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Effects of Nano Selenium on Some Metabolic and Rumen Parameters in Dorper Sheep

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ABSTRACT The aim of the study was to investigate the effects of nano selenium (N-Se) supplementation on some metabolik parameters and rumen fermentation in sheep. In the study, twenty female Dorper sheep, average 60.1±0.44 kg of body weight were used and blood samples and rumen fluid were taken at the end of trial. The animals were randomly divided into two trial groups (n=10). The control animals received the basal ration without Se supplementation, containing a native Se content of 0.06 mg/kg DM. The other sheep were fed the same basal ration supplemented with 3 g/head/day N-Se. Trial were 66 days with 10 days of adaptation to feed and 56 days of feeding period. Serum total protein (p=0.514) and albumin (p=0.126) levels did not change by feeding N-Se. Serum T-cholesterol, triglycerides, AST (p=0.001) and ALT (p=0.030) levels were decreased in the N-Se-treated group when compared with the control. Supplementation of N-Se did not effect ruminal pH values (p=0.792), the molar concentration of acetic acid (p=0.133) and butyric acid (p=0.089), but the ammonia concentration (p=0.001) was decreased, and total VFA concentration (p=0.003) was increased. The ratio of acetate to propionate decreased due to the increasing of propionate concentration (p=0.034). The obtained results indicated that N-Se supplementation positively improved ruminal fermentation and metabolic status. It was concluded that N-Se can be used as an alternatively available selenium source in sheep.

Keywords: *Dorper sheep, Nanomineral, Nano selenium, Rumen.*

ÖZ

Dorper Koyunlarında Bazı Metabolik ve Rumen Parametreleri Üzerine Nano Selenyumun Etkileri

Çalışmanın amacı koyunlarda nano selenyum (N-Se) ilavesinin bazı metabolik parametreler ve rumen fermantasyonu üzerindeki etkilerini araştırmaktır. Çalışmada canlı ağırlıkları ortalama 60.1±0.44 kg olan 20 adet dişi Dorper koyunu ve bu koyunlardan deneme sonunda alınan kan örnekleri ve rumen sıvısı kullanıldı. Hayvanlar rastgele iki deneme grubuna (n=10) ayrıldı. Kontrol hayvanları, Se takviyesi olmadan, 0,06 mg/kg DM'lik doğal Se içeriği içeren temel rasyonla, diğer koyunlar, 3 g/baş/gün N-Se ilaveli temel rasyonla beslendi. Deneme, 10 gün yeme adaptasyon ve 56 gün besi dönemi olmak üzere 66 gün sürdürüldü. Serum toplam protein (p=0.514) ve albümin (p=0.126) düzeyleri N-Se takviyesiyle değişmedi. Serum T-kolesterol, trigliseritler, AST (p=0.001) ve ALT (p=0.030) seviyeleri, N-Se ilave edilen grupta kontrol grubu ile karşılaştırıldığında azaldı. N-Se ilavesi ruminal pH değerlerini (p=0.792), asetik asit (p=0.133) ve bütirik asidin molar konsantrasyonunu (p=0.089) etkilemezken, amonyak konsantrasyonu (p=0.001) azaldı ve toplam VFA konsantrasyonu (p=0.003) arttı. Asetatın propiyonata oranı, propiyonat konsantrasyonunun artmasına bağlı olarak azaldı (p=0.034). Elde edilen sonuçlar, N-Se takviyesinin rumen fermantasyonunu ve metabolik durumu olumlu yönde iyileştirdiğini gösterdi. N-Se'nin koyunlarda alternatif bir selenyum kaynağı olarak kullanılabileceği sonucuna varıldı.

Anahtar Kelimeler: *Dorper koyunu, Nanomineral, Nano selenyum, Rumen.*

INTRODUCTION

Recently, nanominerals have been used especially as feed supplementation to have several benefits on health and to fulfill livestock from the mineral requirements. Researchers try to insert nanominerals using their benefits to aim towards the better health, nutrition and performance of animals. So far, researches have shown that the practise of nanominerals in the immunity, production, and reproduction is promising (Amini and Pirhajati 2018; Osama et al. 2020; Abdelnour et al. 2021; Delir et al. 2022). The nanoparticle form of minerals is to induce changes in the main material's fundamental physical and chemical structure (Singh and Prasad 2017). These are stated to be use the features of quite small

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doses, better bioavailability in metabolism, absorption, and stable connect with other compounds (Abd El-Hack et al. 2017; Uniyal et al. 2017). Nanominerals can act as functional into the animal body by increasing the surface area with biological connect, allowing nutritional compounds to remain in the gastrointestinal tract longer, permeating deeply into the mucosal area. They can enable to fast transportation of nutritional compounds to target tissue and organs (Fesseha et al. 2020; Reddy et al. 2020). So they minimize feces excretion, and can also be used as reducers of environmental pollution (Abdelnour et al. 2021).

The vital trace element selenium is required for diverse physiological and biological processes, especially including skeletal muscles and liver function (Perrone et al. 2016). In addition, selenium is a trace mineral widely used to regulate animal metabolism and some positive effects on rumen fermentation such as total volatile fatty acids (VFA), molar ratio of propionate, acetate-propionate ratio, ruminal ammonia (NH_3-N), pH, and enzymatic activity (Hendawy et al. 2022). In recent years, attention in nano selenium (N-Se), a form of selenium with much smaller particle size, compared to the most common dietary supplements of selenium known organic (selenomethionine, dimethylselenide, selenocystine, Se-methylselenocysteine) or inorganic (selenite, selenate, selenide, elemental selenium) forms has been increasing due to its beneficial effects on health and productivity (Chhabria and Desai 2016; Skalickova et al. 2017; Hendawy et al. 2022; Aljaf and Bolshakova 2023). It has higher bioavailability, catalytic activity, absorption ability, much lower toxicity related to various important characteristics such as particule size, different shape, chemical structure, and surface charge (Hosnedlova et al. 2018; Badgar and Prokisch 2020). Se nanoparticles as an immunostimulator have fascinated great attention due to their unique antimicrobial-antiviral-antioxidant activity, remarkable anticancer activity, in prevention of induced reproductive toxicity and metabolic disorders (Hosnedlova et al. 2018). Studies have been demonstrated on the impact of utilizing N-Se on blood parameters, immune system, renal and liver functions, and liver enzyme levels (Mahmoud et al. 2016; Qin et al. 2016; Surai and Kochish 2020; Bolshakova and Aljaf 2022;). According to the studies, N-Se may help in lowering the levels of liver enzymes like aspartate- aminotransferase (AST) and alanine-aminotransferase (ALT), which are signs of liver damage by increasing the activity of antioxidant enzymes (Shi et al. 2011b; Verstegen and Günther 2023). Moreover, it has been stated that N-Se possesses anti-inflammatory properties, which may aid minimize liver inflammation and improve liver function (Abdou and Sayed 2019; Aljaf and Bolshakova 2023). Supplementing with N-Se may also help to improve lipid profile in terms of cholesterol and triglycerides that could increase the risk of cardiovascular disease when their levels are high (Safdari-Rostamabad et al. 2017). It has been expressed that N-Se has antioxidant activity that may aid in the prevention of lipid peroxidation (Qin et al. 2016). Furthermore, according to Safdari-Rostamabad et al. (2017) N-Se effects the expression of genes associated with lipid metabolism, which may increase the lipid-lowering role of the matter. Information about the effects of N-Se on protein metabolism is quite limited. It is demonstrated that supplementing with N-Se may improve blood levels of proteins, especially albumin the protein most commonly produced by the liver. Albumin is necessary for moving various substances through the blood, like hormones and medications. An improvement in liver function and protein metabolism may be demonstrated by an increase in albumin levels after taking N-Se supplements (Aljaf and Bolshakova 2023). Shi et al. (2011b) stated that nano-Se addition fed (0.3 mg/kg body weight for 12 weeks) male goats Se content in blood, serum, and tissues was improved.

However, the metabolic effects of N-Se on liver function and enzyme levels, may differ depending on daily uptake, solubility, length of addition, other dietary components, age and health status. Additionally, it has been emphasized that the degree of effect of N-Se depends on the size, and the bioactivity and toxicity of selenium nanoparticles may be higher at 36 nm compared to 80 nm and above (Skalickova et al. 2017).

In feeding trials in small ruminants, it was stated that N-Se has a positive effect on rumen development, feed evaluation rate and nutrient digestibility (Shi et al. 2011a; Xun et al. 2012; Skalickova et al. 2017). This effect has been associated to their ability to modificate rumen fermentation, especially fiber digestion in ruminant (Badgar and Prokisch 2020). In some studies, on sheep, it has been suggested that the acetic acid profile in the rumen shifts towards propionic acid with nano selenium supplementation (Shi et al. 2011a; Xun et al. 2012). Most of the propionic acid produced and absorbed by fermentation in the rumen is converted to glucose in the liver and used as an energy source. It was reported that ammonia nitrogen concentration decreased with increasing propionic acid and total VFA in sheep fed N-Se, while ruminal pH level was maintained at optimum limits for cellulolytic activity. It is stated that this situation in the rumen may be due to the rapid absorption and high bioavailability of N-Se (Xun et al. 2012).

To completely perceive the mechanisms and used dosages of nano-selenium for the metabolic and ruminal effects, more research is required. The study aimed to evaluate the effect of N-Se on some metabolic and rumen parameters in sheep.

MATERIAL AND METHODS

This study was carried out with the decision of the local ethics committee of animal experiments in Aksaray University with the date 12.09.2023 and number E-60580050-125.04-11124714.

Twenty healthy female Dorper sheep (60.1±0.44 kg body weight) were randomly divided to one of the two experimental groups (n=10). The animals in the control group (CON) were fed the basal ration (including 0.06 mg Se/kg dry matter) without any supplementation. Basal ration contained a Se concentration, which was lower than the level (2-5 mg/kg diet dry matter) daily intake for sheep (NRC 2007). The animals in experimental second group were taken basal ration added with 3 mg/head/day N-Se. The N-Se was weighed every morning using a precision scale and mixed manually on the concentrated feed into the individual feeder. The basal ration included concentrate feed and, third mowed alfalfa hay as roughage material (Table 1). The animals were consumed 1500 g dust form concentrate and 300 g 3-5 cm size chopped alfalfa hay, in separate feeders at 08:00-18:00, daily. This feeding level fulfilled the nutritional requirement of 60 kg body weight sheep (NRC 2007). The animals were hosted in individual compartments (1.5 m x 1.7 m), had unlimited access to clean water throughout the trial. The experiment lasted for 66 days and consisted of lasting 56 experimental days, including 10 days of adaptation. The concentrate feed was supplied from a commercial feed factory. N-Se (26.80-84.78 nm particule size) biologically synthesized from Vitis vinefera (grape) extract was obtained from Bogazici University Department of Chemistry Laboratory (Figure 1).

Procedures suggested by Association of Official Analytical Chemists (AOAC 1997) were used to determine the values of crude nutrient matters. The amounts acid detergent fibre (ADF) and neutral detergent fibre (NDF) in the feeds were determined according to Van Soest (1994). Metabolizable energy (ME) level was determined according to the method proposed by Turkish Standards Institution (TSI 1991) (Table 1).

Table 1: Chemical compositions of concentrate feed and alfalfa hay used in this study.

Chemical composition	Concentrate feed	Alfalfa hay
Dry matter, %	88.73	91.20
Organic matter[a], %	80.49	83.22
Crude protein, %	13.75	11.29
Crude cellulose, %	10.14	38.12
Ether extract, %	2.37	0.96
Crude ash, %	8.24	7.98
NNE[b], %	54.23	32.85
ADF, %	22.12	33.25
NDF, %	41.04	61.35
HCL[c], %	18.92	28.10
Se, mg/kg DM	0.06	-
ME, Mcal/ kg DM	2.57	1.34

[a]It was measured as the difference between dry matter and the crude ash. [b]Non-nitrogen extract (NNE=DM- (CP+CA+EE+CC)). [c]Hemicellulose (HCL=NDF-ADF).

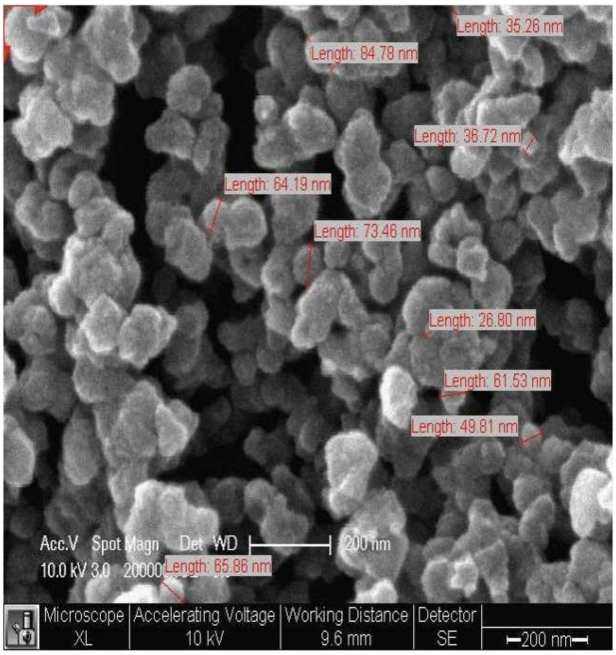

Figure 1: Scanning electron microscopy image of the synthesized N-Se.

The blood samples were taken from the jugular vein of all animals into the hollow tubes (10 mL-Becton Dickinson and Company, New Jersey, USA), without anticoagulant. The samples were collected three hours after the morning feeding on the last day of trial, and they were centrifuged at 3000 rpm/15 min. Afterwards, they were taken to eppendorf tubes and analyzed. Total protein, albumin, total cholesterol (T-cholesterol), triglycerides, AST and ALT levels were determined in serum samples. They were detected using the commercial kits (Randox, UK) and a spectrophotometer (Randox Daytona plus RX 4040, UK) colorimetrically. Selenium (Se) was measured using by plasma mass spectrometry (ICP-MS, Thermo-X Series).

At the last day of fattening, samples of rumen liquid were collected by stomach tube at the same time, blood samples was taken from each animal. After the feeding, feeders were emptied, and all animals were obtained to drink clean water. The pH of the rumen fluid was measured immediately by using automatic pH meter (ADWA AD12). Three sample bottles were used for the rumen fluid of each sheep. Later, they were transported in dry ice to the laboratory.

Gas chromatography was used to detect VFA, acetic, propionic and butyric acids. For this, standards were prepared with 10 mmol / L VFA mixture and 1 µl rumen fluid drawn into 10 µl injector were transfused into chromatography apparatus (Shimadzu GC-2010). The samples were centrifuged at 4000 rpm/15 min. The tubes which 1 ml supernatant and 0.2 ml 25% metaphosphoric acid were added, and then were kept on ice for 30 minutes to precipitate the proteins, later centrifuged again.

Concentration of NH_3-N was measured by using indophenol blue method with spectrophotometer. The samples centrifuged at 14000 rpm/15 min, phenol sodium and ammonia were oxidised in sodium nitroprusside. An intense blue color was created, indicating NH_3-N concentration.

Statistical Analysis

Data were summarized as mean±standard error of mean (SEM) for continuous variables, frequencies (percentiles) for categorical variables. Student's t test or Mann Whitney U test was used for independent group comparisons, depending on the distributional properties of the data (Shapiro Wilk and Kolmogorov Smirnov tests were applied according to groups). Chi-square test was used for proportions and its counterpart Fisher's Exact test was used when the data were sparse. All analyses were performed IBM SPSS Statistics for Windows, Version 20.0. A p value <0.05 was considered as statistically significant.

RESULTS

Table 2 showed the effect of N-Se indicates no significant effect on serum total protein (p=0.514) and albumin (p=0.126) levels. The level of serum T-cholesterol showed a significant decrease from 52.19 to 47.46 mg/dL in the N-Se-treated group when compared with the CON (p=0.001). Additionally, the levels of serum triglycerides were also decreased from 33.34 to 31.01mg/dL (p=0.001). So, it was observed that the lipid profile was decreased using N-Se. The effect of N-Se showed significant alteration in the liver enzymes; the level of serum AST (p=0.001) and ALT (p=0.030) levels were decreased significantly in the N-Se group compared to CON.

Table 2: Effects of N-Se supplementation on metabolic parameters in sheep.

Item	Treatments[1]		SEM[2]	p values[3]
	CON	N-Se		
Total protein, (g/dL)	5.814	6.136	0.186	0.514
Albumin, (g/dL)	2.982	2.958	0.031	0.126
T-cholesterol, (mg/dL)	52.19	47.46	2.911	0.001
Triglycerides, (mg/dL)	33.34	31.01	2.762	0.001
AST, (IU/L)	124.41	119.11	4.838	0.001
ALT, (IU/L)	18.49	17.09	1.072	0.030

[1]Control (CON) sheep were fed a basal diet; N-Se sheep were fed the same basal diet supplemented with nano selenium (N-Se). [2]SEM: Standard error of mean. [3]P values: $p<0.05$ was considered as statistically significant.

The ruminal pH, NH_3-N and VFA are listed in Table 3. Supplementation of N-Se did not affect ruminal pH values (p=0.792), but the ammonia levels from 10.64 to 8.25 mmol/L (p=0.001), while total VFA levels increased from 60.45 to 65.35 mmol/L (p=0.003). The molar concentrations of propionic acid (p=0.017) were increased by N-Se supplementation. The molar concentration of acetic acid (p=0.133) and butyric acid (p=0.089) was similar between the two groups. Ratio of acetate to propionate was decreased with N-Se supplementation (p=0.034).

Table 3. Effects of N-Se supplementation on rumen parameters in sheep.

Item	Treatments[1]		SEM	p values
	CON	N-Se		
Ruminal pH	6.75	6.73	0.04	0.792
NH_3-N, mmol/L	11.25	9.64	0.40	0.001
Total VFA, mmol/L	60.45	65.35	1.74	0.003
VFA, mol/100 mol				
Acetic acid	54.86	55.54	1.69	0.133
Propionic acid	24.37	27.17	0.43	0.017
Butyric acid	10.96	11.08	0.82	0.089
A: P[2]	2.25	2.04	0.05	0.034

[1]Control (CON) sheep were fed a basal diet; N-Se sheep were fed the same basal diet supplemented with nano selenium (N-Se). [2]A:P; Acetic acid: propionic acid. [2]SEM: Standard error of mean. [3]P values: $p<0.05$ was considered as statistically significant.

DISCUSSION AND CONCLUSION

Metabolic Parameters

Blood metabolites may be beneficial in reporting animal health status, may aid in the identification of various metabolic disorders and, these are important indicators for the metabolic activity (Halawa et al. 2023).

Serum total protein levels were quantitatively higher with N-Se supplementation, but this was insignificant. Being supplemented with 3 mg/kg/day N-Se had no appreciable impact on the serum total protein and albumin levels, which agreed with other some studies (Najaf Nejad et al. 2016; Salam et al. 2021; Bolshakova and Aljaf 2022). These results also agreed with Halawa et al. (2023), who showed an unaffecting in serum total protein and albumin, on Ossimi ewes supplementing with 1 mg/kg BW/day N-Se during post-partum. Albumin is a plasma protein with the highest level of concentration and moves various substances in the body (Bolshakova and Aljaf 2022). According to Aljaf and Bolshakova (2023), the drop in albumin level raise the possibility that taking N-Se supplements might affect how proteins are metabolized. On the other hand, Mahmoud et al. (2013) associated the significant increases in serum total protein to the improvement in protein anabolism and reduction of protein catabolism with Se.

In this study, the levels of serum T-cholesterol and triglycerides were decrease with N-Se supplementation. Drop in total cholesterol and triglycerides may indicate the positive role of N-Se on lipid profile and energy metabolism (Ibrahim and Mohamed 2018). Physiological status of animal may also affect to the levels of triglycerides and total cholesterol, and lower their levels are related with an increased energy requirement (Halawa et al. 2023). Halawa et al. (2023) indicated that the triglycerides in N-Se supplemented group showed fluctuated levels on ewes during postpartum periods characterized by significant decrease at day 45 and increase at day 60 of postpartum. The findings of this investigation agreed with those of some other studies regarding the serum T-cholesterol level of N-Se (Bolshakova and Aljaf 2022). However, unlike this study, according to Bolshakova and Aljaf (2022), triglycerides showed no significantly changed in the 0.5 mg/kg N-Se fed lambs. In addition, according to some reports, the amount of organic or inorganic Se supplementation had no appreciable impact on the serum cholesterol and triglycerides of sheep (Novoselec et al. 2022).

AST and ALT levels were significantly reduced on Dorper sheep supplementing with N-Se compared to the CON. These results disagreed with Qin et al. (2016), who showed an raising in ALT, but decreasing AST levels by using N-Se. However, it was consistent with the research results of Halawa et al. (2023), who reported that AST and ALT decreased in the N-Se group. According to Aljaf and Bolshakova (2023), the drop in AST level may be a sign that liver function has possibly improved. It is stated that also dairy cows' serum AST activity were significantly decreased by 3 ppm N-Se administration (Najaf Nejad et al. 2016). Salam et al. (2021), in a study using N-Se supplementation at 0.1 and 0.2 mg/kg levels in sheep diets, emphasized that serum AST levels may also raise as the amount of N-Se in the diet increases. So, results of this study indicate that the dosage and particule size of the N-Se utilized had no impact on female Dorper sheep liver tissue damage, and positively affected liver function.

In addition, Ibrahim and Mohamed (2018) concluded that dietary supplementation of 0.30 mg/kg DM N-Se was more effective than organic and inorganic sources of Se to improve feeding values and some serum metabolic indices of male lambs. Similarly, Khalil et al. (2023) reported that fed the 1.2 mg/kg DM N-Se was more effective than inorganic form as sodium selenite in improving the productive performance of both ewes and their lambs, but did not affect total protein, albumin, triglycerides, cholesterol, AST and ALT levels (except Se concentrations) compared to the control group. According to the researchers, because of increases in the activity of serum antioxidant enzyme and blood Se concentration of ewes fed N-Se compared to the control may confirm the bioavailability of N-Se is higher than inorganic Se. Mousaie et al. (2014) also reported improvements in the general health status and of sheep fed N-Se more than 1 mg/ kg DM without adverse effects on the blood metabolites.

Rumen Parameters

In this study, the ruminal pH values (pH 6.75–6.73) were within the normal range for healty rumen function, because this value lower than 5.5 or higher than 7.0 are accepted abnormal (Hosnedlova et al. 2018). Ruminal pH within optimum limits is an important indicator for cellulolytic bacterial activity (Shi et al. 2011a).

Overally, VFAs from carbohydrates account for approximately 70% of the total energy absorbed by ruminants, therefore, VFA concentrations are important fermentation signaling mechanism in ruminants (Tian et al. 2022). According to Tian et al. (2022); the observed increase in total VFA is likely related to N-Se increasing the relative abundance of rumen microorganisms that primarily degrade carbohydrates, and more propionate is required to provide energy for ruminants. In this study, supplementation with N-Se modified the rumen fermantation in terms of ammonia and total VFA. It was determined that while the NH_3-N concentration decreased, the total VFA concentration increased. It may be related to increased propionate concentration. These positive effects on rumen fermentation could be relatabled to the improved absorption bioavailability, the raised surface area/volume ratio, nano-size, fast and specific action, and catalytic effectiveness (Adegbeye et al. 2019). Similar to these results, Shi et al. (2011a) (0.3, 3 and 6 g elemental N-Se/kg DM instead of non-supplemented basal diet) and Xun et al. (2012) (4 g nano-Se/kg DM instead of Se-yeast) reported that N-Se addition significantly decreased NH_3-N concentration, and total VFA concentration increased. In contrast to the findings of this study, Xun et al. (2012) also found that ruminal pH and molar concentration of propionic acid were significantly reduced. Shi et al. (2011a), confirmed that growing rumen microbial population by N-Se addition would increase the NH_3-N utilization. They also reported that, this was connected with urinary purine involutions which were increased with N-Se addition. N-Se supplementation in Dorper sheep modified rumen fermentation template by shifting to propionic acid, and the increased molar propionic acid ratio resulted in the reduction in the ratio of acetic acid to propionic acid. This finding is consistent with the result of Shi et al. (2011a) who emphasized that this rate was decreased with increasing N-Se addition in sheep.

In addition, in a study conducted on lactating goats reported that similarly with this study's results, oral administration of organic Se with vitamin E (100 mg/goat/day) supported positively the rumen fermentation, enhanced total VFA production especially propionic acid, and reduced NH_3-N concentration compared to the control (Morsy et al. 2019).

In conclusion, in the present study, dietary N-Se supplementation (3 mg/kg DM) effectively reduced serum T-cholesterol, triglycerides, AST and ALT levels in Dorper sheep. It maintained ruminal pH at optimum levels, decreased ammonia nitrogen. These effects were probably due to modifications in the total VFA profile in response to N-Se. In conclusion, the results of the current study indicated that the N-Se supplementation was not only found to positively affect metabolic profile (in terms of T-cholesterol, triglycerides, AST and ALT) both also provide modification on some rumen fermentation parameters. Further investigation is needed to study the mode of action of N-Se supplemented with various particule size and usage doses on blood metabolites and rumen parameters.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

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AUTHOR CONTRIBUTIONS

Idea / Concept: DB
Supervision / Consultancy: DB
Data Collection and / or Processing: DB
Analysis and / or Interpretation: DB
Writing the Article: DB
Critical Review: DB

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