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Research Article

Effect of Supplementation of *Nigella Sativa* Oil on Nutrient Digestibility, Some Blood Metabolites and Rumen Parameters in Karadi Lambs

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Abstract: To examine the effect of supplementation of varying levels of the oil of *Nigella sativa* in Karadi lambs rations on nutrients digestibility, blood metabolites, and some rumen parameters, 18 Karadi lambs were allocated into three groups, and the first group was fed a basal diet as control whereas, the second (T2) and the third (T3) groups fed the basal diet being supplemented with 0.15 and 0.30% of DM *Nigella sativa* oil (NSO) respectively. All animals were fed individually on 1.5 kg/lamb/day. Results showed that dry matter (DM), crude protein (CP), organic matter (OM), and crude fiber (CF) digestibility was not affected (P>0.05) by NSO supplementation. Also, supplementing NSO had no significant effect on serum total protein (TP), albumin (Alb), globulin (Glb), triglycerides (TG), cholesterol (Chol), high density lipoprotein (HDL), and very low-density lipoprotein (VLDL) concentrations. There was an increasing trend (P=0.07) in LDL concentration of lambs fed on T2 and T3 as compared to control. Neither treatment nor interaction between time and treatment had an effect on rumen fluid pH. A significant decrease (P=0.008) was noted in rumen fluid pH value with the advances of time post feeding. The ammonia-nitrogen concentration in rumen fluid was generally lower upon oil supplementation, and it was significantly (P=0.03) decreased in the T2 group at 4 hours following morning feeding. It can be concluded that supplementing with 0.15 and 0.3% /DM of NSO showed a reduction in rumen ammonia-nitrogen while it had no effects on nutrient digestibility and blood metabolites in Karadi lambs.

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1. Introduction

Nowadays, feeding natural additives is appraised as a crucial principle in healthy nutrition. The prohibition of using antibiotics as feed additives in animal nutrition because of their residues in animal products and the elevated level of consumers' awareness on the occurring hazards on health caused more research on safe and natural additives (Khamisabadi et al., 2016) due to improvement caused on animals'

health when used as additives in feeds. Several findings (Calsamiglia et al., 2007; Benchaar et al., 2008; Hart et al., 2008) suggested that adding plants or plant extracts containing many bioactive compounds to ruminant's diets might have a positive effect on ruminal fermentative status, thus supporting the degradation processes and mitigating ruminal methane (CH_4) formation (Calsamiglia et al., 2007; Benchaar et al., 2008; Hart et al., 2008). Essential oils (EO) are known as secondary plant metabolites that can be steam volatilized or extracted using organic solvents (Calsamiglia et al., 2007). Essential oils act as antimicrobial compounds against microbes, counting bacteria, protozoa, and fungi which could result in improved digestibility (Greathead. 2003). *Nigella sativa* (NS) is an annual herb belonging to *Ranunculacae* buttercup family and is globally regarded as an important medicinal plant because of its advantageous actions. It is considered as antibiotics' natural alternative for improving the health of animals and raising the quantitative and qualitative features of animal's products (Longato et al., 2015). It is demonstrated by El-Naggar et al., 2018 that adding oil of NS at 20g kg^1 DM to the diet of ruminants led to improve weight gain, feed conversion, digestibility of nutrients, and nitrogen balance. Similarly, Cherif et al., (2018) found that the addition of *Nigella Sativa* seeds to the concentrate (12g kg^1) significantly improved average daily gain and affected rumen fermentation by rising ammonia nitrogen (NH_3-N) concentration, reducing protozoa count. It is reported that biochemical and hematological measurements are critical indicators during diagnostics (Kaneko et al., 2008 and Nicoll et al., 2004). Furthermore, evaluating the state of metabolism in order to predict and prevent the occurrence of many diseases in critical periods circumstances (Celi et al., 2008). In this regard, Idris et al., (2014) found that the addition of 47g of NSO seeds/kg DM caused a significant rise in Cho, LDL, HDL levels, and live body weight. Since no sufficient information was found available through literature about the effects of NSO on nutrient digestibility, blood metabolites, and rumen parameters in Karadi ewes, therefore, this work pointed to investigate the impacts of dietary addition of NSO on nutrient digestibility, some parameters of rumen and blood in Karadi lambs.

2. Material and Methods

The experiment was performed following the procedure of the Institutional Committee on Animal Use Ethics (Approval No. AEC 06052021). This experiment was conducted at the farm of the Animal Production Department, College of Agricultural Engineering Science, University of Duhok. All procedures were approved by the ethics committee of the College of Agricultural Engineering Sciences, University of Duhok. Eighteen Karadi lambs, with an average body weight of 32.43±0.39 kg, were used and fed individually on three different diets at a rate of 1.5 kg/lamb/day. The concentrate diets consisted of 40.0 % ground barley, 20.0 % ground yellow corn, 15.0% soybean meal, 10.0 % wheat bran, 11.0% wheat straw, 1.5% vitamins and minerals mixture, 1.7 % limestone and 0.8 % salts. The diet composition is shown in Table 1. Three levels (0, 1.5, and 3g kg^1) of oil from NS (as extracted using a hydraulic press system by coaled pressing) were added to concentrate and considered as the three experimental groups; T1, T2, and T3, respectively. N. sativa oil was manually supplemented to the basal diet for the experimental groups. The lambs were individually kept in pens (1×2m) for 14 days as an adaptation period and then transferred to metabolic cages for 7 days as a samples collection period. Fresh drinking water was freely available during the entire experiment. Ruminal fluid was obtained on the 7^{th} day of collection period at 0 and 4 hrs. after morning meal via esophageal tube and suction strainer, and collected into bottles then filtered through a double thickness of cotton gauze and pH was recorded immediately using portable pH meter. Ammonia nitrogen was measured according to MAFF (1986) and kept under -18° C in plastic bottles for further analysis. The daily feed intake and fecal output were recorded, and fecal samples were collected on day 7 of the collection period in metabolism crates. The nutrient digestibility was determined as described by the method of McDonald et al., (2010). The blood samples were collected by draining through Jugular venipuncture into plastic tubes. The samples were refrigerated overnight and then centrifuged at 4500g for 10 minutes. The serum was separated and frozen at -18˚C for subsequent analysis.

2.1 Statistical analysis

The experimental results were analyzed by factorial one-way ANOVA by applying the package of Genstat Statistical Software (Genstat 20^{th} edition, VSN International Ltd, U.K.). All analyzed data

sets were normally distributed. The datasets were analyzed to compare between the three supplemental levels (0, 1.5, and 3g kg^{1}) of NSO experimental groups (T1, T2, and T3) for nutrient digestibility coefficients (%), blood metabolites (mg dl^{1}), NH_3-N (mg/dl) in rumen fluid, and ruminal fluid pH. Repeated measure ANOVA analyses of NH_3-N concentration and pH of rumen fluid datasets were also analyzed to compare between groups of treatment (T1, T2, and T3) during different times (hours) and the treatment x time interaction. Tukey test was used to compare different groups for all parameters. Differences were revealed as significant at $P<0.05$, and trends were revealed when P was between <0.1 and >0.05.

3. Results and Discussion

Table 1. Showed the composition of rations used in experiment

Nutrient%	DM	Crud Protein (CP)	Ether Extract (EE)	Ash	Crude Fiber (CF)	Nitrogen Free Extract (NFE) [1]	Metabolizable energy[2] (MJ Kg^{1} DM) (ME)
T1	93.96	13.01	4.25	8.05	19.77	48.88	11.80
T2	93.75	13.70	4.41	8.12	14.90	52.62	12.21
T3	93.85	13.38	4.21	8.03	16.77	51.46	12.02

Chemical analysis was carried out (on the basis of dry matter) at the nutrition lab. Animal Production Department.
[1]NFE%= 100- (Moisture + Ash + EE + CP + CF contents).
[2]ME was calculated according to MAFF,(1975) ME= (CP*0. 2+EE*0. 31+CF*0.05+NFE*0.14).

3.1. Nutrient digestibility

The effect of dietary oil supplementation of NSO on nutrient digestibility in Karadi lambs is shown in Table 2. Results of the present study revealed that nutrients digestibility of DM, OM, CP, and CF were not affected by NSO supplementation. Such results are in agreement with the findings of Khateri et al., (2017), who noted that the apparent digestibility of DM, CP, OM, and NDF was not influenced by the mixture of essential oil supplementation. Similarly, Khattab et al., (2011) concluded that adding black seed oil did not significantly affect nutrients digestibility in dairy buffaloes. Also, Metwally et al., (2015) found that in cows, the addition of EO caused no significant change to the digestibility of DM, CP, CF, and OM. However, Benchaar et al., (2006) revealed no change in apparent digestibility of DM, NDF, and CP in dairy cows consuming a diet supplemented with EO at 2 g/d. In contrast in sheep, El-Naggar et al., 2018 found that nutrients digestibility and rations nutritive value as supplemented with varying levels of NSO resulted a significant increase ($P<0.05$) in the DM, CP, OM and NFE digestibility. Results of nutrients digestibility in dairy goats fed the diet supplemented with 7.5 g *Nigella sativa*/head are also showing a significant ($P\leq0.05$) escalate in DM, EE digestibility (El-Basiony et al., 2015). Moreover, Klevenhusen et al., (2015) found that 50 mg supplementation of NSO tended to elevate DM and OM disappearance when compared with control as studied *in vitro*. Essential oils as bioactive compounds are complex plant metabolites with variable composition, so they differently affect the rumen microbial growth and activity depending on the dose rate of bioactive compounds and the chemical composition of the diet (Klevenhusen et al., 2012). Therefore, the difference in digestibility coefficients from the present experiment and that of the other studies may be attributed to the variation in essential oil composition as a bioactive compound and also due to the animal species and diet composition.

Table 2. Effect of feeding experimental diets on nutrients digestibility of Karadi lambs

Item	T1	T2	T3	SED	P-value
Nutrient digestibility %					
Dry Matter DM	65.45	67.65	75.00	6.88	0.36
Organic Matter OM	69.04	71.30	78.02	6.50	0.38
Crude Protein CP	62.53	64.79	72.53	6.80	0.32
Crude Fiber CF	64.05	55.81	70.49	8.43	0.25

T1: fed the basal diet. T2: fed the basal diet supplemented with 0.15% of DM *Nigella sativa* oil. T3: fed the basal diet supplemented with 0.30% of DM *Nigella sativa* oil.

3.2. Blood metabolites

The impacts of supplementing the *Nigella sativa* oil on serum blood metabolites are presented in Table (3). Results revealed that supplementing NSO did not affect the TP, Glb, and Alb concentrations in the serum of Karadi lambs. The result is in accordance with the findings shown by Khattab et al., (2011) who stated no effect (P≥0.05) of the NSO on plasma TP, Alb, and Glo concentrations in buffalo calves. Similarly, Abdullah and Farghaly, (2019) in lamb; and El-Hawy et al., (2018) in Barki ewes, observed that feeding *Nigella sativa* meal had no significant impact on concentrations of total TP, Glb, and Alb. These results were in contrast with observations of El-Saadany et al., (2008) who reported that *Nigella sativa* seeds supplementation significantly increased (P<0.05) TP and Glo concentration.

Table 3. Effect of feeding experimental diets on serum metabolites in Karadi lambs.

Parameters	T1	T2	T3	SED	P-value
Total Protein mg dl^{1}	6.39	6.07	6.36	0.48	0.76
Albumin mg dl^{1}	2.83	2.71	2.83	0.13	0.62
Globulin mg dl^{1}	3.56	3.53	3.54	0.42	0.86
Triglyceride mg dl^{1}	18.60	12.00	14.67	4.05	0.29
Cholesterol mg dl^{1}	45.00	46.33	41.00	6.26	0.68
High density lipoprotein HDL mg dl^{1}	24.67	22.50	19.33	3.98	0.42
Low density lipoprotein LDL mg dl^{1}	15.53^{a}	21.43^{b}	18.73^{ab}	0.42	0.07
Very low-density lipoprotein VLDL mg dl^{1}	4.80	2.40	2.93	1.18	0.13

^{a,b}: the difference between the values with different letters in the same raw is significant (P<0.05).
T1: fed the basal diet. T2: fed the basal diet supplemented with 0.15% of DM Nigella sativa oil. T3: fed the basal diet supplemented with 0.30% of DM Nigella sativa oil.

In the present study, NSO supplementation had no effect on serum TG concentration. This result resembled with those obtained by Khattab et al., (2011), who found that buffalo calves fed supplemented ration with NSO had no significant contrast (P>0.05) in TG concentrations. Similarly, Abdullah and Farghaly, (2019) reported that there were no significant deviations (P>0.05) in TG concentrations of lambs due to *Nigella sativa* meal diet. Also, El-Basiony et al., (2015) noted no significant effects on TG concentration when NS was added to the rations of lactating goats. In contrast, Idris et al. (2014) found that the mixing of 47g kg^{1} of NSO in the diets of sheep resulted in a significant (P<0.05) rise in serum triglycerides concentration. In this investigation, serum Chol concentration was not got affected by feeding of NSO. Similarly, Otaru et al., (2011) found that supplementation of palm oil until 16% in a concentrate had no effect on serum Chol of Red Sokoto goats. Also, Khattab et al., (2011) observed no significant effect of black seed oil supplementation on blood plasma Chol concentration of pregnant buffaloes. Unlike our findings, Habeeb and El-Tarabany, (2012) reported that supplementing the diet of growing Zaraibi goats with NS significantly decreased serum total Chol. Saleh, (2005), found that lactating ewes supplemented with NS seeds decreased (P<0.05) total plasma Chol as compared with ewes on a basal diet. This decline in the level of serum Chol could be related to low levels of thymoquinone and monounsaturated fatty acids when hepatocytes synthesize Chol (Padhye et al., 2008).

Serum HDL level was numerically decreased in groups fed NSO as compared to the control animals. This result is in concord with that revealed by (El-Essawy et al., 2019a and 2019b), who noted that plasma levels of HDL were not influenced by the addition of EO to the diet in Barki lambs and ewes; however, El-Hawy et al., (2018) noticed that feeding ration containing *Nigella sativa* meal in Barki ewes led to a significant lessen in HDL level comparing to control. There was a trend (P=0.07) in LDL concentration to be higher in lambs fed on T2 (21.43 mg dl^{1}) compared to the control. In sheep, Idris et al., (2014) showed that NSO, increased significantly (P<0.05) the HDL level and stated that this could be due to the biohydrogenation of the unsaturated fatty acids in the rumen together with a possible effect of the rumen atmosphere to the thymoquinone, which is the active ingredient of NS.

3.3. Rumen fluid

The rumen fluid pH values and ammonia-nitrogen concentrations in Karadi lambs fed black seed oil before the morning feeding and at 4 hrs. post morning feeding are given in Table 4. Neither treatment nor interaction between time and treatment had an effect on rumen fluid pH. A significant decrease (P=0.008) was noticed in rumen fluid pH value at post feeding compared with pre-feeding.

Table 4. Effect of feeding experimental diets on rumen fluid parameters in Karadi lambs

Parameter	Time	T1	T2	T3	SED	P value	Repeated Measures Analysis					
							SED			P value		
							Tr.	Time	Time*Tr	Tr.	Time	Time*Tr
pH												
Before feeding		6.72	6.72	6.72	0.02	0.97	0.02	0.01	0.03	0.97	0.008	0.97
4hrs post feeding		6.67	6.67	6.66	0.04	0.97						
NH_3-N (mg/dl)							5.10	3.89	6.98	0.03	0.95	0.97
Before feeding		37.4	25.1	25.7	7.55	0.22						
4hrs post feeding		38.2a	23.6b	25.8ab	6.36	0.08						

[a,b]: the difference between the values with different letters in the same raw is significant (P<0.1 and >0.05).
T1: fed the basal diet. T2: fed the basal diet supplemented with 0.15% of DM *Nigella sativa* oil. T3: fed the basal diet supplemented with 0.30% of DM *Nigella sativa* oil.

Time post feeding and interaction between time and treatment had no effect on ammonia-nitrogen concentration in rumen fluid, but there was a significant effect of treatment (P=0.03) as it was reduced to 23.6 mg dl^1 in the lambs fed a diet supplemented with 0.15% NSO as compared to those fed control diet (38.2 mg dl^1) (Figure1).

The results are in line with that of Klevenhusen et al., (2015), who found during an in vitro study that supplementing the rumen fluid with either 50 or 500 mg/l resulted in no change in rumen fluid pH, but it caused a significant reduction in NH_3-N. Also, it was reported by Mclntosh et al., (2003) that feeding essential oils caused a reduction in the deamination process in the rumen as a result of the inhibitory effect of EO on most cultures of ruminal bacteria. The same authors showed that *Clostridium sticklansii* and *Prevotella anaerobius,* which are considered hyper-ammonia producing bacterial species, remained sensitive to the inclusion of EO in the diet. In the current study, the decline in NH_3-N concentration may be associated to the suppressing impact of NSO on ammonia-producing bacteria, which might lead to a reduction in the rate of deamination in the rumen.

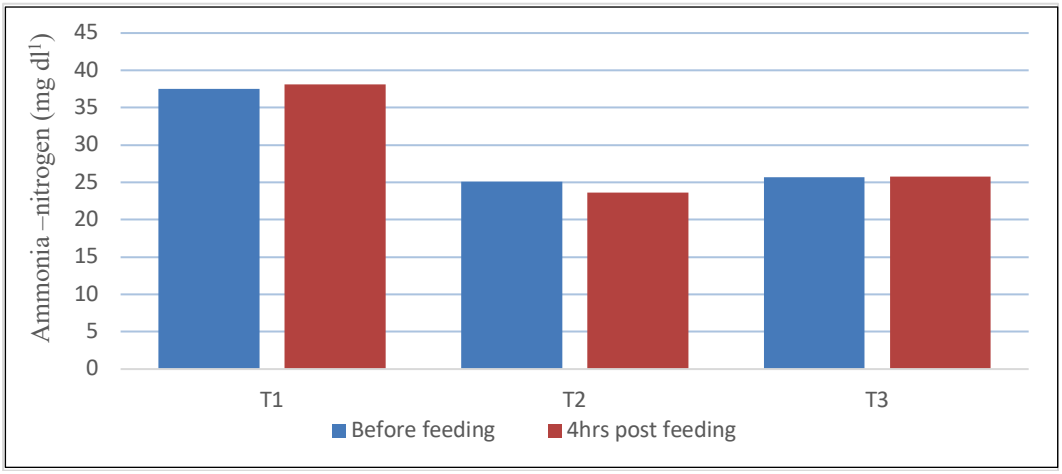

Figure1. Effect of feeding experimental diets on rumen NH_3-N in Karadi lambs.

Conclusion

With the exception of serum HDL concentration, supplementing with 0.15 and 0.30% /DM of NSO had no effects on nutrient digestibility or blood metabolites. *Nigella sativa* oil supplementation caused a reduction in rumen ammonia-nitrogen in Karadi lambs. Further research is required to study NSO supplementation on rumen function at wider time intervals.

References

Abdullah, M. A. M., & Farghaly, M. M. (2019). Impact of partial replacement of cottonseed meal by nigella sativa meal on nutrient digestibility, rumen fermentation, blood parameters, growth

performance of growing lambs. *Egyptian Journal of Nutrition and Feeds, 22*(2) Special Issue: 11-20. 10.21608/EJNF.2019.102283

Benchaar, C., Calsamiglia S., Chaves, A.V., Fraser, G. R., Colombatto, D., McAllister, T. A., & Beauchemin, K. A., (2008). A review of plant-derived essential oils in ruminant nutrition and production. *Animal Feed Science and Technology, 145*, 1–4,: 209-228. https://doi.org/10.1016/j.anifeedsci.2007.04.014

Benchaar, C., Petit, H.V., Berthiaume, R., Whyte, T.D., & Chouinard, P.Y., (2006). Effects of addition of essential oils and monensin premix on digestion, ruminal fermentation, Milk production, and Milk composition in dairy cows. *Journal of Dairy Science*, *89*:4352-64. DOI: 10.3168/jds.S0022-0302(06)72482-1

Calsamiglia, S., Busquet, M., Cardozo, P.W., Castillejos, L., and Ferret A. (2007). Invited Review: Essential Oils as Modifiers of Rumen Microbial Fermentation. *Journal of Dairy Science*, *90*(6). 2580–2595. https://doi.org/10.3168/jds.2006-644

Celi P., Trana, Di., A., & Quaranta, A. (2008) Metabolic profile and oxidative status in goats during the peripartum period. *Australian Journal of Experimental Agriculture, 48*, 1004-1008. https://www.publish.csiro.au/an/EA07410

Cherif, M., Ben Salem, H., & Abidi, S. (2018). Effect of the addition of Nigella sativa seeds to low or high concentrate diets on intake, digestion, blood metabolites, growth and carcass traits of Barbarine lamb. *Small Ruminant Research, 158*: 1–8. https://doi.org/10.1016/j.smallrumres.2017.11.008

El-Basiony, A. Z., Khattab, H. M., Kholif, A.M., Hadhoud, F. I. I., & El-Alamy, H. A. (2015). Effect of using echinacea purpurea, nigella sativa and chicorium intybus in dairy goats' diet on milk production and quality: 2- Effect on digestibility, some blood parameters and milk production and quality. *Egyptian Journal of Nutrition and Feeds*,*18*(2) Special Issue:137-145. DOI: 10.21608/ejnf.2015.104436

El-Essawy, A. M., Abdou, A. R., & EL- Gendy, M. H. (2019a). Impact of Anise, Clove, and Thyme essential oils as feed supplements on the productive performance and digestion of Barki ewes. *Australian Journal of Basic and Applied Sciences*, *13*(1): 1-13. DOI: 10.22587/ajbas.2019.13.6.1

El-Essawy, A. M., Abdou, A. R., Khattab, I. M., & Abdel-Wahed, A. M. (2019b). Effect of addition of anise, clove and thyme essential oils on Barki lambs' performance, digestibility, rumen fermentation, carcass characteristics and intramuscular fatty acids. *Egyptian Journal of Nutrition and Feeds, 22* (3): 465- 477. DOI: 10.21608/ejnf.2019.79415

El-Hawy, A. S., Abdalla, E. B., Gawish, H. A., Abdou, A. & Madany, M. E. (2018). Effects of alternative dietary protein of Nigella sativa on some hematological, biochemical and immunological responses of pregnant Barki ewes. *Australian Journal of Basic and Applied Sciences, 12*(7): 148-154. DOI:10.22587/ajbas.2018.12.7.22

El-Naggar, S., Abou-Ward, G. A., El-Badawi, A. Y. & Ali, A. M. (2018). Commercial oil of Nigella sativa as growth promoter in lambs rations. *Iraqi Journal of Veterinary Sciences, 32* (2) :199-204. 10.33899/ijvs.2019.153850

EL-Saadany, S. A., Habeeb, A. A. M., EL-Gohary, E.S., El-Deeb, M. M. & Aiad, K. M. (2008). Effect of supplementation of oregano or nigella sativa seeds to diet of lactating Zaraibi goats on milk yield and some physiological functions during summer season. *Egyptian Journal of Animal Production, 45*, Suppl. Issue, 469-487.DOI: 10.21608/ejap.2008.104560

Greathead, H. (2003). Plants and plant extracts for improving animal productivity. *Proceeding of the Nutrition Society, 62*(2): 279–290. https://doi.org/10.1079/PNS2002197

Habeeb, A. A. M., & El-Tarabany, A. A. (2012). Effect of Nigella sativa or Curcumin on Daily Body Weight Gain, Feed Intake and some Physiological Functions in Growing Zaraibi Goats during Hot Summer Season. *Journal Radiation Research Application Sciences, 5* (2):60:78.

Hart, K. J., Yanez-Ruiz, D. R., Duval S. M., McEwan. N. R., & Newbold, C. J. (2008). Plant extracts to manipulate rumen fermentation. *Animal Feed Science and Technology, 147*, 8–35.https://doi.org/10.1016/j.anifeedsci.2007.09.007

Idris, M. H., El-Bagir, M. N., & Al-Tayib, O. A. (2014). Effect of commercial oil of *Nigella sativa L.* seeds on lipids parameters and weight in sheep. *The Pharma Innovation Journal, 3*(7): 87-91.

Kaneko, J.J., Harvey, J.W., & Bruss M. L. (2008) *'Clinical Biochemistry of Domestic Animals*. Sixth Edition. (Academic Press: London).

Khamisabadi, H., Kafilzadeh, F. & Charaien, B. (2016). Effect of thyme (*Thymus vulgaris*) or peppermint (*Mentha piperita*) on performance, digestibility and blood metabolites of fattening Sanjabi lambs. *Biharean biologist*, *10* (2): 118-122.

Khateri, N., Azizi, O. & Jahani-Azizabadi, H. (2017). Effects of a specific blend of essential oils on apparent nutrient digestion, rumen fermentation and rumen microbial populations in sheep fed a 50:50 alfalfa hay: concentrate diet. *Asian-Australasian Journal of Animal Sciences, 30* (3):370-378. doi: 10.5713/ajas.15.0865

Khattab, H.M., El-Basiony, A. Z., Hamdy, S. M., & Marwan, A. A. (2011). Immune response and productive performance of dairy buffaloes and their offspring supplemented with black seed oil. *Iranian Journal of Applied Animal Science, 1* (4):227-234. http://ijas.iaurasht.ac.ir

Klevenhusen, F. D. K., Sizmaz, Ö., Wimmer, S., Muro-Reyes, A., Khiaosa-ard, R., Chizzola, R. & Zebeli, Q. (2015). Effects of black seed oil and Ferula elaeochytris supplementation on ruminal fermentation as tested in vitro with the rumen simulation technique (Rusitec). *Animal Production Scienc*e, 55 (6):736-744. http://dx.doi.org/10.1071/AN13332.

Klevenhusen, F., Muro-Reyes, A., Khiaosa-ard, R., Metzler-Zebeli, B. U. & Zebeli, Q. (2012). A meta-analysis of effects of chemical composition of incubated diet and bioactive compounds on in vitro ruminal fermentation. *Animal Feed Science and Technology, 176* (61-69).http://dx.doi.org/10.1016/j.anifeedsci.2012.07.008.

Longato, E., Meineri., G. & Peiretti, P. (2015) Nutritional and zootechnical aspects of Nigella sativa: A review. *Journal of Animal and Plant Sciences*, *25*, 921-934.

MAFF, (1986). *The analysis of agricultural materail* (3rd Ed). London, HMSO.

MAFF, (1975). Ministry of agriculture, fishery and food. *Energy allowances and feeding systems for Ruminants.* Technical Bulletin No. 33. HMSO, London.

McDonald, P., Edwards, R., Greenhalgh, J., Morgan, C., Sinclair, L., & Wilkinson, R. (2010). *Animal Nutrition.* Pearson, Gosport, UK.

McIntosh, F. M., Williams, P., Losa, R., Wallace, R. J., Beever, D. A. & Newbold, C. J. (2003). Effects of essential oils on ruminal microorganisms and their protein metabolism. *Applied and Environmental Microbiology, 69* (8), 5011-4. DOI: 10.1128/AEM.69.8.5011-5014.2003

Metwally, A., Deml, M., Fahn, C., & Windisch, W. (2015). Effects of a specific blend of essential oil on rumen degradability, total tract digestibility and fermentation characteristics in rumen fistulated cows. *Global Veterinarian, 15*(5):441-454. DOI:10.5829/idosi.gv.2015.15.05.101140

Nicoll, D., McPhee, S. & Pignone, M. (2004) *'Pocket Guide to Diagnostic Tests.*' 4th Edition. (McGraw-Hill: NY, USA).

Otaru, S. M., Adamu, A. M., Ehocheb, O. W. & Makun, H. J. (2011). Effects of varying the level of palm oil on feed intake, milk yield and composition and postpartum weight changes of Red Sokoto goats. *Small Ruminant Research, 96*: 25-35.https://doi.org/10.1016/j.smallrumres.2010.11.004

Padhye, S., Banerjee, S., Ahmad, A., Mohammad, R. & Sarkar, F.H. (2008) From here to eternity-the secret of Pharaohs: Therapeutic potential of black cumin seeds and beyond. *Cancer Therapeutics, 6, (b)*: 495-510.

Saleh, S. A. (2005). Effect of black seeds (*Nigella sativa*) supplementation on dairy ewes' performance. *Arab Journal of Nuclear Sciences and Application, 38*: 298-306.