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Short-Chain Fatty Acid Modulation in Chick Gut using Lactobacillus-Fermented Corn and Bean Chaff: A Prebiotic Approach

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Abstract

The poultry industry faces challenges in maintaining chick gut health, impacting growth and productivity. *Lactobacillus*-fermented feed additives have shown promise in promoting gut health. This study aimed to investigate the effects of *Lactobacillus acidophilus*-fermented corn and bean chaff on modulating short-chain fatty acids (SCFAs) in chick gut. The fermenter was isolated and characterized using cultural, morphological, and biochemical tests, and authenticated using molecular techniques. The isolate was identified as *Lactobacillus acidophilus* strain DSM20079. The SCFAs profile of the control and test groups were analyzed using gas chromatography. The results showed that the test group had increased levels of acetate (68.60% ± 0.12 vs 66.22% ± 0.11) and propionate (22.90% ± 0.11 vs 22.40% ± 0.16) compared to the control group, while n-butyrate (0.91 mg/L ± 0.01 vs 2.06 mg/L ± 0.01) and n-valerate (2.02% ± 0.01 vs 3.03% ± 0.01) decreased. Statistical analysis revealed significant differences ($p < 0.05$) in the levels of acetate, n-butyrate, and n-valerate between the test and control groups. The study concluded that *Lactobacillus acidophilus*-fermented corn and bean chaff modulated SCFAs and had the potential to enhance gut health in chicks. This study provides evidence on the prebiotic potential of *Lactobacillus acidophilus*-fermented corn and bean chaff in modulating SCFAs and promoting gut health in poultry.

Keywords: *Lactobacillus acidophilus*, Corn chaff, Bean chaff, Short-chain fatty acids, Gut health, Prebiotic

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Introduction

Microbial fermentation of agricultural produce is capable of yielding highly nutritious compounds for maintaining processes in living organisms (Awad *et al.*, 2017; Okeke *et al*., 2017; Dim *et al*., 2025a). Short chain fatty acid is an essential component found in the gut of living organisms (Awad *et al.*, 2017). This compound contains mainly fat and acid and confers enormous benefits to the organism. The fat and acid components appear in chains, which do not elongate. The roles of short chain fatty involve regulating the integrity of the gut, stabilizing immune function for wading off pathogens, and minimizing inflammation (Latorre *et al*., 2018). Poor production of short chain fatty acid in the gut leads to destabilization of body function in both animals and humans, due to loss of coordination (Gharib-Naseri *et al*., 2019; Amadi *et al*., 2017; Dim *et al*., 2025b).

It is worthy to not that short chain fatty acid is produced naturally in the gut by normal flora, which are vital bacterial species that are capable of digesting dietary fibre (Papatsiros et al., 2013). Digestion of dietary fibre by the normal flora of the gut releases short chain fatty acids as the byproduct such as butyrate, propionate, and acetate (Correa-Oleveira *et al*., 2016; Dim *et al*., 2025c; Chude *et al*., 2020). This indicates that enhancing short chain fatty acid production in both animal and man is essential as source of energy and increased absorption surface in the intestine through the improved proliferation of epithelial cells (Timbermont *et al*., 2010; Stefanello *et al.*, 2020).

Research has revealed that *Lactobacillus* species are capable of enhancing the production of short chain fatty acid in animals such as chick (Dittoe *et al*., 2018). *Lactobacillus* species are lactic acid bacteria that are capable of fermenting dietary fibre, which resists the action of digestive enzymes (Papatsiros *et al.*, 2013). Naturally, indigestible fibre can be found in both plant and animals such as bark of maize and beans chaff. The action of the lactic acid and other metabolites that are produced by *Lactobacillus* species facilitates the breakdown of the indigestible substances to release short chain fatty acids (Khan and Iqbal, 2016).

Several researchers have worked on the role of organic acid in modulating the integrity of short chain fatty acid such as Awad *et al*. (2017), Latorre *et al*. (2018), and Gharib-Naseri *et al*. (2019) but few studies are available on modulation of short chain fatty acid level of chicks gut using *Lactobacillus* fermented corn and beans chaff. Hence, the aim of this study is to evaluate modulation of short chain fatty acid level of chicks gut using *Lactobacillus* fermented chicken corn and beans chaff.

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 ± 2^0C). 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 corn and bean chaff

The corn and bean 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 121^0C 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 supplement was mixed with the feed in the ratio of 1:20. This 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 giving only feed. The experimental animals were fed in the morning, afternoon and night together with water for 4 months.

Short chain fatty acids (SCFAs): This was carried out using gas chromatographic (GC) technique as published by AOAC (2019), Iheukwumere *et al* (2025u), and Iheukwumere *et al* (2025v). One microliter of the prepared stool sample solutions was introduced into the injection chamber; the automated instrument was run in order to generate the values of the acetate, n-butyrate, propionate and n-valerate.

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 cultural and morphological characteristics of the fermenter are shown in Table 1. The result revealed that isolate P showed cream white appearance on MRS agar with a low convex elevation, smooth edge, and surface. The isolate has a Gram-positive rod, non-sporing and non-motile rods.

The biochemical characteristics of the fermenters revealed that isolate P was catalase, citrate, oxidase, urase, gelatin, methyl red, Voges Proskauer negative. The isolate showed complex utilization to glucose, lactose, maltose and fructose while it was unable to utilize xylose and sorbitol. The isolate showed varying utilization to D mannitol, inositol, Trehalose and Dulcitol as shown in table 2. The nucleic acid extracted from the fermenters revealed that it was deoxyribonucleic acid (DNA) at the ratio of 260nm/280nm ranging from 1.80-1.83 as shown in Table 3. The molecular characteristics of the fermenter reveal the presence of Lactobacillus acidophilus strain DSM20079 chromosome with a complete genome (LADSM) as shown in Table 4.

The modulation of short-chain fatty acid using *Lactobacillus* fermented corn and bean chaff revealed that there was an increase in the level of short-chain fatty acid, whereby acetate recorded the highest, followed by Propionate, N-valerate and n-butyrate compared to the control groups, and these changes were statistically significant ($P<0.05$) as shown in Table 5.

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: Short-chain fatty acids values

Short-chain fatty acid	Ref range	Control group	Test Group
Acetate (%)	50 – 72	66.22 ± 0.11	68.60 ± 0.12
n-butyrate (mg/L)	0.80 – 1.00	2.06 ± 0.01	0.91 ± 0.01
Propionate (%)	15.40 – 30.30	22.40 ± 0.16	22.90 ± 0.11
N-valerate (%)	0.80 – 5.00	3.03 ± 0.01	2.02 ± 0.01

Discussion

The results of this study demonstrate that *Lactobacillus acidophilus*-fermented corn and bean chaff modulates short-chain fatty acids (SCFAs) in chick gut. The isolate was identified as *Lactobacillus acidophilus* strain DSM20079, which is a well-known probiotic strain (Kumar *et al.*, 2018). The fermented product increased acetate and propionate levels, indicating a potential prebiotic effect. Acetate is a major SCFA produced by beneficial bacteria and has been shown to have anti-inflammatory properties (Rimm *et al.*, 2019).

The decrease in n-butyrate and n-valerate levels in the test group was unexpected, as these SCFAs are also important for gut health (Koh *et al.*, 2016). However, similar findings have been reported by other researchers, who suggested that the type and amount of substrate used for fermentation can influence SCFA production (Lee *et al.*, 2018). The results suggest that *Lactobacillus acidophilus*-fermented corn and bean chaff may have a specific effect on the gut microbiome, favoring the production of certain SCFAs over others.

The findings of this study are consistent with those of other researchers who have reported the prebiotic potential of Lactobacillus-fermented feed additives (Cutting, 2011; Wong *et al.*, 2019). The results provide evidence that *Lactobacillus acidophilus*-fermented corn and bean chaff can modulate SCFAs and promote gut health in chicks. Further research is needed to explore the mechanisms underlying these effects and to determine the optimal level of inclusion in poultry diets

Conclusion

Lactobacillus acidophilus-fermented corn and bean chaff effectively modulates short-chain fatty acids in chick gut, enhancing gut health and promoting sustainable poultry production. The fermented product increased acetate and propionate levels, showing prebiotic potential. This approach valorizes agricultural waste and offers an alternative to antibiotics. The study provides evidence for using *Lactobacillus acidophilus*-fermented corn and bean chaff as a feed additive to promote gut health and improve poultry productivity.

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