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Synthesis and Characterization of Selenium Nanoparticles and its Effects on *in vitro* Rumen Feed Degradation, Ruminal Parameters, and Total Gas Production

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Abstract
In ruminants, selenium is an essential trace element and has a variety of biological functions, but its importance for rumen microbes is still not fully understood. The goal of the current study is to investigate the effects of prepared selenium nanoparticles (Se-NPs) at various concentrations (0.2, 0.4, 0.6, 1, 2, and 3 mg of Se-NPs/Kg DM on in vitro ruminal feed degradation, fermentation, and total gas production. A supplemented diet with Se-NPs at 0.2 mg/kg DM caused the highest increase ($P<0.05$) in degradability values of dry matter (DM) by 14.3%, organic matter (OM) by 9.1%, neutral detergent fiber (NDF) by 3.2%, acid detergent fiber (ADF) by 6.8%, values for total gas production (TGP), acetic acid by 20.3%, propionic acid by 35%, and butyric acid by 3.2% as compared to the control diet. In comparison to the control diet, supplemented diets with Se-NPs at 2 or 3 mg/kg DM significantly reduced TGP and concentrations of acetic acid, propionic acid, and butyric acid. The supplementation of the diet with 0.2 mg of Se-NPs is sufficient to improve ruminal digestibility and fermentation.
Keywords: Selenium Nanoparticles, *in vitro* Rumen Feed Degradation, Fermentation, Gas Production.

1. Introduction

The rapid development of nanotechnology holds significant potential for applications in medical and nutritional research due to the revelation that nano-materials exhibit distinctive features different from those of bulk materials and microscale materials [1]. On the other hand, the use of nanoparticles can put handlers at risk for injury due to the possibility of inhaling extremely small particles (less than 100 nm) or using levels in animal diets that have not been thoroughly studied [2]. There are efforts worldwide to address and regulate the development of nano-materials and nanotechnology, either through legislation or through advisory and non-binding recommendations [3].

Currently, neither the EU nor any other nation has legislation specifically devoted to regulating nano-materials [4]. According to Meyer et al., [5], selenium is a crucial trace element for farm animal health, immunological function, production, and reproductive efficiency. Selenium nano-elements have received a lot of attention because of their new properties, which include a large specific surface area, high surface activity, high bioavailability, and relatively low toxicity [6].

Recently, selenium nano-particles (Se-NPs) were shown to have both increased bioavailability and decreased toxicity relative to sodium selenite in ewes [7, 8]. These observations make the element of nano-Se an interesting candidate for supplementing the feed of ruminants to maximize health and performance with potentially lower inclusion rates and, at the same time, limit environmental and toxicity risks. Shi et al., [9] reported that the addition of Se-NPs to sheep feedstuffs could improve rumen fermentation and feed utilization.

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However, little is known about the effect of nano-Se at different concentrations on fermentation properties and nutrient digestibility. As a result, the objective of this study was to prepare nano selenium (Se-NPs) and investigate their effects at various concentrations (0.2, 0.4, 0.6, 1, 2, and 3 mg Se-NPs/kg/DM), on in vitro ruminal feed degradation, ruminal parameters, and total gas production, and to determine the proper level of nano-selenium that should be used as an animal feed additive without affecting rumen fermentation.

2. Materials and Methods

All chemicals used in the experiment are analytic reagent grade. Sodium selenite (Na2SeO3.5H2O), ascorbic acid and polyvinyl alcohol (PVA) were purchased from Sigma Aldrich. Deionized water was used throughout the experiment.

2.1. Synthesis of selenium nanoparticles (Se-NPs)
The analytic reagent grade chemicals used in the current experiment were all obtained from Sigma Aldrich. Selenium nanoparticles (Se-NPs) were produced by reducing sodium selenite with ascorbic acid and stabilizing them with polyvinyl alcohol (PVA). Briefly, 50 mg of Na2SeO3.5H2O were added to 100 mL of Milli-Q water. Ascorbic acid (10 mL, 56.7 mM) was added dropwise to the sodium selenite solution with vigorous stirring, and then 10 µL of PVA were added after each 2 mL of ascorbic acid. Selenium nanoparticles were formed after the addition of ascorbic acid. This can be visualized by the color change of the reactant solution from clear white to clear red. Se-NPs were then collected by centrifuging the solution at 12000 rpm. The pellet was re-suspended in sterile, double-distilled water [10].

2.2. Characterization of the prepared Se-NPs

2.2.1. Transmission electron microscopy (TEM)
Using an accelerating voltage of 80 KV, a TEM (JEM-1230; JEOL Ltd., Tokyo, Japan) was used to analyse the shape and particle size of Se-NPs. The transmission electron microscopy specimens were prepared by adding a drop of the nano-Se suspension onto a carbon-coated copper grid.

2.2.2. Dynamic light scattering (DLS)
The particle size distribution of the biosynthesized Se-NPs was tested via a dynamic light scattering (DLS) apparatus (NICOMP 380 ZLS, PSS, Santa Barbara, CA, USA).

2.2.3. X-ray diffraction patterns (XRD)
The biosynthesized Se-NPs' X-ray diffraction pattern was examined using an XRD with a Philips diffractometer (PW 1820 goniometer, PW 1930 generator), stationary through Cu Kα radiation (45 kV, 40 mA, with λ= 0.15418 nm). The prepared Se-NPs' crystalline structure was scanned in a 2θ range of 5 to 80 o with a step size of 0.02 and a step time of 1 s.

2.3. Cytotoxic effect of Se-NPs
Cell viability was assessed by the mitochondrial dependent reduction of yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to purple formazan [11]. All the following procedures were done in a sterile area using a laminar flow biosafety cabinet; class II A2 (manufactured by Labconco). Cells were suspended in DMEM medium, 1% antibiotic-antimycotic mixture (10,000 U/ml Potassium Penicillin, 10,000 µg/ml Streptomycin Sulphate, and 25 µg/ml Amphotericin B), 1% L-glutamine, and 5% fetal bovine serum at 37 ºC under 5% CO2 using a CO2 incubator (Sartorius Stadium, Biotech).
Cells were batch cultured for 10 days, then seeded at a concentration of 10 x 10^3 cells/well in fresh complete growth medium in 96-well plastic plates at 37 ºC for 24 h under 5% CO2 either alone (negative control) or with different concentrations of drugs to give a final concentration of (250, 125, 62.5, 31.25, 15.625, 7.8125, 3.906, 1.953 µg/ml). After 48 h of incubation, medium was aspirated and 20 ul of MTT salt (2.5µg/ml) were added to each well and incubated for a further four hours at 37ºC under 5% CO2. To stop the reaction and dissolve the formed crystals, 200 µL of 10% Sodium Dodecyl Sulphate (SDS) in 0.01M HCL was added to each well and incubated overnight at 37ºC. A positive control composed of 100µg/ml was used as a known cytotoxic natural agent that gives 100% lethality under the same conditions [12, 13]. The absorbance was then measured using a microplate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA) at 595nm and a reference wavelength of 620nm.
Viability = absorbance of drug / absorbance of control x 100
Cytotoxicity = 100- viability.

2.4. *In vitro* rumen fermentation
For the batch fermentation procedure, each treatment required the use of three incubation vessels. Each vessel received 40 ml of a 1:3 (v/v) rumen fluid buffer solution mixture, according to Ismail et al. [14]. Warm rumen fluid, the microorganism's inoculum, was extracted from the rumen of slaughtered rams and placed in a plastic container with free oxygen. The experimental diet's chemical composition is shown in Table 1 and

includes 35% maize grain, 30% Berseem hay, 14% cottonseed meal, 14% wheat bran, and 7% soybean meal. The treatments were as follows: the control diet (400 mg) was used without selenium supplementation, and the control diet was supplemented with Se-NPs for each of the T1, T2, T3, T4, T5, and T6 groups at various amounts of 0.2, 0.4, 0.6, 1, 2, and 3 mg/Kg DM. All vessels were filtered in fibre filter bags with 25-micron porosity (ANKOM-USA) after 24 hours of incubation at 39 °C.

Table (1): Chemical composition of the experimental diet (on dry matter basis)

Diet chemical composition	%
Dry matter (DM)	89.5
Organic matter (OM)	95.0
Crude protein (CP)	15.5
Crude fiber (CF %)	15.67
Ether extract (EE %)	4.9
Ash	5.0
Nitrogen Free Extract (NFE)	58.93
Selenium (Se)	ND

DM: Dry matter, OM: Organic matter, CP: Crude protein, EE: Ether extract, NFE: Nitrogen Free Extract, ND: Not Detected

The residues in the bags were dried at 60 °C for 48 hours to determine the digestibility of dry matter (DM), organic matter (OM), neutral detergent fibre (NDF), and acid detergent fibre (ADF). Following incubation, the AOAC [15] methods were used to assess the chemical composition of the experimental feed and its residue, and the Van Soest [16] method was used to estimate the NDF and ADF. Using a pH meter, the pH of rumen fluid was determined. Hohenheim syringes (100 ml) were used to measure the volume of generated gas by Navarro-Villa et al, [17]. Spectrophotometer was used to measure the ammonia content in accordance with the instructions in the kit pamphlet (Biodiagnostic, Egypt). High-performance liquid chromatography (HPLC) was used to analyze the concentrations of lactic, acetic, propionic, and butyric acids in the Agilent 1260 series. An Eclipse C18 column (4.6 mm x 250 mm i.d., 5 m) was used for the separation at 30 °C.

2.5 Statistical analysis:

The data were statistically analyzed using IBM SPSS [18] Statistics for Windows (2011) and the following equation: $Yij = Ti + eij$.

Where, Yij is the parameter under analysis ij, μ is the overall mean, Ti is the effect due to treatment on the parameter under analysis, eij is the experimental error for ij on the observation. Using a probability level less than 0.05 ($P<0.05$) for significant expression, Duncan's multiple range tests were utilized to examine the significance among means [19].

3. Results and discussion

3.1. Characterization of the prepared selenium nanoparticles (Se-NPs)

The XRD pattern of typical Se-NPs produced chemically using Na_2SeO_3 as a precursor is shown in Fig. 1A. The Se-NPs is a single phase of elemental selenium that is well crystallized and possesses a nanoform. This suggests that ascorbic acid acts as a reducing agent and reduces Na2SeO3 to elemental Se. Accordingly, the diffraction peaks for the (100), (101), (110), (102), (111), (201), (003), (202), (210), and (211) were at 2 = 23.2, 29.5, 41.4, 44.2, 45.9, 51.8, 55.6, 61.3, 65, and 69, respectively. The reflections of selenium nanoparticles in their pure hexagonal phase, with lattice parameters a = 4.366 and c = 4.9536 (JCPDS 06-0362). The presence of Se-NPs was determined by the sharp Bragg reflection, which matches with Selected Area Electron Diffraction (SAED).

Ascorbic acid was used as a reducing agent to reduce the sodium selenite, and according to dynamic light scattering (DLS), the particle size of the synthesized Se-NPs revealed a size distribution ranging from 100 to 900 nm (Fig. 1B).The prepared selenium nanoparticles were characterized as quite stable due to the high negative charge and there were no aggregations formed through the storage period. Furthermore, the obtained results further showed that the prepared Se-NPs particle size is approximately 280 nm. In the recent study, the size of the selenium particles is ranged from 13.95 to 26.26 nm according to El-Zayat et al. [20]. El-Khateeb et al., [21] reported that the selenium nanoparticles showed a spherical shape and had an average particle size of 22.31 to 95.16 nm. The manufacture of Se-NPs from Na_2SeO^3 as a precursor utilizing a chemical process is the current work's attempt to spread the clean technology for the construction of inorganic 1D nanostructure. Producing selenium nanoparticles is an easy, quick, and inexpensive method. Using TEM, the morphology of the materials was studied. The prepared Se-NPs have nanoparticles diameters ranging from 12 to 40 nm, and the TEM examinations of the produced Se-NPs are shown in Fig. 2, showing that the Se nanoparticles have higher aspect ratios.

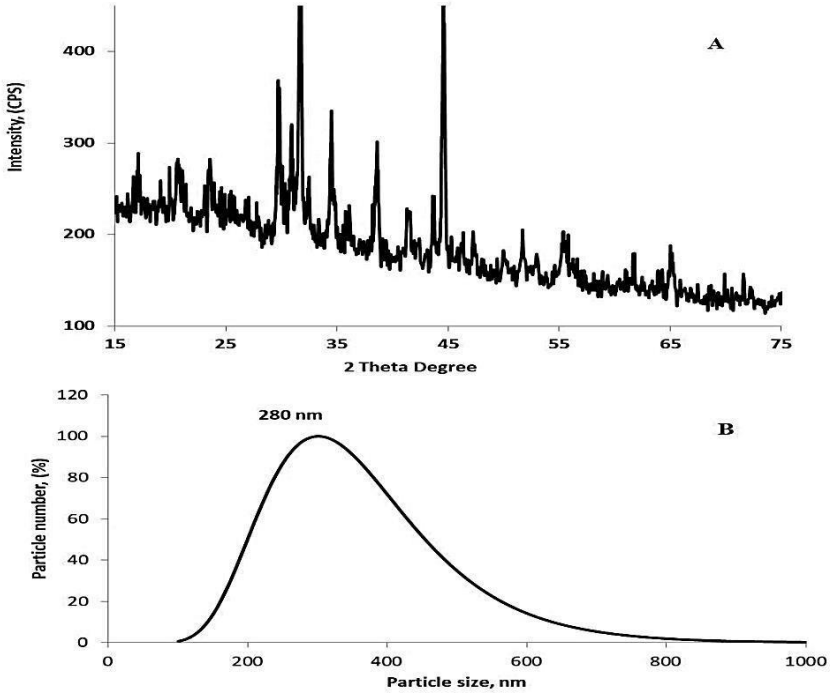

Fig. 1A): XRD of the prepared selenium nanoparticles (Se-NPs), B) Dynamic Light Scattering (DLS) of the prepared Se-NPs

Moreover, no additional morphologies were seen, and the measured morphologies of the chemically generated Se nanostructure were clearly nanoparticles. The morphologies of selenium nanoparticles varied depending on the reductive chemicals utilized in the nanoparticles synthesis process [20]. According to El-Zayat et al. [20], using an extract of Ephedra aphylla stems for the synthesis of selenium nanoparticles revealed that the selenium nanoparticles had higher stability and a variety of desired morphologies because the chemical constituents phenolic, flavonoid, tannin, and alkaloids were present and were in charge of the nanoparticles biosynthesis and stability. The SAED pattern (Fig. 2) also displayed the Se-NPs' diffraction ring pattern, which is indexed as (100), (101), (110), (102), (111), (201), (112), and (202) reflections. This demonstrates the production of hexagonal selenium nanoparticles.

3.2. Cytotoxic effect of Se-NPs on human cell lines

Table 2 shows the cytotoxicity of different concentrations of selenium nanoparticles (Se-NPs) on human skin fibroblast (HSF) cells. The results illustrated that the Se-NPs at a concentration of 125 µg gave a high percentage of cell viability and less toxicity followed by a concentration of 1.98 µg with 88.8% of viability and 11.2% of toxicity. Furthermore, the Fig. 3 illustrated that the cells viability decreased with increasing the concentrations of Se-NPs. Moreover, Serini et al. [22] stated that the prepared nano-materials may be displayed a significant toxicity when cell viability value was less than 50% compared to the control cells. The obtained result s in our study the cytotoxicity was evaluated and the cell viability was around 80%, representative that the fabricated selenium nanoparticles are extremely safe and can be a promising candidate for safe materials for different applications.

3.3 Effects of Se-NPs supplementation levels on the degradability of ruminal diet

The effects of Se-NPs supplementation on in vitro diet degradation by rumen microbes are shown in Table 3. The diets supplemented with Se-NPs at 0.2 mg/kg DM had higher degradability values for dry matter (DM), organic matter (OM), neutral detergent fibre (NDF), and acid detergent fibre (ADF) than the control diet ($P<0.05$). These values were 14.3%, 9.1%, 3.2%, and 6.8%, respectively. Dietary selenium is an essential trace element for livestock and has a variety of biological functions, but its importance for rumen microorganisms is still not clear.

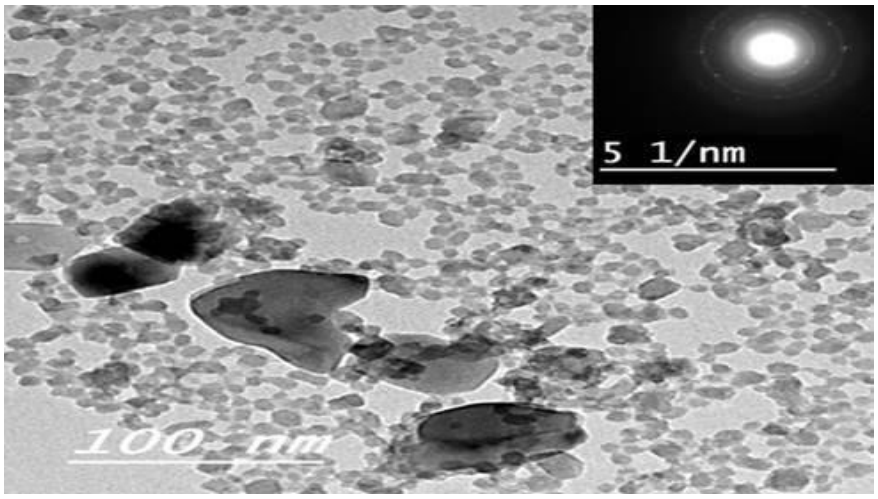

Fig.2. TEM of the prepared selenium nanoparticles (Se-NPs) by reducing sodium selenite in the presence of ascorbic acid and polyvinyl alcohol (PVA)

Table (2): Cytotoxicity of different concentration of selenium nanoparticles (Se-NPs) on HSF cells
HSF: human skin fibroblast

Se-NPs **concentrations (ug/ml)**	**Viability (%)**	**Cytotoxicity (%)**
250	47.8	52.2
125	96.8	3.2
62.5	79.2	20.8
31.25	69.1	30.9
15.625	76.3	23.7
7.8125	76.8	23.2
3.906	68.6	31.4
1.953	88.8	11.2
IC 50 =		
IC 50 = 381.29 ug/ml		

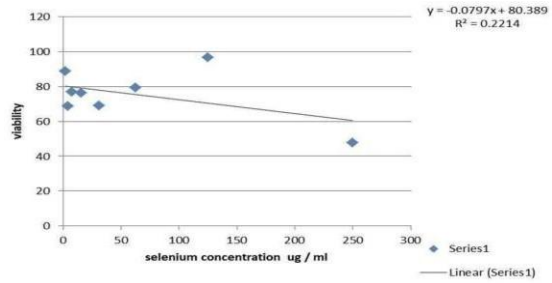

Fig.3. The effect of Se-NPs concentrations on the human skin fibroblast (HSF) cells viability (%(

In this research, the effect of Se-NPs diet supplementation on rumen fermentation characteristics was investigated to determine the most efficient level of Se-NPs on the fermentation activity of the rumen. Ibrahim [23] reported improved ($P<0.05$) DM, OM, and NDF digestibility of ewe's diet supplemented with sodium selenite at 0.2 mg/kg DM, which is consistent with the findings of the current study. Shi et al., [9] also reported that sheep supplemented with 0.3 mg/kg DM of Se-NPs had higher DM, OM, NDF, and ADF digestibility than sheep under control conditions. Moreover, Ibrahim and Mohamed [24] found that Se-NPs were more effective than sodium selenite at increasing the digestibility of all nutrients, and those ewes supplemented with Se-NPs and sodium selenite at 0.3 mg/kg DM showed higher DM, OM, NDF, and ADF digestibility than those of the control.

The superiority of Se-NPs over sodium selenite at the same level of supplementation for improvement of the ruminal diet's DM, OM, NDF, and ADF degradability is reasonable. This may be due to the high bioavailability and low toxicity of nanometric particles compared to inorganic [25], as the nanometric particles exhibit novel characteristics including high surface activity, strong catalytic efficiency, and high adsorption ability [26]. Also, reduction of sodium selenite into insoluble forms (ex: selenides) causes low bioavailability of the inorganic Se sources in the rumen [27]. Increased DM, OM, NDF, and ADF degradability in diets supplemented with Se may be due to increased growth and activity of rumen microorganisms, particularly cellulolytic bacteria, which in turn stimulate ruminal fermentation and microbial digestive enzyme activity [24]. It was demonstrated that when Se-NPs supplementation levels were increased to 1 mg/kg DM in the diet, the digestibility values decreased but remained significantly higher than the control diet. Se-NPs supplementation diets at 2 or 3 mg/kg DM significantly decreased all digestibility values as compared to the control diet. This shows that up to 1 mg/kg DM of Se-NPs supplementation had no

effect on the rumen microbiota in this investigation. In comparison to the control, when Se-NPs were added above 1 mg/kg DM of the diet, the degradability of DM, OM, NDF, and ADF decreased.

The antibacterial activity of selenium nanoparticles may explain the steady decline in ruminal degradability measures with increasing Se-NPs supplementation levels above 1 mg/Kg DM. According to Stolzoff et al., [28], selenium nanoparticles have been efficient in reducing both gram-positive and gram-negative bacteria by depleting glutathione levels, a crucial antioxidant required for bacteria to eliminate reactive oxygen species. On the other hand, Xun et al., [8] reported that sheep supplemented with nano-selenium at a high dose (4 mg/kg DM) had enhanced ruminal fermentation as a result of increased growth and activity of cellulolytic bacteria, as well as higher diet digestibility for DM, OM, NDF, and ADF. The highest tolerable Se level for domestic animals is 2 mg/kg DM, which is important to note [29].

3.4. Effect of Se-NPs supplementation on ruminal basic parameters

Table (4) provides an illustration of the impact of Se-NPs supplementation on ruminal basic parameters. Dietary supplementation with Se-NPs at 0.2 mg/kg DM increased ($P<0.05$) values for total gas production (TGP) and volatile fatty acids (acetic acid by 20.3%, propionic acid by 35%, and butyric acid by 3.2%). Supplementing Se-NPs up to 0.6 mg/Kg DM clearly increased TGP volume and acetic, propionic, and butyric acid concentrations significantly ($P<0.05$) compared to the control. In comparison to the control diet, all ruminal basic measures significantly decreased when Se-NPs supplementation exceeded 1 mg/kg DM of the diet. When diets were supplemented withSe-NPs at 2 and 3 mg/kg DM, the TGP was reduced by 3.2% and 4.8%, respectively, as well as volatile fatty acid concentrations (acetic acid by 30.5 and 32.2%, propionic acid by 30 and 30%, and butyric acid by 33.3 and 40%). No significant change was found in NH3-N concentration due to the supplementation of Se-NPs up to a level of 1 mg/kg DM when compared with the control. The pH levels for all treatments showed no discernible change with Se-NPs diet supplementation.

The beneficial effects of Se-NPs on nutrient digestibility are demonstrated by an increase in the volume of total gas production and volatile fatty acid concentrations (acetic, propionic, and butyric) by supplementing Se-NPs up to 0.6 mg/Kg DM in comparison to the control. Faixová et al., [30] reported that Se-NPs supplements significantly boost the rumen microbial activity and population. Similar findings were observed by Dehghani et al., [25], who discovered that supplemented a diet with sodium selenite or Se-NPs at 0.3 mg/kg DM (*in vitro*) led to a significant increase in both the volume of total gas production and the concentration of volatile fatty acids. In sheep, Shi et al. [26] found that supplementing the diet with Se-NPs at 0.3 mg/kg DM increased total VFA and propionate synthesis, which decreased the ratio of acetate to propionate. Se-NPs' potential to improve rumen fermentation by encouraging rumen microbial growth and digesting enzyme activity may account for these results [25, 9].

In the present work, supplementation of the diet with Se-NPs up to a dose of 1 mg/kg DM did not result in any significant change in the concentration of NH3-N. Additionally, there was no discernible change in the pH levels for any of the treatments when Se-NPs were added to the diets. In line with our findings, Dehghani et al., [25] demonstrated that the ruminal ammonia-N concentration was not affected by sodium selenite or nano Se supplementation at 0.3 mg/Kg DM.

Table (3): Effect of selenium nanoparticles (Se-NPs) supplementation on ruminal diet degradability parameters

Selenium nanoparticles level	DMD %	OMD %	NDFD %	ADFD %
Control	49.02^{c}	54.10^{c}	40.45^{bc}	31.44^{c}
0.2 mg Se-NPs/kg DM	56.04^{a}	61.12^{a}	43.21^{a}	36.99^{a}
0.4 mg Se-NPs/kg DM	53.42^{b}	58.50^{b}	41.78^{ab}	32.71^{bc}
0.6 mg Se-NPs/kg DM	52.39^{b}	57.47^{b}	41.67^{ab}	32.25^{bc}
1.0 mg Se-NPs/kg DM	50.12^{c}	55.20^{c}	40.86^{bc}	31.14^{c}
2.0 mg Se-NPs/kg DM	48.72^{c}	53.80^{c}	39.61^{c}	28.94^{d}
3.0 mg Se-NPs/kg DM	46.66^{d}	51.74^{d}	37.45^{d}	26.13^{e}
SE±	0.63	0.62	0.38	0.64

a,b,c,d and e means at the same column with different superscript are significantly ($P<0.05$) different. DMD: Dry Matter Degradability, OMD: Organic Matter Degradability, NDFD: Neutral Detergent Fiber Degradability and ADFD: Acid Detergent Fiber Degradability. ±SE: standard error

Table (4): Effect of Se-NPs supplementation on ruminal basic parameters

Selenium nanoparticles level	TGP (ml/24h)	pH	Acetic acid (mg/ml)	Propionic acid (mg/ml)	Butyric acid (mg/ml)	NH_3-N (mg/dl)
Control	125.33^{c}	5.97	0.59^{b}	0.20^{b}	0.15^{b}	12.96^{a}
0.2 mg Se-NPs/kg DM	135.67^{a}	5.92	0.71^{a}	0.27^{a}	0.19^{a}	13.38^{a}
0.4 mg Se-NPs/kg DM	135.33^{a}	5.93	0.69^{a}	0.24^{a}	0.18^{ab}	13.07^{a}
0.6 mg Se-NPs/kg DM	132.67^{b}	5.93	0.69^{a}	0.24^{a}	0.18^{ab}	12.93^{a}
1.0 mg Se-NPs/kg DM	124.67^{c}	5.93	0.49^{bc}	0.16^{b}	0.13^{bc}	12.61^{ab}
2.0 mg Se-NPs/kg DM	121.33^{d}	5.97	0.41^{c}	0.14^{c}	0.10^{cd}	12.18^{b}
3.0 mg Se-NPs/kg DM	119.33^{e}	5.9	0.40^{c}	0.14^{c}	0.09^{d}	11.59^{c}
SE±	1.3	0.01	0.03	0.01	0.01	0.13

a,b,c,d and e means at the same column with different superscript are significantly ($P<0.05$) different. TGP: Total gas production (ml/24hr). ±SE: standard error

The diet supplementation with Se-NPs up to 1 mg/kg DM has no significant impact on NH3-N concentration due to the low rate of corn protein degradation by rumen microflora [31].The rumen's microbial community is reflected in part by the pH of the rumen. It has been demonstrated that a lower pH inhibits the attachment of bacteria to plant cell walls, which is detrimental to fibre digestion. It has been thought that, the lack of variations in ruminal pH may be due to higher production of the volatile fatty acids and alkaline ammonia as a result of quick fermentation of the concentrated feed mixture of the tested diet. The mean ruminal pH was in the optimal range for cellulolytic bacterial activity when nano-selenium was present [9]. Similar findings were reported by Liu et al., [32], who stated that different levels of selenium yeast supplementation had no effect on ruminal pH. When Se-NPs supplementation exceeded 1 mg/kg DM of the diet, all ruminal basic measurements decreased significantly compared to the control diet. In the present investigation, dietary additions of Se-NPs at 2 and 3 mg/kg DM significantly reduced the TGP and concentration of volatile fatty acids (acetic acid, propionic acid, and butyric acid). Se-NPs' anti-microbial action may account for the decrease in TGP, VFA, and NH3-N levels following diet supplementation above 1 mg/Kg DM. Se-NP supplementation resulted in significantly lower ruminal pH, NH_3-N concentration, molar proportion of propionate, and ratio of acetate to propionate in sheep when given at levels of 3 and 4 mg/kg DM [8, 26]. On the other hand, Shi et al., [9], found that Se-NPs supplementation up to 6 mg/kg DM increased the synthesis of total VFA and propionate. We suggest that, the inconsistency in the results of the different studies may be due to the different chemical forms and levels of supplementation for selenium, the nature and chemical composition of the diet, the duration and continuity of intake, and the nature of the experiments (*in vivo* or *in vitro*).

4. Conclusion

In the current work, selenium nanoparticles were successfully prepared using a chemical method. It could be concluded that the supplementation diet with 0.2 mg of Se-NPs was sufficient to improve the in vitro ruminal digestibility, rumen parameters, and total gas production. Thus, higher supplementation of Se-NPs than 0.4 mg/kg DM is not recommended.

5. Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request

6. Author's contribution

Shakweer, W.M.E., Azzaz, H.H., EL-Nomeery, Y.A., El-Sayed, S.M., Youssef, A.M., and Noha A. Hassaan: conceptualization, formal analysis, investigation, methodology, resources, supervision, validation, writing (original draft, review, and editing).

7. Conflict of interest:

The authors declare that there was no conflict of interest in carrying out this work.

8. Acknowledgments

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9. References

[1] Albrecht M.A., Evans C.W. and Raston C.L., Green Chemistry and the Health Implications of Nanoparticles. *Green Chemistry*, 8, 417-432 (2006). https://doi.org/10.1039/b51713 1h.

[2] Miller R.J., Lenihan H.S., Muller E.B., Tseng N., Hanna S.K., and Keller A.A., Impacts of Metal Oxide Nanoparticles on Marine

Phytoplankton. *Environmental Science & Technology*, 44 (19), 7329-7334 (2010), DOI: 10.1021/es100247x.

[3] Van der Meulen B., Bremmers H., Purnhagen K., Gupta N., Bouwmeester H., Geyer L.L.,. Governing Nano Foods: Principles-based Responsive Regulation: EFFoST Critical Reviews 3. Academic Press, (2014).

[4] Arts J.H.E., Hadi M., Keene A.M., Kreiling R., Lyon D., Maier M., Michel K., Petry T., Sauer U.G., Warheit D., Wiench K., Landsiedel R., A critical appraisal of existing concepts for the grouping of nanomaterials. *Regul. Toxicol. Pharmacol*, 70 (2), 492-506 (2014).

[5] Meyer U., Heerdegen K., Schenkel H., Da¨nicke S., Flachowsky G., Influence of various selenium sources on selenium concentration in the milk of dairy cows. *J. Verbr. Lebensm*, 9, 101–109 (2014). https://doi.org/10.1007 /s00003- 014-0870-3.

[6] Zhang J., Taylor E.W., Wan X., and Peng D. Impact of heat treatment on size, structure, and bioactivity of elemental selenium nanoparticles. *International journal of nanomedicine*, *7*, 815–825 (2012). https://doi.org/10.2147/IJN.S28538.

[7] Sadeghian S., Kojouri G.A., and Mohebbi, A., Nanoparticles of selenium as species with stronger physiological effects in sheep in comparison with sodium selenite. *Biological trace element research*, *146*(3), 302–308 (2012). https://doi.org/10.1007/s12011-011-9266-8

[8] Xun W., Shi L., Yue W., Zhang C., Ren Y., Liu Q., Effect of High-Dose Nano-selenium and Selenium–Yeast on Feed Digestibility, Rumen Fermentation, and Purine Derivatives in Sheep. *Biol Trace Elem Res*, 150, 130–136 (2012). https://doi.org/10.1007/s12011 -012-9452-3.

[9] Shi L., Yue W.X.W., Zhang C., Ren Y., Liu Q., Wang Q., Shi L., Effect of elemental nano-selenium on feed digestibility, rumen fermentation, and purine derivatives in sheep. *Animal Feed Science and Technology* 163: 136-142 (2011).

[10] Boroumand S., Safari M., Shaabani E., Shirzad M. and Faridi-Majidi, R., Selenium nanoparticles: synthesis, characterization and study of their cytotoxicity, antioxidant and antibacterial activity. *Materials Research Express*, 6, (8), 1-17(2019).

[11] Mosmann T. Rapid colorimetric assays for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Methods.65:55-63. (1983).

[12] Thabrew M.I., Hughes R.D., McFarlane I.G. Screening of hepatoprotective plant components using a HepG2 cell cytotoxicity assay. *J Pharm Pharmacol*.49:1132-5. (1997).

[13] El-Menshawi B.S., Fayad W., Mahmoud K., El-Hallouty S.M., El-Manawaty M., Olofsson M.H., Linder S. Screening of natural products for therapeutic activity against solid tumors. *Indian J Exp Biol*., 48(3):258-64. (2010).

[14] Ismail S.A., Abdel-Fattah A.M., Emran M.A., Azzaz H.H., El-Gamal M.S., and Hashem A.M., Effect of Partial Substitution of Ration's Soybean Meal by Biologically Treated Feathers on Rumen Fermentation Characteristics (*in vitro*). *Pakistan Journal of Biological Sciences, 21,* 110-118 (2018).

[15] AOAC. Official Methods of Analysis of AOAC International. 16th Edn. Vol. 1, "Agricultural, Chemicals, Contaminants, Drugs". Washington, D.C., USA (1995).

[16] Van Soest P.J., Robertson J.B., Lewis B.A., Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. *J. Dairy Sci.*, 74: 3583–3597 (1991).

[17] Navarro-Villa A., O'brien M., López S., Boland T.M. and O'kiely, P., Modifications of a gas production technique for assessing in vitro rumen methane production from feedstuffs. *Anim. Feed Sci. Technol*., 166: 163-174 (2011).

[18] IBM Corp. Released IBM SPSS Statistics for Windows, Version 20 Armonk, NY: IBM Corp (2011).

[19] Duncan, D.B. Multiple ranges and multiple F-test. *Biometrics*. 11:1-42 (1955).

[20] El-Zayat, M.M., Eraqi, M.M., Alrefai, H., El-Khateeb, A.Y., Ibrahim, M.A., Aljohani, H.M. Aljohani, M.M. and Elshaer, M.M., The antimicrobial, antioxidant, and anticancer activity of greenly synthesized selenium and zinc composite nanoparticles using Ephedra aphylla extract. *Biomolecules*, 11(3), p.470 (2021).

[21] El-Khateeb, A.Y., Hamed, E., Ibrahim, F.Y. and Hamed, S.E.,. Eco-Friendly Synthesis of Selenium and Zinc Nanoparticles with Biocompatible Sargassum latifolium Algae Extract in Preservation of Edible Oils. *Journal of Food and Dairy Sciences*, 10 (5), pp.141-146 (2019).

[22] Serini, S., Cassano, R., Facchinetti, E., Amendola, G., Trombino, S., & Calviello, G., Anti-irritant and anti-inflammatory

effects of DHA encapsulated in resveratrol-based solid lipid nanoparticles in human keratinocytes. *Nutrients,* 11(6), 1400 (2019).

[23] Ibrahim E.M., The impact of dietary supplementation of iodine and selenium on nutrients digestibility and productive performance of ewes and their suckling lambs. *Egyptian J. Nutrition and Feeds*. 19 (2): 255-263 (2016).

[24] Ibrahim E.M. and Mohamed M.Y., Effect of different dietary selenium sources supplementation on nutrient digestibility, productive performance and some serum biochemical indics in sheep. *Egyptian J. Nutrition and Feeds*, 21 (1): 53-64 (2018).

[25] Dehghani S, Taghizadeh A and Mohammadzadeh H., The effects of different forms of selenium on gas production parameters, rumen fermentation and rumen protozoa population. *Iranian Journal of Animal Science Research*, 11, (3): 307-317 (2019).

[26] Shi L.G., Xun W.J., Yue W.B. Zhang C.X., Ren Y.S. Shi, L. Wang Q., Yang R.J., Lei F.L. Effect of sodium selenite, Se-yeast and nano-elemental selenium on growth performance, Se concentration and antioxidant status in growing male goats. *Small Ruminant Research*, 96,49–52 (2011a).

[27] Mehdi Y., Hornick J., Istasse L., and Dufranse I., Selenium in the environment, metabolism and involvement in body functions. Molecules, 18: 3292-331 (2013).

[28] Stolzoff M., Wang S.Q., and Webster T.J., Efficacy and mechanism of selenium nanoparticles as antibacterial agents. Front. Bioeng. Biotechnol. Conference Abstract: 10th World Biomaterials Congress, (2016). doi: 10.3389/conf.FBIOE.2016.01.03040.

[29] NRC, Nutrient requirements of sheep. 6th Edition, National Academy of Sciences, National Research Council, Washington, D.C. (1985).

[30] Faixová Z., Faix S., Leng L., Vaczi1 P., Makova Z., Szaboova R., Haematological, blood and rumen chemistry changes in lambs following supplementation with Seyeast. Acta Veterinaria, 76: 3-8 (2007). [26] Liu Q., Wang C., Huang Y. X., Miao C. H., and Gao D.H., Effects of Sel-Plex on rumen fermentation and purine derivatives of urine in Simmental steers. *J. Anim. Feed Sci.* 16 (Supple 2):133-138 (2007).

[31] Azzaz H H., Eman S.A. Farahat and Ebeid H.M, Effect of partial replacement of corn grains by date seeds on rahmani ram's nutrients digestibility and Nubian goat's milk production. *Int. J. Dairy Sci*., 12: 266-274 (2017).

[32] Liu Q., Wang C., Huang Y.X., Miao C.H., Gao D.H., Effects of Sel-Plex on rumen fermentation and purine derivatives of urine in Simmental steers. *J. Anim. Feed Sci.*;16(Suppl. 2):133–138 (2007).