# Impact of dietary supplementation of *Rhamnus prinoides* leaf extract on the growth performance, nutrient retention and intestinal microbial count of “japanese quails”

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**Abstract**

This research was carried out to investigate the impact of dietary supplementation of *Rhamnus prinoides* leaf extract (RPL) on the growth performance, nutrient retention and intestinal microbial count of “japanese quails”. 300-2 weeks old “japanese quails” were allocated sixty birds per group and group treatment had four replicates (15 quails per replicate). Corn-soya meal (basal diet) was formulated according to the nutritional needs of birds (NRC, 1994). Quails in group 1(G1) was fed basal diet with no *R. prinoides* leaf extract, G2, G3, G4 and G5 were fed basal diet with 0.2 mL, 0.4 mL, 0.6 mL and 0.8 mL respectively. Result on the bioactive components in RPL revealed the presence of 15 bioactive compounds with pharmacological properties. Outcome on average daily weight gain showed that G3 (3.20 g), G4 (3.23 g) and G5 (3.24 g) were higher ($P < 0.05$) relative to G2 (3.06 g) and G1 (2.21 g). These groups also had a better feed conversion ratio (G3, G4 and G5). Average daily feed intake which varied from 703.81-708.33 g were not significantly ($P > 0.05$) different among the group. Mortality was recorded only among quails in group 1 (2.0%) ($P < 0.05$). Dry matter, crude protein, ether extract and nitrogen free extracts which varied from 70.12-86.57%, 58.70-68.91% and 50.62-66.05% were higher ($P < 0.05$) in G3, G4 and G5, intermediate in G2 and lowest in G1. Conversely, crude fibre values were higher in G1 relative to the other groups. Population of *Escherichia coli*, *Salmonella* sp. and *Staphylococcus* sp. count in the gut were lower in G2, G3, G4 and G5 relative to G1 while *Lactobacillus* sp. count were lowest in G1 ($P < 0.05$). It was concluded that RPL can be feed to quails up to 0.8 mL/L without causing any negative effect on the growth performance and health status of birds.

**Keywords:** *Rhamnus prinoides*, phytochemicals, performance, microbial count, quails.

# Impacto da suplementação dietética com extrato de folhas de *Rhamnus prinoides* no desempenho de crescimento, retenção de nutrientes e contagem microbiana intestinal de “codornas japonesas”

**Resumo**

Esta pesquisa foi realizada para investigar o impacto da suplementação dietética de extrato de folhas de *Rhamnus prinoides* (RPL) no desempenho de crescimento, retenção de nutrientes e contagem microbiana intestinal de “codornas japonesas”. Para “codornas japonesas” com 300-2 semanas de idade foram alocadas sessenta aves por grupo e o tratamento em grupo teve quatro repetições (15 codornas por repetição). O farelo de milho e soja (dieta basal) foi formulado de acordo com as necessidades nutricionais das aves (NRC, 1994). As codornas do grupo 1 (G1) foram alimentadas com ração basal sem extrato de folhas de *R. prinoides*, G2, G3, G4 e G5 foram alimentadas com ração basal com 0,2 mL, 0,4 mL, 0,6 mL e 0,8 mL respectivamente. O resultado dos componentes bioativos no RPL revelou a presença de 15 compostos bioativos com propriedades farmacológicas. O resultado do ganho de peso médio diário mostrou que G3 (3,20 g), G4 (3,23 g) e G5 (3,24 g) foram maiores ($P < 0,05$) em relação ao G2 (3,06 g) e G1 (2,21 g). Esses grupos também apresentaram melhor conversão alimentar (G3, G4 e G5). O consumo médio diário de ração que variou de 703,81 a 708,33 g não foi significativamente ($P > 0,05$) diferente entre os grupos. A mortalidade foi registrada apenas entre as codornas do grupo 1 (2,0%) (*P*

< 0,05). Matéria seca, proteína bruta, extrato etéreo e extratos isentos de nitrogênio que variaram de 70,12-86,57%, 58,70-68,91% e 50,62-66,05% foram maiores ($P$ < 0,05) no G3, G4 e G5, intermediárias no G2 e menores no G1 . Por outro lado, os valores de fibra bruta foram maiores no G1 em relação aos demais grupos. A população de *Escherichia coli*, *Salmonella* sp. e *Staphylococcus* sp. no intestino foi menor em G2, G3, G4 e G5 em relação ao G1, enquanto a contagem de *Lactobacillus* sp. foi menor em G1 ($P$ < 0,05). Concluiu-se que o RPL pode ser fornecido para codornas até 0,8 mL/L sem causar qualquer efeito negativo no desempenho de crescimento e no estado de saúde das aves.

**Palavras-chave:** *Rhamnus prinoides*, fitoquímicos, desempenho, contagem microbiana, codornas.

## 1. Introduction

Public concern about potential antibiotic resistance threats to human health has sparked research in poultry nutrition and the implementation of antibiotic-free feeding systems. This has resulted in the emergence of feed additives that can be used as in-feed antibiotic alternatives in quail feeding plans (Caroline et al., 2021; Musa et al., 2021). The use of medicinal plants has been proposed as one response to the increasing incidence of antibiotic resistance in animals (Alagbe, 2020).

They also provide nutrients and biological compounds that are necessary for disease treatment (Berhanu, 2014; Asmare et al., 2018). Medicinal plants also include a diverse spectrum of bioactive chemicals or phyto-constituents that have antioxidant, antiviral, antibacterial, anti-inflammatory, hepatoprotective, and immune-stimulatory properties, among other things (Alagbe, 2019; Shittu; Alagbe, 2020).

Rhamnus prinoides are among the widely used medicinal plant with several therapeutic properties (Abebe et al., 2003; Alagbe et al., 2020). The plant belongs to the family Rhamanceae which is made up of about 150 species and are widely distributed in East Africa, Central Africa and some parts of Asia including India (Megersa et al., 2013). *Rhamnus prinoides* contains a variety of secondary metabolites including tannins, flavonoids, alkaloids, saponins, steroids, phenols and anthraquinones which have multiple pharmacological properties (Amabye, 2015; Bosire, 2003).

Traditionally, the extracts from the leaf of *R. prinoides* is used for the treatment of malaria, rheumatism, gastrointestinal disease, body pain, constipation, cold, tooth ache, sexually transmitted disease, brucellosis amongst others (Berhanu, 2014; Bitew et al., 2019). Root infusion can be used to treat cough, respiratory infection, stomach discomfort, joint pain and sore throat (Dzoyem et al., 2016).

The pharmacological screening of the leaf extract has revealed its anti-malarial, anti-hyperglycemic, anti-inflammatory, anti-rheumatic and antipyretic activities (Molla et al., 2016). Its anti-diuretic and anti-bacterial (Pillai et al., 2019), anticonvulsant (Chen et al., 2020), antidiarrheal (Campbell et al., 2020) activities have also been reported. Additionally, its anti-estrogenic activities (Meregi et al., 2007) as well as it antioxidant activities have been documented. It has also been reported that these extracts can inhibit the growth of pathogenic organisms such as: *Streptococcus pneumonia*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Plasmodium falciparum*, *Mycobacterium smegmatim*, *Escherichia coli* and *Mycobacterium aurum* (Gebru, 2010; Giday et al., 2010).

Previous research has shown that plant extract can improve bird growth performance, nutrient digestibility, microbial population in the gastrointestinal tract, feed palatability, fatty acid composition, and blood parameters (Musa et al., 2020; Adewale et al., 2021; Agubosi et al., 2022). However, the majority of the results obtained were inconsistent, implying that processing or extraction methods, species, geographical region, plant age, and dosage supplied may all influence the outcome of the studies (Shittu; Alagbe, 2020). There is relatively limited evidence on the utilization of *R. prinoides* leaf extract with quails.

Given *R. prinoides* vast potential, there is a need to assess its efficacy and determine its tolerable level in birds. This will further contribute to increased poultry output and food security. As a result, the purpose of this study was to see how dietary supplementation with *Rhamnus prinoides* leaf extract affected “japanese quail” growth performance, nutrient retention, and intestine bacteria count.

## 2. Materials and Methods

### *2.1 Experimental location, ethical approval and preparation of Rhamnus prinoides leaf extract*

The study was conducted at the Sumitra Institute's Livestock unit, which is located between 23o 13' N and 72o 41' E and it was conducted in accordance with the guidelines and requirements of procedures that had been

authorized by the research ethics council of India's Sumitra Research Institute (AA/HJ/008C).

*Rhamnus prinoides* leaves were harvested from Lakadiya village, located in Bhachau, Takula of Kachchh district, Gujarat, India and sent to the department of Crop Protection where it was identified and authenticated before it was air dried for 11 days until a constant weight was achieved. Thereafter, it was powdered using an electric blender. 200 grams of *R. prinoides* powder was immersed into 1000 mL of ethanol in a conical flask for 48 h.

The mixture was stirred intermittently every 5 h and filtered into a container to obtain *R. prinoides* extract. *Rhamnus prinoides* extract was evaporated to dryness using a vacuum distillation unit before it was sent to the institute's laboratory for gas chromatography and mass spectrometry (GC-MS) analysis. Gas chromatography and mass spectrometry analysis of *R. prinoides* extract carried out using UV-*Vis* 230 UV-visible spectrophotometer which has an instant helium saver module which allows for an automatic reduction in Helium flow to enable sample transfer to the column. 10 mL of *R. prinoides* extract is injected to the inlet of the machine and to ensure accuracy the kit is adjusted at a wavelength of 190-1100 nm, wavelength accuracy and reproducibility ± 2.0, 1.0 nm, monochromator (Single, C-T, 1200 L/mm$^{-1}$) and photometric accuracy (± 0.5% T).

*2.2 Animal management, experimental diet and design*

In a trial carried out at Sumitra Research Institute in India, 300-2 weeks old "apanese quails" were allocated sixty birds per treatment and each treatment had four replicates (15 quails per replicate). On arrival, quails were unboxed, and stratification of their weights was taken into consideration before they were randomly distributed into a specially constructed all wired battery cage placed in a semi sided pen.

The cages measuring 80 cm by 60 cm by 40 cm (length, breath and height) disinfected with Aquaclean® at the rate 5 mL to 5 L of water two weeks before the arrival of birds. Cages were equipped with automatic feeders and drinkers and 100-watt bulb was used to supply heat to birds. A mixture of Vitamix® and glucose at 15 g to 10 L of water was given for 5 days and experimental diet (basal) were adequate in all nutrients according to the recommendation of NRC (1994). A completely randomized design was adopted, and birds were given unrestricted access to feed and clean water. The experiment lasted for forty-nine days under strict daily and routine management practices. Feed intake, daily gain and feed conversion ratio were measured.

2.2.1 Calculations

1 Final body weight – initial body weight.

2 Weight gain /number of experimental period.

3 Feed served – left over.

4 Total feed intake/number of experimental period.

5 Average daily feed intake/average daily weight gain.

$$\text{Nutrient Digestibility} = \text{Nutrient in feed} - \text{Nutrient in droppings}/\text{Nutrient in feed} \times 100$$

*2.3 Experimental set-up*

A basal diet containing Corn-soya meal was formulated and the treatment was set-up as follows:

Group 1: A corn-soya meal (basal diet) without *R. prinoides* extract; group 2: basal diet with 0.2 mL/L *R. prinoides* extract; group 3: basal diet with 0.4 mL/L *R. prinoides* extract; group 4: basal diet with 0.6 mL/L *R. prinoides* extract and group 5: basal diet with 0.8 mL/L *R. prinoides* extract.

*2.4 Nutrient retention trial (NRT)*

Digestibility retention trial (NRT) was carried out at the end of the experiment. Four birds were selected from each of the replicate, weighed and transferred to a labeled battery cage. Birds were given two days as acclimatization period while five days were used for data collection. Total droppings voided from each replicate were weighed and recorded. Wet droppings were oven dried at 70 ºC for 24 h to determine the dry matter content. Record on daily feed intake was recorded and droppings from the same treatment were thoroughly bulked together and taken to the laboratory for analysis. Proximate analysis of droppings was carried out using near infra- red automated kit (NIR -7000, USA) which uses SensorVu windows® based PC software allowing analyst

to insert set up parameters, perform or adjust calibrator and examine diagnostic values.

*2.5 Intestinal microbial examination*

At the end of the experiment (49th day), four birds were selected from each of the replicate for intestinal microbial examination. Birds were slaughtered very early in the morning and their intestinal content was collected into a sterile sample bottle and transferred immediately to the department of microbiology, Sumitra Research Institute, India. Microbial analysis was carried out using Quantom TxTM (Model Q10002, South Korea) microbial cell counter equipped with automated fluorescence imaging for accurate and objective bacterial cell counts. For accuracy in the results sample volume is adjusted at loading volumes: 5-6 µL, measuring volume: 0.09 µL (10 images).

*2.6 Statistical analysis*

Data obtained from the experiment was subjected to analysis of variance (ANOVA) using the computer software package (SPSS version 25.0) differences among treatment means was compared with *Duncan's* multiple range test (Duncan, 1995).

**3. Results and Discussion**

Table 1 shows the nutritional components and their dietary values in bird feed.

Table 1. Ingredients and chemical composition of experimental diets.

| **Materials** | **Quantity (kilogram)** |
|---|---|
| Maize | 50.40 |
| Wheat bran | 2.00 |
| Soybean meal | 37.05 |
| Fish meal (72 percent) | 3.00 |
| Oyster shell | 2.00 |
| Bone meal | 4.00 |
| Lysine | 0.25 |
| Methionine | 0.25 |
| Vit/Min premix* | 0.25 |
| Salt | 0.35 |
| Total | 100.00 |
| **Determined analysis (%)** | |
| Crude protein | 24.11 |
| Crude fibre | 3.58 |
| Ether extract | 4.26 |
| Calcium | 1.02 |
| Phosphorus | 0.66 |
| Metabolizable energy (Kj/kg$^{-1}$) | 1300.5 |

Note: **Vitamin premix contains: Vtamin A 5,000 I.U; vitamin E 20.0 mg; vitamin D 3,500 I.U, vitamin K 8.00 mg; vitamin B2 5.0 mg; Niacin 80 mg; vitamin B12 21 mg per 2.5 kg. Author, 2023.

As presented in Table 2, bioactive profiling of Rhamnus prinoides leaf extract by GC-MS revealed the presence of 15 chemical compounds with their retention time. The compounds found in *R. prinoides* leaf extract are: β-sorigenin 28%, 3-*O*-Methylquercetin 16%, rhamnocitrin 8%, phytol 7%, emodinanthrone 6%, 3,4 dimethyl 5

hexen-3-ol 5%, 2-myristynoyl pantetheine 4%, palmitoleic acid 4%, Octadecanoic acid 3%, Dodecanoic acid 2%, Quercetin 2%, 3-Butyn-2-ol 1%, Butanic acid 1%, Chrysophanol 0.9%, 4-Allyl-1,2- diacetoxybenzene 0.6% and Allylipo nitrite 0.1% in their order of abundance.

These compounds have several therapeutic or pharmacological properties. For instance, β-sorigenin also found in stem bark of *Annona senegalensis* and leaves of *Cinddoscolus aconitifoliuus*, *Cypraea arabica* exhibits anti-inflammatory and antioxidant properties (Awa et al., 2012; Subavathy et al., 2015). 3-*O*-methylquercetin is present in *Euphorbia grantii* is reported to have antiviral and antioxidant activity (Olajuyige et al., 2011; Mangrove et al., 2014). Chrysophanol also present in *Melia azedarach* has anticancer, antiviral, antiprotozoal, hypolipidemic, hepatoprotective, neuroprotective, antiulcer effects (Sen; Batra, 2012; Nester et al., 2002).

Quercetin are group of flavonoids that possess antioxidant, anti-inflammatory, anti-cancer and hypolipidemic properties (Singh et al., 2022; Alagbe et al., 2024). Rhamnocitrin has antiatherogenic, antioxidant, neuroprotective, antibacterial, anti-inflammatory potential (Bazie et al., 2014; Alagbe; Ushie, 2020). Octadecanoic acid, 3-Butyn-2-ol, dodecanoic acid, palmitoleic acid, butanic acid and 3,4 dimethyl 5 hexen 3-ol, phyto-constituents found in *Prosopis africana*, *Argemone mexicana* and *Ocimum santum* leaves (Namkeleja et al., 2014; Devendran; Balasubramanian, 2011; Doughari et al., 2021).

Phytol has the potential to prevent against different stages arthritis and scavenge against the activities of free radicals (Ogunlesi et al., 2009). It also possesses antimicrobial, anti-inflammatory, antioxidant activity and are found to be present in leaf and stem extract of *Moringa concanensis* (Vadivel et al., 2015). 4-Allyl-1,2-diacetoxybenzene and Allylipo nitrite and emodinanthrone also exhibits antimicrobial, antidiarrheal and antifungal effects (Doughari et al., 2021). The result obtained in this study is in agreement with the findings of Gashew et al. (2021).

Table 2. Bioactive profiling of *Rhamnus prinoides* extract by GC-MS.

| **Compounds** | **% area** | **Molecular formula** | **Molecular weight (g/moL$^{1-}$)** | **Retention time (min$^{-1}$)** |
|---|---|---|---|---|
| Dodecanoic acid | 2.86 | $C_{12}H_{24}O_2$ | 200.31 | 6.12 |
| 2-Myristynoyl pantetheine | 4.33 | $C_{11}H_{22}N_2O_4S$ | 278.36 | 6.98 |
| Phytol | 7.05 | $C_{20}H_{40}O$ | 296.53 | 7.11 |
| Octadecanoic acid | 3.05 | $C_{18}H_{34}O_3$ | 298.46 | 7.60 |
| 4-Allyl-1,2- diacetoxybenzene | 0.60 | $C_{13}H_{14}O_4$ | 234.25 | 10.22 |
| Palmitoleic acid | 4.18 | $C_{16}H_{30}O_2$ | 54.40 | 10.55 |
| Rhamnocitrin | 8.01 | $C_{16}H_{12}O_6$ | 300.26 | 13.40 |
| β-sorigenin | 28.49 | $C_{18}H_{18}O_9$ | 378.09 | 13.86 |
| Emodinanthrone | 6.02 | $C_{15}H_{12}O_4$ | 256.25 | 13.90 |
| 3-*O*-Methylquercetin | 16.48 | $C_{16}H_{12}O_7$ | 316.26 | 17.82 |
| Quercetin | 2.30 | $C_{15}H_{10}O_7$ | 302.23 | 18.08 |
| Chrysophanol | 0.92 | $C_{15}H_{10}O_4$ | 254.24 | 18.64 |
| 3-Butyn-2-ol | 1.77 | $C_4H_6O$ | 70 | 18.90 |
| 3,4 dimethyl 5 hexen 3-ol | 5.08 | $C_8H_{16}O$ | 128 | 20.90 |
| Butanic acid | 1.49 | $C_6H_{12}O_3$ | 132 | 21.22 |
| Allylipo nitrite | 0.17 | $C_6H_{10}N_2O_2$ | 142 | 21.80 |
| **Total** | **92.80** | | | |

Note: (% area) = Expressed as a percentage of relative area. Source: Author, 2023.

Growth performance characteristics of "japanese quails" fed *R. prinoides* leaf extract (Table 3) shows that weight gain and average daily weight gain which ranged from (108.52-158.70 g) and (2.21-3.24 g) in birds fed 0.4 mL/L *R. prinoides* extract (group 3) and 0.6 mL/L *R. prinoides* extract (group 4) were similar ($P > 0.05$) to

those given 0.8 mL/L *R. prinoides* extract (group 5) but significantly higher ($P < 0.05$) than those fed 0 mL (group 1: G1) and 0.2 mL/L *R. prinoides* extract (group 2). The result demonstrates that feeding quails *R. prinoides* extract at 0.4 mL, 0.6 mL and 0.8 mL/L influenced the activities of endogenous enzymes thereby improving the digestion and absorption of nutrient for metabolism.

The presence of bioactive compounds in *R. prinoides* extract as presented in (Table 2) can exert a growth promoting effect on birds. It is clear that at 0.4 mL, 0.6 mL and 0.6 mL more nutrients are up taken and metabolized and then improved body weight was recorded relative to the other groups. According to Sandra (2020), phytogenic feed additives are known for their beneficial effects on animals, from flavouring and sensorial stimulation, antimicrobial, anti-inflammatory, antioxidant properties amongst others. The result obtained on weight gain agrees with the report of Shittu et al. (2022) when *Sida acuta* leaf extract was fed to 8 mL/L. Similar observation was made by Alagbe & Ushie (2022) who reported a significant ($P < 0.05$) difference in average daily weights of broilers fed *Citrus aurantium* stem bark extracts at 4 mL/L. Average daily feed intake which varied from 703.06-708.81 g were not influenced ($P > 0.05$) by the treatment.

Conversely, feed conversion ratio and mortality rate were significantly ($P < 0.05$) different among the groups. The result demonstrates that feeding quails up to 0.6 mL/L *R. prinoides* extract did not improve the palatability of the feed. Part of the explanation could be the presence of β-sorigenin which is responsible for the bitter taste in *R. prinoides* leaf (Gashew et al., 2021). Improved feed conversion ratio and no mortality was recorded among quails fed 0.4 mL, 0.6 mL and 0.8 mL/L *R. prinoides* extract.

This could be as a result of safe gastrointestinal tract morphology. The result obtained agrees with the findings of Alagbe et al. (2022) when Rubia cordifolia root extracts was fed to growing rabbits. Conversely, Alabi et al. (2017) reported that *M. oleifera* leaf extracts influenced the feed intake of Hubbard broiler chicken. This variation in result can be attributed to difference in phyto-constituents or bioactive compounds, dosage feed to birds, specie of plant, extraction techniques amongst others (Alagbe, 2021).

Table 3. Growth performance characteristics of "japanese quails" fed *Rhamnus prinoides* leaf extract.

| **Indices** | **[6]G1** | **[7]G2** | **[8]G3** | **[9]G4** | **[10]G5** | **[11]SEM** |
|---|---|---|---|---|---|---|
| Initial body weight (g) | 42.88 | 42.06 | 42.14 | 42.01 | 42.10 | 0.01 |
| Final body weight (g) | 151.4[c] | 192.11[b] | 198.77[a] | 200.2[a] | 200.8[a] | 8.10 |
| [1]Weight gain (g) | 108.52[c] | 150.05[b] | 156.63[a] | 158.19[a] | 158.7[a] | 7.33 |
| [2]Average daily weight gain (g) | 2.21[c] | 3.06[b] | 3.20[a] | 3.23[a] | 3.24[a] | 0.01 |
| [3]Total feed intake (g) | 703.81 | 706.12 | 708.06 | 708.22 | 708.3 | 16.82 |
| [4]Average daily feed intake (g) | 14.36 | 14.41 | 14.45 | 14.45 | 14.46 | 0.05 |
| [5]FCR | 6.48[a] | 4.70[b] | 4.49[c] | 4.47[c] | 4.46[c] | 0.02 |
| Mortality (%) | 2.00[a] | - | - | - | - | 0.01 |

Note: Means within a row with different letters are significantly different ($P < 0.05$); [5]feed conversion ratio; [6]Corn soya bean meal (basal diet) without *Rhamnus prinoides* extract (Control); [7]basal diet supplemented with 0.2mL/L *Rhamnus prinoides* extract; [8]basal diet supplemented with 0.4mL/L *Rhamnus prinoides* extract; [9]basal diet supplemented with 0.6mL/L *Rhamnus prinoides* extract; [10]basal diet supplemented with 0.8mL/L *Rhamnus prinoides* extract; [11]standard error of mean. Source: Author, 2023.

Nutrient retention indices of "japanese quails" fed *R. prinoides* leaf extract in (Table 4) revealed that dry matter, crude protein, ether extract and nitrogen free extract values which varied from 70-86%, 58-68%, 38-46% and 50-66%. Quails fed 0.4 mL/L *R. prinoides* leaf extract (group 3); 0.6 mL/L *R. prinoides* leaf extract (group 4) were similar ($P < 0.05$) to those fed 0.8 mL/L *R. prinoides* leaf extract (group 5) but significantly higher ($P > 0.05$) than those in other groups.

Crude fibre values in quails fed 0.2 mL/L *R. prinoides* leaf extract (group 2) and 0.4 mL/L *R. prinoides* leaf extract (group 3) were similar ($P < 0.05$) to those fed 0.6 mL/L *R. prinoides* leaf extract (group 4) and 0.8 mL/L *R. prinoides* leaf extract (group 5) but significantly ($P > 0.05$) lower than those in fed 0 mL (group 1). The result obtained demonstrates that *R. prinoides* leaf extract has the potential to improve nutrient absorption and secretion of digestive juices in the gut of quails.

Higher dry matter recorded in G3, G4 and G5 suggests a significant improvement in the nutrient permeability of the gut wall of birds which is made possible as a result of bioactive compounds or phyto-constituents in *R. prinoides* extract. Result obtained in this study is in agreement with the reports of Alagbe et al. (2022) when *Juniperus thurifera* root extract was fed rabbits. Similar outcome was recorded by Esonu et al. (2002) when *Microdesmis puberula* leaf meal was supplemented in the diet of broiler chickens. However, Oloruntola et al. (2016), reported a non-significant ($P > 0.05$) difference in crude fibre and ether extract digestibility of broilers fed diet diets supplemented with *Gliricidia sepium* leaf meal.

Table 4. Nutrient retention indices of "japanese quails" fed *Rhamnus prinoides* leaf extract.

| **Indices (%)** | **[1]G1** | **[2]G2** | **[3]G3** | **[4]G4** | **[5]G5** | **[6]SEM** |
|---|---|---|---|---|---|---|
| Dry matter | 70.12[c] | 78.89[b] | 85.08[a] | 86.22[a] | 86.57[a] | 0.83 |
| Crude protein | 58.70[c] | 61.25[a] | 68.01[a] | 68.55[a] | 68.91[a] | 0.66 |
| Crude fibre | 35.77[a] | 30.86[b] | 30.35[b] | 30.20[b] | 30.17[b] | 0.22 |
| Ether extract | 38.30[a] | 40.98[a] | 46.27[a] | 46.51[a] | 46.42[a] | 0.25 |
| [7]NFE | 50.62[a] | 59.12[a] | 65.11[a] | 65.42[a] | 66.05[a] | 0.68 |

Note: Means within a row with different letters are significantly different ($P < 0.05$); [1]Corn soya bean meal (basal diet) without Rhamnus prinoides extract (Control); [2]basal diet supplemented with 0.2mL/L *Rhamnus prinoides* extract; [3]basal diet supplemented with 0.4mL/L *Rhamnus prinoides* extract; [4]basal diet supplemented with 0.6mL/L *Rhamnus prinoides* extract; [5]basal diet supplemented with 0.8mL/L *Rhamnus prinoides* extract; [6]standard error of mean and [7]Nitrogen free extracts. Source: Author, 2023.

As presented in Table 5, intestinal microbial population of "japanese quails" fed *R. prinoides* leaf extract. *Escherichia coli* counts varied from 1-3 CFU/g$^{-1}$, *Staphyllococus* sp. 1-2 CFU/g$^{-1}$, *Salmonella* sp. 2-4 CFU/g$^{-1}$ and *Lactobacillus* sp. 3-5 CFU/g$^{-1}$. *Escherichia coli*, *Salmonella* sp. and *Staphylococcus* sp. of quails fed 0.2 mL/L *R. prinoides* leaf extract (group 2) and 0.4 mL/L *R. prinoides* leaf extract were similar ($P > 0.05$) to those fed group 4 (0.6 mL/L *R. prinoides* leaf extract) and 0.8 mL/L *R. prinoides* leaf extract (group 5) but significantly ($P < 0.05$) lower than those in group 1.

Conversely, *Lactobacillus* sp. count was higher ($P < 0.05$) in group 2, 3, 4 and 5 relatives to those fed in group 1. According to Kogut & Arsenault (2016), a healthy gut is defined as the absence, prevention and/or avoidance of disease so that the animal is able to perform its physiological functions in order to withstand exogenous and endogenous stressors. The balance of the gut microflora plays an essential role in the health of the animal. Imbalances in the gut microflora will lead to mortality and decreased performance in general (Gwendolyn, 2020).

The result demonstrates that *R. prinoides* leaf extract can interfere with the colonization of harmful microbial species like *E. coli*, *Salmonella* sp. and *Staphylococcus* sp. This may be due to its content of bioactive compounds (Table 2) decreasing the number of available binding sites for pathogenic organisms. *Lactobacillus* sp. is a beneficial bacterium capable of stimulating the gut epithelial cells, reduction of pH, production of antimicrobial agents, inhibiting the growth of potential pathogen, stimulating gut immune functions which aid the absorption of nutrients and synthesize vitamins of the B group (Gwendolyn, 2020). Result obtained is in agreement with the reports of Alagbe et al. (2023) when *P. africana* oil was fed to birds at 800 mg/kg$^{-1}$. According to Goliomytis et al. (2014), dietary supplementation of quercetin in birds prevented dysbiosis in their gastro intestinal tract.

Table 5. Intestinal microbial population of "japanese quails" fed *Rhamnus prinoides* leaf extract.

| Indices (CFU/$g^{-1}$) | [1]G1 | [2]G2 | [3]G3 | [4]G4 | [5]G5 | [6]SEM |
|---|---|---|---|---|---|---|
| *Escherichia coli* | 3.92[a] | 2.05[b] | 2.00[b] | 1.96[b] | 1.90[b] | 0.05 |
| *Salmonella sp.* | 4.71[a] | 3.00[b] | 2.86[b] | 2.80[b] | 2.55[b] | 0.17 |
| *Staphylococcus sp.* | 2.62[a] | 1.71[b] | 1.63[b] | 1.59[b] | 1.53[b] | 0.06 |
| *Lactobacillus sp.* | 3.88[b] | 5.06[a] | 5.11[a] | 5.19[a] | 5.22[a] | 0.21 |

Note: Means within a row with different letters are significantly different ($P < 0.05$); [1]Corn soya bean meal (basal diet) without *Rhamnus prinoides* extract (Control); [2]basal diet supplemented with 0.2mL/L *Rhamnus prinoides* extract; [3]basal diet supplemented with 0.4mL/L *Rhamnus prinoides* extract; [4]basal diet supplemented with 0.6mL/L *Rhamnus prinoides* extract; [5]basal diet supplemented with 0.8mL/L *Rhamnus prinoides* extract, and [6]standard error of mean. Source: Author, 2023.

## 4. Conclusions

In conclusion, *Rhamnus prinoides* leaf extract contains several bioactive compounds with pharmacological properties (anti-helminthic, anti-cancer, anti-malarial, anti-hyperglycemic, anti-inflammatory, anti-rheumatic, immune-stimulatory and anti-pyretic activities amongst others). Feeding quails up to 0.8 mL/L with *R. prinoides* leaf extract has the potential to improve the growth performance, secretion of digestive enzymes, nutrient absorption, increase gut permeability as well as modulating the gastro intestinal tract thereby preventing dysbiosis. Supplementation up to 0.8 mL/L did not negatively affect the performance of quails and this can confer *Rhamnus prinoides* leaf extract as a natural alternative to antibiotics.

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## 6. Authors' Contributions

*John Olujimi Alagbe*: study design, plant collection, sample preparation, laboratory analysis, animal testing, data analysis, study writing, corrections, submission and publication.

## 7. Conflicts of Interest

No conflicts of interest.

## 8. Ethics Approval

Yes applicable. Conducted in accordance with the guidelines and requirements of procedures that had been authorized by the research ethics council of India's Sumitra Research Institute (AA/HJ/008C).

## 9. References

Abebe, D., Debella, A., & Urga, K. (2003). Medicinal plants and other useful plants of Ethiopia. *Bulletin of the Chemical Society of Ethiopia*, 2(1), 15-20.

Agubosi, O. C. P., Alexander, J., & Alagbe, J. O. (2022). Influence of dietary inclusion of Sunflower (*Helianthus annus*) oil on growth performance and oxidative status of broiler chicks. *Central Asian Journal of Medical and Natural Sciences*, 2(7), 187-195. https://ajird.journalspark.org/index.php/ajird/article/view/7

Alabi, O. J., Malik, A. D., Ng'ambi, J. W., Obaje, P., & Ojo, B. K. (2017). Effect of aqueous *Moringa oleifera* leaf extracts on the growth performance and carcass characteristics of hubbard broiler chickens. *Brazilian Journal of Poultry Science*, 19(2), 273-280. https://doi.org/10.1590/1806-9061-2016-0373

Alagbe, J. O (2019). Proximate, mineral and phytochemical analysis of *Piliostigma thonningii* stems bark and roots. *International Journal of Biological, Physical and Chemical Studies*, 1(1), 1-7. https://www.al-kindipublisher.com/index.php/ijbpcs/article/view/366

Alagbe, J. O. (2019). Growth response and bacteria count of broiler starter given *Delonix regia* leaf extract as a natural alternative to antibiotics. *Food and Nutrition: Current Research*, 2(3), 197-203.

Alagbe, J. O (2020). Proximate, phytochemical and vitamin compositions of *Prosopis africana* stem bark. *Journal of Multidimensional Research & Review*, 1(4), 01-12. https://jmrr.org/V1I4/V1I4P01.pdf

Alagbe, J. O., Shittu, M. D., & Ojo, E. A. (2020). Prospect of leaf extracts on the performance and blood profile of monogastric - A review. *International Journal of Integrated Education*, 3(7), 122-127.

Alagbe, J. O., Shittu, M. D., & Ushie, F. T. (2021). GC-MS analysis of methanolic stem bark extract of *Zollingeriana indigofera*. *Asian Journal of Advances in Research*, 4(1), 1265-1267. http://archives.articleproms.com/id/eprint/2064

Alagbe, J. O (2021). Dietary supplementation of *Rauvolfia vomitoria* root extract as a phytogenic feed additive in growing rabbit diets: Growth performance and caecal microbial population. *Concept in Dairy and Veterinary Sciences*, 4(2), 409-416. http://dx.doi.org/10.32474/CDVS.2021.04.000184

Alagbe, J. O., & Ushie, F. T. (2022). Growth performance of broiler chicks fed diets containing different levels of aqueous *Citrus aurantium* stem bark extracts. *Discovery*, 58(319), 735-741. https://www.discoveryjournals.org/discovery/current_issue/v58/n319/A7.pdf?

Alagbe, J. O., Zubairu, H., Adedeji, O. M., Bamigboye, S., & Dora, A. (2022). Influence of Juniperus thurifera root extract on the nutrient digestibility and caecal microbial count of growing rabbits. *Web of Synergy: International Interdisciplinary Research Journal*, 1(1), 5-17.

Alagbe, J. O., Shittu, M. D., Ramalan, S. N., Tanimomo, K. B., & Adekunle, D. A. (2022). Growth performance, semen quality characteristics and hormonal profile of male rabbit bucks fed Rubia cordifolia root extracts. *International Journal of Biological Engineering and Agriculture*, 1(1), 1-13. https://journal.jfpublisher.com/index.php/jpb/article/view/213

Alagbe, J. O (2023). Investigating the effects of dietary supplementation *Eucalyptus camaldulensis* of essential oil on the growth performance, nutrient digestibility and caecal fermentation of weaned rabbits. *Research in: Agricultural and Veterinary Sciences*, 7(3), 139-148.

Alagbe, J. O (2023). Investigating the effects of dietary supplementation of *Eucalyptus camaldulensis* essential oil on haemato-biochemical indices, immune response and oxidative stress of weaned rabbits. *Matrix Science Pharma*, 7(3), 103-108. DOI: 10.4103/mtsp.mtsp_17_23

Alagbe, O. J. (2023). Impact of dietary supplementation of *Carica papaya* essential oil on the blood chemistry of broiler chickens. *Food Science & Applied Microbiology Reports*, 2(2), 58-67. https://doi.org/10.61363/meqmwj94

Alagbe, O. J., Oluchi, C. P. A., Rufus, A. O., Taiwo, O. A., Adegoke, E. A., & Emiola, I. A. (2023). Haemato-biochemical indices and intestinal microbial population of broiler chickens fed diet supplemented with *Prosopis africana* (African mesquite) essential oil. *Brazilian Journal of Science*, 2(9), 98-110. https://doi.org/10.14295/bjs.v2i9.390

Alagbe, O. J. (2024). Novel phytogenics' impact on weaned pigs growth performance, haematology and serum biochemical indicators. *Black Sea Journal of Agriculture*, 7(2), 82-89. https://doi.org/10.47115/bsagriculture.1328386

Amabye, T. G. (2015). Evaluation of phytochemical, chemical composition, antioxidant and antimicrobial screening parameters of *Rhamnus prinoides* (Gesho) available in the market of Mekelle, Tigray, Ethiopia. *Natural Products Chemistry & Research*, 4(1), 1-6. DOI: 10.4172/2329-6836.1000198

Anorue, D. N., Ubong, F., & Alagbe, O. J. (2023). Investigating the effects of pawpaw (*Carica papaya*) essential oil dietary supplementation on the growth performance and carcass characteristics of broilers. *Research in: Agricultural and Veterinary Sciences*, 7(3), 164-174. http://jomardpublishing.com/UploadFiles/Files/journals/RV/V7N3/Anorue_et_al.pdf

Asmare, T., Yilkal, B., Mekuannint, T., & Yibeltal, A. (2018). Traditional medicinal plants used to treat maternal and child health illnesses in Ethiopia: An ethno-botanical approach. *Journal of Traditional Medicine and Clinical Naturopathy*, 7(3), 1-10. DOI: 10.4172/2573-4555.1000277

Awa, E. P., Ibrahim, S., & Ameh, D. A. (2012). GC/MS analysis and antimicrobial activity of dimethyl ether fraction of methanolic extract from stem bark of *Annona senegalensis* Pers. *International Journal of Phamaceutical Sciences and Research*, 3(11), 4213-4218.

https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=92702a6940db651ac2910826bd0355ee11dad4ce

Bazie, S., Ayalew, A., & Woldetsadik, K. (2014). Antifungal activity of some plant extracts against *Colletorichum musae* the cause of post-harvest banana Antracrose. *Journal of Plant Pathology and Microbiology*, 5(2), 1-4. http://dx.doi.org/10.4172/2157-7471.1000226

Berhanu, A. (2014). Microbial profile of Tella and the role of gesho (*Rhamnus prinoides*) as bittering and antimicrobial agent in traditional Tella (Beer) production. *International Food Research Journal*, 21(1), 357-365. http://ifrj.upm.edu.my/21%20(01)%202014/51%20IFRJ%2021%20(01)%202014%20%20Berhanu%20012.pdf

Bitew, H., Gebregergs, H., Tuem, K. B., & Yeshak, M. Y. (2019). Ethiopian medicinal plants traditionally used for wound treatment: a systematic review. *Ethiopian Journal of Health Development*, 33(2), 103-112. https://www.ajol.info/index.php/ejhd/article/view/188853

Bosire, K. O. (2003). An investigation of a traditional herbal therapy used to treat malaria in Kisii [MSc Thesis] University of Nairobi, Nairobi, Kenya. http://erepository.uonbi.ac.ke:8080/xmlui/handle/11295/24728

Caroline, G. V. (2022). Improving feed efficiency and gut integrity of *E. coli* challenged weaned pig's. *International Pig Magazine*, 36(3), 7-8. https://www.cabidigitallibrary.org/doi/full/10.5555/20220444753

Devendran, G., & Balasubramanian, U. (2011). Qualitative phytochemical screening and GC-MS analysis of *Ocimum santum* L. leaves. *Asian Journal of Plant Science and Research*, 1(4), 44-48. https://hal.science/hal-03699060/

Doughari, M., Hamuel, J., & Saa-Aondo, M. (2021). Phytochemical analysis of crude methanol extracts and antimicrobial activity of *n*-hexane fractions of methanol seed and pod extracts of *Prosopis africana* on some selected microoganisms. *Pharmacology Online*, 21(2), 121-137. https://pharmacologyonline.silae.it/files/archives/2021/vol2/PhOL_2021_2_A016_Doughari.pdf

Dzoyem, J. P., Aro, A. O., McGaw, L. J., & Eloff, J. N. (2016). Antimycobacterial activity against different pathogens and selectivity index of fourteen medicinal plants used in southern Africa to treat tuberculosis and respiratory ailments. *South African Journal of Botany*, 102, 70-74. https://doi.org/10.1016/j.sajb.2015.08.002

Esonu, B. O., Iheukwumere, F. C., Emenalom, O. O., & Etuk, B. B. (2002). Performance, nutrient utilization and organ characteristics of broilers fed *Microdemis puberula* leaf. *Livestock Research and Rural Development*, 14(6), 11-16. https://www.lrrd.cipav.org.co/lrrd14/6/eson146.htm

Gebru, M. (2010). Phytochemical and antiplasmodial investigation of Rhamnus prinoides and Kniphofia foliosa [MSc Thesis]. University of Nairobi, Nairobi, Kenya.

Giday, M., Asfaw, Z., & Woldu, Z. (2010). Ethnomedicinal study of plants used by Sheko ethnic group of Ethiopia. *Journal of Ethnopharmacology*, 132(1), 75-85. https://doi.org/10.1016/j.jep.2010.07.046

Goliomytis, M., Tsoureki, D., Simitzis, P. E., Charismiadou, M. A., Hager-Theodorides, A. L., & Deligeorgis, S. G. (2014). The effects of quercetin dietary supplementation on broiler growth performance, meat quality, and oxidative stability. *Poultry Science*, 93(8), 1957-1962. https://doi.org/10.3382/ps.2013-03585

Mangrove-Abayomi, O. E., Kenneth, E., & Mkaparu, K. I. (2014). Chemometric profiling of methanolic leaf extract of *Cinddoscolus aconitifoliuus* (Euphorbiaceae) using UV-VIS, FTIR and GC-MS techniques. *Peak Journal of Medicinal Plant Research*, 2(1), 6-12.

Megersa, M., Asfaw, Z., Kelbessa, E., Beyene, A., & Woldeab, B. (2013). An ethnobotanical study of medicinal plants in Wayu Tuka district, east Welega zone of oromia regional state, West Ethiopia. *Journal of Ethnobiology and Ethnomedicine*, 9(1), 1-18. https://doi.org/10.1186/1746-4269-9-68

Musa, B., Alagbe, J. O., Betty, A. M., & Omokore, E. A. (2020). Growth performance, caeca microbial population and immune response of broiler chicks fed aqueous extract of *Balanites aegyptiaca* and *Alchornea cordifolia* stem bark mixture. *United Journal for Research and Technology*, 1(11), 13-21. https://uijrt.com/articles/v1/i11/UIJRTV1I110002.pdf

Namkeleja, H. S., Tarimo, N. T. C., & Ndakidemi, P. A. (2014). Allelopathic effects of *Argemone mexicana* to growth of native plant species. *American Journal of Plant Sciences*, 5(9), 1336-1344. http://www.scirp.org/journal/PaperInformation.aspx?PaperID=44882

Nester, M. T., Annderson, D. G., Roberts, J. C. E., & Pearlsall, N. N. (2002). Microbiology - A human perspective genitourinary infections and antimicrobial medications. 3rd Ed. McGraw Hill. Madrid, 21-25; 496-664.

Nigussie, G., Alemu, M., Ibrahim, F., Werede, Y., Tegegn, M., Neway, S., & Endale, M. (2021). Phytochemicals, traditional uses and pharmacological activity of *Rhamnus prinoides*: A review. *International Journal of Secondary Metabolite*, 8(2), 136-151. https://www.cabidigitallibrary.org/doi/full/10.5555/20210371881

Oloruntola, O. D., Ayodele, S. O., Agbede, J. O., Oloruntola, D. A., Ogunsipe, M. H., & Omoniyi, I. S. (2016). Effect of *Alchornea cordifolia* leaf meal and enzyme supplementation on growth, haematological, immunostimulatory and serum biochemical response of rabbits. *Asian Journal of Biological and Life Sciences*, 5(2), 190-195.

Sen, A., & Batra, A. (2012). Evaluation of antimicrobial activity of different solvent extracts of medicinal plant: *Melia azedarach* L. *International Journal of Current Pharmaceutical Research*, 4(2), 67-73.

Sharma, S., Alagbe, O. J., Xing, L., Ram, S., & Amita, K. (2022). Comparative analysis of ethanolic *Juniperus thurifera* leaf, stem bark and root extract using gas chromatography and mass spectroemetry. *International Journal of Agriculture and Animal Production*, 2(6), 18-27. https://doi.org/10.55529/ijaap.26.18.27

Shittu, M. D., & Alagbe, J. O. (2020). Phyto-nutritional profiles of broom weed (*Sida acuta*) leaf extract. *Annals of Clinical Toxicology*, 3(2). https://pdfs.semanticscholar.org/4389/c3e04854f79f4a617939de0447197b556040.pdf

Shittu, M. D., Alagbe, J. O., Adejumo, D. O., Ademola, S. G., Abiola, A. O., Samson, B. O., & Ushie, F. T. (2021). Productive performance, caeca microbial population and immune-modulatory activity of broiler chicks fed different levels *Sida acuta* leaf extract in replacement of antibiotics. *Journal of Multidimensional Research & Review*, 2(4), 34-42. https://jmrr.org/V2I4/V2I4P05.pdf

Subavathy, P., & Thilaga, R. D. (2015). GC-MS analysis of bioactive compounds from whole body of methanolic extract of *Cypraea arabica*. *World Journal of Pharmaceutical Research*, 5(3), 800-806. https://wjpr.s3.ap-south-1.amazonaws.com/article_issue/1456804218.pdf

Vadivel, B., Veluchamy, B., & Arjunan, S. (2015). GC/MS analysis of leaf and bark extract of *Moringa concanensis* a siddha medicinal plant of South India. *European Journal of Biotechnology and Bioscience*, 3(12), 57-61.

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