Conventional versus Green ZnO Nanoparticle Pre-treatment Effects on *Mucuna pruriens utilis* Seed Potential Nutritional Value for Beef Cattle

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Abstract

The utility of *Mucuna pruriens* utilis seed meal (MSM) in beef cattle nutrition is limited by high fibre content and antinutritional factors. Hence, this study investigated conventional (C-Nano-ZnO) and green (G-Nano-ZnO) ZnO nanoparticle pre-treatment effects (0, 1, 5, 10, 15, and 20 mg/kg) on the legume's nutritional composition and *in vitro* ruminal fermentation. Both nanoparticle types differentially increased DM, OM, and EE ($P < 0.001$) and tended to increase ash ($P = 0.055$) whilst they decreased NDF, ADF, ADL, cellulose ($P < 0.001$), and hemicellulose ($P < 0.01$) contents of MSM, with 20 mg/kg G-Nano-ZnO inducing greater effects. Also, they increased MSM's Ca ($P < 0.001$), P ($P < 0.001$), Mg ($P < 0.001$), S ($P < 0.001$), Fe ($P < 0.001$), and K ($P < 0.001$), with 20 mg/kg C-Nano-ZnO generally inducing greater effects. However, both nanoparticle types generally induced no effects on MSM's CP, Cr, Mn, Rb, gas production rate, in vitro ruminal gas production kinetics, and cumulative gas production ($P > 0.05$), except for increased 12 h post-incubation gas production rate induced by 20 mg/kg G-Nano-ZnO ($P < 0.001$) and decreased immediately degradable DM fraction a ($P < 0 .001$) and 12 h post-incubation cumulative gas production ($P < 0.001$) induced by, respectively, 20 mg/kg G-Nano-ZnO and 15 mg/kg C-Nano-ZnO. Interestingly, both nanoparticle types increased in vitro ruminal OM degradability and partitioning factors ($P < 0.001$), with 20 mg/kg G-Nano-ZnO inducing greater effects. In conclusion, pre-treatment with 20 mg/kg G-Nano-ZnO mostly enhanced potential nutritional value of MSM.

Keywords: *Mucuna pruriens utilis* seed meal, antinutritional factors, zinc oxide nanoparticles, *in vitro* ruminal fermentation, ruminants.

Introduction.

The global human population continues to rapidly expand creating an ever-growing demand for food especially in developing countries. Due to their natural ability to convert low-quality plant-derived feed resources into nutritious human-edible food (meat and milk), ruminants wield formidable potential to address food and nutrition security in these countries (Greenwood, 2021; Selvan et al., 2023). Beef meat, particularly, is a good source of essential human dietary nutrients especially proteins, minerals, and vitamins (Troy et al., 2016) and health-enhancing antioxidants (Asp et al., 2012; Williams, 2007). Notwithstanding, due to climate change-induced threats to forage quantity and nutritional quality in natural rangelands that serve as the predominant feed source for most beef cattle (Lamega et al., 2021; Tavirimirwa et al., 2019; Vetter et al., 2020), growing numbers of pasture-reared beef cattle in southern Africa are intensively finished off in feedlots where they are fed expensive energy and protein dense commercial diets composed mainly of maize and soyabean meal (DAF, 2019).

Unfortunately, the costs of these commercial diets are prohibitively too high especially for smallholder farmers due to both maize and soyabean being used also as human food, which aggravates the food-feed competition (Abidoye & Mabaya, 2014; Dezah et al., 2021; Sedibe et al., 2023) and climate change-induced drought diminution of the crops' yields (Conway et al., 2015; Shi et al., 2012; Stoddard, 2016). It is therefore necessary to explore cheaper alternative energy and protein rich feed ingredients such as MSM for beef cattle nutrition.

Mucuna pruriens utilis seed meal (MSM) is a nutritious annual legume rich in proteins (21 – 43% CP) and energy (43 – 65 % crude carbohydrate) (Sowdhanya et al., 2023; Theansungnoen et al., 2022). It has a high content of amino acids, minerals and essential fatty acids (Ezeagu et al., 2003; Pugalenthi et al., 2005; Sowdhanya et al., 2023) and possesses anabolic attributes with ability to enhance beef meat production (Alleman Jr et al., 2011; Lieu et al., 2012; Suresh & Prakash, 2012; Yantika et al., 2016). It is derived from drought-tolerant *M. pruriens utilis* plants that are highly adapted to various abiotic stresses (Buckles et al., 1998) and a desirable animal feed resource in this era of climate change-associated recurrent droughts and feed shortages.

However, the nutritional value of MSM is constrained by the presence of relatively high levels of fibre (97 – 193 g/kg DM) (Mthiyane et al., 2018; Pathania et al., 2020) and potentially toxic secondary metabolites [i.e., trypsin inhibitors, haemagglutinins, hydrogen cyanide, phytic acid, tannins, saponins, amylase inhibitors, and 3,4-dihydroxy-L-phenylalanine (L-DOPA)] (Bindu et al., 2023; Deli et al., 2020). A high level of fibre in ruminant diets, inter alia, compromises ruminal fermentative degradation rate, nutrient digestibility, voluntary feed intake, and growth (Jiyana et al., 2022; Tafaj et al., 2005). Of the many antinutritional factors (ANFs) in MSM, L-DOPA's high concentration (4 – 13%) is the major impediment hindering extensive utilization of the indigenous legume (Pulikkalpura et al., 2015). Consequently, complete dietary replacement of soya bean meal with MSM induced adverse effects on dry matter intake in growing lambs (Loyra-Tzab et al.,

2013; Pérez-Hernández et al., 2003). Also, dietary intake of MSM decreased body weight gain in goats (Madzimure et al., 2014) and lambs (Chikagwa-Malunga et al., 2009). Recently, Gamedze et al. (2024a) demonstrated compromised body weight gain, feed intake, feed conversion efficiency, hot carcass weight, cold carcass weight, as well as some haematological variables in beef steers fed diets supplemented with 20% MSM. Hence, there is a necessity for an innovative pre-treatment strategy to address the problem of high fibre and ANFs in MSM prior to its use in beef cattle nutrition.

A growing body of literature has demonstrated nanotechnology to possess enormous ability to enhance the nutritional utility of plant-derived feed resources bedevilled by fibre and ANFs. In this regard, conventionally synthesized nanoparticles have been shown to enhance ruminal degradation of fibre (Chen JunCai et al., 2011; Hosseini-Vardanjani et al., 2020; Shi et al., 2011) and to adsorb and enhance biodegradation of toxic phytochemicals and other biomolecules (Asghar et al., 2020; Remya et al., 2022). In our laboratory, biogenic zinc oxide (ZnO) nanoparticles (G-Nano-ZnO) have been synthesized following the green approach employing *M. pruriens utilis* seed extract as nanoparticle reducing and stabilizing agent.

This type of nanoparticles was characterized, validated and demonstrated to be biologically safe for application in biological systems (Gamedze et al., 2024b). Green nanoparticles synthesized particularly using plant extracts are generally considered to be biologically safe and compatible, economically sustainable, environmentally friendly, and energy efficient (Sardjono et al., 2018). There was therefore interest in evaluating the efficacy of the green G-Nano-ZnO in enhancing the nutritional value of MSM when compared with the conventionally synthesized C-Nano-ZnO. The study hypothesized that MSM pre-treatment with G-Nano-ZnO and C-Nano-ZnO would enhance the legume's nutritional value for beef cattle. Its objective was thus to investigate comparative effects of the two nanomaterials in enhancing the nutritional composition and in vitro ruminal degradability of MSM.

Materials and methods

Animal ethics

It is confirmed that animal management and experimental procedures were carried out following the Guidelines for Care and Use of Laboratory Animals of the Animal Production Research Ethics Committee of North-West University (NWU) (Ethics Number: NWU-00804-22-A5).

Sample acquisition and preparation and experimental design

Dehulled *M. pruriens utilis* seeds were purchased from AGT Foods Africa (Pty) Ltd (Krugersdorp, South Africa) and milled (1 mm) using a milling machine (Polymix-MFC 90D, Switzerland). C-Nano-ZnO (< 50 nm) was purchased from Merck (Pty) Ltd (Modderfontein, South Africa) whilst G-Nano-ZnO was biosynthesized using *M. pruriens utilis* seed extract at North-West University (NWU) Department of Chemistry's Nanomaterials Laboratory (Mahikeng, South Africa) (Gamedze et al., 2024b). Then MSM was pre-treated by homogeneously mixing it (w/w) with C-Nano-ZnO and G-Nano-ZnO at, respectively, 0, 1, 5, 10, 15, and 20 mg/kg application rates of the nanomaterials in a

completely randomized design. Upon pre-treatment, replicate samples of 3 per treatment were stored in labelled bags in a cool dry place for 3 days pending analysis.

Proximate and mineral analyses

The proximate components were determined according to the Official Analytical Chemists Methods (AOAC, 2019) for DM (method no. 930.15), OM (method no. 942.05), CP (method no. 984.13) and ash (method no. 942.05). The detergent fibre system of analysis described by van Soest et al. (1991) was used to determine NDF and ADF contents using the ANKOM2000 Fiber Analyzer (ANKOM Technology, Macedon, NY, USA). Both NDF and ADF were expressed inclusive of residual ash. Acid detergent lignin (ADL) was determined by digesting ADF residues in 72% sulphuric acid for 3 h and then oven-drying them for 2 h. Hemicellulose (HEM) and cellulose (CELL) values were obtained from the differences of NDF and ADF as well as ADF and ADL, respectively. The Soxhlet method was used to determine the ether extract (EE) (AOAC, 2019). The mineral content was analyzed at the Center for Applied Radiation Science and Technology of NWU (Mahikeng, South Africa) using an Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) (Perkin-Elmer, 1982, NexION 300Q, Waltham, USA) following the AOAC method (no. 968.08) (King & Sheridan, 2019).

In vitro ruminal fermentation

The *in vitro* ruminal fermentation experiment was conducted at NWU Molelwane Experimental Farm (25°40.459′ S; 26°10.563′ E) (Mahikeng, South Africa). The Reading Pressure Technique (RPT) of Mauricio et al. (1999) was used to measure in vitro ruminal fermentation dynamics of pre-treated MSM. The rumen inoculum was collected in the morning before feeding from a rumen-cannulated Bonsmara donor cow that was handled, fed [*ad libitum* fresh and clean water plus lucerne (Medicago sativa) hay and blue buffalo grass (Cenchrus ciliaris)], and cared for according to protocols approved (NWU-00804-22-A5) by the NWU Animal Production Research Ethics Committee.

The rumen fluid was immediately collected into pre-warmed (39 °C) thermal flasks under a stream of CO_2 gas and then taken to the laboratory for blending and straining through a white two-layered muslin cloth while being purged with CO_2 to mimic anaerobic conditions of the rumen (Mhlongo et al., 2021). In triplicates, 1 g (+ 0.002) samples of MSM pre-treated with different concentrations of C-Nano-ZnO and G-Nano-ZnO were weighed and added into 125 mL serum bottles, to which were added 90 mL of ANKOM buffer solution [i.e., in g/L: 10.0 potassium dihydrogen phosphate (KH_2PO_4), 0.5 magnesium sulfate heptahydrate ($MgSO4.7H_2O$), 0.5 sodium chloride (NaCl), and 0.1 calcium chloride dihydrate ($CaCl_2.2H_2O$) (Solution A) as well as 15.0 sodium carbonate (Na_2CO_3) and 1.0 sodium sulfide nonahydrate ($Na_2S.9H_2O$) (Solution B), that were then mixed at 5 parts Solution A plus 1 part Solution B (Holden, 1999)]. The bottled samples in ANKOM buffer were then purged with CO_2 followed by rubber stopper sealing of bottles and their incubation (39˚C) overnight. Next morning, the bottle contents were each inoculated with 10 mL rumen fluid through rubber stoppers pierced with a syringe and then transferred into an incubator (39 °C) for 96

h. Additionally, blank serum bottles with buffered rumen inoculum without MSM samples were also incubated at 39 °C. Headspace gas pressure readings were taken at 2, 4, 6, 8, 12, 24, 36, 48, 72 and 96 h post-inoculation by inserting an 18 g needle fitted to a pressure transducer (PX4200-015GI, Omega Engineering Inc., Canada) with a microprocessor that recorded peak gas pressure (psi).

The peak gas pressure readings (psi) were converted to gas volume (mL) using the site-determined equation generated through a calibration process by Mhlongo et al. (2021): y=0.034x^2+6.2325x+1.8143, where y is gas volume (mL) and x is measured gas pressure (psi). Readings from two blank serum bottles were used to correct the gas readings. The rate of gas production per period was calculated by dividing gas volume produced at each period by the corresponding total number of hours.

Data for cumulative gas production were fitted (Datafit 9.0, Oakdale Engineering) into the non-linear model (Ørskov & McDonald, 1979) to estimate fermentation kinetics following the equation: y=a+b(1-e^(-c(t))), where a = gas production from the immediately fermentable fraction, b = gas production from the slowly fermentable fraction, and c = gas production rate constant for the insoluble fraction b. Gas production potential (Pgas) was calculated as the sum of fractions (a+b) whilst effective gas production (Egas) was calculated using the equation: Egas=a+(b×c)/(K+C), where Kis the assumed 2% rumen outflow rate per hour.

In vitro ruminal organic matter degradability

The *in vitro* ruminal OM degradability (ivOMD) of MSM samples was determined using the ANKOM DaisyII incubator consisting of a thermostatic (39 ℃) chamber with 4 jars according to ANKOM Technology method no. 3 for the in vitro true digestibility (ANKOM Technology Corp., Fairport, NY). The samples (0.45 – 0.5 g) were weighed into ANKOM F57 filter bags, heat sealed and placed in ANKOM DaisyII jars to which 1600 mL of pre-warmed buffer solution (39 ℃) described above was added. The collection and processing of the rumen inoculum was as described above. Inoculation was performed by adding 400 mL of the processed rumen fluid into the pre-warmed buffer solution in each DaisyII incubator jar (Mnisi & Mlambo, 2017).

The Daisy jars were then continually purged with CO2 gas to maintain anaerobic conditions prior to them being closed and placed in the incubation chamber (39˚C). The incubation elapsed over a maximum period of 48 h with periodic (12, 24, 36, and 48 h) withdrawals of the bags to determine DM degradability. The withdrawn bags were washed with cold tap water for 15 min, dried in an oven at 105 °C for 12 h, and then incinerated at 550 ℃ to determine ivOMD. Zero hour (0 h) un-incubated samples were washed and oven-dried similarly as incubated samples. Partitioning factors (PFs; g/mL), as a measure of fermentation efficiency, were computed from the ratio of ivOMD to cumulative gas production at 12, 24, 36, and 48 h post-incubation (Cudjoe & Mlambo, 2014; Mhlongo et al., 2022; Tefera et al., 2008).

Statistical analysis

In a completely randomised design, one-way Analysis of Variance (ANOVA) (PROC GLM; SAS, 2013) was employed for the analysis of data on MSM's nutritional composition and in vitro ruminal fermentation, with nanoparticle type as the only factor. To assess the nanoparticle type by nanoparticle concentration (NT x NC) interaction effects on the above-mentioned dependent variables, all data were analysed without the least square means (LSMeans) for the control treatment (i.e., 0 mg/kg) using the model: $Yijk = u + Ni + Cj + (N \times C)ij + Eijk$, where Yijk = response variable, μ = overall mean, Ni = effect of nanoparticle type, Cj = effect of nanoparticle concentration, (N × C)ij = NT x NC interaction effect, and Eijk = random error. For all statistical tests, significance was declared at $P < 0.05$. Where significance was observed, LSMeans were compared using the probability of difference option.

Results

Chemical composition

The proximate composition of MSM pre-treated with or without C-Nano-ZnO and G-Nano-ZnO is shown in Table 1. Both C-Nano-ZnO and G-Nano-ZnO increased the DM ($P < 0.001$) and OM ($P < 0.001$) contents of MSM, with the 15 mg/kg of the green nanoparticles inducing greater effect resulting to the NT x NC interaction effect on these parameters ($P < 0.001$), respectively. Similarly, C-Nano-ZnO and G-Nano-ZnO tended to increase the ash content ($P = 0.055$) of MSM, with 20 mg/kg of green nanoparticles causing greater effect leading to the NT x NC interaction effect on this parameter ($P < 0.001$).

Also, both types of nanoparticles increased the EE content ($P < 0.001$) of MSM, with 10 mg/kg of the biogenic nanomaterials inducing greater effect thus inducing the NT x NC interaction effect on this parameter ($P < 0.001$). In contrast, both C-Nano-ZnO and G-Nano- ZnO decreased the NDF ($P < 0.001$), ADF ($P < 0.001$), ADL ($P < 0.001$), cellulose ($P < 0.001$), and hemicellulose ($P < 0.01$) contents of MSM, with the 20 mg/kg of the phyto-mediated nanoparticles inducing greater effect (except in the case of hemicellulose for which the greatest decrease occurred with the 15 mg/kg of the nanoparticles) resulting to the NT x NC interaction effect on the fibre parameters ($P < 0.001$).

Intriguingly, there were no effects of both C-Nano-ZnO and G-Nano-ZnO pre-treatments on the CP content of the indigenous seed meal ($P > 0.05$) except for the increase in this parameter induced by the 15 mg/kg of the conventional nanomaterials, resulting to a NT x NC interaction effect on the parameter ($P < 0.01$). The effect of MSM pre-treatment with or without C-Nano-ZnO and G-Nano-ZnO on macro- and micro-mineral composition is shown in Table 2. The results showed no particularly clear trend in terms of the effect of the nanoparticles on the minerals. Nevertheless, they generally showed that 20 mg/kg of C-Nano-ZnO induced the highest increase in the MSM concentrations of Ca ($P < 0.001$), P ($P < 0.001$), Mg ($P < 0.001$), S ($P < 0.001$), and Fe ($P < 0.001$). Regarding K, C-Nano-ZnO induced the highest increase in this parameter at 15 mg/kg application rate ($P < 0.001$). Otherwise, there was no effect of the nanoparticles on Cr, Mn, and Rb contents of MSM ($P > 0.05$).

Table 1. Proximate composition (g/kg DM) of mucuna seed meal pre-treated with or without C-Nano-ZnO and G-Nano-ZnO.

Nano type	Nano (mg/kg)	DM	OM	CP	Ash	EE	NDF	ADF	ADL	HEM	CELL
C-Nano-ZnO	0 (Control)	900.09^{d}	868.79^{d}	232.69^{bc}	32.13^{h}	31.38^{d}	225.30^{a}	122.68^{a}	25.51^{a}	102.62^{a}	97.17^{a}
	1	915.31^{cd}	882.58^{cd}	231.04^{c}	32.72^{gh}	33.46^{cd}	203.78^{b}	124.23^{a}	24.80^{b}	79.55^{bc}	99.43^{a}
	5	928.12^{bc}	894.83^{bc}	227.70^{c}	33.29^{ef}	34.29^{cd}	183.97^{b}	110.90^{b}	17.12^{b}	73.07^{c}	93.78^{ab}
	10	914.78^{cd}	880.99^{cd}	234.61^{abc}	33.79^{de}	39.80^{b}	150.36^{d}	94.84^{c}	10.95^{c}	55.52^{d}	83.89^{c}
	15	927.73^{bc}	893.23^{bc}	244.93^{a}	34.50^{bcd}	34.34^{cd}	126.73^{e}	95.94^{c}	8.40^{cd}	30.79^{fg}	87.54^{bc}
	20	924.89^{c}	890.15^{cd}	242.15^{ab}	34.74^{bc}	36.35^{c}	102.27^{fg}	64.22^{e}	8.42^{cd}	38.06^{ef}	55.79^{e}
G-Nano-ZnO	1	914.87^{cd}	881.69^{cd}	230.66^{c}	33.18^{ef}	33.45^{cd}	203.38^{b}	114.75^{b}	16.11^{b}	88.64^{ab}	98.64^{a}
	5	933.45^{bc}	899.93^{bc}	238.31^{abc}	33.53^{efg}	36.17^{c}	173.66^{b}	94.24^{c}	14.12^{b}	79.42^{bc}	80.12^{c}
	10	947.93^{b}	914.83^{b}	232.11^{bc}	33.10^{feg}	46.80^{a}	114.13^{ef}	95.69^{c}	8.46^{cd}	18.44^{g}	87.23^{bc}
	15	978.51^{a}	944.41^{a}	236.40^{abc}	34.10^{bcd}	35.88^{c}	92.78^{gh}	73.67^{d}	8.51^{cd}	19.10^{g}	65.16^{d}
	20	948.27^{b}	913.27^{b}	236.11^{abc}	35.00^{a}	36.04^{c}	83.33^{h}	36.89^{f}	6.14^{d}	46.44^{de}	30.75^{f}
SEM		4.30	4.29	2.09	0.20	0.63	2.54	1.28	0.62	2.90	1.49
***P*-value**											
NT		<0.001	<0.001	0.149	0.055	<0.001	<0.001	<0.001	<0.001	0.002	<0.001
NC		<0.001	<0.001	0.0003	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
NT×NC		<0.001	<0.001	0.002	0.0002	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[a-h] In a column, different superscripts denote significant differences ($P < 0.05$); ADF: acid detergent fibre; ADL: acid detergent fibre; C-Nano-ZnO: conventional zinc oxide nanoparticles; CELL: cellulose; DM: dry matter; EE: ether extract; HEM: hemicellulose; G-Nano-ZnO: green-synthesized zinc oxide nanoparticles; NC: nanoparticle concentration; NDF: neutral detergent fibre; OM: organic matter; NT: nanoparticle type; SEM: standard error of the mean.

Table 2. Macro- and micro-mineral composition (mg/kg DM) of mucuna seed meal pre-treated with or without C-Nano-ZnO and G-Nano-ZnO.

NanoType	Nano (mg/kg)	Ca	P	K	Mg	S	Cu	Fe	Mn	Cr	Rb
	0	6.51^{d}	2.75^{b}	65.52^{d}	1.47^{b}	3.62^{c}	0.03^{bcd}	0.31^{b}	0.02	0.02	0.04
C-Nano-ZnO	1	5.04^{g}	2.24^{de}	67.41^{c}	1.13^{d}	2.62^{g}	0.04^{abc}	0.09^{d}	0.02	0.01	0.03
	5	4.31^{i}	1.74^{f}	52.29^{e}	0.78^{f}	1.93^{i}	0.03^{cd}	0.17^{dc}	0.02	0.01	0.02
	10	5.15^{f}	2.29^{de}	65.38^{d}	1.09^{d}	2.84^{e}	0.03^{bcd}	0.24^{bc}	0.02	0.01	0.03
	15	7.33^{c}	2.29^{de}	71.42^{a}	1.34^{c}	2.74^{f}	0.04^{ab}	0.23^{bc}	0.02	0.01	0.03
	20	9.23^{a}	3.41^{a}	65.40^{d}	2.09^{a}	6.62^{a}	0.03^{bcd}	0.42^{a}	0.02	0.02	0.04
G-Nano-ZnO	1	6.53^{d}	2.67^{bc}	65.67^{d}	1.46^{b}	3.53^{d}	0.02^{d}	0.31^{b}	0.02	0.02	0.03
	5	4.54^{h}	2.23^{de}	68.28^{bc}	1.06^{d}	2.63^{g}	0.04^{abcd}	0.42^{a}	0.02	0.03	0.03
	10	7.82^{b}	2.45^{cd}	68.66^{b}	1.33^{c}	3.52^{d}	0.04^{abc}	0.24^{bc}	0.02	0.01	0.04
	15	3.66^{j}	2.18^{e}	68.41^{b}	0.94^{e}	2.43^{h}	0.03^{abcd}	0.23^{bc}	0.02	0.01	0.03
	20	6.36^{e}	2.84^{b}	68.33^{b}	1.47^{b}	3.72^{b}	0.04^{a}	0.33^{ab}	0.02	0.01	0.03
SEM		0.02	0.04	0.18	0.02	0.01	0.002	0.02	0.002	0.01	0.004
***P*-value**											
NT		<0.001	<0.001	<0.001	0.007	<0.001	0.014	<0.001	0.859	0.219	0.498
NC		<0.001	<0.001	<0.001	<0.001	<0.001	0.029	<0.001	0.370	0.590	0.119
NT×NC		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.164	0.385	0.711

[a-j] In a column, different superscripts denote significant differences ($P < 0.05$); C-Nano-ZnO: conventional zinc oxide nanoparticles; Ca: Calcium; Cr: Chromium; Cu: Copper; Fe: Iron; G-Nano-ZnO: green-synthesized zinc oxide nanoparticles; K: Potassium; Mg: Magnesium; Mn: Manganese; NC: nanoparticle concentration; NT: nanoparticle type; P: Phosphorus; Rb: Rubidium; S: Sulphur; SEM: standard error of the mean.

Table 3 *In vitro* ruminal rate of gas production per period (mL/h) from mucuna seed meal pre-treated with or without C-Nano-ZnO and G-Nano-ZnO at 48 h of incubation.

		Incubation time (h)			
NanoType	Nano (mg/kg)	12	24	36	48
C-Nano-ZnO	0	4.84[b]	4.04	3.58	1.69
	1	5.07[ab]	4.21	2.88	1.72
	5	5.37[ab]	4.13	2.77	1.34
	10	5.32[ab]	3.14	2.66	1.74
	15	2.64[c]	4.21	2.74	1.85
	20	5.81[a]	4.14	2.66	1.77
G-Nano-ZnO	1	4.91[b]	4.54	2.94	1.69
	5	5.30[ab]	4.43	2.75	1.69
	10	4.87[ab]	4.20	2.78	1.66
	15	4.85[b]	4.09	2.89	1.82
	20	5.18[ab]	4.34	1.53	1.77
SEM		0.19	0.31	0.34	0.13
P-value:					
NT		0.48	0.10	0.21	0.31
NC		<0.001	0.38	0.08	0.22
NT ×NC		<0.001	0.61	0.42	0.55

[a-c] In a column, different superscripts denote significant differences ($P < 0.05$); C-Nano-ZnO: conventional zinc oxide nanoparticles; G-Nano-ZnO: green-synthesized zinc oxide nanoparticles; NC: nanoparticle concentration; NT: nanoparticle type; SEM: standard error of the mean.

Table 4 *In vitro* ruminal gas production kinetics (mL/g OM, unless indicated otherwise) of mucuna seed meal pre-treated with or without C-Nano-ZnO and G-Nano-ZnO at 48 h of incubation.

		In vitro Kinetics					
NanoType	Nano (mg/kg)	*a*	*b*	*c*	*lt (%/h)*	*PGas*	*EGas*
C-Nano-ZnO	0	11.12[ab]	171.50	0.03	2.90	182.62	118.83
	1	12.97[a]	169.95	0.04	2.69	182.93	121.51
	5	12.16[a]	161.94	0.04	2.66	174.10	118.23
	10	12.99[a]	155.62	0.03	2.27	168.61	111.49
	15	11.78[a]	162.42	0.03	2.83	174.20	111.87
	20	11.31[ab]	164.93	0.04	2.89	176.24	117.54
						182.48	119.94
G-Nano-ZnO	1	9.82[ab]	172.68	0.04	2.80	182.50	120.14
	5	11.68[a]	168.53	0.04	2.77	180.20	121.54
	10	11.96[a]	170.96	0.04	2.69	182.92	121.62
	15	9.99[ab]	179.50	0.03	2.77	189.49	123.24
	20	7.64[b]	150.82	0.04	2.87	158.46	106.74
SEM		1.270	7.053	0.001	0.16	7.14	4.08
***P*-value**							
NT		0.036	0.278	0.226	0.478	0.490	0.340
NC		0.296	0.330	0.055	0.217	0.265	0.265
NT ×NC		0.595	0.291	0.900	0.566	0.242	0.242

[a-b] In a column, different superscripts denote significant differences ($P < 0.05$); *a* = immediately degradable fraction; *b* = slowly degradable fraction; *c* = degradation rate of fraction; C-Nano-ZnO: conventional zinc oxide nanoparticles; *Egas* = effective gas; G-Nano-ZnO: green-synthesized zinc oxide nanoparticles; *lt*: lag time; NC: nanoparticle concentration; NT: nanoparticle type; *Pgas* = potential gas; SEM: standard error of the mean.

Table 5 *In vitro* ruminal organic matter degradability (mg/g OM), cumulative gas production (mL/g OM) and partitioning factors (mg/mL) of mucuna seed meal pre-treated with or without C-Nano-ZnO and G-Nano-ZnO.

NT	Nano (mg/kg)	OMD12	OMD24	OMD36	OMD48	12 h	24 h	36 h	48 h	PF12	PF24	PF36	PF48
C-Nano-ZnO	0	455.09^{f}	493.85^{f}	525.07^{h}	924.55^{b}	56.49^{a}	94.66	131.80	149.34	8.64^{cd}	5.22^{bc}	4.02^{e}	6.25^{ab}
	1	443.78^{g}	484.77^{f}	549.77^{g}	767.74^{f}	58.34^{a}	102.60	132.90	150.97	7.61^{d}	4.73^{c}	4.14^{de}	5.09^{b}
	5	494.72^{e}	521.12^{e}	638.29^{e}	780.39^{f}	58.39^{a}	102.44	131.99	146.26	8.48^{cd}	5.09^{bc}	4.84^{cde}	5.34^{b}
	10	524.50^{d}	644.45^{bc}	744.86^{c}	844.27^{de}	59.29^{a}	92.32	120.26	138.61	8.88^{cd}	7.10^{a}	6.28^{ab}	6.17^{ab}
	15	548.26^{c}	636.58^{dc}	798.43^{b}	855.55^{de}	46.32^{b}	91.34	120.56	140.37	11.88^{ab}	6.97^{a}	6.63^{ab}	6.10^{ab}
	20	552.65^{c}	667.52^{a}	834.24^{a}	907.43^{bc}	55.86^{ab}	99.89	128.19	146.99	9.97^{c}	6.75^{a}	6.55^{ab}	6.20^{ab}
G-Nano-ZnO	1	457.18^{f}	496.43^{f}	552.33^{g}	838.21^{e}	53.71^{ab}	101.43	132.37	150.12	8.53^{cd}	4.90^{bc}	4.18^{de}	5.59^{b}
	5	521.02^{d}	532.58^{e}	615.29^{f}	874.09^{cd}	57.39^{a}	104.93	134.43	152.53	9.09^{cd}	5.08^{bc}	4.58^{cde}	5.73^{b}
	10	526.90^{d}	619.14^{d}	714.14^{d}	873.20^{d}	57.389^{a}	103.26	133.57	151.68	9.18^{cd}	6.00^{abc}	5.35^{bcd}	5.76^{b}
	15	572.45^{b}	628.40^{dc}	738.11^{c}	911.30^{b}	55.53^{ab}	101.69	134.27	154.77	10.32^{bc}	6.18^{ab}	5.50^{bc}	5.89^{b}
	20	628.05^{a}	662.72^{ab}	847.09^{a}	975.29^{a}	49.70^{ab}	97.12	113.83	133.14	12.68^{a}	6.83^{a}	7.49^{a}	7.35^{a}
SEM		1.81	3.79	3.97	6.71	1.940	3.608	5.191	5.539	0.36	0.26	0.26	0.25
***P*-value**													
NT		<0.001	0.260	<0.001	<0.001	0.920	0.056	0.476	0.304	0.064	0.041	0.153	0.140
NC		<0.001	<0.001	<0.001	<0.001	0.005	0.311	0.209	0.426	<0.001	<0.001	0.0001	0.0001
NT×NC		<0.001	0.001	<0.001	<0.001	0.006	0.311	0.108	0.157	0.0002	0.111	0.007	0.050

[a-h] In a column, different superscripts denote significant differences ($P < 0.05$); C-Nano-ZnO: conventional zinc oxide nanoparticles; G-Nano-ZnO: green-synthesized zinc oxide nanoparticles; NC: nanoparticle concentration; NT: nanoparticle type; OMD: organic matter degradability; PF: partitioning factors; SEM: standard error of the mean.

In vitro ruminal rate of gas production and dry matter degradation kinetics

The effects of MSM pre-treatment with or without C-Nano-ZnO and G-Nano-ZnO on *in vitro* ruminal rate of gas production over 48 h as well as ruminal gas production kinetics at 48 h of incubation are shown in Tables 3 and 4. The results showed that neither C-Nano-ZnO nor G-Nano-ZnO affected the rate of gas production at 24, 36 and 48 h ($P > 0.05$). However, C-Nano-ZnO at 20 mg/kg increased the rate of gas production at 12 h post-incubation resulting to the NT x NC interaction effect on this parameter ($P < 0.001$). It was also noted that the rate of gas production was lowest at 15 mg/kg of C-Nano-ZnO (Table 3). Table 4. shows that both C-Nano-ZnO and G-Nano-ZnO pre-treatment of MSM did not affect all in vitro ruminal gas production kinetic parameters ($P > 0.05$), except for the green nanoparticle-induced decrease in the immediately degradable DM fraction a at 20 mg/kg application rate ($P < 0 .001$).

In vitro ruminal fermentation

The effects of nanoparticle pre-treatment of MSM on ivOMD, cumulative gas production and PFs are presented in Table 5. The results showed that the nanoparticles increased ivOMD at all incubation times especially the green ones particularly at 20 mg/kg application rate ($P < 0.001$). Regarding cumulative gas production, there was no effect of nanoparticle pre-treatment on this parameter at all incubation times ($P > 0.05$). However, it was observed that this was decreased by the 15 mg/kg of C-Nano-ZnO at 12 h post incubation, resulting to the NT x NC interaction effect on this parameter ($P < 0.001$). Table 5 further shows no effect of nanoparticle pre-treatment of MSM on PFs at all incubation times ($P > 0.05$), except at 24 h post-incubation whereby the conventional nanoparticles increased this parameter particularly at 10 mg/kg application rate ($P < 0.05$), though this did not significantly differ from the response at 20 mg/kg

application rate of the green nanomaterials ($P > 0.05$). Notwithstanding, results showed the highest responses of PFs at 20 mg/kg application rate of G-Nano-ZnO at all incubation times ($P < 0.001$) leading to NT x NC interaction effects on this parameter ($P < 0.05$), except at 24 h post-incubation ($P > 0.05$).

Discussion

This study investigated comparative effects of C-Nano-ZnO and G-Nano-ZnO pre-treatment on the nutritional composition and in vitro ruminal degradability of MSM for utilisation in beef cattle nutrition. The observed increased DM, OM, ash, EE and mineral (Ca, P, Mg, S, and Fe) in contrast to decreased fibre (NDF, ADF, ADL, cellulose, and hemicellulose) contents of MSM following its pre-treatment with nanoparticles, particularly the green ones, is unprecedented and there are currently no comparable studies in the literature. However, the results suggest interaction of the nanomaterials probably with endophytic microbiota leading to physiological dynamics within *M. pruriens utilis* seeds. Indeed, plant seeds naturally bear a plethora of bacterial, fungal, viral and oomycete endophytes that facilitate seed germination, promote seed conservation (Rodríguez et al., 2018; Shearin et al., 2018), and enhance plant survival, growth and defence (Rudgers et al., 2009; Shade et al., 2017). These seed-borne endophytes secrete numerous enzymes including amylases, esterases, lipases, protease, phytases, xylanases, cellulases, hemicellulases, ligninases, ß-glucosidases, endopoly galactunorases, pectinases, and others (Khan et al., 2017) and can influence the plant including its seeds' physiological responses to nanoparticle pre-treatment (Minchitha et al., 2024). Their community abundance, diversity, stability as well as metabolite profile can be enhanced by nanoparticles (Shang et al., 2023; Fu et al., 2024). Thus, it is therefore speculated that MSM pre-treatment with nanoparticles in this study altered the seed endophytic microbiota and its enzymatic profiles leading to improved seed cellular metabolic processes. Indeed, Minchitha et al. (2024) observed increased chlorophyll, proline and antioxidant contents in seedlings from endophyte-colonized maize seeds pre-treated with iron-modified multiwalled carbon nanotubes. Also, increased soyabean leaf and pod dry weight was observed following seed pre-treatment with iron oxide nanoparticles (Sheykhbaglou et al., 2010). The speculated nanoparticle-induced alterations in seed endophytic microbiota and fibrolytic enzymes would also explain the observed nanoparticle-induced decrease in NDF, ADF, ADL, cellulose, and hemicellulose contents of MSM.

Bio-synthesized using *M. pruriens utilis* seed extract as phytochemical source for nanoparticle reduction and stabilization (Gamedze et al., 2024b), G-Nano-ZnO would have been encapsulated with generous amounts of seed-derived nutraceutical compounds that would have bolstered the DM and nutritional content of MSM. It is probably for this reason that high application rates (10 to 20 mg/kg) of G-Nano-ZnO induced greater increases in DM, OM, ash, EE and mineral as well as decreases in NDF, ADF, ADL, cellulose, and hemicellulose contents of MSM. In this regard, higher concentrations of the nanomaterials would have translated to greater amounts of encapsulated nutraceutical compounds and endophytic

microbiota secretion of fibrolytic enzymes. However, C-Nano-ZnO and G-Nano-ZnO apparently induced differential effects on the nutritional physiology of *M. pruriens utilis* seeds considering the observed increase in CP and K contents of MSM when pre-treated only with 15 mg/kg of the former nanoparticles.

This study also measured *in vitro* ruminal rate and extent of gas production over 48 h in MSM pre-treated with C-Nano-ZnO and G-Nano-ZnO. These measurements are indicative of ruminal feed degradability (digestibility) and microbial protein synthesis (Elahi et al., 2014; Elghandour et al., 2016). Our results demonstrated lack of effect of both nanoparticle types on the rate of gas production at 24, 36 and 48 h in contrast to the increase in this parameter at 12 h post-incubation consequent to MSM pre-treatment with 20 mg/kg C-Nano-ZnO. These data are in agreement with the observation of lack of effect of both nanomaterial types on all in vitro ruminal gas production kinetic parameters with the exception of the decrease in the immediately degradable DM fraction a induced by 20 mg/kg of the biogenic nanoparticles. Combined, these results suggest varied effects of C-Nano-ZnO and G-Nano-ZnO on early microbial colonizers of MSM, particularly its immediately degradable DM fraction a.

It is known that ruminal microbial biofilms that digest feed particles evolve with time in three successive populations (Huws et al., 2016; McAllister et al., 1994). The first population is composed of loosely associated bacteria that utilize water-soluble proteins and sugars during early digestion and is followed by more stable, mature, diverse and tightly associated populations that ferment plant cell wall materials and starch and, lastly, the tertiary yet less diverse utilizers of fermentation end-products (Watnick and Koltr, 2000; Yang et al., 2018). Our data suggest that C-Nano-ZnO particularly at 20 mg/kg application rate favoured the early bacterial colonizers whilst G-Nano-ZnO had detrimental effects on this population leading to the observed decrease in the immediately degradable DM fraction. Whilst C-Nano-ZnO has been shown to decrease gut bacterial community diversity in chickens (Feng et al., 2017), dietary consumption of this type of nanoparticles even at as high dietary inclusion level as 3000 mg/kg has generally elicited beneficial effects on enteric microbiota (Pei et al., 2019). This may therefore account for the observed increase in the rate of gas production only at 12 h post-incubation of MSM pre-treated with 20 mg/kg C-Nano-ZnO.

On the other hand, G-Nano-ZnO has been demonstrated to induce toxicity to gut bacteria mediated through microbial growth inhibition (Zhou et al., 2021) consequent to dissolution of the nanoparticles into free Zn^{2+} ions and induction of oxidative stress (von Hellfeld et al., 2020). Having been synthesized using *M. pruriens utilis* seed extract (Gamedze et al., 2024b), these biogenic nanomaterials would have been encapsulated with L-DOPA, the toxic phytochemical abundant in the seeds (Huisden et al., 2019), and likely other ANFs. Together, these ANFs would have augmented the free Zn2+ ion-derived toxicity to the early bacterial colonizers of MSM. This may thus explain the observed decrease in the immediately degradable

DM fraction of MSM upon pre-treatment of the legume seed meal with 20 mg/kg of the bio-fabricated nanoparticles. There is obviously a need for investigation of effects of MSM pre-treatment with both C-Nano-ZnO and G-Nano-ZnO on in vitro ruminal microbial diversity and abundance in future studies.

Increased ivOMD suggests improved bacterial fermentative degradation of complex substrates to simpler ones and bioavailability of nutrients whilst increased PFs indicate improved fermentation efficiency owing to increased substrate degradability (Arhab et al., 2009; Mhlongo et al., 2022). Hence, the observed nanoparticle-induced increased ivOMD and PFs particularly when MSM was pre-treated with 20 mg/kg of G-Nano-ZnO suggests that, overall, the green nanoparticles enhanced ruminal bacterial fermentative degradation of complex substrates and efficient incorporation of the degraded nutritional matter into microbial protein mass. Indeed, increased OM degradation suggests enhanced complex substrate release of nutrients including amino acids, fatty acids, and sugars (Rouches et al., 2016; Uwineza et al., 2023) to support growth and metabolic activities of ruminal microbiota. Also, high PFs indicate high digestibility of the plant-derived feedstuff and are correlated with improved DM intake in ruminants (Blümmel et al., 1997).

The increased ivOMD and PFs can be accounted for by the observed increased DM, OM, ash, EE and minerals alongside decreased NDF, ADF, ADL, cellulose, and hemicellulose contents at high application rates (10 – 20 mg/kg) of G-Nano-ZnO. As reported in previous studies, MSM contains a high level of fibre (97 – 193 g/kg DM) that surpasses that of soyabean meal (75 g/kg DM) and many legume seeds (Alabi & Alausa, 2006; Mthiyane et al., 2018). Therefore, its incorporation as a nutrient source in beef cattle diets would increase dietary fibre content. Unfortunately, a high level of fibre in ruminant diets, among other things, reduces ruminal degradation rate and nutrient digestibility (Tafaj et al., 2005) and compromises animal growth performance (Jiyana et al., 2022). Hence, the G-Nano-ZnO-induced decrease in the fibre content of MSM, particularly its lignin, would allow ruminal microbiota to easily access the fermentable substrates in MSM whilst decreasing lignin's inhibitory effect on microbial activity. The decrease in the rate of and cumulative (12 h post-incubation) gas production at 15 mg/kg of C-Nano-ZnO could not be explained.

Conclusion

M. pruriens utilis seed meal (MSM) pre-treatment with 20 mg/kg of G-Nano-ZnO increased the DM, OM, ash, EE and mineral whilst decreasing the fibre contents leading to rapidly improved gas production and ruminal efficiency of fermentation of the legume. The green nanoparticles possess enormous potential to enhance the nutritional value of MSM for beef cattle nutrition. However, they require validation in an in vivo system with live animals prior to large-scale application.

Declarations

Author contribution statement

All authors listed have significantly contributed to the development and writing of this article.

Funding statement

This work was supported by the National Research Foundation (NRF) (Grant number: 150240) as well as NWU Postgraduate and FNAS Faculty Bursaries.

Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgments

The first author gratefully acknowledges the National Research Foundation (NRF) Scholarship (Grant number: 150240) and NWU Postgraduate and FNAS Faculty Bursaries. The authors also acknowledge the NWU School of Agricultural Sciences and Department of Animal Science for financial support to undertake this research.

References

Abidoye, B.O., & Mabaya, E. 2014. Adoption of genetically modified crops in South Africa: effects on wholesale maize prices. *Agrekon*, 53(1):104-123.

Alabi, D., & Alausa, A. 2006. Evaluation of the mineral nutrients and organic food contents of the seeds of Lablab purpureus, *Leucaena leucocephala* and Mucuna utilis for domestic consumption and industrial utilization. *World J. Agric. Sci*. 2(1):115-118.

Alleman Jr, R.J., Canale, R.E., McCarthy, C.G., & Bloomer, R.J. 2011. A blend of *Chlorophytum borivilianum* and velvet bean increases serum growth hormone in exercise-trained men. *Nutr. Metab. Insights.* 2011:4

AOAC, 2019. Official Methods of Analysis of AOAC International (21st ed.), Volume 1.Ed. Latimer, G.W. AOAC International, Gaithersburg, Maryland, USA.

Arhab, R., Macheboeuf, D., Aggoun, M., Bousseboua, H., Viala, D., & Besle, J.M. 2009. Effect of polyethylene glycol on *in vitro* gas production and digestibility of tannin containing feedstuffs from North African arid zone. *Trop. Subtrop. Agroecosyst*. 10(3):475-486.

Asghar, M.A., Zahir, E., Asghar, M.A., Iqbal, J. & Rehman, A.A. 2020. Facile, one-pot biosynthesis and characterization of iron, copper and silver nanoparticles using *Syzygium cumini* leaf extract: As an effective antimicrobial and aflatoxin B1 adsorption agents. *PloS One,* 15(7):e0234964.

Asp, M., Richardson, J., Collene, A., Droll, K., & Belury, M. 2012. Dietary protein and beef consumption predict for markers of muscle mass and nutrition status in older adults. J. *Nutr. Health Aging.* 16(9):784-790.

Bindu, K.H., Sujatha, S., & Tanuja, S. 2023. Genotypic variability in yield, L-Dopa and mineral nutrient profile in velvet bean (*Mucuna pruriens L.*) production systems. *J. Food Compo. Anal.* 118:105152.

Blümmel, M., Steingab, H., & Becker, K. 1997. The relationship between in vitro gas production, *in vitro* microbial biomass yield and 15N incorporation and its implications for the prediction of voluntary feed intake of roughages. *Br. J. Nutr.* 77(6):911-921.

Buckles, D., Triomphe, B., & Sain, G. 1998. Cover crops in hillside agriculture: farmer innovation with Mucuna. 1st Edition. International Development Research Centre, Ottawa, ON, Canada.

Chen JunCai, C.J., Wang Wei, W.W., & Wang ZhiSheng, W.Z. 2011. Effect of nano-zinc oxide supplementation on rumen fermentation *in vitro. Chin. J. Anim. Nutr*. 23 (8): 1415-1421.

Chikagwa-Malunga, S., Adesogan, A., Sollenberger, L., Phatak, S., Szabo, N., Kim, S., Huisden, C.M., & Littell, R. 2009. Nutritional characterization of Mucuna *pruriens*: 4. Does replacing soybean meal with *Mucuna pruriens* in lamb diets affect ruminal, blood and tissue l-dopa concentrations? *Anim. Feed Sci. Technol.* 148(2-4):124-137.

Conway, D., Van Garderen, E.A., Deryng, D., Dorling, S., Krueger, T., Landman, W., Lankford, B., Lebek, K., Osborn, T., Ringler, C. & Thurlow, J. 2015. Climate and southern Africa's water–energy–food nexus. *Nat. Clim. Change* 5(9):837-846

Cudjoe, N., & Mlambo, V. 2014. Buffer nitrogen solubility, in vitro ruminal partitioning of nitrogen and in vitro ruminal biological activity of tannins in leaves of four fodder tree species. *J. Anim. Physiol. Anim. Nutr.* 98(4):722-730.

DAF. 2019. The Queensland beef supply chain.

Deli, M., Nguimbou, R.M., Djantou, E.B., Tatsadjieu Ngoune, L., & Njintang Yanou, N. 2020. Bioactive compounds of velvet bean (*Mucuna pruriens L.)* seeds. *Bioact. Compd. Underutil. Veg. Legumes*. 1-19.

Dezah, T.J., Chikwanha, O.C., Dzama, K., Strydom, P.E., & Mapiye, C. 2021. Comparison of *Acacia mearnsii* and *Medicago sativa* as substitutes for Glycine max in cattle finisher diets: Effects on beef production and quality. *Sci. Afri.* 11:e00673.

Elahi, M.Y., Nia, M.M., Salem, A.Z., Mansouri, H., Olivares-Pérez, J., Cerrillo-Soto, M.A., & Kholif, A.E. 2014. Effect of polyethylene glycol on in vitro gas production kinetics of *Prosopis cineraria* leaves at different growth stages. *Ital. J. Anim. Sci*. 13(2):3175.

Elghandour, M., Kholif, A., Salem, A., De Oca, R.M., Barbabosa, A., Mariezcurrena, M., & Olafadehan, O. 2016. Addressing sustainable ruminal methane and carbon dioxide emissions of soybean hulls by organic acid salts. *J. Clean. Prod*. 135:194-200.

Ezeagu, I., Maziya-Dixon, B., & Tarawali, G. 2003. Seed characteristics and nutrient and antinutrient composition of 12 Mucuna accessions from Nigeria. *Trop. Subtrop. Agroecosyst*. 1(2-3):129-139.

Feng, Y., Min, L., Zhang, W., Liu, J., Hou, Z., Chu, M., Li, L., Shen, W., Zhao, Y., & Zhang, H. 2017. Zinc oxide nanoparticles influence microflora in ileal digesta and correlate well with blood metabolites. *Front. Microbiol.* 8:992.

Gamedze, N.P., Mthiyane, D.M.N., Kgaswane, K.S., Mavengahama, S., & Onwudiwe, D.C. 2024a. Growth, physiological responses, and meat quality of feedlot-finished Bonsmara steers offered unprocessed *Mucuna pruriens* utilis seed meal with or without conventional and green zinc oxide nanoparticles. *Trop. Anim. Health Prod.* 56:379

Gamedze, N.P., Mthiyane, D.M.N., Mavengahama, S., Singh, M., & Onwudiwe, D.C. 2024b. Biosynthesis of ZnO Nanoparticles Using the Aqueous Extract of *Mucuna pruriens* (utilis): Structural Characterization, and the Anticancer and Antioxidant Activities. *Chem. Afr*. 7(1):219-228.

Greenwood, P.L. 2021. An overview of beef production from pasture and feedlot globally, as demand for beef and the need for sustainable practices increase. *Animals* 15:100295.

Holden, L. 1999. Comparison of methods of in vitro dry matter digestibility for ten feeds. *J. Dairy Sci.* 82(8):1791-1794.

Hosseini-Vardanjani, S., Rezaei, J., Karimi-Dehkordi, S., & Rouzbehan, Y. 2020. Effect of feeding nano-ZnO on performance, rumen fermentation, leukocytes, antioxidant capacity, blood serum enzymes and minerals of ewes. *Small Rumin. Res*. 191:106170.

Huisden, C.M., Butterweckb, V., Szaboc, N., Gaskin, J., Arriola, K., Raji, A., & Adesogan, A. 2019. Effects of detoxification of *Mucuna pruriens* on the feed intake, behavior, organ weights, blood cell counts and metabolites of rats. *Trop. Subtrop. Agroecosyst.* 22(2).

Huws, S. A., Edwards, J. E., Creevey, C. J., Rees Stevens, P., Lin, W. & Girdwood, S. E. 2016. Temporal dynamics of the metabolically active rumen bacteria colonising fresh perennial ryegrass. *Front. Microbiol.* 7:1854.

Jiyana, S., Ratsaka, M., Leeuw, K., & Mbatha, K. 2022. Impacts of graded dietary fiber levels on feed efficiency and carbon footprint of two beef breeds. *Livest. Sci*. 258:104867.

Khan, A.L., Shahzad, R., Al-Harrasi, A., & Lee, I.J. 2017. Endophytic microbes: a resource for producing extracellular enzymes. *Endophytes: Crop Prod. Protect*. 2:95-110.

King, T., & Sheridan, R. 2019. Determination of 27 elements in animal feed by inductively coupled plasma-mass spectrometry. J. AOAC Int. 102(2):434-444.

Lamega, S.A., Komainda, M., Hoffmann, M.P., Ayisi, K.K., Odhiambo, J.J.O., & Isselstein, J. 2021. It depends on the rain: smallholder farmers' perceptions on the seasonality of feed gaps and how it affects live stock in semi-arid and arid regions in

Southern Africa. *Clim. Risk Manag*. 343:100362.

Lieu, C.A., Venkiteswaran, K., Gilmour, T.P., Rao, A.N., Petticoffer, A.C., Gilbert, E.V.,Deogaonkar, M., Manyam, B.V., & Subramanian, T. 2012. The antiparkinsonian and antidyskinetic mechanisms of *Mucuna pruriens* in the MPTP-treated nonhuman primate. *Evid.-Based Complement. Altern. Med.* 1: 840247

Loyra-Tzab, E., Sarmiento-Franco, L.A., Sandoval-Castro, C.A., & Santos-Ricalde, R.H. 2013. Nutrient digestibility and metabolizable energy content of *Mucuna pruriens* whole pods fed to growing Pelibuey lambs. *Asian-Australas. J. Anim. Sci.* 26(7):981.

Madzimure, J., Mutema, N., Chimonyo, M., Bakare, A.G., & Mapiye, C. 2014. Performance of Mashona doelings supplemented with different levels of velvet bean (*Mucuna pruriens L.* DC. var. utilis) seed meal. *Trop. Anim. Health Prod*. 46(5):901-904.

Mauricio, R.M., Mould, F.L., Dhanoa, M.S., Owen, E., Channa, K.S., & Theodorou, M.K. 1999. A semi-automated in vitro gas production technique for ruminant feedstuff evaluation. *Anim. Feed Sci. Technol.* 79(4):321-330.

McAllister, T., Bae, H., Jones, G., & Cheng, K.J. 1994. Microbial attachment and feed digestion in the rumen. *J. Anim. Sci*. 72(11):3004-3018.

Mhlongo, G., Mnisi, C.M., & Mlambo, V. 2021. Cultivating oyster mushrooms on red grape pomace waste enhances potential nutritional value of the spent substrate for ruminants. *PLoS One*. 16(2):e0246992.

Mhlongo, G., Mnisi, C., Mlambo, V., & Dlamini, B. 2022. An exogenous fibrolytic enzyme mixture enhances in vitro ruminal degradability of red grape pomace by-product. *S. Afri. J. Anim. Sci.* 52(2):126-133.

Minchitha, Seshagiri, S., Kumar S, H., Venkatesh, S., & Krishna, V. 2024. Influence of Iron nanoparticle and multifaceted endophytic fungus piriformospora indica on *Zea mays* L. - A novel approach toward innovative yet sustainable farming. *Geomicrobiol. J.* 41(3):287-297.

Mnisi, C., & Mlambo, V. 2017. Influence of harvesting site on chemical composition and potential protein value of *Acacia erioloba, A. nilotica* and *Ziziphus mucronata* leaves for ruminants. *J. Anim. Physiol. Anim. Nutr.* 101(5):994-1003.

Mthiyane, D.M., Gamedze, N.P., Dlamini, A.M., Hugo, A. & Nsahlai, I.V. 2018. Effects of dietary replacement of broiler litter with *Mucuna pruriens* utilis forage and seed meal on performance, carcass characteristics, blood biochemical and physiological parameters in indigenous goats. *bioRxiv:*421313.

Ørskov, E.R., & McDonald, I. 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to

rate of passage. *J. Agric. Sci*. 92(2):499-503.

Pathania, R., Chawla, P., Khan, H., Kaushik, R., & Khan, M.A. 2020. An assessment of potential nutritive and medicinal properties of *Mucuna pruriens*: a natural food legume. 3 *Biotech*. 10(6):261.

Pei, X., Xiao, Z., Liu, L., Wang, G., Tao, W., Wang, M., Zou, J., & Leng, D. 2019. Effects of dietary zinc oxide nanoparticles supplementation on growth performance, zinc status, intestinal morphology, microflora population, and immune response in weaned pigs. *J. Sci. Food Agric*. 99(3):1366-1374.

Pérez-Hernández, F., Ayala-Burgos, A., & Belmar-Casso, R. 2003. Performance of growing lambs supplemented with *Mucuna pruriens*. *Trop. Subtrop. Agroecosyst*. 1(2-3):119-112.

Pugalenthi, M., Vadivel, V., & Siddhuraju, P. 2005. Alternative food/feed perspectives of an underutilized legume *Mucuna pruriens* var. utilis-a review. *Plant Foods Hum. Nutri.* 60:201-218.

Pulikkalpura, H., Kurup, R., Mathew, P.J., & Baby, S. 2015. Levodopa in *Mucuna pruriens* and its degradation. *Sci. Rep.* 5(1):11078.

Remya, R., Julius, A., Suman, T., Mohanavel, V., Karthick, A., Pazhanimuthu, C., Samrot, A.V., & Muhibbullah, M. 2022. Role of nanoparticles in biodegradation and their importance in environmental and biomedical applications. *J. Nanomater.* 1:6090846.

Rodríguez, C.E., Mitter, B., Barret, M., Sessitsch, A., & Compant, S. 2018. Commentary: seed bacterial inhabitants and their routes of colonization. *Plant Soil.* 422:129-134.

Rouches, E., Herpoël-Gimbert, I., Steyer, J.P., & Carrere, H. 2016. Improvement of anaerobic degradation by white-rot fungi pretreatment of lignocellulosic biomass: a review. *Renew. Sustain. Energy Rev.* 59:179-198.

Rudgers, J.A., Afkhami, M.E., Rúa, M.A., Davitt, A.J., Hammer, S., & Huguet, V.M. 2009. A fungus among us: broad patterns of endophyte distribution in the grasses. *Ecol.* 90(6):1531-1539.

Sardjono, R., Khoerunnisa, F., Musthopa, I., Qowiyah, A., Khairunisa, D., Erfianty, D., & Rachmawati, R., 2018. Biosynthesis, characterization and anti-Parkinson activity of magnetite-Indonesian velvet beans (*Mucuna pruriens L.*) nanoparticles. *J. Eng. Sci. Technol.* 13 (12): 4258–4270.

Sedibe, M.M., Mofokeng, A.M., & Masvodza, D.R. 2023. Soybean production, constraints, and future prospects in poorer countries: a review.1st Edition. Sher-e-Bangla Agricultural University, Bangladesh.

Selvan, T., Panmei, L., Murasing, K.K., Guleria, V., Ramesh, K.R., Bhardwaj, D.R., Thakur, C.L., Kumar, D., Sharma, P., Digvijaysinh Umedsinh, R., & Kayalvizhi, D. 2023. Circular economy in agriculture: Unleashing the potential

of integrated organic farming for food security and sustainable development. *Front. Sustain. Food Syst.* 7:1170380.

Shade, A., Jacques, M.A., & Barret, M. 2017. Ecological patterns of seed microbiome diversity, transmission, and assembly. *Curr. Opin. Microbiol*. 37:15-22.

Shang, H., Ma, C., Li, C., Cai, Z., Shen, Y., Han, L., Wang, C., Tran, J., Elmer, W.H., & White, J.C. 2023. Aloe vera extract gel-biosynthesized selenium nanoparticles enhance disease resistance in lettuce by modulating the metabolite profile and bacterial endophytes composition. *ACS nano*. 17(14):13672-13684.

Shearin, Z.R., Filipek, M., Desai, R., Bickford, W.A., Kowalski, K.P., & Clay, K. 2018. Fungal endophytes from seeds of invasive, non-native Phragmites australis and their potential role in germination and seedling growth. *Plant Soil.* 422:183-194.

Sheykhbaglou, R., Sedghi, M., Shishevan, M.T., & Sharifi, R.S. 2010. Effects of nano-iron oxide particles on agronomic traits of soybean. *Not. Sci. Bio.* 2(2):112-113.

Shi, L., Xun, W., Yue, W., Zhang, C., Ren, Y., Liu, Q., Wang, Q., & Shi, L. 2011. Effect of elemental nano-selenium on feed digestibility, rumen fermentation, and purine derivatives in sheep. *Anim. Feed Sci. Technol.* 163(2-4):136-142.

Shi, S., Lu, J., Tong, H., Zou, J. & Wang, K., 2012. Effects of graded replacement of soybean meal by sunflower seed meal in laying hen diets on hen performance, egg quality, egg fatty acid composition, and cholesterol content. *J. Appl. Poult. Res.* 21(2):367-374.

Sowdhanya, D., Singh, J., Rasane, P., Kaur, S., Kaur, J., Ercisli, S. & Verma, H. 2023. Nutritional significance of velvet bean (*Mucuna pruriens*) and opportunities for its processing into value-added products. J. *Agric. Food Res.* 100921.

Stoddard, E. 2016. South Africa maize prices scale newpeaks as drought bites [WWW Document]. Moneyweb. https://www.moneyweb.co.za/news-fast-news/samaize-prices-scale-new-peaks-as-drought-bites/. Date of access. 16/05/2024.

Suresh, S., & Prakash, S. 2012. Effect of *Mucuna pruriens* (Linn.) on sexual behavior and sperm parameters in streptozotocin-induced diabetic male rat. *J. Sex. Med*. 9(12):3066-3078.

Tafaj, M., Kolaneci, V., Junck, B., Maulbetsch, A., Steingass, H., & Drochner, W. 2005. Influence of fiber content and concentrate level on chewing activity, ruminal digestion, digesta passage rate and nutrient digestibility in dairy cows in late lactation. *Asian-Austral. J. Anim. Sci.* 18(8):1116-1124.

Tavirimirwa, B., Manzungu, E., Washaya, S., Ncube, S., Mudzengi, C., & Mwembe, R. 2019. Efforts to improve Zimbabwe communal grazing areas: a review. *Afr. J. Rang. Forage Sci.* 36(2):73–83.

Tefera, S., Mlambo, V., Dlamini, B., Dlamini, A., Koralagama, K., & Mould, F. 2008. Chemical composition and in vitro ruminal fermentation of common tree forages in the semi-arid rangelands of Swaziland. *Anim. Feed Sci. Technol*. 142(1-2):99-110.

Theansungnoen, T., Nitthikan, N., Wilai, M., Chaiwut, P., Kiattisin, K., & Intharuksa, A. 2022. Phytochemical analysis and antioxidant, antimicrobial, and antiaging activities of ethanolic seed extracts of four Mucuna species. Cosmetics. 9(1):14.

Troy, D.J., Tiwari, B.K., & Joo, S.-T. 2016. Health implications of beef intramuscular fat consumption. *Korean. J. Food Sci. Anim. Res*. 36(5):577.

Uwineza, C., Bouzarjomehr, M., Parchami, M., Sar, T., Taherzadeh, M.J., & Mahboubi, A. 2023. Evaluation of *in vitro* digestibility of *Aspergillus oryza*e fungal biomass grown on organic residue derived-VFAs as a promising ruminant feed supplement. *J. Anim. Sci. Biotechnol.* 14(1):120.

Van Soest, P.V., Robertson, J.B., & Lewis, B.A. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci*. 74(10):3583-3597.

Vetter, S., Goodall, V.L., & Alcock, R. 2020. Effect of drought on communal livestock farmers in KwaZulu-Natal, *South Africa. Afr. J. Rang. Forage* Sci 37(1):93–106.

von Hellfeld, R., Brotzmann, K., Baumann, L., Strecker, R., & Braunbeck, T. 2020. Adverse effects in the fish embryo acute toxicity (FET) test: a catalogue of unspecific morphological changes versus more specific effects in zebrafish (Danio rerio) embryos. *Environ. Sci. Eur.* 32:1-18.

Watnick, P., & Kolter, R. 2000. Biofilm, city of microbes. *J. Bacteriol*. 182(10):2675-2679.

Williams, P. 2007. Nutritional composition of red meat. *Nutr. & Diet*. 64: S113-S119.

Yang, H.E., Zotti, C.A., McKinnon, J.J., & McAllister, T.A. 2018. Lactobacilli are prominent members of the microbiota involved in the ruminal digestion of barley and corn. *Front. Microbiol.* 9:718.

Yantika, S., Evvyernie, D., Diapari, D., & Winaga, K. 2016. Performance, carcass production, and meat quality of Sumba ongole bulls fed ration supplemented velvet bean (*Mucuna pruriens*). *Media Peternakan*. 39(1):20-26.

Zhou, G., Yu, R., Ahmed, T., Jiang, H., Zhang, M., LV, L., Alhumaydhi, F.A., Allemailem, K.S., & Li, B. 2021. Biosynthesis and characterization of zinc oxide nanoparticles and their impact on the composition of gut microbiota in healthy and attention-deficit hyperactivity disorder children. *Front. Microbiol.* 12:700707.