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Feeding *Nigella sativa* oil to broilers affects their performance, serum constituents and cecum microbiota

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Abstract

The objective of this study was to assess the effect of supplementation of *Nigella sativa* oil (NSO) as a growth promoter in broiler diets. A total of 300 unsexed one-day-old Arbor Acre broiler chicks were randomly divided into five treatment groups (60 chicks/group). Each group was divided into five replicates with 12 chicks per replicate. Group 1 was fed on the basal diet with no additives (control). Group 2 was fed the basal diet supplemented with oxytetracycline (OTC) at the level of 50 ppm. Groups 3, 4 and 5 were fed the basal diet supplemented with 500, 1000 and 1500 ppm NSO, respectively. Birds fed on 1500 ppm NSO diet showed the heaviest body weight at 14, 28 and 42 days of age. Feed conversion ratio was significantly improved at all ages with the inclusions of OTC and NSO generally superior to the control group. The relative weight of spleen was increased significantly by the addition of NSO at various levels. However, relative weights of thymus and bursa were not altered by the treatments. Total bacterial count, total yeast and moulds count, *E. coli* and *salmonella* spp. counts were reduced significantly, whereas the total lactic acid bacteria count was increased in OTC and NSO groups compared with control. *Nigella sativa* oil could be used in broiler chicken feeds as a natural alternative to antibiotic growth promoters to improve gut health and consequently growth performance.

Keywords: growth, feed conversion, immunity, organ weight
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Introduction

In recent decades, the attention of poultry breeders has been on the use of growth promoters to improve performance. As a result, antibiotics were used widely in the poultry industry (Attia & Al-Harthi, 2015). Antibiotics had the desired effects of improving growth performance, but increased resistance of harmful bacteria to antibiotics (Kim *et al.*, 2011; Hassanpour *et al.*, 2013). After the European Union banned the use of antibiotics as growth promoters in animal feeds in January 2006, the quest for natural growth promoters (Saleh, 2014) became critical (Toghyani *et al.*, 2010).

The seeds and oil of *Nigella sativa* (black cumin) have long been used to treat physiological disorders (Attia & Al-Harthi, 2015). *Nigella sativa* is a medicinal plant that could be used as a natural feed substitute in poultry and livestock production to increase efficiency and minimize the risk of antibiotic-resistant bacteria to humans and animals (Doyle, 2001). There is little information in the literature about the effects of NSO on performance and efficiency, carcass traits, and haemato-biochemical and immunological values of broiler chickens, with contradictory findings, depending on the concentration of NSO (Toghyani *et al.*, 2010; Durrani *et al.*, 2007). Hermes *et al.* (2009) found that adding 0.5% and 1% NSO to the broiler diet improved growth, white blood cells (WBCs), packed cell volume (PCV), and feed conversion ratio (FCR) significantly, whereas triglycerides and alanine aminotransferase (ALT) were decreased significantly compared with the untreated control group. Therefore, this study was conducted to evaluate the impact of NSO as a natural alternative to antibiotic growth promoters on growth performance, carcass traits, haemato-biochemical parameters, immune function, antioxidant status, and cecal microbiota in broiler chicks.

Materials and Methods

The current study was conducted at a private poultry farm in San-El-Hagar city, Sharkia Governorate, Egypt, according to the guidelines of the Advisory Committee on the Ethics of Animal Experiments, Poultry

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Department, Zagazig University, Egypt. The laboratory analyses, experiments and protocols were handled in accordance with the ethical standards as stated in the guidelines represented by the Committee of Animal Care and Welfare, Benha University, Egypt, Ethical Approval No. 2020-4.

A total of 300 unsexed one-day-old Arbor Acre broiler chicks were randomly divided into five treatment groups each of 60 chicks. Each group was split into five replicates, each containing 12 chicks. The replicates were placed in separate pens (100 × 120 cm). As a control, the first group was fed only the basal diet with no supplements. The second group was given the basal diet plus 50 ppm oxytetracycline (OTC). The third, fourth, and fifth groups were given the basal diet plus 500, 1000, and 1500 ppm *Nigella sativa* oil, respectively. The NSO was purchased from El Hawag Company for Natural Oils, Cairo, Egypt. The basal diet was created to meet the nutritional requirements of broiler chicks from hatch to 42 days old (NRC, 1994).

Table 1 shows the composition and chemical analysis of the basal diet. All of the birds were reared under the same management and sanitary conditions. A total of 23 hours of light per day were provided by an artificial light source. Feed and water were freely available. The experimental period extended for 42 days (1–42 days old). At 1, 14, 28, and 42 days old, chicks were weighed and feed intake (FI) was measured biweekly. The feed conversion ratio (g feed/g gain) and bodyweight gain were calculated.

Table 1 Feed ingredients of the basal diet during the stages of broiler growth

Ingredient, % as fed	Starter ration (days 1 - 21)	Finisher ration (days 22 - 42)
Maize (8.5% crude protein)	53.03	59.21
Soybean meal (44% crude protein)	35.00	27.00
Maize gluten meal (62% crude protein)	5.00	5.00
Rice bran	0.00	0.00
Soybean oil	2.90	4.82
Limestone	1.40	1.37
Di-calcium phosphate	1.50	1.55
Salt	0.30	0.30
Premix[1]	0.30	0.30
L-Lysine	0.15	0.15
Dl-Methionine	0.12	-
Choline chloride	0.30	0.30
Nutritional composition[2]		
Metabolizable energy, Kcal /kg	3000	3200
Crude protein, %	23.01	20.01
Calcium, %	1.02	1.00
Nonphytate phosphorus, %	0.45	0.45
Lysine, %	1.32	1.10
Total sulfur amino acids, %	0.92	0.72

[1]Amount added for each kg of diet, [2] according to the NRC (1994)

Five birds from each treatment were chosen randomly to represent all replicates, weighed, and slaughtered according to Islamic procedure on day 42. Blood samples were obtained in heparinized tubes for laboratorial analyses. The liver, gizzard, heart, and lymphoid organs (thymus, bursa of Fabricius, and spleen) were all weighed individually in grams. The weights of the total giblets (liver, heart, and gizzard) and of the dressing (carcass + total giblets) were calculated.

Non-enzymatic colorimetric methods were used to determine total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and creatinine. Enzymatic colorimetric methods were used to assess glucose, triglycerides, total cholesterol, high density lipoprotein (HDL) cholesterol, urea, and uric acid. Serum globulin was measured by subtracting serum albumin from serum total protein. The albumin/globulin ratio (A/G ratio) was determined by dividing serum albumin by serum globulin. Serum very low-density lipoprotein (VLDL) cholesterol was calculated by dividing triglycerides by 5. Low density lipoprotein (LDL) cholesterol was calculated by subtracting serum HDL plus VLDL cholesterol from total cholesterol (Friedewald *et al.*, 1972).

Immunoglobulin IgG, IgM, and IgA were measured in plasma using sandwich ELISA kits based on modifications of Bianchi *et al.* (1995). The colorimetric method was used to assess plasma levels of superoxide dismutase (SOD), reduced glutathione (GSH), total antioxidant potential (TAC), and malonaldehyde (MDA) according to Nishikimi *et al.* (1972), Beutler (1963), Koracevic *et al.* (2001), and Ohkawa *et al.* (1979), respectively. The activity of glutathione peroxidase (GPx) was measured with an ultraviolet system (Paglia & Valentine, 1967).

Cecum contents (10 g) were obtained from five birds per treatment and transferred separately to 250 mL Erlenmeyer flasks containing 90 mL sterile peptone (0.1% peptone) and saline solution (0.85% NaCl) and blended thoroughly. The total bacterial count (TBC), total yeasts and moulds count (TYMC), *E. coli, Salmonella* and lactic acid bacteria count were recorded according to Xia *et al.* (2004) and Reda *et al.* (2020a).

Prior to the analysis, data were checked for normality, and all percentages and data on the cecum microbiota count were transformed using logarithmic and arcsine transformations. Data were analysed using one-way analysis of variance according to this statistical model:

$$Y_{ij} = \mu + \alpha_i + e_{ij}$$

where: Y_{ij} = is the observation,
μ = is the overall mean,
α_I = is the fixed effect of the ith treatment (I = 1,2,3,4, and 5), and
e_{ij} = random error.

Duncan's multiple range test (Duncan, 1955) checked the significance (*P* <0.05) of the differences between treatment means. Statistical analyses were performed with SAS software (SAS, version 9.4., SAS Institute Inc., Cary, North Carolina, USA).

Results and Discussion

Chicks fed a diet supplemented with 1500 ppm NSO had higher LBW and BWG at 14 days old (*P* < 0.01) than the control and the other groups (Table 2). Live bodyweight at 28 and 42 days old and BWG through 28 - 42 and 1 - 42 days old were (*P* <0.01) higher in chicks given 50 ppm OTC, and 1000 and 1500 ppm NSO compared with the control and 500 ppm NSO. Bodyweight gain was significantly higher in the groups treated with 1000 and 1500 NSO between 14 and 28 days old. Thus, broiler chicks fed a diet supplemented with 1500 ppm NSO outperformed all other treatments possibly because of the presence of unsaturated fatty acids and especially primary essential fatty acids, which are thought to be necessary for growth (Üstun *et al.*, 1990). The present findings agreed with those of Halle *et al.* (1999), who assessed the effects of diets supplemented with various levels of black cumin essential oil (0.1 or 1 g/kg) or oilseed (10 or 50 g/kg) and found that growth was increased in one experiment, but no positive effects of these levels were found in a second experiment. According to Hermes *et al.* (2009), the inclusion of NSO at 0.5% and 1% improved broiler growth and reduced mortality rates in hot climates. Denli *et al.* (2004) found that feeding quails on diets supplemented with 60 mg/kg of Nigella essential oil improved BWG and feed efficiency. In addition, Saleh (2014) reported that feeding NSO at 1 ml/kg increased BWG compared with the control and antibiotic (avilamycin) groups. According to Attia and Al-Harthi (2015), adding NSO at 0.5 or 1.5 g/kg broiler diet may be used as an alternative growth promoter that is as efficient as or is better than zinc bacitracin.

Feed intake was significantly lower in NSO groups across 28 - 42 and 1 - 42 days old compared with the control. However, it had no impact between the ages of 1 and 14 days and 14 and 18 days. The addition of OTC or NSO at various levels improved FCR substantially over all experimental periods (1 - 14, 14 - 28, 28 - 42, and 1 - 42 days old). In general, chicks fed 1000 ppm NSO had a higher FCR and consumed less feed than the other groups and the control. Similarly, Saleh (2014) found that Nigella seed oil at 1 ml/kg diet had no significant effect on feed consumption in broiler chicks at 1- 21 days, but improved FI and FCR at 1–42 days. Osman (2002) stated that broiler chicks fed a diet containing 1 g NSO had the lowest FI (*P* <0.01), followed by those fed 0.5 g/kg feed. The levels of Nigella seed oil, except for 0.5 g/kg feed, improved FCR significantly compared with the control through all periods. Conversely, Attia and Al–Harthi (2015) found that FI and FCR were not affected substantially by supplementation of antibiotic and NSO levels in broiler chick diets.

Table 2 Growth, feed intake and feed conversion ratio of broiler chicks as affected by dietary supplementation with various levels of *Nigella sativa* oil

Trait	Control	Oxytetracycline, 50 ppm	*Nigella sativa* oil, ppm			SE	*P*-value
			500	1000	1500		
Bodyweight, g							
1 day	39.15	39.13	39.16	39.16	39.20	0.421	1.0000
14 days	312.75^{b}	322.30^{b}	315.20^{b}	319.90^{b}	340.10^{a}	4.324	0.0101
28 days	1131.75^{c}	1166.70^{b}	1151.65^{bc}	1205.15^{a}	1213.95^{a}	7.875	0.0002
42 days	2032.80^{b}	2170.95^{a}	2062.95^{b}	2206.50^{a}	2240.60^{a}	18.406	0.0001
Bodyweight gain, g/day							
1–14 days	19.54^{b}	20.23^{b}	19.72^{b}	20.05^{b}	21.49^{a}	0.296	0.0080
14–28 days	58.50^{b}	60.31^{b}	59.75^{b}	63.23^{a}	62.42^{a}	0.547	0.0012
28–42 days	64.36^{b}	71.73^{a}	65.09^{b}	71.53^{a}	73.33^{a}	0.752	0.0001
1–42 days	47.47^{b}	50.76^{a}	48.19^{b}	51.60^{a}	52.41^{a}	0.436	0.0001
Feed intake, g/day							
1–14 days	32.95	31.17	30.90	31.63	31.89	0.502	0.1388
14–28 days	103.12	99.86	101.25	99.22	101.67	1.272	0.2935
28–42 days	149.57^{a}	144.27^{ab}	139.08^{bc}	136.17^{c}	137.36^{bc}	2.130	0.0090
1–42 days	95.21^{a}	91.76^{ab}	90.41^{b}	89.00^{b}	90.30^{b}	1.055	0.0253
Feed conversion ratio							
1–14 days	1.69^{a}	1.54^{b}	1.57^{b}	1.58^{b}	1.48^{b}	0.027	0.0238
14–28 days	1.76^{a}	1.66^{abc}	1.70^{ab}	1.57^{c}	1.63^{bc}	0.034	0.0282
28–42 days	2.33^{a}	2.01^{bc}	2.14^{b}	1.90^{c}	1.87^{c}	0.046	0.0005
1–42 days	2.01^{a}	1.81^{bc}	1.88^{b}	1.72c	1.72^{c}	0.030	0.0010

^{a,b,c}Within a row, means with a common superscript did not differ with probability *P* =0.05

The positive impact of NSO on feed utilization efficiency because of its antioxidant properties and phenolic compounds could account for the improvements in FCR. In addition, the active ingredients (nigellone and melatonin) had a nutrient-diversifying effect and worked together to increase nutrient utilization (El-Dakhakhny *et al.*, 2002; Abu-Al-Basal 2011; Ali *et al.*, 2012). The antibacterial and antifungal properties of NSO also could protect against hepatotoxicity and improve nutrient utilisation (Rathee, 1982). The NS could also induce thyroid hormone secretion indirectly and directly via the pituitary gland (Mohammed & Al-Suwaiegh, 2016) and thus boost the metabolic rate, resulting in better amino acid use (More *et al.*, 1980).

The additions of 50 ppm OTC and 1500 ppm NSO resulted in substantially higher relative weights of liver, giblets, and dressing percentage compared with the control (Table 3). Supplementation of 50 ppm OTC and 1000 and 1500 ppm NSO resulted in significantly higher gizzard relative weight (Table 3). However, carcass and heart percentages were not altered significantly by the treatments. Changes in carcass composition could be attributed to improvements in BWG and FCR. Other studies revealed that essential oils inhibit pathogens in the digestive system and increase FCR and carcass yield (Alçiçek *et al.*, 2003; Giannenas *et al.*, 2003). The current findings were consistent with those of Osman (2002), who found that when broiler chicks were fed a diet supplemented with 1 g NSO/kg, the dressing percentage increased from 68.92% to 72.78%. Hermes *et al.* (2009) also found that dietary inclusion of NSO at 0.5% and 1% increased the relative weight of caecum and liver of broiler chicks.

Table 3 Carcass traits of broiler chicks as affected by dietary supplementation with various levels of *Nigella sativa* oil

Trait	Control	Oxytetracycline50 ppm	*Nigella sativa* oil, ppm			SE	*P*-value
			500	1000	1500		
Carcass, %	72.40	73.70	72.71	73.49	74.38	0.525	0.1501
Liver, %	2.19^{c}	2.44^{b}	2.20^{bc}	2.24^{bc}	2.75^{a}	0.062	0.0012
Gizzard, %	3.36^{c}	3.82^{a}	3.39^{c}	3.61^{b}	3.92^{a}	0.056	0.0002
Heart, %	0.57	0.52	0.49	0.50	0.54	0.019	0.2642
Giblets, %	6.11^{c}	6.77^{b}	6.09^{c}	6.35^{c}	7.21^{a}	0.086	<0.0001
Dressing, %	78.51^{c}	80.47^{ab}	78.79^{bc}	79.84^{abc}	81.60^{a}	0.525	0.0138

^{a,b,c}Within a row, means with a common superscript did not differ with probability *P* =0.05

Table 4 summarises the effects of OTC and NSO levels on plasma glucose, total protein, and its fractions (albumin and globulin) in broiler chick diets at 42 days old. The findings showed that plasma glucose levels, total protein, and globulin concentrations differed significantly between groups. However, when the treatments were compared with the control, plasma albumin concentration and A/G ratio were not affected. Birds that received 1000 and 1500 ppm NSO had substantially reduced blood glucose compared with control. Plasma total protein and globulin levels were substantially higher in chicks given a 1000 ppm NSO diet supplement. El-Soud *et al.* (2000) also found that birds fed diets containing 1% or 2% NSO had higher total protein, albumin, and globulin levels at all ages compared with control chicks. In birds fed diets containing NSO, Osman (2002) and Hermes *et al.* (2009) recorded small increases in plasma total protein. Saleh (2014) found that when broilers were fed NSO instead of avilamycin or a control diet, plasma total protein, albumin, and the A/G ratio all increased, but plasma glucose decreased. Attia and Al-Harthi (2015) also reported that plasma total protein and albumin levels were significantly lower in chicks fed diets containing 0.5 and 1.5 g NSO/kg compared with those fed zinc bacitracin.

Table 4 Serum glucose, plasma proteins and lipid profile of broiler chicks as affected by dietary supplementation with various levels of *Nigella sativa* oil

Component	Control	Oxytetracycline, 50 ppm	*Nigella sativa* oil, ppm			SE	*P*-value
			500	1000	1500		
Glucose	289.00^{a}	275.55^{ab}	272.85^{ab}	240.02^{c}	257.45^{bc}	6.947	0.0057
Total protein (g/dL)	2.92^{b}	3.14^{ab}	3.12^{ab}	3.45^{a}	3.13^{ab}	0.091	0.0388
Albumin (g/dL)	1.58	1.63	1.65	1.78	1.61	0.044	0.1429
Globulin (g/dL)	1.34^{b}	1.51^{ab}	1.47^{b}	1.67^{a}	1.52^{ab}	0.054	0.0220
Albumin/globulin (%)	1.18	1.08	1.12	1.07	1.06	0.025	0.0917
Total cholesterol (mg/dL)	162.50^{a}	135.20^{b}	126.28^{b}	112.45^{b}	133.35^{b}	6.757	0.0063
Triglycerides (mg/dL)	70.11^{a}	56.33^{b}	51.02^{bc}	41.23^{c}	42.33^{c}	3.834	0.0023
HDL (mg/dL)	42.49^{b}	53.25^{a}	44.07^{b}	54.23^{a}	59.44^{a}	1.949	0.0005
LDL (mg/dL)	106.00^{a}	70.68^{b}	72.01^{b}	49.98^{c}	65.45^{bc}	5.562	0.0007
VLDL (mg/dL)	14.02^{a}	11.27^{b}	10.20^{bc}	8.25^{c}	8.47^{c}	0.767	0.0023

HDL: high-density lipoprotein, LDL: low-density lipoprotein, VLDL: very-low-density lipoprotein
^{a,b,c}Within a row, means with a common superscript did not differ with probability *P* =0.05

An enzyme induced by NSO to increase liver synthetic activity may be responsible for the increase in total protein levels (Al-Jishi & Hozaifa, 2003). Furthermore, the increase in total protein and globulin may be owing to the immunostimulating effect of NSO (Aqel, 1993), which contains macro and micro mineral elements that aid in the growth process, boost the immune system, and boost total protein and albumin levels.

The addition of OTC and NSO to the broiler chick diet decreased plasma total cholesterol, triglycerides, LDL, and VLDL concentrations significantly (Table 4). Chicks fed a diet with 1000 ppm NSO had the lowest values, whereas chicks fed the control diet had the highest values. The concentrations of HDL, which was significantly higher in the plasma of chicks fed the antibiotic diet and NSO, showed the opposite pattern. Hermes *et al.* (2009) stated that NSO at 0.5% and 1% reduced total cholesterol and triglyceride significantly compared with control. Saleh (2014) found that dietary supplementation with NSO decreased plasma triglyceride, total cholesterol, and LDL levels while increasing plasma HDL levels significantly. Moreover, Osman (2002) found that feeding an NSO diet to birds resulted in a substantial increase in plasma cholesterol concentration. The content of unsaturated fatty acids, which may stimulate cholesterol excretion into the intestine and oxidation of cholesterol to bile acids (Tollba & Hassan, 2003), may explain the substantial reduction in cholesterol and triglyceride concentrations by feeding diets supplemented with NSO (El-Dakhakhny *et al.*, 2000).

Plasma AST concentration was reduced significantly with OTC and NSO compared with the control (Table 5). Chicks fed 1500 ppm NSO had the lowest AST value compared with the control and other groups,. However, the addition of OTC and NSO to broiler clicks diets had no effect on plasma ALT, creatinine, urea, and uric acid concentrations. Hermes *et al.* (2009) found that feeding birds diets containing 0.5% and 1% NSO decreased the amount of AST and ALT significantly compared with the control. Saleh (2014) found that feeding broilers NSO reduced AST and ALT activities, which are indices of liver function. Furthermore, Attia and Al-Harthi (2015) found that plasma levels of ALT were higher in the 1.5 g NSO/kg group than the control and the 0.5 g and 1.0 g NSO/kg groups. Additionally, AST was higher in chicks fed 1 g NSO/kg diet compared with the other groups. The urea levels were significantly higher in chicks fed a 1.5 g/kg diet than in the other groups, but lower in chicks fed zinc bacitracin or 1 g NSO/kg diets than in the control (Attia & Al-Harthi, 2015).

Table 5 Liver and kidney function parameters of broiler chicks as affected by dietary supplementation with various *Nigella sativa* oil levels

Parameter	Control	Oxytetracycline, 50 ppm	*Nigella sativa* oil, ppm			SE	*P*-value
			500	1000	1500		
AST, IU/L	75.15[a]	60.41[b]	66.22[ab]	67.08[ab]	59.98[b]	3.086	0.0433
ALT, IU/L	12.49	11.94	12.86	11.73	11.19	1.012	0.8109
Creatinine, mg/dL	0.74	0.96	0.75	1.01	1.13	0.094	0.0634
Urea, mg/dL	1.58	1.76	1.64	1.94	1.93	0.186	0.5795
Uric acid, mg/dL	8.21	7.25	8.14	8.32	8.91	0.636	0.5177

AST: aspartate aminotransferase, ALT: alanine aminotransferase
[a,b]Within a row, means with a common superscript did not differ with probability P =0.05

Table 6 shows the effect of OTC and NSO levels on the relative weight of lymphoid organs. The relative weight of the spleen was increased significantly by NSO supplementation at various amounts. At 42 days old, there were no significant differences in the relative weight of the thymus and bursa of broiler chicks because of OTC and NSO supplementation. However, there was a numerical increase in the relative weights of the thymus and bursa in the OTC and NSO groups compared with the control. The increase of relative weights of lymphoid organs in broiler chicks fed diets containing NSO may be attributed to its role in activating the functions of these organs under normal temperature conditions (Radwan, 2003). The current findings are consistent with those of Osman (2002), who found that 1.0 g NSO/kg feed had a major effect on spleen weight compared with the control. Moreover, Hermes *et al.* (2009) found that birds fed NSO diets had significantly heavier spleens than control birds, and birds fed 1% NSO/kg feed had the highest relative spleen weight at 42 days old.

Table 6 Immune system response and antioxidant status of broiler chicks as affected by dietary supplementation with various levels of *Nigella sativa* oil

Measurement	Control	Oxytetracycline, 50 ppm	*Nigella sativa* oil, ppm			SEM	*P*-value
			500	1000	1500		
Spleen, %	0.09[c]	0.10[c]	0.11[bc]	0.14[a]	0.12[cb]	0.007	0.0043
Thymus, %	0.45	0.49	0.56	0.46	0.56	0.024	0.0563
Bursa of Fabricius, %	0.23	0.33	0.30	0.28	0.31	0.024	0.1772
IgM, mg/ml	0.61	0.63	0.58	0.66	0.61	0.083	0.9709
IgA, mg/ml	0.35[c]	0.56[b]	0.63[b]	0.77[a]	0.54[b]	0.037	0.0002
IgG, mg/ml	0.75[b]	1.06[a]	1.00[a]	1.04[a]	0.97[a]	0.055	0.0222
SOD, U/ml	0.21[c]	0.34[ab]	0.33[ab]	0.30[b]	0.40[a]	0.023	0.0026
MDA, nmol/mL	0.32[a]	0.18[bc]	0.25[ab]	0.16[c]	0.18[bc]	0.022	0.0040
TAC, ng/ml	0.20[b]	0.33[a]	0.20[b]	0.33[a]	0.28[a]	0.020	0.0015
GSH, mg/dl	0.22[c]	0.38[a]	0.25[bc]	0.35[ab]	0.33[abc]	0.031	0.0290
GPX, mg/dl	0.24	0.35	0.28	0.32	0.33	0.032	0.2309

SOD: super oxide dismutase, MDA: malonaldehyede, TAC: total antioxidant capacity, GSH: reduced glutathione, GPX: glutathione peroxidase
[a,b,c]Within a row, means with a common superscript did not differ with probability *P* =0.05

Table 6 shows the effect of dietary OTC and NSO supplementation on immune indices and antioxidant status in broiler chicks. There were no significant differences in average IgM values between trial groups. Groups treated with OTC and NSO had significantly higher IgA and IgG levels compared with the control and chicks fed a diet supplemented with 1000 ppm NSO had the highest IgA levels. This effect of NSO may be because NSO contains 67 constituents capable of inducing pharmacological and beneficial effects against bacteria, including *staphylococcus* and *E. coli* (Hanafy & Hatem, 1991). Antioxidant, antibacterial, and anti-inflammatory properties of black cumin seed active components have been shown to have beneficial effects on immunity and organs (Al Jabre *et al.*, 2003; Al-Saleh *et al.*, 2006; Arslan *et al.*, 2005). Soliman et al. (2017) found a substantial increase in IgG and IgA in birds fed *Nigella sativa* Linn., but no significant changes in IgM in the treated groups or the control. Similarly, Al-Mufarrej (2014) found that supplementing broiler chicks with *Nigella sativa* Linn. seeds at 1% and 1.4% improved immune responsiveness. Table 6 shows that SOD, TAC, and GSH levels were significantly higher in chicks fed OTC and NSO diets than in control chicks. Plasma MAD levels were significantly lower in groups fed OTC and NSO diets relative to the control group, except for the 500 ppm NSO diet. Various supplements, on the other hand, had no major effect on GPx. The antioxidant activity of NSO is well known (M El-Dakhakhny *et al.*, 2002; Mahmoud *et al.*, 2002). Attia and Al-Harthi (2015) found that broiler chicks fed NSO diets had substantially higher SOD levels than the control group. In addition, the 1.5% NSO group had slightly higher GSH levels than the other groups.

Table 7 shows the treatment effects on the intestinal microflora of broilers at the end of the experiment (42 days old). Total bacterial count, TYMC, *E. coli*, and salmonella spp. counts were significantly lower in all groups relative to control. Supplementation with OTC and NSO improved total lactic acid bacteria count in all groups in contrast to the control. There is scientific evidence that herb extracts can reduce the number of pathogenic bacteria in poultry guts (Reda *et al.*, 2020b). The seeds of *Nigella sativa* have antibacterial properties against a wide range of bacteria, including gram-positive and gram-negative bacteria (Kumar & Berwal, 1998). In terms of its bioactive components, thymoquinone has antimicrobial activity against *Pseudomonas aeruginosa*, *Salmonella typhi* and other bacteria, and antifungal, antiviral, and antiparasitic activity against a wide range of gram-positive and gram-negative bacteria, viruses, fungi, *Candida* spp. and parasites (Forouzanfar *et al.*, 2014; Ratz-Łyko *et al.*, 2014).

Table 7 Cecum microbiota of broiler chicks as affected by dietary supplementation with various levels of *Nigella sativa* oil

Cecal microbiota, log CFU/g	Control	Oxytetracycline, 50 ppm	*Nigella sativa* oil, ppm			SE	*P*-value
			500	1000	1500		
Total bacterial count	5.79[a]	5.30[c]	5.50[b]	5.19[c]	5.22[c]	0.054	0.0002
Total yeast and molds count	5.59[a]	4.49[c]	4.88[b]	4.37[c]	4.40[c]	0.078	<0.0001
E. coli	5.94[a]	4.59[c]	4.86[b]	4.30[d]	4.55[c]	0.078	<0.0001
Salmonella spp.	3.49[a]	2.15[d]	2.71[b]	2.23[cd]	2.40[c]	0.057	<0.0001
Lactic acid bacteria	3.69[ab]	3.88[a]	3.46[b]	3.84[a]	3.85[a]	0.071	0.0150

[a,b,c]Within a row, means with a common superscript did not differ with probability *P* =0.05

Alam *et al.* (2010) found that an ethanol extract of *Nigella sativa* was highly effective against *B. subtilis* and *S. aureus*. The total inhibition of *E. coli* growth rate necessitated a high concentration of *Nigella sativa* extract. These findings suggest that black cumin seeds may have antibacterial activity against bacteria that are antibiotic resistant. *Nigella sativa* oil has also been shown to have antifungal activity against *Aspergillus species*, curvula lunata, pathogenic yeast, and Candida albicans (Agarwal *et al.*, 1979; Hanafy & Hatem, 1991). Controlling the gut microflora could affect broiler growth performance positively (Gunal *et al.*, 2006). The added calories from NSO might increase bile flow rate, resulting in enhanced emulsification and stimulating pancreatic lipases which promotes fat digestion and fat-soluble vitamin absorption (Mahmoud *et al.*, 2002). In addition, bioactive compounds in NSO, such as P. cymene, thymol, dithymoquinone, thymoquinone, and carvacrol, may thus improve feed digestibility and nutrient absorption by directly stimulating digestive enzymes (Nasir, 2009; Salam *et al.*, 2013). The antioxidant properties and phenolic compounds in NSO have also been shown to have positive effect on feed utilization, which could explain the improvement in growth performance (Attia & Al-Harthi, 2015).

Conclusions

Dietary supplementation of NSO up to 1500 ppm in broiler diets had positive effects on growth and carcass characteristics, immunological parameters, antioxidant status and cecum microbiota. Thus, itmay be used as a natural alternative to antibiotic growth promoters in broiler chicken diets to encourage gut health and thus enhance growth performance.

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Authors' Contributions

AMS, MSE, AGE and FMR collected the data. AGE and FMR conducted the statistical analyses. AMS, AIA, MSE, AGE and FMR collaborated in interpreting the results, wrote the initial draft of this manuscript, and finalized the manuscript. AIA, and FMR, developed the original hypothesis and designed the experiments. The authors have read and approved the manuscript.

Conflict of Interest Declaration

The authors declare that they have no competing interests.

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