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# Antibacterial effect of *Raphanus sativus* (Radish) root juice on growth performance and caecal bacterial load of broiler chickens

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**ABSTRACT.** Majority of poultry producers have been concerned about the microbial colonization of chicken guts, which usually result in competition for nutrient and poor health. As a way to combat this, antibiotics are used, however, this has led to antibiotic resistance and the presence of antibiotic residues in poultry products. Therefore, the purpose of this study is to ascertain the effect of radish root juice on the microbiota in the caecum of broiler chickens while also monitoring their growth response. This study was a 2 by 3 factorial layout. There were six treatment groups of forty day-old broiler chicks each, consisting of two dosages of radish juice (10 mL $L^{-1}$ or 15mL $L^{-1}$ of the birds' drinking water) and three frequencies of administration (0, 2 and 3 times weekly). The zero frequencies (no juice) were the control groups given Enrofloxacin as antibiotic (1 mL in 4 L of drinking). At the starter and finisher phase, birds were given commercial diets. The growth performance was evaluated at both phases, while the microbial load for each group was determined at the finisher phase. The contents of the birds' caeca were taken for microbial analysis at 42$^{nd}$ day of the experiment. Frequency and nature of microorganism were obtained. Microbial count of broilers administered radish juice ranged from 7.84 x $10^7$ – 1.75 x $10^7$ cfu $mL^{-1}$ while those administered antibiotics had 8.75 x $10^7$ – 8.56 x$10^7$ cfu $mL^{-1}$. Microorganisms isolated include: *Klebsiella Pneumoniae* (27%), *Morganella morganii* (24%), *Bacillus subtilis* (22%), *Providencia rettgerii* (5%), *Staphylococcus saprophyticus* (5%), *Staphylococcus aureus* (4%), *Citrobacter fruendii* (4%), *Proteus mirabilis* (2%), *Micrococcus luteus* (2%), *Escherichia coli* (2%), *Providencia stuartii* (2%). There was no statistical difference (P>0.05) among all the growth parameters in both the radish groups and the antibiotic groups, at the starter and finisher phase. This study suggests radish root juice as an effective antimicrobial agent in reducing microbial load of the gut of broilers which consequently can lead to increased productivity and feed – to – meat conversion ratio.

**Keywords:** Radish; broiler; growth performance; antibacterial activity; caecum bacterial load.

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## Introduction

The gastrointestinal tract (GIT) of broiler chickens is crucial for growth and health throughout the animal's lifecycle (Clavijo and Vives Flórez, 2018). Immediate hatchery and poultry house circumstances, as well as other environmental variables, have a significant impact on the microbial colonization of the chicken GIT (Stamilla et al., 2021). According to Pan and Yu (2013), the health of the intestinal structure and the microbial community in the gut are crucial for immunity, disease resistance, nutrition, and absorption. Numerous investigations have shown that Enterobacteriaceae and, to a lesser extent, *Enterococcus* dominate the early bacterial population. To better understand the connection between the presence of particular bacterial species and their impact on chicken health, several research have recently concentrated on altering the microbiota in the chicken gut. Historically, selective culture-based techniques were used to study microbial diversity in the GIT; however, these methods are highly selective because most bacteria remain uncultured (Apajalahti et al., 2004). Modulation of GIT microbiota in chickens represents the key to production, health, pathogen protection, detoxification, and immune system modulation (Brisbin et al., 2008). Thus, knowledge of what constitutes a healthy microbiota and the acceptable number of microorganisms is relevant for improving poultry meat production while maintaining animal welfare under appropriate nutritional and environmental condition.

*Raphanus sativus* (radish) is a popular vegetable all over the world. Radishes are members of the Brassicaceae family and come in a variety of skin colors or variations, including red, purple, black, yellow, white, and pink radishes (Nakamura et al., 2008). It is a winter crop with a high concentration of secondary chemicals with medicinal and pharmacological characteristics. The major bioactive compounds found in radish are glucosinolates and isothiocyanates (Ishida et al., 2015; Malik et al., 2010; Baenas et al., 2016). The radish plant is high in vitamin C and A, as well as various minerals such as iodine, sulfur, calcium, magnesium, and potassium. Radishes are therefore plants with great nutritional content that are both tasty and easy to digest.

Radish plants (roots, leaves, and seeds) have a variety of medical applications, including the treatment of tuberculosis, whooping cough, asthma attacks, laxative to treat constipation, fracture of kidney and bladder stones, thinner, cholesterol-lowering, antiradical, and analgesic pain (joint medical problems affecting the joints), and textile (Gutierrez and Perez, 2004; Jha, 2007). The radish plant can be used to cure diarrhea (Al Thwani et al., 2010). Coumarins, alkaloids, nitrogenous chemicals, gibberellins, glycosinolate, organic acids, Phenolic compounds, and sulphur pigments are among the active substances found in radish root and leaves. Perez and Rosalinda (2004) discovered sulforaphane. It has antioxidant properties (Lugasi et al., 2005). Because of their potent secondary compounds, radish root and leaf extracts are useful against antimicrobial microbes.

When radish root juice is used, it functions as an antibacterial agent against a variety of bacteria, including *Salmonella typhi, Bacillus subtilis,* and *Pseudomonas aeruginosa* (Jaafar et al., 2020). A few studies have also found that radish has antifungal properties (Truta et al., 2011). Tetracycline, amoxicillin, and ampicillin are antibiotic feed additives that have been used to boost growth and control infections in chicken. However, in recent years, similar feeding strategies have been linked to the development of antibiotic resistance and the occurrence of antibiotic residues in chicken products. As a result, different tactics such as the usage of natural products (such as radish) in treating bacterial infections and improving growth performance are required. These natural products are free of toxins and residues in poultry products. Thus, the aim of this study is to determine the effect of radish root juice on the bacterial caecum count as well as growth rate of broiler chicken.

## Materials and methods

### Experimental site

This research was carried out at Directorate of University Farms (DUFARMS), Federal University of Agriculture, Abeokuta (FUNAAB), Ogun state, Nigeria. The site is located on the rainforest zone of south-west Nigeria Latitude 7°13'49.46"'N, Longitude 3°26'11.98"*E and altitude 98m above sea level (Google earth, 2021). The climate is humid with a mean precipitation of 1003 mm annum. The annual mean temperature and humidity is 34.7 and 82% respectively (Google Earth, 2021).

### Preparation of test ingredient

White radish (*Raphanus sativus*) roots were purchased from organic farms within Abeokuta, Ogun State, Nigeria. They were blended without water being added after which a muslin cloth was used to separate its juice from the chaff. The radish juice was bottled and kept refrigerated in batches until when needed.

### Animals and management practices

A total of 240 unsexed day-old broiler chicks (Arbor acre strain) were used for this study. The chicks were brooded for two weeks. On arrival, chicks were weighed and randomly distributed into six treatments (forty birds per treatment). The birds were raised under intensive system of management using deep litter system. The room temperature and humidity were monitored using a digital thermo-hygrometer. Feed and water were offered *ad libitum* during the experiment Commercially produced broiler starter diet (containing 2900 kcal $kg^{-1}$ ME, 21% crude protein) was offered for the first four weeks while finisher diet (containing 3000 kcal $kg^{-1}$ ME, 18% crude protein) was offered from week five to six. Vaccination against infectious bursal disease and Newcastle disease was done on days 7 & 14 and 21 & 28. All procedures used in the experiment strictly adhered to the research Ethics guidelines of the Federal University of Agriculture, Abeokuta (FUNAAB, 2016). The nutrient composition of both diets is shown in Table 1.

**Table 1**. Nutrient composition of the commercial diet as declared by the manufacturer.

| Ingredient | Starter (%) | Finisher (%) |
|---|---|---|
| Crude protein | 21.00 | 18.00 |
| Fats and oil | 6.00 | 6.00 |
| Crude fibre | 5.00 | 6.00 |
| Calcium | 1.00 | 1.00 |
| Available phosphorus | 0.45 | 0.40 |
| Lysine | 1.00 | 0.86 |
| Methionine | 0.50 | 0.30 |
| Salt (NaCl) | 0.30 | 0.30 |

## Experimental design

The experimental layout was a 2×3 factorial arrangement (two dosages and three frequencies of administration). Birds were randomly assigned to each of six treatments in a Completely Randomized Design. A treatment was replicated four times with 10 birds per replicate.

Below are the six treatments:

T1 - Control for 10 mL $L^{-1}$ radish juice group

T2 - 10 mL radish juice per L of drinking water twice weekly

T3 - 10 mL radish juice per L of drinking water thrice weekly

T4 - Control for 15 mL $L^{-1}$ radish juice group

T5 - 15 mL radish juice per L of drinking water twice weekly

T6 - 15 mL radish juice per L of drinking water thrice weekly

Birds in the two control levels (T1 and T4) were administered conventional antibiotic, Enrocare® (containing 20% Enrofloxacin and bromhexine hydrochloride solution) at a dosage of 1 mL in 4 L of drinking water thrice per week. The administration of the test ingredients was done on consecutive days per week, and it commenced on the second day of brooding and spanned through the six weeks of the experiment.

## Enumeration of microorganism

Pour plate technique was used for the enumeration of bacteria from the caecum of the test groups. Serial dilution was the first step used in the isolation of organisms from the caecum sample after which 1ml was taken from each dilution factor in the test tubes prepared and dispensed into different sterile petri dishes and 18 ml of sterile molten plate count agar (Oxoid). The plates were then swirled gently for proper mixing of the sample with the agar. The agar was allowed to set on the bench and then incubated in an inverted position in the incubator at 37°C for 24 hours. The colonies obtained after incubation was then counted and recorded. The experiment was repeated in triplicates and the mean microbial load was obtained in Colony Forming Unit per Milliliter (CFU $mL^{-1}$) (Mongi, 2023).

## Isolation of microorganisms

Several different colonies obtained from the serial dilution was taken using a sterile inoculating loop and sub – cultured on sterile solidified nutrient agar and incubated for 24 hours at 37°C. The growth obtained was further sub – cultured until a pure culture is obtained.

## Identification of Isolates

Bacteria obtained from the sample were identified using cultural (colour, opacity, consistency), morphological (Gram stain) and biochemical identification techniques (Catalase test, Coagulase, Indole, Urease test, Voges Proskaeur, Methyl red test, Citrate test, Oxidase test) (Cheesebrough, 2006)

**Determination of growth performance**: Data were collected on the following growth performance parameters; feed intake, water intake, weight gain, and feed conversion ratio was calculated. The relevant formulae are as stated:

$$Total\ feed\ intake\ (g) = \frac{Total\ feed\ given\ (g) - Total\ feed\ leftover(g)}{Total\ number\ of\ birds}$$

$$Total\ water\ intake\ (l) = \frac{Total\ water\ given\ (l) - Total\ water\ leftover(l)}{Total\ number\ of\ birds}$$

$$Total\ weight\ gain\ (g) = \frac{Final\ weight\ (g) - Initial\ weight\ (g)}{Total\ number\ of\ birds}$$

$$Feed\ Conversion\ Ratio = \frac{Feed\ Intake}{Weight\ Gain}$$

### Statistical analysis

Growth performance data were subjected to Analysis of Variance in a 2 by 3 factorial layout using General Linear Model procedure in MINITAB statistical software package (MINITAB, 2018). Means were separated using Tukey test of the software package at 5% level of significance.

## Result

### Bacterial count from broiler caecum

The mean caecum bacterial count showed a steady decrease when radish root juice concentration was given to the broiler chicken as indicated in Figure 1. The highest bacterial count was observed in test group 1 and 4 (Control) while the lowest count was seen in test group 6.

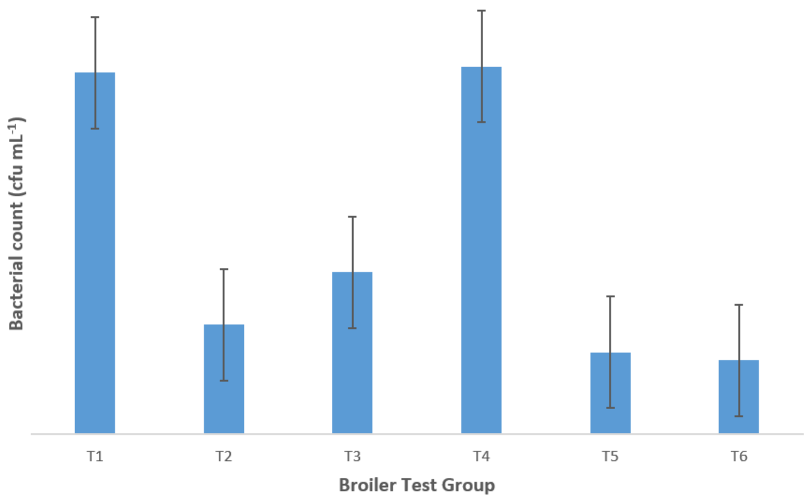


**Figure 1**. Mean caecum bacterial count in different test groups.

### Distribution of bacteria among test groups

The bacterial diversity of the different groups as indicated in Table 2 shows a variation between the test groups treated with antibiotics and those with Radish root juice. Also, Figure 2 indicates the frequency of occurrence of bacteria within the caecum of broiler chicken. *Klebsiella pneumoniae* had the highest frequency of occurrence (27%) followed by *Morganella morganii* (24%) and *Bacillus subtilis* (22%) which is the only probiotic among the organisms isolated.

### Growth performance of broiler chickens

Tables 3, 4, 5, and 6 shows the main and interactive effect of the dosages and frequencies of administration of radish root juice on the growth performance of broiler chickens at the starter and finisher phase of growth. All the growth parameters measured were not significantly (P>0.05) influenced, that is, both the birds in the conventional antibiotics and the radish root juice group performed similarly.

**Table 2**. Bacteria identified in each test groups of broilers.

| Test Group | Administration |
|---|---|
| T 1 | *Bacillus subtilis, Klebsiella pneumoniae, Proteus mirabilis, Morganella morganii, Staphylococcus aureus* |
| T 2 | *Klebsiella pneumoniae, Escherichia coli, Morganella morganii, Staphylococcus saprophyticus* |
| T 3 | *Morganella morganii, Klebsiella pneumoniae, Bacillus subtillis, Micrococcus luteus* |
| T 4 | *Kelbsiella pneumoniae, Morganella morganii, Bacillus subtilis Providencia retgerri, Providencia stuartii* |
| T 5 | *Bacillus subtilis, Klebsiella Pneumoniae, Staphylococcus saprophyticus, Morganella morganii, Klebsiella pneumoniae* |
| T 6 | *Bacillus subtilis, Klebsiella pneumoniae, Morganella morganii, Citrobacter freundii* |

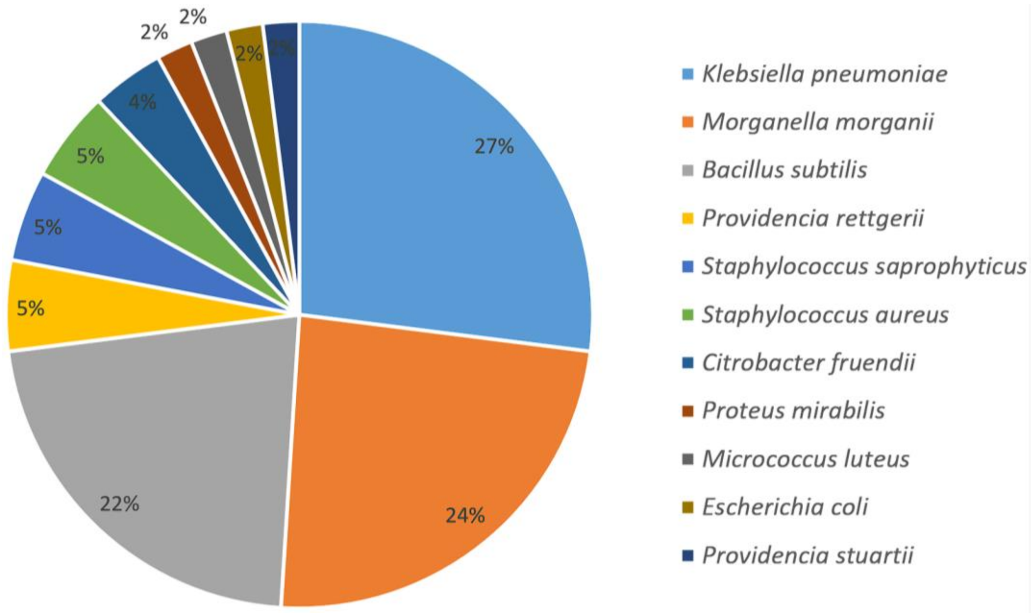


**Figure 2**. Frequency of occurrence of bacteria within the caecum of broiler chicken.

**Table 3**. Main effect of dosages and frequencies of administration of radish root juice on the growth performance of broiler chickens at the starter phase.

| Parameter | Dosages of RRJ | | | | Frequencies of administration | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 10 mL $L^{-1}$ | 15 mL $L^{-1}$ | SEM | P-value | 0/wk | Twice/wk | Thrice/wk | SEM | P-value |
| IW (g $bird^{-1}$) | 42.05 | 40.79 | 1.14 | 0.44 | 42.00 | 40.63 | 41.63 | 1.39 | 0.77 |
| FW (g $bird^{-1}$) | 1066.90 | 1094.40 | 14.90 | 0.21 | 1102.50 | 1046.30 | 1093.10 | 18.30 | 0.09 |
| TWG (g $bird^{-1}$) | 1024.80 | 1053.60 | 15.20 | 0.20 | 1060.50 | 1005.70 | 1051.40 | 18.60 | 0.11 |
| TFI (g $bird^{-1}$) | 2082.90 | 2056.10 | 41.80 | 0.66 | 2086.30 | 2110.80 | 2011.90 | 51.20 | 0.39 |
| FCR | 2.04 | 1.95 | 0.05 | 0.20 | 1.97 | 2.11 | 1.92 | 0.06 | 0.07 |
| TWI (l $bird^{-1}$) | 3.61 | 3.60 | 0.06 | 0.91 | 3.58 | 3.66 | 3.57 | 0.08 | 0.68 |

SEM: Standard error of mean, IW: Initial weight, FW: Final weight, TWG: Total weight gain, TFI: Total feed intake, FCR: Feed conversion ratio, TWI: Total water intake, /wk: per week, RRJ: Radish root juice.

**Table 4**. Main effect of dosages and frequencies of administration of radish root juice on the growth performance of broiler chickens at the finisher phase.

| Parameter | Dosages of RRJ | | | | Frequencies of administration | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 10 mL $L^{-1}$ | 15 mL $L^{-1}$ | SEM | P-value | 0/wk | Twice/wk | Thrice/wk | SEM | P-value |
| IW (g $bird^{-1}$) | 42.05 | 40.79 | 1.14 | 0.44 | 42.00 | 40.63 | 41.63 | 1.39 | 0.77 |
| FW (g $bird^{-1}$) | 1831.40 | 1787.10 | 31.70 | 0.34 | 1778.00 | 1858.00 | 1790.60 | 38.80 | 0.32 |
| TWG (g $bird^{-1}$) | 1789.30 | 1746.30 | 31.70 | 0.35 | 1736.80 | 1817.80 | 1748.90 | 38.90 | 0.31 |
| TFI (g $bird^{-1}$) | 4751.00 | 4562.00 | 166 | 0.43 | 4641.00 | 4825.00 | 4504.00 | 204.00 | 0.55 |
| FCR | 2.66 | 2.63 | 0.12 | 0.83 | 2.68 | 2.66 | 2.59 | 0.13 | 0.88 |
| TWI (l $bird^{-1}$) | 8.40 | 8.24 | 0.18 | 0.55 | 8.13 | 8.61 | 8.23 | 0.23 | 0.30 |

SEM: Standard error of mean, IW: Initial weight, FW: Final weight, TWG: Total weight gain, TFI: Total feed intake, FCR: Feed conversion ratio, TWI: Total water intake, /wk: per week, RRJ: Radish root juice.

**Table 5**. Interactive effect of dosages and frequencies of administration of radish root juice on the growth performance of broiler chickens at the starter phase.

| Parameters | 10 mL $L^{-1}$ RRJ | | | 15 mL $L^{-1}$ RRJ | | | SEM | P-value |
|---|---|---|---|---|---|---|---|---|
| | 0/wk | Twice/wk | Thrice/wk | 0/wk | Twice/wk | Thrice/wk | | |
| IW (g $bird^{-1}$) | 41.88 | 41.88 | 42.40 | 42.12 | 39.38 | 40.87 | 1.97 | 0.78 |
| FW (g $bird^{-1}$) | 1082.50 | 1025.60 | 1092.50 | 1122.50 | 1067.10 | 1093.60 | 25.80 | 0.68 |
| TWG(g $bird^{-1}$) | 1040.60 | 983.70 | 1050.10 | 1080.40 | 1027.70 | 1052.70 | 26.40 | 0.69 |
| TFI (g $bird^{-1}$) | 2116.10 | 2190.90 | 1941.80 | 2056.50 | 2029.80 | 2082.00 | 72.40 | 0.14 |
| FCR | 2.03 | 2.23 | 1.85 | 1.90 | 1.98 | 1.98 | 0.08 | 0.06 |
| TWI (l $bird^{-1}$) | 3.57 | 3.71 | 3.54 | 3.60 | 3.60 | 3.59 | 0.11 | 0.61 |

SEM: Standard error of mean, IW: Initial weight, FW: Final weight, TWG: Total weight gain, TFI: Total feed intake, FCR: Feed conversion ratio, TWI: Total water intake, /wk: per week, RRJ: Radish root juice.

**Table 6**. Interactive effect of dosages and frequencies of administration of radish root juice on the growth performance of broiler chickens at the finisher phase.

| Parameters | 10 mL $L^{-1}$ RRJ | | | 15 mL $L^{-1}$ RRJ | | | SEM | P-value |
|---|---|---|---|---|---|---|---|---|
| | 0/wk | Twice/wk | Thrice/wk | 0/wk | Twice/wk | Thrice/wk | | |
| IW (g $bird^{-1}$) | 41.88 | 41.88 | 42.40 | 42.12 | 39.38 | 40.87 | 1.97 | 0.78 |
| FW (g $bird^{-1}$) | 1790.00 | 1824.20 | 1880.00 | 1767.50 | 1892.70 | 1701.10 | 54.90 | 0.10 |
| TWG (g $bird^{-1}$) | 1748.10 | 1782.30 | 1837.60 | 1725.40 | 1853.30 | 1660.20 | 55.00 | 0.10 |
| TFI (g $bird^{-1}$) | 4736.00 | 5143.00 | 4374.00 | 4545.00 | 4506.00 | 4634.00 | 288.00 | 0.32 |
| FCR | 2.71 | 2.90 | 2.38 | 2.66 | 2.43 | 2.80 | 0.19 | 0.09 |
| TWI (l $bird^{-1}$) | 8.08 | 8.87 | 8.26 | 8.18 | 8.35 | 8.20 | 0.32 | 0.61 |

SEM: Standard error of mean, IW: Initial weight, FW: Final weight, TWG: Total weight gain, TFI: Total feed intake, FCR: Feed conversion ratio, TWI: Total water intake, /wk: per week, RRJ: Radish root juice

# Discussion

This study has been able to reveal the different groups of bacteria in the cecum of broiler chickens. The bacteria in the Enterobacteriaceae family had the highest population in this study. *Morganella morganii* has been regarded as a normally opportunistic pathogen, however some strains carry antibiotic resistant plasmids and have been associated with nosocomial outbreaks of infections (Singla et al., 2010), In the same vein, *Klebsiella pneumoniae* was also isolated in this study which is in agreement with the study of Franklin-Alming et al., (2021) and it is one of the microorganisms commonly known to cause infections such as urinary tract infections, pneumonia, septicaemia and liver abscesses. On the other hand, *Bacillus subtilis* is a non–pathogenic, Gram-positive rod–shaped bacterium usually found in the soil. It is usually a normal flora and considered harmless. It has been used as feed additive to improve broiler performance, modulate immune response and act as a prophylactic agent against bacterial disease by balancing gut microbiota (Hayashi et al., 2018). *Bacillus subtilis* was isolated in all the treatment groups (antibiotic-treated and radish-juice-treated) except the group given the lowest concentration of radish root juice (10mL L-1 twice per week). This could suggest that a higher concentration of the radish root juice is more beneficial to the non-pathogenic microbes.

The addition of antimicrobial agents as feed additive has in the past contributed to the growth and development of poultry birds. However, the recent spike in antimicrobial resistance of microorganisms has necessitated the search for other viable agents. Radish root juice used in this study showed promising results as there was a steady decline in the microbial load as the concentration and exposure time increases. Presence of microorganisms in birds may lead to synthesis of toxins by the microbes, which can decrease performance or alter the health depending on the toxins and the dose in addition, colonization of the animal's gut may lead to complex effects from beneficial effect (gut colonization and development of gut microbiota) by the beneficial microbes (*Bacillus subtilis*) to detrimental effects such as reduced feed efficiency by pathogenic microorganisms (*Klebsiella pneumoniae, Morganella morganii*). These negative effects may result to financial loss to the farmers due to poor feed conversion and reduced growth rate/weight gain, damage to body organs/tissues, organoleptic changes, poor quality of meat, and mortality (Ezekiel et al., 2012). This is in consonance with Szmigiel et al., (2021) who reported a significant decrease in microbial load when the broilers are fed with antimicrobial additive. This could be due to the presence of phytochemical compounds such as flavonoids, glycosides and alkaloids. The similarity in the values obtained for the growth performance indices (final weight, weight gain, feed intake, FCR) from both the antibiotic-treated groups and the radish-root-juice-treated groups could further reflect the growth – promoting potential as well as antibacterial effect of radish. This can be attributed to the phytochemicals present in radish (Bonacucina *etal.,* 2009). Fernandes et al*.,* (2013) opined that dietary supplementation with phytochemical-rich materials can enhance digestive secretions, increase nutrient uptake, stimulate blood circulation and exhibit antioxidant effects.

# Conclusion

Taking into account the results obtained so far, it can be concluded that radish root juice is potent in inhibiting colonization of the gut of poultry by pathogenic bacteria, reducing the microbial load and competing favorably with conventional antibiotics as a substitute growth promoter to increase the productivity of broiler chickens, which in fact is the ultimate goal in poultry farming i.e. reducing disease outbreak and increasing body weight gain. It is also important to note that this dietary supplement is natural hence, there is no concern about its residual effect both on the broilers as well as on man (via biomagnification).

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