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RESEARCH

Compatible alternative to antibiotics in broilers: evaluating *Nigella sativa* as a natural growth promoter

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Abstract The restriction or ban of antibiotics as feed additives in poultry production in many countries, including those in the European Union, has prompted the need for safe and effective natural alternatives. This study evaluated the potential of *Nigella sativa* L. (black cumin) seed extract as a feed additive in broiler chickens, focusing on its effects on performance, carcass traits, blood biochemistry, digestive microbiota, and immune response. A total of 200 one-day-old Ross 308 male chicks were randomly divided into five treatment groups: a control group receiving Virginiamycin (0.2 g/L) and four groups supplemented with 0, 150, 300, or 450 mg/l of *N. sativa* extract in drinking water from day 1 to 42. Birds receiving 150 mg/l of *N. sativa* showed significantly higher weight gain, final live weight, carcass yield, and breast and drumstick mass ($p < 0.05$) compared to other treatments. This group also exhibited lower total cholesterol and LDL levels at day 42, alongside reduced coliform counts and increased Lactobacilli, indicating beneficial effects on gut microbiota. Although feed intake remained unaffected, *N. sativa* influenced immune parameters: IgG levels decreased at 300 mg/l, while IgM levels increased at 450 mg/l. These results suggest that supplementing broiler diets with 150 mg/l of *N. sativa* extract may serve as an effective alternative to antibiotics, promoting growth performance, improving metabolic health, and supporting intestinal microbial balance. Further studies are warranted to confirm its efficacy,

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assess long-term safety, and clarify the mechanisms involved in its growth-promoting and cholesterol-lowering effects.

Keywords *Nigella sativa* · Broiler chicks · Feed additive · Growth promoter · Performance

Introduction

Antibiotics play an essential role in animal health by preventing diseases and improving the efficiency of production as they reduce mortality and stimulate weight gain (Dhama et al. 2014; Gadde et al. 2017).The use of subtherapeutic levels of antibiotics included in the formulation of animal feed has shown a relevant role in poultry production benefiting producers and consumers (Dhama et al. 2014). Therefore, they have been used to sustain health and improve growth performance and feed conversion efficiency (Markowiak and Ślizewska 2018) by reducing pathogenic microorganisms. However, the risk posed by antibiotics as growth promoters (GPA) to create cross-resistance to antibiotics used in human medicine and their presence in animal products and the environment has been recognized as a global health problem. Thus, their use has been restricted in many countries (Gaucher et al. 2015), as it has been banned in the European Union since 2006 (Gadde et al. 2017; Muaz et al. 2018). Furthermore, consumer perceptions and demands are increasingly aware of the importance of the quality and safety of the consumed animal products, urging the manufacture of feed to be more respectable and balanced with the environment and to obtain healthy and safe animal by-products for consumers (Committee on Improving the Health; Safety and Well-Being of Young Adults et al. 2015).

Some investigations stated the need to find alternatives to antibiotics and use new plant feed and additives to replace antibiotics (Gaucher et al. 2015; Seidavi et al. 2020).These additives have been demonstrated to be safer, healthier, and biodegradable, allowing the maintenance of intestinal health in poultry production (Salaheen et al. 2017), and contributing to the absorption of nutrients that improve growth performance. Among these alternatives, the most used are probiotics, prebiotics, enzymes, essential oils, herbs, spices, and plant extracts (Dhama et al. 2014; Omolere and Alagbe 2020). Recently, plant extracts have received extensive attention due to concerns regarding food safety, synthetic additives, and the growing interest in natural and healthy foods. Some of them can be considered substitutes for GPAs thanks to their antimicrobial properties due to the presence of polyphenolic compounds, and essential oils. Moreover, herbs, plant extracts, and essential oils used as food additives include different bioactive ingredients such as alkaloids, flavonoids, tannins, and saponins that act on the appetite of animals and their intestinal microbiota, by stimulating the production of digestive enzymes (Fahmideh et al. 2019; Adegbeye et al. 2020). In addition, they can promote intensification of endogenous enzyme activity and the immune system along with antimicrobial and antioxidant properties benefiting the health and weight gain of farm animals (Reyes-Munguía et al. 2016; Aziz and Karboune 2018; Mahfuz et al. 2021; Obianwuna et al. 2024) and consequently improving the quality of meat.

Concerning black cumin (*Nigella sativa L*) seed and oil, several studies have detailed their positive effects on animal and human health and in the healthier production of animal products for humans (Hassanien et al. 2015; El-Hack et al. 2016; Kumar et al. 2017). Therefore, *Nigella sativa* (*N. sativa*) has been described to have many biological properties, including antiparasitic (Ali et al. 2016), antidiabetic (Alimohamadi et al. 2014), anticancer (Hidayati and Habib 2015; AlAttas et al. 2016), antiatherosclerosis and hypotensive effects (Shakeri et al. 2018) and antiviral and antibacterial activity (Sudesh et al. 2019). According to Ahmad et al. (2013) another property of *N. sativa* seeds is their ability to stimulate the pancreatic digestive enzymes' activities and the liver stimulation to produce and secrete bile. This property may be due to the 38 bioactive compounds such as thymoquinone, which demonstrate broad-spectrum antimicrobial effects recognized in *N. sativa*. It also contains about 3% essential oils which are carvone, limonene, as well as sabinene, flavonoids, polysaccharides, coumarin, and cumin aldehyde (Seidavi et al. 2020).

Advances in poultry research have led the search for phytobiotics to improve the efficiency of feed use, increasing the desired attributes of the carcass. In recent years, different studies (El-Bahr et al. 2021; Laudadio et al. 2022) have reported the positive effects of *N. sativa*

on broilers. Thus, El-Bahr et al. 2021 reported that *Nigella sativa* supplementation in six-week-old broilers improved body weight, total protein, albumin, globulin levels, and antibody titers against Newcastle disease and infectious bovine diarrhoea. Similarly, Laudadio et al. (2022) observed significant weight gain between days 22 and 42, along with increased immune titers in chickens treated with *Nigella sativa.*

Moreover, some investigations compared a diet with pre and probiotics with one that included *N. sativa*, observing that both diets led to similar increases in body weight, but the *N. sativa* diet had a better feed conversion rate and lower cholesterol levels (Alimohamadi et al. 2014). Additionally, broilers fed *N. sativa* had increased blood cell count, haemoglobin concentration, and hematocrit percentage. Similarly, other studies showed increased body weight, feed intake, metabolizability of nutrients, range of immunology titers, and total protein (Alimohamadi et al. 2014; Kumar and Patra 2017; Laudadio et al. 2022; Yesuf et al. 2023; Zaazaa et al. 2023). Meanwhile, the levels of *Salmonella* spp., *E. coli*, and total coliform, as well as cholesterol decreased linearly. Other studies performed on broilers at 42 days, concluded that a diet including 20 g/kg of *N. sativa* may lead to an improvement in slaughter body weight, fatty acid concentrations, and properties of broiler meat (Kumar and Patra 2017).

The present study aims to investigate the effects of different doses of *N. sativa* extract compared to virginiamycin on the diet of broilers. This approach investigates the potential replacement of natural supplements for the systematic use of antibiotics. Although other investigations reported the use of *N. sativa* on the growth performance in poultry, here we have assessed not only the effect on weight gain, feed intake and feed conversion ratio but also on carcass characteristics, blood chemistry parameters, digestive bacterial count and immune response of broilers. In addition, this study innovatively compares the effects of *Nigella sativa* at various doses with a control group of chickens treated with antibiotics, using an oily extract administered through drinking water and evaluating *Nigella sativa* as a single compound to determine the optimal concentration.

Materials and methods

Animals, diets and study design

A total of 200 Ross 308 broiler chicks one day-old male were purchased and raised under thermo-neutral ambient temperature according to the standard brooding practice for the rearing stages of the birds. The birds were vaccinated against influenza (H5) on day 1, infectious bronchitis on days 1 and 8, Gumboro on days 16 and 32, and Newcastle disease on days 1 and 8 of age (Aviagen 2018). Broiler diets were formulated for three growth phases (starter: day 1 to 14; grower: day 15 to 28, and finisher: day 29 to 42) (Table 1).

Broilers (n = 200) were randomly allotted to 20-floor pens (2.0 m × 2.0 m) containing ten broilers each. Broilers were assigned into five experimental treatments with four replicates and 10 chicks each. From day 1 to day 42 of age, broiler chickens were supplied with drinking water containing *Nigella sativa* extract at concentrations of 0, 150, 300, and 450 mg per liter of water. The extract (oil) was homogenized in drinking water using mechanical stirring to ensure uniform dispersion and was offered daily and ad libitum. To ensure accurate estimation of extract intake, daily water consumption was monitored for each replicate pen. A control treatment included Virginiamycin antibiotics (Pouyan Zarin Mehr Co, Iran) at concentration of 0.2 g per liter of water. The preparation of the alcoholic extract of *N.*

Table 1 Ingredients (%), chemical composition (%), and metabolizable energy(kcal/kg) of the starter, grower, and finisher diets

Treatment	Starter (from 1 to 14 day)	Grower (from 15 to 28 day)	Finisher (from 29 to 42 day)
Ingredients (%)			
Corn	54.5	58.5	62.7
Soybean meal	37.5	33.2	29.5
Soybean oil	4.0	4.0	4.0
Calcium carbonate	1.2	1.2	1.1
Dicalcium phosphate	1.6	1.5	1.5
Crude protein	21	20	18
Metabolisable energy (kcal/kg)	3010	3050	3100

sativa consisted of a mixture of fifty grams of *Nigella* seed powder mixed with 500 mL of ethanol (70%, v/v). We made provision of feed and water ad libitum during the growth period. We observed weekly data on weight gain, feed intakes and conversion ratios over six weeks. Carcass characteristics, blood chemistry and digestive bacterial count of broilers were evaluated on the last day of the experiment (day 42), and one broiler per pen (n = 20) was randomly chosen. The immune response was assessed at 28 and 42 days of age when blood samples were collected and pooled per replicate.

Growth performance: analysis of weight gain, feed intake and feed conversion rate

Body weight and feed intake (difference between offered and refused feed) were measured weekly. Feed intake, weight gain, and feed conversion ratio were calculated for each replicate within each treatment during the study period (days 1 to 42).

Carcass characteristics

On the last day of the experiment (day 42), one broiler per pen (n = 20) was randomly chosen, weighed, and sacrificed to evaluate the characteristics of the carcass. The weight of the entire de-feathered carcass, the carcass without the head and drumsticks was recorded. Viscera and abdominal fat were removed, and the carcass yield and the relative weights (percentage of eviscerated carcass) of abdominal fat and anatomical parts (breast, drumsticks, wings, and neck) were calculated.

Clinical biochemistry

On day 42, one broiler per pen (n = 20) was used to evaluate immune responses were sacrificed to calculate the relative weights of organs related to the immune system function (spleen and the bursa of Fabricius) (Shabani et al. 2015). In addition, we collected a blood sample (1.5 mL) per replicate from 1 broiler per pen (n = 20) into EDTA (ethylene diamine tetraacetic acid) tubes from a wing vein. Blood plasma was separated by centrifugation (Rotofix 32 A centrifuge, Hettich, Germany) at 1500 rpm for 10 min and stored at –20 °C until analysis. The levels of uric acid, total cholesterol, triglycerides, low and high-density lipoprotein (LDL; HDL), very low-density lipoprotein (VLDL), total protein, and albumin were measured using commercial laboratory kits (Pars Azmoon Co., Tehran, Iran). In the same way we performed glucose analysis on fresh plasma samples using the Pars Azmoon glucose oxidase kit, as described previously. Moreover, to evaluate the immune-response, one broiler per replicate (n = 20) was randomly chosen for the blood collection from the brachial vein. Blood serum was pooled per replicate and incubated at room temperature for one hour, separated by centrifugation (as above), and stored at −20 °C until analysis.

Cecum microbiota

The cecum microbiota counted from one broiler per pen (n = 20) was determined on day 42 based on the method described by Dibaji et al. (2014). Briefly, agar plates were streaked on the site with ileal content to determine the bacterial growth (*Lactobacilli and E. coli*) and colony counts. The sample collection tubes were weighed and wrapped with an aluminum sheet before processing with an autoclave for 10 min. We prepared the culture media and poured it into the sterile Petri plates 24 h before sample collection. The collection tubes were weighed empty and with the sample, and the weight difference was used to determine the weight of the sample in each tube. The tubes were shaken for 30 min to isolate the gastrointestinal contents and bacteria to obtain the suspension. About 1 ml of the resulting suspension was collected and mixed with 9 ml of phosphate-buffered saline (PBS) in a separate tube. Aliquots were plated on MacConkey agar for coliform determination and on MRS agar for *Lactobacillus* spp., incubated under aerobic conditions for 48 h and under anaerobic conditions for 72 h, respectively. The bacteria count was carried out using a colony counter. These counts were obtained as the logarithm of bacteria number per gram of the sample.

Immune response

To evaluate the systemic antibody response (Dibaji et al. 2014), broilers at 14 and 35 days (one per replicate), with weights similar to the replicate average, were vaccinated against sheep red blood cells (SRBC) by subcutaneous administration of SRBC suspension in 5% of phosphate-buffered saline (PBS). At 28 and

42 days of age, blood samples were collected and pooled per replicate, and the total antibody against SRBC was determined using a hemagglutination assay. In U-bottom microtiter plates, two-fold serial dilutions of heat-inactivated serum (at 56 °C) were added to PBS (0.01 mol/L; pH 7.4) for assessment of the total antibodies or to PBS with 1.4% 2-mercaptoethanol for assessment of IgG antibodies. All antibody titers were recorded as $\log_{10}$ of the highest dilution of serum that agglutinated an equal volume of a 0.5% SRBC suspension in PBS. We determined the IgM titer as the difference between the total and the IgG titers.

Statistical analyses

Analysis of weight gain, feed intake and feed conversion rate

Systematic observations were conducted on identical broiler units over six consecutive weeks, resulting in repeated measures datasets on weight gains, feed intakes, and feed conversion ratios. A univariate approach was consistently employed using mixed models. Various ad-hoc models were evaluated for each dataset to identify the most suitable one based on Bayesian Information Criterion (BIC) optimization. For weight gains, incorporating the week as a continuous variable through orthogonal polynomials proved advantageous. Conversely, treating the week as a categorical factor yielded better results for analyzing feed intakes and conversion ratios.

Weight gain

Weekly weight gain data were log-transformed (ln) to reduce skewness, and thus, the results are presented as geometric means. In addition, repeated measures on the broiler flocks were modelled using linear mixed models with orthonormal polynomials (Crowder and Hand 2017; Edwards and Simpson 2014; Motil et al. 2022; Rovadoscki et al. 2016; Nolte et al. 2020; Conover and Iman 1979). A broad class of models for growth analysis is of the form **[M1]:**

$$\ln\left(y_{i,w,T}\right) = \beta_0 + \beta_1\phi_1(w) + \beta_2\phi_2(w) + \beta_3\phi_3(w) + \gamma_T + \delta_{T,w} + batch_i + e_{i,T,w}$$

Here, $y_{i,w,T}$ denotes the weight gains corresponding to ith batch, treated with T at week w, $\phi_1(w)$, $\phi_2(w)$ and $\phi_3(w)$ are orthonormal polynomials of degrees 1, 2 and 3 respectively, γ_T is the effect of treatment T (Virginiamycin is taken as reference and thus $\gamma_{\text{virg}}=0$), $\delta_{T,w}$ is the interaction treatment-week, $batch_i$ is the random effect of the ith batch and finally $e_{i,T,w}$ is the random error. We assume that $batch_1$ are independent and identically distributed random variables distributed $N(0,\sigma_b)$, and the random errors $e_{i,w,\text{T}}$ independent and identically distributed $N(0,\sigma_e)$. From all models of the M1 shape, we selected the one that optimized the Bayesian Information Criterion (BIC). Comparisons between treatments were carried out by estimating all geometric mean ratios using 95% confidence intervals.

Feed intake and feed conversion ratio

For the outcomes *feed intakes* and *feed conversion ratio*, we consider mixed models of the form **[M2]**:

$$\ln\left(y_{i,w,T}\right) = \alpha + \beta_w + \gamma_T + batch_i + e_{i,T,w}$$

where $y_{i,w,T}$ denotes the value of outcome (*feed intakes* or *feed conversion ratio*) for the *i*th batch, during week (*w)* and treatment (*T*). The parameter β_w denotes the effect of week (*w*) ($\beta_1=0$ [reference]). Similarly, γ_T denotes the effect of treatment (*T*) ($\gamma_{\text{virg}}=0$ [reference]). Likewise, $batch_i$ is the random effect corresponding to ith batch and $e_{i,\text{w},T}$ are the random of errors. We assume that the random effects $batch_1$, …, $batch_{20}$ are independent and identically distributed random variables $N(0,\sigma_b)$ and the random errors $e_{i,w,T}$ independent and identically distributed random variables $N(0,\sigma_\text{e})$.

Carcass characteristics, blood chemistry and cecum microbiota analysis

Carcass characteristics, blood constituents, and gut microflora were measured on broilers. To evaluate the effects of treatments on these variables, we performed an analysis of variance with a variation factor (ANOVA-1). Therefore, for each of these variables, means were compared by treatment group using the F test. In cases where the test showed statistical significance (at least two means were significantly

different), a multiple comparisons analysis was performed using the Scheffe test. For each variable, the corresponding model was summarized in means by treatment group and the *p*-value corresponding to the F test. When it was necessary to perform a multiple comparisons analysis, different superscripts were assigned to the means that showed to be significantly different.

Immune-response analysis

The *IgG* and *IgM* levels (logarithm_{10} scale) by day and treatment were summarized as medians and interquartile ranges (IQR = 25th—75th percentile). Comparisons according to treatments were carried out using the Kruskal–Wallis test. Where appropriate, multiple comparisons were performed using Conover's All-Pairs Rank Comparison Test for medians (Conover and Iman 1979).

Statistical significance was set at $p < 0.05$. Data were analyzed using the R package, version 4.2.1 (R Core Team 2022).

Results

Growth performance

Weight gain

We summarized the estimated mixed model for the weight gains in Table 2. This model was optimal according to the BIC among the mixed models explored. The effect of the week across the polynomials $\phi_1(w)$, $\phi_2(w)$ and $\phi_3(w)$ showed statistical significance ($p < 0.001$). Given that $\phi_1(w)$, $\phi_2(w)$ and $\phi_3(w)$ are of degrees 1, 2, and 3 respectively, the expression, $\beta_1\phi_1(w) + \beta_2\phi_2(w) + \beta_3\phi_3(w)$ forms a polynomial of degree three. Consequently, the effect of the week on the logarithm of weight gain is cubic. The interactions treatment-week that showed statistical significance ($p < 0.001$) is explained by the fact that in the sixth week, *N. sativa* (150 mg/L) and *N. sativa* (450 mg/L) changed in their relationship with the other treatments.

Table 2 Mixed model fixed effects estimation for weekly weight gain

Parameters	Coefficient (SE)	*p*-value
(Intercept)	**5.90 (0.03)**	< 0.001
Week effects: Orthogonal polynomials [1]		
$\phi_1(w)$	**4.00 (0.18)**	< 0.001
$\phi_2(w)$	**−3.06 (0.18)**	< 0.001
$\phi_3(w)$	**−1.03 (0.17)**	< 0.001
Treatments		
Virginiamycin (0.2 g/L)	0 (Reference)	-
N. sativa (0 mg/L)	0.02 (0.05)	0.612
N. sativa (150 mg/L)	−0.02 (0.05)	0.706
N. sativa (300 mg/L)	−0.02 (0.05)	0.727
N. sativa (450 mg/L)	−0.12 (0.05)	0.022
Interactions six week with [2]**:**		
N. sativa (150 mg/L)—week six	0.60 (0.10)	< 0.001
N. sativa (450 mg/L)—week six	0.39 (0.1.0)	< 0.001

[1]The model shows the effect of the week on the *weekly weight gain*[2] For the full model, Bayesian Information Criterion (BIC) = −18.67; if the interactions are removed, BIC = 3.76 (worse model). In general, among all the [M1] form models, the present model minimized the BIC

The geometric means of the weekly weight gains (95% CI) adjusted by the mixed model [M1] are presented in Table 3.

Concerning to the two treatments that differed in week 6 (Table 2) from the rest of the treatments, it is observed in Table 3 that the greatest weight gain occurs in the 150 mg/L and in the 450 mg/L of *N. sativa* treatments, where the weight gain remains stable and similar to the previous two weeks.

Comparisons between pairs of treatments were carried out by means of the ratios of the corresponding geometric means, which were estimated by multivariate linear contrasts. Given the existing interactions, comparisons were made separately for weeks 1 to 5 (Table 4) and week 6 (Table 5).

For weeks 1 to 5 (Table 4), *N. sativa* at 450 mg/L showed a lower gain than the other treatments, among which no significant differences were observed.

At week 6, Table 5 shows the greatest significant weekly weight gain with the use of 150 mg/L of *N. sativa* compared to the rest of the treatments. On the other hand, the dose of 450 mg/L significantly

Table 3 Adjusted geometric means (95% Confidence Interval) by the mixed model of **weekly weight gain** (g) according to **treatment** and **week**

Week	*Treatment*				
	Virginiamycin (0.2 g/L)	*Nigella sativa*			
		0 mg/L	150 mg/L	300 mg/L	450 mg/L
1	160 (146; 175)	164 (150; 179)	166 (143; 172)	157 (144; 172)	141 (129; 155)
2	252 (233; 273)	258 (239; 279)	248 (228; 269)	248 (230; 268)	223 (205; 242)
3	411 (382; 443)	421 (391; 454)	404 (374; 436)	405 (376; 436)	364 (336; 393)
4	584 (542; 630)	598 (555; 645)	573 (531; 619)	575 (533; 620)	516 (478; 557)
5	608 (563; 658)	623 (576; 673)	597 (549; 649)	599 (554; 647)	538 (495; 584)
6	392 (354; 434)	401 (363; 444)	**702 (601; 820)**	386 (349; 427)	513 (439; 599)

Table 4 Geometric mean ratios (95% Confidence Interval) of weekly weight gains (g) between treatments for **weeks 1 to 5**

	Virginiamycin (0.2 g/L)			
N. sativa (0 mg/L)	1.024 (0.936; 1.120)	*N. sativa* (0 mg/L)		
N. sativa (150 mg/L)	0.981 (0.892; 1.080)	0.958 (0.871; 1.054)	*N. sativa* (150 mg/L)	
N. sativa (300 mg/L)	0.984 (0.899; 1.076)	0.961 (0.878; 1.051)	1.002 (0.911; 1.103)	*N. sativa* (300 mg/L)
N. sativa (450 mg/L)	**0.884 (0.803; 0.972)**	**0.863 (0.784; 0.949)**	**0.900 (0.816; 0.993)**	**0.898 (0.816; 0.988)**

Data are geometric mean ratios corresponding to row versus column treatment for the weeks 1 to 5. A ratio greater than one means that the row treatment has a higher geometric mean than the column treatment. When the confidence interval does not contain the unit (**highlighted),** it implies that the difference is significant. *N. sativa* (450 mg/L) showed a lower gain in weeks 1–5 than the rest of the treatments, among which no significant differences were observed

Table 5 Geometric mean ratios (95%Confidence Interval) of weekly weight gains (g) between treatments **at week six**

	Virginiamycin (0.2 g/L)			
N. sativa (0 mg/L)	1.024 (0.936; 1.120)	*N. sativa* (0 mg/L)		
N. sativa (150 mg/L)	**1.792 (1.488; 2.157)**	**1.750 (1.454; 2.106)**	*N. sativa* (150 mg/L)	
N. sativa (300 mg/L)	0.984 (0.899; 1.076)	0.961 (0.878; 1.051)	**0.549 (0.456; 0.661)**	*N. sativa* (300 mg/L)
N. sativa (450 mg/L)	**1.308 (1.087; 1.574)**	**1.277 (1.061; 1.538)**	**0.730 (0.586; 0.909)**	**1.329 (1.104; 1.600)**

Data are geometric mean ratios corresponding to row versus column treatment for the week six. A ratio greater than one means that the row treatment has a higher geometric mean than the column treatment. When the confidence interval does not contain the unit **(highlighted**), it implies that the difference is significant. Weight gains were up to week 5 lower for *N. sativa* (450 mg/L), but in the last week, there were trade-offs which justifies the interactions included in the model. However, *N. sativa* (150 mg/L) continued to show higher growth in the sixth week

improved the weight gain of the chickens compared to the different treatments, except for *N. sativa* 150 mg/L (Table 5).

Feed intake

Figure 1 shows the observed feed intake and it's fit by the [M2] model throughout the six weeks of

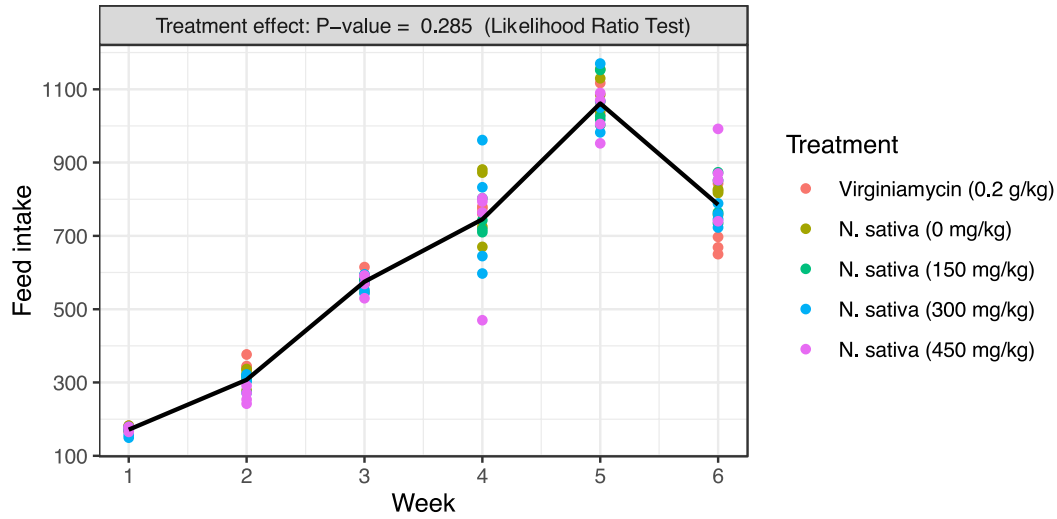

Fig. 1 Feed intake throughout the six weeks of follow-up period: observed data and geometric means adjusted by the mixed model. Treatments did not show statistical significance ($p=0.285$). *N. sativa*: *Nigella sativa*

Table 6 Feed intake (g): geometric means adjusted by the mixed model along with their corresponding 95% confidence intervals, categorized by week

Week	*Feed Intake*: geometric mean (95% CI)
1	171.2 (164.5; 178.2) [a]
2	307.3 (295.2; 319.8) [b]
3	575 (552.4; 598.5) [c]
4	745.4 (716.2; 775.9) [d]
5	1062 (1020; 1105) [e]
6	785 (754.2; 817) [d]

Treatments did no show significant differences (likelihood ratio test: $p=0.285$)

[a, b, c, d, e] Multiple linear comparisons: different superscripts indicate significant differences at $p<0.001$

follow-up. The treatment did not show a statistically significant effect on the feed intake ($p=0.285$). Table 6 summarizes the fifted geometric means (95% CI) for each week. Since the week effect was significant ($p<0.001$), we perform multiple comparisons by means of linear contrast.

Table 6 and Fig. 1 indicate that feed intake was significantly lower in week 1 and higher in week 5 for all animals across all treatments tested. This result is a normal growth behavior of broilers since the animals increase their intake as they gain weight. Additionally, in week 6, once the peak fattening phase had been reached, feed intake decreased for all animals regardless of the treatment.

Feed conversion ratio (FCR)

The treatment did not show a significant statistical effect on the feed conversion ratio ($p=0.204$). Figure 2 displays the observed feed conversion ratio (FCR) and it's fit by the [M2] model throughout the six weeks of follow-up.

Figure 2 shows a better result (lower FCR) of broilers supplemented with 150 mg/L of *N. sativa* compared to the other treatments at week 6. However, according to the mixed model [M2], the overall treatment effect was not statistically significant (likelihood ratio test: $p=0.204$).

Therefore, we only estimate from the model [M2], the fitted geometric means (95% CI) per week summarized in Table 7. Since the week effect was significant ($p<0.05$), multiple comparisons were performed using linear contrast.

In week 1, the chickens that received either the control or the experimental treatments demonstrated the best results, with a significantly lower FCR compared to the rest of the study period. The FCR remained similar during weeks 2,3, and 4. However, in weeks 5 and 6, there was a significant increase in this ratio (Fig. 2 and Table 7).

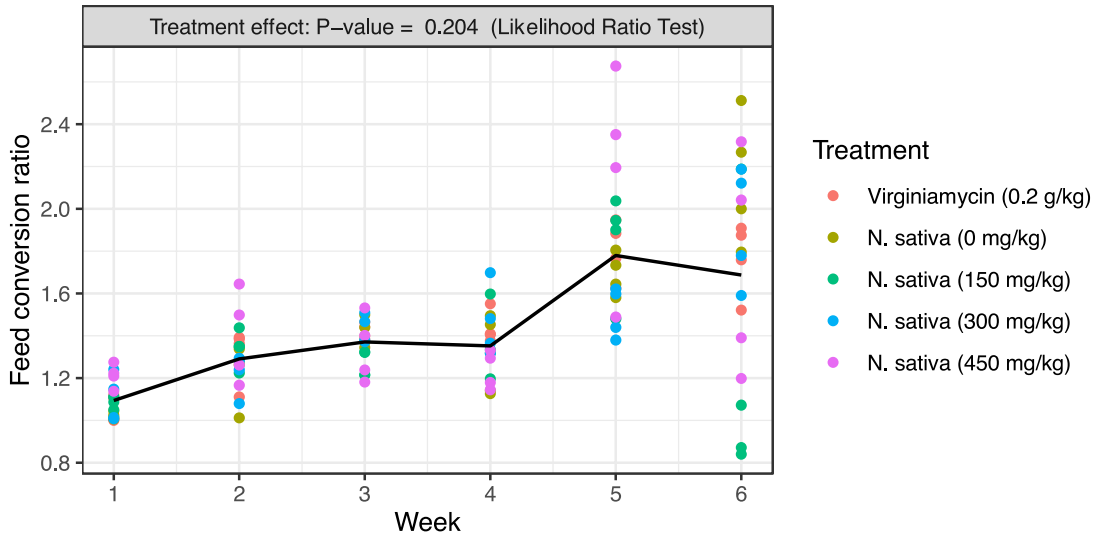

Fig. 2 Feed Conversion Ratios over the six weeks follow-up period: observed data and geometric means adjusted by the mixed model. Treatments did not show statistical significance ($p = 0.204$)

Table 7 Feed Converse Ratios: geometric means adjusted by the mixed model along with their corresponding 95% confidence intervals, categorized by week

Week	Geometric means (95% CI)
1	1.09 (1.01; 1.17) [a]
2	1.29 (1.20; 1.38) [b]
3	1.37 (1.27; 1.47) b
4	1.35 (1.26; 1.45) b
5	1.77 (1.65; 1.91) [c]
6	1.68 (1.57; 1.81) [c]

Treatments did no show significant differences (likelihood ratio test: $p = 0.204$)

[a, b, c...] Multiple linear comparisons: different superscripts indicate significant differences at $p < 0.05$

Carcass characteristics

The average weights of the carcasses, organs, and other organs related to the immune system of broilers at day 42 are shown in Table 8. Except for the weight of the bursa of Fabricius, wings, abdominal fat, liver, and gizzard in which no significant findings were found, the weight of the final live body, chicken breasts and drumsticks were significantly higher in those chickens fed supplemented with 150 mg/L of *N. sativa* ($p < 0.05$). The weight of the empty abdomen was significantly higher in those chickens fed with 150 mg/L of *N. sativa* ($p < 0.05$) and antibiotics group, and heart weight was significantly higher in those chickens fed supplemented with 450 mg/L of *N. sativa* ($p < 0.05$). It is important to point out that no significant differences were found in final body weight or eviscerated carcass yield between chickens supplemented with 150 mg/L of *N. sativa* and those given antibiotics.

On day 42, the broilers that incorporated the virginiamycin antibiotics and chickens supplemented with 150 mg/L of *N. sativa* showed values of the spleen significantly higher ($p < 0.05$) than chickens fed with doses of 0, 300 and 450 mg/L of *N. sativa.*

Blood chemistry

The mean values of the blood parameters described in Table 9 show that on day 42, the levels obtained for glucose, triglycerides, HDL, VLDL, albumin, and LDL/HDL did not show a significant effect between the different study groups. However, values in total cholesterol and LDL levels ($p < 0.05$) were lower in chickens fed with *N. sativa* compared with diets without it. Total cholesterol and LDL ($p < 0.05$) were the lowest in broilers fed 300 mg/L of *N. sativa.* Otherwise, total protein concentrations were significantly higher for diet-fed broilers with the addition of 150, 300 and 450 mg/L of *N. sativa* than without.

Table 8 Mean (± SEM) carcass and organ weights (g) of broilers at 42nd day with different levels of *Nigella sativa* (*N. sativa)* extract

	Final Live body	Empty abdomen Carcass	Breast	Spleen	Bursa of Fabricius	Drumsticks	Wings	Abdominal fat	Heart	Liver	Gizzard
Virginiamycin (0.2 g/L)	2645^{ab}	1940^{a}	710^{ab}	4.5^{a}	4.0	548^{ab}	56.5	40.2	12.2^{bc}	63.7	44.0
N. sativa (0 mg/L)	2415^{b}	1747^{b}	656^{abc}	3.0^{bc}	3.2	508^{b}	53.2	41.5	10.2^{c}	56.2	41.2
N. sativa (150 mg/L)	$2745.^{a}$	$1932.^{a}$	729^{a}	4.0^{ab}	4.0	599^{a}	58.5	33.7	14.0^{ab}	55.0	46.5
N. sativa (300 mg/L)	2402^{b}	1697^{b}	635^{bc}	3.2^{bc}	3.2	571^{ab}	54.2	32.5	12.7^{ab}	56.5	39.0
N. sativa (450 mg/L)	2417^{b}	1715^{b}	622^{c}	2.7^{c}	3.2	532^{b}	53.0	33.0	14.7^{a}	51.5	45.2
p-value	0.040	0.019	0.040	0.010	0.098	0.043	0.483	0.123	0.008	0.326	0.701
SEM	47.779	34.006	14.089	0.198	0.135	10.792	1.090	1.462	0.467	1.824	1.746

Means within each column without common superscript have significant difference at $p < 0.05$. SEM = Standard error of the mean. *N. sativa*: *Nigella sativa* extract

Table 9 Mean (±SEM) of blood parameters (mg/dl) at 42^{nd} days of age in Ross 308 broilers fed with different levels of *Nigella sativa* (*N. sativa)* extract

	Glucose (mg/dl)	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	Albumin (mg/dl)	Total protein (mg/dl)	Uric acid (mg/dl)	LDL/HDL
Virginiamycin (0.2 g/L)	211.5	150^{a}	81	61.3	75^{a}	16.3	1.7	4.9^{b}	5.2^{a}	0.83
N. sativa (0 mg/L)	223	143^{a}	92	61	62.1^{ab}	18	1.5	3.5^{c}	5.7^{a}	0.99
N. sativa (150 mg/L)	213.3	134.3^{ab}	76.5	57.7	62.1^{ab}	15.6	1.3	5.3^{ab}	4.1^{b}	0.93
N. sativa (300 mg/L)	194.7	114.8^{b}	87.3	56.6	43.8^{b}	18.2	2.1	6.0^{a}	4.2^{b}	1.78
N. sativa (450 mg/L)	215	139.8^{ab}	91.5	56.5	62.9^{ab}	18.1	1.4	5.3^{ab}	5.3^{a}	0.94
p-value	0.414	0.043	0.830	0.169	0.045	0.852	0.321	0.001	0.001	0.302
SEM	4.537	4.249	4.656	0.829	3.384	0.885	0.119	0.212	0.164	0.156

HDL:High-Density Lipoproteins; LDL: Low-Density Lipoproteins; VLDL: very Low-Density Lipoproteins. Means within each column without common superscript have a significant difference at $p<0.05$.SEM= Standard error of the mean

Moreover, the uric acid (with 150 and 300 mg/L of *N. sativa*) decreased significantly ($p < 0.05$).

Cecum microbiota

We observe the mean of cecum microbiota parameters at 42 days of age in broilers fed with different levels of *N. sativa* extract (Table 10). The count of coliform bacteria at 42 days in broilers fed diets incorporating virginiamycin diet was higher than in chickens fed on *Nigella sativa* extract or without treatment (0 mg/L) diet ($p < 0.05$). The *coliform* bacteria (cfu/gr) were lower ($p < 0.05$) on broilers fed on *N. sativa* (150 mg/L) while they were higher on broilers fed on Virginiamycin (0.2 g/L). Statistically significant results were also obtained regarding the counts of *Lactobacillus* spp, which were higher on broilers fed *N. sativa* extract (0, 150, 300, 450 mg/L) and were lower on broilers fed on Virginiamycin ($p < 0.05$).

Immune-response

The antibody levels (IgG, IgM) according to day and treatment are summarized in Table 11. For the day 28, the treatment effects were significant for both IgG ($p = 0.026$) and IgM ($p = 0.01$). The concentration of IgG antibodies was significantly lower in chickens treated with 300 mg/L of *N. sativa*, while a higher concentration of IgM was observed with the 450 mg/L treatment. However, the effect of treatment

Table 10 Mean of cecum microflora parameters (cfu/gr) at 42nd days of age in Ross 308 broilers fed with different levels of *Nigella sativa* (*N. sativa)* extract

	Coliform bacteria (cfu/gr)	*Lactobacillus* spp (cfu/gr)
Virginiamycin (0.2 g/L)	2.42^{a}	1.03^{c}
N. sativa (0 mg/L)	1.99^{ab}	2.27^{a}
N. sativa (150 mg/L)	1.42^{b}	2.06^{ab}
N. sativa(300 mg/L)	1.61^{ab}	1.67^{b}
N. sativa(450 mg/L)	1.86^{ab}	2.43^{a}
p-value	0.001	0.048
SEM	0.131	0.133

Means within each column without common superscript have a significant difference at $p<0.05$. SEM= Standard error of the mean. cfu/gr:colony-forming units per gram

cfu/gr: colony-forming units per gram

Table 11 IgG and IgM antibodies ($\log_{10}$). Median of immunity parameters against sheep red blood cells at different days of age in broilers fed with different levels of *Nigella sativa* extract

	IgG		IgM	
	Day 28	Day 42	Day 28	Day 42
Virginiamycin (0.2 g/L)	4.5 (4.0; 5.0)^{a}	3.5 (2.8; 4.8)	2.5 (2.0; 3.0)^{a,b}	6.0 (4.2; 7.2)
N. sativa(0 mg/L)	5.0 (4.8; 5.0)^{a}	4.5 (3.8; 5.2)	2.0 (1.8; 2.0)^{b}	5.0 (4.8; 5.0)
N. sativa(150 mg/L)	4.5 (4.0; 5.0)^{a}	4.0 (2.8; 5.0)	1.5 (1.0; 2.0)^{b}	4.0 (4.0; 4.8)
N. sativa(300 mg/L)	1.0 (1.0; 1.0)^{b}	5.0 (4.2; 5.5)	3.0 (2.5; 3.0) ^{a,b}	6.0 (4.2; 7.2)
N. sativa (450 mg/L)	4.5 (4.0; 5.0) ^{a}	4.5 (4.0; 5.5)	4.0 (4.0; 4.0) ^{a}	6.0 (5.2; 6.0)
p-value*	0.026	0.806	0.01	0.837

Data are medians (IQR)

(*) Kruskal–Wallis test to compare antibody levels according to treatment

(^{a,b}) Multiple comparisons between treatments: different superscripts indicate significant differences at $p < 0.05$

did not show significance for day 42 for both concentrations of immunoglobulins IgG and IgM.

Discussion

Growth performance

Weight gain

The weight gain determination in chickens helps in assessing the nutritional and health status of broiler chickens. Several studies have reported that dietary supplementation of *N. sativa* seeds improves the performance of broilers (Islam et al. 2016; Seidavi et al. 2020; El-Bahr et al. 2021, Elbaz et al. 2025). They found that the mean body weight and body weight gain were enhanced by the addition of *N. sativa* seeds. Similarly, our results at 42nd days of age indicated that the weight gains in broilers increased in those broilers fed a dose of 150 mg/L of *N. sativa* in week 6 of the trial, presenting better results than broilers fed other treatments and with the antibiotics. However, broilers fed 450 mg/L of *N. sativa* had worse results for the entire experimental period but improved the weight gain of the broilers in the last (sixth) week compared to the other treatments. After adaptation time, 150 mg/L of *Nigella* acts better than broilers fed with antibiotics. It could be due to better cecum microbiota parameters (*Lactobacillus* spp higher and the coliform bacteria lower) on broilers fed on *N. sativa*, compared with the antibiotics. *Lactobacillus* spp couldact upon intestinal microflora, stimulating the production of digestive enzymes with benefits on growth gain. Similar results reported that feeding with *N. sativa* significantly increased the body weight of broilers at 21 and 42 days of age, reducing the feed conversion ratio during the trial period (Talebi et al. 2021). It has been suggested that these results were produced by the effect of black cumin seeds on the activity of pancreatic digestive enzymes and the liver's production and secretion of bile (Shakeri et al. 2016; Singh and Kumar 2018).

However, other studies found that diets containing *N. sativa* seed did not significantly influence weight gain and feed efficiency as measured for the entire experimental period (Fathi et al. 2023). These differences could be due to the quantities used and the different preparations of the samples of *N. sativa*. Thus, these authors evaluated different levels of black cumin *(N. sativa*) seed meal and only observed improvements in body weight and FCR in chickens fed the highest levels of *(N. sativa*), suggesting that at low doses, the effects on growth are limited. However, we also found that high doses of *N. sativa* (450 mg/L) showed a lower weight gain of chicks at 36 days compared to the other treatments, suggesting that the amount of *Nigella* supplied is important. Interestingly, other reports presented different results depending on the presentation or mode of administration of the *N. sativa* (Rahman and Kim 2016 and Kumar et al. 2018), found that its *Nigella* seeds improve the performance of the broilers compared to feeding on the extract. The authors suggest that ground seeds improve the release of active compounds compared to whole seeds and oily extract. *Nigella* seeds contain a combination of fibre, oils, and phenolic compounds that enhance the effects compared to oil, however, oils have a higher concentration of thymoquinone. Although they suggest some evidence that the mode of administration (ground seeds vs. oil) influences growth performance, with seeds showing better results, no comparison or trial comparing both presentation formats and mode of administration of *Nigella* was conducted.

Feed intake

On day 36, all the broilers exhibited similar responses to food intake, including those in the groups receiving the *N. sativa* extract, indicating that this extract did not influence their diet intake. Some studies have found that an increased amount of *N. sativa* in diets resulted in decreased feed intake. Rahman and Kim (2016) support the affirmation by reporting a reduction in feed intake with a moderate dose of *N. sativa,* although it does not evaluate multiple levels of supplementation. At a young age, the addition of the *N. sativa* to their diet may not have been sufficiently tasty or palatable for the birds. Hence, the animals might require some time to adapt to the new taste before increasing their intake. In our case, this is not a significant result because the *N. sativa* extract was added to their water. Our findings align with those of other researchers, who observed that daily feed intake remained stable when *N. sativa* was included in the diet (Laudadio et al 2022). These results reinforce the notion that *N. sativa* does not affect the

animals'appetite, as feed intake was similar across all treatments, with an overall increase observed as the birds grew over the weeks.

However, during the final week of the study, both feed intake and weight gain declined (except for the animals fed 150 mg/L of *N. sativa*). Feed intake decreased across all groups studied. Although this could be attributed to health or management issues, interestingly, the animals fed with 150 mg/L of *N. sativa* exhibited a significant weight gain increase at 42 days compared to the other groups. This outcome suggests that the effects of health and management issues should be discarded, as all the animals reared under identical conditions and would have been equally affected. This is likely explained by the fact that the chicks had already reached their expected body weight, around day 42 according to the production cycle, age, and genetic selection of the animals, after which intake and growth decrease (Bell and Weaver 2002; Zuidhof et al. 2014).

Feed conversion rate

As reported by Al-Mufarrej (2014) none of the treatments affected the feed conversion rate and efficiency. However, the feed conversion rate was lower and more favorable during the first 4th weeks of the study, worsening as values increased during the 5th and 6th weeks. This trend is typical, as age affects growth; adult birds generally gain weight more slowly than younger ones, which impacts the feed conversion rate. Zuidhof et al. (2014) found that rates of weight gain were significantly higher in the first few weeks (1–21 days) and decrease by day 42, when birds reach their target weight. This decrease in weight in adult birds was accompanied by an increase in the feed conversion rate (lower feed efficiency), as a higher proportion of feed is used for maintenance rather than growth. The authors point out that metabolic efficiency was higher in young birds due to their rapid muscle growth and lower body mass.

However, at the end of the study period, we observed the best results in broilers fed 150 mg/L of *N. sativa* compared to other treatments.

Carcass characteristics

The final live body weights, empty abdomen carcass weight, chicken breasts and drumsticks, were higher in those chickens' fed diets supplemented with 150 mg/L of *N. sativa*. These results correspond to an increase in the body weight gain observed in broilers receiving this dosage. The inclusion of antibiotics in the diet, compared to supplementation with 150 mg/L of *N. sativa*, did not result in any significant differences in final body weight or eviscerated carcass yield.

Other parameters, such as the weights of the bursa of Fabricius, wings, abdominal fat, liver, and gizzard, showed no significant differences with any of the diets used in our study. Nevertheless, previous studies using *N. Sativa* at concentrations of 0.5 and 1% reported increases in the growth and relative weights of the cecum, liver, spleen, and thymus, as well as overall organ weight (Rahman and Kim 2016; Seidavi et al. 2020; Laudadio et al. 2022). In the present study, notably, the heart weight was higher in broilers supplemented with 450 mg/L of *N. sativa.* Laudadio et al. (2022) also suggest that the reduction in *Escherichia coli,* could be related to the development of the cecum. The same study reported similar findings, noting increases in live body weight, as well as breast, wing, and thigh weights when using powdered *N. sativa* seeds.

Regarding the spleen, an organ related to the immune system, we observed that on day 42, groups incorporating virginiamycin and 150 mg/L of *N. sativa* had higher spleen weights compared to broilers fed with 0, 300, and 450 mg/L of *N. sativa*. The effects on lymphoid organ weights were not conclusive, as the weight of the bursa of Fabricius showed no significant differences. However, spleen weight decreased as the levels of *N. sativa* increased in the diet. Oppositely, Fathi et al. (2023) reported increase in relative spleen weight at the highest doses of *N. sativa* was added to the diet. Other investigations (Al-Mufarrej 2014; Laudadio et al. 2022), reported that increasing the amount of *N. sativa* seed in the diet correlates with an increase in the weight of lymphoid organs. Further studies are necessary to clarify the *N. sativa* effects on organ weights related to the immune system.

Blood chemistry

The mean blood parameter values for glucose, triglycerides, HDL, VLDL albumin and LDL/HDL at day 42 did not show significant differences among

the groups. However, the total cholesterol and LDL levels were lower in birds fed *N. sativa*. These results align with other studies that found diets supplemented with *N. sativa* seeds significantly reduced cholesterol and triglycerides (Siddiqui et al. 2015; Seidavi et al. 2020; Laudadio et al. 2022). This effect could be attributed to the high content of polyunsaturated fatty acids in *N. sativa*, which supports its cholesterol-lowering potential (Attia and Al-Harthi 2015; Rahman and Kim 2016; Singh and Kumar 2018; Fathi et al. 2023). Also, these authors suggest that the high content of antioxidant compounds such as thymoquinone together with anti-inflammatory properties of *N. sativa* contribute to the reduction of lipids by improving lipid metabolism and reducing lipid oxidation. Additionally, *N. sativa* contains sterols, particularly β-sitosterol, which can inhibit dietary cholesterol absorption (Khan et al. 2021).

This finding suggests that *N. sativa* may be beneficial for managing cholesterol levels in both animals and humans. Regarding glucose values, our study found no significant changes, consistent with several reports indicating that *N. sativa* supplementation in diets does not affect plasma glucose concentrations, although it does lower triglyceride and plasma cholesterol levels (Khalil et al. 2020; Laudadio et al. 2022).

In terms of total protein concentration, it was significantly higher in broilers fed a diet containing 300 mg/L of *N. sativa,* which agrees with other researchers (Alagawany et al. 2015; Rahman and Kim 2016; El-Bahr et al. 2021; Fathi et al. 2023). Additionally, the total protein concentrations were notably higher in the *N. sativa* diet-fed broilers compared to those without it. However, some studies reported decreasing total plasma protein and albumin levels in broilers on *N. sativa* supplemented diets compared to those fed antibiotics (Attia and Al-Harthi 2015). This difference may be due to various physiological and pathological factors described in broilers, such as feed consumption, metabolic changes, and overall body condition during growth.

Regarding the uric acid level, a decrease was observed in the group fed with 150 and 300 mg/L of *N. sativa,* which could be attributed to improved protein utilization and metabolism, resulting in less waste production.

However, another report (Attia and Al-Harthi 2015) indicated significantly higher plasma urea levels in groups fed *N. sativa* compared with other groups, while a different investigation (Khalil et al. 2020) found no significant changes in uric acid content with varying levels of *N. sativa*. Although more research is needed, the use of *N. sativa* may prove interesting for treating and preventing visceral gout or avian urolithiasis, a common condition associated with arthritis due to severe renal failure (Hayatdavoudi et al. 2016; Nirumand et al. 2018).

Cecum microbiota

Intestinal bacteria play a crucial role in the health of animals, including poultry (Islam et al. 2016). In this study, coliform bacteria levels were lower in broilers fed *N. sativa* at 150 mg/L, while higher levels were observed in broilers that received antibiotics.

Other investigations have also reported that *N. sativa* inhibits the growth of harmful bacteria, such as *E. coli* in the faeces of broilers (Kumar et al. 2017; Soliman et al. 2017; Seidavi et al. 2020; Laudadio et al. 2022). The authors attribute these effects to *Nigella sativa's* antimicrobial compounds (e.g., thymoquinone) that inhibit pathogens and promote a healthy microbiome and suggest that their use may be an effective approach to maintaining gut health by promoting a favorable microbial balance. While virginiamycin exhibits a narrow-spectrum antimicrobial activity mainly targeting Gram-positive bacteria, it may allow the overgrowth of Gram-negative coliforms due to microbial imbalance. However, the thymoquinone in *N. sativa* has demonstrated broad-spectrum antimicrobial effects against both Gram-positive and Gram-negative bacteria, including *Escherichia coli.* These results suggest that dietary supplementation with *N. sativa* can reduce the number of coliform bacteria in the intestines.

Another important aspect of animal health is maintaining the appropriate levels of beneficial bacteria, such as *Lactobacillus* spp. in the cecal microbiota. *Lactobacillus* spp. was lower on broilers that received antibiotics, but significant increases in *Lactobacillus* spp. counts were obtained in broilers with *N. sativa*, indicating a positive effect of *N. sativa* on the intestinal flora. This could be due prolonged or routine use of antibiotics in poultry production has been associated with intestinal dysbiosis and a marked reduction in beneficial bacterial populations, such as *Lactobacillus* and *Bifidobacterium,* which are crucial for

gut health and competitive exclusion of pathogens (Gadde et al. 2017).

However, the changes obtained in gut microbiota in broilers with *N. sativa* were associated with improved host health, including increased immune organ weights (spleen and thymus) and improved antibody titres (Rahman and Kim 2016; Laudadio et al. 2022; Fathi et al. 2023).

Therefore, *N. sativa* could be used as an effective approach to maintaining host health by increasing the number of beneficial bacteria and inhibiting the colonization of harmful pathogens.

Immune-response

Concerning immune parameters, differences were found in IgG and IgM levels performed on day 28. The concentration of IgG antibodies was significantly lower in chickens treated with 300 mg/L of *N. sativa*, while a higher concentration of IgM was observed with the 450 mg/L treatment. In addition, other researchers (Al-Mufarrej 2014; Laudadio et al. 2022) have reported a significant dose-dependent improvement in antibody titers against Newcastle disease. They also noted that *N. sativa* enhancesantibody titer against the infectious bronchitis virus. These effects were associated with an increase in the weight of lymphoid organs (spleen and thymus) and a reduction in intestinal pathogens, suggesting that *N. sativa* enhances humoral immunity. These effects may also be attributed to the immunostimulant compounds in *N. sativa,* such as thymoquinone, which enhance the humoral immune response.

Conclusions

Our findings suggest that incorporating *Nigella sativa* into the drinking water of broiler chicks does not affect feed intake and is easily administered within poultry production systems. At a dose of 150 mg/L, *N. sativa* positively influenced growth performance, improved biochemical parameters (e.g., reduced cholesterol levels), and supported gut microbiota health by decreasing coliform bacteria while increasing *Lactobacillus* spp populations. These preliminary results showed improved outcomes in growth performance, cholesterol levels, and gut microbiota balance compared to those observed with virginiamycin, suggesting that *N. sativa* could serve as a potential alternative to routine antibiotic use. However, further studies with larger sample sizes are needed to strengthen these findings and establish *N. sativa* as an effective growth promoter. Additionally, future research should explore the potential of N. sativa to improve LDL cholesterol, total cholesterol, and uric acid levels.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interests The authors report no conflicts of interest.

Statement on welfare of animals The Animal Ethics Committee of the Islamic Azad University ratified the experimental protocol. The protocol number is: 11750103972001-1400-03-30.

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