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Impact of *Nigella sativa* meal on blood metabolites and immune status of growing lambs

Heba A. Nasr[1*], Fares A. Eldeeb[2], Abdelbaset Ahmed[3,4], Ghada S.E. Abdel-Raheem[3]

[1]*Department of Clinical Pathology, Faculty of Veterinary Medicine, Assiut University, Assiut 71526, Egypt.*
[2]*Department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Aswan University, 81528 Aswan, Egypt.*
[3]*Department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Assiut University, 71526 Assiut, Egypt.*
[4]*Department of Veterinary and Public Health, School of Veterinary Medicine, Badr University in Assiut, Assiut 71526, Egypt.*

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*Correspondence:

Corresponding author: Heba A. Nasr
E-mail address: hebabaset@aun.edu.eg

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ABSTRACT

The aim of the present study was to assess the impact of *Nigella sativa* meal (NSM) at different levels of inclusion on blood metabolites, antioxidant status and immunomodulatory of growing lambs. Thirty-five Rahmani lambs were randomly allocated to five diet treatments for 90 days. Group 1 received a basal diet without *Nigella sativa* meal and considered as control, while groups 2, 3, 4 and 5 fed on diets contained 6, 12, 18 and 24 % NSM, respectively. The parameters measured were blood metabolites, oxidative stress indicators, immune response, and inflammatory cytokines levels. Blood metabolites indicated increased total protein, albumin, and globulin levels, while the levels of creatinine, liver enzymes, lipids, and glucose were reduced. There were increases in antioxidants and immune markers and a decrease in inflammatory markers. It could be concluded that supplementation of NSM enhance blood metabolites, antioxidant status, and immune status of growing Rahmani lambs and therefore suggests the use of NSM as a sustainable and ideal alternative for antibiotics.

Introduction

Sheep and goats are valuable for investment and protection due to their high fertility, short reproductive cycles, and ability to thrive in harsh conditions. In Egypt, a major issue for animal farming is the shortage of adequate feed to meet the nutritional needs of the current animal population (Kisku and Singh, 2022). Antibiotics have been investigated to reduce diseases and mortality, stricter regulations in livestock nutrition, such as the EU ban on antibiotics and ionophores and the US Veterinary Feed Directive, have interested a shift towards alternative strategies (Hayajneh *et al.* 2024). Consequently, natural feed additives, such as medicinal plants (Batool *et al.* 2023; Khan *et al.* 2023), have more attention in recent decades as a scientific approach to promoting optimal growth, development, and health in early life (Rashid *et al.* 2024; Wang *et al.* 2024).

Nigella sativa, an annual herb from the Ranunculaceae family, is widely cultivated, especially in the Middle East (Obeidat and Alqudah 2023). *Nigella sativa* seeds were served as a potential alternative feed source. This herbal plant is used both as a feed additive which is rich in various bioactive compounds and essential nutrients (Ciesielska-Figlon *et al.* 2023) containing thymoquinone (TQ), which is found to be responsible for many of its therapeutic effects (Dabeer *et al.* 2022). *Nigella sativa* seeds contain bioactive compounds like thymohydroquinone (TQ), and nogelleone, which have antioxidant, antimicrobial, and immune-boosting properties (Majeed *et al.* 2021). In addition, *Nigella sativa* has a potent antioxidant and immune-stimulating effects (Ciesielska-Figlon *et al.* 2023; Meddah *et al.* 2024), which may offer potential benefits in mitigating stress induced by diseases. Thymoquinone (TQ), a key polyphenol in *Nigella sativa*, along with other compounds like p-cymene, carvacrol, α-thujene, and β-pinene, has been shown to enhance rumen metabolism, leading to improve the productivity of ruminants (Kabir *et al.*, 2020; Sadarman *et al.*, 2021; Singh *et al.*, 2022). These bioactive compounds also possess antioxidant, antimicrobial, anti-inflammatory, immunomodulatory, and anticancer properties (Ahmad *et al.*, 2021).

Nigella sativa seed meal has been produced from its seeds for extracting *Nigella sativa* oil for human health supplies (Fathi *et al.* 2023). It is recognized for its rich content of natural compounds and secondary metabolites. Packed with nutrients such as crude protein (CP), essential amino acids, and healthy fats, it stands out as a promising alternative in animal feed (Obeidat, 2020, 2021). As a result, *Nigella sativa* meal can be an effective ingredient in lamb feed, lowering feed costs and boosting the economic efficiency of livestock production without compromising animal health (Abdel-Magid *et al.*, 2007). Since no available data was found through literature about the impacts of *Nigella sativa* meal on the blood metabolites, and immune status in Rahmani lambs, consequently, this study sought to examine the effect of *Nigella sativa* meal in diets of Rahmani growing lambs on the blood metabolites, antioxidant status, immunoglobulins and inflammatory cytokines.

Materials and methods

Ethical approval

The Ethics Committee of the Faculty of Veterinary Medicine, Assiut University approved the animal care and use procedures during running this experiment in compliance with Egyptian laws and regulations (Ethical Approval No. 06/2024/0262).

Animals and experimental ration

This research involved 35 healthy Rahmani ram lambs at Faculty of Veterinary Medicine, Assiut University, Assiut city, Egypt (Latitude and longitude coordinates are: 27.180134, 31.189283.). The average age of lamb was 10.0±1.3 months and weighing about 30.0±1.5 kg. Lambs were in good health and exhibited typical signs of health prior to and through-

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out the experiment. They were vaccinated and dewormed based on a veterinarian's guidance before the experiment. Each lamb was housed in its own space with separate feeding and drinking arrangements. Natural light cycles and temperatures were maintained, with constant access to fresh water. Daily rations for lambs were determined by the National Research Council (2007) guidelines for sheep (Table 1), provided at 4% of body weight per day, split into two feedings at 8:00 a.m. and 5:00 p.m., over a 90-day period. Feed remnants were collected and weighed consistently.

Experimental design

Before the experiment began, lambs were given two weeks to adjust and were then randomly distributed to one of five groups (7 lambs per each). Specifically, group 1 received a basal diet without *Nigella sativa* meal and considered as a control. Group 2 diet included 6% NSM, group 3 diet contained 12% NSM, group 4 diet had 18% NSM, and group 5 diet had 24 % NSM, respectively.

Chemical analysis

The proximate analysis of both feeding stuffs and experimental rations for crude fiber, dry matter, crude protein, ether extract, and ash was determined according to the AOAC guidelines (AOAC, 2023).

Blood metabolites

By the end of the study, blood samples were collected from each lamb via the jugular vein, two hours following their morning meal. The samples underwent centrifugation at 3000 x g for 15 minutes, and the resulting serum was kept at −20°C for subsequent analysis. The serum was examined for total protein, albumin, uric acid, urea, and creatinine levels. To calculate serum globulin, albumin subtracted from total protein.

Enzymatic activities for various markers, including ALT (alanine aminotransferase), AST (aspartate aminotransferase), ALP (alkaline phosphatase) and GGT (γ-Glutamyl transferase) were assessed. The metabolic profile, which included glucose, cholesterol, triglycerides, HDL (high-density lipoprotein), LDL (low-density lipoprotein), and VLDL (very-low-density lipoprotein), was evaluated using commercial kits from Spectrum Diagnostics (Cairo, Egypt), following the provided protocols. The VLDL and LDL concentrations were calculated using the formulas from Oliveira *et al*. (2013). Furthermore, the levels of serum calcium (Ca), phosphorus (P), and magnesium (Mg) were analyzed with commercial kits from Biolabo Merieux (France) using an automatic spectrophotometer.

Immunological assay

The serum levels of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and malondialdehyde (MDA) were measured using colorimetric methods with Spectrum and Bio-diagnostic kits (Bio-diagnostic Company, Egypt). Immunoglobulin concentrations, specifically IgA and IgG, were determined using bovine sandwich ELISA kits, following the protocols provided by CUSAbio Biotech Inc. (Wuhan, China).

Inflammatory cytokines

Inflammatory biomarkers, including interferon gamma (IFN-γ), interleukin-1β (IL-1β), tumor necrosis factor (TNF-α), interleukin-6 (IL-6), and interleukin-4 (IL-4), were quantitatively measured using bovine sandwich ELISA kits, following the protocols provided by CUSAbio Biotech Inc. (Wuhan, China).

Statistical analysis

The statistical analysis was performed using SPSS version 16 (SPSS Inc., Chicago, IL, USA). Prior to analysis, the data were assessed for normality, confirming that all measurements are normally distributed. A one-way analysis of variance (ANOVA) was employed to analyze the

Table 1. Ingredients and composition (%) of the experimental diets.

	NSM0	NSM6	NSM12	NSM18	NSM24	NSM
Corn, ground	48.24	47.37	46.5	45.55	44.61	
Soybean meal	20	15	10	5	0	
Nigella sativa meal	0	6	12	18	24	
Wheat straw	30	30	30	30	30	
Limestone, ground	0.96	0.83	0.7	0.59	0.46	
Monosodium phosphate	0	0	0	0.06	0.13	
Common salt	0.5	0.5	0.5	0.5	0.5	
Premix*	0.3	0.3	0.3	0.3	0.3	
Total	100	100	100	100	100	
Chemical composition:						
Dry matter	87.43	87.91	88.39	88.79	89.21	95
Crude protein	14.7	14.7	14.7	14.71	14.72	37.5
Ether extract	2.62	3.12	3.63	4.14	4.65	10.26
Crude fiber	13.54	13.95	14.36	14.77	15.18	12.2
Nitrogen free extract	65.04	63.98	62.97	61.93	60.9	32.93
Ash	4.14	4.25	4.35	4.45	4.55	7.11
Calcium	0.51	0.51	0.51	0.51	0.51	1.13
Phosphorus	0.28	0.27	0.25	0.24	0.24	0.35
ME (Mcal/Kg)	2.45	2.47	2.49	2.5	2.52	3.19

*Premix consist of (per 3kg): 20,000,000 IU vitamin A, 10,000 mg vitamin E, vitamin 200,000 IU D3, 10,000 mg Fe, 2500 mg Cu, 100 mg Mo, 20,000 mg Mn, 100 mg Co, 800 mg I, 20,000 mg Zn and 100 mg Se.

data, followed by Tukey's test to determine significant differences among treatments. The findings are reported as mean ± standard error (SE), with statistical significance defined at P < 0.05.

Results

Blood metabolites

Lambs in the fourth group diet had the highest total protein, however, the fifth group had the highest albumin levels and albumin-to-globulin ratio, whereas the control group had the lowest values (Table 2). The highest globulin concentration was observed in the fourth group (P<0.05), in comparison with experimental groups and control. *Nigella sativa* meal significantly affected serum creatinine and urea levels (P<0.05). The lowest creatinine levels were seen in lambs that were fed NSM diets, while the control group showed the highest. Additionally, serum urea level was higher in NSM (P<0.05). Dietary NSM significantly (P<0.05) reduced liver enzyme concentrations, including ALT, AST, ALP, and GGT, as compared to control group (Table 2). Lambs fed the 24% NSM diet exhibited the lowest concentrations, while the control group had the highest values.

Lipid Parameters and Glucose

Diets containing NSM significantly lowered total lipids, triglycerides, cholesterol, LDL, VLDL, and glucose levels (P<0.05) while HDL level showed significant increase (P<0.05) in the fourth group only compared to the control group (Table 3). The results showed significant differences in calcium, phosphorus, and magnesium levels (P<0.05). The fourth group had the highest levels of these minerals compared to the other experimental groups and the control.

Immunological Assay

Dietary NSM significantly elevated T-SOD and GSH-Px, levels (P<0.05), with the highest values found in the 24%NSM group and the lowest in the control group (Table 4). Conversely, MDA levels decreased significantly in NSM-fed groups (P<0.05), with the lowest values in the NSM group and the highest in the control. NSM also improved serum immunoglobulin concentrations (IgA and IgG). Lambs fed 24%NSM, followed by 18%NSM had the highest IgA and IgG levels (P<0.05), while the control group showed the lowest.

Inflammatory Cytokines

The levels of inflammatory cytokines in lambs fed NSM diets are shown in Table 5. The levels of IFN-γ, IL-1β, TNF-α, and IL-6 were significantly lower (P<0.05) in the NSM-fed groups, while IL-4 levels showed a significant increase (P<0.05) compared to the control group.

Table 2. Effect of *Nigella sativa* meal on the protein parameters, kidney and liver functions.

Items	Experimental groups					SEM	P-Value
	NSM0	NSM6	NSM12	NSM18	NSM24		
Protein parameters							
Total protein (g/dl)	7.50 ^{b*}	7.78 ^{b}	8.25 ^{a}	8.52 ^{a}	8.28 ^{a}	0.09	0.00
Albumin (g/dl)	3.51 c	3.61^{bc}	3.79^{bc}	3.95 ^{b}	4.64 ^{a}	0.09	0.00
Globulin (g/dl)	3.99 ^{d}	4.17 ^{c}	4.46 ^{b}	4.57 ^{a}	3.64 ^{e}	0.01	0.00
A/G ratio**	0.88 ^{b}	0.87 ^{b}	0.85 ^{b}	0.86 ^{b}	1.27 ^{a}	0.02	0.00
Kidney functions:							
Creatinine (mg/dl)	1.85 ^{a}	1.52^{ab}	1.45^{ab}	1.27 ^{b}	1.05 ^{b}	0.12	0.01
Urea (mg/dl)	12.45^{c}	12.75^{c}	13.35^{b}	14.15^{a}	13.85^{ab}	0.11	0.00
Liver functions:							
ALT (U/L)	45.23 ^{a*}	43.34 ^{b}	41.11 ^{c}	39.21 ^{d}	35.53 ^{e}	0.12	0.00
AST (U/L)	65.43 ^{a}	62.13 ^{b}	59.53 ^{c}	56.65 ^{d}	55.79 ^{e}	0.13	0.00
ALP (U/L)	8.11 ^{a}	7.75^{ab}	7.41^{bc}	6.93 ^{c}	6.24 ^{d}	0.14	0.00
GGT (U/L)	30.58 ^{a}	29.15 ^{b}	27.75 ^{c}	26.38 ^{d}	24.31 ^{e}	0.12	0.00

*Means in the same row with different superscripts are significantly different (P<0.05).
**A/G: Albumin/Globulin ratio, ALT: Alanine aminotransferase; AST: Aspartate transaminase; ALP: Alkaline phosphatase; GGT: Glutamyl transferase

Table 3. Effect of *Nigella sativa* meal on the serum lipid profile, glucose and minerals.

Items	Experimental groups					SEM	P-Value
	NSM0	NSM6	NSM12	NSM18	NSM24		
Total lipids (mg/dl)	45.32 ^{a*}	43.51 ^{b}	41.25 ^{c}	39.15 ^{d}	35.75 ^{e}	0.14	0.00
Triglycerides (mg/dl)	30.13 ^{a}	28.97 ^{b}	27.45 ^{c}	25.80 ^{d}	23.54 ^{e}	0.20	0.00
TC (mg/dl)**	43.12 ^{a}	41.15 ^{b}	39.25 ^{c}	37.26 ^{d}	34.11 ^{e}	0.21	0.00
HDL (mg/dl)	13.25 ^{c}	13.75^{bc}	14.51^{abc}	15.11^{ab}	16.13 ^{a}	0.39	0.00
LDL (mg/dl)	23.84 ^{a}	21.61 ^{b}	19.25 ^{c}	16.99 ^{d}	13.27 ^{e}	0.27	0.00
VLDL (mg/dl)	6.03 ^{a}	5.79 ^{b}	5.49 ^{c}	5.16 ^{d}	4.71 ^{e}	0.04	0.00
Glucose (mg/dl)	75.53 ^{a}	73.75 ^{b}	67.65 ^{c}	65.48 ^{d}	60.67 ^{e}	0.30	0.00
Minerals							
Calcium (mg/dl)	8.16 d*	8.43 c	8.75 b	9.25a	8.77 b	0.02	0.00
Phosphorus (mg/dl)	6.48 d	6.69 c	7.10 b	7.32 a	7.13 b	0.02	0.00
Magnesium (mg/dl)	3.15 d	3.25 c	3.44 b	3.57 a	3.45 b	0.02	0.00

*Means in the same row with different superscripts are significantly different (P<0.05).
**TC: Total Cholesterol; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein; VLDL: Very Low Density Lipoprotein

Table 4. Effect of *Nigella sativa* meal on the serum antioxidants and immune status.

Items	Experimental groups					SEM	P-Value
	NSM0	NSM6	NSM12	NSM18	NSM24		
T-SOD (U/ml)**	12.15 ^{d*}	12.75 ^{cd}	13.26^{bc}	13.81 ^{b}	14.75 ^{a}	0.14	0.00
GSH-Px (U/ml)	130.11 ^{d}	134.73 ^{cd}	141.58^{bc}	147.48 ^{a}	157.15 ^{a}	1.94	0.00
MDA (nmol/ml)	3.85 ^{a}	3.27^{ab}	2.65^{bc}	2.31^{bc}	2.01 ^{c}	0.25	0.00
IgA (U/L)	0.93 ^{b}	1.18^{ab}	1.32^{ab}	1.87^{ab}	2.15 ^{a}	0.22	0.01
IgG (U/L)	1.35 ^{c}	1.48^{bc}	1.89^{abc}	2.56 ^{a}	2.51^{ab}	0.23	0.01

*Means in the same row with different superscripts are significantly different (P<0.05).
T-SOD: Total superoxide dismutase; GPx: Glutathione peroxidase; MDA: Malondialdehyde; IgA: Serum immunoglobulin A, IgG: Serum immunoglobulin G.

Table 5. Effect of *Nigella sativa* meal on the serum inflammatory cytokines.

Items	Experimental groups					SEM	P-Value
	NSM0	NSM6	NSM12	NSM18	NSM24		
IFN-γ (pg/ml)**	65.50 ^{a*}	63.15^{ab}	60.60^{ab}	56.69^{bc}	51.65 ^{c}	1.74	0.00
IL-β (pg/ml)	125.70 ^{a}	120.93^{ab}	114.72^{bc}	107.95 ^{c}	98.65 ^{d}	1.75	0.00
TNF-α (pg/ml)	75.80 ^{a}	73.11^{ab}	68.56^{ab}	65.45^{bc}	59.34 ^{c}	1.95	0.00
Il-6(pg/ml)	115.20 ^{a}	110.36^{ab}	104.12^{ab}	98.88^{bc}	90.25 ^{c}	2.51	0.00
IL-4 (pg/ml)	82.60 ^{c}	85.53^{bc}	89.95^{abc}	93.73^{ab}	98.97 ^{a}	2.06	0.00

*Means in the same row with different superscripts are significantly different (P<0.05).
**IFN-γ: Interferon gamma; IL-1β, interleukin-1β; TNF-α, tumor necrosis factor-alpha; IL-6, interleukin-6; IL-4, interleukin 4

Discussion

Feeding lambs on diet supplemented with *N. sativa* meal (NSM) significantly increased total protein and albumin levels (P<0.05). Compounds found in *N. sativa*, such as thymoquinone and essential amino acids, likely boosted protein synthesis and thyroid hormone secretion (Amin and Hosseinzadeh, 2016). Lambs fed on 18% NSM ration exhibited higher globulin levels, indicating enhanced immune response (Ghasemi *et al*., 2014), potentially due to *N. sativa*'s immunomodulatory properties and reduced oxidative stress (Ahmed *et al*., 2021). However, Abd El-Hack *et al*. (2016) reported that 10 and 15% BCSM increased serum total protein, albumin, and A:G ratio. However, some studies reported no significant changes in protein or globulin levels with supplementation of NSM in the diets of lambs (Yavari *et al*., 2021; Obeidat *et al*., 2023).

In terms of kidney function, NSM decreases serum creatinine and increase urea level, reflecting protein degradation in the rumen (Nauroze *et al*., 2023). Increased blood urea nitrogen indicated enhanced protein breakdown, though some studies noted decreased blood urea, suggesting more efficient protein metabolism (Odhaib *et al*., 2018). The nephroprotective effects of NS could be by decreased creatinine levels in lambs (Al-dain and Jarjeis 2015).

Lambs fed on diets contained *Nigella sativa* meal (NSM) exhibited a significant (P<0.05) reduction in liver enzymes (ALT, AST, ALP, and GGT) when compared to control group, likely due to the liver-protective effects of thymoquinone, the main active compound in *N. sativa*, which helps maintain liver cell membrane integrity and reduces enzyme activity (Shaterzadeh-Yazdi *et al*., 2018). Elsayed *et al*. (2025) reported that calves fed on the diets supplemented with NS resulted in a significant linear decrease in creatinine level and liver enzymes (AST and ALT) in comparison with the control diet. These results are aligning with those of El-Nagar *et al*. (2023), who found significant reductions in AST, ALT, and creatinine levels in calves fed on the diets supplemented with NS. Our results agreed with the findings of Zaher *et al*. (2020) who found significantly lower serum ALT activity in goats supplemented with NS. These results suggested that NS have hepato-renal protective effects. The authors hypothesized that effect might be attributed to the presence of TQ in NS. Previous studies supporting its hepatoprotective properties these findings, indicating decreased ALT, AST, and ALP levels in lambs with *N. sativa* supplementation (Yavari *et al*., 2021; Nauroze *et al*., 2023). However, other studies reported no significant changes in ALT and AST in goats and lambs (Odhaib *et al*., 2018).

The findings from this study demonstrated that *Nigella sativa* meal (NSM) supplementation significantly reduced serum lipid profile, including total lipids, triglycerides, and cholesterol in lambs in comparison with the control group as found by Hassan *et al*. (2024). Our results align with Elsayed *et al*. (2025) who found lower plasma cholesterol and triglyceride levels when calves fed on the diets supplemented with NS compared control group This effect may be attributed to NSM high content of unsaturated fatty acids and bioactive compounds like Nigellone and β-sitosterol, which inhibit cholesterol absorption and reduce 3-hydroxy-3-methyl-CoA reductase r activity (Weerawatanakorn *et al*. 2024). Additionally, NSM increased HDL cholesterol levels and decreased LDL cholesterol, potentially due to the biohydrogenation of unsaturated fatty acids and the influence of thymoquinone (El-Hawy *et al*., 2018; Hassan *et al*., 2024). Abdullah and Farghaly (2019) reported lowered levels of cholesterol and liver enzymes when feeding lambs on diets supplemented with NSM.

Serum glucose levels were decreased linearly with increased level of NSM in this study, supporting *N. sativa*'s anti-diabetic effects, which may result from improved glucose utilization, enhanced insulin sensitivity, and inhibition of α-glucosidase enzyme activity (Loh *et al*., 2019). In contrast, dietary inclusion of NS significantly reduced serum glucose and total lipids as reported by Abd El-Hafeez *et al*. (2014).

Blood mineral profiles were also positively affected by different levels of NSM, with increased levels of calcium, phosphorus, and magnesium observed at moderate NSM inclusion levels (Al-Jasass and Al-Jasser, 2012). However, excessive supplementation of NSM led to a decline, highlighting the importance of optimal dosing.

In terms of antioxidant activity, the study found that lambs fed NSM had significantly higher levels of antioxidant enzymes such as GSH-Px and T-SOD and lower levels of the oxidative stress marker malondialdehyde (MDA) as reported by Hassan *et al*. (2024). This antioxidant effect is linked to thymoquinone and other phenolic compounds in *N. sativa*, which enhanced the expression of antioxidant enzymes like catalase and superoxide dismutase, reducing oxidative stress and lipid peroxidation (Shahin *et al*., 2018; Rashwan *et al*., 2023). Previous research has also confirmed that *N. sativa* supplementation improved antioxidant capacity in livestock, as evidenced by reduced MDA levels (Selim *et al*., 2019). Polyphenols found in NSM, which can act as an excellent superoxide anion scavenger for free radicals (Ruwali *et al*., 2022). In addition, polyphenols act on the cellular antioxidant signaling pathway, which activate related transcription factors and regulate the expression of downstream genes as reported by Zeng *et al*. (2024). In addition, Selim *et al*. (2019) reported that dietary NS contained essential oils which improved blood oxidative stability, through reducing MDA levels which aligns with that reported by Desai *et al*. (2015).

The vital defense mechanism through the immune system protected the body from various diseases. It considered safeguards against foreign invaders and microorganisms, thereby maintaining internal balance and overall health. Regarding the immune response, NSM significantly increased serum immunoglobulin levels (IgA and IgG) in comparison with control group in this study. This immune-boosting effect is likely due to the anti-inflammatory and antibacterial properties of *N. sativa*, as well as its ability to modulate both T cell- and B cell-mediated immune responses (El-Gindy *et al*., 2020; Abd El-Hack *et al*., 2021). Calves fed on NS diets recorded increase in immunoglobulin G (IgG) and M (IgM) as found by Elsayed *et al*. (2025). Immunological levels included IgG and IgM were improved significantly in Friesian calves fed on diets supplemented by NS (El-Nagar *et al*. 2023). Previous studies have reported that NS increased the production of IgG and IgM in calves (Abd El-Hafeez *et al*. 2014; Fathi *et al*. 2024). Odhaib *et al*. (2018) found an increase in IgA, IgG, and IgM concentration in Dorper lambs fed on the diet supplemented with *Nigella sativa*. In another study reported that serum immunoglobulins (IgG, IgM, and IgA) concentrations in kids were significantly ($P<0.05$) increased in kids groups fed on *Nigella sativa* and the higher level of NS showed significantly ($P<0.05$) the highest values. In addition, NS supplementation improved immunity status of small ruminants (Singh *et al*., 2022). Several studies have reported the immunomodulatory effects of NS, due to the presence of bioactive compounds like TQ which can activate the immune modulatory agent NF-κB pathway by upregulating phosphorylated P65 and IκBα, as well as phosphorylating JNK, ERK, and p38 to activate the MAPK signaling pathway (Wei *et al*. 2022).

Inflammation is a natural response to the infection, a process involving different immune cells and signaling molecules which workings together to protect the body from harm. In our study, NSM inhibited pro-inflammatory cytokines (IFN-γ, IL-1β, TNF-α, and IL-6) and increased anti-inflammatory markers (IL-4), primarily due to the action of thymoquinone, which suppresses inflammatory pathways and neutralizes reactive oxygen species (Khalifa *et al*., 2021). Our results agreed with that reported by Elsayed *et al*. (2025) who found increased in T-AOC and decreased MDA in the plasma of calves fed on the diets supplemented with NS. Several studies reported that NS and its active compounds, such as TQ, can modulate the immune system (Ciesielska-Figlon *et al*. 2023).

Conclusion

This research highlights the potential of 18% *Nigella sativa* meal (NSM) supplementation to optimize growing Rahmani lambs health and performance. We found significant improvements in blood health, antioxidant levels, and immunity, coupled with a reduction in oxidative and inflammatory stress making NSM a promising alternative feed option to antibiotics. These results suggest that supplementation of NSM into lamb diets can bolster lamb productivity. While these results are promising, future molecular investigations are needed to strengthen our understanding of NSM mechanisms of action.

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Conflict of interest

The authors declare that they have no conflicts of interest to disclose.

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