

Physiological Response of Broiler Chicken Fed Different Levels of Neem Leaf Powder on Gut Microbial Composition

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Abstract

This study evaluated the effects of graded levels of neem (*Azadirachta indica*) leaf powder on growth performance, gut microbial count, digestible feed kinetics, vital organs, and intestinal characteristics of Arbor Acre broiler chickens. A total of 100 day-old mixed-sex Arbor Acre broiler chicks were randomly allotted to five dietary treatments in a completely randomized design. The treatments consisted of a control diet without neem leaf powder and diets supplemented with 0.3%, 0.4%, 0.5%, and 0.6% neem leaf powder. The feeding trial lasted for 42 days. Data were collected on growth performance, microbial count, gastrointestinal parameters, vital organ weights, and intestinal morphology, and were subjected to one-way analysis of variance. The results showed that dietary neem leaf powder had no significant effect ($p > 0.05$) on initial weight, final weight, weight gain, feed conversion ratio, digestible feed kinetics, or vital organ weights. However, feed intake differed significantly ($p < 0.05$), with the control group recording the highest value and birds fed 0.5% neem leaf powder recording the lowest value. Total bacterial count and faecal contaminant count were significantly affected ($p < 0.05$) by dietary treatment, while total fungal count and total coliform count were not significantly different. Significant differences ($p < 0.05$) were also observed in jejunum and ileum lengths, suggesting possible improvement in intestinal development in neem-supplemented birds. No growth of *Escherichia coli* and *Salmonella* was observed during microbial incubation. The findings indicate that neem leaf powder can be included in broiler diets up to 0.6% without adverse effects on growth performance or organ development. Neem leaf powder may also contribute to gut microbial

modulation and improved intestinal characteristics. Therefore, neem leaf powder has potential as a natural phyto-genic feed additive and possible alternative to antibiotic growth promoters in broiler production.

Keywords: Arbor Acre broiler; *Azadirachta indica*; gut microbiota; intestinal morphology; neem leaf powder; phyto-genic feed additive.

1. Introduction

Broiler chicken production is an important component of the global poultry industry because it provides a relatively affordable and high-quality source of animal protein for human consumption [1]. In both developed and developing countries, the demand for poultry meat continues to increase due to population growth, urbanization, and the rising preference for animal protein. However, the broiler industry faces persistent challenges related to feed cost, disease pressure, production efficiency, and the need to maintain bird health under intensive management systems [2]. Feed remains the largest single cost in broiler production; therefore, improving feed utilization and growth efficiency is essential for profitable and sustainable poultry farming [3]. The performance of broiler chickens is influenced by several factors, including nutrition, genetics, environmental management, health status, and gut microbial balance [4]. Among these factors, gut health has received considerable attention because of its direct relationship with nutrient digestion, immune function, feed conversion, and overall productivity [5]. The gastrointestinal tract of chickens contains a diverse microbial population that contributes to digestion, nutrient metabolism, pathogen exclusion, and immune system development [6]. A balanced gut microbiota

improves intestinal integrity and enhances the ability of broilers to convert feed into body mass efficiently [7]. Conversely, microbial imbalance or dysbiosis may reduce nutrient absorption, increase disease susceptibility, and negatively affect growth performance [8]. For many years, antibiotic growth promoters have been used in poultry diets to improve feed efficiency, suppress pathogenic microorganisms, and enhance growth performance [9]. However, the continuous and indiscriminate use of antibiotics in livestock production has raised serious public health concerns, particularly the development of antimicrobial-resistant bacteria and antibiotic residues in animal products [10]. As a result, there is growing interest in natural, safe, and sustainable feed additives that can support broiler productivity without contributing to antimicrobial resistance [11]. Phytogetic feed additives, including medicinal plants and their derivatives, have been widely investigated as alternatives to antibiotic growth promoters because of their antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory properties [12].

Neem (*Azadirachta indica*) is a tropical evergreen tree widely distributed in Asia and Africa, including Nigeria, where it is commonly known as dogonyaro [13]. The plant is rich in bioactive compounds such as azadirachtin, nimbin, salanin, gedunin, meliacin, nimbolide, and other limonoids, which have been associated with antibacterial, antifungal, antiparasitic, anti-inflammatory, antioxidant, and immunomodulatory activities [14]. These biological properties suggest that neem leaf powder may improve gut microbial balance, enhance immune response, and promote better nutrient utilization in broiler chickens [15]. Previous studies have reported that dietary inclusion of neem leaf meal or powder may influence growth performance, carcass traits, blood biochemical indices, and microbial populations in poultry [16]. Nevertheless, the response of broilers to neem supplementation may vary depending on inclusion level, bird strain, diet composition, management conditions, and the physiological parameters evaluated [17]. In Nigeria and other tropical regions, neem leaves are readily available, inexpensive, and locally adaptable, making them a potentially valuable feed additive for small- and medium-scale poultry farmers. The use of neem leaf powder in broiler nutrition could reduce dependence on synthetic antibiotics, lower production costs, and support the production of antibiotic-free poultry meat. However, there is still

a need for more empirical information on the effects of graded levels of neem leaf powder on gut microbial composition, intestinal characteristics, organ development, and growth performance of broiler chickens.

Therefore, this study evaluated the effects of varying dietary inclusion levels of neem leaf powder on the growth performance, gut microbial count, digestible feed kinetics, vital organs, and intestinal characteristics of Arbor Acre broiler chickens. The study was designed to determine whether neem leaf powder could serve as a natural feed additive for improving gut health and broiler productivity without adverse effects on performance.

2. Literature Review

2.1 Broiler production and poultry management

Poultry production is one of the fastest-growing livestock enterprises because of its short generation interval, high feed conversion potential, and ability to provide affordable animal protein for human consumption [1]. In many developing countries, including Nigeria, poultry production contributes significantly to household income, food security, and employment generation. Broiler chickens are particularly important because they are genetically selected for rapid growth, high meat yield, and efficient feed utilization [2]. The productivity of broiler chickens depends on several interacting factors, including breed or strain, feed quality, health management, environmental conditions, housing, stocking density, and disease prevention practices [3]. In intensive systems, birds are usually raised under controlled feeding, lighting, brooding, vaccination, and sanitation programmes to maximize growth performance and reduce mortality. However, poor feed quality, disease outbreaks, high feed cost, and inefficient management remain major constraints to profitable broiler production [4]. In Nigeria, poultry production includes both traditional scavenging systems and commercial intensive production. Indigenous chickens are commonly reared under extensive systems, while improved broiler strains such as Arbor Acre, Cobb, Ross, Hubbard, and other commercial lines are used in intensive meat production systems [5]. Commercial broilers have been selected mainly for fast growth, improved carcass yield, and better feed conversion efficiency. However, their high growth potential requires

adequate nutrition and proper health management to achieve optimum performance [6].

2.2 Broiler strains and growth performance

Broiler strains differ in growth rate, feed intake, feed conversion ratio, carcass yield, disease tolerance, and adaptability to environmental conditions. Arbor Acre broilers are widely used in commercial poultry production because of their rapid growth, good feed efficiency, and high meat yield [7]. Other broiler strains such as Cobb, Ross, Hubbard, Cornish Cross, Delaware, Red Broiler, and Noiler have also been used in different production systems depending on the production objective and environmental conditions [8]. Growth performance in broilers is commonly evaluated using parameters such as initial body weight, final body weight, weight gain, feed intake, and feed conversion ratio. Feed conversion ratio is particularly important because it indicates the efficiency with which birds convert consumed feed into body mass [9]. Genetic improvement has greatly enhanced broiler performance over the years, leading to faster growth, earlier market weight, and improved feed efficiency [10]. However, these improvements have also increased the need for balanced diets, effective disease control, and proper gut health management. Sex, age, strain, nutrition, and environmental conditions can influence broiler growth performance. Male broilers often grow faster and attain higher body weights than females due to differences in hormonal and metabolic activity [11]. Age also affects nutrient utilization, intestinal development, and growth rate, especially during the starter and finisher phases. Similarly, strain differences may affect body weight, feed intake, carcass composition, abdominal fat deposition, and organ development [12].

2.3 Feeding and nutrient utilization in broiler chickens

Feed is the most expensive input in broiler production and may account for a large proportion of total production cost [13]. Therefore, improving feed efficiency is essential for sustainable and profitable poultry production. Broiler diets are formulated to provide adequate energy, protein, amino acids, vitamins, minerals, and other nutrients required for growth, maintenance, immunity, and carcass development [14]. The efficiency of nutrient utilization depends not only on diet

composition but also on digestive capacity, enzyme activity, intestinal morphology, gut microbial balance, and health status of the birds [15]. Poor nutrient digestibility may result in reduced growth, poor feed conversion, increased waste output, and higher susceptibility to disease. Consequently, nutritionists are increasingly interested in feed additives that can improve digestion, stabilize gut microbiota, enhance immune function, and support intestinal development [16]. Feed additives such as probiotics, prebiotics, enzymes, organic acids, amino acids, phytobiotics, and herbal products have been used to improve poultry performance [17]. Phytogetic feed additives are especially gaining attention because they contain bioactive compounds that may exert antimicrobial, antioxidant, anti-inflammatory, digestive-stimulating and immunomodulatory effects [18].

2.4 Chicken digestive tract and gut microbiota

The gastrointestinal tract of chickens plays a central role in digestion, absorption, metabolism, immune defence, and microbial regulation. The digestive system includes the crop, proventriculus, gizzard, small intestine, caeca, large intestine, and cloaca [19]. Feed is temporarily stored in the crop before moving to the proventriculus, where digestive secretions begin chemical digestion. The gizzard mechanically grinds feed particles, while the small intestine is the main site of enzymatic digestion and nutrient absorption [20]. The chicken gut contains a complex and diverse microbial community made up of bacteria, fungi, and other microorganisms. These microorganisms are found in different sections of the digestive tract, with the caeca generally containing the highest microbial density and diversity [21]. The gut microbiota contributes to nutrient digestion, fermentation of indigestible carbohydrates, production of short-chain fatty acids, synthesis of vitamins, immune modulation, and protection against pathogens [22]. A healthy gut microbiota is associated with improved nutrient utilization, stronger intestinal barrier function, and better growth performance. Beneficial bacteria may inhibit pathogenic organisms through competitive exclusion, production of antimicrobial substances, and stimulation of host immune responses [23]. In contrast, dysbiosis, which refers to an imbalance in microbial composition, can reduce digestive efficiency, damage intestinal tissues, impair immunity, and increase the risk of enteric diseases [24]. Several factors influence the composition and

function of the chicken gut microbiota, including age, diet, breed, sex, environment, litter quality, medication, stress, and management practices [25]. Dietary interventions are among the most practical methods for modulating gut microbiota in broiler chickens. Therefore, the use of natural feed additives such as neem leaf powder may help improve gut microbial balance and promote better intestinal health.

2.5 Role of gut microbiota in digestion and immunity

Gut microbiota contributes significantly to feed digestion and nutrient metabolism in poultry. Since chickens have limited capacity to digest some complex polysaccharides, microbial fermentation in the caeca helps break down undigested feed components and produces metabolites such as acetate, propionate, butyrate, and other short-chain fatty acids [26]. These metabolites provide energy to intestinal cells, support gut barrier integrity, and influence immune responses. The microbiota also participates in nitrogen metabolism, amino acid transformation, and vitamin synthesis [27]. Although not all microbial metabolites are fully absorbed by the host, their presence can influence intestinal physiology and overall bird health. In addition, gut microorganisms interact with mucus layers, epithelial cells, and immune tissues, thereby contributing to immune system development and pathogen resistance [28]. The intestinal barrier is protected by mucus layers, epithelial tight junctions, antimicrobial peptides, and immune cells. Goblet cells produce mucins that form a protective mucus layer over the intestinal epithelium [29]. This mucus layer traps pathogens, supports beneficial microbial colonization, and protects the gut wall from harmful substances. Therefore, maintaining microbial balance is important for preventing intestinal inflammation, improving nutrient absorption, and enhancing broiler productivity.

2.6 Antibiotic growth promoters and the search for alternatives

Antibiotics have historically been used in poultry production to prevent disease, improve feed efficiency, and promote growth [30]. However, the continuous use of antibiotics as growth promoters has raised global concerns due to antimicrobial resistance, antibiotic residues in animal products, and potential risks to human and animal health [31]. These concerns have led to restrictions or bans on

antibiotic growth promoters in several countries and have increased the demand for natural alternatives. Phytogetic feed additives are among the most promising alternatives to antibiotic growth promoters. These plant-derived products may improve appetite, stimulate digestive enzyme secretion, suppress harmful microorganisms, reduce oxidative stress, and enhance immune responses [32]. Common phytogetic additives used in poultry include neem, garlic, ginger, turmeric, moringa, black cumin, peppermint, fennel, and fenugreek [33]. Unlike synthetic antibiotics, many plant-based additives are locally available, biodegradable, culturally acceptable, and less likely to contribute to antimicrobial resistance when properly used. However, their effectiveness depends on plant species, processing method, inclusion level, diet composition, and bird age or strain [34]. Therefore, experimental evaluation is necessary to determine safe and effective inclusion levels in broiler diets.

2.7 Neem leaf as a phytogetic feed additive

Neem (*Azadirachta indica*) is a tropical evergreen tree widely distributed in Asia and Africa. In Nigeria, it is commonly known as dogonyaro and is often found in rural and urban environments [35]. Neem has long been used in traditional medicine because of its wide range of biological activities. Different parts of the plant, including leaves, seeds, bark, and oil, contain numerous bioactive compounds with medicinal and agricultural value [36]. Neem leaves contain compounds such as azadirachtin, nimbin, salanin, gedunin, meliacin, nimbolide, valassin, flavonoids, tannins, saponins, and other limonoids [37]. These compounds have been associated with antibacterial, antifungal, antiviral, antiparasitic, anti-inflammatory, antioxidant, hepatoprotective, antimalarial, and immunomodulatory effects [38]. Because of these properties, neem leaf powder has been investigated as a natural growth promoter and health-supporting feed additive in poultry. In broiler production, neem leaf supplementation may help suppress harmful gut microorganisms, enhance immune response, improve liver function, reduce disease incidence, and support nutrient utilization [39]. Some studies have reported that neem leaf meal or powder can improve growth performance, carcass characteristics, blood biochemical parameters, and profitability in broilers when included at appropriate levels [40]. However, high inclusion levels may reduce feed intake due to the bitter taste

and presence of anti-nutritional factors such as tannins, oxalates, phenolic compounds, and triterpenoids [41].

2.8 Effects of neem leaf powder on broiler performance and gut health

The effect of neem leaf powder on broiler performance may depend on the inclusion level and the physiological response of the birds. At moderate inclusion levels, neem may improve gut microbial balance, reduce pathogenic bacterial load, and support better nutrient absorption [42]. Its antimicrobial activity may help control organisms such as *Escherichia coli*, *Salmonella*, and other enteric pathogens, thereby improving intestinal health and reducing disease pressure [43]. Neem leaf powder may also influence intestinal morphology. Improved intestinal length, villus development, or absorptive surface area may enhance nutrient uptake and growth performance. The jejunum and ileum are particularly important because they are major sites of nutrient digestion and absorption in poultry [44]. Any improvement in these intestinal segments may contribute to better feed conversion and overall productivity. However, responses to neem supplementation are not always consistent. Some studies have found improvements in growth performance, while others reported no significant changes in body weight gain, feed conversion ratio, or organ weight [45]. These differences may be due to variation in neem processing, nutrient composition, experimental design, broiler strain, age, management, and environmental conditions. Therefore, determining the optimum level of neem leaf powder is necessary to maximize benefits while avoiding possible negative effects on palatability and nutrient utilization.

The available literature suggests that neem leaf powder has potential as a natural phytochemical feed additive for broiler chickens due to its antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory properties. It may help improve gut microbial balance, intestinal development, immune response, and production performance. However, the effects of graded levels of neem leaf powder on gut microbial composition, intestinal characteristics, digestible feed kinetics, organ development, and growth performance in Arbor Acre broilers require further investigation. This study therefore contributes to existing knowledge by evaluating the response of Arbor Acre broiler

chickens fed diets containing different levels of neem leaf powder. The findings may support the use of neem as a locally available alternative to antibiotic growth promoters in sustainable poultry production.

3. Materials and Methods

3.1 Experimental site

The experiment was conducted at the Poultry Unit of the Teaching and Research Farm, University of Ibadan, Ibadan, Oyo State, Nigeria. Laboratory analyses were carried out at the General Laboratory of the Department of Animal Science, University of Ibadan. The study area is located at approximately latitude 07°26'N and longitude 03°54'E. The experimental procedure was designed to evaluate the effects of graded levels of neem (*Azadirachta indica*) leaf powder on growth performance, gut microbial count, digestible feed kinetics, vital organs, and intestinal characteristics of Arbor Acre broiler chickens.

3.2 Pre-experimental management

Before the arrival of the birds, the poultry pen was thoroughly cleaned and disinfected. The preparation involved sweeping, washing, scrubbing, fumigation, removal of unwanted materials, repair of damaged nets and electrical fittings, and disinfection of feeders and drinkers. Clean wood shavings obtained from the Forestry Department, University of Ibadan, were used as litter material. Proper sanitation and biosecurity measures were maintained to reduce the risk of disease outbreak during the experiment.

3.3 Brooding management

The chicks were brooded in a prepared brooding unit under controlled environmental conditions. Adequate temperature, ventilation, lighting, humidity, and sanitation were maintained throughout the brooding phase. Brooding temperature was initially maintained at about 35 °C and gradually reduced as the birds advanced in age. Feed and clean drinking water were supplied ad libitum. The brooding unit was cleaned regularly to minimize microbial contamination and disease risk.

3.4 Collection and processing of neem leaves

Fresh green neem leaves were collected within the University of Ibadan environment. The leaves were sorted to remove foreign materials and damaged portions before processing. The selected leaves

were weighed and air-dried at room temperature until they became crispy to touch. The dried leaves were milled into powder using a locally fabricated milling machine. The neem leaf powder was then stored in airtight plastic containers until incorporation into the experimental diets.

3.5 Experimental birds and management

A total of 100 day-old mixed-sex Arbor Acre broiler chicks were obtained from a commercial hatchery in Ibadan, Oyo State, Nigeria. On arrival, the chicks were given vitamin-electrolyte solution to reduce transportation stress and were allowed to acclimatize. Routine vaccination against Newcastle disease and infectious bursal disease was

administered according to standard broiler management practice. The feeding trial lasted for 42 days and covered both starter and finisher phases. Body weight was recorded at the beginning of the experiment and subsequently on a weekly basis. Feed intake, weight gain, and feed conversion ratio were calculated during the experimental period.

3.6 Experimental diets and treatments

The experimental diets were formulated to meet the nutrient requirements of broiler chickens at the starter and finisher phases, following standard broiler feeding recommendations. Neem leaf powder was added to the diets at different graded levels. Five dietary treatments were used as follows:

T1:	Basal	diet	without	neem	leaf	powder,	serving	as	the	control
T2:	Basal	diet	supplemented	with	0.3%	neem	leaf	powder		
T3:	Basal	diet	supplemented	with	0.4%	neem	leaf	powder		
T4:	Basal	diet	supplemented	with	0.5%	neem	leaf	powder		
T5:	Basal	diet	supplemented	with	0.6%	neem	leaf	powder		

Due to the low inclusion levels, neem leaf powder was added on top of the formulated diet and mixed manually to ensure even distribution. The starter and finisher diets contained maize, soybean meal, wheat, fish meal, palm oil, bone meal, premix, limestone, methionine, lysine, and salt as major ingredients.

3.7 Experimental design

The experiment was arranged in a completely randomized design. The 100 broiler chicks were randomly allotted to five dietary treatments. Each treatment had four replicates, with five birds per replicate. The birds were managed under similar environmental and husbandry conditions throughout the study to ensure that observed differences were mainly due to dietary treatment effects.

3.8 Data collection

Data were collected on growth performance, gastrointestinal parameters, microbial count, intestinal characteristics, and vital organ weights.

3.8.1 Growth performance

The birds were weighed at the beginning of the experiment and weekly thereafter. Weekly weight gain was calculated as the difference between the current week’s body weight and the previous

week’s body weight. Feed intake was determined by subtracting leftover feed from the quantity of feed offered. Feed conversion ratio was calculated by dividing total feed intake by total body weight gain.

3.8.2 Gastrointestinal parameters and organ measurements

At the end of the experiment, representative birds were selected from each treatment for gastrointestinal and organ evaluation. The birds were slaughtered, and the whole gastrointestinal tract was carefully removed. The proventriculus, gizzard, liver, heart, spleen, duodenum, jejunum, ileum, caecum, and colon were weighed using a sensitive scale. Intestinal segments were measured using a measuring tape. Digesta samples were collected from the caecum into sterile bottles for microbial analysis.

3.8.3 Materials used for microbial analysis

The materials used for microbial analysis included Petri dishes, potato dextrose agar, plate count agar, eosin methylene blue agar, MacConkey agar, sterile test tubes, sterile cotton wool, conical flasks, test tube stands, distilled water, funnels, measuring cylinders, autoclave, paper tape, foil paper, Pasteur pipettes, stirring rods, incubator, syringes, gloves, and beakers.

3.8.4 Preparation of culture media

Potato dextrose agar was prepared by dissolving the required quantity of the powdered medium in distilled water. The medium was sterilized by autoclaving and allowed to cool before antibiotics were added to inhibit bacterial contamination during fungal isolation. Plate count agar was prepared by suspending the powdered medium in distilled water, stirring thoroughly, autoclaving, cooling, and pouring into sterile Petri dishes. MacConkey agar and eosin methylene blue agar were similarly prepared according to standard microbiological procedures and sterilized before use.

3.8.5 Microbial count estimation

Total viable bacterial count was determined using the pour plate method. One millilitre of each caecal digesta sample was serially diluted, inoculated into sterile Petri dishes, and mixed with prepared agar medium. The plates were allowed to solidify and were incubated at 37 °C for 18–24 h. Colony-forming units were counted after incubation and expressed as microbial counts.

3.8.6 Total fungal count

Potato dextrose agar was used for fungal isolation. The prepared medium was sterilized by autoclaving and modified with antibiotics to prevent bacterial growth. The samples were inoculated using the pour plate method and incubated at 27 °C for 48–72 h. Fungal colonies were observed, counted, and identified based on cultural and morphological characteristics.

3.8.7 Statistical analysis

Data obtained from the experiment were subjected to one-way analysis of variance using the completely randomized design model. Where significant differences were observed, treatment means were separated using Duncan's multiple range test. Statistical significance was declared at $p < 0.05$.

4. Results

4.1 Growth performance

The growth performance of Arbor Acre broiler chickens fed diets containing varying levels of neem (*Azadirachta indica*) leaf powder is presented in Table 4.1. The parameters evaluated included initial weight, final weight, weight gain, feed intake, and feed conversion ratio. There were no significant differences ($p > 0.05$) among treatments for initial weight, final weight, weight gain, and feed conversion ratio. However, feed intake differed significantly ($p < 0.05$) across the dietary treatments. Birds in the control treatment recorded the highest feed intake value of 8.66 g, while birds fed 0.5% neem leaf powder had the lowest feed intake value of 7.55 g. Birds fed 0.3%, 0.4%, and 0.6% neem leaf powder had intermediate feed intake values of 7.77 g, 7.95 g, and 8.29 g, respectively. Although feed intake was significantly affected by dietary treatment, the inclusion of neem leaf powder did not significantly reduce final body weight, weight gain, or feed conversion ratio. This indicates that neem leaf powder could be included in broiler diets up to 0.6% without adverse effects on growth performance.

Table 1. Mean values of growth performance traits of Arbor Acre broilers fed varying levels of neem leaf powder

Variables	T1 0%	T2 0.3%	T3 0.4%	T4 0.5%	T5 0.6%	SEM
Initial weight	1.10	0.89	1.00	0.96	1.11	0.16
Final weight	7.15	6.84	6.83	6.49	6.74	0.52
Weight gain	6.05	5.95	5.83	5.53	5.63	0.43
Feed intake	8.66 ^a	7.77 ^{ab}	7.95 ^{ab}	7.55 ^b	8.29 ^{ab}	0.22
Feed conversion ratio	1.44	1.30	1.37	1.38	1.48	0.11

Values with different superscripts within the same row differ significantly at $p < 0.05$. T1 = control diet; T2 = 0.3% neem leaf powder; T3 = 0.4% neem leaf powder; T4 = 0.5% neem leaf powder; T5 = 0.6% neem leaf powder.

4.2 Gut microbial count

The microbial count of Arbor Acre broiler chickens fed different inclusion levels of neem leaf powder is shown in Table 4.2. Significant differences ($p < 0.05$) were observed in total bacterial count and faecal contaminant count among the treatments. Total fungal count and total coliform count were not significantly affected by dietary treatment. The control treatment recorded a total bacterial count of 10.35 CFU, while birds fed neem leaf powder recorded lower bacterial counts in most supplemented groups. Birds fed 0.3%, 0.5%, and

0.6% neem leaf powder recorded total bacterial counts of 5.37, 2.58, and 3.65 CFU, respectively. The result suggests that neem leaf powder may have reduced bacterial load in the gut of broiler chickens. However, the value reported for T3 requires confirmation because the narrative section of the source document reported 10.97, while Table 4.2 showed 1.97. Faecal contaminant count was significantly higher in T5, while T1, T2, T3, and T4 recorded no faecal contaminant growth. No growth of *Escherichia coli* and *Salmonella* was reported during incubation, suggesting that neem leaf powder may support microbial control in broiler gut contents.

Table 2. Mean values of microbial count of Arbor Acre broilers fed varying levels of neem leaf powder

Parameters	T1 0%	T2 0.3%	T3 0.4%	T4 0.5%	T5 0.6%	SEM
Total bacterial count	10.35 ^a	5.37 ^b	1.97 ^a	2.58 ^b	3.65 ^b	0.87
Total fungal count	0.18	0.25	0.30	0.45	0.38	0.05
Total coliform	1.43	1.90	0.10	0.63	1.17	0.38
Faecal contaminant	0.00 ^b	0.00 ^b	0.00 ^b	0.00 ^b	0.43 ^a	0.05

Values with different superscripts within the same row differ significantly at $p < 0.05$. TBC = total bacterial count; TFC = total fungal count; TC = total coliform; FC = faecal contaminant.

4.3 Digestible feed kinetics

The digestible feed kinetics of broilers fed different inclusion levels of neem leaf powder is presented in Table 4.3. The parameters measured included weight of the gastrointestinal tract, proventriculus weight, gizzard weight, eviscerated gizzard weight, digesta difference, and percentage digestible feed in the stomach. There were no significant differences ($p > 0.05$) among treatments for all measured

parameters. The weight of the gastrointestinal tract ranged from 214.14 g in T3 to 243.50 g in T5. Gizzard weight ranged from 40.63 g in T4 to 49.81 g in T5, while eviscerated gizzard weight ranged from 26.80 g in T1 to 32.81 g in T5. Although numerical differences were observed, these differences were not statistically significant. This indicates that neem leaf powder inclusion did not negatively affect gastrointestinal tract development or digestible feed kinetics in the broiler chickens.

Table 3. Mean values of digestible feed kinetics of Arbor Acre broilers fed varying levels of neem leaf powder

Variables	T1 0%	T2 0.3%	T3 0.4%	T4 0.5%	T5 0.6%	SEM
Weight of gastrointestinal tract	226.71	236.67	214.14	215.35	243.50	8.40
Proventriculus	5.99	6.49	6.33	6.13	5.90	0.22
Gizzard	41.85	46.07	46.73	40.63	49.81	1.90

Eviscerated gizzard	26.80	31.23	32.29	28.32	32.81	1.32
Difference	15.05	14.84	14.44	12.31	17.00	2.74
Digestible feed in stomach (%)	35.97	32.19	30.72	30.37	33.97	1.22

T1 = control diet; T2 = 0.3% neem leaf powder; T3 = 0.4% neem leaf powder; T4 = 0.5% neem leaf powder; T5 = 0.6% neem leaf powder.

4.4 Vital organ characteristics

The effects of neem leaf powder on the vital organs of Arbor Acre broiler chickens are shown in Table 4.4. The organs evaluated included the heart, spleen, pancreas, and liver. There were no significant differences ($p > 0.05$) among treatments for all organ parameters measured.

Heart weight ranged from 6.56 g in the control group to 9.14 g in birds fed 0.6% neem leaf powder. Liver weight also showed numerical variation, ranging from 33.14 g in T1 to 43.47 g in T5. However, the differences were not statistically significant. This suggests that neem leaf powder did not cause abnormal enlargement or reduction of the measured organs and may be safely included in broiler diets at the evaluated levels.

Table 4. Mean values of vital organs of Arbor Acre broilers fed varying levels of neem leaf powder

Variables	T1 0%	T2 0.3%	T3 0.4%	T4 0.5%	T5 0.6%	SEM
Heart	6.56	7.66	7.04	8.06	9.14	0.51
Spleen	1.56	1.95	2.00	1.66	1.78	0.08
Pancreas	3.60	3.99	3.50	4.60	3.83	0.19
Liver	33.14	34.53	38.77	34.76	43.47	1.45

T1 = control diet; T2 = 0.3% neem leaf powder; T3 = 0.4% neem leaf powder; T4 = 0.5% neem leaf powder; T5 = 0.6% neem leaf powder.

4.5 Intestinal characteristics

The intestinal characteristics of Arbor Acre broiler chickens fed varying levels of neem leaf powder are presented in Table 4.5. The parameters measured included duodenum weight, duodenum length, jejunum weight, jejunum length, ileum weight, ileum length, caecum weight, caecum length, and colon weight.

Significant differences ($p < 0.05$) were observed in jejunum length and ileum length. Birds fed 0.3% and 0.6% neem leaf powder recorded higher jejunum length values of 89.67 cm and 86.00 cm,

respectively. The control group recorded the lowest jejunum length value of 47.83 cm. Similarly, ileum length was highest in birds fed 0.4% neem leaf powder, with a value of 82.33 cm, while the control group recorded the lowest value of 54.00 cm. The increase in jejunum and ileum length may suggest improved intestinal development and absorptive surface area in birds fed neem leaf powder. Since the jejunum and ileum are major sites of nutrient digestion and absorption, improved development of these segments may contribute to better nutrient utilization. Other intestinal parameters did not differ significantly among treatments.

Table 4.5. Mean values of intestinal characteristics of Arbor Acre broilers fed varying levels of neem leaf powder

Variables	T1 0%	T2 0.3%	T3 0.4%	T4 0.5%	T5 0.6%	SEM
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Duodenum weight	15.24	15.02	14.84	13.93	18.52	0.73
Duodenum length	31.33	35.33	30.83	29.17	31.93	0.92
Jejunum weight	57.50	23.29	37.38	47.20	58.78	5.01
Jejunum length	47.83 ^b	89.67 ^a	63.80 ^{ab}	82.83 ^{ab}	86.00 ^a	6.51
Ileum weight	29.01	32.69	31.46	28.49	23.10	2.56
Ileum length	54.00 ^b	62.33 ^{ab}	82.33 ^a	67.67 ^{ab}	70.67 ^{ab}	3.32
Caecum weight	17.17	13.50	14.68	13.58	20.93	1.37
Caecum length	19.10	17.67	21.17	17.43	18.50	0.67
Colon	5.57	12.39	4.61	6.03	14.66	1.99

Values with different superscripts within the same row differ significantly at $p < 0.05$. T1 = control diet; T2 = 0.3% neem leaf powder; T3 = 0.4% neem leaf powder; T4 = 0.5% neem leaf powder; T5 = 0.6% neem leaf powder.

4.6 Fungal isolates

The occurrence of fungal isolates from broiler chickens fed varying levels of neem leaf powder showed that *Fusarium oxysporum* had the highest frequency of occurrence, with 79.06%. *Aspergillus* spp. and *Rhizopus stolonifer* had lower frequencies of occurrence, each at 6.98%, while other fungal isolates accounted for 2.32%. The result indicates that neem leaf powder may have contributed to reduced fungal population in some treatment groups, particularly for *Aspergillus* spp. and *Rhizopus stolonifer*. No growth of *E. coli* and *Salmonella* was observed during incubation. This observation supports the possible antimicrobial effect of neem leaf powder and suggests that it may help reduce the proliferation of harmful microorganisms in the gut of broiler chickens.

5. Discussion

5.1 Growth performance

The results of this study showed that neem leaf powder did not significantly affect final weight, weight gain, or feed conversion ratio of Arbor Acre broiler chickens. This suggests that neem leaf powder could be included in broiler diets at levels between 0.3% and 0.6% without compromising growth performance. However, feed intake was

significantly influenced by dietary treatment, with the lowest intake recorded in birds fed 0.5% neem leaf powder. The reduction in feed intake observed in some neem-supplemented groups may be associated with the bitter taste and bioactive constituents of neem leaves. Neem contains compounds such as phenolics, tannins, oxalates, and triterpenoids, which may affect palatability when included at higher levels. However, despite the reduction in feed intake, body weight gain and feed conversion ratio were not significantly impaired. This implies that the birds were able to utilize the diets efficiently.

The findings agree with earlier reports that neem leaf meal may support growth performance when included at moderate levels but may reduce feed intake when used at higher levels. The bioactive compounds in neem may enhance nutrient utilization by improving gut microbial balance and reducing pathogenic microbial activity. Therefore, the absence of negative effects on growth performance indicates that neem leaf powder has potential as a natural phytochemical feed additive in broiler production.

5.2 Gut microbial count

The reduction in total bacterial count observed in neem-supplemented treatments suggests that neem leaf powder may possess antimicrobial activity in the gastrointestinal tract of broiler chickens. Neem is known to contain bioactive compounds with antibacterial, antifungal, and anti-inflammatory properties. These compounds may inhibit the

proliferation of harmful microorganisms and support a healthier microbial balance in the gut. The absence of *E. coli* and *Salmonella* growth during incubation is particularly important because these organisms are major enteric pathogens in poultry production. Their absence may indicate improved gut hygiene and possible antimicrobial effects of neem supplementation. However, faecal contaminant count was higher in birds fed 0.6% neem leaf powder, suggesting that higher inclusion levels may not necessarily provide additional microbial benefits. The microbial results indicate that neem leaf powder may contribute to gut microbial modulation in broiler chickens. Nevertheless, the inconsistency between the reported T3 value in the narrative and the table should be corrected before final publication.

5.3 Digestible feed kinetics

Neem leaf powder did not significantly affect the weight of the gastrointestinal tract, proventriculus, gizzard, eviscerated gizzard, digesta difference, or percentage digestible feed in the stomach. This suggests that dietary neem leaf powder did not negatively influence the digestive organ structure or feed passage characteristics of the broilers. The absence of significant changes in gizzard and proventriculus weights indicates that neem leaf powder did not impose abnormal physiological stress on the upper digestive tract. Similarly, the lack of significant differences in percentage digestible feed in the stomach suggests that feed digestion was not impaired by neem supplementation. These results support the safe use of neem leaf powder within the tested inclusion levels.

5.4 Vital organs

The weights of the heart, spleen, pancreas, and liver were not significantly affected by dietary inclusion of neem leaf powder. Organ weight is often used as an indicator of physiological response, metabolic stress, or possible toxicity in feeding trials. The absence of significant changes in these organs suggests that neem leaf powder did not produce harmful effects on organ development or function. The liver is especially important because it plays a major role in metabolism and detoxification. Although birds fed 0.6% neem leaf powder had a numerically higher liver weight, the difference was not statistically significant. This indicates that the inclusion levels used in this study were not

detrimental to the birds. The findings support the potential safety of neem leaf powder as a dietary additive for broiler chickens.

5.5 Intestinal characteristics

The significant increase in jejunum and ileum length observed in neem-supplemented birds suggests that neem leaf powder may positively influence intestinal development. The jejunum and ileum are important sites for nutrient digestion and absorption in poultry. Therefore, increased length of these intestinal segments may enhance absorptive surface area and improve nutrient utilization. Birds fed 0.3% and 0.6% neem leaf powder recorded higher jejunum length, while birds fed 0.4% neem leaf powder recorded the highest ileum length. This suggests that neem leaf powder may stimulate intestinal development at moderate inclusion levels. Improved intestinal structure may be linked to the antimicrobial and anti-inflammatory properties of neem, which could reduce intestinal microbial stress and support gut integrity. However, other intestinal parameters such as duodenum weight, duodenum length, jejunum weight, ileum weight, caecum weight, caecum length, and colon weight were not significantly affected. This shows that the major effect of neem leaf powder was observed in the length of the jejunum and ileum rather than in overall intestinal mass.

5.6 Fungal morphology and occurrence

The fungal isolation result showed that *Fusarium oxysporum* had the highest occurrence among the fungi identified. Lower occurrence was recorded for *Aspergillus* spp. and *Rhizopus stolonifer*. The relatively low occurrence of some fungal species may be associated with the antifungal properties of neem leaf powder. Neem contains bioactive compounds that can inhibit fungal growth, which may explain the reduced occurrence of certain fungal organisms. The reduction or absence of some harmful microbial organisms supports the potential of neem leaf powder as a natural antimicrobial additive in broiler production. However, further studies using molecular microbial analysis would provide more detailed information on the specific bacterial and fungal communities affected by neem supplementation.

6.0 Conclusion

This study evaluated the effects of graded levels of neem (*Azadirachta indica*) leaf powder on the

growth performance, gut microbial count, digestible feed kinetics, vital organs, and intestinal characteristics of Arbor Acre broiler chickens. The findings showed that dietary inclusion of neem leaf powder had no adverse effect on final body weight, weight gain, feed conversion ratio, digestible feed kinetics, or vital organ weights. This indicates that neem leaf powder can be safely incorporated into broiler diets at the tested levels without negatively affecting general performance or organ development. Neem leaf powder significantly influenced feed intake, total bacterial count, faecal contaminant count, jejunum length, and ileum length. The reduction in bacterial count observed in some neem-supplemented groups suggests that neem leaf powder may possess antimicrobial properties capable of supporting gut microbial balance. In addition, the increased jejunum and ileum lengths recorded in neem-fed birds indicate a possible improvement in intestinal development and absorptive capacity. Overall, the results suggest that neem leaf powder has potential as a natural phyto-genic feed additive in broiler production. Its inclusion may help improve gut health, support intestinal development, and reduce dependence on synthetic antibiotic growth promoters. Based on the findings, neem leaf powder may be included in broiler diets at levels of 0.3% to 0.6%, although moderate inclusion levels should be considered to balance gut health benefits with feed intake and overall performance.

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