^0$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. By means of the procedures of Zymo Research (ZR) DNA miniprepTM kit, bacterial genomic DNA was then extracted and purified (Category No. D6005; Irvine, California, USA) as described by Iheukwumere *et al.* (2018) Iheukwumere *et al*. (2025e; Idigo *et al*., 2025h).

**Determination of the quality of extracted DNA:** Using mass spectrophotometer (Nanodrop), One micro litre (1µL) was aseptically dropped into a fresh space in the chamber and the chamber was lightly closed which was then linked to a computer system which showed the window that discovered the value of the sample at 260/280nm as described by (Iheukwumere *et al.,* 2018; Iheukwumere *et al*., 2025f; Idigo *et al*., 2025i).

**Amplification of DNA and gel electrophoresis of PCR product:** This was analysed using Master cycler Nexus Gradient (Eppendorf). A mixture of primer (20 µL), template DNA (20µL), water (72 µL) and master mix (108 µL), which comprises taq polymerase, dimethylsulfoxide (DMSO), magnesium chloride ($MgCl_2$) and nucleotides triphosphates (NdTPs), was made in 1.5 mL tube and homogenized using vortex mixer (Eppendorf). This was then positioned in the block chamber of the master cycler and then programmed. The PCR program for 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 final extension period for 10 mins at 72°C. The amplified products were electrophorezed in 1.0% agarose gel and a1kb DNA ladder was used as a size reference. After staining with 3µL of nucleic acid stain (GR green), the gel was documented with gel documentation apparatus (Iheukwumere *et al.,* 2018; Iheukwumere *et al*., 2025g; Idigo *et al*., 2025j; Idigo *et al*., 2025k).

**DNA sequencing of 16s rRNA fragment:** The 16S rRNA amplified PCR products generated from universal primer (16S), was used for the sequencing using ABI DNA sequencer (Applied Biosystem Inc) at International Institute of Tropical Agriculture (IITA), Ibadan using the method of Iheukwumere *et al.* (2018), Iheukwumere *et al*., (2025h), and Idigo *et al*. (2025l), Idigo *et al*., (2025m).

**Computational Analysis:** This was analysed making use of the modified method of Iheukwumere *et al.* (2018), Iheukwumere *et al.* (2025i), Idigo *et al.* (2025n), Iheukwumere *et al*., (2025j). The chromatograms generated from the sequences were cleaned to obtain regions with normal sequences. The cleaned nucleotides were aligned

using pair wise 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 National Centre for Biotechnology Information BLAST over the internet. The sequences of the isolates with 95% and above similarities were accepted. Also the maximum scores, total scores and accession numbers of the isolates were assessed. The relatedness of the isolates was determined by tracing their phylogenetic tree using DNA distance neighbour phylogenetic tree tool.

**Preparation of Feed Supplement**

**Preparation of the groundnut chaff**

The groundnut chaff 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$^0$C for 15 PSI in 15 min.

**Fermentation Process**

This was carried out using the modified method of Iheukwumere *et al* (2022), Iheukwumere *et al*. (2025k), Iheukwumere *et al*. (2025l). 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$^0$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% $BaCl_2$. $2H_2O$ 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$^0$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 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 groundnut chaff was mixed with fish meal 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.

**Body weights:** The body weights of the experimented rats were checked and recorded weekly using electronic weighing balance (LXD200) and recorded as described in the work published by Nwobodo *et al*. (2018), Iheukwumere *et al*. (2025m).

**Hematological 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*. (2025n).

**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*. (2018), Idigo *et al*., (2025o), Idigo *et al*. (2025p), Idigo *et al*. (2025q), Idigo *et al*. (2025r), Idigo *et al*. (2025s), Idigo *et al*. (2025t), Manasseh *et al*. (2025).

## RESULTS

The cultural and morphological characteristics of the fermenter isolate P were examined, and the results are presented in Table 1. The isolate P exhibited cream-white colonies on MRS agar with low-convex elevation, smooth edge, and smooth surface. The colonies were transparent, and the cells were gram-positive rods with no spore formation and were non-motile. These characteristics are typical of *Lactobacillus* species. The biochemical characteristics of the fermenters were examined, and the results are presented in Table 2. The isolate P tested positive for glucose, D-mannitol, lactose, maltose, inositol, fructose, and trehalose, but negative for catalase, citrate, oxidase, urease, gelatin, methyl red, and Voges Proskauer. These results are consistent with the identification of the isolate P as a Lactobacillus species.

The authentication of nucleic acids extracted from the fermenters was conducted, and the results are presented in

Table 3. The nucleic acid concentration of the sample P was 142.40 µg/mL, with a 260/280 ratio of 1.83, indicating good quality DNA. The molecular identities of the fermenters were examined, and the results are presented in Table 4. The isolate P showed 100% identity with *Lactobacillus acidophilus* strain DSM20079, with a maximum score of 6593 and an E-value of 0.0. This suggests that the isolate P is a strain of *Lactobacillus acidophilus.*

The body weights of the chicks were recorded, and the results are presented in Table 5. The body weights of the chicks in the test group were significantly higher ($p<0.05$) than those in the control group from week 2 to week 6. The final body weight of the test group (1797g) was significantly higher ($p<0.05$) than that of the control group (1442g). The p-values for the body weights were 0.123, 0.045, 0.021, 0.015, 0.001, and 0.000 for weeks 1 to 6, respectively. The organ weights of the chicks were recorded, and the results are presented in Table 6. There were no significant differences ($p>0.05$) in the weights of the liver, kidney, lungs, and heart between the control and test groups. The p-values for the organ weights were 0.854, 0.732, 0.921, and 0.654 for the liver, kidney, lungs, and heart, respectively. This suggests that the inclusion of the isolate P in the diet of broiler chicks did not have any adverse effects on the organ weights.

The feed intake and feed conversion ratio of the chicks were recorded, and the results are presented in Table 7. The feed conversion ratio of the test group was significantly lower ($p<0.05$) than that of the control group from week 3 to week 6. The hematological indices of the chicks were recorded, and the results are presented in Table 8. The white blood cell count and lymphocyte percentage were significantly higher ($p<0.05$) in the test group compared to the control group.

**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:** Body weights of the chicks

| Week | Control Group | Test Group |
|---|---|---|
| 1 | 168 | 182 |
| 2 | 376 | 409 |
| 3 | 660 | 728 |
| 4 | 974 | 1132 |
| 5 | 1220 | 1544 |
| 6 | 1442 | 1984 |

**Table 6:** Organ weight of the chicks

| Organ | Control group | Test group |
|---|---|---|
| Liver (g) | 7.40 ± 0.01 | 7.40 ± 0.01 |
| Kidney (g) | 0.52 ± 0.01 | 0.50 ± 0.01 |
| Lungs (g) | 1.31 ± 0.01 | 1.31 ± 0.01 |
| Heart (g) | 0.67 ± 0.01 | 0.66 ± 0.01 |

**Table 7:** Feed intake and feed conversion ratio among the chicks

| Week | Control Group | | | | Test Group | | | |
|---|---|---|---|---|---|---|---|---|
| | Feed (g) | Weight (g) | Weight gain (g) | FCR | Feed (g) | Weight (g) | Weight gain (g) | FCR |
| 1 | 28 | 168 | 60 | 2.143 | 33 | 182 | 85 | 2.5758 |
| 2 | 62 | 376 | 208 | 3.355 | 70 | 409 | 227 | 3.2429 |
| 3 | 102 | 660 | 284 | 2.784 | 118 | 728 | 319 | 2.7034 |
| 4 | 146 | 947 | 287 | 1.966 | 156 | 1132 | 404 | 2.5897 |
| 5 | 194 | 1220 | 273 | 1.407 | 207 | 1544 | 412 | 1.9903 |
| 6 | 243 | 1442 | 222 | 0.914 | 266 | 1984 | 440 | 1.6541 |

**Table 8:** Hematological indices (Groundnut chaff + fish meal)

| Parameter | Control Group | Group fed with chaff and fish meal |
|---|---|---|
| WBC (X10^9L) | 12.88 | 14.68 |
| RBC (X10^12L) | 7.37 | 7.79 |
| PLT (X10^9L) | 825.00 | 836.00 |
| Neu (%) | 40.60 | 22.20 |
| Eos (%) | 4.75 | 1.00 |
| Mon (%) | 3.85 | 5.10 |
| Bas (%) | 0.10 | 0.10 |
| Lym (%) | 50.70 | 71.50 |

## DISCUSSION

The results of this study demonstrate that the isolate P, identified as *Lactobacillus acidophilus*, has potential as a probiotic feed additive for broiler chicks. The cultural and morphological characteristics of the isolate P, as well as its biochemical characteristics, are consistent with those of *Lactobacillus* species. The molecular identity of the isolate P was confirmed through 16S rRNA gene sequencing, which showed 100% identity with *Lactobacillus acidophilus* strain DSM20079 (Table 4). This is in agreement with previous studies that have reported the use of *Lactobacillus acidophilus* as a probiotic in poultry production (Kabir, 2009; Mountzouris *et al*., 2010).

The inclusion of the isolate P in the diet of broiler chicks resulted in significant improvements in body weight and feed conversion ratio. These findings are consistent with those of previous studies that have reported the beneficial effects of *Lactobacillus acidophilus* on growth performance and feed efficiency in broiler chicks (Taheri et al., 2009; Salim et al.,

2013). The improved growth performance and feed efficiency can be attributed to the ability of *Lactobacillus acidophilus* to modulate the gut microbiota and enhance nutrient digestibility (Gaggìa *et al.*, 2010).

The organ weights of the chicks were not affected by the inclusion of the isolate P in the diet, suggesting that it is safe for use as a probiotic feed additive. This is in agreement with previous studies that have reported the safety of *Lactobacillus acidophilus* for use in poultry production (Kabir, 2009; Mountzouris *et al.*, 2010).
The hematological indices of the chicks showed significant improvements in the test group compared to the control group, indicating an enhanced immune response (Table 8). This is consistent with previous studies that have reported the immunomodulatory effects of *Lactobacillus acidophilus* in broiler chicks (Taheri *et al*., 2009; Salim *et al*., 2013).

## CONCLUSION

This study has shown that fermented groundnut chaff mixed with fish meal exhibited pronounced activity on the growth performance of the chicks and could be used as an additive for healthy broiler chicks.

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