Research Article

Efficacy of Methanol and Hexane Extracts of *Moringa oleifera* Seeds on Avian *Escherichia coli* and their Effects on Zootechnical Performance, Biochemical Parameters and the Gut Microbiota of Broilers

Mekuiko Watsop Hippolyte[1]*, Messi Etoundi Janvier Sosthene[1], Vougat Ngom Ronald[1], Moffo Frédéric[1], Nenba Sambo Jean Jacques[2], Danut Nicolae Enea[3], Mingoas Kilekoung Jean-Pierre[1]

[1]School of Veterinary Medicine and Sciences, University of Ngaoundere, P.O. BOX 454, Ngaoundere, Cameroon; [2]Agriculture Research Center, Institute of Agriculture Research for Development, P.O. BOX 65 Wakwa, Cameroon; [3]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania.

Abstract | Due to rising bacterial resistance and heightened public awareness of health and food-safety issues, antibiotics are no longer allowed as growth promoters in the chicken industry. There is the need to search for safe antibiotic alternatives and to improve long-term feed management practices that support better chicken health and growth. This study examined the comparative efficacy of methanol and hexane extracts of *M. oleifera* seeds on avian *Escherichia coli* blse and their effects on zootechnical, biochemical and gut microbiota parameters in broilers. A total of 250 21-day-old Cobb 500 male broilers (500.16 ± 0.73 g live weight) were randomly allocated into five treatments with five replicates and 10 chickens per replicate. Animals received basic diet without extracts and antibiotics (negative control or T0); with 1 g doxycycline (positive control or T1); or 74.73 mg hexane extract (T2); or 5.03 mg methanol extract (T3); or 19.40 mg combined methanol and hexane extract (T4). The extract doses in the feed were calculated according to the minimal inhibitory concentration. Compared with the hexane extract (291.9 mg/ml) and its combination (75.78 mg/ml), the methanol extract of *M. oleifera* seeds showed the best antibacterial activity at a low dose (19.69 mg/ml). Birds receiving *M. oleifera* seed extracts had significantly greater carcass weights than those in the negative control group. The hexane extract of *M. oleifera* promoted greater ($p = 0.044$) total protein (6.34 g/dl) than the other treatments did. The results for total coliforms revealed that the addition of *M. oleifera* extracts did not lead to significant variations ($p > 0.05$) among the different experimental groups. Overall, the methanol extract of *M. oleifera* seeds had the best low-dose antibacterial activity against avian *E. coli* blse, and the different extracts used significantly improved carcass weight. Based on this, the *M. oleifera* seed extracts may therefore be used as a potential feed additive in poultry farming.

Keywords | Antibacterial activity, Broilers, Gut microbiota, *Moringa oleifera,* Zootechnical performance

Received | March 11, 2026; **Accepted** | June 05, 2026; **Published** | July 06, 2026
***Correspondence** | Mekuiko Watsop Hippolyte, Department of Animal Production, School of Veterinary Medicine and Sciences, University of Ngaoundere, Cameroon; **Email:** hippolytewatsop87@gmail.com
Citation | Hippolyte MW, Sosthene MEJ, Ronald VN, Frédéric M, Jacques NSJ, Enea DN, Jean-Pierre MK (2026). Efficacy of methanol and hexane extracts of *Moringa oleifera* seeds on avian *Escherichia coli* and their effects on zootechnical performance, biochemical parameters and the gut microbiota of broilers. Adv. Anim. Vet. Sci., 14(7):1426-1436.
DOI | https://dx.doi.org/10.17582/journal.aavs/2026/14.7.1426.1436
ISSN (Online) | 2307-8316

INTRODUCTION

Antibiotic growth promoters (AGPs) are antibiotics added to animal feed at sub-therapeutic doses to improve growth and feed efficiency (Niewold, 2007). While they initially increased productivity in intensive livestock systems, later studies raised concerns about possible risks to human health (Wickramasuriya *et al*., 2024). After more than seven decades of use in food-producing animals such as poultry (Dibner and Richards, 2005; Miyakawa *et al*., 2024) widespread antibiotic use contributed to the emergence and spread of antimicrobial resistance genes in the gut microbiota, with potential downstream risks for humans (Kim and Ahn, 2022). Given the regulatory restrictions on AGPs and the increasing expectations from consumers, producers and researchers have been prompted to identify strategies that can sustain intensive animal production while still ensuring an adequate and continuous supply of meat and eggs. In line with this need, studies evaluating potential substitutes for AGPs have gained prominence as an important research priority (Kurt *et al*., 2019). Finding substitutes for antibiotics, previous studies have focused mainly on phytobiotics or phytogenics. Plant extracts and their biologically active constituents represent some of the most commonly applied phytogenic products in poultry nutrition (Hashemi and Davoodi, 2012). Several uses of these plants have been reported (Renata *et al*., 2021) because of their potential to improve production performance (Rahimi *et al*., 2011; Costa *et al*., 2020), as well as animal health (stability of the gut microbiota) (Urban *et al*., 2024). They are also used for their antimicrobial (Walter *et al*., 2011; Renata *et al*., 2021), biochemical and immunomodulatory (Renata et al., 2021; Abd El-Ghany, 2020; Khan *et al*., 2021) properties. Moreover, in addition to their antifungal properties (Aondo *et al*., 2018; Khan *et al*., 2021), plant extracts used as phytogenic feed additives improve zootechnical performance, intestinal wall histomorphology, biochemical profiles, carcass characteristics and the intestinal bacterial profile of broilers more than antibiotics do (Renata et al., 2021).

As a promising plant, *M. oleifera*, is classified as an important herbal plant due to its immense medicinal and nonmedicinal benefits (Pareek *et al*., 2023). It is a Moringaceae family of trees and shrubs comprising 14 species, of which *Moringa oleifera* is the most known and widely used (Rakotomamonjy, 2016). *M. oleifera* contains antinutritional substances such as trypsin inhibitors, phytates, tannins, oxalates, cyanide, and saponins that can hinder the absorption of protein, minerals, vitamins, and polyphenols (Richter *et al*., 2003; Abd - Elhakim et al., 2018). To address this, specific solvents are used during extraction (methanol and hexane extraction) to reduce the concentration of these antinutritional factors and enhance nutrient availability. Plant extracts (leaves, seeds, roots, flowers, pods and stems) of *M. oleifera* are known to be rich in polyphenols and flavonoids. Recent modern microbiological studies have shown that the hexane extracts of *M. oleifera* and *M. stenopetala* strongly inhibited three bacterial species (*Salmonella typhii*, *Vibrio cholerae* and *Escherichia coli*) that cause water-borne diseases (Walter *et al*., 2011; Pareek *et al*., 2023), indicating that *M. oleifera* and *M. stenopetala* possess antibacterial properties, particularly at low doses.

In view of the various studies carried out on phytobiotics in general and, more specifically, on the versatile virtues of *M. oleifera,* the use of this plant and its extracts could constitute one of several strategies to be included in national or even global action plans to combat antimicrobial resistance. This could have a greater impact when a formula is included for the administration of doses of extracts for targeted action directly in the gut, enabling treatments to be controlled by the administration of so-called adequate doses, as it is essential to know the exposure to the drug at the site of infection (Nielsen and Friberg, 2023). The polarity of the solvent affects the efficiency of extraction of bioactive compounds (Kaczorová *et al*., 2021). Thus, methanol, a polar solvent, would extract a higher amount of phenolic and flavonoid compounds, leading to a stronger antibacterial activity than that obtained with hexane, a non-polar solvent. The present study aimed to evaluate the comparative efficacy of methanol and hexane extracts of *M. oleifera* seeds on avian *E. coli* blse, as well as their effects on zootechnical parameters, the intestinal microbiota and some biochemical parameters of Cobb 500 broilers.

MATERIALS AND METHODS

Study site

The present study was carried out between April and November 2024 in three different areas in Ngaoundéré (Cameroon): at the Laboratory of Chemistry and Environment of the Faculty of Science, University of Ngaoundere; at the Laboratory of the Institute of Agriculture Research for Development (IARD), and at a private experimental poultry farm located in the peri-urban area.

Sources of treatments and preparation of *M. oleifera* seed extracts

The plant material, consisting of *M. oleifera* seeds, was collected from Ngaoundere town, Cameroon, and air-dried in the shade at room temperature. botanical identification keys were used for species identification (Spichiger *et al*., 2002), with reference to the International Code of Botanical Nomenclature (Greuter, 2003). The dried *M. oleifera* seeds were cleaned and ground to powder via an

electric grinder and a fine sieve (1.25 mm mesh) to enable hexane and methanol extractions, which were carried out in the Laboratory of Chemistry and Environment of the Faculty of Science, University of Ngaoundere. The recovered powder was sieved and stored in hermetically sealed glass vials protected from light until use. 500 g of powder was macerated in 3 L of methanol. A similar quantity of powder was macerated in 3 L of hexane. After 24 hours of maceration at room temperature, the products obtained were filtered through Whatman (N° 1) filter paper and concentrated in a rotary evaporator. The extracts obtained were weighed and stored in a refrigerator at 4°C until use (Gata-Gonçalves *et al.*, 2003). The extraction yield (EY) of *M. oleifera* seeds, estimated as a percentage (Figure 1), was calculated in relation to the weights of the plant material used, according to the formula proposed by Ambang *et al.* (2011):

$$EY\ (\%) = \frac{Extract\ mass(g)}{Mass\ of\ plant\ material\ (g)} x\ 100$$

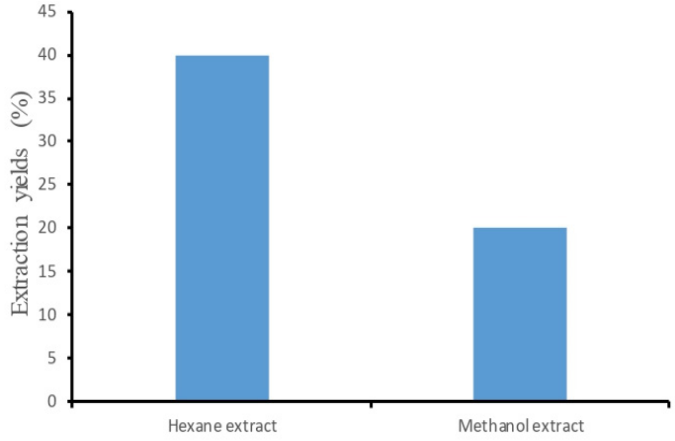

Figure 1: Extraction yield according to silvent. It appears that the extraction yield is higher with the hexane extract (40%) compared to the methanol extract (20%).

Phytochemical screening of *Moringa oleifera* extracts

This phytochemical screening was carried out to characterize the alkaloids (Dohou *et al.*, 2004), phenolic compounds (Talla *et al.*, 2016), triterpene steroids and flavonoids (Fankam *et al.*, 2011), tannins saponins, glycosides and anthraquinones (Fankam *et al.*, 2011) present in *M. oleifera* extracts. Our phytochemical study was qualitative.

In vitro susceptibility studies

Determination of the minimum inhibitory concentrations

The minimum inhibitory concentration is defined as the lowest concentration able to prevent any visible growth after an incubation period of 18-24 hours. The minimum inhibitory concentrations (MIC) of the methanol and hexane extracts of *M. oleifera* seeds were determined via the microdilution method using Muller Hinton broth (MHB) in 96-well microplates. The plant extracts (methanol, hexane and mixed extracts (v/v)) were first dissolved in 10% dimethyl sulfoxide (Naz *et al.*, 2020) to reach a concentration of 900 microlitres per millilitre, and twofold dilutions were made with the culture broth. The methanolic and hexanic extracts were prepared separately and then mixed in equal proportions (1:1, v/v) to produce the final combined extract for testing, with 450 µL of each (Mohammad *et al.*, 2016). Each test sample and growth control (broth plus dimethyl sulfoxide, either without plant extract or with antimicrobial compounds) was inoculated with 100 µl of bacterial suspension containing 5×10^6 UFC/ml and incubated for 24 h at 37°C. After incubation, a 40 µL solution of resazurin (0.1 g resazurin tablet dissolved in 1000 ml sterile distilled water) was added to each sample, and the mixture was incubated for 30 min at 37°C. Bacterial proliferation was assessed by an observable shift in color from violet to pink or colorless (scored visually). All tests were performed in triplicate to evaluate each dilution for the target bacterial strain and to establish the MIC.

Determination of minimum bactericidal concentration (MBC)

The Minimum Bactericidal Concentration (MBC) is the lowest concentration of crude *M. oleifera* seed extract that killed 99.99% of the organisms tested. The MBC was determined by adding 50 µl aliquots of the serial dilution that showed no visible growth after incubation in the MIC test and adding 50 µl developer (resazurin) to 100 µl Muller–Hinton broth (Nkala *et al.*, 2019). After incubation at 37°C for 24 hours, the presence of bacterial growth was indicated by a pink color, and colorless or purple wells indicated the inhibition of bacterial growth by the extract. Control streaks from each well were run on Mueller–Hinton agar medium to visually observe the lowest concentration that indicated growth inhibition.

Determination of the combined action of methanol and hexane extracts

The values of the different fractional inhibitory concentrations (FIC) were determined according to the method proposed by Alshareef (2021).

In vitro antibacterial activity of *M. oleifera* seed extracts against *Escherichia coli*

The antimicrobial activity of *M. oleifera* seed extract against *E. coli* was tested via the microdilution method. The organism was identified at Cameroon's national veterinary laboratory and inoculated at the IARD, Wakwa, Cameroon. On the basis of IARD, it was inoculated into nutrient broth (Mueller Hinton) and incubated for 12 h

at 37 °C to reach a turbidity of 0.5 McFarland. A uniform microbial culture broth was used.

In vivo study

Diets formulation and experimental design

A total of 250 21-day-old male Cobb 500 chicks (500.16 ± 0.73 g live weight) were purchased from the *Société de Provenderie du Cameroun* (SPC) already vaccinated against Newcastle disease and infectious bronchitis, and a routine check was carried out. Using a completely randomized design, the chicks were allocated into 25 pens (each pen serving as an experimental unit). The five dietary treatments were randomly distributed to the pens, with five replicates per treatment; thus, each replicate pen contained 10 birds. Birds were reared on the floor in pens of 2.5 m×1.0, with partitions made from galvanized wire mesh. Each pen was lined with white wood shavings as litter. This study was carried out following the applicable guidelines and regulations, and received ethical approval from the Scientific Research and Ethics Committee of the School of Veterinary Medicine and Sciences, University of Ngaoundéré, Cameroon (Ref. No. 2021/2022/061/UN/ESMV/DAACRS/SSFC of 27 March 2024).

The centesimal composition of the ingredients and the chemical composition of the experimental diet are presented in Table 1.

Doses of extracts in chicken diets

The dose of extracts to be incorporated into the diet was determined by the following formula, adapted from the formulas proposed by Medbullets Step 1 and the *Collège National de Pharmacologie Médicale* (CNPM). In our approach, we adapted pharmacological models described in the literature to obtain an exploratory estimate of the dietary doses based on the MIC values determined in vitro (Medbullets Step 1 and CNPM) (Ricard *et al.*, 1967; Velmurugu and Abdollahi, 2021). Since the intended site of action was primarily intestinal, we assumed, for the sake of simplification, a theoretical bioavailability of 1. This assumption was not meant to reflect true systemic bioavailability; rather, it was used to presume that the entire administered extract remained available within the intestinal lumen, which represented the main target site against enteric pathogens.

$$Dose\ (mg/kgDM) = \frac{Target\ concentration \times VDT}{Bioavailability}$$

VDT: volume of the digestive tract, representing 3.2% of the chicken's live weight (Ricard *et al.*, 1967; Velmurugu and Abdollahi, 2021).

$$Target\ concentration = MIC \times 4$$

Bioavailability: represents the fraction of extract that reaches the intestine.

Table 1: Experimental diets.

	Growth (21-35 d)					Finishing (35-42 d)				
Ingredients (kg/100 kg)	**T0**	**T1**	**T2**	**T3**	**T4**	**T0**	**T1**	**T2**	**T3**	**T4**
Corn meal	48	48	48	48	48	56	56	56	56	56
Remoulage	17.5	17.5	17.5	17.5	17.5	11.5	11.5	11.5	11.5	11.5
Soya cake 49	15	15	15	15	15	14	14	14	14	14
Groundnut cake	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Doxycycline	0	1	0	0	0	0	1	0	0	0
Hexane extract	0	0	0.07473	0	0	0	0	0.07473	0	0
Methanol extract	0	0	0	0.00503	0	0	0	0	0.00503	0
combined methanol and hexane extract	0	1	0	0	0.0194	0	1	0	0	0.0194
Concentrate 10%	10	10	10	10	10	10	10	10	10	10
Bone meal	2	2	2	2	2	1	1	1	1	1
Belgotox	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Total	100.15	100.15	100.224	100.155	100.169	100.15	100.15	100.224	100.155	100.169
Nutritive values										
Dry matter (%)	89.47					89.47				
Crude protein(% DM)	20.59					19.00				
Fibre (% DM)	1.62					1.71				
Crude cellulose (% DM)	3.47					3.66				
Metabolizable energy (kcal/kg DM)	2820.85					2998.45				

On the basis of the calculations via this formula, birds in each group received one of the following treatments during the study period: base diet without extracts or antibiotics (negative control or T0); base diet with 1 g doxycycline/kg DM (positive control or T1); base diet with 74.73 mg hexane extract/kg DM (T2); base diet with 5.03 mg methanol extract/kg DM (T3); and base diet with 19.40 mg combined methanol and hexane extract/kg DM (T4).

Growth performance

Chicks were first weighed to obtain the initial mean body weight (500.16 ± 0.73 g). Thereafter, body weights were recorded weekly over a 3-week period using digital scales (SH-125; range 1–7000 g) to determine weekly body weight gain. For feed utilization, the diet offered each day was weighed prior to feeding, and the remaining feed (refusals) was collected and weighed before the next feeding. Daily feed intake (FI) was determined as the difference between the amount offered and the amount refused. The feed conversion ratio (FCR) was then computed as FI divided by body weight gain.

Carcass characteristics

On the 42nd day of age, 2 broilers per experimental unit × 5 replications were slaughtered to record carcass characteristics. Body weight was averaged per compartment or replication. The calculated variables were carcass percentage (%) and the relative weights of several organs (gizzard, liver, heart, legs, abdominal fat and intestinal weight). The relative weight of each organ was calculated by dividing the weight of the carcass or that of the corresponding organ by the live body weight of the bird (Gorenz *et al*., 2024).

Biochemical parameters

At 42 days of age, blood samples were collected randomly from five birds per replicate pen. After puncturing the birds' branchial veins with sterile needles and using 5 mL syringes, approximately 4 mL of blood was drawn into two sets of sterile tubes for serum biochemical determination. The samples designated for serum analyses were then centrifuged at room temperature for 10 min at 3000 rpm to separate the serum (Iyaode *et al*., 2020). The serum biochemical indices (total cholesterol, glucose and total protein) were analysed at the IARD Physiology and Biochemistry Laboratory in Wakwa, Cameroon. Proportioning was colorimetric, and the absorbance was measured via a spectrophotometer.

Evaluation of the effects of different *M. oleifera* seed extracts on the intestinal microbiota

On the last day of the feeding study, one bird from each replicate, i.e., a total of five chickens per treatment, was randomly selected and transported in an appropriate car to the IARD Laboratory. The chickens were slaughtered, and the caecal and rectal contents were collected to determine the total number of viable coliforms. The determination of *E. coli* and coliform populations followed the procedure of Sudatri (2021) via the scatter method in Eosin Methylene Blue (EMB) agar media. Five grams of the digested sample was put into an Erlenmeyer flask containing 0.1% peptone water solution with a volume of 45 ml, resulting in a $^{10-1}$ dilution. Planting was carried out at dilution levels of 10^{-1} to 10^{-7}, to count the growing bacterial colonies via the cup count method, namely, by selecting the number of colonies that grew ranging from 30–300 colonies. We used total coliforms as a general indicator of enteric bacterial load and intestinal microbial balance. The in vitro study had already demonstrated antibacterial activity of the extracts against isolated *Escherichia coli* strains. The in vivo assessment of total coliforms was therefore used as a complementary indicator of the overall intestinal impact of the treatments.

Statistical analysis

The recorded data were entered into an Excel spreadsheet (version 2019) and analysed via SPSS (ver. 20.0, Chicago, IL, USA). The normality and homogeneity of variance of all the data collected were first examined via the Kolmogorov–Smirnov test and Levene's test. One-way analysis of variance (ANOVA) followed by Duncan's test was subsequently performed to investigate the differences in growth performance, carcass characteristics, relative proportions of organs, biochemical parameters and the gut microbiota of broilers among the different treatments. As for the biochemical parameters, we average the 5 birds per replicate pen first. For all the analyses, a significance level of 5% was fixed.

RESULTS

Phytochemical screening of *Moringa oleifera* seed extracts

The results of the phytochemical screening of *M. oleifera* seed extracts revealed the presence of several chemical compounds belonging to various families of secondary metabolites. The presence of these compounds varied according to the type of solvent used (Table 2). Indeed, alkaloids, triterpenes and flavonoids were present in all the different *M. oleifera* seed extracts analysed (methanol and hexane). However, tannins, anthraquinones and glycosides were absent from all the extracts. Saponins and phenolic compounds were found only in the methanol extract.

Compared with the hexane extract, the methanol extract was found the richest in chemical compound families (with a strong presence of flavonoids, triterpenes, saponins and phenolic compounds).

Table 2: Phytochemical screening of *Moringa oleifera* seed extracts.

Compound family	Methanol extract	Hexane extract
Alkaloids	+	+
Flavonoids	+++	+
Triterpenes	+++	+++
Tannins	-	-
Saponins	+++	-
Phenolic compounds	++	-
Anthraquinones	-	-
Glycosides	-	-

(-) : Absence, (+) : presence, (++):strong presence

Antibacterial activity of *Moringa oleifera* seed extracts

The *in vitro* antibacterial activity of *M. oleifera* seed extracts was assessed against *E. coli* Blse. Minimal inhibitory concentration (MIC) values for the antibacterial activity of *M. oleifera* seed extracts were obtained (Table 3). The methanol extract of *M. oleifera* seeds had significantly greater antibacterial activity, with a minimum MIC value of 19.69 mg/ml. In comparison, the highest MIC value was obtained for the hexane extract of *M. oleifera* seeds (291.9 mg/ml). An intermediate MIC value (75.78 mg/ml) was recorded for the mixture of hexane and methanol extracts.

Table 3: *In vitro* antibacterial activity of *M. oleifera* seed extracts against *E. coli*.

Extracts	MIC (mg/ml)	CMB (mg/ml)
Hexane	291.9	/
Methanol	19.64	78.57
Hexane+ Methanol	75.78	303.21

/: no bactericidal effect; MIC: Minimal inhibitory concentration; MBC: minimum bactericidal concentration.

The methanol extracts and the combination of extracts had bactericidal effects, with MBC values close to those of the MIC, highlighting their bactericidal characteristics. In contrast, the hexane extract had no bactericidal effect.

Effects of *Moringa oleifera* seed extracts on broiler growth performance

Table 4 shows the performance of broilers according to treatment. The incorporation of *M. oleifera* seed extracts (methanol, hexane or hexane + methanol mixture) into the broiler diet had no significant effect ($P > 0.05$) on feed intake and weight gain. The feed conversion ratio of broiler chickens fed diets T0, T1, T3, and T4 were comparable and were significantly ($p<0.05$) higher than those in chickens fed diet T2.

Effects of *Moringa oleifera* seed extracts on the carcass characteristics of broilers

The effects of *M. oleifera* seed extracts on broiler carcass characteristics are shown in Table 5. Carcass weight was significantly ($p < 0.05$) greater in birds fed *M. oleifera* seed extracts than in those in the negative control group (T0). On the other hand, live weights and carcass yields remained comparable ($P > 0.05$) irrespective of diet.

Effects of *Moringa oleifera* seed extracts on the relative proportions of organs

Table 6 shows that the addition of moringa extracts to the broilers' diet did not significantly ($p>0.05$) influence the relative proportions of organs studied (heart, gizzard, legs, head and liver).

Effects of *Moringa oleifera* seed extracts on biochemical parameters

Table 7 shows that blood glucose and cholesterol concentrations were not significantly influenced by the incorporation of *M. oleifera* extracts into broiler diets. However, The total serum protein levels in broiler chickens fed diets T0, T1, T2, and T3 were comparable and were significantly ($p<0.05$) higher than those in chickens fed diet T4.

Effects of *Moringa oleifera* seed extracts on the gut microbiota

The effects of *M. oleifera* seed extracts on the gut microbiota, more specifically on coliform bacteria, are presented in Figure 2. The results revealed that the addition of extracts at different specific concentrations resulted in no significant difference ($p>0.05$) between treatments. Nevertheless, it was observed that the lowest coliform population (7.46 log cfu/g) was recorded in birds that received the hexane-methanol extract mixture (T4).

Table 4: Performance of broilers according to treatment.

Parameters	Treatments					p
	T0	T1	T2	T3	T4	
DFI (g/day)	107.18± 3.31[a]	120.37 ± 5.7[a]	103.7 ± 7.68[a]	115.25 ± 5.02[a]	105.83 ± 6.56[a]	0.09
DIWG (g/day)	45.19 ± 1.36[a]	55.23 ± 3.38[a]	55.08± 1.35[a]	52.38 ± 4.01[a]	51.14 ± 1.2[a]	0.27
FCR	2.44 ± 0.0[3]b	2.45 ± 0.1[1]b	2.03 ± 0.1[2] a	2.50 ± 0.0[7]b	2.20 ± 0.1[7] [a]b	0.02

[a,b]Value within line with different superscripts differ at $p < 0.05$; T0: Base diet without extracts or antibiotics; T1: Base diet with 1 g Doxycycline/kg DM; T2: Base diet with 74.73 mg hexane extract/kgDM; T3: Base diet with 5.03 mg methanol extract/kg DM; T4: Base diet with 19.40 mg combined methanol and hexane extract/kgDM; DFI= daily feed intake; DIWG: daily individual weight gain; FCR= feed conversion ratio.

Table 5: Effect of *M. oleifera* seed extracts on carcass characteristics of broilers.

Parameters	Treatments					p
	T0	T1	T2	T3	T4	
LW (g)	1826.66 ± 90.61 [a]	1918.33 ± 19.64 [a]	1878.33 ± 41.26 [a]	1850 ± 108.27 [a]	1815 ± 83.11 [a]	0.946
CW (g)	1272.33 ± 32.34 [a]	1408.33 ± 6.74[c]	1310.67 ± 17.18[ab]	1355.3 ± 41.16[b]	1309.67 ± 18.25[ab]	0.001
CY (%)	69.58 ± 1.22 [a]	73.41 ± 0.99 [a]	69.80 ± 1.22 [a]	72.86 ± 3.62 [a]	72.05 ±1.92 [a]	0.151

[a,b,c]value within line with different superscripts differ at p<0.05; LW: live weights; CW: Carcass weight; CY: carcass yields; T0: Base diet without extracts or antibiotics; T1: Base diet with 1 g Doxycycline/kg DM; T2: Base diet with 74.73 mg hexane extract/kgDM; T3: Base diet with 5.03 mg methanol extract/kg DM; T4: Base diet with 19.40 mg combined methanol and hexane extract/kgDM.

Table 6: Effect of *M. oleifera* seed extracts on relative proportion of organs.

Variables (%)	Treatments					p
	T0	T1	T2	T3	T4	
Heart	0.80 ± 0.02	0.70 ± 0.01	0.91 ± 0.02	0.77 ± 0.03	0.67 ± 0.04	0.784
Liver	3.26 ± 0.10	2.93 ± 0.13	3.22 ± 0.32	2.93 ± 0.07	3.04 ± 0.13	0.556
Gizzard	3.42 ± 0.39	2.47 ± 0.12	2.66 ± 0.1	2.45 ± 0.23	3.28 ± 0.55	0.178
Legs	5.63 ± 0.47	5.20 ± 0.24	6.25 ± 0.01	5.63 ±0.41	5.62 ± 0.45	0.422
Head	3.29 ± 0.21	2.98 ± 0.08	3.34 ± 0.26	3.28 ±0.1	3.10 ± 0.25	0.677

T0: Base diet without extracts or antibiotics; T1: Base diet with 1 g Doxycycline/kg DM; T2: Base diet with 74.73 mg hexane extract/kgDM; T3: Base diet with 5.03 mg methanol extract/kg DM; T4: Base diet with 19.40 mg combined methanol and hexane extract/kgDM.

Table 7: Effect of M. oleifera seed extracts some biochemical parameters.

Parameters	Treatments					p
	T0	T1	T2	T3	T4	
Glucose (mmol/l)	6.22 ± 0.34[a]	5.98 ± 0.27[a]	6.24 ± 0.71[a]	6.33 ± 0.23[a]	5.44 ± 0.11[a]	0.103
Total proteins (g/dl)	6.21 ± 0.51[b]	5.81 ± 0.93[ab]	6.34 ± 0.59[b]	5.85 ± 0.14 [ab]	4.89 ± 0.17[a]	0.044
Cholesterol (mg/dl)	200.85 ± 23[a]	203.28 ±37[a]	199.47 ± 25[a]	197.85 ± 12[a]	207.20 ± 15[a]	0.140

[a,b] Value within line with different superscripts differ at p<0.05; T0: Base diet without extracts or antibiotics; T1: Base diet with 1 g Doxycycline/kg DM; T2: Base diet with 74.73 mg hexane extract/kgDM; T3: Base diet with 5.03 mg methanol extract/kg DM; T4: Base diet with 19.40 mg combined methanol and hexane extract/kgDM.

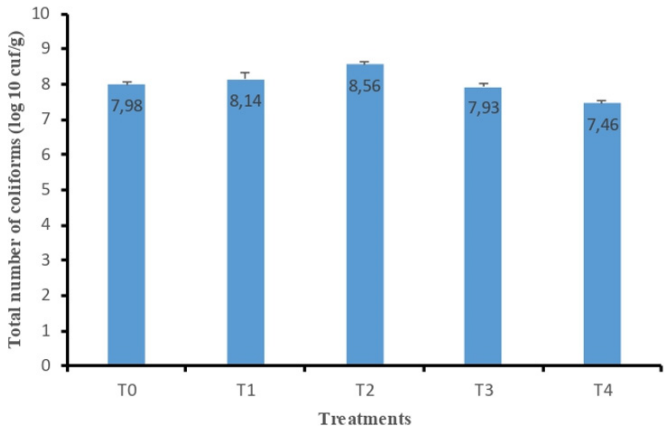

Figure 2: Effects of *M. oleifera* seed extracts on the gut microbiota

DISCUSSION

Antibiotics have been used as a growth promoter for decades to increase meat production, accelerate poultry growth and prevent disease. The intensive use of antibiotics has led to the development of resistant pathogens with serious effects on public and environmental health, making it imperative to find alternatives to antibiotics as growth promoters in this sector. For this reason, the use of *M. oleifera* seeds and extracts could be a strategy to combat antimicrobial resistance and increase poultry production. The aim of the present study was to evaluate the comparative antibacterial efficacy of methanol (a highly polar solvent) and n-hexane (a nonpolar solvent) extracts of *M. oleifera* seeds and their combination on avian *E. coli* and their effects on zootechnical and biochemical parameters and on the gut microbiota of Cobb 500 broilers. Phytochemical screening of *M. oleifera* seed extracts revealed the presence of several chemical compounds belonging to various families of secondary metabolites. Alkaloids, triterpenes and flavonoids were present in all the extracts (methanol and hexane) of the *M. oleifera* seeds analysed. However, tannins, anthraquinones and glycosides were absent from all the extracts. Saponins and phenolic compounds were found only in the methanol

extract. These results are similar to those of Ayirezang *et al.* (2020), whose analysis of phytochemical compounds found in *M. oleifera* seeds independently of the extraction solvent used revealed the presence of alkaloids, resins, terpenoids, tannins, flavonoids, glycosides and saponins, among others. The differences observed are probably because the phytobiotic components of plants vary according to the environmental conditions of the growing area (Radkowska, 2013). Determination of the chemical compounds present in *M. oleifera* seeds clearly revealed that plants synthesize bioactive or phytobiotic compounds to protect themselves against invasive pathogens such as bacteria, viruses and fungi (Kikusato, 2021).

The results of the antibacterial activity revealed that the methanol extract of *M. oleifera* seeds (EMGMO) had the maximum antibacterial activity, with the lowest MIC of 19.69 mg/ml, in contrast to the hexane extract of *M. oleifera* seeds (EHGMO) (291.9 mg/ml), whereas the mixture of hexane and methanol extracts (EHMGMO) (v/v) achieved an intermediate MIC of 75.78 mg/ml. These MIC results corroborate those of Walter *et al.* (2011), who showed that both methanol and hexane extracts inhibited the *E. coli* responsible for waterborne diseases but contradict that hexane extracts at higher concentrations were numerically more effective against *E. coli* than methanol extracts were. The opposite was true in this study, probably due to differences in the phytobiotic compositions responsible for the antibacterial effect. The results of this study are similar to those of Romanucci *et al.* (2020), whose MIC and MBC values for tri-terpenes generally ranged from 17.1 to 21.6 mg/ml against *E. coli,* demonstrating the effective bactericidal activity of at least one phytobiotic compound present in the methanol extract of *M. oleifera* seeds. The significant variation in chemical composition might explain the variations observed in the antibacterial activity of extracts from the same part of the plant used. Similarly, a trend has been shown indicating that extracts richer in flavonoids exhibit the highest antibacterial activities (Beddou, 2015). It may be assumed that the antagonistic effect is due mainly to the excessively high MIC value of the hexane extract and the consequent reduction in the content of phytobiotic active compounds, which in this case reduces the antibacterial effect.

The incorporation of the different extracts into the broiler diet did not significantly influence ($p > 0.05$) the feed intake. This lack of a significant effect contrasts with the findings of some studies but is consistent with those of other authors. Tekce *et al.* (2020) and Abou-Elkhair *et al.* (2020) reported improved feed intake in response to different forms and doses of *M. oleifera* in the diet. However, Macambira *et al.* (2022) reported that the inclusion of Moringa leaf powder at concentrations up to 6% did not significantly affect feed intake in broilers. The results of this study are in line with the latter work, suggesting that seed extracts, such as leaves, do not significantly influence feed intake at certain doses. Paul *et al.* (2018) reported a reduction in feed intake with an aqueous extract of *M. oleifera* leaves, suggesting that the type of extract (aqueous extract, methanol or hexane extract, powder) and the part of the plant used (leaves or seeds) could lead to variations. The average daily gain (ADG) was not significantly influenced by the incorporation of the different extracts in the broiler diet. These results are in line with the studies of Wahab *et al.* (2020). However, Dey and Partha (2013), suggested that Moringa may improve digestibility and nutrient absorption, which may be reflected in an increase in the ADG and total weight gain of the subjects. Compared with the other groups, the group receiving hexane extract from the diet presented the best feed conversion ratio, although no significant differences were observed. These results corroborate the observations of Paul *et al.* (2018), who reported an improvement in FCR with an aqueous extract of Moringa leaves compared with antibiotic treatment. On the other hand, these results disagree with those of Khan et al. (2012), who reported that the addition of Moringa meal to the broiler diet reduced feed conversion efficiency. These variations are thought to be because the bioactive components of the hexane extracts of the seeds differ from those of the leaves or their aqueous extracts, which reduces their potential to improve feed efficiency.

The carcass yield was not significantly influenced ($p>0.05$) by the different treatments, although the diet containing doxycycline had the highest value (73.41%). On the other hand, the carcass weights of batches that consumed a base diet with 5.03 g methanol extract/kg DM were significantly greater ($p<0.05$) than those of the negative control. These results are partially consistent with the work of Mousa *et al.* (2017), who reported that *M. oleifera* may be responsible for the increase in carcass weight and yield, which could be explained by the presence of antioxidants (ascorbic acid, tocopherol) that reduce oxidative stress and increase protein absorption (Qwele *et al.*, 2013).

The different extracts did not significantly affect ($p > 0.05$) the serum glucose concentration or cholesterol level. On the other hand, significant differences ($p<0.05$) were observed in the total protein values. The highest total protein values (6.34 g/dl) were recorded in birds fed a diet containing hexane extract of *M. oleifera* seeds. Similar results were reported by Khan *et al.* (2021), who reported that *M. oleifera* can increase serum and plasma total protein concentrations. The serum cholesterol content of birds fed *M. oleifera* seed extracts in their diet was not significantly different from that of the Doxy batch or the negative control. These results diverge from the work of Dey and

Partha (2013), who noted a decrease in plasma cholesterol with *M. oleifera*. This discrepancy could be attributed to differences in the active compounds in the leaves and seeds or to variations in the blood products used.

The addition of *M. oleifera* seed extracts to the broiler diet did not result in significant differences in coliform populations between the different experimental groups ($p > 0.05$). However, notable variation was observed between the treatments. This result agrees with the latter study (hexane + methanol) of *M. oleifera* seeds presented the lowest coliform population (7.46 log cfu/g), whereas the batch treated with the hexane extract presented the highest population (8.56 log cfu/g). This observation could be linked to the variability of the antimicrobial properties of the extracts, which depend on their specific chemical compositions, including the polyphenols and triterpenes that inhibit the growth of pathogens such as *E. coli* (Romanucci *et al*., 2020). The results of this study are in agreement with those of Farahat *et al*. (2021), whose supplementation with plant extracts significantly reduced the number of coliform bacteria.

CONCLUSION

This study aimed to assess the comparative efficacy of methanol and hexane extracts of *M. oleifera* seeds on avian *Escherichia coli* bacteria and their effects on zootechnical, biochemical and gut microbiota parameters in broilers. The methanol extract of *M. oleifera* seeds had the best low-dose antibacterial activity against avian *E. coli* bacteria. *M. oleifera* seed methanol extracts contribute significantly to improving the total protein content of birds. Birds receiving *M. oleifera* seed extracts had significantly greater carcass weights than those in the negative control group. However, it does not significantly influence the others production performance or total coliforms. On the basis of the results obtained, the *M. oleifera* seed extracts could therefore be used as a feed additive in poultry farming, especially in broilers.

ACKNOWLEDGMENTS

The authors would like to thank and appreciate the School of Veterinary Medicine and Sciences, University of Ngaoundere, Cameroon, for the facilities used to conduct the present study.

NOVELTY STATEMENTS

The novelty of our work entitled "Efficacy of methanol and hexane extracts of *Moringa oleifera* seeds on avian *Escherichia coli* and their effects on zootechnical performance, biochemical parameters and the gut microbiota of broilers" can be summarized as the methanol extract of *M. oleifera* seeds the best low-dose antibacterial activity against avian *E. coli* blse, and the different extracts used significantly improved carcass weight. The study suggest *M. oleifera* as a potential natural feed additive in poultry diet, especially in broilers.

AUTHOR'S CONTRIBUTION

MWH: Conceptualization, data curation, supervision, formal analysis, resources, funding acquisition, investigation, methodology, project administration, writing original draft, writing review and editing. EMJS: Conceptualization, data curation, formal analysis, funding acquisition, methodology, software, writing review and editing. VNR: Data curation, formal analysis, funding acquisition, investigation, methodology, resources, software, visualization, writing review and editing. MF: Data curation, formal analysis, funding acquisition, software, writing review and editing. NSJJ: Visualization, writing review and editing. DNE: Visualization, data curation, methodology, writing review and editing. MKJ-P: Project administration, supervision, validation, visualization, writing original draft, writing review and editing.

Funding

This project was supported by the authors.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Abd-El-Ghany WA (2020). Phytobiotics in Poultry industry as growth promoters, antimicrobials and immunomodulators. Rev. World's Poult. Sci., 10(4): 571-579. https://doi.org/10.36380/jwpr.2020.65

Abd-Elhakim YM, El-Bohi KM, Hassan SK, El Sayed S, Abd-Elmotal SM (2018). Palliative effects of *Moringa olifera* ethanolic extract on hemato-immunologic impacts of melamine in rats. Food Chem. Toxicol., 114: 1– 10. https://doi.org/10.1016/j.fct.2018.02.020

Abou-Elkhair R, Abdo BH, Slouma HAEN, Ajarem JS, Maodaa SN, Allam AA, Naiel MAE (2020). Effect of a diet supplemented with the *Moringa oleifera* seed powder on the performance, egg quality, and gene expression in Japanese laying quail under heat-stress. Animals, 10(5): 809. https://doi.org/10.3390/ani10050809

Alshareef F (2021). Protocol to evaluate antibacterial activity MIC, FIC and time kill method. Acta Sci. Microbi., 4(5): 2-6. https://doi.org/10.31080/ASMI.2021.04.0825

Ambang Z, Essono G, Kosma P, Ndongo B, Ngoh JP (2011). Control of leaf spot disease cause by *Cercospora* sp. on groundnut (*Arachis hypogea*) using Méthanolic extracts of yellow oleander (*Thevetia peruviana*) seeds. Aust. J. Crop Sci., 5(3): 227-232.

Aondo TO, Odiaka NI, Akesa TM, Olaleye OO (2018). Efficacité phytochimique et antifongique de différentes parties d'extraits de plantes de *Moringa oleifera*. Asian J. Biotechnol. Bioresour. Technol., 3(2): 1-8. https://doi.org/10.9734/AJB2T/2018/40198

Ayirezang FA, Azumah BK, Achio S (2020). Effects of *Moringa oleifera* leaves and seeds extracts against food spoilage fungi. Adv. Microbiol., 10(1): 27-38. https://doi.org/10.4236/aim.2020.101003

Beddou F (2015). Phytochemical study and biological activities of two Saharan medicinal plants *Rumex vesicarius* L. and *Anvillae radiate* Coss. Dur. Doctoral thesis. Université Abou Bekr Belkaid, Tlemcen, Algérie. pp. 143.

Callaway TR., Lillehoj H, Chuanchuen R, Gay CG (2021). Alternatives to antibiotics: a symposium on the challenges and solutions for animal health and production. Antibiotics, 10(5): 471. https://doi.org/10.3390/antibiotics10050471

Costa TF, Gouveia ABVS, Nunes FC, Sampaio SA, Silva NGD, Abreu JM, Júnior EMA, Costa KO, Feitosa TJO, Paulo LM, Souza CS, Minafra-Rezende CS, Santos FR, Minafra CS (2020). Phytogenic additives: Essential oils for broilers - review. Res. Soc. Dev., 9(3): 20p. https://doi.org/10.33448/rsd-v9i3.2325

Dey A, Partha S (2013). Influence of *Moringa oleifera* leaves as a functional feed additive on the growth performance, carcass characteristics and serum lipid profile of broiler chicken. Indian J. Anim. Res., 47(5): 449-452.

Dibner JJ, Richards JD (2005). Antibiotic growth promoters in agriculture: History and mode of action. Poult. Sci., 84(4): 634-643. https://doi.org/10.1093/ps/84.4.634

Dohou N, Yamni K, Gmiran N, Idrissi HLM (2004). Etude de polyphénols des feuilles d'une endémique ibéro Marocaine, *Thymelaea lychroides*. Acta Bot. Malacit., 29: 233-239. https://doi.org/10.24310/abm.v29i0.7221

Fankam AG, Kuete V, Voukeng IK, Kuiate RJ, Pages J-M (2011). Antibacterial activities of selected cameroonian spices and their synergistic effects with antibiotics against multidrug-resistant phenotypes. Complement. Altern. Med., 11: 104-109. https://doi.org/10.1186/1472-6882-11-104

Farahat M, Ibrahim D, Kishawy ATY, Abdallah HM, Hernandez-Santana A, Attia G (2021). Effect of cereal type and plant extract addition on the growth performance, intestinal morphology, caecal microflora, and gut barriers gene expression of broiler chickens. Animal, 15(3): 2021. https://doi.org/10.1016/j.animal.2020.100056

Gata-Gonçalvès L, Nogueira JMF, Matos O, Sousa B (2003). Phytoactive extract from Thevetia peruviana with antifunal properties agains *Cladosporuim cucumerinum*. J. Photochem. Photobiol., 70(1): 51-54. https://doi.org/10.1016/S1011-1344(03)00024-1

Gorenz B, Maci LO,Julianna C.J, Chang C., Alexandra HS, Roderick I.M, Ryan ND (2024). Organ growth and fermentation profiles of broilers differing in body growth rate. Poult. Sci., 103(5): 103628. https://doi.org/10.1016/j.psj.2024.103628

Greuter W (2003). The Euro+Med treatment of Cardueae (Compositae)-generic concepts and required new names. Willdenowia, 33(1): 45-47. https://doi.org/10.3372/wi.33.33103

Hashemi SR, Davoodi H (2012). Herbal plants as new immunostimulator in poultry industry: A review. Asian J. Anim. Vet. Adv., 7: 105-116. https://doi.org/10.3923/ajava.2012.105.116

Iyaode II, Ibrahim HO, Uwade F, Shittu MW (2020). Haematology and serum biochemistry of broiler strains (Cobbs and Arbor-acre) fed ginger (*Zingiber officinale*). GSC Biol. Pharm. Sci., 11(2): 320-326. https://doi.org/10.30574/gscbps.2020.11.2.0145

Kaczorová D, Karalija E, Dahija S, Bešta-Gajević R, Parić A, Ćavar Zeljković S (2021). Influence of extraction solvent on the phenolic profile and bioactivity of two *Achillea* species. Molecules. 13; 26(6): 1601. https://doi.org/10.3390/molecules26061601

Khan A, Tahir M, Alhidary I, Abdelrahman M, Swelum AA, Khan RU (2021). Role of dietary *Moringa oleifera* leaf extract on productive parameters, humoral immunity and lipid peroxidation in broiler chicks. Anim. Biotechnol., 33(6): 1353-1358. https://doi.org/10.1080/10495398.2021.1899936

Khan RU, Khan A, Naz S, Ullah Q, Laudadio V, Tufarelli V, Ragni M (2012). Potential Applications of Moringa oleifera in Poultry Health and Production as Alternative to Antibiotics: A Review. Antibiotics (Basel). 10(12): 1540. https://doi.org/10.3390/antibiotics10121540

Kikusato M (2021). Phytobiotics to improve health and production of broiler chickens: functions beyond the antioxidant activity. Anim. Biosci., 34(3): 345–353. https://doi.org/10.5713/ab.20.0842

Kim J, Ahn J (2022). Emergence and spread of antibiotic-resistant foodborne pathogens from farm to table. Food Sci. Biotechnol., 31(12): 1481-1499. https://doi.org/10.1007/s10068-022-01157-1

Kurt T, Wong N, Fowler H, Gay C, Lillehoj H, Plummer P, Scott HM, Hoelzer K (2019). Strategic priorities for research on antibiotic alternatives in animal agriculture-results from an expert workshop. Front. Vet. Sci., 6: 429. https://doi.org/10.3389/fvets.2019.00429

Macambira GM, Rabello CBV, Navarro MIV, Lopes CC, Lopes EC, Nascimento GR, Oliveira HSH, Silva. JCR (2022). Effects of *Moringa oleifera* leaf meal on performance and carcass yield of broilers. Rev. Bras. Zootec., 51: e20210203. https://doi.org/10.37496/rbz5120210203

Miyakawa MEF, Natalia AC, Michael HK (2024). How did antibiotic growth promoters increase growth and feed efficiency in poultry? Poult. Sci., 103(2): 103278. https://doi.org/10.1016/j.psj.2023.103278

Mohammad M, Hamed AN, Maziar H, Mehrzad F, Manouchehr V, Davood R (2016). Experimental study and thermodynamic modeling for determining the effect of non-polar solvent (hexane)/polar solvent (methanol) ratio and moisture content on the lipid extraction efficiency from Chlorella vulgaris. Bioresour. Technol., 201: 304-311. https://doi.org/10.1016/j.biortech.2015.11.066

Mousa MA, Osman AS, Hady HA (2017). Performance, immunology and biochemical parameters of *Moringa oleifera* and/or Cichorium intybus addition to broiler chicken ration. J. Vet. Med. Anim. Health, 9(10): 255-263. https://doi.org/10.5897/JVMAH2017.0611

Naz R, Roberts TH, Bano A, Nosheen A, Yasmin H, Hassan MN, Rumana K, Sami U, Wajiha K, Zahid A (2020). GC–MS analysis, antimicrobial, antioxidant, antilipoxygenase

and cytotoxic activities of Jacaranda mimosifolia methanol leaf extracts and fractions. PLoS One, 15(7): e0236319. https://doi.org/10.1371/journal.pone.0236319

Nielsen EI, Friberg LE (2023). Pharmacokinetic-pharmacodynamic modelling of antibacterial drugs. Pharmacol. Rev., 65(3): 1053–1090. https://doi.org/10.1124/pr.111.005769

Niewold TA (2007). The Nonantibiotic anti-inflammatory effect of antimicrobial growth promoters, the real mode of action? A hypothesis. Poult. Sci., 86(4): 605-609. https://doi.org/10.1093/ps/86.4.605

Nkala BA, Mbonwa HP, Qwebani-Ogunleye T (2019). The *in vitro* evaluation of some South African plant extracts for minimum inhibition concentration and minimum bactericidal concentration against selected bacterial strains. Int. J. Sci. Res. Publ., 9(7): 998. https://doi.org/10.29322/IJSRP.9.07.2019.p91132

Pareek A, Pant M, Gupta MM, Kashania P, Ratan Y, Jain V, Pareek A, Chuturgoon AA (2023). *Moringa oleifera*: An updated comprehensive review of its pharmacological activities, ethnomedicinal, phytopharmaceutical formulation, clinical, phytochemical, and toxicological aspects. Int. J. Mol. Sci., 24: 2098. https://doi.org/10.3390/ijms24032098

Paul SYA, Tarafder M, Rimon MA, Haque MA, Sikder MH (2018). Water supplementation of *Moringa oleifera* as a substitute for antibiotics on performance and blood parameters of broiler chickens. J. Bangl. Agric. Univ., 16: 266-270. https://doi.org/10.3329/jbau.v16i2.37981

Qwele K, Hugo A, Moyo SOOB, Masika PJ, Muchenje V (2013). Chemical composition, fatty acid content and antioxidant potential of meat from goats supplemented with Moringa (*Moringa oleifera*) leaves, sunflower cake and grass hay. Meat Sci., 93(3): 455-462. https://doi.org/10.1016/j.meatsci.2012.11.009

Radkowska I (2013). The use of herbs and phytogenic feed additives in livestock nutrition. Zootech. News., 4: 117–124.

Rahimi S, Kathariou S, Grimes JL, Siletzky RM (2011). Effect of direct-fed microbials on performance and *Clostridium perfringens* colonization of turkey poults. Poult. Sci., 90(11): 2556–2562. https://doi.org/10.3382/ps.2011-01342

Rakotomamonjy VN (2016). Pool water treatment trials using *Moringa oleifera* seed residue as a flocculant. Master's thesis, University of Antananarivo. 79p.

Ren H, Vahjen W, Dadi T, Saliu E, Boroojeni FG, Jürgen, Zentek J (2019). Synergistic effects of probiotics and phytobiotics on the intestinal microbiota in young broiler chicken. Microorganisms, 7(12): 684. https://doi.org/10.3390/microorganisms7120684

Renata G, Silveira D, Camila M, Elizangela BO, Antônio APGJ, Ronaldo VFF, Bruno BD (2021). Systematic review of the use of phytobiotics in broiler nutrition. J. Agroveterinary Sci., 20(1): 98-106. https://doi.org/10.5965/223811712012021098

Ricard F-H, Rouvier R, Marche G, Lafont F (1967). Study of the anatomical composition of the chicken. I. - Variability in the distribution of different body parts in "Bresse-Pile" cockerels. Ann. Zootech., 16(1): 23-39. https://doi.org/10.1051/animres:19670104

Richter N, Siddhuraju P, Becker K (2003). Evaluation of nutritional quality of Moringa (*Moringa oleifera* Lam.) leaves as an alternative protein source for Nile tilapia (*Oreochromis niloticus* L.). Aquaculture, 217(1-4): 599–611. https://doi.org/10.1016/S0044-8486(02)00497-0

Romanucci V, Maria G, Sergio D, Cinzia DM, Afef L, Anna DM (2020). Antimicrobial Effect and Antioxidant Activity of Triterpenes Isolated from *Gymnema sylvestre* R. Br. Rec. Nat. Prod., 14(3): 213-218. https://doi.org/10.25135/rnp.165.19.10.1448

Spichiger R., Savolainene V, Figeat M, Jeanmonod D (2002). Systematic botany of flowering plants. Presses polytechniques et universitaires romandes. pp. 413.

Sudatri NW (2021). Identification of active compounds of Turmeric rhizome (*Curcuma domestic* Val.) and Tamarind fruit (*Tamarindus indica* L.) and their implementation to improve health performance and broiler production. Dissertation, Doctoral Program, Faculty of Animal Husbandry, Udayana University, Denpasar, Indonesia.

Talla E, Tamfu NA, Gade SI, Yanda L, Mbafor TJ, Laurent S, Vander EL, Popova M, Bankova V (2016). New mono-ether of glycerol and triterpenes with DPPH radical scavenging activity from cameroonian propolis. Nat. Prod. Res., 31(12): 1379-1389. https://doi.org/10.1080/14786419.2016.1253077

Tekce E, Bayraktar B, Aksakal V, Dertli E, Kamiloğlu A, Çinar TK., Kaya H (2020). Response of Japanese quail (*Coturnix coturnix japonica*) to dietary inclusion of *Moringa oleifera* essential oil under heat stress conditions. Ital. J. Anim. Sci., 19(1): 514-523. https://doi.org/10.1080/1828051X.2020.1760740

Urban J, Karwan YK, Arkadiusz M, Damian B, Patrycja C, Kamil L, Monika M (2024). Enhancing broiler chicken health and performance: the impact of phytobiotics on growth, gut microbiota, antioxidants, and immunity. Phytochem. Rev., 24: 2131-2145. https://doi.org/10.1007/s11101-024-09994-0

Velmurugu R, Abdollahi MR (2021). Nutrition and digestive physiology of the broiler chick: State of the art and outlook. Animals, 11(10): 2795. https://doi.org/10.3390/ani11102795

Wahab OAA, Sobhy HM, Badr AM, Ghazalah AA (2020). Effect of *Moringa oleifera* seeds powder on performance and immunity of broiler chicks. AIMS Agric. Food, 5(4): 896–910. https://doi.org/10.3934/agrfood.2020.4.896

Walter A, Samuel W, Pierre A, Joseph O (2011). Activité antibactérienne des extraits au méthanol et de seedes de *Moringa oleifera* et Moringa stenopetala et au n-hexane sur les bactéries impliquées dans les maladies d'origine hydrique. Afr. J. Microbiol. Res., 5(2): 153-157.

Wickramasuriya SS, Jessica A, Stephanie R, Cyril GG, Hyun SL (2024). Alternatives to antibiotic growth promoters for poultry: A bibliometric analysis of the research journals. Poult. Sci., 103(9): 103987. https://doi.org/10.1016/j.psj.2024.103987

Yang Y, Paul IJI, Choct M (2009). Gut microflora and alternatives to in-feed antibiotics. World's Poult. Sci. J., 65(1): 97-114. https://doi.org/10.1017/S0043933909000087