JWPR
Journal of World's Poultry Research

2021, Scienceline Publication

J. World Poult. Res. 11(3): 338-343, September 25, 2021

Research Paper, PII: S2322455X2100040-11

DOI: https://dx.doi.org/10.36380/jwpr.2021.40

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

Sahar Omar Mohamed[1*], Mohammed Ahmed Kandiel[2], Omayma Ahmed Ragab Abo Zaid[3], Mahmoud Mohamed Arafa[4], and Ghada Mohamed Safwat[2]

[1]*Postgraduate Student of Biochemistry Department, Animal Health Research Institute, Beni Suef Branch, 62511, Dokki, Egypt*
[2]*Biochemistry Department, Veterinary Medicine, Beni Suef University, 62511, Beni Suef, Egypt.*
[3]*Biochemistry Department, Veterinary Medicine, Banha University, 13736, Moshtohor, Qalyuobia, Banha, Egypt*
[4]*Chief Researcher of Biochemistry, Animal Health Research Institute, Dokki, Giza, Nadi El-Seid Street, PO Box 264 - Giza, 12618 Cairo, Egypt*

*Corresponding author's Email: saharalhagry303@gmail.com; ORCID: 000-0001-9952-4063

Received: 19 June 2021
Accepted: 06 September 2021

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 Mandarah 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 phytogenic 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	*Nigella sativa* (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	*Nigella sativa* (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 Mandarah 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 adlibitum 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^{o}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^{o}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[a]	105± 2.9[a]	32.7 ± 1.5[b]	117.8 ± 2.1[a]	21 ± 0.6[a]
Nigella sativa group	160 ± 2[b]	85 ± 2.9[b]	50.3 ± 0.9[a]	93 ± 2.1[b]	18 ± 0.6[b]

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[a]	61 ± 3.1[a]	58 ± 0.6[b]	222 ± 6.9[a]	11.8 ± 0.7[a]
Nigella sativa group	260 ± 2.9[b]	54 ± 1.4[b]	69 ± 1.5[a]	198 ± 3.7[b]	10.6 ± 0.2[b]

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[a]	6.4 ± 0.2[a]	2.9 ± 0.2[b]	24.6 ± 1.6[b]	731.8 ± 7.1[b]
Nigella sativa group	6.4 ± 0.2[b]	4.9 ± 0.1[b]	7.7 ± 0.3[a]	40 ± 0.7[a]	912± 1.7[a]

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[a]	1.9 ± 0.05[a]	1.5 ± 0.06[b]	8.1 ± 0.5[b]	114 ± 4.3[b]
Nigella sativa group	3.2 ± 0.1[b]	1.2 ± 0.04[b]	2.7 ± 0.2[a]	11.3 ± 0.4[a]	160 ± 3.3[a]

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

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 Yalçin 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.

REFERENCES

Ahmad S, and Beg ZH (2013). Hypolipidemic and antioxidant activities of thymoquinone and limonene in atherogenic suspension fed rats. Food Chemistry, 138(2): 1116-1124. DOI: https://www.doi.org/10.1016/j.foodchem.2012.11.109.

Akhtar MS, Nasir Z, and AR, and Abid (2003). Effect of feeding powdered *Nigella Sativa* L. seeds on poultry egg production and their suitability for human consumption. Veterinarski Arhiv, 73(3): 181-190. Available at: https://hrcak.srce.hr/74865

Ali BH, and Blunden G (2003). Pharmacological and toxicological properties of *Nigella sativa*. Phytotherapy. Research, 17(4): 299-305. DOI: https://www.doi.org/10.1002/ptr.1309.

Ali OAA, Suthama N, and Mahfud LD (2014). The Effect of feeding black cumin (*Nigella Sativa*) and Vitamin C on blood lipid profiles and growth performance of broilers. International Refereed Journal of Engineering and Science, 3(4): 28-33. Available at: https://api.semanticscholar.org/CorpusID:11593494

Al-Naqeep G, Al-Zubairim AS, Ismail M, Amom ZH, and Esa NM (2011). Antiatherogenic potential of *Nigella sativa* seeds and oil in diet-induced hypercholesterolemia in rabbits. Evidence-Based Complementary and Alternative Medicine, Article ID: 213628. DOI: https://www.doi.org/10.1093/ecam/neq071.

Al-Shiekh AM, Al-Shati AA, and Sarhan MAA (2014). Effect of white tea extract on antioxidant enzyme activities of streptozotocin-induced diabetic rats. Egyptian Academic Journal of Biological Sciences, 6(2): 17-30. DOI: https://www.doi.org/10.21608/eajbsc.2014.13710

Bavelaar FJ, and Beynen AC (2004). Relationships between the intake of n-3 polyunsaturated fatty acids by hens and the fatty acid composition of their eggs. International Journal of Poultry Science, 3: 690-696. DOI: https://www.doi.org/10.3923/IJPS.2004.690.696

Beutler E, Duron O, and Kelly MB (1963). Improved method for the determination of blood glutathione. Journal of Laboratory and Clinical Medicine, 61: 882-888. Available at: https://pubmed.ncbi.nlm.nih.gov/13967893/

Boka J, Mahdavi AH, Samie AH, and Jahanian R (2014). Effect of different levels of black cumin (*Nigella sativa* L.) on performance, intestinal Escherichia coli colonization and jejunal morphology in laying hens. Journal of Animal Physiology and Animal Nutrition, 98: 373-383. DOI: https://www.doi.org/10.1111/jpn.12109.

Bourgou S, Pichette A, Marzouk B, and Legault J (2010). Bioactivities of black cumin essential oil and its main terpenes from Tunisia. South African Journal of Botany, 76(2): 210-216. DOI: https://www.doi.org/10.1016/j.sajb.2009.10.009

Burstein M, Scholnick H R, and Morfin R (1970). Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. Journal of Lipid Research, 11(6): 583-595. Available at: https://pubmed.ncbi.nlm.nih.gov/4100998/

Cheikh-Rouhou S,Besbes S, Hentati B, Blecker C, Deroanne C, and Attia H(2007). *Nigella sativa* L.chemical composition and physicochemical characteristics of lipid fraction. Food Chemistry, 101(2): 673-681. DOI: https://www.doi.org/10.1016/j.foodchem.2006.02.022

Djoussé L, Hunt SC, and Arnett DK (2003). Dietary linoleic acid is inversely associated with plasma triacylglycerol: the National Heart, Lung, and Blood Institute Family Heart Study. The American Journal of Clinical Nutrition, 78(6): 1098-10102. DOI: https://www.doi.org/10.1093/ajcn/78.6.1098.

El Bagir NM, Hama AY, Hamed RM, Abd El Rahim AG, and Beynen AC (2006). Lipid composition of egg yolk and serum in laying hens fed diets containing black cumin (*Nigella sativa*). International Journal of Poultry Science, 5(6): 574-578. DOI: https://www.doi.org/10.3923/ijps.2006.574.578

El-Beshbishy HA, Singab ANB, Sinkkonen J, and Pihlaja K (2006). Hypolipidemic and antioxidant effects of Morus alba L. (Egyptian mulberry) root bark fractions supplementation in cholesterol-fed rats. Life Sciences, 78: 2724-2733. DOI: https://www.doi.org/10.1016/j.lfs.2005.10.010

Farag RS, Ali MN, and Taha SH (1990). Use of some essential oils as natural preservation for butter. Journal of the American Oil Chemists Society, 67(3): 188-191. DOI: https://www.doi.org/10.1007/BF02638965

Fassati P, and Prencipe L (1982). Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clinical Chemistry, 28(10): 2077-2080. Available at: https://pubmed.ncbi.nlm.nih.gov/6812986/

González-Muñoz MJ, Bastida S, Jiménez O, Lorenzo de C, Vergara G, and Sánchez-Muniz FJ (2009). The effect of dietary fat on the fatty acid composition and cholesterol content of the eggs from Hy-line and Warren hens. Grasas Y Aceites, 60(4): 350-359. DOI: https://www.doi.org/10.3989/gya.108208

Grobas S, Mendez J, Lazaro R, De Blas C, and Mateos GG (2001). Influence of source and percentage of fat added to diet on performance and fatty acid composition of egg yolks of two strains of laying hens. Poultry Science, 80: 1171-1179. DOI: https://www.doi.org/10.1093/ps/80.8.1171

Guler T, Ertas ON, Kizil M, Dalkilic B, and Ciftci M (2007). Effect of dietary supplemental black cumin seeds on antioxidant activity in broilers. Medycyna Weterynaryjna, 63(9): 1060-1063. Available at: https://www.cabdirect.org/cabdirect/abstract/20073199138

Hassan SM, and Alaqil AA (2014). Effect of adding different dietary levels of black cumin (*Nigella sativa* L.) seed on productive performance of laying hens. Asian Journal of Poultry Science, 8(2): 41-48. DOI: https://www.doi.org/10.3923/ajpsaj.2014.41.48

Herber SM, and Van Elswyk ME (1996). Dietary marine algae promotes efficient deposition of n-3 fatty acids for the production of enriched shell eggs. Poultry Science, 75: 1501-1507. DOI: https://www.doi.org/10.3382/ps.0751501.

Hosseinzadeh H, Parvardeh S, Asl MN, Sadeghnia HR, and Ziaee T (2007). Effect of thymoquinone and *Nigella sativa* seeds oil on lipid peroxidation level during global cerebral ischemia-reperfusion injury in rat hippocampus. Phytomedicine: International Journal of Phytotherapy and Phytopharmacology, 14: 621-627. DOI: https://www.doi.org/10.1016/j.phymed.

Khan SH, Ansari J, Ahsan UH, and Ghulam A (2012). Black cumin seeds as phytogenic product in broiler diets and its effects on performance, blood constituents, immunity and caecal microbial population. Italian Journal of Animal Science, 11: 438-444. Available at: https://www.tandfonline.com/doi/full/10.4081/ijas.2012.e77

Koracevic D, Koracevic G, Djordjevic V, Andrejevic S, and Cosic V (2001). Method for the measurement of antioxidant activity in human fluids. Journal of Clinical Pathology, 54(5): 356-361. DOI: https://www.doi.org/10.1136/jcp.54.5.356.

Lee SM, Kim JK, Shin HJ, and Baik JH (2008). GCG -Rich tea catechins are effective in lowering cholesterol and -triglyceride concentrations in hyperlipidemic rats. Lipids, 43(5): 419-429. DOI: https://www.doi.org/10.1007/s11745-008-3167-4

Leskanish CO, and Noble RC (1997). Manipulation of the n-3 polyunsaturated fatty acid composition of avian meat. World's Poultry Science Journal, 53: 156-182. DOI: https://www.doi.org/10.1079/WPS19970015

Lykkesfeldt J, and Svendsen O (2007). Oxidants and antioxidants in disease: Oxidative stress in farm animals. The Veterinary Journal, 173: 502-511. DOI: https://www.doi.org/10.1016/j.tvjl.2006.06.005.

Mahdavi R, Namazi N, Alizadeh M, and Farajnia S (2015). Effects of *Nigella sativa* oil with a low-calorie diet on cardiometabolic risk factors in obese women: A randomized controlled clinical trial. Food and Function, 6(6): 2041-2048. DOI: https://www.doi.org/10.1039/c5fo00316d

Mendez AJ, Cabeza C, and Hsia SL (1986). A fluorometric method for the determination of triglycerides in nanomolar quantities. Analytical Biochemistry, 156(2): 386-389. DOI: https://www.doi.org/10.1016/0003-2697(86)90269-1

Montgomery HAC, and Dymock JF (1961). The compustion of organic compounds by ignition in oxygen: the determination of carbon and hydrogen. Analyst. 86: 414-416. DOI: https://www.doi.org/10.1039/AN9618600411

Moruisi KG, Oosthuizen W, and Opperman AM (2006). Phytosterols/stanols lower cholesterol concentrations in familial hypercholesterolemic subjects: A systematic review with meta-analyses. Journal of the American College of Nutrition, 25(1): 41-48. DOI: https://www.doi.org/10.1080/07315724.2006.10719513.

Muhammad Torequl I, Guha B, Hosen S, Thoufiqul Alam R, Shahadat S, Leonardo da Rocha S, Jose Victor de Oliveira S, Josemar José da Silva J, Rosália Maria T, Antonio Lima B et al. (2017). Nigellalogy: A review on *Nigella Sativa*. MOJ Bioequivalence & Bioavailability, 3(6): 167-181. DOI: https://www.doi.org/10.15406/mojbb.2017.03.00056

Najmi A, Nasiruddin M, Khan RA, and Haque SF (2012). Therapeutic effect of *Nigella sativa* in patients of poor glycemic control. Asian Journal of Pharmaceutical and Clinical Research, 5(3): 224-228. Available at: https://www.cochranelibrary.com/central/doi/10.1002/central/CN-00908800/full

Nishikimi M, Roa NA, and Yogi K (1972). The Occurrence of Supeoxide Anion in the Reaction of Reduced Phenazine Methosulfate and Molecular Oxygen. Biochemical Biophysical Research Communications, 46: 849-854. DOI: http://www.dx.doi.org/10.1016/S0006-291X(72)80218-3

National Research Council (NRC) (1994). Nutrient requirements of poultry. 9th revised edition. Washington: National Academy Press. Washington, DC., USA. Available at: https://www.nap.edu/catalog/2114/nutrient-requirements-of-poultry-ninth-revised-edition-1994

Rahman M, and Kim SJ (2016). Effects of dietary *Nigella sativa* seed supplementation on broiler productive performance, oxidative status and qualitative characteristics of thighs meat. Italian Journal of Animal Science, 15: 241-247. DOI: https://www.doi.org/10.1080/1828051X.2016.1159925

Richmond W (1973). Preparation and properties of a cholesterol oxidase from Nocardia sp. and its application to the enzymatic assay of total cholesterol in serum. Clinical Chemistry, 19(12): 1350-1356. Available at: https://pubmed.ncbi.nlm.nih.gov/4757363/

Saleh AA, Eid Z, Tareak E, and Hayashi K (2012). The modification of the muscle fatty acid profile by dietary supplementation with Aspergillus awamori in broiler chickens. British Journal of Nutrition, 108: 1596-1602. DOI: https://www.doi.org/10.1017/S0007114511007069

Satoh K (1978). Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clinica Chimica Acta, 90: 37. DOI: https://www.doi.org/10.1016/0009-8981(78)90081-5.

Swamy SM, and Tan BK (2000). Cytotoxic and immune potentiating effects of ethanolic extract of *Nigella sativa* seeds L. Journal of Ethnopharmacology, 70(1): 1-7. DOI: https://www.doi.org/10.1016/s0378-8741(98)00241-4.

Talati R, Baker WL, Pabilonia MS, White CM, and Coleman CI (2009). The effects of barley-derived soluble fiber on serum lipids. Annals of Family Medicine, 7(2): 157-163. DOI: https://www.doi.org/10.1370/afm.917

Tollba AH, and Hassan MH (2003). Using some natural additives to improve physiological and productive performance of broiler chicks under high temperature conditions 2-black cumin (*Nigella sativa*) or garlic (Allium sativum). Egyptian Poultry Science Journal, 23: 327-340. Available at: https://www.sid.ir/en/journal/ViewPaper.aspx?ID=485001

Wieland H, and Seidel D (1983). A fully enzymatic colorimetric determination of LDL cholesterol in serum. Journal of Lipid Research, 24(7): 904-909. Available at: https://pubmed.ncbi.nlm.nih.gov/6631224/

Yalçin S, Yalçin S, Erol H, Bugdayc K, Ozsoy B, and Çakir S (2009). Effects of dietary black cumin seed (*Nigella sativa* L.) on performance, egg traits, egg cholesterol content and egg yolk fatty acid composition in laying hens. Journal of the Science of Food and Agriculture, 89: 1737-1742. DOI: https://www.doi.org/10.1002/jsfa.3649

Zaidi SK, Ansari SA, Tabrez S, Naseer MI, Shahwan MJ, Banu N, and Al-Qahtani MH (2019). Antioxidant potential of Solanum nigrum aqueous leaves extract in modulating restraint stress-induced changes in rRat's liver. Journal of Pharmacy and Bioallied Sciences, 11(1): 60-68. DOI: https://www.doi.org/10.4103/jpbs.JPBS_58_18