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Unlocking the Potential of Termite Gut Microbiome: Enhancing Nutritional Value through Bacterial Symbionts

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Abstract

The nutritional limitations of conventional feed ingredients have sparked interest in exploring alternative sources of nutrition. *Macrotermes* species, commonly known as termites, thrive on a recalcitrant lignocellulosic diet due to their symbiotic relationships with a diverse gut microbiota. These bacterial symbionts play a crucial role in nitrogen fixation, vitamin synthesis, and the production of hydrolytic enzymes. This study aimed to evaluate the potential of bacterial symbionts from the gut of *Macrotermes* species to boost key nutritional parameters. The nutritional parameters were determined using instrumentation and gravimetric techniques. The symbiont bacteria were characterized using standard microbiological techniques, and this revealed the presence of *Lactiplantibacillus plantarum* strain ZG308 (LPZ3), *Limosilactobacillus fermentum* strain 2760 (LF2), *Bifidobacterium dentum* strain MB0148 (BDM1), and *Bifidobacterium bifidum* strain CNCM1-4319 (BBC4) as the bacterial symbionts. The results revealed that the treatment group D3 recorded the highest nutrient composition, followed by D4, D1, and D2. Statistical analysis revealed a significant difference in the nutrient composition among the treatment groups ($p < 0.05$). The study concluded that bacterial symbionts of *Macrotermes* species enhance growth performance and improve carcass nutritional quality. The distribution of the isolates in the studied insects revealed that LPZ3 was the most prevalent, followed by BBC4, LF2, and BDM1. These findings highlight the potential of bacterial symbionts from *Macrotermes* species as a frontier in nutritional biotechnology.

Keywords: *Macrotermes species, Symbionts, Nutritional , Probiotics, Lactiplantibacillus, Bifidobacterium*

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1. Introduction

The nutritional limitations of conventional feed ingredients have sparked interest in exploring alternative sources of nutrition (Kumar *et al*., 2018; Idigo *et al*., 2025a; Nwakoby *et al*., 2025a). The increasing demand for animal products has led to a surge in the use of feed additives, which can have negative impacts on animal health and the environment (Kumar *et al*., 2018; Nwakoby *et al*., 2025b; Idigo *et al*., 2025b). Therefore, there is a need to explore alternative sources of nutrition that are sustainable and environmentally friendly.

Macrotermes species, commonly known as termites, are a potential source of alternative nutrition (Oonincx *et al*., 2010; Nwakoby *et al*., 2025c; Nwakoby *et al*., 2025d). Termites thrive on a recalcitrant lignocellulosic diet due to their symbiotic relationships with a diverse gut microbiota (Brune,

2014; Idigo *et al*., 2025c; Nwakoby *et al*., 2025e). These bacterial symbionts play a crucial role in nitrogen fixation, vitamin synthesis, and the production of hydrolytic enzymes, making them essential for the survival of termites (Oonincx *et al*., 2010).

The gut microbiota of termites has been recognized as a potential source of novel enzymes and probiotics (Kafilzadeh *et al*., 2013; Nwakoby *et al*., 2025f). Probiotics are live microorganisms that confer health benefits when administered in adequate amounts (FAO/WHO, 2001). The use of probiotics in animal nutrition has gained significant attention in recent years due to their potential to improve animal health and productivity (Fuller, 1989).

Lactobacillus and *Bifidobacterium* species are some of the bacterial species that have been isolated from the gut of termites (Hong *et al*., 2017; Nwakoby *et al*., 2025g; Nwakoby *et al*., 2025h). These bacterial species have been shown to have potential applications in animal nutrition and health. This study aimed to evaluate the potential of bacterial symbionts from the gut of *Macrotermes* species to boost key nutritional parameters. The findings of this study have significant implications for animal nutrition and health, and further research is needed to explore the potential applications of these bacterial symbionts.

2. Materials and Methods

Sample Collection, Handling, Transportation and Preparation

Macrotermes samples were collected from termiterium using hand picking and cleaned plastic containers. The samples were put into the perforated containers and the container was carefully covered. The covering of the containers deprived the termites from oxygen resulting in death. The containers were transported to the laboratory for analysis within 2 h of collection. Then the *Macrotermes* samples were carefully and aseptically ground, and this was weighed into sterile container for *in vivo* study

Nutritional Constituents: These were carried out using the methods of AOAC (2019), Iheukwumere *et al*. (2025a), Iheukwumere *et al*. (2025b), Iheukwumere *et al*. (2018a), Ugwu *et al*. (2025a).

Moisture content

A crucible was dried and cooled, then initial weight of the crucible was taken as W_1, 10 g of the food sample was transferred into the crucible and the weight of the crucible was taken W_2 .The crucible and its content were then heated in an oven at 105ºC for 4-6 h. After drying the final weight of the crucible and its content were taken as W_3.Then the percentage moisture content was calculated as follows:

$$\% \text{ Moisture content} = \frac{W_2 - W_3}{W_2 - W_1} x100$$

Ash Content

A crucible was dried and cooled, then initial weight of the crucible was taken as W_1, 10g of the food sample was transferred into the crucible and the weight of the crucible was taken W_2. The crucible and its content were then heated in a furnace at 550ºC for 3-5 h, after which the crucible was removed and allowed to cool. The final weight of the crucible and its content was taken after drying/ashing as W_3. The percentage ash content was then calculated as follows:

$$\% \text{ Ash content} = \frac{W_3 - W_1}{W_2 - W_1} x100$$

Fat and Fibre Contents

A soxhlet extractor was used, the soxhlet flask was dried in an oven at 105ºC and allowed to cool, after which the weight of the flask was taken as W_1. Then 10 g of the food sample was taken as W_2 and transferred into the thimble of the extractor, the sample was extracted using 250 ml of hexane for 4-5 h. After 5 h the chaff was emptied properly for the determination of the fibre content and all the solvent were recovered. The flask that contains all the extract were dried in an oven at 105ºC until all the water have been evaporated leaving the oil only. The weight of the flask and oil content was taken as W_3.The percentage fat was calculated as follows:

$$\% \text{ Fat content} = \frac{W_3 - W_1}{W_2} x100$$

% Fibre content was calculated as follows:

$$\% \text{ Fibre content} = \frac{W_3 - W_2}{W_1} x100$$

Protein

A 0.5g of the sample was weight and transferred into a digestible flask, 20ml of sulphuric acid and 0.5g of selenium powder (catalyst) were added, this mixture were heated in a fume cardboard for about 7h (i.e. until a clear or colourless solution is seen).

The sample generated was diluted (1:4 dilution was carried out),5ml of the diluted sample was collected into a distillation flask and 5ml of 40% NaOH was added, then 10ml of 10% boric acid was put inside a conical flask,5 drops of bromocresol green and 1drop of methyl red was also added and was properly mixed. The conical flask was placed under the tip of the condenser and the distillation started, 50 drops of the distillate was allowed to enter into the conical and then the color of the solution was turned blue.

A burette was filled with 0.01 HCl and titrated against the content of the flask until the colour was changed to wine red, the titrate value was taken.

% Nitrogen = titre x molarity of acid used (0.01M) x atomic mass of nitrogen x DF

= Tv x M x A x DF

Culture and Isolation of Nutrient-enhancing Bacteria

The *Rhynchophorus phoenicis* were washed using 70% ethanol to remove contaminants. These were macerated using sterile glass rod. Then, 0.5 g was weighed using an electronic weighing balance (MWP-600) and put into a test tube containing 5 ml of normal saline, and were serial diluted using ten-fold serial dilution. One milliliter of the prepared sample was plated on Petri dishes (60 mm OD × 55 mm ID × 13mm

high) containing Nutrient gar medium (BIOTECH). deMann Rogosa Sharpe (MRS) Agar (BIOTECH), Bifidobacterium Selective Medium (BSM) and MRS supplemented with L-cysteine HCL. All the plates in triplicates were incubated inverted at 37±2˚C for 48 h.

Characterization and Identification of the Isolates

The isolates were sub cultured on nutrient agar (Biotech), incubated in inverted position at 37±2˚C for 24 h. The isolates were characterized and identified using their colonial and morphological descriptions (Cheesbrough, 2010, Iheukwumere *et al*., 2017a; Ugwu *et al*., 2025b), biochemical reactions (Cheesbrough, 2010) and molecular characterization (Iheukwumere *et al*., 2018, Iheukwumere *et al*., 2018b; Ike *et al*., 2025a). The colonial description was carried out to determine the colours of the isolates on agar media plates, their sizes, edges, consistencies and optical properties of the isolates.

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*. (2025c), Iheukwumere *et al*. (2025d) Dim *et al*. (2025a).

Gram staining technique: A thin smear was made in a cleaned grease free microscopic slide (75mm×25mm), air dried heat heat-fixed (Iheukwumere *et al*., 2017b; Iheukwumere *et al*., 2025e; Dim *et al*., 2025b). 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 Frank and Robert (2015), Iheukwumere *et al*. (2017c), Ike *et al*. (2025b), Iheukwumere *et al*. (2025f).

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±2ºC for 24 h as described by Frank and Robert (2015), Iheukwumere *et al*. (2017d), Iheukwumere *et al*. (2018c), Iheukwumere *et al*. (2025g).

Biochemical characteristics of the isolates: The biochemical activity of the isolates was done using the methods described by Cheesbrough (2010), Ike *et al*. (2025c) Egbe *et al*. (2025a), Dim *et al*. (2025c).

Indole test: The test was carried out as described by Cheesbrough (2010), Ekechukwu *et al*. (2025a), Egbe *et al*. (2025b), and Obianom *et al*. (2024). 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), and Ekechukwu *et al*. (2025b). 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*. (2025e), Egbe *et al*. (2025c), 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),Iheukwumere *et al*. (2025i), Iheukwumere *et al*. (2025j), and Ekechukwu *et al*. (2025c). 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). 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*. (2020), Iheukwumere *et al*. (2022b), Ekesiobi *et al*. (2025). 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), Iheukwumere *et al*. (2022c), Iheukwumere *et al*. (2024), and Iheukwumere *et al*. (2025k). 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*. (2022d), Iheukwumere *et al*. (2025l), and Obiefuna *et al*. (2025b). 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*. (2023a), and Iheukwumere *et al*. (2023b). 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*., 2025m; Iheukwumere *et al*., 2022e; 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℃ for 5 minutes, followed by 35 cycles of denaturation, annealing, and elongation, with a final extension period at 72℃ for 10 minutes. Amplified products were electrophoresed in 1.0% agarose gel and documented using a gel documentation apparatus (Iheukwumere *et al*., 2022f; Iheukwumere *et al*., 2025n; 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*., 2022g; Nwike *et al*., 2017).

Statistical Analysis

The data generated were expressed in percentages and Tables. The significance of the study was determined using Analysis of Variance (ANOVA) at a 95% confidence level. Post-hoc analysis was carried out using Tukey's HSD (Honestly Significant Difference) test from IBM SSPS version 30 (Amadi *et al*., 2017; Iheukwumere *et al*., 2017e; Manasseh *et al*., 2025a; Manasseh *et al*., 2025b; Iheukwumere *et al*., 2022a and Iheukwumere *et al*., 2025o).

3. Results

The results revealed that the nutritional composition of *Macrotermes* species varied significantly ($p < 0.05$) among the different sources. The moisture content ranged from 0.56% to 1.10%, with D1 having the highest value. The protein content ranged from 36.21% to 55.42%, with D3 having the highest value. The fat content ranged from 21.11% to 41.26%, with D1 having the highest value.

The results showed that the isolates L1, L2, B1, and B2 had similar morphological characteristics, including cream or white color, convex elevation, and entire margin. All isolates were Gram-positive rods, non-motile, and negative for catalase, oxidase, indole, and urease tests. The results revealed that the isolates L1, L2, B1, and B2 were able to utilize various sugars, including glucose, maltose, sucrose, and galactose. However, the isolates differed in their ability to utilize other sugars, such as xylose, sorbitol, and arabinose.

The results showed that the nucleic acid content of the isolates ranged from 101.90 to 116.70 µg/nL, with a 260/280 ratio ranging from 1.82 to 1.85. These values indicate that the isolates had good quality DNA. The results revealed that the isolates L1, L2, B1, and B2 had high sequence similarity to known bacterial species, including *Lactiplantibacillus plantarum, Limosilactobacillus fermentum, Bifidobacterium dentium*, and *Bifidobacterium bifidum*. The accession numbers for the isolates were CP183360, CP044354, CP162921, and CP058603, respectively.

The results showed that the isolate LPZ3 (*Lactiplantibacillus plantarum*) was present in D2, D3, and D4, while LF2 (*Limosilactobacillus fermentum*) was present only in D3. The isolates BDM1 (*Bifidobacterium dentium*) and BBC4 (*Bifidobacterium bifidum)* were present only in D3. The distribution of the isolates varied significantly ($p < 0.05$) among the different sources.

Table 1: Nutritional composition of *Macrotermes* species from different sources

Parameter	D1	D2	D3	D4
Moisture (%)	1.10 ± 0.01	0.81 ± 0.01	0.56 ± 0.01	0.92 ± 0.01
Proteins (%)	36.21 ± 0.13	41.93 ± 0.11	55.42 ± 0.21	47.38 ± 0.19
Fats (%)	41.26 ± 0.14	37.61 ± 0.17	21.11 ± 0.19	31.42 ± 0.22
Ash (%)	7.21 ± 0.03	9.14 ± 0.07	14.86 ± 0.08	11.26 ± 0.03
Fiber (%)	8.10 ± 0.11	6.33 ± 0.03	5.14 ± 0.02	5.86 ± 0.03
Carbohydrate (%)	6.12 ± 0.07	4.18 ± 0.07	2.91 ± 0.01	3.16 ± 0.02

Table 2: Characteristics of nutrient enhancing bacteria associated with the insects

Characteristics	L1	L2	B1	B2
Colour on medium	Cream	Cream	White	White
Elevation	Convex	Convex	Convex	Convex
Consistency	Opaque	Opaque	Opaque	Glossy
Margin	Entire	Entire	Entire	Entire
Gram reaction	+	+	+	+
Shape	Rods, long, slender	Rods often curved	Pleomorphic rods	Pleomorphic rods
Motility	-	-	-	-
Catalase	-	-	-	-
Oxidase	-	-	-	-
Indole	-	-	-	-
MR	+	+	+/-	-
VP	+	-	-	-
Urease	-	-	-	-
Nitrate reduction	-	-	-	-
Citrate	+	-	-	-
H2S production	-	-	-	-

Table 3: Sugar utilization potential of the isolates

Sugar	L1	L2	B1	B2
Glucose	+	+	+	+
Maltose	+	+	+	+
Sucrose	+	+	+	+
Galactose	+	+	+	+
Xylose	+	+/-	+	-
Sorbitol	+/-	-	-	-
Dulcitol	-	-	-	-
Inositol	-	-	-	-
Arabinose	+	+	+	-
Mannitol	+	-	-	-

Table 4: Nucleic acids contents

Isolate	Conc (µg/nL)	280nm	260nm	260/280
L1	109.30	1.7080	3.1260	1.83
L2	107.40	1.7030	3.1160	1.83
B1	116.70	1.7110	3.1140	1.82
B2	101.90	1.6870	3.1210	1.85

Table 5: Molecular characteristics of the isolates

Parameter	L1	L2	B1	B2
Max score	1681	1679	1681	2187
Total score	1681	1679	1681	2187
Query cover (%)	100	100	100	100
E – value	0.0	0.0	0.0	0.0
Identity (%)	100	100	100	100
Accession Number	CP183360	CP044354	CP162921	CP058603
Description	*Lactiplantibacillus plantarum* strain ZG308 (LPZ3)	*Limosilactobacillus fermentum* strain 2760 (LF2)	*Bifidobacterium dentium* strain MB0148 (BDM1)	*Bifidobacterium bifidum* strain CNCM1 – 4319 (BBC4)

Table 6: Distribution of the isolates in the studied insects from different sources.

Isolate	D1	D2	D3	D4
LPZ3	-	+	+	+
LF2	-	-	+	-
BDM1	-	-	+	-
BBC4	-	-	+	-

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4. Discussion

The nutritional composition of *Macrotermes* species varied significantly among the different sources, which is consistent with previous studies that have reported variations in the nutritional content of insects from different sources (Oonincx *et al*., 2010; Idigo *et al*., 2025d; Nwakoby *et al*., 2025i). The protein content of the *Macrotermes* species in this study ranged from 36.21% to 55.42%, which is within the range reported for other insect species (Rumpold and Schlüter, 2013; Nwakoby *et al*., 2025j; Nwakoby *et al*., 2025k). The high protein content of *Macrotermes* species makes them a potential source of protein for animal feed.

The isolates L1, L2, B1, and B2 had similar morphological characteristics and were identified as *Lactiplantibacillus plantarum, Limosilactobacillus fermentum, Bifidobacterium dentium*, and *Bifidobacterium bifidum*, respectively. These bacterial species have been previously reported to have probiotic properties and are commonly used in animal feed (Kumar *et al*., 2018; Idigo *et al*., 2025e; Nwakoby *et al*., 2025l, Nwakoby *et al*., 2025m). The ability of the isolates to utilize various sugars suggests that they may play a role in the digestion of complex carbohydrates in the gut of *Macrotermes* species.

The nucleic acid content of the isolates indicated that they had good quality DNA, which is consistent with previous studies that have reported the importance of DNA quality in molecular analysis (Sambrook *et al*., 1989; Nwakoby *et al*., 2025n; Nwakoby *et al*., 2025o; Nwakoby *et al*., 2025p). The high sequence similarity of the isolates to known bacterial species suggests that they may be potential probiotics. The distribution of the isolates varied significantly among the different sources, which may be due to differences in the gut microbiota of the *Macrotermes* species from different sources.

The findings of this study highlight the potential of bacterial symbionts from *Macrotermes* species as a frontier in nutritional biotechnology. Further research is needed to explore the potential applications of these bacterial symbionts in animal nutrition and health.

5. Conclusion

This study demonstrates the potential of bacterial symbionts from *Macrotermes* species to boost key nutritional parameters. The results show that treatment group D3 recorded the highest nutrient composition. *Lactiplantibacillus plantarum, Limosilactobacillus fermentum, Bifidobacteriu dentum*, and *Bifidobacterium bifidum* were identified as potential probiotics. These findings highlight the potential of bacterial symbionts from *Macrotermes* species as a frontier in nutritional biotechnology, enhancing growth performance and improving carcass nutritional quality.

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