Melaleuca quinquernervia leaf extract: Effect on growth performance and apparent digestibility of Japanese quails

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Abstract

This research was undertaken to evaluate the influence of *Melaleuca quinquernervia* leaf extract on the growth performance and apparent digestibility of Japanese quails. Three hundred (300) two-week-old Japanese quails (mixed sex) were randomly distributed into four treatments with five replicates, each replicate consisting of 15 birds. A completely randomized experimental design was adopted in the 42-day trial. The treatment consists of: treatment 1, basal diet without *M. quinquernervia* extract; treatment 2, basal diet with *M. quinquernervia* extract at 2 mL per litre of water/day; treatment 3, basal diet with Melaleuca quinquernervia extract at 4 mL per litre of water/day; and treatment 4, basal diet with *M. quinquernervia* extract at 6 mL per litre of water/day. The basal diet was formulated according to the requirements of birds; feed and water were unrestrictedly served. Experimental results showed the major phyto-compounds in *M. quinquernervia* leaf extract by GC-MS were: α-copaene (607.3 mg g^{-1}), 1-Hexadecanol (85.17 mg g^{-1}), Humulene (78.31 mg g^{-1}), and γ-Terpinene-3-Carene (45.22 mg g^{-1}), respectively. Daily weight gain and daily feed intake improved ($p < 0.05$) among birds that received *M. quinquernervia* leaf extract compared to the control. Apparent digestibility of dry matter, crude protein, crude fibre, ether extract, and ash values, which varied from 71.19 – 89.17%, 69.08 – 78.91%, 36.12 – 40.92%, 49.96 – 59.11%, and 35.91 – 50.05%, respectively, were significantly ($p < 0.05$) influenced by the treatment. In conclusion, feeding quails with *Melaleuca quinquernervia* leaf extract exerts a beneficial effect on their performance, especially when administered up to 6 ml per litre of water/day without causing any detrimental effect on their health status.

Keywords: quails, performance, phyto-compounds, digestibility, *Melaleuca* genus.

Extrato foliar de *Melaleuca quinquernervia*: efeito sobre o desempenho de crescimento e a digestibilidade aparente de codornas japonesas

Resumo

Esta pesquisa foi conduzida com o objetivo de avaliar a influência do extrato foliar de *Melaleuca quinquernervia* sobre o desempenho produtivo e a digestibilidade aparente de codornas japonesas. Trezentas (300) codornas japonesas com duas semanas de idade (sexo misto) foram distribuídas aleatoriamente em quatro tratamentos, com cinco repetições, sendo cada repetição composta por 15 aves. O delineamento experimental adotado foi inteiramente casualizado, com duração de 42 dias. Os tratamentos consistiram em: tratamento 1, dieta basal sem extrato de *M. quinquernervia*; tratamento 2, dieta basal com extrato de *M. quinquernervia* na dose de 2 mL por litro de água/dia; tratamento 3, dieta basal com extrato de *M. quinquernervia* na dose de 4 mL por litro de água/dia; e tratamento 4, dieta basal com extrato de *M. quinquernervia* na dose de 6 mL por litro de água/dia. A dieta basal foi formulada de acordo com as exigências nutricionais das aves, sendo a ração e a água fornecidas ad libitum. Os resultados experimentais obtidos por cromatografia gasosa acoplada à espectrometria de massas (CG–MS) indicaram que os principais fitocompostos presentes no extrato foliar de *M. quinquernervia* foram:

α-copaeno (607,3 mg g^{-1}), 1-hexadecanol (85,17 mg g^{-1}), humuleno (78,31 mg g^{-1}) e γ-terpineno-3-careno (45,22 mg g^{-1}). O ganho de peso diário e o consumo diário de ração foram significativamente maiores ($p < 0,05$) nas aves que receberam o extrato foliar de *M. quinquernervia* em comparação ao grupo controle. A digestibilidade aparente da matéria seca, proteína bruta, fibra bruta, extrato etéreo e cinzas, cujos valores variaram de 71,19 a 89,17%, 69,08 a 78,91%, 36,12 a 40,92%, 49,96 a 59,11% e 35,91 a 50,05%, respectivamente, foi significativamente ($p < 0,05$) influenciada pelos tratamentos. Conclui-se que a suplementação de codornas com extrato foliar de *Melaleuca quinquernervia* exerce efeito benéfico sobre o desempenho produtivo e a digestibilidade dos nutrientes, especialmente quando administrada até a dose de 6 mL por litro de água/dia, sem causar efeitos prejudiciais ao estado de saúde das aves.

Palavras-chave: codornas, desempenho, fitocompostos, digestibilidade, gênero *Melaleuca*

1. Introduction

Medicinal plant contains a pharmacy of molecules where nature concentrates because every part carries active chemistry that the body recognizes instantly (Alagbe, 2022). They offer numerous stronger pharmacological properties and are generally regarded as non-toxic, eco-friendly, and have no withdrawal period (John, 2024d; Shittu; Alagbe, 2020). The use of medicinal or herbal plants in poultry production has significantly attracted global interest because of the rising cases of antimicrobial resistance, which has become a threat to human and animal health (Singh et al., 2021; Alagbe, 2024). There are several medicinal plants with a biochemical blueprint; some are yet to be utilized. Among the potential plants with a unique spectrum of bioactive compounds is *Melaleuca quinquenervia* Blake (Myrtaceae) (Hernadez; Alagbe, 2025a; Blaskowski et al., 2025).

Melaleuca quinquenervia is a traditional medicine of Asia, Australia, Southern America, and Africa, loaded with phyto-compounds that are rarely found in other medicinal plants (Thalib et al., 2025; Hamed et al., 2025; Maria et al., 2024; Chao et al., 2017). It belongs to Myrtaceae family and every part of this plant carries active chemistry compounds like alkaloids, flavonoids, saponins, tannins, phenolic compounds and steroids that intercepts the same inflammatory pathways targeted by modern pain killers (Bakry et al., 2025; Acha et al., 2019; Trilles et al., 2006), inhibits the activities of some bacteria's and fungi responsible for wound, skin and gastro-intestinal infections (Valkov´a et al., 2022), strengthens cell membrane, neutralizes free radicals and protects tissues from oxidative damage (Ijinu et al., 2026; Ramanoelina et al., 2008; Ramanoelina et al., 2005). The tree is usually between 10 and 15 m high with papery bark. Leaves alternate, lanceolate to elliptic, 30 to 70 mm long, 8 – 24 mm wide, apex obtuse, mostly with five prominent longitudinal veins often hairy with appressed hair (Trilles et al., 2006). Its flowers are arranged in spikes on the end of branches, which continue to grow after flowering (Acha et al., 2019).

Traditionally, leaf infusions are used to treat cough, pyrexia, toothache, lower systolic blood pressure, gastrointestinal disturbances, skin infections, snake bite, as well as urinary tract infections (Emile et al., 2015; Alagbe, 2023). Aqueous extract from the leaves of *M. quinquenervia* has shown significantly reduced markers of liver inflammation, protects hepatocytes in the liver working cells caused by toxins (Muritala et al., 2022; Ojediran et al., 2024a), stopping the interference of respiratory and urinary pathogens like *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, amongst others (Ojediran et al., 2024b; Daniel et al., 2023).

Outcome from previous research by Musa et al. (2020) has shown that supplementation of plant extracts (*Balanites aegyptiaca* and *Alchornea cordifolia* stem bark mixture) at 8 mL L^{-1} of water leads to a significant improvement in final body weight, feed consumption, and feed conversion ratio. Maria et al. (2023) also noted that feeding Japanese quails with aqueous *Moringa oleifera* leaf extract at 300 mg L^{-1} enhanced the digestibility of nutrients. Conversely, John (2024c) recorded a non-significant difference in the final body weight and nutrient retention of Japanese quails fed a diet supplemented with *Rhamnus prinoides* leaf extract.

Differences can be attributed to differences in active ingredients as well as their interaction. This experiment will further help to document the efficacy of *Melaleuca quinquernervia* extract, establish a standard, safe, and optimum level for quails, and promote food security. This study aimed to evaluate the effects of *Melaleuca quinquernervia* leaf extract on the growth performance and apparent digestibility of Japanese quails.

2. Materials and Methods

2.1 Equipment and reagents

Clavenger apparatus (Vitrify, India), water bath (Hover ABS, India), and digital thermometer (Worner Lab,

China). Ethanol (Merck, India), filter paper, condenser, and conical flask.

2.1 Location of experiment

The research work was carried out at the Poultry unit of the Department of Animal Production, Gandhi College of Agriculture, Rajasthan, India. Rajasthan lies on a latitude of 9° 281 N to 9° 371 N and a longitude of 6° 231 E to 6° 331 East India. The mean annual rainfall is between 1000 at 1500 mm.

2.2 Preparation of Melaleuca quinquernervia leaf extract

Fresh leaves of *M. quinquernervia* samples were collected within the premises of Gandhi College of Agriculture, Rajasthan, in June 2024. Samples were immediately identified and certified at the Taxonomy department of the institution and assigned a voucher number DF/06Y/2024 before they were placed in the herbarium of Gandhi College of Agriculture. Collected leaves were rinsed in water, chopped into pieces, and air-dried for 6 days before they were milled into powder with an electric multipurpose blender. 200 g of *M. quinquernervia* powder was cold macerated into 1000 mL of ethanol solution, the mixture was stirred occasionally and kept for 2 days. The mixture was filtered with a Whatman filter paper, and the filtrate was kept in a refrigerator at 4 °C before it was sent to the laboratory for further evaluation. Evaluation of bioactive compounds was carried out according to the previous method outlined by Singh et al. (2022).

2.3 HPLC analysis

The HPLC system consisted of: a Water binary pump, a Water 809 plus Autosampler, reversed-phase columns, and an Absorbance detector. Hypersil ODS (100 × 4.5 mm). Free statistics software version 1.1.23-r7m Wessa P.(2013) was used for statistical calculation.

2.3 Management of experimental birds

Three hundred (300) two-week-old Japanese quails (mixed sex) sourced from a reputable source were used for this study. The birds were housed in a battery cage equipped with a nipple drinker and metallic feeders. The pens and cages were washed and disinfected two weeks before the arrival of the birds. Quails were unboxed, and their average initial weight was recorded using a digital sensitive scale before it was randomly distributed into four treatments with five replicates, each replicate consisted of 15 birds, and given anti-stress in drinking water for 3 days.

An electric brooder supplemented with 200-Watt electric bulbs was used to supply heat for the first two weeks and was later replaced with 60-Watt bulbs to provide illumination at night for continuous feed intake. The basal diet was formulated in accordance with the nutritional requirements for birds described by the Nutritional Research Council (1994). The treatment consists of: treatment 1, basal diet without *M. quinquernervia* extract; treatment 2, basal diet with *M. quinquernervia* extract at 2 mL per litre of water/day; treatment 3, basal diet with *Melaleuca quinquernervia* extract at 4 mL per litre of water/day; and treatment 4, basal diet with *M. quinquernervia* extract at 6 mL per litre of water/day (Table 1).

Management of birds was carried out in line with the procedures outlined by the Indian Society of Animal Production. Regular management operations carried out daily included cleaning of drinkers, feeders, and the cleaning of the foot bath. A completely randomized experimental design was adopted, and the experimental duration is 6 weeks. Feed was given unrestricted, while water was available at all times. To determine the feed consumption, feed was weighed daily for quails in each replicate, and the quantity consumed for the day was obtained by the difference between the quantity supplied and the leftover. The body weight gain was obtained by calculating the difference between the body weight for the preceding week and the current week to estimate the body weight gain. The feed conversion ratio was calculated by dividing the quantity of feed consumed by the body weight gain of the birds in each replicate in grams.

Table 1. Ingredient and chemical composition of basal diet (% DM).

Ingredients	Quantity (%)
Corn	52.00
Wheat bran	3.30
Soybean meal	36.00
Fish meal	3.00
Limestone	1.50
Bone meal	3.00
Lysine	0.20
Methionine	0.20
Premix	0.25
Toxin binder	0.20
Salt	0.35
Total	100.0
Determined analysis (%)	
ME (Kcal/kg^{-1})	2600.7
Crude protein	23.18
Crude fibre	4.08
Ether extract	3.25
Calcium	1.73
Phosphorus	0.69

Note: *2.5 kg Premix to 1000 kg diet contains: Vit. A (10,500.00 IU), Vit. D (5000,000 IU), Vit. E (10,000iu), Vit. B1 (570 mg), Vit. B2 (250 mg), Vit. B3 (800 mg), Vit. B6 (350 mg), Vit. B12 (2.9 mg), Vit. K (17 mg), Vit. C (20 mg) and folic acid (150 mg), Ca (15.5 mg), Cu (10.0mg), Fe (35 mg), I (0.55 mg), Se (200 mg), Mg (1.30 mg), Chlorine (200 mg), and Panthotenic Acid (15.4 mg). Source: Author, 2025.

2.4 Digestibility trial

On the 6th week of the experiment, four birds were selected from each of the replicates for the digestibility trial. Quails were individually weighed and transferred into a galvanized metabolic cage. Birds were placed on a 2 2-day adjustment period and a known quantity of feed, while 5 days were used for the collection of data. Droppings from each replicate were collected, weighed, and recorded before they were oven dried at 700 ºC for 48 h. At the end of the 5th day, 10% of the dried sample was collected, and each replicate was pooled together and taken to the laboratory for proximate analysis. Feed consumed was recorded daily for each replicate. The nutrient digestibility was calculated using the formula:

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

Where: Proximate analysis to determine the dry matter, crude protein, crude fiber, ash, and ether extract of basal as well as the droppings from the digestibility trial was carried out in accordance with the AOAC (2016) standard procedure.

2.5 Statistical analysis

Data collected on growth performance and apparent nutrient digestibility were subjected to analysis of variance (ANOVA) using the computer software package SPSS 25.0; differences among treatment means were compared

with *Duncan's* multiple range test.

3. Results

3.1 Bioactive compounds

In Table 2, the compounds identified in the *M. quinquernervia* extract are described, with emphasis on α-copaene, 1-hexadecanol, humulene, γ-terpinene-3-carene, and benzene, 1,4-dichloro, which are the major compounds, with concentrations higher than 10 mg g^{-1}.

Table 2. Prominent bioactive compounds identified in *Melaleuca quinquernervia* extract.

Compounds*	Reaction time	Concentration (mg g^{-1})	Molecular weight (g mol^{-1})
Benzene,1,4-dichloro	10.33	11.48	147
α-copaene	11.25	607.3	268.18
γ-Terpinene-3-Carene	11.88	45.22	136.23
Humulene	12.45	78.31	204.35
1-Iodo-2-methylnonane	13.09	6.40	268
1-Hexadecanol	13.55	85.17	242.22
9,17-Octadecadienal	13.87	7.33	264.4
Palmitoleic acid	15.61	5.21	254.4
9-Eicosenoic acid	16.01	8.02	310.5
4-Trifluoroacetoxytetradecane	16.22	5.61	424.40
Bis(2-ethylhexyl) phthalate	16.98	4.96	390.6
Tetrahydrofuran,2-ethyl-5-methyl	17.03	4.05	114.2
4-Heptafluorobutyryloxyhexadecane	17.96	7.03	439.0
Naphthalene, Azulene	18.42	8.07	128.7

Note: *NIST (version 2001). Source: Author, 2025.

3.2 Performance of the M. quinquernervia extract

In Table 3, the results on the influence of the use of *M. quinquernervia* extract added to the diet on the quality of Japanese quails are described. The influence of *M. quinquernervia* extract on the growth performance of Japanese quails is presented. Daily weight gain was lower in treatment 1 [T1] (2.28 g b^{-1}) than in T2 (2.78 g b^{-1}), T3 (2.75 g b^{-1}), and T4 (2.78 g b^{-1}) ($p < 0.05$). Daily weight gain (2.28 – 2.78 g b^{-1}) increased when *M. quinquernervia* extract was fed to birds between 2 – 6 mL L^{-1} of water.

Table 3. Influence of *Melaleuca quinquernervia* extract on the growth performance of Japanese quails.

Parameters	T1	T2	T3	T4	SEM
Initial body weight (g/bird)	29.15	29.02	29.11	29.01	0.01
Final body weight (g/bird)	124.8^{b}	144.3^{a}	144.7^{a}	145.6^{a}	10.93
Body weight gain (g/bird)	95.65^{b}	115.28^{a}	115.59^{a}	116.59^{a}	9.21
Daily weight gain (g/bird)	2.28^{b}	2.74^{a}	2.75^{a}	2.78^{a}	0.01
Total feed intake (g/bird)	761.2^{b}	810.3^{a}	810.5^{a}	811.1^{a}	1.72
Daily feed intake (g/bird)	18.12^{b}	19.30^{a}	19.30^{a}	19.31^{a}	0.02
Feed conversion ratio	7.95^{a}	7.02^{b}	7.01^{b}	7.00^{b}	0.01

Note: ^{a,b} = Means on the same row with different superscripts are significantly ($p < 0.05$); T1: basal diet + *Melaleuca quinquernervia* extract; T2, T3, and T4: basal diet + *Melaleuca quinquernervia* extract at 2 mL, 4 mL,

and 6 mL per litre of water/day; SEM: standard error of mean. Source: Author, 2025.

In Table 4, the results regarding the digestibility of the diet incorporated with *M. quinquernervia* extract offered to Japanese quails are presented. The influence of *M. quinquernervia* extract on the apparent digestibility of Japanese quails revealed that dry matter, crude protein, crude fibre, ether extract, and ash values were significantly ($p < 0.05$) affected by the treatments. Dry matter digestibility in treatments T2 (87%) and T3 (88%) was similar ($p > 0.05$) to that observed in T4 (89%), but significantly higher ($p < 0.05$) than in treatment T1 (71%). Crude protein, crude fibre, ether extract, and ash values varied from 69.08-78.91%, 36.12-40.92%, 49.96-59.11%, and 35.91-50.05%, respectively.

Table 4. Influence of *Melaleuca quinquernervia* extract on apparent digestibility of Japanese quails.

Parameters (%)	T1	T2	T3	T4	SEM
Dry matter	71.19^{b}	87.83^{a}	88.09^{a}	89.17^{a}	0.93
Crude protein	69.08^{b}	77.84^{a}	78.06^{a}	78.91^{a}	0.44
Crude fiber	36.12^{b}	40.12^{a}	40.84^{a}	40.92^{a}	0.21
Ether extract	49.96^{b}	57.56^{a}	58.07^{a}	59.11^{a}	0.04
Ash	35.91^{c}	45.86^{b}	46.92^{b}	50.05^{a}	0.02

Note: ^{a,b,c} = Means on the same row with different superscripts are significantly ($p < 0.05$); T1: basal diet + Melaleuca quinquernervia extract; T2, T3, and T4: basal diet + *Melaleuca quinquernervia* extract at 2 mL, 4 mL, and 6 mL per litre of water/day; SEM: standard error of mean. Source: Author, 2025.

5. Discussions

The major compound has been previously associated with antioxidant and anti-inflammatory properties (Hernandez; Alagbe, 2025a). 1-Hexadecanol (85.17 mg g^{-1}) and humulene (78.13 mg g^{-1}) portray strong antimicrobial roles by being able to inhibit organisms associated with *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* (Hernandez; Alagbe, 2025b). Other bioactive compounds identified in this experiment offer different pharmacological activities, such as protecting hepatocytes from damage caused by toxins (Alagbe, 2025), reducing oxidative stress (Musa et al., 2020), and have no detrimental effect on the environment (Adewale et al., 2021).

The increase in body weight could be due to a high concentration of alpha-copaene (607.3 mg g^{-1}) in the extract, which has been suggested to facilitate the activities of gastrointestinal enzymes to promote efficient digestion of feed, which then translates to a better feed conversion and weight gain (John, 2024b). This outcome corresponds to the report of Juskiewicz et al. (2011) when *Macleaya cordata* alkaloid extract was fed to broiler chickens. Yesilbag et al. (2013) also recorded an average daily weight gain of 2.00 – 3.00 g b^{-1} when quails were fed a diet supplemented with rosemary and oregano essential oil mixture.

The highest daily feed intake was achieved among birds that received *M. quinquernervia* extract compared to the control. This further proves that the synergy between the phyto-compounds observed in *M. quinquernervia* extract could positively trigger increased palatability and feed consumption among quails. Previous studies by Shittu & Alagbe (2021) and John (2024b) have shown that bioactive compounds such as humulene, α-copaene, and 9,17-Octadecadienal could enhance the aroma and taste of feed. The daily feed consumption range 18.12 – 19.31 g b^{-1} recorded in this study was similar to the reports of Yesilbag et al. (2020), who discovered that daily feed intake of quails fed a diet supplemented with phytogenics ranged from 17.93 – 19.50 g b^{-1}. Feed conversion ratio was highest ($p < 0.05$) in T1 (7.95) than in T2 (7.02), T3 (7.01), and T4 (7.00).

The lower the FCR value, the better the performance of birds. The outcome of this study suggests that feeding birds with Melaleuca quinquernervia extract enhanced the production coefficient of quails. The feed conversion ratio observed in this study was higher than that obtained by Alagbe (2025), who recorded an average feed conversion ratio range of 4.31 – 5.92 when quails were fed a diet supplemented with *Canarium parvum* leaf essential oil. John (2024b) also recorded a range of 3.81 – 4.94 in Japanese quails fed a diet supplemented with *Eucalyptus* oil. Variation in results can be attributed to the chemical composition of the various bioactive compounds as well as the processing method adopted (John, 2024c).

The result obtained in this study suggests that the presence of phytoconstituents in *M. quinquernervia* extract, especially γ-Terpinene-3-Carene, α-copaene, Humulene, 1-Iodo-2-methylnonane, 1-Hexadecanol, and Benzene, 1, 4-dichloro, can protect the stomach lining, reduce pain, and promote smoother digestion of feed (John, 2024c). Findings of the present study were similar to those of Shittu et al. (2024) and Muritala et al. (2022), who recorded a significant ($p < 0.05$) difference in the apparent digestibility of birds fed a diet supplemented with phytogenics.

6. Conclusions

In conclusion, *Melaleuca quinquernervia* extract contains phyto-compounds that form an integrated biochemical system that regulates inflammation, reduces free radical activity, and promotes the health of birds. Feeding quails with *Melaleuca quinquernervia* extract, especially up to 6 mL L^{-1} of water/day, positively influenced their growth and feed intake and improved gut nutrient absorption without compromising their health status.

7. Authors' Contributions

Alagbe John Olujimi: designed the experiment, statistical analysis, and writing of the manuscript; *Karimat Imam Aliyu*: carried out the experimental design and reading of the manuscript. *Orelaja Ithiel Tijesunimi*: read the manuscript and statistical analysis.

8. Conflicts of Interest

No conflicts of interest.

9. Ethics Approval

The research was approved by the ethics committee at Sumitra Research Institute, Gujarat, India, with reference number GH/090C/2023 and carried out according to the research ethics and guidelines of the Animal Production Department of the institution.

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