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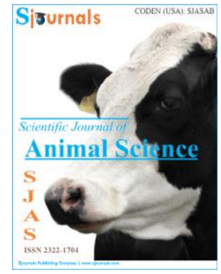

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Original article

Effects of neem oil (*Azadirachta indica*) on feed digestibility, growth performance and gut microbiota of broilers chickens

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ABSTRACT

Antibiotics residues were reported to accumulate in animal farm products and equally develop resistance in pathogenic microorganisms. The present study was designed to evaluate the effects of neem seeds oil as antibiotic substute on feed digestibility, growth performance and gut microbiota of broiler chickens. A total of 336 one-day-old Cobb 500 chicks were randomly assigned to 7 treatments replicated 3 times with 16 chicks each (8 males, 8 females). The experimental rations consisted of a control ration without supplementation (R_0), a positive control ration containing 1 g antibiotic/kg feed (R_0+) and five other rations supplemented with 1, 2, 4, 6 and 8 g of neem oil/kg feed respectively. No significant differences were observed on the digestibility of dry matter, organic matter and crude protein at any oil level compared to the control rations. Following digestibility, the highest values of dry matter (78.53 ±2.54 %), organic matter (83.77 ± 1.14 %) and crude protein (91.28 ±1.15 %) were respectively recorded in animals fed 6 g neem oil/kg feed.

With the exception of birds fed 1 g neem oil/kg feed, feed intake, live weight, weight gain and feed conversion ratio were improved with this oil as feed additive. The lowest FCR value (2.01 ± 0.03) was recorded with 6 g neem oil/kg feed. Carcass characteristics were not significantly (*P > 0.05*) affected compared to the control ration. Neem oil induced an increase in *Lactobacilli* counts compared to *Salmonella* and *Escherichia coli* counts. In conclusion, neem oil at 6 g/kg feed, improved feed palatability, stabilize gut microbiota thereby favoring a better absorption of nutrients and thus improve growth performance of broilers.

1. Introduction

The sub-therapeutic use of antibiotics in poultry nutrition for decades helped to balance broilers gut and improve growth performance (Huyghebaert et al., 2011). This practice, which was found to be very effective, has been prohibited due to the resistance developed by pathogenic microorganisms and the accumulation of antibiotic residues in animal products (Kana et al., 2011; Chardon and Brugere, 2014). This banning was put in place as a precautionary measure to limit the consequences on the health of the animal and that of the consumer. Thus, farmers are increasingly looking for the alternative in poultry farming by using active principles from plant origin that can have the same properties as antibiotics with least or no negative consequences on consumer's health and the environment (Mafouo et al*.,* 2019b). Thus, a significant number of products have been considered, including phytobiotics. In this group, essential oils (Khattak et al., 2014; Ngouana et al., 2017), plant extracts and spices (Kana et al., 2017) are evaluated with the view of solving public health problems without compromising the production efficiency of animals. Among phytobiotics, neem seeds oil has been used as feed additive in several studies in animal nutrition (Ogbuewu et al., 2011; Mafouo et al., 2019a; 2019b).

Neem, a tropical plant widely distributed in Africa and available throughout year, is well adapted to the climatic and edaphic conditions of tropical forest and Sahelian rainforest areas (Koona and Budida, 2011; Ogbuewu et al., 2011). Neem seeds contain about 20-32% blackish to pale yellow oil from the seed (Munoz-Valenzuela et al., 2007; Faye, 2010); 30-52% from kernel (Faye, 2010). Neem oil contains numerous terpenoic compounds such as azardirachtin, gedunin, nimbine and nimbidin which have antibacterial and antifungal properties (Valarmathy et al., 2010). Some of these bioactives molecules are responsible for its pungent smell, bitterness and strong oxidation. This oil is harmless to warm-blooded animals and humans (Aribi et al., 2020) and like many other vegetable oils, it contains compounds that can stimulate the animal's immune system (INRA, 2013). Ogbuewu et al. (2010) reported that, the effects of neem oil on weight gain, linear measurements and blood chemistry in prepubertal rabbits showed that they could tolerate up to 15% dietary inclusion of this oil without deleterious effects. Recent works on the effects of neem oil as a feed preservative and on broiler growth performance revealed respectively that this oil is a promising natural preservative for reducing poultry feed losses in stocked (Mafouo et al., 2019a), and at levels of 15; 20 and 25 g/kg feed this oil has depressive effects on broiler growth characteristics (Mafouo et al., 2019b). The latter authors concluded that the poor performance obtained was due to the high levels of this oil. With regards to the phytochemical composition of neem oil, the objective of the present study was to determine the effects of its low in-feed doses on feed components digestibility, growth performances and gut microbiota composition of broilers.

2. Materials and methods

2.1. Study area

This study was conducted at the University of Dschang Teaching and Research Farm (TRF) located at 05°26' North latitude, 10°26' East longitude and at an altitude of 1420 m. The prevailing climate is equatorial, characterized by two seasons. A rainy season lasted from March to November and a dry season that covers the rest of the year. Rainfall varies between 1500 and 2000 mm per year. The average temperature is around 21°C, the average annual insolation is 1837 hours and the average relative humidity is 76.8%.

2.2. Experimental birds

A total of 336 day-old Cobb 500 chicks were used in this study for a period of 49 days. They were randomly assigned following a completely randomized design to 7 treatments replicated 3 times with 16 chicks each (8 males and 8 females). Water and feed were provided *ad libitum* to the animals. An anti-stress was given in drinking water during the first 3 days after the chicks' arrival. The latter was equally given before, during and after each handling and transfer of chicks from the brooder to the finishing house. An anti-coccidian [Vetacox®] and vitamins [AMINTOTAL®] were given through drinking water on 3 consecutive days each week, and from the fourth week onwards only anti-coccidian was given. The birds were vaccinated against infectious bronchitis [H120®] and Newcastle disease [Hitchner B1®] at seven day old and against Gumboro disease [IBA Gumboro®] at tenth day old. The vaccination recall was done on the eighteenth day.

2.3. Experimental rations

Fresh neem fruits were harvested in the Northern Region of Cameroon, washed under running water and air-dried to a constant moisture content. Then crushed to remove the pulp, and oil was extracted using cold extraction method described by Nkouam et al. (2017). Phytochemical analysis of the extracted oil sample was carried out by the chromatographic method as described by Talukdar et al. (2010). It revealed the presence of flavonoids, terpenoids, phenols, alkaloids, tannins, anthocyanin's, anthraquinones and steroids, and the absence of saponins (Table 1). The chicks were litter brooded at a density of 20 chicks/m² at the starter phase (1-21 days) and 10 chicks/m² at the finisher phase (22-49 days).

A negative control ration (R_0) without supplement (Table 2), and a positive control ration (R_0^+) containing 1 g antibiotic (Doxycycline)/kg feed were formulated, and the other five treatments consisted of incorporating neem oil (NO) at doses of 1, 2, 4, 6 and 8g/kg of in the control ration (R_0).

Table 1
Phytochemical composition of neem seed oil.

Phytochemicals	Result
Alkaloids	+
Phenols	+
Flavonoids	+
Sterols	+
Terpenoids	+
Tannins	+
Saponins	-
Anthocyanins	+
Anthraquinones	+

Key: + Present; - absent

Table 2
Composition of control ration.

Ingredients (kg)	Starter phase	Finisher phase
Maize	60	67
Cotton seed meal	05	05
Soybean meal	22	15
Fish meal	05	05
Shell	01	01
Wheat bran	02	02
*Premix 5%	05	05
Total	100	100

Calculated chemical composition		
Metabolisable energy (kcal/kg)	2963.70	3021.73
Crude protein (%)	23.31	20.48
Crude cellulose (%)	3.29	3.25
Energy / Protein	127.14	148.61
Lysine (%)	1.41	1.21
Methionine (%)	0.49	0.45
Calcium (%)	1,17	1.16
Phosphorus (%)	0.56	0.53
Price of feed (FCFA/kg)	293.50	276
Analysed chemical composition		
Crude protein (%)	22.11	19.44
Crude cellulose (%)	3.08	3.07

*Premix 5%: Mineral, Nitrogen and Vitamin Complex: Crude Protein = 40%, Lysine = 3.3%, Methionine = 2.40%, Calcium = 8%, Phosphorus = 2.05%, Metabolisable energy = 2078 kcal/Kg.

2.4. Data collection

2.4.1. Feed digestibility

At 42 days old, 6 birds per treatment (3 males and 3 females) were housed individually in wire meshed cages. Faeces of each bird per experimental unit was collected over a period of 3 days. The faeces were then oven-dried at 50°C to a constant weight for proximate analysis. Dry matter (DM), organic matter (OM), crude cellulose (CC) and crude protein (CP) content were determined according to the method described by AOAC (1990). The apparent digestibility utilization coefficient (aDUC) of the CP, DM, OM and CC of the experimental rations were calculated with the following formula:

$$\mathbf{aDUC}\,\% = \frac{\text{Quantity ingested (g)} - \text{Quantity excreted (g)}}{\text{Quantity ingested (g)}} \times 100$$

2.4.2. Growth performance

Data were collected every 7 days on feed intake, live weight, weight gain and feed conversion ratio. Feed was weighed and distributed to the animals daily and at the end of each week, the left overs were collected then weighed. Feed intake was calculated as the difference between the quantity served and the left over in each experimental unit. At the beginning of the trial and every 7 days thereafter, birds in each experimental units were weighed and weekly weight gain was calculated as the difference between two consecutive week's weights. Feed conversion ratio (FCR) was calculated as the ratio of the amount of feed intake during the week and the weight gain of the same week.

2.4.3. Carcass characteristics

At 49 days old, 10 chickens per treatment (05 males and 05 females) were fasted for 24 hours, weighed, sacrificed, plucked and eviscerated for carcass evaluation (Kana et al*.,* 2014). Carcass yield and relative weights of organs were respectively calculated by dividing the weights of carcass and that of organs by the live body weight of the bird.

2.4.4. Gut microbiota

Before the chickens were sacrificed, faeces samples were aseptically collected using sterile swabs from the cloaca of 4 chickens per treatment and immediately transported to the laboratory for the identification and quantification of lactic acid bacteria, *Salmonella* and *Escherichia coli* respectively in the specific culture media. The culture medium used was respectively MRS AGAR, SS AGAR and Mac Conkey AGAR.

2.4.5. Statistical analysis

All data collected were submitted to one-way analysis of variance (ANOVA). In case of a significant differences between treatments groups, Duncan's multiple range test was used to separate mean at the 5%

significance level. The statistical software SPSS 20.0 (Statistical Package for Social Science) was used for the analyses.

3. Results

3.1. Feed digestibility

The digestibility of feed components as affected by different levels of neem oil is summarized in Table 3. No significant difference was observed on dry matter, organic matter and crude protein digestibility irrespective of the treatments. However, there was an upward trend with the antibiotic and neem oil irrespective of the level of incorporation compared to the control ration (R_0). The highest values of DM (78.53 ±2.54 %), OM (83.77 ± 1.14 %) and CP (91.28 ±1.15 %) were recorded with 6 g neem oil/kg feed compared to the other experimental rations. As compared to the control ration, crude cellulose digestibility was significantly higher with antibiotic and neem oil irrespective of inclusion level. The highest value (35.94 ± 4.40 %) was obtained with the antibiotic treatment though comparable to those induced by 1, 6 and 8 g neem oil/kg feed. The digestibility of crude cellulose tend to increase with the increasing level of neem oil in the ration.

Table 3
Variation of feed components digestibility as affected by neem oil incorporation level in broilers rations.

Feed components	Control rations		Incorporation levels of neem oil					
	R_0	**R_0 +**	**1**	**2**	**4**	**6**	**8**	***p***
DM ingested (g)	547.64±11.00	562.06 ± 88.02	494.01±64.62	500.69±81.24	477.86±63.61	533.64±33.36	519.85±34.06	0.430
DM excreted (g)	147.03 ± 8.72	133.49±25.13	106.96 ± 7.96	129.83±30.61	133.03±21.97	114.15±10.23	123.28±31.85	0.225
aDUC DM (%)	73.16 ± 1.23	75.89 ± 5.56	78.22 ± 1.46	74.24 ± 2.15	74.19 ± 0.80	78.53 ± 2.54	75.91 ± 7.94	0.406
OM ingested (g)	587.56±11.80	603.04±94.44	530.03±69.33	537.19±87.16	512.70±68.24	572.54±35.79	557±75±36.54	0.430
OM excreted (g)	123.64±10.21	105.61±18.12	90.84±8.74	105.86±25.05	108.78±18.42	92.68±4.22	108.47±23.25	0.178
aDUC OM(%)	78.97 ± 1.36	82.18 ± 4.24	82.68 ± 2.46	80.45 ± 1.37	80.25 ± 0.51	83.77 ± 1.14	80.28 ± 5.67	0.302
CC ingested (g)	19.64 ± 0.39	20.16 ± 3.16	17.72 ± 2.32	17.96 ± 2.91	18.66 ± 3.23	19.14 ±1.20	18.65 ± 1.22	0.748
CC excreted (g)	18.54 ± 0.43^a	13.56 ± 1.54^b	12.51 ± 1.41^b	13.92 ± 0.85^b	13.76 ± 1.75^b	13.46 ± 0.74^b	12.72 ± 0.93^b	0.000
aDUC CC (%)	5.49 ± 2.52^d	35.94 ± 4.40^a	29.19 ± 4.65^{ab}	15.99 ± 1.00^c	25.79 ± 4.43^b	29.56 ± 5.01^{ab}	31.59 ± 9.33^{ab}	0.000
CP ingested (g)	123.59±7.69	126.85±7.53	111.49±14.35	113.00±18.33	107.84±14.58	120.43±19.87	117.32 ±2.48	0.430
CP excreted (g)	12.84 ±1.62	11.72 ±2.20	9.31 ±0.49	10.68 ±3.17	10.66 ±1.17	10.49 ±1.36	11.70 ±2.85	0.328
aDUC CP (%)	89.63 ± 1.13	90.64 ±1.95	91.08 ± 0.74	90.68 ±1.20	90.09 ± 0.40	91.28 ± 1.15	89.87 ± 3.15	0.742

a, b, c, d: means with identical letters on the same line are not significantly different ($p > 0.05$), R_0 = basal ration, R_0 += R_0 + 1 g Antibiotic,; DM = dry matter, OM = organic matter, CC = crude cellulose, CP = crude protein, aDUC = apparent digestive utilization coefficient.

3.2. Growth performances

The effects of neem oil on feed intake, live weight, weight gain and feed conversion ratio of broilers are summarized in Table 4.

Table 4
Variation of broiler's growth performance with respect to in-feed incorporation level of neem oil.

Period (Days)	Control rations		Incorporation levels of neem oil					
Feed intake (g)	**R_0**	**R_0^+**	**1**	**2**	**4**	**6**	**8**	***P***
1-21	1027.33±78.53	1011.67±16.20	1042.00±65.00	1033.67±53.20	991.67±17.62	1001.00±34.04	1004.33±39.51	0.838
22-49	4053.33 ± 82.11^b	4278.67 ± 206.74^{ab}	3838.67 ± 211.83^c	4176.33 ± 90.60^{ab}	4406.67 ± 85.33^a	4188.33 ± 169.18^{ab}	4231.67 ± 161.40^{ab}	0.013
1-49	5080.00 ± 147.90^b	5290.33 ± 217.45^{ab}	4881.33 ± 253.57^c	5209.67 ± 60.05^{ab}	5398.00 ± 80.02^a	5189.33 ± 137.45^{ab}	5235.67 ± 139.54^{ab}	0.037
Live weight (g)								
1-21	733.33±24.11	752.33±25.70	734.00±26.23	730.33±92.52	719.00±39.66	739.33±19.60	735.33±21.03	0.983
22-49	2429.67 ± 99.68^b	2612.00 ± 46.81^a	2226.00 ± 62.64^c	2484.33 ± 110.23^{ab}	2622.33 ± 100.27^a	2625.33 ± 25.42^a	2554.33 ± 103.89^{ab}	0.000
1-49	3163.12 ± 112.29^b	3363.94 ± 69.09^a	2959.72 ± 62.15^c	3214.86 ± 71.89^{ab}	3341.34 ± 125.19^a	3365.17 ± 42.07^a	3289.94 ± 83.18^{ab}	0.000
Weight gain (g)								
1-21	689.33±24.11	708.00±25.98	690.00±26.23	686.33±92.52	675.00±39.66	695.33±19.60	691.33±21.03	0.984
22-49	1696.67 ± 91.22^b	1859.33 ± 31.39^{ab}	1491.67 ± 73.23^c	1753.33 ± 190.18^{ab}	1903.33 ± 88.00^a	1886.00 ± 17.59^{ab}	1819.00 ± 124.74^{ab}	0.003
1-49	2385.67 ± 99.68^b	2567.33 ± 47.25^a	2181.67 ± 62.88^c	2440.33 ± 110.23^{ab}	2578.33 ± 100.27^a	2581.33 ± 25.42^a	2510.33 ± 103.89^{ab}	0.000
Feed conversion ratio								
1-21	1.49±0.14	1.43±0.05	1.51±0.15	1.52±0.14	1.47±0.11	1.44±0.08	1.45±0.06	0.926
22-49	2.39 ± 0.08^{ab}	2.30 ± 0.11^b	2.57 ± 0.09^a	2.40 ± 0.20^{ab}	2.32 ± 0.08^b	2.22 ± 0.08^b	2.33 ± 0.08^b	0.043
1-49	2.13 ± 0.04^{ab}	2.06 ± 0.07^b	2.24 ± 0.12^a	2.14 ± 0.07^{ab}	2.10 ± 0.05^b	2.01 ± 0.03^b	2.09 ± 0.03^b	0.023

a,b,c: means with identical letters on the same line are not significantly different ($P > 0.05$), R0 = Basal ration, R_0 += R_0 + 1 g Antibiotic.

No significant (*P > 0.05*) effect was recorded amongst treatments means for all characteristics irrespective of the incorporation level considered at the starter phase (1-21 days). At the finisher phase (22-49 days) and throughout the trial period (1-49 days), this oil had a significant (*P < 0.05*) effect on feed intake, except for 1 and 4 g of neem oil/kg feed, which induced the lowest and the highest values respectively compared to the control ration (R_0). Antibiotic and neem oil at 4 and 6 g/kg feed significantly (*P < 0.05*) improved live weight and weight gain of broilers compared to the control ration. The highest values of these two characteristics were obtained with 6 g neem oil/kg feed. However, except for the lowest level (1 g) of neem oil in the ration, all treatments containing this oil were comparable (*P > 0.05*) for the live weight and weight gain. The same trend was recorded in feed conversion ratio which was comparable to all treatments except with the lowest oil level (1 g).

3.3. Carcass characteristics

Carcass yield and relative weights of broilers organs as affected by graded level of neem oil in feed are presented in Table 5. From this Table, it can be seen that neem oil had no significant (*P > 0.05*) effect at any level on carcass characteristics and relative weight of organs compared to antibiotic and control ration.

Table 5
Variation of carcass characteristics of broilers as affected by neem oil incorporation levels in feed.

	Control rations		Incorporation levels of neem oil					
Parameters (% lw)	**R_0**	**R_0^+**	**1**	**2**	**4**	**6**	**8**	***P***
Carcass yield	74.30±1.16	74.80±1.14	73.50±1.27	74.20±1.32	73.80±1.48	73.50±0.97	73.70±1.34	0.195
Head	2.18±0.18	2.07±0.29	2.19±0.06	2.16±0.23	1.99±0.21	2.13±0.23	2.12±0.28	0.366
Legs	3.69±0.45	3.52±0.52	3.77±0.48	3.89±0.54	3.64±0.54	3.67±0.37	3.59±0.32	0.480
Liver	1.61±0.19	1.61±0.15	1.91±0 .27	1.64±0.14	1.69±0.13	1.66±0.12	1.49±0.38	0.309
Heart	0.51±0.07	0.49±0.05	0.51±0.09	0.49±0.06	0.52±0.08	0.51±0.07	0.49±0.04	0.905
Pancreas	0.22±0.04	0.18±0.05	0.20±0.05	0.19±0 .08	0.18±0 .06	0.19±0.05	0.19±0.05	0.634
Abdominal fat	1.80±0.40	1.94±0.60	1.69±0.36	1.58±0.61	2.01±0.53	2.03±0.58	2.02±0.76	0.429

R_0 = Basal ration, R_0^+ = R_0 + 1 g Antibiotic.

3.4. Gut microbiota

The results from Table 6 showed that, whatever the incorporation level considered, neem oil had no significant (*P > 0.05*) effect on *Lactobacilli* and *Salmonella* counts in broiler's gut. This oil at 6 g/kg feed tend to increase (*P > 0.05*) *Lactobacilli* and decrease (*P > 0.05*) *Salmonella* counts. The same oil level significantly (*P < 0.05*) reduced *Escherichia coli* counts compared to all other oil levels and antibiotic.

Table 6
Effects of neem oil incorporation rate on the gut microbiota count of broilers.

Bacteria counts (Log_{10}CFU)	Control rations		Incorporation levels of neem oil					
	R_0	**R_0^+**	**1**	**2**	**4**	**6**	**8**	***P***
Lactobacilli	8.47 ± 0.25	8.51 ± 0.68	8.49 ± 0.34	8.51 ± 0.54	8.82 ± 0.09	9.16 ± 0.29	8.40 ± 0.34	0.292
Escherichia coli	8.96 ± 0.32^{ab}	8.92 ± 0.38^{ab}	9.02 ± 0.25^{ab}	8.55 ± 0.19^{b}	8.85 ± 0.26^{ab}	8.37 ± 0.12^{c}	9.20 ± 0.11^{a}	0.017
Salmonella	7.84 ± 0.12	7.64 ± 0.26	7.73 ± 0.31	7.46 ± 0.50	7.50 ± 0.05	7.27 ± 0.19	7.62 ± 0.22	0.279

a, b, c: means with identical letters on the same line are not significantly different (P>0.05), R0 = Basal ration, R_0 += R_0 + 1 g Antibiotic.

4. Discussion

The present study revealed a non-significant increase in dry matter, organic matter and crude protein digestibility regardless of the treatment considered. This results contradicts those of Egbeyale et al. (2018) who showed that incorporating neem leaf powder at 0.5 and 1% in broiler feed had a significant effect on dry matter and crude protein digestibility. Similarly, the work of Oluwafemi et al. (2020) revealed that, dietary supplementation of neem oil at 0.1 to 0.4% in weaned rabbit feed resulted in a significant increase in dry matter and crude protein digestibility. However, the digestibility of crude fiber was improved with this oil in feed. This result is in agreement with those of Shaahu et al. (2020) and Egbeyale et al. (2018) who reported that the incorporation of aqueous neem leaf extract and neem leaf powder in feed at 0.2; 0.4; 0.6% and 0.5; 1%

respectively in broilers feed significantly increase crude fiber digestibility. The present result could be attributed to the phenolic compounds present in this oil that improved digestion by stimulating endogenous enzymes in broiler's gut (Wafaa et al., 2012) thus promoting the absorption and utilization of the available component in the consumed feed.

Throughout the entire trial period, graded levels of neem oil significantly improved feed compared to the control ration. This result is not consistent with those of Mafouo et al. (2019b) who reported a non-significant effect on feed intake with the incorporation of 15, 20 and 25 g neem oil/kg in broiler's feed. This observed difference could be resulted from the different oil level used. However, it is in agreement with the findings of Shihab et al. (2017) who reported that the incorporation of neem leaf powder at 1, 2 and 3 g/kg feed significantly increased feed intake in broilers at 5 weeks of age. Patil (2016) reported a significant increase in feed intake with the incorporation of 0.1; 0.2; 0.3 and 0.4% neem oil in broiler's feed. Similarly, Oluwafemi et al. (2020) stated that, dietary supplementation of neem oil at 0.1; 0.2; 0.3 and 0.4% in weaned rabbits feed resulted in a significant decrease in total feed intake.

Neem oil in feed significantly improved live weight and weight gain throughout the entire trial period. This result is in line with those of Oluwafemi et al. (2020) who reported that dietary supplementation of neem seed oil at 0.1; 0.2; 0.3 and 0.4% in feed significantly improved live weight and total weight gain in weaned rabbits. In the same line, Shihab et al. (2017) reported that these characteristics were significantly higher with 1; 2 and 3 g neem leaf powder/kg in broiler's feed. These results differ from those of Patil (2016) and Mafouo et al. (2019b) who reported a significant decrease in live weight and weight gain when neem oil was incorporated in broiler's feed at 0.1; 0.2; 0.3; 0.4% and 15; 20; 25 g/kg respectively. The significant improvement in weight gain and feed intake could be due to the presence of bioactive phytochemicals in this oil which, through their antibacterial, antiprotozoal, anti-inflammatory, antioxidant, antifungal and immunomodulatory properties, did not only increase feed palatability, but also improve the intestinal microflora by stimulating digestive secretions which increased the height of the jejunal mucosa. Thus increases in the intestine surface favorable for the absorption of nutrients in the intestine. The lowest feed conversion ratio was recorded with 6 g neem oil/kg feed, indicating better feed conversion by the broiler birds in this treatment.

The inclusion of neem oil in feed had no significant effect on carcass characteristics. In the same line, Egbeyale et al. (2018) reported no significant effect on carcass yield, lung, heart, liver, gizzard and abdominal fat weights when 0.5 and 1% neem leaf powder was incorporated in broiler's feed. According to these authors, air-drying of neem reduces anti-nutritional elements that can impact liver and lung health. Nodu et al. (2016) reported that neem leaf extract can prevent liver and lung hypertrophy in broiler chickens. Similarly, Shaahu et al. (2020) reported a non-significant effect on carcass yield, relative weights of gizzard, liver, lung, kidney, head and abdominal fat with the inclusion of 0.2; 0.4 and 0.6% of aqueous neem leaf extracts in broiler's feed. Contrarily, Mafouo et al. (2019b) reported a significant increase on liver weight of broilers when adding 15; 20 and 25 g neem oil/kg feed. This observed difference could be related to the excessively high levels of this oil which leads to an intense activity of the hepatic and secretory cells of the liver due to the presence of important quantities of anti-nutritional factors. Graded levels of small quantities of this oil in feed had no significant effect on abdominal fat. This result corroborates those of Egbeyale et al. (2018) who reported that the incorporation of neem leaf powder at 0.5 and 1% in broiler's feed resulted in a non-significant decrease in abdominal fat. This suggests that the presence of fatty acids in neem oil of such as oleic acid (43.68%), linoleic acid (19.78%) and linolenic acid (0.45%) modulate lipid metabolism (Mafouo et al., 2019b).

Regardless of the inclusion level considered, this oil had no significant effect on *Lactobacilli a*nd *Salmonella* counts compared to the control ration. However, neem oil significantly decreased the number of *Escherichia coli* compared to the control ration. This result contradicts the finding of Mafouo et al. (2019b) who reported a significant increase in the number of *Escherichia coli* in broilers when 15; 20 and 25 g neem oil/kg were added in feed. The antimicrobial and anti-protozoal properties of neem oil is due to the presence of numerous secondary bioactive compounds, which can explain this result. Indeed, they help to reduce pathogenic microorganisms in birds by causing a disruption of the cell membrane of gram-negative microorganisms, as they are lipophilic compounds. Moreover, when the living conditions of the intestinal environment become favourable, lactic acid bacteria multiplies and selectively eliminate pathogenic bacteria due to their acidifying power and production of antibacterial substances such as organic acids and colicin (Elaroussi et al., 2008; Riyazi et al., 2015).

5. Conclusion

Neem oil at 6 g/kg feed, can substitute synthetic antibiotics feed additive, improve feed palatability, stabilize gut microbiota thereby hence favoring better absorption of nutrients and thus improve growth performances of broiler chickens.

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