



## Biochemical Effect of *Nigella sativa* Seeds on Fatty Acids, Lipid Profile, and Antioxidants of Laying Hens

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### ABSTRACT

This study aimed to evaluate the biochemical effect of *Nigella sativa* (NS) seeds as feed additives on serum and egg yolk lipids, antioxidants, and fatty acids in laying hens. The experiment was conducted on 42 Commercial Mandarrah strain laying hens at 31 weeks old with uniform body weight which were assigned to 2 groups with 21 hens per group. Control group and NS group (basal diet + 2% NS seeds) were examined for 12 weeks. The findings indicated that NS fed group showed a significant decrease in cholesterol, triglycerides, LDL, and VLDL concentrations in serum and egg yolk with a significant increase in HDL concentration. In addition, the antioxidant status of NS hens improved as MDA and NO concentrations significantly decreased in serum and egg yolk, while SOD, GSH, and TAC increased. Moreover, an increase in egg yolk concentration of unsaturated fatty acid linolenic, with a decrease in palmitic fatty acid concentration in egg yolk. Conclusively, NS has beneficial effects on antioxidants and different lipid fractions of serum and egg yolk of laying hens.

**Keywords:** Antioxidants, Egg yolk, Fatty acids, *Nigella sativa* seeds

### INTRODUCTION

Several studies on phytogetic plants illustrated their effect as alternatives to antibiotics with antioxidant capacity, growth-promoting efficacy, and immune-stimulating effects (Ahmad and Beg, 2013). *Nigella sativa* (NS) is a plant that is grown worldwide and commonly known as black seed or black cumin (Ahmad and Beg, 2013), that have antioxidant, antihyperlipidemic, and anti-diabetic effects (Mahdavi et al., 2015).

Egg lipids are confined to the yolk. The fatty acid content of the diet can influence the egg lipids in laying hens (Bavelaar and Beynen, 2004). González-Muñoz et al., (2009) demonstrated that the quantity and type of fatty acids present in the diet could also influence egg yolk cholesterol content.

The present study aimed to use natural feed additives in laying hens to produce a high-quality egg.

### MATERIALS AND METHODS

#### Ethical approval

All animal procedures used in this study were carried out in accordance with the Guide for Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee (IACUC) of Beni-Suef University (021-163).

#### Diet and *Nigella sativa*

As indicated in (Table 1), the diet was iso-caloric and iso-nitrogenous, covering the nutritional requirements of laying hens (NRC, 1994). *Nigella sativa* seeds are produced by Alwatanya for seeds, Giza, Egypt, and it was analyzed for fatty acid profiles using gas chromatography-mass spectrometry (GC-MS), illustrated in (Table 2) (Saleh et al., 2012b).

**Table 1.** The ingredients, nutrient concentration of the basal diet used in the experiment

Ingredient	Amount (g)
Corn	635
Soya bean 44%	210
Ca carbonate	93.5
Full fat soya	40
Methionine	1.25
Bone meal	10
NaCl	4
Tega Ad Extra (probiotic)	0.25
Coccistac	0.5
Premix S	4
Na sulphate	0.2
Gro- k- pro (antifungal)	0.3
Lysine	1
Total	1000

**Table 2.** Fatty acids composition of *Nigella sativa*

Fatty acid	<i>Nigella sativa</i> (g/ 100 g)
Myristic (C 14:0)	0.23
Palmitic (C 16:0)	9.5
Stearic (C 18:0)	3.23
Oleic (C 18:1 n9)	17.24
Linoleic (C 18:2 n6)	45.49
Linolenic (C 18:3 n3)	0.36
Arachidic (C 20:0)	0.016

**Table 3.** The composition of the different experimental diets

Chemical composition (%)	Control	<i>Nigella sativa</i> (2%)
Protein (%)	15.5	14.1
Fat (%)	6.13	5.3
Moisture (%)	6.2	7.85
Ash (%)	12.14	11.8
Fiber (%)	2.96	5
Carbohydrate (%)	50.1	55.95
Total energy (%)	3440	3337

### Laying hens

A total of 42 commercial Mandarrah strain laying hens aged 31 weeks with uniform body weight (1.7 kg) were assigned into 2 equal groups (21 hens per group) with 3 replicates and each replicate contained 7 hens. The groups were the control group that fed on a basal diet and the NS seed group that fed on a basal diet supplemented with 2% NS seed as indicated in Table 3 (Hassan and Alaqil, 2014). Feed and water were provided ad libitum throughout the experimental period (12 weeks). Hens were vaccinated

with necessary and common vaccines before the study period.

### Sampling collection

At the end of the experiment, 10 hens were randomly selected from each group and bled from the wing vein, then the blood was allowed to clot for one hour at room temperature and was then centrifuged at 1300 g for 15 minutes, then the serum was collected and kept frozen at -20°C until analysis. Eggs were collected during the last three days of the experimental period (43 weeks of age). The yolks were separated and 10 samples of the pooled yolks for each treatment were frozen and stored at -20°C until analysis.

### Biochemical analysis

Serum and yolk samples were analyzed for cholesterol and triacylglycerol (Cell Biolalabs, San Diego, USA), high-density lipoprotein, low-density lipoprotein, and very low-density lipoprotein concentration (Biodiagnostics Company, Cairo, Egypt) according to methods described by Burstein et al. (1970); Richmond (1973); Fassati and Prencipe (1982); Wieland and Seidel (1983); Mendez et al. (1986) and Lee et al. (2008). Total antioxidant capacity, MDA, GSH, No, and SOD concentrations (Biodiagnostics Company, Cairo, Egypt) of serum and egg yolk were measured according to Montgomery and Dymock (1961), Beutler et al. (1963), Nishikimi et al. (1972), Satoh (1978), and Koracevic et al. (2001). All chemical reactions were measured by using Hitachi spectrophotometry, Model U - 2000 (Hitachi Ltd. Tokyo, Japan). The extracted total lipids of the pooled yolk samples were used for the isolation of fatty acids (Farag et al., 1990). Fatty acid profiles were analyzed by gas chromatography-mass spectrometry (GC-MS) (Saleh et al., 2012).

### Statistical analysis

Results were expressed as means ± SEM. The results were analyzed by one-way analysis of variance ANOVA followed by Tukey test using Graph Pad Instate software (version 3). Differences were considered significant at  $p < 0.05$ .

## RESULTS AND DISCUSSION

Results indicated a significant ( $p < 0.05$ ) decrease in cholesterol, triacylglycerol, LDL, and VLDL concentrations with a significant ( $p < 0.05$ ) increase in HDL in serum and egg yolk of the NS group, compared to the control group (Table 4 and 5).

**Table 4.** Effect of *Nigella sativa* seeds on serum cholesterol, triacylglycerol, high-density lipoproteins, low-density lipoproteins, and very low-density lipoproteins concentrations of laying hens

Parameters Groups	Cholesterol (mg/dl)	TAG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Control	177 ± 3.8 <sup>a</sup>	105 ± 2.9 <sup>a</sup>	32.7 ± 1.5 <sup>b</sup>	117.8 ± 2.1 <sup>a</sup>	21 ± 0.6 <sup>a</sup>
<i>Nigella sativa</i> group	160 ± 2 <sup>b</sup>	85 ± 2.9 <sup>b</sup>	50.3 ± 0.9 <sup>a</sup>	93 ± 2.1 <sup>b</sup>	18 ± 0.6 <sup>b</sup>

Values are represented as mean ± standard error. The different superscript letters mean a significant difference between different groups ( $p < 0.05$ ). TAG: Triacylglycerol, HDL: High-density lipoproteins, LDL: Low-density lipoproteins, VLDL: Very low-density lipoproteins

**Table 5.** Effect of *Nigella sativa* seeds on egg yolk cholesterol, triacylglycerol, high-density lipoproteins, low-density lipoproteins, and very low-density lipoproteins concentrations in laying hens

Parameters Groups	Cholesterol (mg/dl)	TAG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Control	281 ± 4.4 <sup>a</sup>	61 ± 3.1 <sup>a</sup>	58 ± 0.6 <sup>b</sup>	222 ± 6.9 <sup>a</sup>	11.8 ± 0.7 <sup>a</sup>
<i>Nigella sativa</i> group	260 ± 2.9 <sup>b</sup>	54 ± 1.4 <sup>b</sup>	69 ± 1.5 <sup>a</sup>	198 ± 3.7 <sup>b</sup>	10.6 ± 0.2 <sup>b</sup>

Values are represented as mean ± standard error. The different superscript letters mean a significant difference between different groups ( $p < 0.05$ ). TAG: Triacylglycerol, HDL: High-density lipoproteins, LDL: Low-density lipoproteins, VLDL: Very low-density lipoproteins

This finding agreed with [Yalçin et al. \(2009\)](#) who reported that feeding of diets with 1 and 1.5% or 1 and 3% black cumin seeds reduced serum and egg yolk total cholesterol. The decrease in egg yolk cholesterol is secondary to the decrease in serum cholesterol which is the precursor for egg yolk cholesterol. The liver of the layer hen produces most of the lipids found in egg yolk which are transported to the ovary by serum lipoprotein ([El Bagir et al., 2006](#)). Thus, the decrease in egg-yolk cholesterol by supplementation of black cumin seed may be due to a lesser deposition of cholesterol by the liver in egg-yolk during yolk synthesis ([Akhtar et al., 2003](#)). The hypolipidemic effect of black seed is due to the synergistic action of its constituents, including thymoquinone (TQ), nigellamine, soluble fiber (e.g. mucilage), sterols, flavonoids, and high content of polyunsaturated fatty acids (PUFAs) ([Ali and Blunden, 2003](#)). TQ significantly reduced total cholesterol, LDL, triglycerides while increased HDL-cholesterol concentration ([Al-Naqeep et al., 2011](#)) through decreasing cholesterol synthesis or increasing bile acid excretion ([Swamy and Tan, 2000](#)).

The soluble dietary fibers ([Talati et al., 2009](#)) and sterols ([Moruisi et al., 2006](#)) can inhibit the intestinal reabsorption of dietary cholesterol. *Nigella sativa* seeds reduce cholesterol synthesis by hepatocytes or decrease its fractional reabsorption from the intestine and also increase primary bile acid synthesis and its fecal losses ([Moruisi et al., 2006](#)) and both actions were known to reduce serum cholesterol levels ([Najmi et al., 2012](#)). Flavonoids help liver cells to remove LDL-C from blood, either by increasing LDL receptor densities or by binding to apolipoprotein B ([El-Beshbishy et al., 2006](#)).

*Nigella sativa* contains monounsaturated fatty acids which may stimulate cholesterol excretion into the intestine and its oxidation. It has been documented that MUFAs may reduce LDL cholesterol, while it might increase HDL cholesterol ([Tollba and Hassan, 2003](#)). *Nigella sativa* contains PUFAs that are well-known to decrease serum total cholesterol ([Djoussé et al., 2003](#)). Nigellone, the effective substance in NS, is mainly responsible for the depression of 3-hydroxy-3-methylglutaryl Co-A (HMG-CoA) reductase activity, the key regulatory enzyme in cholesterol synthesis ([Khan et al., 2012](#)).

Phytosterol found in NS can inhibit the formation of micelles due to the absorption of bile acids into the intestine, so inhibit cholesterol and causes a decrease in serum cholesterol levels ([Ali et al., 2014](#)). NS seeds inhibit the flux of acetyl-CoA into the lipogenic pathway in the liver leading to reductions in the concentrations of triacylglycerol and phospholipids in serum and egg yolk ([Leskanish and Noble, 1997](#)).

Poultry in intensive farming systems is frequently exposed to oxidative stress which leads to reduced performance and health ([Lykkesfeldt and Svendsen, 2007](#)). Oxidative stress defense depends on the synergism between the exogenous and endogenous antioxidants. The stability of a living organism must be maintained by its balance between oxidative and antioxidant defense ([Zaidi et al., 2019](#)). Antioxidant enzymes, as well as, non-enzymatic antioxidants are the first line of defense against ROS, inducing oxidative damage, in a living organism ([Al-Shiekh et al., 2014](#)).

**Table 6.** Effect of *Nigella sativa* seeds on antioxidants and oxidative stress parameters in serum of laying hens

Parameters Groups	MDA (nmol / ml)	NO (µmol / L)	SOD (U/ml)	GSH (mmol/L)	TAC (mM / L)
Control	12.2 ± 0.8 <sup>a</sup>	6.4 ± 0.2 <sup>a</sup>	2.9 ± 0.2 <sup>b</sup>	24.6 ± 1.6 <sup>b</sup>	731.8 ± 7.1 <sup>b</sup>
<i>Nigella sativa</i> group	6.4 ± 0.2 <sup>b</sup>	4.9 ± 0.1 <sup>b</sup>	7.7 ± 0.3 <sup>a</sup>	40 ± 0.7 <sup>a</sup>	912 ± 1.7 <sup>a</sup>

Values are represented as mean ± standard error. The different superscript letters mean a significant difference between different groups (p < 0.05). MDA: Malondialdehyde, NO: Nitric oxide, SOD: Superoxide dismutase, GSH: Glutathione reduced, TAC: Total antioxidant capacity

**Table 7.** Effect of *Nigella sativa* seeds on antioxidants and oxidative stress parameters in egg yolk of laying hens

Parameters Groups	MDA (nmol/ gm tissue)	NO (µmol / gm)	SOD (U/gm tissue)	GSH (mmol /g.tissue)	TAC (mM / gm)
Control	4.6 ± 0.2 <sup>a</sup>	1.9 ± 0.05 <sup>a</sup>	1.5 ± 0.06 <sup>b</sup>	8.1 ± 0.5 <sup>b</sup>	114 ± 4.3 <sup>b</sup>
<i>Nigella sativa</i> group	3.2 ± 0.1 <sup>b</sup>	1.2 ± 0.04 <sup>b</sup>	2.7 ± 0.2 <sup>a</sup>	11.3 ± 0.4 <sup>a</sup>	160 ± 3.3 <sup>a</sup>

Values are represented as mean ± standard error. The different superscript letters mean a significant difference between different groups (p < 0.05). MDA: Malondialdehyde, NO: Nitric oxide, SOD: Superoxide dismutase, GSH: Glutathione reduced, TAC: Total antioxidant capacity

**Table 8.** Effect of *Nigella sativa* on fatty acids concentration in egg yolk of laying hens

Parameter	Control	<i>Nigella sativa</i> group
C 14:0 (Myristic)	0.22 ± 0.01 <sup>a</sup>	0.17 ± 0.1 <sup>a</sup>
C 16:0 (Palmitic)	20.6 ± 0.45 <sup>a</sup>	19.1 ± 0.5 <sup>b</sup>
C 18:0 (Stearic)	7.00 ± 0.29 <sup>a</sup>	7.36 ± 0.4 <sup>a</sup>
C 18:1 n-9 (Oleic)	39.33 ± 0.5 <sup>a</sup>	40.3 ± 0.9 <sup>b</sup>
C 18:2 n-6 (Linoleic)	10.41 ± 0.82 <sup>a</sup>	10.1 ± 0.8 <sup>a</sup>
C18:3 n-3 (Linolenic)	0.55 ± 0.11 <sup>a</sup>	0.78 ± 0.14 <sup>b</sup>
C 20:0 (Arachidic)	0.2 ± 0.05 <sup>a</sup>	0.2 ± 0.01 <sup>a</sup>

Values are represented as mean ± standard error. The different superscript letters mean a significant difference between different groups (p < 0.05).

Results showed an improvement in antioxidant parameters after NS administration, which was indicated by a significant (p < 0.05) decrease in MDA and NO with a significant (p < 0.05) increase in SOD, GSH, and TAC in serum (Table 6) and egg yolk (Table 7). These results agreed with that of Boka et al. (2014) and Rahman and Kim (2016) who reported that black cumin significantly decreased both serum and egg yolk MDA concentrations. Thymoquinone, dithymoquinone, carvacrol, anethole, and 4-terpinol are the main active components of NS (Bourgou et al., 2010) which reduce lipid peroxidation and the release free radicals, so decrease MDA concentrations of serum and egg yolk (Guler et al., 2007; Hosseinzadeh et al., 2007). Muhammad et al. (2017) reported that TQ effectively changed the parameters of catalase, myeloperoxidase, reduced glutathione, superoxide dismutase, and nitric oxide through a number of *in vitro* and *in vivo* antioxidant studies that have been conducted with NS extracts, seed oil, and TQ. Polyunsaturated fatty acids in NS enhance the oxidative stability of food

products (Ahmad and Beg, 2013). Polyphenols are one of the most effective anti-oxidative constituents in NS which suppress reactive oxygen and nitrogen species formation.

Grobas et al. (2001) found that the source and number of fatty acids in diet markedly modified the fatty acid composition of egg yolks. Herber and Van Elswyk (1996) found that dietary n-3 fatty acids increased yolk total n-3 fatty acids.

Present results revealed that NS seeds supplementation resulted in a significant decrease in palmitic concentration (p < 0.05) and a significant increase in linolenic concentration (p < 0.05) as indicated in (Table 8). That was agreed with Yalçın et al. (2009) who reported that total saturated fatty acids and the ratio of saturated/unsaturated fatty acids in egg yolk samples were decreased significantly by black cumin seed supplementation. This effectiveness may be because of a combination of fatty acids (85% unsaturated fatty acids), volatile oils, and trace elements composition of NS seeds (Cheikh et al., 2007).

## CONCLUSION

Supplementation of NS in laying hens' diet for three months, improved lipid profile, antioxidant parameters in serum and egg yolk, and also developed the fatty acid concentrations in egg yolk beneficially. It can be concluded that NS can be used safely as a feed additive in layer diets.

## DECLARATIONS

### Consent to publish

All authors agree to publish this manuscript.

### Competing interests

The authors have declared no competing interests.

### Ethical considerations

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by the authors.

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