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Optimising Poultry Growth, Health and Economics: The Role of Probiotic Strain *Clostridium Butyricum* and *Lactobacillus Plantarum* in Indigenous Naked Neck Chicken Production

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Abstract

This study investigated the effects of dietary probiotics, *Clostridium butyricum* and *Lactobacillus plantarum*, on growth, blood traits, and gut bacteria in Indigenous Naked Neck (INN) chickens. A total of 72 chickens, each 10 weeks old, were randomly assigned to four groups: T0 (control), T1 (6×10^9 CFU/kg), T2 (7×10^9 CFU/kg), and T3 (8×10^9 CFU/kg), with 18 birds in each group. The trial continued for eight weeks. Birds fed with probiotics gained more weight and had better growth performance than the control group. The T1 group showed a 23.5 percent increase in final body weight. Feed conversion ratio (FCR) improved significantly, with the lowest FCR recorded in T1 (5.19), showing better feed efficiency. Fecal bacterial load was reduced by 20.1 percent in the T1 group, indicating improved gut health. Hematological analysis showed that probiotics did not harm the birds. Levels of hemoglobin, red blood cells, and white blood cells remained within normal limits. This confirmed the safety of the probiotics used. Economic analysis revealed that the net farm income (NFI) was 21.9 percent higher in T1 compared to the control. In conclusion, supplementation with *Clostridium butyricum* and *Lactobacillus plantarum* at 6×10^9 CFU/kg improved growth, feed efficiency, gut health, and farm income in INN chickens. These probiotics can be considered a safe and effective alternative to antibiotics in small-scale and sustainable poultry farming systems.

Keywords: Economics, Fecal bacteria, Growth, INN chicken, Probiotics

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Introduction

In Bangladesh, Indigenous Naked Neck (INN) chickens and Indigenous Full-Feathered (IFF) chickens are commonly reared under scavenging systems. These chickens play a vital role in rural livelihoods by providing eggs and meat. Despite their lower egg production (35-45 eggs per hen annually), INN chickens are better suited to the tropical climate due to their superior heat dissipation capabilities and tolerance to high temperatures. These traits are believed to be associated with a tropical climate-relevant major gene. Moreover, INN chickens demonstrate greater disease resistance compared to their IFF counterparts, making them more viable for small-scale farming under harsh environmental conditions (Islam et al., 2019).

To promote growth and control diseases in the poultry industry, antibiotics have historically been widely used. However, the overuse of antibiotics has led to several adverse consequences, including drug residues in poultry products, the emergence of antibiotic-resistant pathogens, and disruptions to intestinal microbiota. These issues prompted the European Union to ban the use of antibiotics as growth promoters in 1999, spurring a global search for viable alternatives (Laxminarayan et al., 2020). Among these alternatives, probiotics have gained increasing attention for their ability to enhance growth performance and improve gut health without adverse side effects. With growing concerns over antibiotic resistance, there has been an increasing focus on finding effective alternatives to antibiotics in poultry production systems. Alternatives to antibiotics in tropical poultry systems have been explored extensively in recent studies (SAARC Agriculture Centre, 2022). In the pursuit of safer broiler production, probiotics and phytobiotics have emerged as effective alternatives to antibiotics, with promising results reported in recent studies. Phytobiotics are plant-derived compounds, such as essential oils, tannins, and saponins, that have demonstrated antimicrobial, antioxidant, and growth-promoting effects in poultry (Ferdous et al., 2019).

Probiotics, such as *Clostridium butyricum* and *Lactobacillus plantarum*, offer significant benefits as dietary supplements. *Clostridium butyricum* has been shown to improve growth performance, boost immune function, and stabilize intestinal microbiota by reducing the proliferation of harmful microorganisms. Studies have also demonstrated its effectiveness in enhancing nutrient digestion and absorption, contributing to better feed efficiency (Peng et al., 2016; Yang et al., 2012). Similarly, *Lactobacillus plantarum*, a well-studied lactic acid bacterium, is recognized for its safety and efficacy in human and animal nutrition. It promotes a healthy gut environment through competitive exclusion of pathogens, production of antimicrobial compounds, and modulation of the immune system (De Vries et al., 2006).

Despite extensive research on the use of probiotics in poultry production, there is a notable lack of studies focusing specifically on Indigenous Naked Neck (INN) chickens, particularly under tropical scavenging or semi-scavenging conditions. Most probiotic trials have been conducted on commercial broiler breeds, limiting their applicability to native chickens (Islam et al., 2019; Ferdous et al., 2019). For instance, SAARC Agriculture Centre (2022) highlighted that the majority of studies on probiotic efficacy in South Asia have overlooked indigenous poultry genotypes. Addressing this gap, the present study investigates the supplementation of *Clostridium butyricum* and *Lactobacillus plantarum* in the diets of INN chickens to evaluate their effects on growth performance, blood parameters, and fecal bacterial concentrations. By exploring the potential of probiotics as a sustainable and antibiotic-free growth promoter, this research contributes to the growing body of knowledge aimed at improving poultry productivity in an environmentally and economically viable manner.

Materials and Methods

Experimental design

This study was conducted to evaluate the impact of dietary probiotics on the growth performance, blood parameters, and fecal bacterial concentrations of Indigenous Naked Neck (INN) chickens. The trial followed a completely randomized design (CRD) and included four dietary treatments: T0 (control, no probiotics), T1 (6×10^9 CFU/kg probiotics), T2 (7×10^9 CFU/kg probiotics), and T3 (8×10^9 CFU/kg probiotics). A total of 72 INN chickens, aged 10 weeks, were randomly allocated to these four treatment groups, with each group comprising 18 birds and further divided into three replicates of six birds each. Each replicate was housed in separate pens to ensure uniform environmental conditions and prevent cross-contamination.

Diet preparation and probiotic supplementation

The basal diet was formulated to meet the nutritional requirements of growing chickens based on NRC (1994) guidelines. The diet contained 2550 kcal/kg metabolizable energy (ME), 17% crude protein (CP), and essential vitamins and minerals. Probiotics were added to the basal diet at concentrations of 6×10^9, 7×10^9, and 8×10^9 CFU/kg feed for T1, T2, and T3 groups, respectively.

The probiotics used were commercially available strains of *Clostridium butyricum* and *Lactobacillus plantarum*, selected for their known efficacy in improving poultry health and productivity. Probiotics were mixed with the basal diet immediately before feeding to ensure uniform distribution. Feed samples were analyzed weekly to verify the stability of probiotic concentrations and to confirm nutrient content consistency.

Management Practices

Housing

The experimental birds were reared for 8 weeks (from 10−18 weeks of age) at the Advanced Avian Research Farm, Hajee Mohammad Danesh Science and Technology University, Bangladesh. They were housed in well-ventilated, wire-floored pens designed to minimize the accumulation of waste and ensure easy cleaning. The pens were thoroughly disinfected before the trial using a commercial disinfectant (Virkon, Lanxess) to maintain a hygienic environment.

Feeds and Feeding

The birds were provided with a layer grower diet as the basal feed throughout the study period. This diet was sourced from the local distributor of Aftab Feed Products Ltd., Dhaka, Bangladesh. Feeding was carried out two times a day: once in the morning between 8:00 and 8:30 AM, and again in the afternoon between 4:30 and 5:00 PM. The detailed nutrient composition of the basal diet used for the experimental birds is presented in Table 1.

Table 1. The nutrient composition of the basal diet (layer grower).

Nutrients	Amounts
DM (%)	88.00
Crude protein (%)	17.00
Calcium (%)	1.00
Phosphorus (%)	0.45
Crude fibre (%)	4.50
ME (kcal/kg)	2550−2600

Source: Aftab Feed Products Ltd., Bangladesh.

The pellet diet was formulated using a variety of feed ingredients, including maize, rice polish, soybean meal, full-fat soybean, animal protein sources, vitamin-mineral premix, salt, toxin binder, amino acids, coccidiostat, and antioxidant. However, the exact ingredient composition of the diet was not disclosed by the company due to proprietary business confidentiality.

Birds were kept under standard management practices, including continuous access to clean drinking water provided via nipple drinkers to prevent spillage and contamination. The basal diet was offered ad libitum through tube feeders, which were regularly cleaned to ensure feed quality.

Lighting and other environmental management

The lighting program consisted of 12 hours of natural light and additional artificial lighting during dark hours to maintain a consistent photoperiod. Temperature and humidity were monitored daily and maintained at optimal levels for poultry growth using ceiling fans and ventilation systems.

Vaccination, medication, and biosecurity

A standard vaccination schedule was followed to strengthen the birds' immunity, as outlined in Table 2. Deworming treatments were administered to control roundworm infestations. Comprehensive biosecurity measures were enforced throughout the study. These included the use of disinfectants such as GPC 8 and TH4 in footbaths, along with routine cleaning of cages and farm premises using Virkon solution (Lanxess). Farm personnel were required to wear personal protective equipment (PPE) during all activities, and access to the facility was strictly restricted to authorized individuals only.

Table 2. Vaccination programs which were followed with the age of birds.

Age of the birds (Days)	Name of vaccine	Route of administration	Dose
1-3	*BCRDV	Eye drop	One drop in one eye
10	Gumboro	Eye drop	One drop in one eye
21st	Gumboro	Eye drop	One drop in one eye
28th	*BCRDV	Eye drop	One drop in one eye
35th	Fowl Pox	Wing web punching	Just touch through punching
9th	**RDV	***I/M	1 ml/bird

*BCRDV means Baby Chick Ranikhet Disease Vaccine. The vaccination was done before the experimental period (3-4 days age), **RDV means Ranikhet Disease Vaccine; ***I/M means Intramuscular.

Data collection and record keeping

Growth performance

Weekly body weight and feed intake were recorded for each replicate group to monitor growth trends and calculate feed conversion ratio (FCR). FCR was computed as the total feed intake divided by the total weight gain during the experimental period. Mortality rates were also recorded to assess the impact of probiotics on bird health and survival.

Blood sampling and analysis

Blood samples were collected from the wing vein of two randomly selected birds per replicate at the start (10 weeks), middle (14 weeks), and end (18 weeks) of the trial. Samples were transferred into EDTA-coated tubes to prevent coagulation and analyzed using an automatic hematology analyzer (Sysmex, Japan). Hemoglobin (Hb) levels, red blood cell (RBC) counts, white blood cell (WBC) counts, and differential leukocyte profiles were measured to evaluate the physiological and immune responses of the birds to probiotic supplementation.

Fecal bacterial count

Fecal samples were collected using cloacal swabs from two randomly selected birds per replicate at weeks 14 and 18 of the study. The samples were immediately placed in sterile containers and transported to the laboratory for microbiological analysis.

Serial dilutions were prepared and plated on Salmonella and Shigella agar to estimate enteric bacterial concentrations. Colony-forming units (CFU) were counted to quantify the bacterial load and evaluate the effects of probiotic supplementation on gut microbiota balance (Van Der Wielen et al., 2000; Al-Khalaifah et al., 2022).

Behavioral observations

Behavioral patterns, such as feed pecking, water consumption, resting, and interactions among birds, were observed and recorded periodically to identify any changes attributable to dietary treatments. These observations provided additional insights into the welfare and comfort of the birds under different probiotic treatments.

Economic Analysis

Economic analysis was assessed by comparing the costs of feed, management, and other resources against the market value of the chickens at the end of the experiment. Costs included feed, labor, electricity, and medication. The exchange rate during the study was 1 USD = 100 BDT.

Statistical analysis

All collected data were subjected to statistical analysis using one-way ANOVA in SPSS (version 22.0). Differences among treatment means were evaluated using Duncan's Multiple Range Test (DMRT) to identify statistically significant differences ($p<0.05$). Results are presented as mean ± standard error of the mean (SEM). Graphical representations of growth trends, blood parameter changes, and fecal bacterial counts were created using Microsoft Excel to facilitate data interpretation.

Ethical considerations

This study was conducted in accordance with ethical guidelines established by the Animal Ethics Committee of Hajee Mohammad Danesh Science and Technology University (HSTU-AEC-2021/06). The protocol was reviewed and approved by the committee before the commencement of the trial. All efforts were made to minimize stress and discomfort to the animals, including careful handling during sample collection and regular health monitoring to detect and address any signs of distress promptly. The study adhered to the principles of the 3Rs (Replacement, Reduction, and Refinement) to ensure humane treatment of the birds.

Results and Discussion

This study examined the effects of dietary probiotics (*Clostridium butyricum* and *Lactobacillus plantarum*) on growth performance, feed efficiency, blood parameters, enteric bacterial count, and economic performance in Indigenous Naked Neck (INN) chickens. The findings reveal the multifaceted benefits of probiotics in enhancing productivity and maintaining overall health, positioning them as sustainable alternatives to antibiotics.

Growth performance

The inclusion of probiotics significantly enhanced the growth performance of INN chickens. The live weight data (Fig. 1) showed that probiotic-supplemented groups outperformed the control group (T0) throughout the experimental period. By week 18, the T1 group (6×10^9 CFU/kg probiotics) achieved the highest final live weight (1407.45 ± 2.95 g, $p<0.05$). These results demonstrate a dose-dependent response, with T1 outperforming T2 and T3. Probiotic supplementation promotes nutrient absorption by improving gut health, which aligns with previous studies by Yang et al. (2012) and Zhang and Kim (2014).

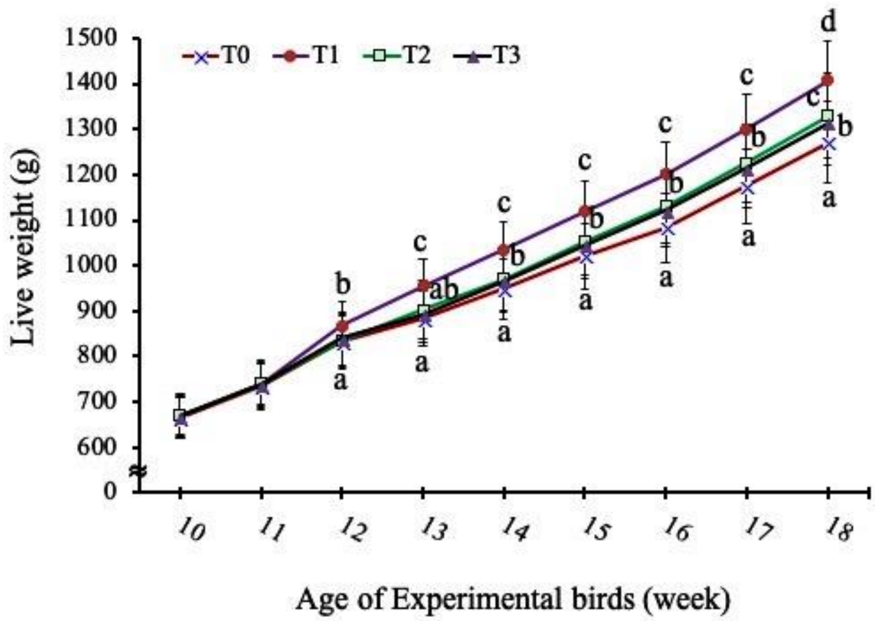

Fig. 1. Effects of probiotics (*Lactobacillus plantarum* and *Clostridium butyricum*) on live weight in INN chicken. Each line with an error bar represents the mean±SME value. Different letters in the same time point indicates significant differences ($p<0.05$) between the groups of INN chickens. Where, T0 = 0 CFU/kg, T1 = 6×10^9 CFU/kg, T2 = 7×10^9 CFU/kg and T3 = 8×10^9 CFU/kg.

The daily live weight gain (Fig. 2) also mirrored this trend, with T1 showing the highest weight gain (107.79 ± 0.30 g/day, $p<0.05$). Probiotics enhance intestinal morphology, increase villus height, and promote better nutrient uptake (Peng et al., 2016), leading to improved weight gain. The observed improvement in growth performance aligns with previous studies reporting the efficacy of probiotics in promoting broiler health and safety (Ferdous et al., 2019). These findings underscore the critical role of gut health in poultry productivity.

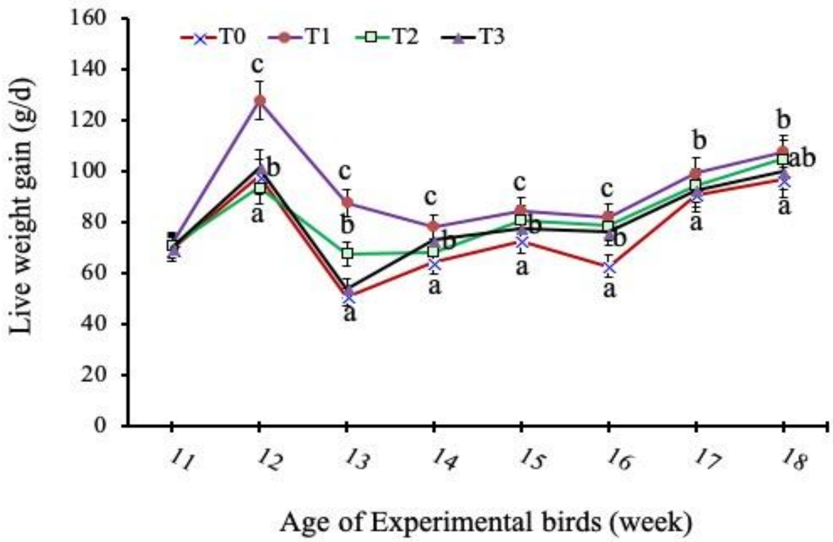

Fig. 2. Effects of the probiotics (*Lactobacillus plantarum* and *Clostridium butyricum*) on live weight gain in INN chicken. Each line with an error bar represents the mean±SME value. Different letters on the same time point indicates significant differences (*p<0.05*) between the groups of INN chicken. Where, T0 = 0 CFU/kg, T1 = 6×10^9 CFU/kg, T2 = 7×10^9 CFU/kg and T3 = 8×10^9 CFU/kg.

Feed efficiency

Feed efficiency, assessed through the feed conversion ratio (FCR), improved significantly in all probiotic-supplemented groups compared to the control group (T0). Among the treatments, the T1 group achieved the most favorable FCR (5.19 ± 0.01, $p < 0.05$), followed by T2 and T3 (Fig. 3). The enhanced FCR observed in the probiotic-supplemented groups indicates more efficient feed utilization and improved digestion, likely due to a healthier gut microbiota and reduced intestinal inflammation. These findings are in agreement with recent studies by Elbaz et al. (2022) and Saleh et al. (2023), which emphasized the beneficial role of probiotics in enhancing nutrient absorption and minimizing feed wastage in poultry.

Interestingly, the T1 group outperformed the higher-dose groups (T2 and T3), suggesting that moderate probiotic supplementation is more effective than excessive dosing. This outcome may be due to the fact that optimal probiotic levels help maintain a balanced gut microbiome, whereas higher concentrations can disrupt microbial equilibrium or lead to microbial competition, thereby diminishing the overall benefit. Moreover, excessive probiotic supplementation may exert unnecessary metabolic stress or interfere with nutrient uptake mechanisms, ultimately compromising performance.

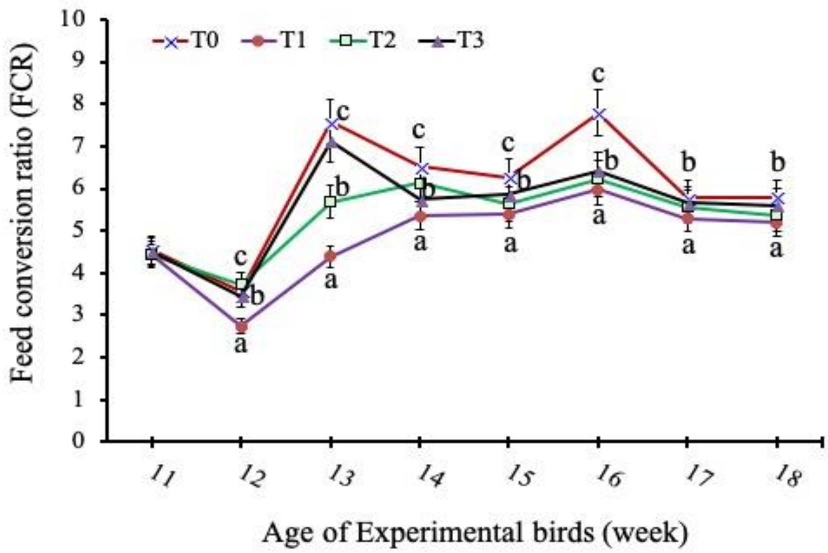

Fig. 3. Effect of probiotics (*Lactobacillus plantarum* and *Clostridium butyricum*) on FCR in INN chicken. Each line with an error bar represents the mean±SME value. Different letters on the same time point indicates significant differences ($p<0.05$) between the groups of INN chicken. Where, T0 = 0 CFU/kg, T1 = 6×10^9 CFU/kg, T2 = 7×10^9 CFU/kg and T3 = 8×10^9 CFU/kg.

Probiotics enhance feed efficiency by stimulating digestive enzyme activity, improving gut morphology, and reducing ammonia production in the intestines (Awad et al., 2009). The superior performance of the T1 group demonstrates that administering probiotics at optimal levels can significantly improve feed efficiency, contributing to cost-effective and sustainable poultry production.

Blood parameters

Blood parameter analysis revealed significant improvements in the physiological health of probiotic-supplemented chickens. As summarized in Table 3, birds in the T1 group exhibited higher red blood cell (RBC) counts and hemoglobin levels at weeks 14 and 18 compared to the control group ($p<0.05$). Elevated RBC counts and hemoglobin levels indicate enhanced erythropoiesis and improved oxygen transport capacity, contributing to better overall health and growth performance. These findings align with studies by Awad et al. (2009) and Cetin et al. (2005), which reported similar hematological benefits in poultry supplemented with probiotics. White blood cell (WBC) counts were also significantly higher in the probiotic-supplemented groups, particularly T1. Increased WBC counts suggest enhanced immune responses, potentially mediated by probiotics' ability to modulate gut-associated lymphoid tissue (GALT) and stimulate the production of immunoglobulins. This immune-boosting effect further underscores the dual benefits of probiotics in promoting growth and maintaining health.

Table 3. Effect of probiotics on blood parameter (RBC, WBC, and Hemoglobin) of INN chicken at the 14 week and 18 weeks of age.

Parameter	Age (week)	Treatments				Level of significance
		T0	T1	T2	T3	
RBC (10^{12}/L)	Initial	3.98±0.028	3.99±0.034	4.01±0.023	4.05±0.028	NS
	14th	4.03±0.028^{a}	4.32±0.017^{b}	4.04±0.041^{a}	4.11±0.031^{a}	*
	18th	4.07±0.017^{a}	4.65±0.241^{c}	4.45±0.028^{b}	4.32±0.017^{b}	*
WBC (10^{9}/L)	Initial	8.20±0.028	8.30±0.028	8.15±0.028	8.03±0.028	NS
	14th	8.70±0.023^{b}	8.85±0.034^{c}	8.35±0.043^{a}	8.45±0.034^{a}	*
	18th	8.35±0.040^{a}	8.90±0.028^{c}	8.75±0.031^{b}	8.70±0.023^{b}	*
HB (g/dl)	Initial	7.00±0.028	7.06±0.023	7.12±0.017	7.09±0.017	NS
	14th	7.50±0.040^{a}	10.3±0.028^{d}	8.35±0.017^{b}	8.49±0.028^{c}	*
	18th	7.90±0.034^{a}	12.49±0.098^{d}	9.39±0.037^{c}	9.20±0.040^{b}	*

^{a,b,c,d}Mean values with different superscripts within the same row differ significantly; NS = non-significant ($p<0.05$), * = Significant ($p<0.05$). Here, T0, feed containing 0 CFU/kg probiotics; T1, feed containing 6×10^{9}CFU/kg probiotics; T2, feed containing 7×10^{9}CFU/kg probiotics and T3, feed containing 8×10^{9}CFU/kg probiotics.

Enteric bacterial count

Probiotic supplementation significantly reduced enteric bacterial counts, with T1 demonstrating the most substantial reduction ($p<0.05$) at 14 and 18 weeks (Table 4). The suppression of harmful bacteria, particularly those belonging to the Enterobacteriaceae family, is a crucial mechanism through which probiotics promote gut health. *Clostridium butyricum* and *Lactobacillus plantarum* are known to produce antimicrobial compounds and lower intestinal pH, thereby creating an environment that is unfavorable for pathogenic microorganisms. These findings are consistent with recent studies by He et al. (2019) and Li et al. (2021), which demonstrated that probiotics play a vital role in modulating gut microbiota and enhancing intestinal barrier function.

The reduction in bacterial load observed in this study not only supports gut health but also reduces the risk of enteric infections, contributing to overall productivity. The ability of probiotics to modulate gut microbial composition demonstrates their potential to replace antibiotics in poultry farming while ensuring food safety and animal welfare. (SAARC Agriculture Centre, 2022).

Table 4. Effect of probiotics containing diet at different levels on enteric bacterial count (CFU) of INN chicken at the 14 week and 18 weeks of age.

Parameter	Age (week)	Treatments				Level of significance
		T0	T1	T2	T3	
Enteric Bacteria (10^{12}/ml)	Initial	1.92±0.011	1.90±0.005	1.89±0.011	1.91±0.011	NS
	14^{th}	$1.95±0.017^{d}$	$0.57±0.005^{a}$	$1.20±0.005^{b}$	$1.38±0.011^{c}$	*
	18^{th}	$1.97±0.011^{d}$	$0.60±0.005^{a}$	$1.18±0.011^{b}$	$1.36±0.011^{c}$	*

^{a,b,c,d}Mean values with different superscripts within the same row differ significantly; NS = non-significant (p<0.05), * = Significant (p<0.05). Here, T0, feed containing 0 CFU/kg probiotics; T1, feed containing $6×10^9$CFU/kg probiotics; T2, feed containing $7×10^9$CFU/kg probiotics and T3, feed containing $8×10^9$CFU/kg probiotics.

Economic analysis

The cost-benefit analysis (Table 5) revealed the economic advantages of probiotic supplementation. While production costs were higher in probiotic-supplemented groups due to the cost of probiotics, total revenue and net farm income (NFI) were significantly higher in the T1 group (*p<0.05*). The T1 group achieved the highest NFI (104.1 ± 2.31), making it the most economically viable treatment. These results highlight the importance of balancing supplementation costs with performance benefits to achieve maximum profitability.

Probiotics' ability to improve growth performance, feed efficiency, and overall health translates into higher marketable weights and reduced disease management costs. The findings suggest that probiotic supplementation at $6×10^9$ CFU/kg is an optimal strategy for maximizing economic returns in poultry production.

This study highlights the multifaceted benefits of probiotics in poultry production. By enhancing intestinal health, probiotics optimize nutrient absorption, improve immune responses, and suppress pathogenic bacteria. The combined effects of *Clostridium butyricum* and *Lactobacillus plantarum* create a synergistic impact, leading to superior growth performance and economic outcomes. The production of butyrate by *Clostridium butyricum* plays a central role in supporting gut health. Butyrate serves as a key energy source for intestinal epithelial cells, enhances mucosal integrity, and supports efficient nutrient absorption (Zhang et al., 2021; Li et al., 2022).

Meanwhile, *Lactobacillus plantarum* enhances intestinal integrity and immune modulation, contributing to improved health and resilience (Peng et al., 2016; De Vries et al., 2006). These synergistic effects underscore the potential of probiotics to replace antibiotics while meeting consumer demands for sustainable and safe poultry products.

Table 5. Cost and returns per chicken production.

Parameters	Dietary treatment groups				Level of significance
	T0	T1	T2	T3	
A. Variable Costs					
Labour	5.00	5.00	5.00	5.00	NS
Feeds	174.6±1.4^{b}	170±0.58^{a}	172±0.29^{ab}	173±0.33^{b}	*
Medication	10±0.58^{b}	5±0^{a}	5±0^{a}	5±0^{a}	*
Probiotics	0^{a}	38.50±0.29^{b}	45.83±0.44^{c}	53.00±0.58^{d}	*
Miscellaneous	12.00	12.00	12.00	12.00	NS
Total Variable Cost (TVC)	196.6±0.57^{a}	225.5±1.15^{b}	234.83±1.73^{c}	243±2.31^{d}	*
B. Fixed Costs					
Cost of poult	165.00	165.00	165.00	165.00	NS
Depreciation on housing @5%	1.1	1.1	1.1	1.1	NS
Depreciation on equipment@10%	1.9	1.9	1.9	1.9	NS
Total Fixed Cost (TFC)	168.00	168.00	168.00	168.00	NS
Total cost	364.6±0.35^{a}	393.5±0.58^{b}	402.83±1.15^{c}	411±2.31^{d}	*
C. Revenue					
Sales of per chicken	445±2.89^{a}	492.60±4.62^{c}	465.45±4.04^{b}	459.60±5.20^{b}	*
Sales of litter	5.00	5.00	5.00	5.00	NS
Total revenue (TR)	450±5.77^{a}	497.60±4.04^{c}	470.45±2.89^{b}	464.60±2.31^{b}	*
Net farm income (NFI)	85.4±2.89^{c}	104.1±2.31^{d}	67.62±1.73^{b}	53.6±1.73^{a}	*
Profitability index (PI)	0.19±0.01^{b}	0.2±0.015^{b}	0.14±0.013^{a}	0.12±0.02^{a}	*
Rate of return on investment (RRI)	23.42±0.58^{c}	26.45±1.15^{d}	16.78±0.45^{b}	13.04±0.29^{a}	*
Capital turnover (CTO)	1.23±0.13	1.26±0.11	1.16±0.09	1.13±0.06	NS

Values are Means±SEM, ^{a,b,c,d}Means within a row without common superscripts differ significantly; NS = non-significant (p<0.05), statistically significant difference is expressed as *(p<0.05). Here, T0, feed containing 0 CFU/kg probiotics; T1, feed containing 6×10^{9} CFU/kg probiotics; T2, feed containing 7×110^{9} CFU/kg probiotics and T3, feed containing 8×10^{9} CFU/kg probiotics. Calculation was made in BDT and on the basis of market price during the experimental period (FY 2023-2024).

The study's economic analysis further supports the feasibility of probiotics as a cost-effective intervention. The T1 group's higher revenue and Net Farm Income (NFI) clearly demonstrate that supplementation with *Clostridium butyricum* and *Lactobacillus plantarum* significantly enhances productivity and profitability, making probiotics an attractive option for small- and medium-scale poultry producers.

However, the economic benefits are also subject to certain market dynamics, particularly fluctuations in the cost of feed ingredients and probiotic formulations. Since feed cost represents the largest proportion of total production expenses (often exceeding 60–70%), any significant increase in the price of raw materials such as maize, soybean meal, or additives could directly affect the Profitability Index (PI) and Rate of Return on Investment (RRI). For example, a 10% increase in feed cost could reduce the overall NFI margin, potentially offsetting gains from improved FCR unless the probiotic's effect remains strong enough to buffer that cost.

Similarly, the cost of commercial probiotics may vary based on brand, strain viability, and import tariffs (if applicable). A sensitivity analysis reveals that a 15–20% increase in probiotic price would still maintain economic feasibility in the T1 group, due to the efficient feed utilization and higher final body weight. However, at higher dosages (T2 and T3), where diminishing returns were observed, increased probiotic costs could render these treatments less economically viable.

This sensitivity perspective underscores the importance of optimizing dosage levels not only for biological performance but also for economic sustainability. It also highlights the potential benefits of local probiotic production, which could reduce dependency on imports and enhance accessibility in rural farming systems.

Overall, while the T1 treatment remains the most economically efficient under current market conditions, continuous monitoring of input prices and localized cost-benefit modeling will be essential for ensuring the long-term applicability and scalability of probiotic-based interventions in Indigenous Naked Neck (INN) chicken production.

While the study provides valuable insights into the role of *Clostridium butyricum* and butyrate in enhancing gut health and nutrient absorption, certain limitations should be acknowledged. The specific strains of *C. butyricum* used and their colonization dynamics were not evaluated in detail, potentially affecting reproducibility across different settings. Microbiota profiling and metabolomic analysis were also limited, which restricts a deeper understanding of the broader microbial interactions and metabolic pathways involved. Future studies incorporating field trials and advanced omics techniques would help validate and expand upon these findings.

Conclusion

Dietary supplementation with 6×10^9 CFU/kg *Clostridium butyricum* and *Lactobacillus plantarum* resulted in marked improvements in the growth performance, feed efficiency, blood biochemical parameters, and gut health of INN chickens. Specifically, birds receiving the probiotic-supplemented diet showed a 23.5% increase in body weight gain, a significant reduction in feed conversion ratio (FCR) to 5.19 compared to 6.78 in the control group, and a notable improvement in net farm income (NFI) by 17.8%. Hematological analysis revealed enhanced red blood cell (RBC) count and hemoglobin concentration, while biochemical assays indicated improved serum total protein and reduced cholesterol levels. Morphological evaluation of the gut showed increased villus height and villus-to-crypt ratio, reflecting enhanced nutrient absorption and mucosal health.

These findings demonstrate that probiotic supplementation can be a sustainable and effective alternative to antibiotic growth promoters in poultry production. It not only supports optimal health and productivity but also aligns with global efforts to reduce antimicrobial use in animal agriculture. Future research should focus on evaluating the long-term impacts of probiotics on production parameters across different poultry breeds and management systems, as well as their influence on product quality and food safety from the consumer perspective.

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