



Bacillus subtilis Fermented Bean Chaff and Chicken Feather: A Potential Strategy for Modulating Short-Chain Fatty Acid Levels in Chicks' Gut

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Abstract	Article History
<p>Poultry industry struggles with gut health and productivity optimization. Chicken feather waste and bean chaff are underutilized. Limited studies explore <i>Bacillus subtilis</i> fermented feed impact on chicks' gut SCFA levels, warranting investigation into its gut health potential. This study was carried out to evaluate the effect of <i>Bacillus subtilis</i> fermented corn and bean chaff on the modulation of short-chain fatty acid (SCFA) levels in the gut of chicks, and to investigate its potential as a gut health modulator. Fermenter used in this study was obtained following the standard microbiological techniques. Corn and bean chaff were collected, processed, homogenized and fermented using solid state fermentation method, and this was incorporated as feed additive and assessed for the impact on SCFAs of chicks' gut using <i>in vivo</i> technique. The fermenter (<i>Bacillus subtilis</i> PK5-17) was characterized culturally, morphologically, and biochemically. Results showed increased acetate (69.10% vs 66.22%) and propionate (26.03% vs 22.40%) levels ($p < 0.05$) in test vs control groups. n-butyrate decreased (0.92 vs 2.06 mg/L, $p < 0.05$); n-valerate was similar ($p > 0.05$). The fermented feed shows potential as a gut health modulator in chicks.</p> <p>Keywords: <i>Bacillus subtilis</i>, Fermented, Short-chain fatty acids, Gut health, Chicks</p>	<p>Received: 29 Dec 2025 Accepted: 08 Feb 2026 Published: 13 Feb 2026</p> <p>Scan QR code to view*</p>  <p>License: CC BY 4.0</p>  <p>Open Access article.</p>
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1. Introduction

Short chain fatty acid is an essential component found in the gut of living organisms (Awad *et al.*, 2017; Iheukwumere *et al.*, 2025a; Dim *et al.*, 2025a). 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).

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; Iheukwumere *et al.*, 2022a; and Nwike *et al.*, 2017). 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-Oliveira *et al.*, 2016). This indicates that enhancing short chain fatty acid production in both animal and man is essential for regulation of the intestinal mucosa and productivity and the product enhances absorption of nutrients and stimulation of vibrant

immune response in chicks ((Timbermont *et al.*, 2010; Stefanello *et al.*, 2020; Amadi *et al.*, 2017).

Research has revealed that *Bacillus* species are capable of enhancing the production of short chain fatty acid in animals such as chick (Dittoe *et al.*, 2018; Ejike *et al.*, 2017). *Bacillus subtilis* is a lactic acid bacterium that can ferment 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, beans and chicken feathers. The action of the lactic acid and other metabolites that are produced by *Bacillus* species facilitates the breakdown of the indigestible substances to release short chain fatty acids (Khan and Iqbal, 2016; Ekechukwu *et al.*, 2025a; Obianom *et al.*, 2024; Dim *et al.*, 2025b).

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), Iheukwumere *et al.* (2025b), Dim *et al.* (2025c), but few studies are available on the modulation of short-chain fatty acid levels of chicks gut using *Bacillus subtilis*-fermented chicken feather and beans chaff. Hence, the aim of this study is to evaluate the modulation of short chain fatty acid level of chicks' gut using *Bacillus subtilis*-fermented beans chaff and chicken feather.

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 microaerophilic 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^oC).

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^oC 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^oC 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 added 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 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 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 (H₂O₂) 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°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 miniprep™ 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 (MgCl₂) 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 electrophoresed in 1.0% agarose gel and a 1kb 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 bean chaff and Chicken feather

The bean chaff and chicken feather 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°C for 15 min at 15psi.

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°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% BaCl₂. 2H₂O and 99.4 mL of 1% Conc. H₂SO₄. This was allowed for 7 days.

Storage and packaging

After fermentation, the fermented samples were aseptically dried using an electric oven at 80°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

Experimental Protocols for the *In vivo* Models: A total of 36 broiler chicks were used for this study. The broiler chicks were grouped into six groups, and each group comprises 6 chicks. . A 0.5 g/100 g of fermented corn mixed with fish meal was orally administered to each of group of broiler chicks, and the remaining group was giving only feed and water as control group. The body weights and blood absolute lymphocytes were assessed from the blood samples drawn from the chicks after 11 days.

Short chain fatty acids (SCFAs): This was carried out using gas chromatographic (GC) technique as published by AOAC (2019), Iheukwumere *et al.* (2025m), Iheukwumere *et al.* (2025n). 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.* (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 is shown in Table 1. The results revealed that isolate Q appeared white on Nutrient agar with a flat elevation, irregular edge and a rough surface. The isolate was a gram-positive cocci bacillus and a spore former with a central positioning of the spore.

The biochemical characteristics of the fermenter are shown in Table 2. The results revealed that isolate Q was positive to catalase, citrate, oxidase, gelatin, and Voges-Proskauer, while negative to urease and methyl red. The isolate showed complete utilization of glucose, D-mannitol, maltose, xylose, inositol, fructose, sorbitol, trehalose and dulcitol while unable to utilize lactose.

The nucleic acid extracted from the fermenter revealed that it was deoxyribonucleic acid (DNA) at the ratio of 260nm/280nm with a range of 1.80-1.81, as shown in Table 3. The molecular characteristics of the fermenter revealed the presence of the *Bacillus subtilis* strain PK5-17 chromosome with a complete genome (BSPK5) as shown in Table 4.

The modulation of short-chain fatty acid using *Bacillus subtilis* fermented bean chaff and feather fed to the test group showed an increase in the level of short-chain fatty acid, whereby acetate recorded the highest, followed by propionate, N-valerate and n-butyrate, and these effects were statistically significant ($P < 0.05$) as shown in Table 5.

Table 1: Cultural and morphological characteristics of the fermenters

Parameter	Isolate Q
Appearance	Cream/white on Nutrient agar
Elevation	Flat
Edge	Irregular
Surface	Rough
Optical Nature	Opaque
Gram Reaction	+
Cell Morphology	Rods with round ends
Spore	+
Position of Spore	Central
Motility	+

++ Positive; - = Negative

Table 2: Biochemical characteristics of the fermenter

Parameter	Isolate Q
Catalase	+
Citrate	+
Oxidase	+
Urease	-
Gelatin	+
Methyl Red	-
Voges Proskauer	+
Glucose	+
D-mannitol	+
Lactose	-
Maltose	+
Xylose	+
Inositol	+
Fructose	+
Sorbitol	+
Trehalose	+
Dulcitol	+
Possible Isolate	<i>Bacillus</i> species

Table 3: Authentication of nucleic acids extracted from the fermenter

Sample ID	Nucleic Acid Conc($\mu\text{g/mL}$)	260 nm	280 nm	260/280
Q	126.20		1.7220	1.81

Table 4: Molecular identities of the fermenter

Parameter	Isolate Q
Max Score	4783
Total Score	4783
Query Cover (%)	100
E-Value	0.0
Identity (%)	100
Accession Length	4086149
Accession Number	CP026037.1
Description	<i>Bacillus subtilis</i> strain PK5-17 Chromosome Complete genome (BSPK5)

Table 5: Short-chain fatty acids values

Short-chain fatty acid	Ref range	Control group	Test Group
Acetate (%)	50 – 72	66.22 \pm 0.11	69.10 \pm 0.17
n-butyrate (mg/L)	0.80 – 1.00	2.06 \pm 0.01	0.92 \pm 0.01
Propionate (%)	15.40 – 30.30	22.40 \pm 0.16	26.03 \pm 0.11
N-valerate (%)	0.80 – 5.00	3.03 \pm 0.01	3.07 \pm 0.01

DISCUSSION

Bacillus subtilis is the type species for the genus *Bacillus* which are gram-positive, spore-forming bacilli which are capable of producing enzymes which break down proteins, starch and lipids into amino acids, sugars, fatty acids and glycerol (Bayasal and Yildiz, 2019). Their ability to break down proteins, starch and lipids into amino acids, sugars, fatty acids and glycerol supports gut health and boosts the immune response, has gained significant insight (Kovacs, 2019). This present study focuses on the modulation of short-chain fatty acid levels using *Bacillus subtilis* fermented chicken feather in broiler chicks. The cultural, morphological and biochemical characteristics of the *Bacillus subtilis* corroborated with the findings of many researchers (Das and Prasad, 2010; Vijayalakshmi *et al.*, 2019; Baysal and Yildiz, 2019) who isolated and characterized *Bacillus subtilis* from banana. The ability of the isolates to utilize common sugars such as glucose, lactose, sucrose, maltose and some alcoholic sugars such as mannitol, sorbitol and xylitol indicates their potential to produce beneficial metabolites which support gut health and aid food fermentation. These potentials were also described by many researchers (Das and Prasad, 2010; Vijayalakshmi *et al.*, 2019; Baysal and Yildiz, 2019). The molecular characterization of the *Bacillus subtilis* strain revealed the presence of *Bacillus subtilis* strain PK5-17 (LADSM). The occurrence of *Bacillus subtilis* strain in banana had been reported by Haroun (2011) and Ragavi *et al.* (2019), who stated that the occurrence may be due to the enzyme richness of banana.

The results obtained from the study revealed that *Bacillus subtilis* fermented bean chaff and chicken feather significantly increased the short-chain fatty acid levels such as acetate, n-butyrate, propionate and N-valerate. These findings were in line with the findings of most researchers (Omar *et al.*, 2021; Chang, 2022; and Zhang *et al.*, 2023) who investigated the effect of *Bacillus subtilis* fermented meal on the modulation of short-chain fatty acid levels in broiler chicks.

CONCLUSION

The study has shown that *Bacillus subtilis* fermented bean chaff and chicken feather exhibited pronounced activity on the level of short-chain fatty acid of the broiler chicks. Hence could be used as additive for healthy chicks,

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