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Effect of Supplementation of Different Forms of Selenium on *In Vitro* Dry Matter Digestibility and Microbial Biomass Production

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ABSTRACT

Nano selenium was synthesized by wet chemical method at laboratory level. In this study particle size, particle shape, zeta potential and selenium content were characterized by using particle size analyser (PSA) and inductively coupled plasma mass spectrometry (ICP-MS). The result revealed that selenium nano particle is spherical in shape with mean particle size of 31.8 ± 2.31 nano meter (nm) and concentration of selenium is 98.34 ± 2.8 per cent selenium that ensured the purity of nano selenium. The toxicity was analysed by MTT assay against *Vero* cell line. The nano selenium effectively inhibited the growth of *Vero* cells in a dose dependent manner. In vitro digestibility and microbial biomass production also evaluated on different form of selenium on basal diet at different levels. We used sodium selenite, selenocysteine and nano selenium. Based on the calculations, IC_{50} for nano selenium derived from selenium powder was 89.11 µg/ml. when selenium is added at graded level to the basal diet in any form resulted in significant increase ($P<0.05$) in digestibility parameter such as *in vitro* apparent dry matter digestibility, in vitro true dry matter digestibility and microbial biomass production at all levels of addition compared to when no selenium was added. From the results it can be inferred that spherical shaped, nano-selenium particles of size ranging 31.8 nm could be produced by wet chemical method at laboratory level.

HIGHLIGHTS

- Nano selenium can be produced by wet chemical method at laboratory level.
- Nano selenium has all the required properties and safe to use.

Keywords: Nano selenium, *In vitro* digestibility, Microbial biomass, Particle size

Selenium is an essential trace element for animal health, immune function, productivity, and reproductive performance in farm animals and found in both organic and inorganic forms in nature, has a specific place among the nutrients in animal feed because of its role in animal body (Mehdi and Dufrasne, 2016).

Selenium added to the feeding ration is not fully utilized by the animal's organism, as its significant part is absorbed and metabolized by ruminal bacteria, thus the highest selenium concentrations are found in ruminal biomass, whereas the lowest are in the ruminal fluid (Panev *et al.,* 2013).In ruminants, inorganic Se compounds are partly reduced by ruminal microbes to unabsorbable elemental selenium resulting in a lower Se apparent absorption. Selenoamino acids are subject to ruminal microbial degradation also decreasing apparent selenium absorption from the digestive tract. Rumen microorganisms have been shown to incorporate Se from both inorganic and organic forms into their own protein and into the microbial cell wall components.

Faixova *et al.* (2016) concluded that feeding diets

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supplemented with selenium from organic or inorganic sources significantly increased the Se concentrations in the total rumen fluid and blood. Shahid *et al.* (2020) showed that dietary selenium yeast supplementation improved ruminal fermentation pattern, induced ruminal epithelial growth and increased GSH-Px activity in ruminal epithelium of goats.

So, keeping all this point in mind this experiment was carried out to assess the effect of different selenium sources on microbial biomass production and *in vitro* digestibility with the objective of arriving for optimum dose of different selenium in animal trial.

MATERIAL AND METHODS

Nano selenium synthesis

Nano Se was prepared using sodium selenite and ascorbic acid according to the modified method of Qian Le *et al*. (2010). Finally, the gray precipitant obtained was filtered and washed by water and ethanol, respectively.

Characterization of nano selenium

When the particle size is reduced to nano size, the properties of the materials are likely to be far different from the bulk materials. In the present study, Transmission electron microscopy, particle size analyser, and inductively coupled plasma mass spectrometry were used to analyse the properties like morphology, particle size distribution etc.

In vitro cytotoxicity assay

In vitro cytotoxicity assay was carried out in vero cell lines to ensure the safety of nano particle source of selenium as per the method of Mosmann, 1983. The parameters like per cent cell inhibition exhibited under different concentration of nano forms of selenium was studied.

In vitro dry matter digestibility

The rumen liquor needed for the experiment was collected in slaughter house from six sheep immediately prior to slaughter and brought to the laboratory by maintaining the temperature of rumen liquor at 39 ºC and under anaerobic condition during the transit. Ruminal fluid was filtered through 4 layers of muslin cloth and stored in pre warmed thermos container at 39°C till its use.

The basal diet used for the experiment had roughage: concentrate in the ratio of 60:40. Roughage used in this study was Bajra Napier hybrid grass (CO_4). The concentrate mixture had maize (48.8 per cent), Soya bean oilcake (21.6 per cent) de oiled rice bran (25.6 per cent), salt (1.6 per cent) and calcite (2.4 per cent). The roughage component was ground to pass through 1.0 mm sieve. The roughage: concentrate mixture was well mixed to form the basal diet. There is also addition of sodium selenite, nano selenium and selenocysteine at different levels.

The accurately weighed individual feed ingredients were subjected to *in vitro* gas production studies. 500 mg of feed in 40 ml of buffered rumen fluid were incubated in pre-warmed (40ºC) 100 ml calibrated glass syringes placed in an incubator as described by Menke *et al*. (1979).

An incubation period of 24 h was adopted for this study. At the end of the incubation, *in vitro* apparent dry matter digestibility (IVADMD), *in vitro* true dry matter digestibility (IVTDMD) and microbial biomass production were determined.

In vitro apparent digestibility of dry matter (IVADM) was determined as per Blummel *et al.* (1997_b). True digestibility was calculated as the weight of substrate incubated minus the weight of the residue after neutral detergent solution treatment.

The microbial biomass was calculated using the equation quoted by Blummel *et al.* (1997_a).

Microbial biomass = Substrate truly digested – Substrate apparently digested

STATISTICAL ANALYSIS

All the data were statistically analysed with analysis of variance (ANOVA) through the procedure of statistical analysis system (IBM SPSS version 22.0 for windows) as per Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

Nano selenium

The size and zeta potential of nano particle synthesized

from sodium selenite is 31.8 ± 2.31 nm and -33.1 ± 6.5 mV respectively while product yield is 40.97%. The synthesized nano selenium powder contains 98.62% selenium, free from impurities, nano in nature and spherical in shape. Result is presented in table 1.

Table 1: Product yield (recovery %), particle size, zeta potential and selenium content in nano form of selenium (Mean* ± SE)

Sl. No.	Characterization parameters	
1	Chemical name of source	Sodium selenite
2	Recovery (%)	40.97 ± 0.79
4	Size (assessed through particle size analyser) nm*	31.8 ± 2.31
5	Zeta potential (mV) *	-33.1 ± 6.50
6	Selenium content (%)	98.34 ± 2.80
7	Shape	Spherical

*Mean of six observations

Similarly, Zhang *et al.* (2017) synthesized nano selenium with mean particle size of 36.8±4.1 nm using beta lactoglobulin as a stabilizer in redox system of ascorbic acid and selenite. Gangadoo *et al.* (2017) use solution phase synthesis approach for selenium nanoparticles by reducing selenium tetrachloride in the presence of ascorbic acid. Nanoparticle spherical in shape and have size 46 nm.

In vitro cytotoxicity assay

In order to determine safe level of inclusion of nano selenium, *in vitro* cytotoxicity test was done on *in vero* cell line with concentrations, 0.25, 0.5, 1, 2, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 µg/ml for nano selenium. Based on the calculations, IC_{50} for nano selenium derived from selenium powder was 89.11 µg/ml. The IC_{50} is defined as the sample concentration that is required to reduce the absorbance to half that of the control and which would give the 50 % cell death. Since our inclusion level of nano selenium is 0.3 mg/kg and it revealed that the nano selenium is safe up to the level of 30 mg/kg to use as feed supplement as source of selenium in lamb ration.

Hashem *et al.* (2021) showed that IC50 of mycosynthesized Se-NPs was 316.73 µg/ml towards *Vero* cell line CCL-81. Salem *et al*. (2020) showed that IC_{50} value of selenium nanoparticle against two different cell cultures, namely; human normal lung fibroblast (Wi 38) and human cancer colorectal adenocarcinoma epithelial (Caco-2) was 171.8 and 104.3 µg/ml respectively,

In vitro dry matter digestibility

The results of the *in vitro* studies to assess effect of different forms of selenium on *in vitro* dry matter degradability (IVDMD) are presented in the table 2. From the results it can be inferred that when selenium is added at graded level to the basal diet in any form (inorganic, organic and nano) resulted in significant increase ($p<0.05$) in digestibility parameter such as *in vitro* apparent dry matter digestibility, *in vitro* true dry matter digestibility and microbial biomass production at all levels of addition compared to when no selenium was added. Rumen fermentation is a fundamental and special process of the ruminant metabolism and it determines the assimilation and intake of nutrients from ingested food.

Table 2: Effect of supplementing different forms of selenium at graded levels in basal diet on *in vitro* apparent dry matter digestibility, *in vitro* true dry matter digestibility and microbial biomass production by *In Vitro* Gas Production Technique (Mean*± SE)

	IVADMD %	IVTDMD %	Microbial biomass %	SEM	P Value
Control	29.67±1.44^{a}	37.20±1.56^{a}	7.53±0.13^{a}	1.21	0.21
Inorganic selenium (ppm)					
0.150	35.27±1.73^{ab}	55.06±1.51^{b}	19.80±2.51^{b}	1.43	0.04
0.225	35.47±2.21^{ab}	55.80±2.12^{b}	20.33±3.09^{b}	2.41	0.046
0.300	35.60±1.20^{ab}	56.00±2.89^{b}	20.40±1.86^{b}	2.52	0.049
0.375	36.00±2.30^{b}	56.53±1.79^{b}	20.53±3.80^{b}	1.45	0.023
0.450	37.00±1.73^{b}	56.27±2.08^{b}	19.27±0.37^{b}	1.80	0.03
Organic selenium (ppm)					
0.150	37.20±1.27^{b}	55.267±2.87^{b}	18.07±4.00^{b}	2.34	0.025
0.225	37.80±1.33^{b}	56.07±3.12^{b}	18.27±3.06^{b}	2.21	0.045
0.300	37.90±1.67^{b}	56.47±4.22^{b}	18.57±5.88^{b}	1.54	0.035
0.375	37.40±0.87^{b}	57.10±2.89^{b}	19.70±3.76^{b}	2.10	0.041
0.450	38.00±0.91^{b}	56.67±2.33^{b}	18.67±3.17^{b}	1.76	0.040
Nano selenium (ppm)					
0.150	37.80±0.58^{b}	56.33±3.84^{b}	18.53±3.88^{b}	2.54	0.034
0.225	38.00±0.42^{b}	57.17±2.80^{b}	19.17±2.24^{b}	1.34	0.042
0.300	38.20±0.42^{b}	57.73±3.70^{b}	19.53±3.81^{b}	1.88	0.02
0.375	38.60±0.42^{b}	58.67±2.33^{b}	20.07±2.19^{b}	1.11	0.033
0.450	39.00±1.15^{b}	57.67±4.10^{b}	18.67±2.96^{b}	0.67	0.044

Means with different superscripts within a column differ significantly ($p<0.05$); *Mean of six observations.

In this trial, Inorganic, Organic and Nano selenium at graded levels when added to basal diet significantly ($p<0.05$) increased *in vitro* apparent dry matter digestibility, *in vitro* true dry matter digestibility and microbial biomass production at all levels of addition compared to unsupplemented group.

Consistent with our result Shi *et al.* (2011) stated that addition of nano selenium to the basal diet of sheep significantly ($p<0.001$) increased the feed utilization and rumen fermentation pattern through improvement in nutrient digestibility, whereas Ibrahim *et al*. (2018) reported that Nano selenium supplementation at 0.3 mg/kg level improved nutrient digestibility, feeding value and growth performance in ossimi lambs compared to Sodium selenite and Selenium yeast form of supplementation.

CONCLUSION

The result of the present study indicated that spherical shaped, nano-selenium particles of size ranging 31.8 nm could be produced by wet chemical method at laboratory level. Based on the calculations, IC_{50} for nano selenium derived from selenium powder was 89.11 µg/ml. All the forms (Inorganic, Organic and Nano) of selenium supplementation resulted in significantly higher *in vitro* apparent dry matter digestibility (IVADMD), *in vitro* true dry matter digestibility (IVTDMD) and microbial biomass production compared to control. Hence, thus synthesized selenium nano particles could be used as a feed supplement for lambs as per the standard recommended dosage (0.3mg/kg of feed NRC, 2007 recommendation).

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