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SHORT COMMUNICATION

Production of probiotic-fortified composite poultry feed from food and agricultural waste material

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ABSTRACT

Objective: The objective of the study was to ascertain the feasibility of fortifying composite poultry feed from food and agricultural waste material with the probiotic organism *Lactobacillus fermentum* and determine the efficiency of formulated probiotic-fortified feed via animal feeding tests.

Materials and Methods: Probiotic-fortified feed (G3) was formulated using proximate analysis values of waste materials. Alternative diets were G1—Feed Mill of Nigeria starter mash and G2—Ground corn. For growth comparison test, 30 1-day-old Agricol broiler chicks were randomized into three groups of 10 chicks each with each group being placed on a separate diet (G1, G2, and G3). Probiotics antimicrobial efficacy feeding assay consisted of the treatment diets T1—Feed Mill of Nigeria starter mash and T2—probiotic-fortified feed. Twenty 1-day-old unvaccinated chicks were placed into two groups of 10 chicks each and fed 0.5 ml of 9.0×10^8 CFU/ml *Escherichia coli* 0157:H7 on day 1 after which they were placed on treatment diets. Data collected were analyzed and interpreted using the SPSS Statistical tool version 25.

Results: Chicks fed G1 and G3 diets performed similarly ($p < 0.05$) in terms of measured parameters (weight, height, and wingspan) and had better performance compared to chicks on G2. In the *E. coli* treatment group, chicks placed on treatment diets T1 and T2 showed similar levels of *E. coli* cell reduction every week. Performance based on measured parameters was also similar ($p < 0.05$).

Conclusion: Feasibility of fortifying composite animal feed with the probiotic organism *L. fermentum* was ascertained and the efficiency of the feed via animal feeding tests was proven.

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INTRODUCTION

Food waste is produced at every stage of food production from the farm to fork (harvest, transportation, storage, distribution, and consumption). In developing countries, most waste occurs during harvest, transportation, and storage [1]. With food waste being intrinsically linked to food insecurity, loss of resources and adverse environmental impacts, there is the need to channel wastes being produced into alternative channels for utilization. Studies investigating the feasibility of feeding animals' food waste have been carried out with positive findings [2–5].

Conventionally produced feed has been implicated in the spread of antibiotic resistance genes [6] and bioaccumulation of toxic feed additives [7]. It is also often expensive thereby making it inaccessible to small-scale producers and farmers. Hence, there is the urgent need to move from the use of conventionally produced feed to safer, cheaper, and environmentally friendly alternatives. One such way is in the use of beneficial microorganisms as feed additives or direct-fed microbials [8].

The use of microorganisms as additives in feed and direct-fed microbials in animal feeding tests has been shown to reduce intestinal pathogen colonization, improve rumen pH stabilization, improve productivity (egg production, body weight, milk production), facilitate the production of antibacterials [9,10], and produce other health

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benefits [11–13]. This study aims to ascertain the feasibility of fortifying composite animal feed formulated from food and agricultural waste materials with the probiotic organism *Lactobacillus fermentum*, as well as its effectiveness via animal feeding tests.

Materials and Methods

Ethical approval

Ethical approval for use of experimental animals (broiler chicks) feeding test was sought for and approved by the Health Research Ethics Committee, Covenant University, Ogun State, Nigeria (NHREC/25/10/2018).

Waste material collection and treatment

Leftover food waste was collected from Cafeteria 2, Covenant University, Ogun State, Nigeria. Plantain peels, corn husks, and yam peels were collected from domestic kitchen waste. The materials were aseptically collected in zip lock bags and transported to the Microbiology laboratory of Covenant University, Ogun State. The waste materials were sorted into their respective nutrient classes and heat treated at 90°C for 4 h to decrease moisture content to 10%–12% which is the moisture content recommended in the feed.

Isolation and identification of microbial isolate

Microorganism of interest was isolated from Ogi and Yoghurt samples. Upon serial dilution, aliquots were plated on de-Mann, Rogosa and Sharpe (MRS) agar (HiMedia, Mumbai, India) and incubated in 4% CO_2 at 37°C for 5 days. Sub-culturing was carried out to obtain pure cultures which were also incubated at 37°C for 5 days in 4% CO_2. Morphological (gram staining and colonial morphology), biochemical (catalase, sugar fermentation, oxidase, and milk coagulation), cultural (endospore test), and molecular characterization were carried out [14].

Bacterial genomic DNA was extracted by the protocol of Trindade et al. [15]. The stock DNA was run on a 1.5% Agarose gel at a voltage of 120 V for 40 min. This was later viewed under UV light to confirm that bacterial DNA was present. The stock DNA was also quantified on a Nanodrop spectrophotometer 2000 (Applied Biosystem Inc., USA) to determine the quantity and also the purity of the samples before polymerase chain reaction (PCR). Working DNA solution was diluted at 1:50 for subsequent PCR assay. PCR was carried out with the GeneAmp 9700 PCR System Thermal cycler (Applied Biosystem Inc., USA). Amplified DNA fragment was run on 2% Agarose gel at a voltage of 120 V for 60 min. This was later viewed under UV light to confirm the presence of the amplified PCR products.

Formulation of composite feed

Proximate analysis test was carried out on heat treated waste materials. Waste materials were ground and feed was formulated according to the nutritional requirement of broilers. Materials were compounded into composite feed using the values obtained from the proximate analysis. The Pearson's square was used in feed formulation.

Probiotic fortification of composite feed

The probiotic organism *L. fermentum* was grown in 500 ml MRS broth (HiMedia, Mumbai, India) at 4% CO_2 and incubated for 5 days at 37°C. After incubation, broth containing *L. fermentum* was centrifuged at 4,000 rpm for 20 min. Pellets were resuspended in 10% normal saline after a phosphate-buffered saline wash and standardized to 1×10^5 colony forming unit (CFU) using McFarland's standard. 1.5×10^5 CFU of resuspended *L. fermentum* in normal saline was added per gram of formulated feed (1.5×10^5 CFU/gm). Fortified feed was incubated at 37°C for 12 h [16] to acidify to the pH of 4.2 after which it was refrigerated (G3).

Animal feeding test

Animal feeding test was split into two parts—(1) Growth comparison group (G1, G2, and G3) and (2) Probiotics antimicrobial efficacy feeding assay (T1 and T2). Day-old AGRICOL broiler chicks ($n = 50$) were purchased from Y2F farms, Ibadan, Nigeria. Upon arrival, chicks were randomized into groups (10 chicks per group) and weighed.

Growth Comparison Group

Diets were formulated for chicks in the growth comparison group. Diet G1 was Feed Mill of Nigeria starter mash purchased from Nuga Pet store, Abule-Egba, Lagos, Nigeria. Diet G2 was ground corn purchased from the Oju-Ore market, Ogun State and diet G3 was formulated probiotic-fortified broiler feed. Chicks in each group (G1, G2, and G3) were fed twice daily by placing 600 gm (300 gm morning and 300 gm evening) of designated diet into each group feeder. Feeding occurred over a period of 4 weeks during which the following parameters were observed and measured—weight, height, and wing span.

Probiotics antimicrobial efficacy feeding assay

Serotyped *Escherichia coli* O157:H7 was obtained from the Nigerian Institute of Medical Research, Yaba, Lagos State and cultured in MacConkey broth at 37°C for 24–48 h. MacConkey broth was centrifuged for 15 min at 4,000 rpm and a bacterial suspension of *E. coli* 0157:H7 at 9.0×10^8 CFU/ml was obtained [17]. Birds were assigned to two groups of 10 chicks each and were orally inoculated

with 0.5 ml of *E. coli* at 9.0 × 10^8 CFU/ml. The chicks were monitored after inoculation with *E. coli* and mortality was observed [17].

Treatment schemes

Infected chicks in each group (T1 and T2) were fed twice daily with 600 gm (300 gm morning and 300 gm evening) of treatment meals over a period of 4 weeks post inoculation. Every week, bird droppings were cultured on MacConkey agar to observe for reduction in *E. coli* cell count as a result of treatment meals being fed and MRS agar to check for the presence of *L. fermentum* in the gastrointestinal tract. Weight, height, and wing span are also measured in each treatment group. Mortality and morbidity were observed.

Monitoring feed viability

pH of probiotic-fortified formulated feed was taken at bi-weekly intervals to ascertain the production of lactic acid by *L. fermentum* used to fortify feed. Culturing of the feed material on MRS agar was done to ensure the viability of the probiotic organism in the feed material.

Statistical analysis

Data on body weight, height, and wingspan among groups were analyzed using analysis of variance and Tukey's multiple comparison post-hoc tests in statistical software SPSS version 25. Results were considered significant when $p < 0.05$.

Results and Discussion

Feed was formulated using proximate analysis values listed in Table 1. In Figure 1, chicks fed G1 and G3 diets performed similarly ($p < 0.05$) in terms of measured parameters (weight, height, and wingspan) and had better performance as compared to chicks of G2. In the *E. coli*

Table 1. Results of proximate analysis value of dried food and agricultural waste samples (average mean).

Item	Moisture content (%)	Ash content (%)	Crude fat (%)	Protein (%)	Crude fiber (%)	Non-fat extract/ Carbohydrates (%)
Food waste	0.73	4.50	16.52	1.20	0.12	76.93
Corn husks	0.50	1.86	4.11	4.50	21.45	67.58
Yam peels	2.19	4.80	3.12	3.80	7.12	78.97
Plantain peels	0.14	2.80	8.10	11.0	3.58	74.38

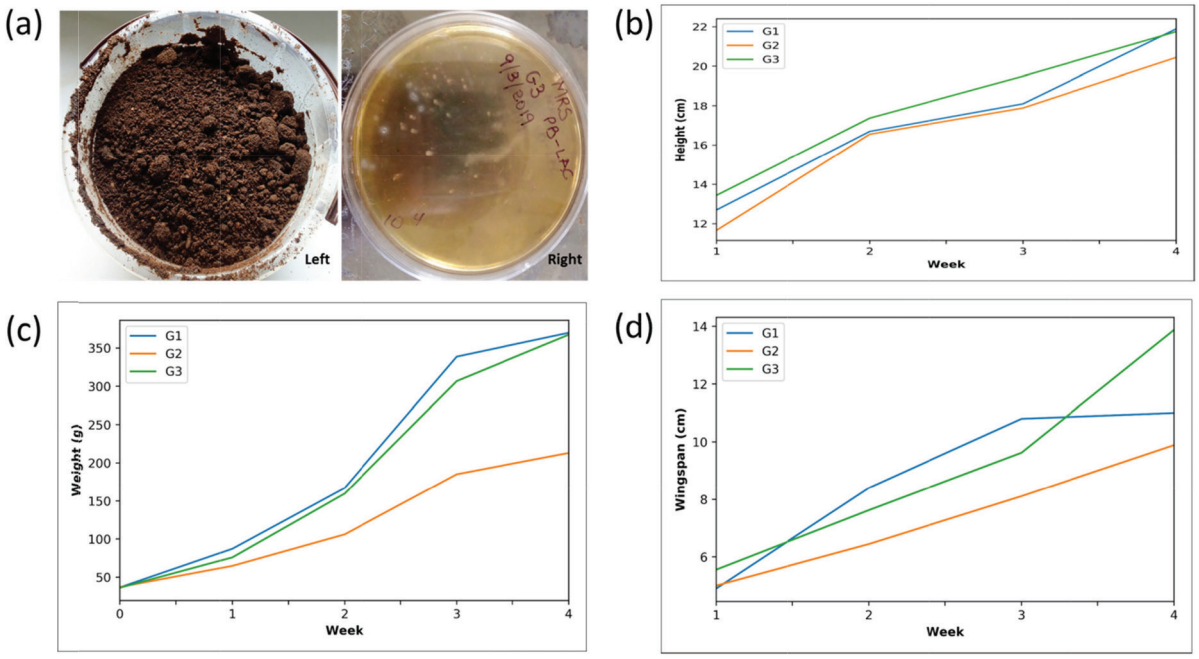

Figure 1. Probiotic fortified feed ingredients and their effects on the broiler. (a-left): probiotic fortified feed materials, (a-right): *L. fermentum* culture, (b) effect of probiotic fortified feed on height, (c) effect of probiotic fortified feed on weight, and (d) effect of probiotic fortified feed on the wing span of the broiler.

treatment group, chicks placed on treatment diets T1 and T2 showed similar levels of *E. coli* cell reduction every week (Table 2). Performance based on measured parameters was also similar ($p < 0.05$).

Probiotic fortification of feed material has been shown to improve growth performance, animal immunity, and overall animal health [18,19] and has shown promising results as an alternative to conventional feed in improving poultry performance. Probiotic organisms have been used as animal feed material separately and have also been used to supplement conventional feed [20]. Weight gain in chicks on probiotic-fortified feed is proposed to be as a result of the ability of probiotic organisms to adhere to intestinal walls, thereby enhancing nutrient utilization and increasing the digestion rate of feed consumed, thus resulting in higher feed conversion [21]. With probiotic-fortified feed performing as well as conventionally produced feed in the growth comparison test which is in line with results obtained by Gadde et al. [22], there is the possibility of substituting conventional feed which has been implicated in adverse health benefits to the animal being fed and ultimately to the consumer of the animal, with the probiotic-fortified feed formulated in this study. A study by Kim and Lillehoj [23] revealed that antibiotics, metals, and other additives are included in the conventional feed to promote animal growth and control pathogen population, but this has resulted in the spread of antibiotic resistance genes and bioaccumulation of toxins. However, in our study, *L. fermentum* included in the formulated feed (G3) exhibited antimicrobial properties and adequately reduced the pathogen cell population (Table 2). However, no pathogen was detected in the feed. This is in line with a study by Liao and Nyachoti [24] on the immunomodulatory effects of probiotic organisms which revealed the ability of probiotics to reduce pathogen cell colonization by the release of short-chain fatty acids, chemical inhibition, and competitive exclusion without the possibility of antibiotic resistance genes being transferred or bioaccumulation of toxins.

Table 2. *E. coli* cell count cultured from chick dropping in treatment groups T1 (Feed Mill of Nigeria) and T2 (Probiotic-fortified feed).

Week	Treatment Groups	Mean *E. coli* cell count (CFU/gm)
1	T1	0.5×10^3
	T2	1.0×10^3
2	T1	1.3×10^5
	T2	3.1×10^4
3	T1	1.0×10^4
	T2	2.2×10^3
4	T1	1.2×10^2
	T2	1.5×10^2

Conclusion

The results obtained from feed viability tests show the feasibility of fortifying composite animal feed with the probiotic organism *L. fermentum*. Data analyzed from measured parameters via animal feeding tests were interpreted. Conventionally produced feed can also be substituted by probiotic-fortified feed thereby reducing and ultimately eliminating the adverse health benefits associated with feeding conventionally produced feed.

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Conflict of interest

The authors' declared that they have no conflicts of interest.

Authors' contributions

Research was carried out by Ihuoma Onu-Okpara under the supervision of Solomon Oranusi. Experimental design was conceived and drafted by Solomon Oranusi. Hilary Okagbue carried out statistical analysis and language editing of the manuscript. All authors reviewed and approved the final manuscript.

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