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Effect of dietary black cumin seed (*Nigella sativa*) on performance, immune status, and serum metabolites of small ruminants: A meta-analysis

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ABSTRACT

A growing number of publications are identified toward the use of black cumin seeds (*Nigella sativa* L) on small ruminant animals since many beneficial evidences have been reported in humans and animals. This study used a meta-analysis approach to quantify the effect of black cumin seeds (BCS) supplementation on the productive performance, nutrients utilization, and blood metabolites profile of small ruminant animals. Following the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) protocols, a total of 23 studies were aggregated in a database and were analyzed using mixed model methodology classifying the studies as random effects and levels of BCS as fixed effects in the models. Results found that interaction between BCS levels and animal species tended to be significant whereas increasing dietary BCS levels linearly increased average daily gain (ADG) ($p < 0.01$, $R^2 = 0.538$) and dry matter intake in lambs ($p < 0.01$, $R^2 = 0.958$) but had no effect on sheep. Nitrogen (N) intake, and N digested were found to be significantly increased ($p < 0.01$) as inclusion rates of BCS increased while organic matter (OM), crude protein (CP), and ether extract (EE) intakes tended to increased ($p < 0.10$). In addition to other immune responses, strong positive relationships were found on the increase of concentration of IgA ($R^2 = 0.922$) and IgG ($R^2 = 0.939$) ($p < 0.05$) in response to increasing BCS supplementation. To conclude, black cumin seeds can be used as a safe and beneficial feed supplement to promote the growth of small ruminant animals such as lambs, sheep, and goats.

1. Introduction

Investigations on the importance of medicinal feedstuff from lesser plants on ruminant animals have been well documented over the last decades. Black cumin (*Nigella sativa* L) seed (BCS) is among plant seeds that recently received a growing interest as a feed supplement because it is rich in oils and contains diverse phytochemical compositions (Ahmad et al., 2021; Cherif et al., 2018b). The major constituent of BCS is thymoquinone (TQ) (Sahak et al., 2016) with a considerable amount of polyphenols and essential oil compounds such as p-cymene, thymoquinone, α-thujene, carvacrol, β-pinene, and many more (Kabir et al., 2020; Odhaib et al., 2018b).

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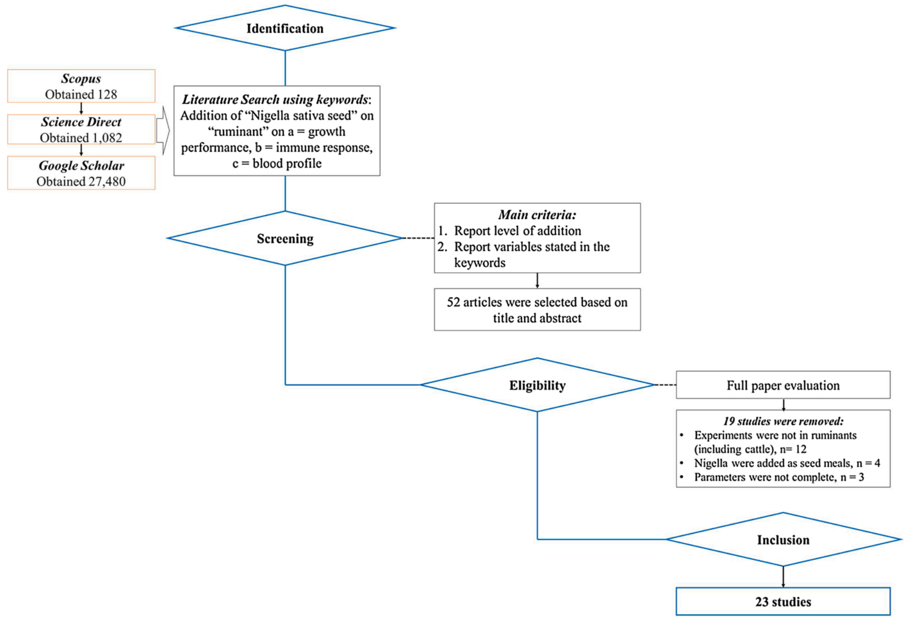

Fig. 1. Flow chart of literature selection process following PRISMA protocols.

As widely explained, phytochemical compounds are advantageous for ruminant productivity since they can positively modulate rumen metabolism hence improve growth performance. A recent review described that active compounds of BCS possess many biological roles and perform as antioxidant, antidiabetic, antimicrobial, antitussive, anticancer, hepatoprotective, neuroprotective, anti-inflammatory, gastroprotective, immunomodulator, analgesic, spasmolytic, and bronchodilator activity (Ahmad et al., 2021; Kooti et al., 2016). Many shreds of evidence from meta-analyses showed that essential oils are effective to improve growth performance and nutrient digestibility in cattle and small ruminants (Khiaosa-Ard and Zebeli, 2013; Torres et al., 2020) and broiler chickens, and it was effective to replace antibiotics as growth promoters (Irawan et al., 2021).

Recent studies reported that supplementation of 1.2 % BCS either in low or high concentrate diets increased protein digestibility and growth rate of lambs as well as decreased ruminal protozoa (Cherif et al., 2018a, b). On the other hand, there was another study reported that dietary treatment with BCS impaired ruminal fermentation and nutrient digestibility but it had a positive effect to enhance the immune status of Dorper lambs (Odhaib et al., 2018a). Others suggested that the effect may differ due to different circumstances, basal diet, and dietary level (Habeeb and El-Tarabany, 2012; Zanouny et al., 2013). Since available studies used a large variety of inclusion rates of Nigella seeds, determination of optimal level is important to help future experiments.

In addition, relying on the individual study is often conflicting to generalize or synthesize scientific evidence due to a high heterogeneity among experimental conditions. In addition, a conventional-narrative review is also prone to subjectivity and publication bias. Therefore, utilizing meta-analysis as a method to aggregate publicly available papers is increasingly popular (Sauvant et al., 2020) because it requires authors to conduct rigorous article selection based on a specific protocol to reduce publication bias using a robust statistical method. As the publication on the utilization of black cumin seed in small ruminant animals continuously increased, the present meta-analysis aimed to summarize the relationship of dietary BCS levels on growth performance, nutrient digestibility, blood metabolites, and immune status of small ruminants.

2. Materials and methods

2.1. Literature search

A dataset was constructed using studies reporting the use of Nigella seeds in the lambs, sheep, and goat diets. The articles included were obtained from online scientific platforms Science Direct, PubMed central, and Google scholar. Several terms were employed to generate the data, including "lambs", "sheep", "goat", "Nigella sativa", "black seed", and "performance". In this initial stage, all results were transferred to the reference manager for selection purposes.

2.2. Inclusion criteria and selection process

The selection of generated-papers was conducted to assure that the quality of the paper complies with the criteria that had previously been determined. In the reference manager, we removed those articles with inappropriate titles and duplicated articles. In this step, a total of 52 papers were suitable according to the title and abstract and were retained for further selection. Eligibility of studies was selected following the PRISMA-P (Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols) (Liberati et al., 2009) according to the following criteria: (1) the study is published in a reputable journal in the English language with a report of an ethical clearance; (2) the study reported clear methodology such as experimental design, diet composition and specification, replication, and details of animals used for the experiment; (3) The study explicitly described the type and level of black cumin seed used; (3) the study reported biological effect on animals at least the performance variables such as average daily gain or growth rate and feed intake, or allow to calculate these variables. Finally, we selected 23 studies to be included in the database and removed 19 papers because these did not meet the criteria. Detail for the selection process is presented in the PRISMA-P flowchart in Fig. 1 and studies included in this meta-analysis are presented in Table 1.

Information about the authors, year, country, diet composition, experimental period, level, and type of black cumin seed as well as all variable outcomes available in the paper were extracted in the Microsoft excel datasheet. Data were arranged with each column representing response variables and each row representing the treatment. Variable outcomes listed in the database were productive performance (average daily gain, ADG; daily feed intake, DFI; feed conversion ratio, FCR),

Table 1
Studies selected to be included in the meta-analysis.

Study	Reference	Levels (% in DM Basis)	Animal	Breed	Design	Type of Diet
1	(Obeidat, 2020)	0, 15	Lamb	Awassi	RBD	TMR
2	(Abdullah and Farghaly, 2019)	0, 7, 14	Lamb	Farafra	RBD	TMR
3	(Hassan and Hassan, 2009)	0, 0.75	Lamb	Karadi	RBD	TMR
4	(Cherif et al., 2018a)	0, 1.2	Lamb	Barbarine	RBD Factorial	TMR
5	(Cherif et al., 2018b)	0, 1.2	Lamb	Barbarine	RBD Factorial	TMR
6	(El-Hawy et al., 2018)	0, 13, 25	Sheep	Barki	RBD	TMR
7	(Odhaib et al., 2018a)	0, 1	Lamb	Dorper	RBD	TMR
8	(Odhaib et al., 2018b)	0, 1	Lamb	Dorper	RBD	TMR
9	(Retnani et al., 2019)	0, 10, 20	Lamb	Domestic	RBD	TMR
10	(Aqil et al., 2017)	0, 0.5	Goat	–	RBD	TMR
11	(Alragubi, 2017)	0, 5, 10	Sheep	Barki	RBD	TMR
12	(El-Basiony et al., 2015)	0, 0.64	Goat	Damascus	RBD	TMR
13	(Abdalla et al., 2015)	0, 13, 25	Sheep	Barki	RBD	TMR
14	(Abd-El Moty et al., 2015)	0, 0.02	Lamb	Ossimi	RBD	TMR
15	(Mahmoud and Bendary, 2014)	0, 12.5	Lamb	Barki	RBD	TMR
16	(El-Far et al., 2014)	0, 0.15	Sheep	–	RBD	TMR
17	(Nanda et al., 2013)	0, 0.25	Goat	–	RBD	TMR
18	(Zanouny et al., 2013)	0, 0.23, 0.46	Lamb	Ossimi	RBD	TMR
19	(Habeeb and El-Tarabany, 2012)	0, 0.2	Goat	Zaraibi	RBD	TMR
20	(Hassan et al., 2011)	0, 0.75	Lamb	Karadi	RBD Factorial	TMR
21	(El-Ghousein, 2010)	0, 0.58	Sheep	Awassi	RBD	TMR
22	(Hassan et al., 2010)	0, 0.75	Lamb	Karadi	RBD Factorial	TMR
23	(Jain and Sahni, 2010)	0, 0.05	Goat	–	RBD	TMR

Exp, experiment; RBD, randomized block design; TMR, total mixed ration.

nutrient digestibility and nitrogen metabolism, blood metabolite compositions, and immune responses (Immunoglobulin A, IgA; Immunoglobulin g, IgG). In the spreadsheet, reports of measurement units were transformed into the same units to allow the calculation purposes.

2.3. Statistical analysis

The meta-analysis was performed following the linear mixed models methodology (Sauvant et al., 2008; St-Pierre, 2001) where the "study" was declared as random effects and the term "level" of dietary supplementation of Nigella seeds was set as fixed effects. The assessment of black cumin seeds concentration in the diets was conducted by employing the following mathematical models:

$$Y_{ai} = \beta_0 + (\beta_1 \times A)X_i + A_{0a} + (A_1S_0)_{ai} + A_3{*}X_i + e_{ai} \quad (1)$$

$$Y_{ai} = \beta_0 + \beta_1X_i + \beta_1X_i^2 + A_{0a} + (A_1S_0)_{ai} + A_3{*}X_i + e_{ai} \quad (2)$$

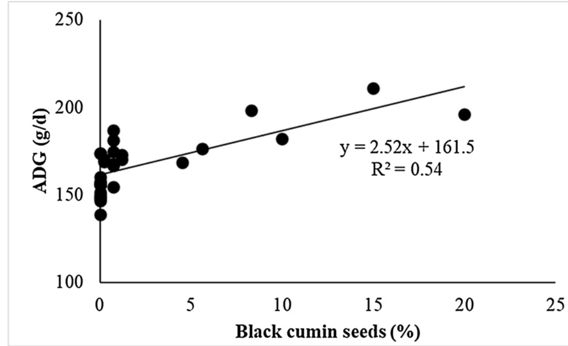

Fig. 2. Relationship between levels of dietary black cumin seed supplementation (% DM) on average daily gain of lambs (g/day) based on the results of the meta-analysis (n= 30; $p < 0.01$; RMSE = 1.071; $R^2 = 0.538$).

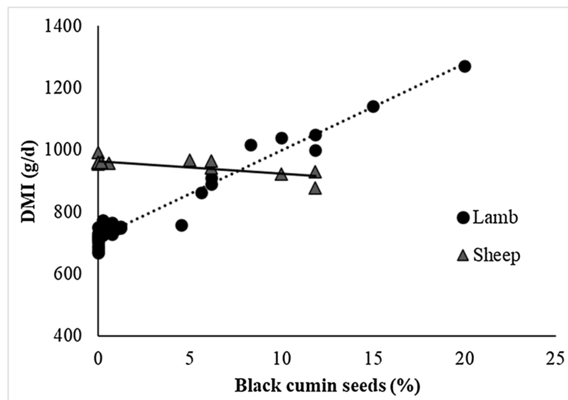

Fig. 3. Relationship between levels of dietary black cumin seed supplementation (% DM) on dry matter intakes (g/day) (● = lamb; ▲ = sheep) based on the results of the meta-analysis. Equation for lamb (n = 30): DMI (g/d) = 28.02x + 717.9 ($p < 0.05$; $R^2 = 0.958$), and for sheep (n = 6): DMI (g/d) = -4.125x + 964.5 ($P > 0.05$; $R^2 = 0.5803$).

Where the first model (1) is linear mixed model form while the second (2) is a quadratic mixed model. Fixed effects components are $\beta_0 + \beta_1X_i$ (order 1) and $\beta_0 + \beta_1X_i + \beta_1X_i^2$ (order 2), respectively while the random effects are $A_{0a} + (A_1S_0)_{ai}$. Y_{ai} = response variable, β_0 = overall intercept, A_{0a} = random effect of the a th study, $(A_1S_0)_{ai}$ = random interaction between the a th study and the ith levels, β_1 = coefficient of linear regression of Y on X (fixed effect), X_i = value of the continuous predictor (levels of BCS supplementation), $(\beta_1 \times A)X_i$ = overall regression coefficient [slope (β1)] for the extension of Y_{ai} change as affected by the concentrate proportion in the diets, $A_3 * X_i$ interaction beetwen animal anad level, and e_{ai} = the unexplained residual error.

Data for all response variables were weighted by the number of animals used in each experiment using a command “weights” in the “nlme” library. Levene’s test was performed to identify the heterogeneity between-studies of the variants (Fox and Weisberg, 2019). We recognized that animal species and types of diets among experiments may interfere and/or interact with BCS inclusion output. Thus, we performed meta-regression analysis to test the effects of animal species and concentrate levels as covariates. Interactions between BCS supplementary levels and animal species as well as with concentrate proportion (level × animal; level × concentrate) are presented in the Tables 3–5. The relationships between BCS inclusion levels with ADG and DMI are visualized using the Y adjusted value as described by St-Pierre (2001) and are presented in Figs. 2 and 3.

Statistical models were summarized by p-value, root mean square errors (RMSE), and conditional r-squared value (Nakagawa et al., 2017).

Table 2
Descriptive statistic of the database.

Response variable	Unit	Mean	SD	Max.	Min.
Level of black cumin seed (BCS)	g/Kg of feed as DM Basis	16.9	38.7	200	0
Ration					
Concentrate	%	57.3	17.3	75	24.1
Forage and roughage	%	42.7	16.9	76	25.0
Chemical composition of black cumin seed					
Dry matter	%	91.6	1.08	92.6	88.6
Ash	% DM	3.84	1.48	8.43	2
Organic matter	% DM	96.2	1.48	98	91.6
Crude protein	% DM	23.3	7.07	33.1	7.5
Ether extract	% DM	9.67	2.32	12.7	4.7
Crude fiber	% DM	9.84	4.45	19.9	6.6
Nitrogen free extract	% DM	52.0	11.6	67.9	34.8
Neutral detergent fiber	% DM	42.7	12	55.1	22.8
Acid detergent fiber	% DM	23.7	5.95	29.3	11.4
Hemicellulose	% DM	19.0	6.33	25.8	11.4
Chemical composition of total mixed ration					
Dry matter	%	92.3	2.28	97.1	89.9
Ash	% DM	8.68	3.26	14.6	5.17
Organic matter	% DM	91.3	3.26	94.8	85.4
Crude protein	% DM	15.2	2.92	22	9.30
Ether extract	% DM	4.29	2.15	9.02	1.90
Crude fiber	% DM	12.1	6.82	21.2	3.07
Nitrogen free extract	% DM	60.6	8.64	70	39.6
Neutral detergent fiber	% DM	42.2	7.36	55.7	29.3
Acid detergent fiber	% DM	17.6	6.44	27.4	6.98
Cellulose	% DM	15.5	0.0577	15.5	15.4
Hemicellulose	% DM	24.6	9.87	39.6	9.30
Lignin	% DM	5.31	1.42	7.31	3.94
Metabolizable energy	Mcal/Kg	2.74	0.313	3.05	2.04
Performance					
Average daily gain (ADG)	g/head/d	153	58.0	272	54.2
Average daily intake (ADI)	g/head/d as DM Basis	1,180	381	2,188	443
Feed conversion ratio (FCR)		7.37	1.92	12.7	3.9
Nutrient digestibility and nitrogen metabolism					
Dry matter	%	67.8	7.71	79.2	56.6
Organic matter	% DM	67.0	7.96	80.3	55.8
Crude protein	% DM	68.9	7.58	78.1	50.6
Ether extract	% DM	65.9	12.2	85.6	39.0
Nitrogen free extract	% DM	71.9	7.98	80.1	62.3
Neutral detergent fiber	% DM	66.3	6.04	72.8	56.8
Acid detergent fiber	% DM	55.1	6.55	60.7	43.8
Nitrogen intake	g/d	20.1	7.69	34.2	11.2
Nitrogen in faces	g/d	5.52	0.98	6.9	4.18
Nitrogen in urine	g/d	5.44	4.17	9.7	0.05
Nitrogen digested	g/d	14.8	6.81	27.3	7.8
Nitrogen retention	g/d	9.33	5.52	19.5	3
Blood metabolites composition					
Albumin	g/dL	3.26	0.653	4.85	2.21
Globulin	g/dL	3.38	1.46	5.89	0.72
Albumin/globulin ratio	g/dL	1.52	1.48	5.55	0.58
Blood urea nitrogen	mg/dL	55.5	31.8	111	12.3
Cholesterol	mg/dL	98.0	55.5	187	29.5
Total protein	g/dL	6.09	1.41	9.85	4.05
Creatinine	mg/dL	0.85	0.62	2.83	0.49
Glucose	mg/dL	59.0	21.1	82.9	17.7
Triglyceride	mg/dL	70.7	48.5	177	12.3

SD, Standard deviation; Max., Maximum; Min., Minimum.

Significance was declared when the p-value was less than 0.05 while a tendency was declared at a p-value of between 0.05 and 0.10. Data were analyzed according to the script developed by R statistical software version 3.6.3 with the “nlme” library (Pinheiro et al., 2021).

3. Results

3.1. Productive performance

The effect of dietary levels of *Nigella sativa* seeds on the growth performance of small ruminants is displayed in Table 3. Meta-regression analysis suggested that there was no interaction effect between BCS inclusion levels and animal species on ADG and FCR ($p > 0.05$). The interaction between BCS inclusion levels and concentrate proportion in the diets was also not significant. However, interaction between BCS levels and animal species tended to be significant on dry matter intake (DMI) ($p < 0.10$). In lambs, increasing levels of BCS in the diets showed a moderate linear increased on ADG (Fig. 2, $p < 0.01$, $R^2 = 0.538$) and substantial increased on DMI (Fig. 3, $p < 0.01$, $R^2 = 0.958$). However, BCS levels had no effect on sheep for DMI (Fig. 3).

3.2. Nutrient digestibility and nitrogen metabolism

All response variables associated with nutrient digestibility and nitrogen metabolism are presented in Table 4. No interaction effect was observed between BCS inclusion and animal species and between BCS inclusion and concentrate proportion ($p > 0.10$) in regard to nutrient digestibility and nitrogen metabolism. Positive effects of increasing BCS level in the small ruminant diets were observed on the increase of dry matter (DM) digestibility ($p < 0.05$) and a tendency to increase the organic matter (OM), ether extract (EE), and crude protein (CP) digestibility ($p < 0.10$). It was found that increasing dietary inclusion of BCS did not associate with changes in fiber fractions of neutral detergent fiber (NDF) ($p > 0.10$). Regarding the effect on nitrogen (N) metabolism, a higher level of BCS was associated with a linear increase on N intake and digested N ($p < 0.05$) but N in feces and N in urine were not influenced ($p > 0.10$).

3.3. Blood metabolites

The relationship between BCS inclusion level and blood metabolites are summarized in Table 5. A higher level of BCS inclusion was associated with higher albumin concentration ($p < 0.05$). In addition to other metabolites, there was no relationship between levels of dietary BCS on globulin, blood urea nitrogen (BUN), cholesterol, creatinine, glucose, and triglyceride concentrations ($p > 0.10$). Increasing dietary BCS had strong relationship on immune status of small ruminants were observed on the increased the concentration of IgA ($R^2 = 0.922$) and IgG ($R^2 = 0.939$) ($p < 0.05$) although the sample sizes for these outcomes are small.

4. Discussion

4.1. Performance and nutrient utilization

The present meta-analysis summarized that feeding Nigella seeds improved the productive traits of small ruminants, especially in lambs because they are in the growing period. This could be attributed to the role of phytochemicals composition and protein level from the composition of the seeds. Black cumin seed used in the present meta-analysis contains 23.3 % crude protein (CP) on average (Table 2). There have been strong evidence from previous studies that feeding functional seeds rich in phenolic compounds is beneficial both in ruminants and poultry (Lillehoj et al., 2018). Several modes of action regarding their positive effects as growth promoter have been provided, including their role to modulate rumen fermentation and microbiota, enhance enzyme secretion, nutrients digestion and absorption as well as improve immunity (Costa et al., 2021; Griss et al., 2020; Patra and Yu, 2012).

A number of phytochemical groups from Nigella seeds have been reported. Odhaib et al. (2018b) reported that black cumin seeds contained 35–37 mg/g tannins and non-tannins phenols to be supplemented

Table 3
Regression equation of the relationship between dietary levels of black cumin seed (g/kg DM) on performance of small ruminant animals.

Response variables	Animal	M	N	Parameter estimates				Model estimates					Levene's test[4]
				β0		β1		BCS[1]	BCS vs Animal[2]	BCS vs CON[3]	RMSE	R^2*	
				Value	SE	Value	SE						
ADG, g/d	Lamb	L	30	161.5	13.05	2.52	0.793	0.005	0.690	0.246	1.071	0.538	0.519
DMI, g/d	Overall	L	36	889	345	21.65	0.189	0.004	0.081	0.313	1.203	0.921	0.383
	Lamb		30	717.9	344	28.02	1.426						
	Sheep		6	964.5	403	−4.125	4.871						
Feed conversion	Lamb	L	32	7.42	0.482	−0.0048	0.0044	0.292	0.100	0.158	1.211	0.172	0.542

M, Model (L = linear); N, Number of data; Q, Quadratic; SE, Standard error; β_0 = intercept; β_1 = slope for level of BCS.
1 p-value of the effect of BCS inclusion level.
2 p-value for the interaction effect between BCS inclusion levels and animal species.
3 p-value for the interaction effect between BCS inclusion levels and concentrate proportion in the diets.
4 p-value of Levene's test for equality of variances, p > 0.05 indicates equal population variances (Fox and Weisberg, 2019).
* R^2 = The conditional r-squared value of the mixed effect model according to Nakagawa et al. (2017).

Table 4
Regression equation of the relationship between dietary levels of black cumin seed (g/kg DM) on nutrient digestibility and nitrogen metabolism of small ruminant animals.

Response variables	Animal	M	N	Parameter estimates				Model estimates				R^2*	Levene's test[4]
				β0		β1		BCS[1]	BCS vs Animal[2]	BCS vs CON[3]	RMSE		
				Value	SE	Value	SE						
Digestibility													
Dry matter	All	L	17	66.1	2.61	0.066	0.0291	0.0522	0.850	0.452	1.031	0.717	0.400
	Lamb		15	58.5	6.16	0.169	0.1139						
Organic matter, %	Lamb	L	15	65.5	2.74	0.103	0.0506	0.0812	0.966	0.947	1.025	0.774	0.910
Crude protein, %	Lamb	L	17	67.5	2.37	0.0548	0.025	0.06	0.931	0.316	1.203	0.793	0.878
Ether extract, %	Lamb	L	13	62.7	3.81	0.116	0.0625	0.114	0.630	0.423	1.138	0.420	0.721
Neutral detergent fiber, %	Lamb	L	10	66.7	3.51	−0.0238	0.0257	0.406	–	0.849	0.887	0.775	0.656
N utilization													
N intake, g/d	All	L	41	17.7	4.46	0.0105	0.0037	0.0097	0.269	0.716	1.457	0.960	0.709
	Lamb		23	22.8	4.22	0.278	0.264						
	Sheep		12	17.6	7.51	−0.099	0.103						
	Goat		6	15.8	0.53	0.004	0.011						
N in feces, g/d	Lamb	L	9	5.76	0.562	−0.0025	0.0016	0.1964	–	0.255	0.735	0.921	0.564
N in urine, g/d	Lamb	L	9	6.34	2.08	−0.0051	0.003	0.1648	–	0.660	0.700	0.983	0.555
N digested, g/d	Lamb	L	9	14.3	3.68	0.0207	0.0034	0.0036	–	0.916	0.833	0.994	0.641
N retention, g/d	Lamb	Q	9	7.79	2.55	0.0526 −0.000149	0.0029 0.000015	0.0004 0.0024	–	0.647	0.586	0.991	0.753

L, Linear; M, Model; N, Number of data; Q, Quadratic; SE, Standard error; β_0 = intercept; β_1 = slope for level of BCS.
1 p-value of the effect of BCS inclusion level.
2 p-value for the interaction effect between BCS inclusion levels and animal species.
3 p-value for the interaction effect between BCS inclusion levels and concentrate proportion in the diets.
4 p-value of Levene's test for equality of variances, p > 0.05 indicates equal population variances (Fox and Weisberg, 2019).
* R^2 = The conditional r-squared value of the mixed effect model according to Nakagawa et al. (2017).

in the lamb diets. In addition, the most current review summarized that BCS contained several groups of chemical constituents: essential oils (terpenes group), alkaloids, saponins, flavonoids, amino acids, and trace minerals (Ahmad et al., 2021) where they all benefit small ruminant health and production (Lillehoj et al., 2018). Studies in lambs showed that administration with BCS consistently improved growth rate and nitrogen metabolism either in a high concentrate diet or low concentrate diet (Cherif et al., 2018b; Majdoub-Mathlouthi et al., 2013).

Several reasons can explain their positive effect on the growth of lambs. First, it could be attributed to the nutrient use efficiency as confirmed in this study whereas BCS inclusion increased the DM intake and digested N. Previous studies also reported that dietary BCS in small amount improved appetite and increased nutrient utilization in lambs (Habeeb and El-Tarabany, 2012). This was confirmed in the present meta-analysis where significant increased on DMI was found in lambs. Moreover, additional contribution on the increase of growth rate could also be associated with the nutritional profile of Nigella seeds which contained a high amount of polyunsaturated fatty acids such as linolenic, oleic, and linoleic acids as an energy source (Ramakrishna Rao et al., 2003). These explanations might be more acceptable regarding the positive effects showing in this meta-analysis rather than explaining the effect of phytochemical contents, particularly essential oils. A recent meta-analysis summarizing essential oils' effects on small ruminants failed to show any advantages (Torres et al., 2020). Thus, the present meta-analysis suggests feeding whole black cumin seeds is more promising to stimulate the higher growth performance of small ruminant livestock because they are more nutritious.

4.2. Blood biochemical parameters

The present meta-analysis confirmed previous evidence both in humans and animals that Nigella seed supplementation could stimulate immune response as shown in increasing IgA and IgG concentrations without any adverse effect on blood metabolites. This immunostimulatory effect could be ascribed to its antioxidant, antibacterial, and anti-inflammatory properties (Ahmad et al., 2021). Numerous studies

Table 5
Regression equation of the relationship between dietary levels of black cumin seed (g/kg DM) on blood metabolites and immune response of small ruminant animals.

Response variables	Animal	M	N	Parameter estimates				Model estimates					Levene's test[4]
				β0		β1		BCS[1]	BCS vs Animal[2]	BCS vs CON[3]	RMSE	R^2*	
				Value	SE	Value	SE						
Albumin, g/dL	All	L	28	3.27	0.167	0.00195	0.00374	0.009	0.330	0.986	0.582	0.593	0.375
	Lamb		10	3.04	1.595	0.0047	0.1595						
	Sheep		6	4.01	1.062	−0.001	0.016						
	Goat		12	3.77	1.845	−0.118	0.886						
Globulin, g/dL	All	L	20	3.22	0.755	0.0052	0.00408	0.235	0.362	0.158	0.927	0.582	0.489
Albumin/globulin ratio	All	L	20	1.67	0.847	−0.000681	0.00721	0.926	0.645	0.834	0.743	0.927	0.985
BUN, mg/dL	All	L	24	57.4	9.96	0.0447	0.0382	0.884	0.148	0.924	0.964	0.743	0.865
Cholesterol, mg/dL	All	L	19	109	30.5	−0.2	0.0957	0.062	0.303	0.196	0.971	0.964	0.396
	Lamb		10	41	23.47	7.82	4.65						
	Sheep		5	110	65.46	0.411	0.957						
	Goat		4	151	58.28	−3.09	13.59						
Total protein, g/dL	All	L	32	5.93	0.366	0.0319	0.0142	0.114	0.554	0.658	0.883	0.971	0.188
Creatinine, mg/dL	All	L	15	0.851	0.294	0.000574	0.0035	0.874	0.060	–	0.781	0.883	0.974
Glucose, mg/dL	All	L	30	56.3	14.7	0.0283	0.0296	0.354	0.643	0.461	0.955	0.781	0.508
Triglyceride, mg/dL	All	L	19	70.1	16.1	0.0068	0.0541	0.902	0.485	0.219	0.967	0.955	0.661
IgA, mg/L	All	L	7	387	74.1	2.95	0.617	0.017	–	–	0.787	0.922	0.794
IgG, mg/L	All	L	7	28.7	6.32	0.209	0.024	0.003	–	–	0.696	0.939	0.939

L, Linear; M, Model; N, Number of data; Q, Quadratic; SE, Standard error; β_0 = intercept; β_1 = slope for level of BCS.
[1] p-value of the effect of BCS inclusion level.
[2] p-value for the interaction effect between BCS inclusion levels and animal species.
[3] p-value for the interaction effect between BCS inclusion levels and concentrate proportion in the diets.
[4] p-value of Levene's test for equality of variances, $p > 0.05$ indicates equal population variances (Fox and Weisberg, 2019).
* R^2 = The conditional r-squared value of the mixed effect model according to Nakagawa et al. (2017).

claimed that Nigella seeds and their extract had a strong antioxidant activity corresponded to their bioactive compounds (Bourgou et al., 2012; Sultan et al., 2012).

Thymoquinone (TQ), the most active constituent of black cumin seed, has shown an effective suppressing effect on oxidative stress by maintaining the glutathione (GSH) and nitric oxide (NO) levels (El Gendy et al., 2007). TQ also serves as a superoxide scavenger and free radicals as well as preserves major antioxidant enzymes such as glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD) (Ahmad et al., 2021). Additionally, they were involved in many metabolic pathways and effective to ameliorate many diseases due to their anti-inflammatory properties(Umar et al., 2012). Regarding their role as an immunomodulator, *Nigella sativa* has been reported to affect cellular immunity through T cells which could be promoted by reducing oxidative stress (Salem, 2005). Bioactive constituents of the seeds were reported to augment their immunomodulatory effect (Ahmad et al., 2021). Regarding this, (Odhaib et al., 2018a) found an increase in IgA, IgG, and IgM concentration on Dorper lambs fed dietary treatment with Nigella seeds. Other studies also reported that *Nigella sativa* supplementation positively altered immune response in ruminant animals (Gupta et al., 2016).

No effect of dietary Nigella seeds on some blood metabolite indices such as cholesterol, glucose, and triglycerides was similar with (Yalçin et al., 2012) who found that Nigella seeds did not alter blood serum parameters. Previous studies also suggested that using several plants rich in bioactive compounds is pro-performance and pro-health benefits without altering the hematological and biochemical indices of goat (Chaturvedi et al., 2013). Since blood parameters are an important indicator for the health status of animals, this finding implies that there is no detrimental effect associated with Nigella seeds supplementation. It was suggested that the inclusion of black cumin seeds in the diet is safe.

5. Conclusion

In conclusion, the present meta-analysis highlighted that growth performance, nutrients utilization, and metabolism of small ruminants' responses on dietary supplementation of Nigella seeds were linearly improved, especially for lambs. Nigella seeds could also be considered as immunomodulator as they can enhance IgA and IgG of the animals without adversely affecting blood metabolites parameters.

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CRediT authorship contribution statement

Sadarman: Conceptualization, Investigation, Methodology, Project administration, Resources, Validation, Writing - original draft. **Dewi Febrina:** Writing - original draft. **Yendraliza:** Resources, Writing - original draft. **Miftahush Shirothul Haq:** Resources, Writing - original draft. **Rizki Amalia Nurfitriani:** Resources. **Nisa Nurmilati Barkah:** Investigation, Methodology. **Muhammad Miftakhus Sholikin:** Conceptualization, Data curation, Formal analysis. **Yunilas:** Project administration. **Novia Qomariyah:** Resources. **Anuraga Jayanegara:** Supervision, Writing - review & editing. **Rondius Solfaine:** Writing - original draft. **Agung Irawan:** Conceptualization, Data curation, Validation, Writing - review & editing.

Declaration of Competing Interest

The authors report no declarations of interest.

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