The effects of supplementation *Nigella sativa* seeds as a natural substance on growth rate, some serum indices, carcass quality and antibody titers of broiler birds

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ABSTRACT

The present study was conducted to investigate the effects of feeding low and high levels of *Nigella sativa* seed (NSS) as a natural substance on growth rate, carcass traits, some serum blood indices and antibody titers of broiler birds under the environmental conditions of Yemen. A total of one hundred eighties unsexed (day-old) chicks were housed into five equal groups and each group contains three replicates with 12 chicks each. There were 5 dietary treatments for feeding birds; first one was the control (T1), without any natural substances and NSS were added in diets 2, 3, 4 and 5 (T2, T3, T4 and T5) at levels 0.25, 0.5, 1% and 2% respectively. Results at 5 weeks indicated that the addition of NSS up to 1% in the diets enhanced linearly body weight, weight gain and feed conversion ratio. The higher level of the seeds 2% not necessarily caused a higher degree of improvement. Birds fed diets containing 1% NSS (T4) achieved a higher ($P<0.05$) body weight and weight gain compared with the control (T1) and 0.25% NSS (T2) and better ($P<0.05$) feed conversion than other treatments. Similarly, the dietary treatment T4 (1% NSS) recorded the best ($P<0.05$) carcass dressing, breast and thigh percentage compared with the control. However, there were no significant variances in giblets and abdominal fat among all dietary groups. On the other hand, feeding birds' diets supplemented by diverse levels of NSS decreased blood total cholesterol. Dietary group T4 significantly increased ($P<0.05$) serum total protein and albumin as compared to the control group. Additionally, data showed that NSS treatments except T5 enhanced ($P<0.05$) the antibody titers against GD and ND virus.

KEYWORDS: *Nigella sativa*; growth; serum indices; antibody titers; broiler birds**.**

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INTRODUCTION

It is clear that develop new techniques in the livestock and poultry nutrition like using feed additives, particularly antibiotics led to increasing the nutritional value of the feed. Antibiotics as a chemotherapeutic drug have been used in animal diets for many years to enhance production performance and health (Dibner and Richards, 2005). Despite the witnessed progress, the use of antibiotics has been complained due to the fear of the existence of antimicrobial resistance in human (Parreto *et al.,* 2008**).** It was also reported that antibiotics were banned from administration in the European Union and United Kingdome (Ansari, *et al.,* 2008). Therefore, many investigators are searching for natural alternatives, which have desirable properties on performance and health status of animals**.**

Currently, the use of natural feed additives such as probiotics, prebiotics, medicinal plants and herbs has become a modern tendencies worldwide as a replacement for antibiotics in poultry diets (Revington, 2002: Guo *et al.,* 2004). In Yemen, there is a growing trend in practice some traditional methods like using medicinal plants for the prevention and treatment of many illnesses. Black seeds (*Nigella sativa* Linn.) Is one of the medicinal plants and could be used as natural feed alternatives in the livestock and poultry production to enhance performance and reduce the incidence of developing antibiotic resistant bacteria in human and animals (Doyle, 2002). Recently, some studies have been conducted to explore the effects of the seeds of broiler birds, and the results showed that NSS had a positive effect on growth performance **(**Tollba and Hassan, 2003: Guler *et al.,* 2006**)** and livability (El-Ghamry *et al*., 2002). Feeding NSS improved performance and egg quality of layer chickens (Akhtar *et al.,* 2003; Aydin *et al.,* 2008). Furthermore, Guler *et al.,* (2007) reported that black seeds could be considered as a natural antioxidant developer for poultry.

In fact, several research works were conducted globally to explore the possible effects of NSS on poultry. The results were promising and opened a new opportunity for more examination to specify the best dosage for the potentially different benefits on poultry production and health. Subsequently, the present research work aimed to investigate the effects of the low and high levels of *Nigella sativa* seed on growth rate, carcass traits, some serum blood indices and antibody titers of broiler birds under the environmental conditions of Yemen.

MATERIALS AND METHODS

Birds and management

The experimental work was conducted in a floor area in a small open sided unit under similar management and hygienic conditions. A total of one hundred eighties unsexed (day-old) commercial broiler chicks (Hubbard, JV) was used. The chicks were weighed and distributed into 5 dietary treatment groups, and each group contains three replicates with 12 chicks each. The chicks were raised in a small pen (2x1 meter square) using barriers of wood. Each individual pen was supplied with a feeder and automatic drinker used for raising one replicate of the experiment groups. All chicks were vaccinated against Newcastle disease (ND) on day 6 and 21, and Gumboro disease (GD) on 10 and 18 days. Feed and water were offered free *(ad Libitum)* throughout the experimental period that continued for six weeks.

Experimental feeds

A commercial mash feed was offered free (*ad libitum*) in two stages; a starter from 1 to 3 weeks and a grower diet from 4 to 6 weeks of age for broiler birds (Table 1). Experimental diets were formulated according to the strain manual recommendation (Hubbard JV). There were 5 dietary treatments for feeding birds; first one was the control (T1), free of any natural additives, Nigella sativa seeds (NSS) were added at the levels 0.25, 0.5, 1% and 2% in the diets T2, T3, T4 and T5 respectively. NSS of Yemeni origin was purchased from a local herb market, Sanaa, Yemen. The chemical composition of the seeds was analyzed according to the method detailed in AOAC (1990), and the result showed that the *Nigella sativa* seeds cultivated in Yemen contain 21.3% protein, 36.6% oil, 31.7% carbohydrate and 4.2% ash.

Table 1: Ingredients and composition of the experimental birds' diet

Ingredients %	Starter (1-3weeks)					Grower (4-5weeks)				
	0%	0.25%	0.5%	1%	2%	%0	0.25%	0.5%	1%	2%
Yellow corn	60	60	59.75	59.5	59	65	65	64.75	64.5	64
Soybean meal 46%	30	29.75	29.75	29.5	29	25	24.75	24.75	24.5	24
Protein concentrate10%*	10	10	10	10	10	10	10	10	10	10
Nigella sativa seeds	0	0.25	0.50	1	2	0	0.25	0.50	1	2
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
Calculated analyses:										
Metabolize energy (K Cal. / Kg)	2911	2914	2915	2919	2927	2969	2972	2973	2977	2985
Crude protein %	22.0	21.96	21.99	21.96	21.90	20.15	20.09	20.12	20.09	20.03
Calcium %	0.95	0.95	0.96	0.96	0.97	0.74	0.74	0.74	0.75	0.75
Available phosphor %	0.42	0.42	0.42	0.42	0.43	0.29	0.29	0.29	0.29	0.30
Methionine %	0.54	0.54	0.54	0.54	0.54	0.48	0.48	0.48	0.48	0.48
Lysine %	1.14	1.14	1.14	1.14	1.15	0.96	0.96	0.96	0.96	0.96
Methionine + Sistine %	0.96	0.96	0.96	0.96	0.96	0.87	0.87	0.87	0.87	0.87

*Protein concentrate 30% protein, 2000 metabolize able energy (K Cal. /Kg), Calcium 8.20%, Phosphor 1.50%, Methionine 1.85%, Lysine 2.20% and Methionine + Sistine 2.40 %.

Growth rate measurements

During the experiment, growth rate measurement were evaluated as a fellow; weekly live body weight (LBW), body weight gain (BWG) and feed intake (FI) were recorded. Feed conversion ratio (FCR) was calculated (kg feed consumed/kg of live weight gain).

Carcass traits

At the end of the experimental period, six broiler chicken birds within each treatment (two chickens/each replicate) were randomly taken and weighed to document live weight. The birds were slaughtered and waited for whole bleeding, and then their feathers were removed. The weights of the carcass, dressing percentage, breast, thigh, giblets (liver, heart and gizzard) and abdominal fat around the gizzard were separately recorded for each bird and reflected as the ratio of live weight.

Serum blood inspections

At the end of the experiment, six birds from each dietary group were randomly taken for blood inspects. Blood samples were collected and slanted away from the sunshine. The serum clear yellow colored was separated and centrifuged at a rate of 1000 RPM for 15

minutes, then stored at –20^{0}C till next application. Serum total protein, albumin and cholesterol were examined by spectrophotometer examination using commercial reagents, while Serum globulin constituent was computed as the difference among the values of protein and albumin. Regarding immunity indices. Serum ND Antibodies were explored using the test Haemagglutination inhibition (HI) according to the procedure described by the investigators Thayer and Beard, (1998). The serum antibody response to GD was assayed by ELIZA inspection using BioCheck commercial kits, Netherlands.

Statistical analysis

Experimental data were statistically evaluated using the general linear model (GLM) described in the software program SAS (2004). Static significant variances between the experimental treatments were calculated by the method multiple range test (Duncan, 1955).

RESULTS AND DISCUSSIONS

Growth rate

The results of the data analyses at the age of 5 weeks revealed that addition of different levels of *Nigella sativa* seeds (NSS) in broiler diets increased both live body weight, body weight gain and enhanced feed conversion ratio (Table, 2). This improvement was linearly increased as NSS levels increased in the diet up to 1% and the higher level of the seeds 2% not necessarily caused a higher degree of improvement. Birds fed diets containing 1% NSS (T4) had a higher ($P<0.05$) live body weight, body weight gain compared with the Control (T1) and 0.25% NSS (T2) and better ($P<0.05$) feed conversion ration than other treatments. These results were supported by the findings of Guler *et al.,* (2006) and Abu-Dieyeh and Abu-Darwish, (2008) who stated that using 1 or 1.5% black seeds had a better average of body weight and feed conversion ratio. While, Durrani *et al.,* (2007) found that 4% NSS had a significant effect on the growth rate of broiler birds.

In terms of cumulative feed intake, there were no significant effects ($P >0.05$) of all dietary treatments at 5 weeks. These results are closely with those findings by Guler *et al.,* (2006) in broilers. However, other studies indicated that *Nigella sativa* supplementation in the broiler chicken diets increased significantly feed intake (Hassan *et al.,* 2004; Hermes *et al,* 2009).

This improvement in growth rate can be attributed to the nutritional effects of the main components of Nigella sativa. NSS contain high percentages of fatty acids and all essential amino acids that may promote growth rate of birds (El-Deek, *et al.,* 2009). NSS has also a positive effect on digestive enzymes to enhance the utilization of dietary nutrients (Hermes *et al,* 2009). Pharmacological effects of NSS and its active components may consider as another active mechanism that contributes to the enhancement of broiler bird. Nigella sativa has an important influence as antibacterial and antifungal (Rathee *et al.,* 1982; Maraka *et al.,* 2007). Roy *et al.,* (2006), who stated that Black seed extracts had a positive activity against several types of bacteria like *E. coli* and *Staphylococcus aureus* and attributed this effect to the active ingredients of Nigella seeds particularly the compound Thymoquinone.

Table 2: Means ±SE of growth rate of the experimental birds at 5 weeks of age

Treatment	Live body weight (g.)	Body weight gain (g.)	Feed intake (g.)	Feed conversion ratio
Control (T1)	^{c}1259.64 ±15.30	^{c}1218.14 ±10.75	^{a}2429.72 ±25.90	^{a}1.99 ±0.02
NSS 0.25 % (T2)	^{cb}1313.34 ± 40.55	^{cb}1272.34 ± 23.90	^{a}2402.78 ±42.94	^{b}1.89 ±0.02
NSS 0.5% (T3)	^{b}1323.47 ± 41.12	^{b}1281.97 ±18.02	^{a}2402.36 ±42.26	^{b}1.87 ±0.03
NSS 1 % (T4)	^{a}1402.80 ± 14.94	^{a}1361.30 ±15.11	^{a}2463.02 ±19.10	^{c}1.81 ±0.01
NSS 2 % (T5)	^{ab}1367.08 ± 33.24	^{ab}1325.75 ±13.81	^{a}2529.71 ±29.32	^{b}1.91 ±0.03

The Means within the same column having different subscript are significantly different (P<0.05).

Carcass traits

The results of the data analyses in table 3 revealed that dietary treatment T4 recorded the best (P<0.05) dressing, breast, and thigh percentages was observed for T4 compared with the control. The present results are in harmony with those obtained by Guler *et al.,* (2006) who cleared that cold carcass, thigh and breast were higher in broiler birds fed diets containing 1% black seed. The reason for this enhancement in carcass percentage may be attributed to that Nigella seeds are rich in its content of fatty acids that enhance the metabolism of dietary nutrients. Additionally, there were no significant differences in abdominal fat and giblets between dietary treatments as a result of application different levels of NSS. Similar results were obtained by Hermes *et al,* (2009) and AL-Beitawi and El-Ghousein, (2008) who noticed an absence of any significant differences in the percentage of giblets and abdominal fat as a result of feeding broilers with various levels of NSS.

Table 3: Means ±SE of carcass traits of the experimental birds

Treatment	Dressing (%)	Breast (%)	Thigh (%)	Giblets* (%)	Abdominal fat (%)
Control (T1)	**b** 70.92 ±0.74	**b** 19.98 ±0.33	**b** 10.37 ±0.36	**a** 4.59 ±0.13	**a** 0.73 ±0.06
NS 0.25 %(T2)	**ab** 72.24 ±0.38	**ab** 20.50 ±0.42	**ab** 11.01 ±0.38	**a** 4.59 ±0.10	**a** 0.63 ±0.02
NSS 0.5% (T3)	**ab** 71.65 ±0.71	**ab** 20.82 ±0.40	**ab** 11.00 ±0.22	**a** 4.64 ±0.06	**a** 0.73 ±0.04
NSS 1 % (T4)	**a** 73.45 ±0.53	**a** 21.30 ±0.22	**a** 11.29 ±0.28	**a** 4.62 ±0.13	**a** 0.62 ±0.02
NSS 2 % (T5)	**ab** 72.63 ±0.48	**ab** 20.97 ±0.55	**ab** 10.96 ±0.26	**a** 4.48 ±0.14	**a** 0.64 ±0.03

The Means within the same column having different subscript are significantly different (P<0.05).
*Giblets: (liver, heart and Gizzard).

Some serum blood indices

The results of the statistical analyses in table 4 showed that the serum total cholesterol decreased significantly (p<0.05) as a result of NSS supplementation in broiler diets as cholesterol level reached 124.33, 119.00, 115.67, 119.00 (mg/dl) for treatments T2, T3, T4 and T5 respectively, compared with the T1 which recorded 137.67 mg/dl (table 5). The present results support the findings of other researchers in broilers (Siddig and Abdelati, 2001; AL-Betawi and El-Ghousein, 2008). Another researcher also proved the reducing

effect of NSS for both serum and egg yolk cholesterol in layers (El-Bagir *et al.*, 2006; Yalsin *et al.*, 2009). The reduction in the total serum cholesterol as a result of the addition of NSS may be due to the high content of unsaturated fatty acids, mainly linoleic acid and oleic acid, which may contribute to lower cholesterol synthesis (Cheikh-Rouhou *et al.*, 2007). On the other hand, the reason might be attributed to the compound β-sitosterol of NSS oil that may inhibit the absorption of dietary cholesterol (Atta, 2003).

The results of the data in table 3 indicated that the treatment T4 achieved a significant difference ($P<0.05$) in the serum levels of both total protein and albumin compared with the treatment T1 the control, while there were not significant statistical differences between treatment T1 and other treatments T2, T3 and T5. In terms of globulin levels, there was noticed numerical differences (no significant effects) for the NSS treatments. The current results are in agreement with the findings of (Hassan and Ragab, 2007), who reported that adding 1% NSS to layer chickens increased plasma total protein and albumin ($P<0.05$). Additionally, they found no significant effects on serum components; total protein, albumin and globulin when adding 0.2, 0.4% NSS (El-Ghamry *et al.*, 2002), as well as 2% NSS (Al-Homidan *et al.*, 2002) in broiler diets. The increase in the albumin and protein levels might be attributed to an enzyme induced by NSS to increase liver synthetic activity (Al-Jishi and Abuo Hozaifa, 2003).

Table 4: Means ±SE of some serum indices for broiler birds at the end of the experiment

Treatment	Cholesterol (mg/dl)	Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)
Control (T1)	^{a} 137.67 ±1.45	^{b} 3.03 ±0.03	^{b} 1.36 ±0.03	^{a} 1.67 ±0.05
NSS 0.25 % (T2)	^{ab} 124.33 ±3.88	^{ab} 3.23 ±0.04	^{ab} 1.43 ±0.04	^{a} 1.80 ±0.05
NSS 0.5% (T3)	^{b} 119.00 ±3.00	^{ab} 3.21 ±0.05	^{ab} 1.44 ±0.08	^{a} 1.77 ±0.03
NSS 1 % (T4)	^{b} 115.67 ±6.64	^{a} 3.35 ±0.08	^{a} 1.56 ±0.02	^{a} 1.79 ±0.07
NSS 2 % (T5)	^{b} 119.00 ±7.65	^{ab} 3.18 ±0.09	^{ab} 1.49 ±0.06	^{a} 1.69 ±0.04

The Means within the same column having different subscript are significantly different ($P<0.05$).

Antibody titers

The results in table 5 appeared an increase ($P<0.05$) in the means of antibody titers of Gumboro disease (GD) for *Nigella sativa* treatments except T5 as compared with the control at 6 weeks. The data also stated that NSS treatments; T2, T3 and T4 improved antibody response to ND virus, and the dietary treatment T4 achieved the highest level ($P<0.05$) compared with T1 and T5. The current results are in agreement with the findings of AL-Beitawi *et al.*, (2009) who documented a significant improvement ($P<0.05$) in the levels of antibody titer for ND and GD disease by using black seeds instead of antibiotic in broiler diets. The same trends were noticed by Al-Mufarrej, (2013) who reported that feeding broiler diets containing 1.4% NSS enhanced immune responses against ND and GD virus.

This improvement in the level of antibody titers against GD and ND diseases may be due to the active compounds found in the *Nigella sativa* essential oil mainly Thymoquinone and other antioxidant components; carvacrol, trans-enethole, and 4-terpinol (Guler *et al.*, 2007). Farah, *et al.*, (2004), also showed that NS oil significantly increased the phagocytic activity, and the potential immunity mechanism is mediated through stimulation activity of macrophage phagocytic or by activation of lymphocytes. Furthermore, application different

doses of NSS extracts (2.5, 5 and 7.5ml/kg) significantly decreased mortality rates of the partridge bird groups as compared with 1% flavomycin treatment (Cetin *et al.*, 2008).

Table 5: Means ±SE of antibody titers for broiler birds at the end of the experiment

Treatment	Antibody titers GD (ELIZA Means)	Antibody titers ND (lg2)
Control (T1)	5850^{b} ±652.6	3.8^{c} ±0.4
NSS 0.25 % (T2)	8199^{a} ±619.9	4.5^{abc} ±0.3
NSS 0.5 % (T3)	8968^{a} ±844.7	5.0^{ab} ±0.32
NSS 1 % (T4)	9123^{a} ±598.7	5.5^{a} ±0.22
NSS 2 % (T5)	5547^{b} ±641.1	4.0^{bc} ±0.45

The Means within the same column having different subscript are significantly different (P<0.05)

CONCLUSION

Based on these results, it could be concluded that NSS particularly 1% seems to have a great effect on immunity, therefore enhance the performance and quality of broiler birds. Further studies are needed in broilers to determine the influences of the active ingredients of Nigella sativa seed and the potential mechanisms.

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