2026 SCIENCELINE

Online Journal of Animal and Feed Research

Volume 16, Issue 2: 109-117; March 30, 2026

ISSN 2228-7701

DOI: https://dx.doi.org/10.51227/ojafr.2026.13

EFFECT OF *Nigella sativa* OIL ON LIVER FUNCTION AND LIPID PROFILE IN BROWN, ALBINO AND LOCAL RABBITS: A COMPARATIVE STUDY

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Supporting Information

RESEARCH ARTICLE

PII: S2228770126000013-16

Received: February 02, 2026

Revised: March 27, 2026

Accepted: March 28, 2026

ABSTRACT: This study investigated the effects of *Nigella sativa* oil (NSO) on the metabolism of three rabbit breeds: Albino, Brown, and Local. A total of 48 rabbits were assigned to six groups and fed either a control diet or an NSO-supplemented diet (5 g/kg DM). Diets were alternated between groups every 15 days. Blood samples were analyzed for liver enzymes, kidney function parameters, lipid profile, thyroid hormones, and blood proteins. The results showed that NSO had no significant effect (P>0.05) on blood glucose, liver enzymes, or lipid profile. However, numerical differences were observed among breeds. Albino rabbits showed higher total cholesterol 83.6% and low-density lipoprotein (LDL) cholesterol (177.6%) compared with the Local breed (P≤0.01). Brown rabbits exhibited higher aspartate aminotransferase (AST) activity (60-64%) than Albino rabbits (P≤0.02). Additionally, brown rabbits showed higher (51.2%) creatinine levels compared with Albinos (P≤0.0001). Uric acid levels slightly increased (15-18%) during the second treatment period. Protein levels remained relatively constant among groups; however, the brown breed had higher (P≤0.02) albumin levels (9%) than other breeds. In conclusion, rabbit breed had a greater influence on the measured parameters than dietary treatments which indicates that breed-specific genetic variations play an important role in such studies.

Keywords: Breeds, Liver function, Lipid profile, *Neglia sativa*, Rabbits.

INTRODUCTION

In recent years, there has been growing interest worldwide in the use of black seed oil (*N. sativa*) as a therapeutic agent due to its reported health benefits. These effects are attributed to its bioactive compounds, particularly thymoquinone and nigellone. These compounds may possess anti-inflammatory properties, reduce cellular oxidative damage, and support immune system function (Adam et al., 2022).

Researchers have investigated the effects of *Nigella Sativa* (NS) on various organs, especially the liver heart (Mat et al., 2011 and Safithri et al., 2023). Evidence suggests that dietary supplementation with *N. Sativa* can lower blood glucose levels in animals, possibly by enhancing insulin secretion and improving pancreatic function (Tiwari et al., 2022 and Shaukat et al., 2023). The liver is a vital organ responsible for metabolism, detoxification of harmful substances, and synthesis of essential proteins (Ozougwu, 2017). Liver damage caused by exposure to chemicals or infections typically results in elevated levels of specific enzymes in the blood, which serve as indicators of hepatic injury (Guo et al., 2025). Enzymes such as AST, and alkaline phosphatase (ALP) are commonly used biomarkers of liver dysfunction (Al-Razzuqi et al., 2011).

Several studies have reported that dietary supplemented with NSO reduces AST and ALP levels in rats with fatty liver disease or chemically induced liver damage caused by carbon tetrachloride (Hashim et al., 2025). These findings suggest that NSO may help protect liver cells and improve overall liver function (Rounagh et al., 2024; Derosa et al., 2024). Elevated cholesterol and triglyceride levels are well-known risk factors for cardiovascular disease. NSO has been reported to reduce these risks (Alu'datt et al., 2024). Studies in humans and animals have shown that N. sativa can decrease low-density lipoprotein (LDL) and triglyceride levels while increasing high-density lipoprotein (HDL), possibly by enhancing lipid metabolism and fat breakdown in the body (Zhang et al., 2021). Different rabbit breeds, such as albino, brown, and local, exhibit distinct physiological characteristics that may influence their response to dietary supplementation with NS (Ye et al., 2021). Breed-specific growth responses have been reported with *N. Sativa* was used as a feed additive in rabbit diets (Al-Shaar, 2020). In addition to its anti-inflammatory and immunomodulatory properties, *N. Sativa* also exhibits strong

Citation: Hasan WI, Hidayet HM, Azeez OH, Sefdeen SM, Yateem CA, Mustafa KN (2026). Effect of *Nigella sativa* oil on liver function and lipid profile in brown, albino and local rabbits: a comparative study. *Online J. Anim. Feed Res.*, 16(2): 109-117. DOI: https://dx.doi.org/10.51227/ojafr.2026.13

antioxidant activity (Alberts et al., 2024), which may help protect the body against conditions such as liver inflammation and cardiovascular complications (Bhavikatti et al., 2024).

Antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), have been shown to increase in activity following NS supplementation (Hannan et al., 2021; Aslani et al., 2024). Evidence also suggests that NSO may protect kidney function, as reduced levels of creatinine, urea, and uric acid have been reported in animals receiving diets supplemented with different levels of NS (Alghesheiry et al., 2025). In rats with kidney injury, NS oil improved both circulating biochemical parameters and renal tissue structure, likely due to its antioxidant properties (Naderi et al., 2022; Al-Hamdany and Raoof, 2023). In rabbit diets, NS supplementation has been associated with increased total protein, albumin, and globulin levels, indicating improved liver function and enhanced immune support. These changes occurred without evidence of liver or kidney damage, suggesting its safety as a dietary supplement (Al-Asadi et al., 2025). Several studies have also examined the effects of NS oil on thyroid hormones. In rats with thyroid dysfunction, NSO increased triiodothyronine (T3) and thyroxine (T4) levels while reducing thyroid-stimulating hormone (TSH), suggesting that NSO may help regulate thyroid hormone balance in both hypothyroid and hyperthyroid conditions, indicating a modulatory rather than stimulatory effect (Elghareeb et al., 2024).

Therefore, the present study aimed to evaluate the potential protective effects of NSO on liver enzyme activity and selected blood biochemical parameters in three rabbit breeds. Additionally, the effects of NSO on kidney function markers (creatinine, urea, and uric acid) and thyroid hormones (T3 and T4) were investigated.

MATERIALS AND METHODS

Experimental Design: A total of 48 rabbits (16 Albino, 16 Brown, and 16 Local) with an initial body weight ranging from 4 to 8 kg were used in the present study. The rabbits were acclimatized for one week in cages located at the Animal Production Department, College of Agricultural Engineering Sciences, University of Duhok prior to the start of the experiment. Each breed was equally divided into two dietary groups (n = 8 per group): Control group, fed basal diet, and Treatment group, fed basal diet supplemented with 5 g NSO/kg dry matter (DM). The experimental period lasted five weeks including a five-day dietary adaptation period (basal diet only) followed by two experimental phases of 15 days each.

Housing Conditions: All rabbit groups were housed in clean dry cages bedded with soil and maintained under identical environmental conditions. Animals were provided with free access to water source and adequate lighting throughout the experimental period (Trocino and Tolini 2024; Wang et al., 2025).

Feeding Routine: Each young rabbit fed 30 g/d standardized feed mixture. Feed ingredients and the chemical composition of the diets are given in Table 1. Feed samples were chemically analyzed according to the methods described by AOAC (2000). Rabbits were fed twice a daily. Adult rabbits received 15 - 20 g of diet per kg of body weight per day. Feeding times and quantities were kept consistent throughout the study to prevent digestive disturbances and nutritional disorders.

Table 1 - Feed ingredients and the chemical composition of the diets.

Feed Ingredients, g/kg	Basal diet	Basal diet + 5 g NSO	Chemical composition of the diets	Basal diet	Basal diet + 5 g NSO
Barley	400	400	Moisture (%)	4.07	6.26
Wheat bran	200	200	Ash (g/kg)	61.1	59.1
Soybean meal	200	200	CP (g/kg)	130.4	130.9
Corn	150	150	EE (g/kg)	54.2	60.1
Vitamins-minerals premix	20	20	CF (g/kg)	35.6	36.6
Lysine	10	10	NFE (g/kg)	678.0	650.7
Methionine	10	10	-	-	-
Iodized salt	10	10	-	-	-
Nigella sativa oil (g/kg DM)	–	5	-	-	-

NSO: *Nigella sativa* oil

Blood sample collection

Blood samples were collected from the jugular vein into plain glass tubes without anticoagulants three times during the study period (days 0, 15, and 30) following the second round. Blood samples were allowed to clot at room temperature for approximately 30- 60 minutes and were then centrifuged at 3000 rpm for 15 minutes. The serum was subsequently separated into a 2-mL Eppendorf tubes and sorted at -20°C until further analysis. Blood sampling and processing procedures were conducted in accordance with standard rabbit blood collection and serum preparation techniques described by Nelson et al. (2010).

Biochemical analysis

Biochemical parameters were measured using Biolabo standard kits (France) through colorimetric methods. The evaluated parameters included liver enzymes (ALT, AST), creatine kinase (CK), lipid profile markers (total cholesterol, LDL, HDL, and triglycerides), kidney function markers (creatinine, urea, and uric acid), protein profile indicators (total protein, albumin, and globulin), and thyroid hormones (T3 and T4).

Statistical analysis

Data were analyzed as a change-over design procedure using SAS software (SAS Institute Inc., 2016) to assess the effects of treatment, breed, and round on the measured blood parameters. Least squares means were compared using the Tukey–Kramer test, and statistical significance was set at P≤0.05.

RESULTS

A comprehensive evaluation of NSO supplementation at a dose 5gm/kg DM across three rabbit breeds (Albino, Brown, and Local) revealed distinct patterns in biochemical parameters over two experimental rounds. Effect of NS Oil on Blood Glucose: Blood glucose levels remained stable across all breeds and treatments throughout the period of the study. No significant differences were observed due to dietary treatment, breed, or experimental round (P>0.05). In all groups, blood glucose concentrations fluctuated by less than 5% from baseline, with Albino rabbits showing particularly stable levels (variation < 3% between rounds) (Table 2).

Effect of NS Oil on lipid profile

The lipid profile of the rabbit breeds included in the present study varied considerably (Table 3). Albino rabbits consistently exhibited the highest total cholesterol levels compared with the other breeds. In the first round, cholesterol levels in Albino rabbits were approximately 35% higher than those of Brown rabbits (P≤0.05) and 84% higher than those of local rabbits (P≤0.007). The same pattern persisted in the second round with cholesterol concentrations remaining 42% and 45% higher than those of Local and Brown rabbits, respectively. Albino rabbits also showed markedly higher LDL cholesterol levels than the other two breeds. In round 1, LDL levels were 70% higher than those of Brown rabbits (P≤0.05) and approximately 178% higher than those of the Local rabbits (P≤0.005). Elevated LDL concentration in Albino rabbits remained persisted across rounds, remaining 60% and 65% higher than those of local rabbits and 21% and 69% higher than those of brown rabbit, respectively. However, neither breeds nor treatments produced statistically significant differences in HDL or triglyceride levels. Although a modest increase in HDL levels (P≤0.08) and a slight reduction in triglycerides (P≤0.07) were observed in the treatment group, these changes did not reach statistical significance. Furthermore, no significant interaction effects were detected overall.

Effect of NS Oil on liver enzymes

As shown in Table 4, Brown rabbits exhibited 60% higher AST activity in round 1 and 64% higher activity in round 2 compared with Albino rabbits (P≤0.02). Additionally, AST activity in Brown rabbits was 70.67% higher than those of local rabbits in round 1 (P≤0.05). A consistent pattern was observed across rounds, with local rabbits exhibiting AST values 50% higher in round 2 and 40% higher in round 2 compared with Albino rabbits (P≤0.05). When data from both rounds were combined, AST levels in Brown rabbits were 174.07% higher than those in Albino rabbits. A round effect was also observed, with AST values in round 1 being 24.38% higher than those in round 2 (P=0.045). Although ALT showed no significant breed effect (P=0.06) and no treatment or interaction effects, creatine kinase (CK) and LDH activities varied among breeds by up to 59%. However, no statistically significant differences attributable to treatment or breed were detected (P>0.05).

Effect of NS Oil on kidney function

Analysis of renal parameters revealed clear breed differences (Table 5). In round 1, the creatinine levels in brown rabbits were approximately 51.16% higher than those of albino rabbits (P≤0.0001). Although the difference was not statistically significant, Brown rabbits also appeared to have higher creatinine levels than Local rabbits during round 1. In round 2, Brown rabbits exhibited significantly higher creatinine concentrations (P≤0.05) than both Albino and Local rabbits, exceeding their levels by 41.57% and 57.36%, respectively. Local rabbits also showed significantly higher creatinine levels than Albino rabbits in both rounds (25.33% in round 1 and 21.56% in round 2; P≤0.05), indicating a consistent inter-breed trend in creatinine clearance. A continuous inter-breed trend in creatinine clearance was observed when local rabbit's creatinine was significantly higher (P≤0.05) than those of Albino rabbits in both rounds (25.33% and 21.56% in round 1 and 2, respectively). Urea concentrations in Local rabbits were significantly higher than those in Albino rabbits during round 1 by 15.6% (P≤0.05). In round 2, both Brown and Local rabbits showed higher urea levels compared with Albino rabbits (25.37% and 14.73%, respectively; P≤0.05). No significant differences were observed between Brown and Local rabbits in either round. Uric acid levels demonstrated a significant round effect, with values in round 2 being 46.43% higher than those in round 1 (P≤0.042). However, no treatment- or breed-related differences were detected for this parameter.

Table 2 - Effect of NSO on blood glucose (mg/dl) in different rabbit breeds

Breeds	Round 1		Round 2	
	Control	Treatment	Control	Treatment
Albino	113.75 ± 11.69	111 ± 12.86	102.75 ± 2.39	109 ± 7.78
Brown	115 ± 10.54	107.33 ± 7.88	109 ± 3.71	101 ± 4.73
Local	92.50 ± 6.12	109.33 ± 6.17	116.50 ± 2.36	92 ± 6

Data are presented as mean ± standard error (SE). Significant differences between groups are indicated by P (P ≤0.05).

Table 3 - Effect of NSO on lipid profile (mg/dl) in different rabbit breeds

Breeds		Round 1		Round 2	
		Control	Treatment	Control	Treatment
Total Cholesterol	Albino	105.25 ± 9.78^{a}	110.25 ± 10.50^{a}	128.50 ±12.8^{a}	101.25 ± 15.35^{a}
	Brown	97 ± 13.97^{b}	77.33 ± 17.70^{b}	74 ± 9.29^{b}	89 ± 23.43^{b}
	Local	88 ± 30.35^{b}	69.67 ± 12.03^{b}	70 ± 6^{b}	65.67 ± 13.30^{b}
HDL	Albino	20.75 ± 1.25	23 ± 1.15	27.25 ± 1.97	25.75 ± 2.46
	Brown	22.25 ± 4.61	20 ± 2	21 ± 2.52	26 ± 6.08
	Local	15.50 ± 1.55	22.33 ± 2.19	26.33 ± 0.88	21.67 ± 2.40
LDL	Albino	78.50 ± 10.74^{a}	81.75 ± 9.57^{a}	105.50 ± 9.53^{a}	79.50 ± 18.54^{a}
	Brown	65.50 ± 13.57^{b}	47.33 ± 22.82^{b}	39 ± 12.00^{b}	47 ± 14.53^{b}
	Local	67 ± 35.77^{b}	39 ± 12.42^{b}	38 ± 6.56^{b}	39.33 ± 16.18^{b}
Triglycerides	Albino	94.25 ± 7.66	100.25 ± 22.97	82.25 ± 20.03	70.75 ± 5.59
	Brown	103.75 ± 10.42	103.33 ± 4.48	123 ± 19.08	86.67 ± 15.01
	Local	82.25 ± 17.99	79 ± 5.03	81.33 ± 6.69	68.67 ± 2.60

*Data are Mean ± SE. Superscript letters (^{a}, ^{b}) indicate significant breed differences for that parameter are indicated by (P≤0.05). Significant breed effect was found in Total Cholesterol (P≤0.007) and LDL (P≤0.005).

Table 4 - Effect of NSO on liver enzymes (IU/L) in different rabbit breeds

Breeds		Round 1		Round 2	
		Control	Treatment	Control	Treatment
AST (IU/L)	Albino	22.50 ± 4.73^{b}	26.50 ± 3.52^{b}	13.50 ± 1.85^{b}	17.25 ± 1.55^{b}
	Brown	32.75 ± 5.78^{a}	34 ± 160.1^{a}	37 ± 11.00^{a}	26.33 ± 9.84^{a}
	Local	38.25 ± 8.93^{a}	44.33 ± 9.33^{a}	26.67 ± 4.91^{a}	21 ± 4.16^{a}
ALT (IU/L)	Albino	41.25 ± 9.51	60 ± 8.53	55.75 ± 10.24	56.50 ± 3.97
	Brown	54.50 ± 8.70	60.33 ± 6.12	52.50 ± 15.50	39.67 ± 14.89
	Local	57 ± 14.91	79.33 ± 10.84	92.33 ± 13.04	59.67 ± 18.70
CK (IU/L)	Albino	1934.50 ± 763	1101 ± 82.12	875.50 ± 162.73	1129.50 ± 197
	Brown	941.75 ± 121	704.33 ± 96.44	2302 ± 87	2338.33 ± 364
	Local	3605.50 ± 175	1712.33 ± 139	1403.50 ± 43.28	1615 ± 210
LDH (IU/L)	Albino	461.25 ± 158.39	429.50 ± 21.30	257.25 ± 38.21	279.50 ± 49.16
	Brown	485 ± 153.57	237.67 ± 50.85	682.67 ± 108.51	1047.67 ± 74.16
	Local	710.75 ± 178.32	579 ± 29.54	467.25 ± 43.91	471.33 ± 99.78

*Data are Mean ± Standard Error (SE). Superscript letters (^{a}, ^{b}) denote significant differences between breeds (P≤ 0.05). Significant breed effect was detected for AST (P≤ 0.02).

Table 5 - Effect of NSO on kidney function parameters in different rabbit breeds

Breeds		Round 1		Round 2	
		Control	Treatment	Control	Treatment
Creatinine (mg/dl)	Albino	0.86 ± 0.02^{c}	0.80 ± 0.07^{c}	0.76 ± 0.07^{c}	0.82 ± 0.09^{c}
	Brown	1.30 ± 0.03^{a}	1.04 ± 0.02^{ab}	1.25 ± 0.04^{a}	1.21 ± 0.03^{a}
	Local	0.96 ± 0.08^{b}	1.08 ± 0.02^{b}	1.07 ± 0.01^{b}	0.89 ± 0.08^{b}
Urea (mg/dl)	Albino	45.00 ± 4.88^{b}	40.50 ± 2.18^{b}	46.25 ± 2.56^{b}	47.25 ± 5.48^{b}
	Brown	59.00 ± 2.86^{a}	42.67 ± 4.84^{ab}	56.00 ± 1.73^{a}	53.33 ± 1.33^{a}
	Local	51.75 ± 5.02^{a}	51.67 ± 3.18^{a}	46.50 ± 3.20^{b}	55.00 ± 3.46^{a}
Uric Acid (mg/dl)	Albino	0.28 ± 0.07	0.31 ± 0.04	0.32 ± 0.04	0.33 ± 0.04
	Brown	0.35 ± 0.07	0.15 ± 0.02	0.58 ± 0.18	0.41 ± 0.18
	Local	0.36 ± 0.07	0.21 ± 0.02	0.41 ± 0.03	0.36 ± 0.06

Data are Mean ± SE. Superscript letters (^{a}, ^{b}, ^{c}) indicate significant breed differences (P≤0.05). Significant differences found in Creatinine (P≤0.0001) and Urea (P≤0.01). Uric acid showed a significant round effect only (P≤0.042), but no breed/treatment significance.

Effect of NS Oil on protein profile

Protein metabolism remained generally stable across all groups, with albumin being the most responsive parameter (Table 6). During round 1, albumin levels of Brown rabbits were 4.85% and 7.27% higher than those in Albino and local rabbits, respectively (P≤0.05). This pattern persisted in round 2, with Brown rabbits maintaining significantly higher albumin concentrations than Albino and Local rabbits by 6.65% and 0.66%, respectively (P≤0.05).

The consistently higher albumin levels observed in Brown rabbits may indicate breed-related variation in protein synthesis or absorption capacity. Across both rounds, albumin concentrations in Brown rabbits were approximately 8–9% higher than those in Albino rabbits. Breed and dietary treatment did not significantly affect total protein or globulin concentrations throughout the experimental period (P>0.05). Although mild breed-related variation in globulin levels was observed (P≤0.047), this difference did not reach statistical significance. Total protein concentrations fluctuated within 3% of baseline values, suggesting maintained protein homeostasis across all groups.

Effect of NS Oil on thyroid hormones

Endocrine assessment revealed no significant differences (P>0.05) in T3 or T4 concentrations attributed to treatment, breed, or experimental round (Table 7). Thyroid hormone levels remained stable, with T3 and T4 varying by less than 22% across all groups. T4 concentration in Brown rabbits remained particularly stable, deviating by less than 5% from baseline values.

Table 6 - Effect of NSO on serum protein profile (g/dl) in different rabbit breeds

Breeds		Round 1		Round 2	
		Control	Treatment	Control	Treatment
Total Protein	Albino	6.25 ± 0.07	6.20 ± 0.16	6.29 ± 0.19	6.19 ± 0.07
	Brown	6.96 ± 0.22	6.73 ± 0.29	6.59 ± 0.41	6.71 ± 0.48
	Local	6.93 ± 0.64	6.47 ± 0.23	6.62 ± 0.17	6.19 ± 0.18
Albumin	Albino	5.77 ± 0.09^{b}	5.71 ± 0.13^{b}	5.71 ± 0.11^{b}	5.74 ± 0.04^{b}
	Brown	6.05 ± 0.22^{a}	6.28 ± 0.29^{a}	6.09 ± 0.01^{a}	5.76 ± 0.18^{a}
	Local	5.64 ± 0.23^{b}	5.90 ± 0.23^{b}	6.05 ± 0.11^{b}	5.62 ± 0.16^{b}
Globulin	Albino	0.48 ± 0.14	0.49 ± 0.12	0.58 ± 0.27	0.44 ± 0.07
	Brown	0.91 ± 0.20	0.75 ± 0.66	0.50 ± 0.40	0.95 ± 0.32
	Local	1.30 ± 0.46	0.56 ± 0.04	0.57 ± 0.14	0.57 ± 0.16

Data are presented as mean ± standard error (SE). Superscript letters (^{a}, ^{b}) indicate significant breed differences (P≤0.05). Breed effect was significant for Albumin (P≤0.02).

Table 7 - Effect of NSO on thyroid hormones (nmol/L) in different rabbit breeds

Breeds		Round 1		Round 2	
		Control	Treatment	Control	Treatment
T3	Albino	2.74 ± 1.83	0.91 ± 0.04	0.79 ± 0.01	1.27 ± 0.34
	Brown	0.74 ± 0.06	0.78 ± 0.04	0.81 ± 0.07	0.84 ± 0.16
	Local	1.21 ± 0.17	1.00 ± 0.07	0.71 ± 0.10	0.99 ± 0.12
T4	Albino	38.52 ± 7.53	34.39 ± 5.35	36.49 ± 3.02	39.27 ± 2.46
	Brown	31.80 ± 1.70	30.97 ± 4.96	32.11 ± 3.20	33.33 ± 6.19
	Local	40.74 ± 4.15	41.36 ± 0.75	32.18 ± 4.72	32.69 ± 1.75

Data are presented as mean ± standard error (SE). Significant differences between groups are indicated by P ($P \leq 0.05$).

DISCUSSION

This study investigated the effect of dietary NSO supplementation on selected biochemical markers in three rabbit breeds: Albino, Brown, and Local. The findings indicate that several observed differences were primarily breed-dependent, reflecting inherent metabolic variation rather than treatment effects. Overall, NSO supplementation did not produce large or consistent alterations across the measured biochemical parameters.

Blood glucose concentrations remained stable across breeds and dietary treatments. These findings are consistent with Mahomoodally et al. (2022), who found that NS reduces blood glucose primarily in diabetic animals rather than in healthy individuals with normal insulin regulation. Similarly, Wang et al. (2023) suggested that metabolic differences among rabbit breeds do not necessarily affect glucose homeostasis in healthy animals.

Significant breed-related variation was observed in total cholesterol and LDL cholesterol levels, with Albino rabbits exhibiting higher concentrations than Brown and Local breeds. These findings are consistent with the study of Funes et al. (2024), who attributed elevated lipid profiles in Albino rabbits to differences in hepatic cholesterol synthesis and lipoprotein metabolism. Although NSO has been widely reported to exert hypolipidemic effects largely attributed to thymoquinone-mediated inhibition of HMG-CoA reductase (Kareem et al., 2022) no significant treatment-related lipid changes were detected in the present study. This may be explained by the absence of experimentally induced hyperlipidemia, a condition under which NSO effects are typically more pronounced. Comparable improvements in lipid and antioxidant status have been reported primarily under oxidative or metabolic stress conditions (Ahmed and Abdul-Rahman, 2023; Fatima et al., 2024). Nevertheless, a slight reduction in triglycerides and a modest round-related increase in HDL were observed, suggesting minor improvements in lipid turnover over time, consistent with previous experimental observations (Atufe et al., 2022).

Breed-related differences were also evident in liver enzyme activity. Specifically, AST levels were significantly elevated in Brown rabbits, whereas ALT exhibited a borderline trend among breeds. Abdel-Hamid and Dawod (2020) documented natural inter-breed variations in baseline liver enzyme activity, supporting the interpretation that the observed differences likely reflect physiological characteristics rather than dietary effects. Previous studies have demonstrated hepatoprotective properties of Nigella sativa under toxic stress conditions (Hassan et al., 2012; Hatipoğlu et al., 2023). The generally normal liver enzyme profiles observed here may therefore indicate limited scope for antioxidant intervention under physiologically stable conditions (Abdelsalam and Fathi, 2023).

Higher creatinine and urea concentrations observed in Brown rabbits may reflect breed-related differences in renal clearance efficiency or muscle mass. Similar baseline variation among breeds has been reported by Sze-Yu et al. (2025), who noted that inherent physiological differences may obscure treatment effects in healthy animals. Conversely, Dollah et al. (2013) demonstrated nephroprotective effects of Nigella sativa through reductions in serum creatinine without evidence of nephrotoxicity. The round-related increase in uric acid observed in the present study, particularly during round 2, may reflect time-dependent metabolic or handling stress, as previously suggested by De la Fuente et al. (2004).

Albumin concentrations were consistently higher in Brown rabbits, potentially reflecting enhanced hepatic synthetic protein synthesis or improved protein assimilation. Differences in total protein and globulin among breeds were minimal, consistent with findings by Umar et al. (2018) and Cimrin et al. (2023), who reported improved protein status in rabbits receiving Nigella sativa supplementation, possibly due to enhanced nitrogen utilization and liver function. These findings suggest a potential protein-sparing or anabolic role of NSO, particularly during prolonged supplementation or nutritional stress (El-Gindy et al., 2020). Similar improvements in protein metabolism have been observed with antioxidant-rich supplements such as coenzyme Q10 and wheat germ oil in stressed rabbits (Al-Samarai and Al-Janabi, 2021).

Thyroid hormones (T3 and T4) remained unaffected by treatment, breed, or experimental round, supporting findings by Elfaki and Elkhair (2023), who reported minimal endocrine disruption following phytogenic supplementation. Avci et al. (2021) further suggested that Nigella sativa may act as a hormonal modulator primarily under pathological thyroid conditions rather than altering euthyroid physiological states.

Biological variability should also be considered when interpreting the findings. High standard errors were observed for several parameters, particularly liver enzymes, lipid fractions, and thyroid hormones. Such variability is common in rabbit models and may arise from genetic diversity, limited sample size, or stress responses related to handling and environmental factors. Previous studies have similarly documented fluctuations in enzymes such as AST, ALT, CK, and LDH associated with breed and stress exposure (Washington and Van Hoosier, 2012). Hormonal and metabolic markers are likewise influenced by circadian rhythms, feed intake, and adaptive physiological responses (Liang et al., 2022). Despite this variability, statistically significant differences were detected in key parameters such as creatinine, cholesterol, and albumin, suggesting that the observed effects are biologically meaningful. Future studies incorporating larger sample sizes and refined sampling protocols may help reduce variability and strengthen these findings.

CONCLUSION

Dietary supplementation with N.S oil (5 g/kg DM) produced breed-dependent biochemical responses among the three rabbit breeds studied. Albino rabbits exhibited higher cholesterol and LDL concentrations, whereas Brown rabbits showed elevated creatinine and urea levels. AST activity decreased more markedly in Albino and Local rabbits compared with the Brown breed. Overall, NSO supplementation appeared to support liver stability while maintaining normal kidney function without inducing adverse biochemical effects. Future research should investigate the effects of different inclusion levels of Nigella sativa oil to determine the optimal dosage for each rabbit breed, particularly in relation to lipid profile and kidney function parameters.

DECLARATIONS

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Ethical approval statement

The study received ethical approval from the Animal Ethics Committee at the College of Veterinary Medicine, University of Duhok, Iraq (Ethical code No. DR2996919CV, approved January 11, 2021).

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Funding statement

This research did not receive specific grants from funding agencies in public, commercial or not-for-profit sectors.

Authors' contribution

W.I. Hasan and H.M. Hidayet conceived and designed the study. O.H. Azeez, C.A. Yateem, and K.N. Mustafa executed the experiment and analyzed the sera and tissue samples. S.M. Sefdeen analyzed the data. All authors interpreted the data, critically revised the manuscript for important intellectual content, and approved the final version.

Acknowledgements

The authors are grateful to the help given by the College of Agricultural Engineering Sciences, especially Department of Animal Production, University of Duhok, Duhok-IRAQ.

Competing interests

The authors declare no competing interests in this research and publication.

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