

Effect on performance, serum biochemistry and haematological components of feeding “japanese quails” phytogenic feed additions comprising *Megaphrynium macrostachyum* leaves

John Olujimi Alagbe¹

¹ Department of Animal Nutrition and Biochemistry, Sumitra Research Institute, Gujarat, India

Correspondence: Alagbe Olujimi John, Department of Animal Nutrition and Biochemistry, Sumitra Research Institute, Gujarat, India. E-mail: dralagbe@outlook.com

Received: March 07, 2024

DOI: 10.14295/bjs.v3i5.571

Accepted: April 25, 2024

URL: <https://doi.org/10.14295/bjs.v3i5.571>

Abstract

This experiment was carried out to examine the effect on performance, serum biochemistry and haematological components of feeding quails phytogenic feed additions comprising *Megaphrynium macrostachyum* leaves. A total of 400-1 day old “japanese quails” were randomly distributed into five treatments and each treatment had four replicates (20 birds per replicate) in a completely randomized design. Basal diets were adequate in all nutrients and quails in treatment 1 was fed basal diet with no antibiotics, treatment 2 was fed basal diet supplemented with neomycin at 0.2 g/kg⁻¹ while treatment 3, 4 and 5 were fed basal diet supplemented with *M. macrostachyum* leaf meal (MML) at 2 g, 4 g and 6 g/kg⁻¹ respectively. The experiment lasted for 42 days, feed and fresh clean water were offered ad libitum. Experimental result showed that MML contained several phyto-constituents viz: tannins (318.62 mg/g⁻¹), terpenoids (620.11 mg/g⁻¹), flavonoids (1205.3 mg/g⁻¹), steroids (51.79 mg/g⁻¹), glycosides (42.55 mg/g⁻¹), alkaloids (200.8 mg/g⁻¹) and phenols (1402.4 mg/g⁻¹). Average daily weight gain was higher ($P < 0.05$) in treatment 4 (3.00 g/bird⁻¹) and 5 (3.02 g/bird⁻¹) relative to the other treatments. Similarly, average daily feed intake and feed conversion ratio were higher ($P < 0.05$) in treatment 4 and 5, intermediate in treatment 2 and lowest in treatment 1 and 2. Mortality was recorded only among birds in treatment 1 (2.56%) and 2 (1.16%) ($P < 0.05$). Red blood cell, white blood cell, haemoglobin, pack cell volume, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, lymphocytes and heterophil values were influenced ($P < 0.05$) by the treatments except for monocyte count ($P > 0.05$). Total protein, Creatinine, alanine phosphatase, aspartate transaminase and alanine transaminase were not significantly ($P > 0.05$) influenced by the diet. However, all values were within the established ranges for healthy quails. In conclusion, MML can be supplemented up to 6 g/kg⁻¹ in the diet of quails without causing any deleterious effect on the health status and performance of birds.

Keywords: *Megaphrynium macrostachyum*, japanese quails, performance, haematology, serum.

Efeito sobre o desempenho, bioquímica sérica e componentes hematológicos da alimentação de “codornas japonesas” com adições fitogênicas de rações contendo folhas de *Megaphrynium macrostachyum*

Resumo

Este experimento foi realizado para examinar o efeito no desempenho, na bioquímica sérica e nos componentes hematológicos da alimentação de codornas com adições fitogênicas de ração contendo folhas de *Megaphrynium macrostachyum*. Um total de 400 codornas japonesas de 400-1 dia de idade foram distribuídas aleatoriamente em cinco tratamentos e cada tratamento teve quatro repetições (20 aves por repetição) em um delineamento inteiramente casualizado. As dietas basais foram adequadas em todos os nutrientes e as codornas do tratamento 1 foram alimentadas com dieta basal sem antibióticos, o tratamento 2 foi alimentado com dieta basal suplementada com neomicina a 0,2 g/kg⁻¹ enquanto os tratamentos 3, 4 e 5 foram alimentados com dieta basal suplementada com folha de *Megaphrynium macrostachyum* refeição (MML) a 2 g, 4 g e 6 g/kg⁻¹ respectivamente. O experimento teve duração de 42 dias, sendo oferecidas ração e água limpa e fresca *ad libitum*. O resultado

experimental mostrou que o MML continha vários fitoconstituintes, a saber: taninos (318,62 mg/g⁻¹), terpenóides (620,11 mg/g⁻¹), flavonóides (1205,3 mg/g⁻¹), esteróides (51,79 mg/g⁻¹), glicosídeos (42,55 mg/g⁻¹), alcalóides (200,8 mg/g⁻¹) e fenóis (1402,4 mg/g⁻¹). O ganho de peso médio diário foi maior ($P < 0,05$) nos tratamentos 4 (3,00 g/ave⁻¹) e 5 (3,02 g/ave⁻¹) em relação aos demais tratamentos. Da mesma forma, o consumo médio diário de ração e a taxa de conversão alimentar foram maiores ($P < 0,05$) nos tratamentos 4 e 5, intermediários no tratamento 2 e menores nos tratamentos 1 e 2. A mortalidade foi registrada apenas entre as aves do tratamento 1 (2,56%) e 2 (1,16%) ($P < 0,05$). Glóbulos vermelhos, glóbulos brancos, hemoglobina, volume globular, volume corpuscular médio, hemoglobina corpuscular média, concentração média de hemoglobina corpuscular, linfócitos e valores de heterófilos foram influenciados ($P < 0,05$). Os tratamentos, exceto contagem de monócitos ($P > 0,05$), proteína total, creatinina, alanina fosfatase, aspartato transaminase e alanina transaminase não foram influenciados significativamente ($P > 0,05$) pela dieta. Concluindo, o MML pode ser suplementado em até 6 g/kg⁻¹ na dieta de codornas sem causar qualquer efeito deletério ao estado de saúde e ao desempenho das aves.

Palavras-chave: *Megaphrynium macrostachyum*, codornas japonesas, desempenho, hematologia, soro.

1. Introduction

The growing awareness that plant-based medications are safe, non-toxic, widely accessible, and reasonably priced has led to a surge in demand for herbal medicine throughout the world (Mith et al., 2014). Medicinal plants are intricate matrices that yield bioactive substances with various polarity and functional groups. Alkaloids, tannins, steroids, phenols, saponins, flavonoids, and terpenoids are among the phytochemical categories that are frequently encountered (Barba et al., 2015).

In addition to being novel medications, pharmaceuticals generated from medicinal plants can also be used as drug leads that medicinal and synthetic chemists can optimize (Balunas; Kinghorn, 2005). Herbs are extensively used in traditional medicine, and there is ample evidence of their therapeutic benefits (Nascimento et al., 2000).

In Ayurveda, a number of therapeutic herbs are frequently employed. *Megaphrynium macrostachyum*, a perennial semi-woody herb in the marantaceae family that is extensively distributed in west and central African rainforests as well as some regions of Asia, is one of the promising plants (Jennings et al., 2001). Folk medicine uses it extensively (Ibironke; Olusola, 2013; Ley; Claßen-Bockhoff, 2013; Meva et al., 2016). Leaf is often utilized as a roof-thatch and is papery. They are utilized as packaging material and to wrap items such as meat, kolanut, tapioca roots, and food (Jennings et al., 2000). Ayurvedic treatments for a range of illnesses, including blood cleaning, memory enhancement, diarrhea, internal pile, cough, chest, discomfort, waist pain, irregular menstruation, malaria, headache, and scars, employed the leaf and stem bark extract (Oyegade et al., 1999).

The plant extract's anti-helminthic, anti-cancer, anti-malarial, anti-hyperglycemic, anti-inflammatory, anti-rheumatic, anti-measles, and anti-pyretic properties were discovered through pharmacological screening (Ondo et al., 2013). There have also been reports of its antiviral (Vernon et al., 1999), antibacterial (Sabu and Kattan, 2002), and antidiarrheal (Sabu; Kattan, 2002) properties. There have also been reports of *M. macrostachyum* antioxidant qualities (Bruneton, 2009; Zongo et al., 2010).

According to Arnovet-Grant (1994), and Adjanohoum et al. (2001) the plant's phytochemical examination reveals that it includes saponins, alkaloids, flavonoids, triterpenoids, diterpenoids, tannins, and steroids. Numerous potential advantages of phytochemicals have been reported, including enhanced nutrient utilization (Daniel; Alagbe, 2023); higher digestive tract enzymatic activity (Alagbe, 2018); and well-documented favorable effects on feed conversion ratio and live weight gain (Swann, 1969). The reason for contradictory findings, however, is that the chemical components found in phytochemicals contribute to some variance in their constituents as well as other influencing elements such as climate, location, harvest, stage, and storage conditions, which can account for variations in the effectiveness of different medicinal plants (Jan Dirk, 2020).

The main factor influencing whether a medicinal plant or pharmaceutical medicine is harmful or therapeutic is its dose (Adewale et al., 2021). Therefore, it is necessary to investigate the effects of feeding quails phytochemical feed additives that include *Megaphrynium macrostachyum* leaves, as well as the performance, serum biochemistry, and haematological components of this feed. Food safety will be further supported by this research, which will also help to curb the rising rate of antibiotic resistance.

2. Materials and Methods

2.1 Study area (location)

The Poultry unit of the Sumitra Institute, situated between 23° 13' N and 72° 41' E, is where the study was carried out. The experiment, which took place in January and February of 2021, followed the rules and specifications of protocols approved by the research ethics council of India's Sumitra Research Institute.

2.2 Sample collection, authentication and preparation

Fresh *M. macrostachyum* leaves were collected on the Campus of a Gujarati Research Institute, India. The sample was brought to the Department of Crop Protection at the Sumitra Research Institute in Gujarat, India, for identification and authentication. There, it was given the voucher number FD/08A/2023. The leaves were then cleaned under running tap water and allowed to air dry in the open for 12 days, or until a consistent weight was reached. Using an electric blender, the dried leaves were ground into powder. The resulting samples were then placed in a labeled, clear polythene bag and transported to the laboratory for additional analysis at the organization.

2.3 Management of experimental birds and design

Four hundred one day old Japanese quails were purchased from a reputable farm in India and housed in a battery cage measuring 95 cm by 65 cm by 50 cm (length, breath and width) equipped with automated nipple drinkers and feeders in a semi sided pen. The cages, feeders and pen was cleaned, washed and disinfected with Aquaclean® two weeks before the arrival of the birds. Cages were also labeled for proper identification using a permanent marker. 60 watts' electric bulbs were used to supply heat and illumination at night for continuous feed intake.

On arrival, the average weight of the birds was recorded with a digital scale, and they were gently unboxed into the cages that had previously been heated few hours before the arrival of the birds. Quails were given Glucomol® (an anti-stress) at the rate of 2 g to 10 L of water and given for 7 days. Eighty birds were allocated to five treatments and each treatment had four replicates (20 birds per replicate). A completely randomized design was adopted, and experimental diet was formulated according to the requirements of birds recommended by NRC (1994) as presented in (Table 1). After one week of acclimatization, the test ingredient was introduced to birds in the following order:

Diet 1: A corn soya bean meal (basal diet) without antibiotics (Control);

Diet 2: Basal diet supplemented with antibiotic growth promoter (Neomycin at 0.2 g/kg) (positive control);

Diet 3: The basal diet supplemented with 2g/kg dried *Megaphrynium macrostachyum* leaf meal;

Diet 4: Basal diet supplemented with dried 4g/kg *Megaphrynium macrostachyum* leaf meal,

Diet 5: Basal diet supplemented with dried 6g/kg *Megaphrynium macrostachyum* leaf meal.

Table 1. Ingredients and chemical composition of experimental diets.

Feedstuffs	Inclusion (kg)
Yellow Corn	50.00
Wheat bran	2.00
Soybean meal	37.40
Fish meal	2.00
Limestone	2.50
Bone meal	5.00
Lysine	0.25
Methionine	0.25
***Mineral/Vitamin premix (Starter)	0.25
Salt	0.35
Total	100.00
Determined analysis (%)	
Crude protein	24.02
Crude fibre	3.65
Ether extract	4.02
Calcium	1.03
Phosphorus	0.62
Metabolizable energy (Kj/kg ⁻¹)	1261.8

Source: Author, 2023.

Fresh clean water and feed were offered ad libitum and other routine management practices were strictly observed.

2.4 Performance traits examined

Feed intake (g): Feed was weighed daily for quails in each replicate and the quantity consumed for the day was obtained by difference between the quantity supplied and the left over.

Average daily feed intake (g) was obtained by dividing the total feed intake by the number of experimental periods.

Body weight gain (g): The body weight gain was obtained by calculating the difference between the body weight for the preceding week and current week.

Average daily weight gain (g): Average daily weight gain was obtained by dividing the body weight gain by the number of experimental periods.

Feed conversion ratio: the feed conversion ratio was determined by dividing the quantity of feed consumed by the body weight gain of the birds in each replicate in grams.

2.5 Phytochemical evaluation of *Megaphrynium macrostachyum* leaf meal

Megaphrynium macrostachyum leaf meal was subjected to phytochemical examination using the methodology previously described by Alagbe et al. (2024). The following reagents are needed for the analysis: potassium hydroxide, sodium nitroprusside, sodium dodecyl sulphate, ferric chloride (FeCl₃) solution, sulphuric acid (H₂SO₄), sodium hydroxide (NaOH), copper sulphate (CuSO₄), sodium carbonate, *Folin-Ciocalteu* reagent, aluminum chloride, *Folin-Denis* reagent, and zinc acetate solution.

2.6 Estimation of flavonoid contents in *Megaphrynium macrostachyum* leaf meal

4 mL of distilled water were placed in a 10-mL volumetric flask, and 2.0 grams of leaf meal of *M. macrostachyum* was added. Three milliliters of 5% NaNO₂ were added to the mixture above. 0.3 mL of 10% AlCl₃ was added after 5 min. After adding 2 mL of 1 M sodium hydroxide (NaOH) at the sixth minute, the amount was increased to 10 mL using distilled water. After thoroughly mixing the solution, the absorbance at 510 nm was measured in comparison to the prepared reagent blank.

2.7 Estimation of phenolic compounds in *Megaphrynium macrostachyum* leaf meal

In a test tube, 2.5 g of *M. macrostachyum* leaf meal was added to 1 mL of distilled water. Subsequently, the test tube was filled with 2.5 mL of sodium carbonate solution (20%) and 0.5 mL of *Folin-Ciocalteu* reagent (1:1 v/v with water). The tubes were quickly left in the dark for 40 min after the reaction liquid was vortexed, and the absorbance at 725 nm was measured in comparison to the reagent blank.

2.8 Estimation of alkaloid content in *Megaphrynium macrostachyum* leaf meal

To make a smooth paste, 20 mL of 80% pure alcohol were added to 2.0 g of finely ground sample that had been weighed into a 100 mL beaker. After transferring the mixture to a 250 mL flask, additional alcohol was added to bring the total amount of magnesium oxide to 1 g. For one and a half hours, the mixture was broken down in a boiling water bath with periodic shaking and a reflux air condenser. The mixture was run through a *Büchner* funnel while it was still hot. After pouring the residue back into the flask and digesting it again for thirty minutes with 50 mL of alcohol, the alcohol evaporated. The lost alcohol was replaced with distilled water. Three drops of 10% HCl were added once all of the alcohol had evaporated. The entire solution was then transferred to a 250 mL volumetric flask, and to create a uniform mixture, 5 mL of zinc acetate solution and 5 mL of potassium ferricyanide solution were well mixed.

2.9 Estimation of tannin content in *Megaphrynium macrostachyum* leaf meal

2.0 g of the material was measured into a 50 mL beaker. Add 20 mL of 50% methanol, cover with parafilm, and place in a water bath at 77-80 °C for 1 h. It was shaken vigorously to achieve even mixing. The extract was filtered into a 100 mL volumetric flask through a double-layered Whatman No. 41 filter paper. Add 20 mL of water, 2.5 mL of *Folin-Denis* reagent, and 10 mL of 17 percent Na₂CO₃. Mix well. The mixture was prepared to the mark with water, thoroughly mixed, and allowed to stand for 20 min. At the end of the range, a bluish-green tint will appear. The 0-10ppm sample was processed similarly to the 1 mL sample above. The solution was well mixed, and the absorbance was measured against the prepared reagent blank at 700 nm.

2.10 Total steroid estimation in *Megaphrynium macrostachyum* leaf meal

2.5 g of each sample was weighed into a 100 mL beaker. On a shaker, 20 mL of a chloroform-methanol (2:1, v/v) combination was added to dissolve the extract after 30 min of shaking. The overall concoction should be free of steroids. 1 mL of the filtrate was pipetted into a 30 mL test tube, followed by 5 mL of alcoholic KOH. The mixture was thoroughly shaken to ensure homogeneity. The mixture was then placed in a water bath heated to 37-40 °C for 90 min. It was chilled to room temperature before 10 mL of petroleum ether was added, followed by 5 mL of distilled water.

2.11 Proximate composition of *Megaphrynium macrostachyum* leaf meal (MML)

Proximate composition of *M. macrostachyum* leaf meal was determined using a DA 7250 near infrared (NIR) tester, which can analyze samples in six minutes. Each sample weighed 200 g and was passed through a sample collecting vat linked to a monitor. To demonstrate findings efficiently, the equipment was tuned to a wavelength range of 570-1100 nm.

2.12 Collection of blood and analysis

On the 42nd day of the experiment, blood samples were drawn from sixteen randomly selected birds per treatment and sent to the laboratory for hematological and serum biochemical analysis. four milliliters of blood was obtained from each bird's wing vein, with 2 mL put to a sterile bottle containing anticoagulant for

haematological tests and the remaining two ml collected into anticoagulant-free vials for serum biochemical evaluation. To minimize degradation, all samples were stored in a pack of ice. The XN-1500 advanced diagnostic auto-analyzer (HD-066DC, Netherlands) was used to analyze red blood cells, pack cell volume, haemoglobin, white blood cells, leucocytes, and monocytes. It was fitted with closed and open tube volumes of 100 μL each, a workstation (intel pentium dual core 2.00 GHz 200 W desktop/tower), a 3Gb/s 7200 RPM 16 MB cache hard drive, a 2 GB memory module CD-RW, and an 11-inch torch screen with LCD monitor.

Serum biochemical evaluation was performed using a 200T/H automatic chemistry tester with a sample and reagent volume of 70 μL and 350 μL , post-spectral spectrophotometry, LAN port access, thirteen operational wave lengths (305, 340, 450, 480, 505, 546, 570, 600, 630, 686, 712, 705, 722 nm), and humidity of 40 to 85%. The outcomes were displayed within 120 seconds.

2.13 Statistical analysis

The data from the experiment was subjected to analysis of variance (ANOVA) using the computer software package (SPSS version 25.0), and differences between treatment means were examined using *Duncan's* multiple range test.

3. Results and Discussion

Table 2 and Figure 1 describes the results of the phytochemical parameters of *M. macrostachyum* leaf composition found in this study. The phyto-constituents in *M. macrostachyum* leaf meal revealed that it contained the highest concentration of phenolic compound (1402 mg/g^{-1}), followed by flavonoids (1205 mg/g^{-1}), terpenoids (620 mg/g^{-1}), tannins (318 mg/g^{-1}), alkaloids (200 mg/g^{-1}), steroids (51 mg/g^{-1}), and glycosides. The phytochemical contents reported in this investigation are consistent with the findings of (Ibironke; Olusola, 2013; Adeogun et al., 2017). However, the amounts measured were higher than those reported by Adeogun et al. (2017). This suggests that factors such as plant age, geographical location, species, and processing method, among others, may alter phytoconstituents in plants (Ahmad et al., 2016; Alagbe, 2023).

The presence of these phytochemicals suggests that *M. macrostachyum* leaf meal has a variety of pharmacological properties, including anti-helminthic, anti-cancer, anti-malarial, anti-hyperglycemic, anti-inflammatory, anti-rheumatic, immune-stimulatory, antidiarrheal, antiviral, antioxidant, analgesic, and hepatoprotective properties (Musa et al., 2021; Alagbe, 2023). Plants produce a variety of chemical compounds or phytochemicals to perform biological tasks such as insect defense, fungal attack, and herbivore protection (Akinyeye et al., 2010; Alagbe, 2019). These chemical compounds serve as the foundation for modern medications. For example, alkaloids exhibit a wide range of pharmacological activity, including analgesics, anti-malarial, vasodilatory, anti-tumor, and antiarrhythmic properties (Gupta et al., 2003).

Gupta et al. (2004) reported that phenols had antibacterial, antioxidant, and anti-inflammatory properties. Flavonoids can act as external antioxidants, defending cells against excessive free radical generation and protecting their contents from oxidative damage. Because of their ability to chelate metal ions and free radicals, they have a lower redox potential and can thermodynamically reduce highly oxidizing free radicals. Tannins and terpenoids can have antibacterial, antidiarrheal, anti-inflammatory, and antioxidant properties (Dwivedi et al., 2011; EL-Mahmood, 2009). Glycosides have been shown to have antidiarrheal effects in animals (Aritra; Sumana, 2012).

Table 2. Phytochemical composition of *Megaphrynium macrostachyum* leaf meal.

Variables	Concentrations (mg/g ⁻¹)
Tannins	318.62
Terpenoids	620.11
Flavonoids	1205.3
Steroids	51.79
Glycosides	42.55
Alkaloids	200.8
Phenols	1402.4

Source: Author, 2023.

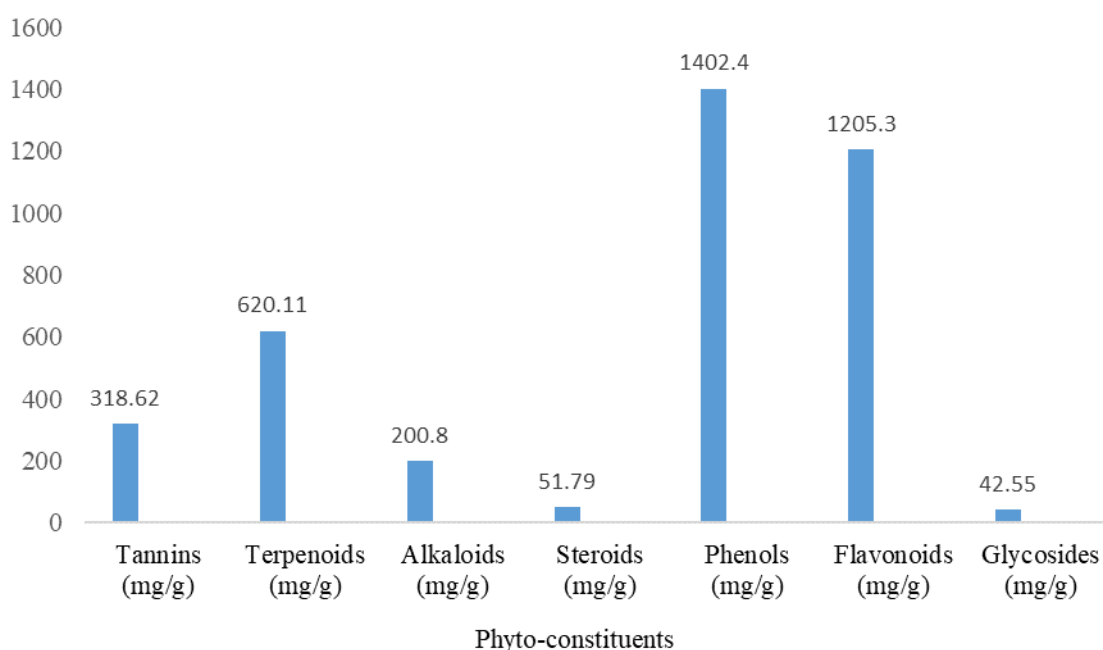


Figure 1. Phyto-constituents in *Megaphrynium macrostachyum* leaf meal. Source: Author, 2023.

In Table 3 which reveals the performance characteristics of quails fed dried *M. macrostachyum* leaf meal. The final body weight and average daily weight gain values which ranged from 131-151 g and 2-3 g were similar ($P > 0.05$) in quails fed 4 g MML/kg⁻¹ (diet 4) and diet 5 (6g MML/kg⁻¹) but significantly higher ($P < 0.05$) than the other groups. Total feed intake and average daily feed intake which ranged from 685-711 g and 16-16 g were highest in quails fed diet 4 (4g MML/kg⁻¹) and diet 5 (6g MML/kg⁻¹), intermediate in diet 2 (0.2 g neomycin/kg⁻¹) and diet 3 (2 g MML/kg⁻¹), lowest in diet 1 (0 g MML) ($P < 0.05$).

In this present study, results obtained revealed that feeding quails 4 g MML/kg⁻¹ in diet 4 and 6 g MML/kg⁻¹ (diet 5) can potentially act in support of modulating growth and enzyme responses in quails. The presence of phytochemicals in *M. macrostachyum* leaf meal can positively influence the secretion of digestive juices and permeability of the gut wall leading to improved absorption of nutrients in birds. For instance, Phytochemical compounds, such as flavonoids, have been shown to exert a beneficial effect on gut morphology, their ability to modulate barrier permeability by maintaining the integrity of tight junctions (Alagbe; Ushie, 2022).

They preserve the mucus layer by promoting mucin production, regulating the intestinal immune system with differentiation and proliferation of immune cells, and increasing IgA secretion (Alagbe, 2022). Though quails fed diet 2 (0.2 g/kg neomycin⁻¹) and 3 (2 g MML/kg⁻¹) had similar body weight compared those fed diet 1 (control). This suggest that neomycin and *M. macrostachyum* leaf meal can be used as growth promoters for birds. Average daily feed intake and feed conversion ratio were higher ($P < 0.05$) in quails fed *M. macrostachyum* leaf meal

relative to the other groups. This indicates that *M. macrostachyum* leaf meal can improve feed palatability, intake and to guarantee the consistent result on animal performance.

The result obtained is consistent with the reports of Gumus et al. (2017); Ebile et al. (2018) who recorded a significant improvement ($P < 0.05$) in body weight, intake via stimulating the secretion of endogenous enzymes and slight reduction in the digesta transit time of quails fed *Dichrostachys glomerata* fruit powder at 6g/kg⁻¹. Conversely, Oloruntola et al. (2021) recorded a non-significant ($P > 0.05$) difference in the intake of broilers fed *Ocimum gratissimum* leaf powder. This discrepancy can be attributed to variation in phytochemical composition or phyto-constituents, age of plant used, species as well as levels supplemented in the diet of animals (Musa et al., 2021; Adewale et al., 2021).

No mortality was recorded in quails fed *M. macrostachyum* leaf meal. This suggests that phyto-constituents in the sample can reshape the homeostasis of bile acids via up-regulation of the liver-gut axis and have been shown to counteract dysbiosis and help to recover intestinal permeability. The presence of flavonoids, saponins, terpenoids, tannins and alkaloids in *M. macrostachyum* leaf meal can modulate intestinal immunity and further improve local and systemic inflammatory conditions. The result obtained is in agreement with the findings of Kana et al. (2017b) when broiler chickens fed on diets supplemented with *Afrostryax lepidophyllus* fruit and bark.

Table 3. Performance characteristics of quails fed dried *Megaphrynium macrostachyum* leaf meal.

Parameters	¹ D1	² D2	³ D3	⁴ D4	⁵ D5	⁶ SEM
Number of birds	60.00	60.00	60.00	60.00	60.00	-
Experimental period (days)	42.00	42.00	42.00	42.00	42.00	-
Initial body weight (g)	25.11	25.08	25.05	25.01	25.00	2.02
Final body weight (g)	131.88 ^c	140.2 ^b	145.8 ^b	150.2 ^a	151.8 ^a	16.94
Weight gain (g)	106.77 ^c	115.12 ^b	120.75 ^b	125.19 ^a	126.8 ^a	12.03
Average daily weight gain (g)	2.54 ^c	2.74 ^b	2.88 ^b	3.00 ^a	3.02 ^a	0.01
Total feed intake (g)	685.11 ^c	691.22 ^c	702.8 ^b	711.2 ^a	711.2 ^a	40.52
Average daily feed intake (g)	16.31 ^c	16.46 ^c	16.73 ^b	16.93 ^a	16.93 ^a	1.71
⁷ FCR	6.42	6.00	5.82	5.68	5.60	0.08
Mortality (%)	2.56	1.16	-	-	-	0.04

Note: ¹Corn soya bean meal (basal diet) without antibiotics (Control); ²basal diet supplemented with antibiotic growth promoter (Neomycin at 0.2 g/kg⁻¹) (positive control); ³basal diet supplemented with 2g/kg⁻¹ dried *Megaphrynium macrostachyum* leaf meal; ⁴basal diet supplemented with dried 4g/kg⁻¹ *Megaphrynium macrostachyum* leaf meal; ⁵basal diet supplemented with dried 6g/kg⁻¹ *Megaphrynium macrostachyum* leaf meal; ⁶standard error of mean, and ⁷feed conversion ratio. Source: Author, 2023.

In Table 4, hematological characteristics of quails fed dried *M. macrostachyum* leaf meal showed that pack cell volume, haemoglobin, red blood cell, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and lymphocytes whose values ranged correspondingly from 28-32%, 9-14 (g/dL⁻¹), 4-6 ($\times 10^6/\mu\text{L}^{-1}$), 49-64 fl, 39-47 pg, 22-40 (g/dL⁻¹) and 46-68% of quails fed diet 2 (0.2 g Neomycin/kg⁻¹) and diet 3 (2 g MML/kg⁻¹) were similar ($P > 0.05$) to those of diet 4 (4 g MML/kg⁻¹) and diet 5 (6 g MML/kg⁻¹) but significantly ($P < 0.05$) higher than those fed diet 1 (control). White blood cell values of birds fed diet 3 and 4 were similar ($P > 0.05$) to those given diet 5 but significantly higher ($P < 0.05$) than the other group.

Heterophils and heterophil/lymphocytes ratio were higher in diet 1 relative to the other groups while monocytes values were not significantly ($P > 0.05$) influenced by the diet. Analysis of blood can be used in disease diagnosis, nutritional deficiency and metabolic disorders (Musa et al., 2021). Red blood cell, pack cell volume and haemoglobin concentration were within the normal range of 3-8 ($\times 10^6/\mu\text{L}$), 27-34% and 6-15 g/dL reported for healthy quails by Agina et al. (2017); Gumus et al. (2017) and Fudge (1997).

Reduction in red blood cell value could be a possibility of bone marrow disorder in animals (Alagbe, 2020)

while decreased pack cell volume indicates anaemia, hemodilution or overhydration as well as acute kidney infection (Coles, 1986). Mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentrations were within the established range 31.00-78.00 fl, 22.50-50.00 pg and 18.00-43.00 g/dL⁻¹ cited by Suman et al. (2022) and Ebile et al., 2018. This results further confirms that all the birds across the group were healthy.

White blood cell plays an important role in the production of antibodies, thus preventing the entry of pathogens and strengthens the immune system of quails (Alagbe, 2018; Coles, 1986). The values of white blood cell were within the normal range 9-25 ($\times 10^3/\mu\text{L}$) cited by Jain (1986). The values of lymphocytes in quails fed control diet (diet 1) were lower than the normal range of 48.00-81.00% for quails (Alagbe, 2019). Lymphocytes, heterophil and monocytes values obtained in this study was within the normal values for quails 40.0-75.00%, 10.2-25.00% and 1.50-4.00% reported by Kalio et al. (2016).

The values of monocytes obtained in diet 1 were lower than the normal range 1.50-4.00% cited by Alagbe (2018). Lymphocytes, monocytes and heterophil are involved in antibody proliferation in birds (Banks, 1974). Heterophils/lymphocytes (H/L) ratio reflects bird's robustness and immune system status. Quails with low Heterophils/lymphocytes ratio are superior to the birds with high H/L ratio in survival, immune response, and resistance to pathogenic organisms (Alagbe, 2018).

Table 4. Haematological characteristics of quails fed dried *Megaphrynium macrostachyum* leaf meal.

Parameters	¹ D1	² D2	³ D3	⁴ D4	⁵ D5	⁶ SEM
Red blood cell ($\times 10^6/\mu\text{L}$)	4.81 ^b	6.30 ^a	6.38 ^a	6.40 ^a	6.56 ^a	0.92
Heamoglobin (g/dL)	9.62 ^b	13.80 ^a	13.95 ^a	14.10 ^a	14.15 ^a	0.18
Pack cell volume (%)	28.46 ^b	32.00 ^a	32.17 ^a	32.60 ^a	32.68 ^a	0.35
MCV (fl)	49.18 ^b	61.08 ^a	62.62 ^a	63.50 ^a	64.01 ^a	0.21
MCH (pg)	39.22 ^b	43.50 ^a	45.95 ^a	46.61 ^a	46.72 ^a	0.14
MCHC (g/dL)	22.10 ^b	33.51 ^a	39.81 ^a	39.85 ^a	40.91 ^a	0.61
White blood cell ($\times 10^3/\mu\text{L}$)	10.61 ^c	13.88 ^b	16.02 ^a	16.71 ^a	16.30 ^a	0.48
Lymphocytes (%)	46.80 ^b	65.41 ^a	67.00 ^a	68.04 ^a	68.12 ^a	0.16
Heterophils (%)	24.00 ^a	22.70 ^b	22.85 ^b	22.90 ^b	22.96 ^b	0.02
Monocytes (%)	1.91	2.00	2.18	2.17	2.22	0.10
Heterophils/lymphocytes ratio	0.51 ^a	0.34 ^b	0.34 ^b	0.33 ^b	0.33 ^b	0.04

Note: ¹Corn soya bean meal (basal diet) without antibiotics (Control); ²basal diet supplemented with antibiotic growth promoter (Neomycin at 0.2 g/kg⁻¹) (positive control); ³basal diet supplemented with 2g/kg⁻¹ dried *Megaphrynium macrostachyum* leaf meal; ⁴basal diet supplemented with dried 4g/kg⁻¹ *Megaphrynium macrostachyum* leaf meal; ⁵basal diet supplemented with dried 6g/kg⁻¹ *Megaphrynium macrostachyum* leaf meal and ⁶standard error of mean. Source: Author, 2023.

Serum biochemical indices of quails fed dried *Megaphrynium macrostachyum* leaf meal (Table 5). Total protein, albumin and globulin values of quails fed diet *Megaphrynium macrostachyum* leaf meal (MML) at 2 g/kg⁻¹ (diet 3) and diet 4 (4 g/kg MML⁻¹) were similar ($P > 0.05$) to those fed diet 5 (6 g/kg MML⁻¹) but significantly higher ($P < 0.05$) than those fed the diet 1 (control) and diet 2 (0.20 g/kg neomycin⁻¹). Albumins are responsible for controlling the colloidal osmotic pressure in the body (Alagbe; Adegbite, 2020). It also aids to move fatty acids, bilirubin, hormones, cations as well as drugs in an animal's body (Alagbe; Grace, 2020) while globulin function in immune defense, transportation of substances in the body and enzymatic processes (Adewale et al., 2021).

This suggests that the experimental diet was adequate in all nutrients according to the requirements of quails by NRC (1994). According to Manu (2021); Alagbe and Ushie (2022), saponins have the potential to increase the rate of nutrient absorption in the gut, this explains why birds fed diet 3, 4 and 5 had a higher serum protein relative to the other groups. The outcome of this experiment is in agreement with the reports of Alagbe (2018) when *Coriandrum sativum* leaves meal was added in the diet of quails. Low levels of serum globulin may be a sign of liver or kidney disease as well as malnutrition (Alagbe, 2024). However, values of albumin and globulin

were within the normal range 2.0-6.0 g/dL and 1.8-4.0 g/dL cited by Lubran (1978) and Prakash (2013), respectively.

Albumin/globulin ratio and cholesterol levels whose values ranged from 1-1 g/dL and 110-191 mg/dL follow a similar pattern and were higher ($P < 0.05$) among quails fed diet 1 and 2 relative to the other treatments. Values obtained for albumin/globulin ratio and cholesterol were within the range 0.8-1.5 g/dL and 100-220 mg/dL reported by Lumeij (1997). High albumin/globulin ratio can occur when there is over production of albumin in the liver, severe dehydration or diarrhea (Suman et al., 2022).

Low serum cholesterol recorded among quails fed MML (diet 3 to 5) indicates that the test ingredient has hypolipidemic properties thereby reducing the risk of cardiovascular disease (Suman et al., 2022). Creatinine, alanine phosphatase, aspartate transaminase and alanine transaminase were not significantly ($P > 0.05$) influenced by the diet. However, values obtained were within the established ranges reported by Mercks Veterinary Manual (2001). High creatinine level is a sign of impaired kidney function (Sulastri; Basri, 2019). Elevation in aspartate transaminase and alanine phosphatase values are signs of hepatic disorder and obstruction of the bile duct (Daniel; Alagbe, 2023). The result obtained suggests that alanine phosphatase, aspartate transaminase and alanine transaminase showed that MML was not toxic to the birds. Suman et al. (2022) recorded a similar result when thyme and turmeric essential oil were fed to growing “japanese quails”.

Table 5. Serum biochemical indices of quails fed dried *Megaphrynium macrostachyum* leaf meal.

Parameters	¹ D1	² D2	³ D3	⁴ D4	⁵ D5	⁶ SEM
Total protein (g/dL)	5.11 ^b	5.52 ^b	6.87 ^a	6.92 ^a	6.98 ^a	0.26
Albumin (g/dL)	3.00 ^b	3.00 ^b	3.84 ^a	3.90 ^a	3.96 ^a	0.12
Globulin (g/dL)	2.11 ^b	2.52 ^b	3.03 ^a	3.02 ^a	3.02 ^a	0.05
Albumin/Globulin ratio (g/dL)	1.31 ^a	1.30 ^a	1.27 ^b	1.29 ^b	1.29 ^b	0.07
Cholesterol (mg/dL)	191.5 ^a	187.8 ^a	106.5 ^b	110.2 ^b	110.8 ^b	0.04
Creatinine (mg/dL)	0.81	0.85	0.73	0.85	0.92	0.33
⁷ ALP (U/L)	80.90	83.55	86.10	88.71	88.96	2.40
⁸ AST (U/L)	23.76	23.08	23.56	23.93	24.00	1.52
⁹ ALT (U/L)	55.20	54.60	55.11	55.18	55.90	0.96

Note: ¹Corn soya bean meal (basal diet) without antibiotics (Control); ²basal diet supplemented with antibiotic growth promoter (Neomycin at 0.2 g/kg⁻¹) (positive control); ³basal diet supplemented with 2g/kg⁻¹ dried *Megaphrynium macrostachyum* leaf meal; ⁴basal diet supplemented with dried 4g/kg⁻¹ *Megaphrynium macrostachyum* leaf meal; ⁵basal diet supplemented with dried 6g/kg⁻¹ *Megaphrynium macrostachyum* leaf meal; ⁷alanine phosphatase; ⁸aspartate transaminase and ⁹alanine transaminase. Source: Author, 2023.

4. Conclusions

Megaphrynium macrostachyum leaf meal (MML), when employed as a phyto-genic feed addition, has the potential to modulate growth and enzyme responses in quails. MML has been proven to include various phyto-constituents with medicinal characteristics and functionality to eliminate reactive oxygen species, perhaps leading to lower lipid peroxidation and enhanced performance and blood counts in birds. It was determined that MML can be supplied in the food of quails at a dose of up to 6g/kg⁻¹ without negatively impacting growth performance or animal health.

5. Acknowledgments

Thanks to the Department of Animal Nutrition and Biochemistry, Sumitra Research Institute, Gujarat, India.

6. Authors' Contributions

John Olujimi Alagbe: scope of the study, experimental design, analysis, plant collection, feed preparation and analysis, study writing, statistical analysis, submission and publication.

7. Conflicts of Interest

No conflicts of interest.

8. Ethics Approval

Yes applicable. Experimental procedures and management were carried out according to the guidelines of the ethic committee of Sumitra Research Institute, India (ASD/08C/2023).

9. References

- Adewale, A. O., Alagbe, J. O., Adeoye, Adekemi. O. (2021). Dietary supplementation of *Rauvolfia vomitoria* root extract as a phyto-genic feed additive in growing rabbit diets: Haematology and serum biochemical indices. *International Journal of Current Researches in Ciencias, Social Sciences and Languages*, 1(3), 15-26. <https://www.ijcrsssl.com/papers/v1/i3/IJCRSSSLV1I30003.pdf>
- Adeogun O. O., Adekunle, A. A., Adongbede, E. M., & Ashafa, T. A. (2017). Chemical composition, toxicity and antifungal activities of *Megaphrynium macrostachyum* (K. Schum) leaf extract against foodborne fungi. *Notulae Scientia Biologicae*, 9(3), 361-370. <https://doi.org/10.15835/nsb9310121>
- Adjanohoum, J. E., Aké-Assi, L., Floret, J. J., Guinko, S., Koumaré, M., Ahyi, A. M., & Raynal, J. (2001). *Medicine traditionnelle et Pharmacopée; contribution aux études ethnobotaniques et floristiques au Mali*. ACCT, Paris, 291 p. <https://agris.fao.org/search/en/providers/122621/records/647396d83ed73003714cfa42>
- Ahmad, I., Aqil, F., Ahmad, F., & Owais, M. (2006). Herbal medicines: prospects and constraints. In: *Modern phytomedicine: turning medicinal plants into drugs*. Ed., Ahmad I., Aqil F., & Owais M., Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, 59-76 p. <https://onlinelibrary.wiley.com/doi/book/10.1002/9783527609987#page=79>
- Agwah, E. D., Raphaël, K. J., Wilfried, E. N. L., Joël, P. M. H., Gildas, N. D., Ruben, N. T., Hervé, M. K., & Florence, F. A. (2018). Growth performance, gut microbiota and haemato-biochemical profile of quails fed diet supplemented with graded levels of *D. glomerata* Fruit Powder. *Animal and Veterinary Sciences*, 6(5), 80-87. <https://doi.org/10.11648/j.avs.20180605.13>
- Agina, O. A., Ezema, W. S., & Iwuoha, E. M. (2017). The haematology and serum biochemistry profile of adult Japanese quail (*Coturnix coturnix japonica*). *Notulae Scientia Biologicae*, 9(1), 67-72. <https://doi.org/10.15835/nsb919928>
- Akinyeye, R. O., Oluwadunsin, A., & Omoyeni, A. (2010). Proximate, mineral, anti-nutrients and phytochemical screening and amino acid composition of the leaves of *Pterocarpus mildbraedi* Harms. *Electronic Journal of Environmental, Agricultural and Food Chemistry*, 9(8), 1322-1333.
- Alagbe, J. O. (2018). Effect of different levels of feed added Coriander (*Coriandrum sativum*) leaves meal on the performance, carcass quality, immune response and blood profile of quails (*Coturnix coturnix japonica*). *Pacific International Journal*, 1(4), 142-150. <https://doi.org/10.55014/pij.v1i4.46>
- Alagbe, J. O. (2018). Growth and haematological parameters of Japanese quails (*Coturnix coturnix japonica*) fed dried Corn silk *Polyalthia longifolia* leaf meal mixture. *Pacific International Journal*, 1(3), 80-86. <https://doi.org/10.55014/pij.v1i3.45>
- Alagbe, J. O. (2018). Effect of different levels of feed added Coriander (*Coriandrum sativum*) leaves meal on the performance, carcass quality, immune response and blood profile of quails (*Coturnix coturnix japonica*). *Pacific International Journal*, 1(4), 142-150. <https://doi.org/10.55014/pij.v1i4.46>
- Alagbe, J. O., & Grace, F. R. (2019). Effect of *Albizia lebbek* seed oil dietary supplementation on the haematological and serum biochemical parameters of weaner rabbits. *Sumerianz Journal of Agriculture and Veterinary*, 2(10), 96-100. [https://www.sumerianz.com/pdf-files/sjav2\(10\)96-100.pdf](https://www.sumerianz.com/pdf-files/sjav2(10)96-100.pdf)
- Alagbe, J. O., Sharma, D., & Liu, X. (2019). Effect of aqueous *Piliostigma thonningii* leaf extracts on the haematological and serum biochemical indices of broiler starter chicks. *HSOA Journal of Protein Research and Bioinformatics*, 2, 1-6. <http://doi.org/10.24966/PRB-1545/100006>
- Alagbe, J. O. (2019). Growth performance and haemato-biochemical parameters of broilers fed different levels of *Parkia biglobosa* leaf extracts. *Academic Journal of Life Sciences*, 5(12), 107-115. <http://doi.org/10.32861/ajls.512.107.115>

- Alagbe, J. O., & Betty, A. M. (2019). Haematological and serum biochemical indices of starter broiler chicks fed aqueous extract of *Balanites aegyptiaca* and *Alchornea cordifolia* bark mixture. *International Journal of Biological, Physical and Chemical Studies*, 1(1), 8-15. <https://www.al-kindipublisher.com/index.php/ijbpcs/article/view/415>
- Alagbe, J. O. (2020). Effect of dietary supplementation of *Cymbopogon citratus* oil on the haematology and serum biochemical parameters of broiler chicks. *Journal of Multidimensional Research & Review*, 1(3), 41-60. <https://jmrr.org/V1I3/V1I3P05.pdf>
- Alagbe, J. O. (2020). Performance, hematology and serum biochemical parameters of weaner rabbits fed different levels of fermented *Lagenaria breviflora* whole fruit extract. *Advances in Research and Reviews*, 1,5.
- Alagbe, J. O. (2022). *Prosopis africana* (African mesquite) oil as an alternative to antibiotic feed additives on broiler chickens diets: performance and nutrient retention. *Discovery*, 58(314), 134-142. https://www.discoveryjournals.org/discovery/current_issue/v58/n314/A7.pdf?
- 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. (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.
- Alagbe, O. J., Anuore, D. N., Daniel, S. M., Adewale, E., Taiwo, A., Emmanuel, A. A. (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. (2024). Novel phytochemicals' 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>
- 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
- Arvouet-Grant, A., Vennat, B., Pourrat, A., & Legret, P. (1994). Standardisation d'un extrait de propolis et identification des principaux constituants. *Journal de la Pharmacology Belge*, 49(6), 462-468. <https://europepmc.org/article/med/7884635>
- Balunas, M. J., & Kinghorn, A. D. (2005). Drug discovery from medicinal plants. *Life Sciences*, 78(5), 431-441. <https://doi.org/10.1016/j.lfs.2005.09.012>
- Banks, W. I. (1974). Histology and comparative organology. A text atlas. The Williams and Wilkins Company Baltimore, USA, 79-83 p.
- Barba, F., Criado, M., Belda-Galbis, C., Esteve, M., & Rodrigo, D. (2015). *Stevia rebaudiana* Bertoni as a natural antioxidants/antimicrobial for high pressure processed fruit extract: Processing parameter optimization. *Food Chemistry*, 148, 261-267. <https://doi.org/10.1016/j.foodchem.2013.10.048>
- Bruneton, J. (2009). Pharmacognosie, Phytochimie, plantes médicinales. In: EM inter, ed. Tec & Doc, 4ème édition.
- Chatterjee, A., & Chatterjee, S. (2012). proximate analysis, phyto-chemical screening and anti-inflammatory activity of *Coccinia indica*. *International Journal of Pharmaceutical Chemical Biological Sciences*, 2(3), 299-304.
- Coles, E. H. (1986). Veterinary Clinical Pathology, 4th Edition. W. B. Saunders, Philadelphia.
- David, O., Adesanya, O., Ayodeji, F., Bamidele, A., & Joseph, O. (2021). Performance of broiler chicken fed diets supplemented with *Irvingia gabonensis* kernel powder and *Ocimum gratissimum* leaf powder. *Slovak Journal of Animal Science*, 54(1), 7-20. <https://office.sjas-journal.org/index.php/sjas/article/view/646>
- Dwivedi, S. C., Dudey, R., Tyagi, R., Masand, M., & Advani, U. (2011). Medicinal bioactives as antimicrobial agents: an overview. *International Journal of Pharmaceutical Research and Development*, 3(7), 24-30.

- El-Mahmood, M. A. (2009). Efficacy of crude extracts of garlic (*Allium sativum* Linn.) against nosocomial *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Pseudomonas aeruginosa*. *Journal of Medicinal Plants Research*, 3(4), 179-185. <https://academicjournals.org/journal/JMPR/article-full-text-pdf/E869B3014958>
- Fudge, A. M. (1997). Avian clinical pathology, haematology and chemistry. In: Altman, R. B., Clubb, S. L., Dorrestein, G. M., Quesberry, K. (Eds). Avian Medicine and Surgery. WB Saunders Co., Philadelphia, 142-157 p.
- Gumus, R., Ercan, N. A. Z. L. I., & Imik, H. (2017). The effect of thyme essential oil (*Thymus vulgaris*) added to quail diets on performance, some blood parameters, and the antioxidative metabolism of the serum and liver tissues. *Brazilian Journal of Poultry Science*, 19(2), 297-304. <https://doi.org/10.1590/1806-9061-2016-0403>
- Gupta, M., Mazumder, U.K., Kumar, R.S. & Kumar, T.S. (2003). Studies on anti-inflammatory, analgesic and antipyretic properties of methanol extract of *Caesalpinia bonducella* leaves. *Iranian Journal of Pharmacology and Therapeutics*, 2, 30-34.
- Gupta, M., Mazumder, U. K., Kumar, R. S., Sivakumar, T., & Vamsi, M. L. (2004). Antitumor activity and antioxidant status of *Caesalpinia bonducella* against Ehrlich ascetic carcinoma in Swiss albino mice. *Journal of Pharmacological Sciences*, 94(2), 177-184. <https://doi.org/10.1254/jphs.94.177>
- Ibironke, A. A., & Olusola O. O. (2013). Phytochemical screening, proximate analysis and antimicrobial activity of aqueous extract of *Megaphrynium macrostachyum* seeds. *International Journal of Engineering Research and Technology*, 2(9), 2123-2131.
- Jan-Dirk. (2022). Phytochemicals be one step lead with plant derived feed additives. *International Poultry Magazine*, 2(2), 5-6.
- Jain, N. C. (1986). Schalm's Veterinary Haematology Lea and Febiger. Philadelphia, USA, 1221 p.
- Jennings, S. B., Brown, N. D., Boshier, D. H., Whitmore, T. C., & Lopes, J. A (2001). Ecology provides a pragmatic solution to the maintenance of genetic diversity in sustainably managed tropical rainforest. *Forest Ecology and Management*, 154(1-2), 1-10. [https://doi.org/10.1016/S0378-1127\(00\)00637-X](https://doi.org/10.1016/S0378-1127(00)00637-X)
- Kalio, G. A., Wariboko, O. N., & Okafor, B. B. (2016). Growth performance and haematological profile of growing Japanese quails fed graded levels of *Azadirachta indica* leaf meal. *Journal of Animal Science Advances*, 6(2), 1586-1594. <http://dx.doi.org/10.5455/jasa.196912310400000>
- Kana J. R., Mube, K. H., Ngouana, T. R., Yangoue, A., Kmguep, R., Tsafong, F., & Tegua A. (2017b). Growth performance and serum performance profile of broiler chickens fed on diets supplemented with *Afrotyrax lepidophyllus* fruit and bark as alternative to antibiotic growth promoters. *Journal of Veterinary Medicine and Research*, 4(6), 1-7.
- Ley, A. C., & Claßen-Bockhoff, R. (2013). Breeding system and fruit set in African Marantaceae. *Flora – Morphology, Distribution, Functional Ecology of Plants*, 208(8-9), 532-537. <https://doi.org/10.1016/j.flora.2013.07.011>
- Lubran, M. M. (1978). The measurement of total serum proteins by the Biuret method. *Annals of Clinical Laboratory Science*, 8, 106-110. <http://www.annclinlabsci.org/content/8/2/106.short>
- Lumeij, J. T. (1997). Avian clinical biochemistry. In: Kaneko, K. K., Harvey, J. W., Bruss, M. L. (Eds). Clinical Biochemistry of Domestic Animals. Academic Press, San Diego, CA, 857883 p.
- Meva, F. E., Segnou, M. L., Ebongue, C. O., Ntoumba, A. A., Kedi, P. B. E., Deli, V., Etoh, M-A., & Mpondo, E. M. (2016). Spectroscopic synthetic optimizations monitoring of silver nanoparticles formation from *Megaphrynium macrostachyum* leaf extract. *Revista Brasileira de Farmacognosia*, 26, 640-646. <https://doi.org/10.1016/j.bjp.2016.06.002>
- Mith, H., Dure, R., Delcenserie, V., Zhiri, A., Daube, G., & Clinquart, A. (2014). Antimicrobial activities of commercial essential oils and their components against food-borne pathogens and food spoilage bacteria. *Food Science and Nutrition*, 2(4), 403-416. <https://doi.org/10.1002/fsn3.116>
- 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>
- Nascimento, G. G. F., Locatelli, J., Freitas, P. C., & Silva, G. L. (2000). Antibacterial activity of plant extracts

- and phytochemicals on antibiotic-resistant bacteria. *Brazilian Journal of Microbiology*. 31(4), 247-256. <https://doi.org/10.1590/S1517-8382200000400003>
- National Research Council, NRC. (1994). *Nutrient Requirements of Poultry*. 9th Edition National Academy Press. Washington, D.C., 1-114 p.
- Ondo, J. P., Obame, L. C., Andzi, B. T., Akoué, N., Nsi Emvo, E., & Lebibi, J. (2013). Phytochemical screening, total phenolic content and antiradical activity of *Asplenium africanum* (Aspleniaceae) and fruit of *Megaphrynium macrostachyum* (Marantaceae). *Journal of Applied Pharmaceutical Science*, 3(8), 092-096. <http://dx.doi.org/10.7324/JAPS.2013.3816>
- Oyagade, J. O., Awotoye, J. T., Adewunmi, A. & Thorpe, H. T. (1999). Antimicrobial activity of some Nigerian medicinal plants: Screening for antibacterial activity. *Biological Recourses Community*, 11(3), 193-197.
- Prakash, D., Suri, S., Adhyay, G. U., & Singh, B. N. (2007). Total phenol, antioxidant and free radical scavenging activities of some medicinal plants. *International Journal of Food Sciences and Nutrition*, 58(1), 18-28. <https://doi.org/10.1080/09637480601093269>
- Prakash, R. K. (2013). Serum biochemical profile of Japanese quails (*Coturnix coturnix japonica*). *Indian Journal of Fundamental and Applied Life Sciences*, 3(1), 182-183.
- Ranwa, S., Palod, J., Rabendra, K., & Kumar, S. (2022). Effect of supplementation of thyme and turmeric essential oils on haemato-biochemical parameters of Japanese quails. *The Indian Journal of Veterinary Sciences and Biotechnology*, 18(5), 14-18. <https://doi.org/10.48165/ijvsbt.18.5.03>
- Sabu, M., & Kattan, R. (2002). Antidiabetic activity of medicinal plants and its relationship with their antioxidant property. *Journal of Ethnopharmacology*, 81(2), 155-160. [https://doi.org/10.1016/S0378-8741\(02\)00034-X](https://doi.org/10.1016/S0378-8741(02)00034-X)
- Sulastri, M. P., & Basri, H. (2019). The effect of turmeric and ginger powder on Japanese quail (*Coturnix coturnix*) serum lipid profile. *Biosaintifika: Journal of Biology and Biology Education*, 11(2), 243-248.
- Swann, M. M. (1969). *Use of antibiotics in animal husbandry and Veterinary medicine*. UK Joint Committee report London: H. M Stationery Office.
- Vernon, L. S., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, 299, 152-178.
- Zongo, C., Savadogo, A., Ouattara, L., Bassolé, I. H. N., Ouattara, C. A. T., Ouattara, A. S., Barro, N., Koudou, J., & Traoré, A. S. (2010). Polyphenols content, antioxidant and antimicrobial activities of *Ampelocissus grantii* (Baker) Planch. (Vitaceae), a medicinal plant from Burkina Faso. *International Journal of Pharmacology*, 6(6), 880-887.
- Yokozawa, T., Dong, E. B., Kawai, Y., Gemba, M., & Shimizu, M. (1999). Protective effects of some flavonoids on the renal cellular membrane. *Experimental and Toxicologic Pathology*, 51(1), 9-14. [https://doi.org/10.1016/S0940-2993\(99\)80050-5](https://doi.org/10.1016/S0940-2993(99)80050-5)

Funding

Not applicable.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Copyrights

Copyright for this article is retained by the author(s), with first publication rights granted to the journal.

This is an open-access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/4.0/>).