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Research Article
Growth Performance, Blood Metabolites and Nitrogen Utilization of Lambs Fed with *Nigella sativa* Meal

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Abstract
Background and Objectives: *Nigella sativa* is the scientific name for black cumin or habbatussauda. *Nigella sativa* seeds contain oil commonly used for medicinal purposes to treat various diseases. *Nigella sativa* meal (NSM) is a by-product from the industry that extracts the oil. NSM contains a high protein content and can be used as a source of protein in the diet. The purpose of this research was to evaluate the effect of using NSM as a feed on the growth performance, metabolite and blood profile and nutrient digestibility of lambs. **Materials and Methods:** This study used a randomized block design with 5 replicates of 3 treatments using 15 local Indonesian male lambs. The concentrations of NSM in the rations were 0, 10 and 20%, with a maintenance period of 56 days. The forage to concentrate ratio was 30:70. **Results:** The average daily weight gain and blood urea nitrogen (BUN) concentration of the experimental animals were significantly ($p<0.05$) higher for the diets that contained 10 and 20% NSM (T1 and T2) compared to those for all other treatment groups. Additionally, the use of NSM in the diet improved the digestibility of crude protein, nitrogen retention and nitrogen use efficiency, with the mean biological value of protein being higher for the diet that contained NSM T1 (99.51) and T2 (99.44) compared to that for the control treatment group T0 (98.09). **Conclusion:** When the concentration of NSM reaches 20% in lamb rations, it has the most effect on growth performance and nutrient efficiency without affecting the metabolite and blood profiles, which remain in the normal range.

Key words: Black cumin, feed alternative, feed protein source, lamb rations, *Nigella sativa* meal

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Feed is one of the most important components of a farm. This is because feed is used to meet the nutrient needs for maintenance, growth, production and reproduction. The provision of the appropriate feed in accordance with the needs of the animal will produce animals with optimal productivity. Retnani *et al.*[1] stated that the main constraints of ruminant feed are low quality and low digestibility. In Indonesia, the availability of feed is currently constrained by the high price of feed ingredients, especially feed ingredients that are protein sources. The high demand for feed protein necessitates exploration to obtain alternative feed ingredient protein sources with a high nutrient content that are safely consumed by the animal, are cheap and do not compete with human consumption.

Nigella sativa, commonly called black cumin or black seed, contains 32-40% fixed oil, 0.4-0.45% volatile oil, 8-9 types of essential amino acids, vitamins and carbohydrates[2]. This oil has been used as a medicine for various diseases due to the presence of active compounds[3]. The *N. sativa* oil processing industry produces waste that has not been optimally utilized. *Nigella sativa* meal (NSM) is the residue from extracting the oil from the seeds of *N. sativa*. According to Wahyuni[4], in Indonesia, as many as 144 817 kg year^{-1} of *Nigella sativa* seeds are used as raw material in the *N. sativa* processing industry. Gokdogan *et al.*[5] stated that 70% of NSM is generated from the total raw materials.

NSM contains 90.12% dry matter, 8.94% ash and 37.40% crude protein[6]. According to Ali *et al.*[7], NSM generated from oil extracted by the cold press method contains 91.8% dry matter, 9.6% ash and 23.3% crude protein. In addition, NSM also contains a variety of macro and microminerals that can be utilized by animals. Cheikh-Rouhou *et al.*[8] stated that NSM contains the minerals Mg, Fe, Cu, Ca and K. The utilization of NSM as feed in Indonesia is still rare even though NSM contains nutrients that are able to support the growth of animals. Abdel-Magid *et al.*[6] stated that substituting 30% or 60% of the crude protein in a feed ration with NSM can increase average daily gain up to 10% compared with a control that did not contain NSM. Mansour *et al.*[9] recommend the use of NSM in calf feed because it can reduce the risk of diseases by strengthening the immune system that protects the body. The objectives of this research were to evaluate the effects of NSM inclusion in feed on the growth performance, metabolite and blood profile and nitrogen utilization of lambs.

MATERIALS AND METHODS

Experimental site: This research was conducted in the Department of Nutrition and Feed Science, Faculty of Animal Science, Bogor Agricultural University located in Bogor, West Java, Indonesia.

Climatic and weather conditions: The climate at the experimental site was tropical, with an average temperature and relative humidity (RH) in the morning, afternoon and evening during the study of 24.2°C and 87.2%, 30.4°C and 77.8% and 28.4°C and 78.2%, respectively. The average annual rainfall in this area was 3993 mm.

Experimental animals: Fifteen local male lambs below 5 months of age were divided into 5 groups based on initial body weight. The average initial body weight was 13.93±1.63 kg head^{-1}. The lambs were randomly assigned to 3 treatment groups designated T0, T1 and T2 comprising 5 replicates with 1 animal per replicate. The lambs were kept in individual pens for 77 days. Water was provided ad libitum and was given twice in the morning and in the evening. The animals were allowed to adapt to the new concentrate feed for 2 weeks. In the first 3 days of adaptation, a new concentrate was given to replace 25% of the previous concentrate. Then, the replacement increased every 3 days until the new concentrate replaced the whole of the previous concentrate.

Experimental feeds: Three experimental diets were prepared for this study. Rations P0, P1 and P2 contained 0, 10 and 20% NSM, respectively. The concentrate used in this research contained pollard, onggok (tapioca by products), coconut meal, NSM, molasses, $CaCO_3$ and a premix. The forage given was elephant grass obtained from the Field Garden Faculty of Animal Science, Bogor Agricultural University. The forage to concentrate ratio was 30:70. The feed material was weighed according to the formulation in Table 1 and mixed using a machine mixer (Bogor, West Java, Indonesia). Before all the ingredients were mixed, the NSM was first milled using a grinder (Bogor, West Java, Indonesia) with a diameter die of 6 mm. The mixed concentrate feed was packed into sacks and stored in a clean place. The nutrient content of NSM, the feed ingredients used and the concentrate are presented in Table 2-4, respectively.

Experimental design: The experimental design used was a randomized block design (RBD). Fifteen local Indonesian male lambs above 4 months of age and with an average weight of

13.93 kg were used for this study. The lambs were randomly assigned to 3 treatments designated T0, T1 and T2 comprising 5 replicates with 1 animal per replicate.

Table 1: Feed ingredients in each treatments

	Treatments[a]		
Raw material	T0	T1	T2
Elephant grass (%)	30.00	30.00	30.00
Pollard (%)	24.50	24.50	24.50
Onggok (tapioca by products)	13.65	17.85	22.05
Nigella sativa meal (%)	0.00	10.00	20.00
Coconut meal (%)	28.00	14.00	0.00
Molasses (%)	2.80	2.80	2.80
$CaCO_3$ (%)	0.70	0.70	0.70
Premix (%)	0.35	0.35	0.35

T0: 30% forage+70% concentrate (without NSM), T1: 30% forage+70% concentrate (10% NSM), T2: 30% forage+70% concentrate (20% NSM)

Table 2: Proximate analysis and level of thymoquinone in *Nigella sativa* meal

Nutrient contents (%)	Percentage[a]
Dry matter	92.300
Ash	8.100
Crude protein	29.880
Crude fat	5.370
Crude fiber	3.100
Non nitrogen extract	53.550
Thymoquinone content (%)	
Thymoquinone	0.015

[a]Analyzed by Laboratory of Research Center for Biological Resources and Biotechnology, IPB and Center Laboratory for Biopharmaceutical Studies, Bogor Agricultural University (2017)

Table 3: Nutrient content of concentrate in each treatments (100% dry matter)

	Treatment[a]		
Nutrient content[a] (%)	T0	T1	T2
Dry matter	91.64	90.80	89.92
Ash	6.13	7.52	9.42
Crude protein	9.30	12.73	15.69
Crude fat	9.02	6.15	2.61
Crude fiber	12.19	9.81	10.73
Non nitrogen extract	63.36	63.79	61.55

T0: 30% forage+70% concentrate (without NSM), T1: 30% forage+70% concentrate (10% NSM), T2: 30% forage+70% concentrate (20% NSM), [a]Analyzed by Laboratory of Research Center for Biological Resources and Biotechnology and Laboratory of Center for Biopharmaceutical Studies, Bogor Agricultural University (2017)

Feeding trial: The experimental lambs were fed at 4% of their body weight. The lambs were fed 4 times per day by performing a rotation between giving concentrate and forage. The remaining feed was weighed every morning to determine the amount of feed intake. Prior to data retrieval, an adaptation period was conducted for 14 days, followed by the study period in accordance with the treatments provided. Body weight was measured before the adaptation period and once a week during the experimental period.

Blood metabolite profiles: At the end of week 8, blood samples were taken from each lamb to analyze the blood metabolites and blood profiles. Five milliliters of blood was taken from the jugular vein with a 5 mL syringe; part of the neck was cleansed with 70% alcohol-treated cotton. The blood that was taken was inserted into a tube containing EDTA (ethylenediaminetetraacetic acid) anticoagulants. The blood was placed into a cooler and brought to the laboratory for metabolite and blood profile analyses. The blood metabolites that were measured were glucose, triglycerides and BUN, which were analyzed using Microlab 300 based on enzymatic reactions by the KIT method and measured using a spectrophotometer. The measurements of glucose and blood triglyceride levels were performed at a wavelength of 500 nm and the BUN measurements were performed at a wavelength of 578 nm. The blood hematological analysis measured the number of erythrocytes and leucocytes, leukocyte differentiation and hemoglobin levels according to the method of Sastradipradja *et al.*[10].

Digestibility trial: At week 9, 12 lambs were transferred to metabolic cages for the analysis of feed digestibility. Four experimental lambs were taken from each experimental treatment group at the termination of the growth study. Each lamb was placed in a metabolic pen that has been equipped with a container for urine and feces. The lambs were fed the same feed as the feed in the feed trial. Prior to urine and fecal data collection, a 2 week cage adaptation period was performed. Fecal collection was performed for seven days at

Table 4: Average nutrient intake of 15 Indonesian male local lambs in 56 days of research periods

	Treatments[a]		
Variables (g $head^{-1}$ day^{-1})	T0	T1	T2
Dry matter	707.30±100.98	851.32±60.81	864.57±149.09
Crude protein	67.44±9.21[b]	104.34±7.68[a]	125.30±21.30[a]
Crude fat	51.62±10.43[a]	46.00±3.65[a]	21.80±3.74[b]
Carbohydrate	531.70±76.58	627.31±44.77	628.23±108.44
Crude fiber	114.93±9.30	113.23±6.51	126.06±22.95
Non nitrogen extract	416.77±67.37	514.09±38.34	502.17±85.70

T0: 30% forage+70% concentrate (without NSM), T1: 30% forage+70% concentrate (10% NSM), T2: 30% forage+70% concentrate (20% NSM), [a]The value n the same row with different alphabet showed significantly different p<0.05 (Duncan's multiple tests) week 12. Fecal collection was performed once every hour by weighing the resulting feces to determine the fresh fecal weight. Then, as much as 10% of the total fresh feces collected each day was used as fecal samples that were dried using sunlight. When the feces were no longer wet, the feces were dried again in a 60°C oven for 24 h. The sample was weighed and then composited for subsequent analysis. The calculations for nutrient digestibility included dry matter, crude protein, crude fiber, crude fat, nonnitrogen extracted material and total digestible nutrients (TDN). Urine collection was performed for 7 days along with the collection of feces. Urine was collected using a container placed under the cage. Every day after the bucket was emptied, 10 mL of 10% H_2SO_4 was added to the bucket to prevent evaporation of urine N-NH_3. Then, 10% of the collected urine was used for analysis. The urine sample was stored in the freezer until analyzed. The calculations of nutrient digestibility and nitrogen retention were performed in accordance with McDonald *et al.*[11]. The concentrate, forage, and fecal samples were analyzed for dry matter, ash, crude fat, crude protein, crude fiber, and nitrogen extracts according to the AOAC method[12].

Statistical analysis: The data obtained from this study were statistically analyzed by using one-way ANOVA in SPSS v15.0. The results are expressed as the mean±standard deviation. Differences were considered significant at $p<0.05$. When significant differences were detected, the analysis was followed with Duncan's new multiple range test[13].

RESULTS AND DISCUSSION

Chemical composition of feed ingredients: The nutrient content of the NSM is listed in Table 2. NSM contains high crude protein, so NSM is suitable for use as an alternative source of feed protein. NSM also contains thymoquinone but in very low amounts. This indicates that the thymoquinone content is present in parts of the oil that have previously been extracted for industrial use. Additionally, the nutrient content of each concentrate used in this study is listed in Table 3. The concentrates had the same protein and energy content but the results from the subsequent analysis show the differences in the crude protein content among treatments. The addition of NSM to the feed replaced the use of coconut meal as one source of feed protein. The higher the level of NSM added to the feed, the higher the crude protein content was. This showed that NSM contains more crude protein than coconut meal. The high protein content of the feed has a good impact on growth performance.

Nigella sativa oil can be obtained through two methods, extraction using a hexane solvent or a pressing method. The NSM used in this study was obtained from extracting the oil from the *Nigella sativa* seeds using the pressing method. NSM has a fragrant odor and black color and it comes in the form of a slab. Table 3 shows that the NSM used in this study contained 29.88% crude protein (100% DM). The content of crude protein was different from the crude protein content mentioned by Ali *et al.*[7] (23.3%) and Abdel-Magid *et al.*[6] (37.4%). Table 4 shows the nutrient content of the concentrates containing NSM at different levels. The higher the NSM content used, the higher the crude protein content of the diet will be. NSM contains high crude protein. The *Nigella sativa* processing industry strives to keep a low temperature, (30°C maximum), for the extraction of *Nigella sativa* seeds. This is done to maintain the content of active substances such as thymoquinone that exist in *Nigella sativa* oil. This could be one cause for the greater stability of the crude protein content of *Nigella sativa* compared to that of other raw materials of protein feed sources.

Nutrient intake: The data in Table 4 shows that the use of NSM in the diet did not affect dry matter (DM) intake. Therefore, the use of NSM did not interfere with the palatability of the feed for the lambs. According to Retnani *et al.*[1], the limiting factors of ruminant feed were the low quality of the forage, low digestibility and level of palatability. Wibowo *et al.*[13] stated that palatability was related to DM intake because it attracted the lambs and stimulated the consumption of the feed they liked. The DM intake of the lambs in this study was in the range of 707-864 g head^{-1} day^{-1} and it met the DM intake that the NRC[14] recommended for 4-month-old lambs weighing 20 kg and with a weight gain of 100-150 g head^{-1} day^{-1} was 650-780 g head^{-1} day^{-1}. Abd El-Ghani[15] reported that the use of NSM significantly increased the average feed intake and body weight. As in the results of this study, the use of NSM did not increase the average DM intake but significantly increased the body weight of the lambs.

Body weight gain: The data in Table 5 indicates that the utilization of NSM in the diet had the most effect ($p<0.05$) on the daily body weight of lambs. The feed containing NSM resulted in higher body weight gains (126.35 g^{-1} day^{-1}) than did the control feed (103.81 g^{-1} day^{-1}). However, these body weight gains were lower than those obtained by Mahmoud and Bendary[16] who studied the use of NSM with crude protein at 14.85% in lambs, which resulted in an average daily weight gain of 174 g head^{-1} day^{-1}. This result occurred because the T1 and T2 feeds had a good quality and palatability, which caused total dry matter and feed protein intake to be higher

Table 5: Average body weight of the beginning, end and daily weight gain of 15 lambs for 56 days fed with NSM in different levels

	Treatments[a]		
Variables	T0	T1	T2
Initial body weight (kg head^{-1})	17.52±2.19	17.94±1.63	17.56±2.40
Final body weight (kg head^{-1})	24.06±2.52	25.02±2.35	25.52±3.39
Body weight gain (kg head^{-1})	6.50±0.59	7.10±1.05	8.00±1.13
Average Daily Gain (g head^{-1} day^{-1})	103.81±9.30[a]	112.38±16.70[b]	126.35±17.98[b]

T0: 30% forage+70% concentrate (without NSM), T1: 30% forage+70% concentrate (10% NSM), T2: 30% forage+70% concentrate (20% NSM), [a]The value on the same row with different alphabet show significantly different p<0.05 (Duncan's multiple tests)

Table 6: Average of blood metabolites from 15 Indonesian male local lambs fed with NSM in different levels

	Treatments			
Variables (mg dL^{-1})	T0	T1	T2	Normal level
Glucose	68.55±5.89	78.94±10.70	74.80±8.68	50-100[b]
Triglyceride	57.50±24.02	41.00±7.98	31.33±4.92	21-49[c]
BUN	12.25±3.52[c]	18.27±3.03[b]	24.25±3.05[a]	10-26[d]

T0: 30% forage+70% concentrate (without NSM), T1: 30% forage+70% concentrate (10% NSM), T2: 30% forage+70% concentrate (20% NSM), The value in the same row with different alphabet show significantly different p<0.05 (Duncan's multiple tests), [b]Kaneko (2014), [c]Mojabi (2000), [d]Kramer (2000)

Table 7: Average blood profiles from 15 Indonesian male local lambs fed with NSM in different levels

	Treatments[a]			
Variables	T0	T1	T2	Normal level[a]
Erythrocytes (million mm^{-3})	14.98±1.90	15.47±1.27	14.32±2.37	9-15
Hemoglobin (g%)	11.74±1.00	12.18±1.03	13.01±1.00	9-15
Leukocytes (thousand mm^{-3})	11.43±1.93	10.73±1.97	10.74±1.89	4-12

T0: 30% forage+70% concentrate (without NSM), T1: 30% forage+70% concentrate (10% NSM), T2: 30% forage+70% concentrate (20% NSM), [a]Kramer (2000)

than those in T0. According to the NRC[14], one of the factors that could influence weight gain is the total protein obtained daily from the feed consumed.

Blood metabolites: The data in Table 6 indicates that the use of NSM in the diet did not affect the blood glucose or blood triglyceride levels of the lambs. The blood glucose levels in this study were in the range of 68-78 mg dL^{-1} and this was around the normal range (50-100 mg dL^{-1}) according to the opinion of Kaneko[17]. When carbohydrates are consumed, they are digested by glycolytic enzymes into glucose. Blood glucose levels are regulated by insulin and glucagon, which are regulatory hormones, so that they remain stable. The lamb blood triglyceride level in the study was in the range of 31-57 mg dL^{-1} and this level was in the normal range (21-49 mg dL^{-1}) for lamb blood triglycerides[18]. According to Soehardi[19], the level of triglycerides in the blood is influenced by a high fat intake, which causes digested fat to be accompanied by a high intake of fat from outside the body. In addition, triglycerides in the blood are affected by the digestion of some feed ingredients (VFA, glucose, free fatty acids, amino acids and triglycerides) absorbed by the liver and converted into triglycerides[20]. Furthermore, these triglycerides enter the blood circulation or are stored in the liver or other tissues.

The use of NSM in the diet increased the BUN content of the local Indonesian lamb blood (p<0.05). The average nutrient intake in Table 4 shows that the use of NSM increased the crude protein intake. Coles[21] stated that the protein content in the feed affects the BUN content. The BUN data in Table 7 shows that the BUN concentration of lambs in this study was in the range of 12-24 mg dL^{-1} and according to Kramer[22] this was within the normal BUN range (10-26 mg dL^{-1}). In the rumen, crude protein is degraded into ammonia. Then, ammonia is used by rumen bacteria for microbial protein synthesis. Inside the liver, ammonia is converted to urea with the help of urea cycle enzymes[23]. Then, this urea is secreted into the blood and could be measured through BUN. The high levels of BUN in lambs fed NSM may be due to the crude protein contained in the NSM which is dominated by rumen degradable protein (RDP) or by non-protein nitrogen (NPN). This was in accordance with Wahjuni and Bijani[24], suggesting that increased protein metabolism will increase BUN levels. The increased NH_3-N concentration in the rumen would increase the concentration of BUN[25].

Blood profiles: The data in Table 7 indicates that the use of NSM in the diet had no effect on the amount of erythrocytes, hemoglobin and leukocyte in the blood of the lambs. This was in accordance with the opinion of Abd El-Halim *et al.*[26]

Table 8: Average intake of N, N feces, N urine, nitrogen retention, nitrogen utilization efficiency and biological value of N

	Treatments[a]		
Variables	T0	T1	T2
N intake (g head^{-1} day^{-1})	12.70±1.48	15.81±1.13	16.34±2.95
N feces (g head^{-1} day^{-1})	4.90±1.36	4.42±0.58	4.18±0.63
N urine (g head^{-1} day^{-1})	0.15±0.03[a]	0.05±0.05[b]	0.06±0.03[b]
N retention (g head^{-1} day^{-1})	7.65±0.64[b]	11.34±0.78[a]	12.10±2.33[a]
Nitrogen utilization efficiency (%)	60.58±6.54[b]	71.71±2.65[a]	72.92±1.51[a]
Biological value of N (%)	98.09±0.33[b]	99.51±3.71[a]	99.44±0.18[a]

T0: 30% forage+70% concentrate (without NSM), T1: 30% forage+70% concentrate (10% NSM), T2: 30% forage+70% concentrate (20% NSM), [a]The value in the same row with different alphabet show significantly different p<0.05 (Duncan's multiple tests)

who found that supplementing as much as a 47 g kg^{-1} concentration of *Nigella sativa* oil in lambs feed did not significantly affect the amount of erythrocytes in the blood. According to Astuti *et al.*[20], the amount of blood erythrocytes tends to remain the same due to homeostatic processes. When protein intake is reduced, the body will remodel the protein reserves to form erythrocytes and hemoglobin. This result indicated that the presence of NSM in the diet did not interfere with blood hemoglobin levels, so the function of blood metabolism, especially binding oxygen from the lungs and dispersing it to the whole body, was not disturbed. Leukocytes play a role in the cellular and humoral defense of organisms against foreign substances. The use of NSM did not affect the number of blood leukocytes and total blood leukocytes of lambs in this study, which was in the normal range (i.e., 4-12 thousand mm^{-3})[22]. Antibacterial use should enhance immunity by increasing the number of leukocytes as the body's active defense system. However, due to the low content of thymoquinone, which is an antibacterial compound, in the NSM used, there was only a 0.015% level of thymoquinone in it cause in effective work as an antibacterial.

Nitrogen utilization: The data in Table 8 shows that the use of NSM significantly affected (p<0.05) nitrogen levels in the urine, increasing the nitrogen retention, nitrogen use efficiency and biological value of the feed proteins. NSM has good quality crude protein that is easy to digest. Nitrogen retention is the amount of nitrogen retained in the body of the livestock and it is assumed that the remaining nitrogen is utilized by livestock. Muhtarudin[27] reported that nitrogen retention correlates positively with protein synthesis and that the retention value is an indicator of the quality of feed proteins. Nitrogen is used for protein synthesis in various organs of lambs. The nitrogen use efficiency is a method used to calculate the quality of the nitrogen/protein in the rations used; the more efficient the nitrogen is used by the body, the more the feed proteins can be used by the livestock[28]. The biological value of the protein is the percentage of the nitrogen in the body per the nitrogen digested. The biological value of the protein will determine how much protein can be absorbed and can be used by livestock. According to Piliang and Djojosoebagio[29], the biological value is an index of protein quality derived from food, reflecting the percentage of protein that is absorbed. The greater of the amount of protein that stays in the body, the greater the biological or protein quality is.

CONCLUSION

When the concentration of NSM reaches 20% in lamb rations, it has the most effect on growth performance and nutrient efficiency without affecting the metabolite and blood profiles, which remain in the normal range.

SIGNIFICANCE STATEMENT

This study found that NSM can be developed as an alternative feed protein source that can be used as a substitute for conventional feed protein. NSM contains a high concentration of crude protein and when NSM is included in lamb rations at 10% or 20%, it can support the growth performance of lambs and cause higher body weight gains diet without negatively affecting blood metabolites and feed digestibility.

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