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Evaluating the Prebiotic Effects of Fermented Cocoyam Peel and Fish Meal on Broiler Chick Performance

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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 cocoyam peel 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 cocoyam peel mixed with fish meal as a chicken additive for healthy broiler chicks. The fermenter, designated isolate M, was identified as *Lactobacillus acidophilus* strain DSM20079 based on cultural, morphological, biochemical, and molecular characteristics (100% 16S rRNA gene identity). The mixture of cocoyam peel and fish meal, fermented by this strain, was incorporated into the diet of broiler chicks, and its effects on growth performance, organ weights, feed intake, feed conversion ratio (FCR), and hematological indices were evaluated using *in vivo* techniques. Results showed that body weights of chicks in the test group were significantly higher ($p < 0.05$) than those in the control group from week 2 to week 6. The FCR of the test group was significantly lower ($p < 0.05$) than that of the control group from week 3 to week 6, indicating better feed efficiency. Organ weights (liver, kidney, lungs, and heart) were not significantly affected by the inclusion of the feed additive ($p > 0.05$). Haematological indices, including white blood cell count and lymphocyte percentage, were significantly higher ($p < 0.05$) in the test group compared to the control group, while all values remained within normal ranges, indicating no adverse health effects. This study suggests that the mixture of cocoyam peel and fish meal, fermented by *Lactobacillus acidophilus* strain DSM20079, 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, cocoyam peel, fish meal, feed conversion ratio, haematological indices.

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Introduction

The poultry industry is a significant contributor to the global food supply, with broiler chickens being a major source of protein (Adeola *et al*., 2018; Madubueze *et al*., 2025a; Anekwe *et al*., 2025a). However, the increasing demand for poultry products has led to concerns about animal welfare, food safety, and environmental sustainability (Herrero *et al.*, 2016; Egberi *et al.*, 2025a; Mbanefo *et al*., 2025a). One approach to addressing these concerns is the use of prebiotics, which are non-digestible feed ingredients that promote the growth of beneficial microorganisms in the gut, thereby enhancing

animal health and performance (Gibson *et al.*, 2017; Nwadiogbu *et al*., 2025a; Madubueze *et al*. 2025b).

Cocoyam (*Colocasia esculenta*) is a tropical tuber crop that is widely cultivated in Nigeria and other parts of West Africa (Onyeka *et al.*, 2015; Anekwe *et al*., 2025b; Egberi *et al*., 2025b). The peel of cocoyam is a rich source of dietary fiber, including prebiotic oligosaccharides, which can be fermented by beneficial microorganisms (Adeyemo *et al.*, 2018; Mbanefo *et al*., 2025b; Nwadiogbu *et al*. 2026a). Fish meal, on the other hand, is a high-quality protein source that is commonly used in poultry feed (NRC, 1994; Madubueze *et al*., 2026a; Anekwe *et al*., 2026a). Fermentation of cocoyam peel and fish meal blend has the potential to enhance their prebiotic properties and improve the nutritional value of broiler chick feed.

Despite the potential benefits of fermented cocoyam peel and fish meal blend as a prebiotic, there is a dearth of information on its effects on broiler chick performance (Egberi *et al*., 2025c; Mbanefo *et al*., 2025c; Nwadiogbu *et al*., 2026b). Most studies have focused on the use of prebiotics from plant sources, such as inulin and fructooligosaccharides (FOS), with limited attention given to the potential of fermented agro-industrial by-products like cocoyam peel and fish meal blend (Patterson *et al.*, 2014; Madubueze *et al*., 2026b; Anekwe *et al*., 2026b). This study aims to evaluate the prebiotic effects of fermented cocoyam peel and fish meal blend on broiler chick performance, thereby addressing the research gap in this area.

The objective of this study is to investigate the effects of fermented cocoyam peel and fish meal blend on the growth performance, gut health, and microbial population of broiler chicks. The findings of this study will contribute to the development of sustainable and eco-friendly poultry production systems, while also providing insights into the potential use of fermented cocoyam peel and fish meal blend as a prebiotic supplement in broiler chick diets.

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 (using a candle to remove all traces of oxygen, thereby creating an atmosphere of only carbon dioxide). The incubation was carried out for 24 – 48 h at (30±2°C).

Purification of the Isolates

The plate that showed discrete colonies was selected after 24 - 48 h, and each colony was aseptically streaked onto a sterile poured plate (90mm x 15mm) containing nutrient agar (BIOTECH), prepared according to the manufacturer's instructions. Afterwards, it was incubated under the required growth conditions.

Characterization of the Bacteria Pure Isolates

The pure isolates were characterized using the 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 cocoyam peel

The cocoyam peel was properly collected from the appropriate sites, washed and air-dried. The material was ground using an electrical blender, packed in a 500 ml beaker (PYREX) sealed with aluminium foil, and then autoclaved at 1210C for 15 PSI for 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 samples were aseptically dried in an electric oven at 80 °C for 7 days**.** After drying, the 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 (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 the poultry market located at Ihiala market, Ihiala L. G. A. in Anambra State, and were used for the study. The chicks were kept in separate, thoroughly cleaned and disinfected houses and provided with feeds and water ad libitum. All the chicks were vaccinated against Newcastle disease using the Lasota vaccine strain at 6 and 19 days of age, against infectious bronchitis using the live H120 strain at 6 days of age, and against avian influenza (A1) disease using the inactivated H5N1 virus vaccine strain at 7 days of age. All the vaccines were administered via eye-drop instillation, except the A1 vaccine, which was administered subcutaneously at the back of the neck, according to the folder report collected from the poultry farmer.

Feed Additive

The fermented cocoyam peel was mixed with fish meal and the feed in a ratio of 1:20 (fish meal: fermented cocoyam peel). 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 experimental rats were checked and recorded weekly using an electronic weighing balance (LXD200) and recorded as described in the work published by Nwobodo *et al*. (2018) and Onwuasonya *et al*. (2026b).

*Hematological Indices***:** The blood samples collected from the broiler chicks were examined using an Automated Haematology Analyser (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), Abba *et al*. (2026b) and Ezeoke *et al*. (2026b)

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 the Bonferroni correction test. Trend analysis was conducted using the Cochran-Armitage test for dose response. Pairwise comparison was performed 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 cultural and morphological characteristics of the fermenter were examined, and the results are presented in Table 1. The isolate M appeared as cream-white colonies on MRS agar, with a low-convex elevation, smooth edge, and smooth surface. The colonies were transparent, and the Gram reaction was positive, indicating that the isolate was a Gram-positive, rod-shaped bacterium.

The biochemical characteristics of the fermenter were also investigated, and the results are shown in Table 2. The isolate M was catalase-, citrate-, oxidase-, urease-, and gelatin-negative. It was positive for glucose, D-mannitol, lactose, maltose, inositol, fructose, and trehalose, but negative for xylose, sorbitol, and dulcitol. Based on these characteristics, the isolate was tentatively identified as a Lactobacillus species.

The molecular identity of the fermenter was confirmed through nucleic acid extraction and sequencing, and the results are presented in Tables 3 and 4. The nucleic acid concentration was found to be 142.40 µg/mL, with a 260/280 ratio of 1.83, indicating good-quality DNA. The sequencing results showed 100% identity to Lactobacillus acidophilus strain DSM20079, confirming the isolate's identity.

The effects of the fermented cocoyam peel and fish meal blend on broiler chick performance were evaluated, and the results are presented in Tables 5-8. From week 2 onwards, the body weights of chicks in the test group were significantly higher than those in the control group ($P < 0.05$). Organ weights, including liver, kidney, lungs, and heart, did not differ significantly between the two groups ($P > 0.05$). Feed intake and feed conversion ratio (FCR) were also assessed, and the test group had a lower FCR than the control group, indicating

better feed efficiency. The haematological indices, including WBC, RBC, PLT, Neu, Eos, Mon, Bas, and Lym, were within normal ranges in both groups, indicating no adverse effects on the chicks' health.

Table 1: Cultural and morphological characteristics of the fermenter

Parameter	Isolate M
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 M
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
M	142.40	3.1915	1.7440	1.83

Table 4: Molecular identities of the fermenter

Parameter	Isolate M
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	167	173
2	303	388
3	586	642
4	794	912
5	903	1244
6	1232	1542

Table 6: Organ weight of the chicks

Organ	Control group	Test group
Liver (g)	7.38 ± 0.01	7.39 ± 0.01
Kidney (g)	0.54 ± 0.01	0.53 ± 0.01
Lungs (g)	1.32 ± 0.01	1.32 ± 0.01
Heart (g)	0.67 ± 0.01	0.67 ± 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	167	60	2.7833	31	173	63	2.7460
2	61	373	140	2.6643	66	435	162	2.6852
3	99	586	222	2.6396	112	696	272	2.5588
4	134	781	298	2.6208	146	938	384	2.4427
5	168	1003	398	2.5201	186	1212	502	2.4143
6	202	1196	488	2.4508	236	1386	601	2.3061

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

Parameter	Control Group	Group fed with chaff and fish meal
WBC (X10^9L)	12.88	14.72
RBC (X10^12L)	7.37	7.52
PLT (X10^9L)	825.00	814.00
Neu (%)	40.60	35.00
Eos (%)	4.75	1.00
Mon (%)	3.85	3.00
Bas (%)	0.10	0.10
Lym (%)	50.70	60.90

Discussion

The results of this study demonstrate that isolate M, identified as *Lactobacillus acidophilus*, has potential as a probiotic feed additive for broiler chicks. The cultural, morphological, and biochemical characteristics of isolate M were consistent with those of *Lactobacillus* species. Its molecular identity was confirmed through 16S rRNA gene sequencing, showing 100% identity with *Lactobacillus acidophilus* strain DSM20079 (Table 4). This finding aligns with previous studies reporting the use of *Lactobacillus acidophilus* as a probiotic in poultry production (Gumus and Demirci, 2022; El-shal *et al.*, 2023; Egberi *et al*., 2026a; Mbanefo *et al*, 2026a).

Regarding growth performance, the inclusion of isolate M in the diet of broiler chicks resulted in significant improvements in body weight and feed conversion ratio. These results are consistent with earlier reports on the beneficial effects of *Lactobacillus acidophilus* on growth performance and feed efficiency in broilers (Vantsawa *et al.*, 2019; Attiia *et al.*, 2023; Nwadiogbu *et al*., 2026c; Anekwe *et al*., 2026d). The improved growth and feed efficiency can be attributed to Lactobacillus acidophilus's ability to modulate the gut microbiota and enhance nutrient digestibility (Liu *et al.*, 2025; Madubueze *et al*., 2026c; Anekwe *et al*., 2026c; Madubueze *et al*., 2026d).

In terms of safety, organ weights in chicks were not affected by the dietary inclusion of isolate M, suggesting that it is safe for use as a probiotic feed additive. This agrees with previous studies that have reported the safety of *Lactobacillus acidophilus* in poultry production (Hossain *et al.*, 2015; Vantsawa *et al.*, 2017; Egberi *et al*., 2026b; Mbanefo *et al*., 2026b; Nwadiogbu *et al*., 2026d).

Furthermore, haematological indices showed significant improvements in the test group compared to the control group, indicating an enhanced immune response (Table 8). This finding supports previous work demonstrating the immunomodulatory effects of *Lactobacillus acidophilus* in broiler chicks (Vantsawa *et al.*, 2017; Attia *et al.*, 2025; Egberi *et al*., 2026c; Nwadiogbu *et al*., 2026e).

Conclusion

This study has shown that fermented cocoyam peel 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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