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Facile green preparation of zinc oxide nanoparticles *and its effects on in-vitro feed degradation, ruminal fermentation, and total gas production*

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Abstract

In ruminants, the rumen provides the nutrients needed for animal physiological requirements and for facing ambient environmental effects. Therefore, any rumen manipulation affects animal biological functions. The goal of the present study is to investigate the effects of different levels of zinc nanoparticles (nano-ZinO) on *in vitro* ruminal feed degradation, ruminal fermentation, and total gas production. The fig leaf extract was used as a reducing agent for zinc ions to prepare nano-ZnOs instead of chemical reducing agents (green chemistry). The X-ray diffraction (XRD) pattern, scanning electron microscope (SEM), transmission electron microscope (TEM), Fourier transform infrared (FT-IR) spectroscopy, and dynamic light scattering (DLS) were used to evaluate the nano-ZnOs. The batch culture technique was used to evaluate the impact of supplemented diets with different levels of nano-ZnO; 30, 60, 90, 120, 150 and 180 mg/Kg dry matter (DM) diet on *in vitro* rumen fermentation characteristics. The findings demonstrated that fig leaf extract has the capacity to produce spherical zinc oxide nanoparticles with an average size of 25 nm. The crystallographic structure of the green synthesized nano-ZnO was confirmed using XRD analysis. The DLS showed that the manufactured nano-ZnO particles had an average diameter of roughly 215 nm. The diet supplemented with 30 mg nano zinc/kg DM increased the degradability values ($P<0.05$) of the dry matter (DM), organic matter (OM), neutral detergent fibre (NDF), acid detergent fibre (ADF), and rumen fermentation parameters, including total gas production (TGP), lactic, acetic, propionic, and butyric acids, and ammonia-nitrogen (NH3-N) concentrations to the maximum compared to the control. The increased level of nano-ZnO supplementation gradually led to a decline in rumen fermentation and diet degradability. It could be concluded that the supplemented diet with 30 mg nano-ZnO/kg DM is the optimal level for the utilization to guarantee the highest feed nutrient digestion and enhancement of fermentation efficiency in the rumen.

Keywords: Eco-friendly, zinc nanoparticle, XRD, rumen gas production, volatile fatty acids, pH, ammonia.

1. Introduction

Livestock owners always aim to improve the health and productivity of their herds in an economical manner. Previously, minerals were considered minor dietary components, and therefore they were not given enough attention as feed additives. However, it is now recognized that minerals are required for animal genomic stability, metabolism, performance, immunity, and antioxidant protection [1]. Zinc (Zn) is a micro-mineral that plays an important role in DNA and RNA synthesis and in metabolic processes of lipids, carbohydrates, and proteins as it makes up many enzymes as a cofactor [2]. Additionally, zinc deficiency has been associated with imbalances of the immune system, males reproductive system and dairy cows mastitis [3].

Zinc is traditionally added to ruminants' diets in either organic (chelated Zn-amino acids) or inorganic form (zinc oxide). These forms are poor in solubility and bioavailability; therefore, breeders are forced to add them over the recommended level to avoid zinc deficiency [4]. Therefore, recently, scientists are increasingly focusing on nanoparticles in animal nutrition [5] because of their intriguing properties, such as higher bioavailability, nanoscale size, specific and rapid movement, high surface activity, high area

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surface, high catalytic efficacy, and high absorption percentage [6,7,8]. Animal performance, feed efficiency, health, and total gas output have all been proven to improve using nano-ZnO in previous tests [7,8,9]. On the other hand, nano-ZnO has been found to be hazardous to animals in other research [10,11]. According to Swain et al. [12], nano-ZnO affects rumen fermentation dynamics in ruminants and can change volatile fatty acids, which could affect gas production. More research is needed to assess the effect of nano-ZnO on ruminal variables that impact animal performance. The purpose of this study was to better understand how different quantities of nano-ZnO (30, 60, 90, 120, 150, and 180 mg/Kg DM) affect *in vitro* ruminal feed degradation, ruminal parameters, and total gas production.

Experimental

Zinc acetate dihydrate Zn $(CH3COO)_2.2H_2O$ and sodium hydroxide (NaOH) were used as precursors for zinc oxide nano formation. Fresh fig leaves were collected from farms in the Menofya government. The fig leaf extract was used as a reducing agent for zinc ions to prepare nano-ZnOs instead of chemical reducing agents (green chemistry). All the chemicals used in the experiment were purchased from Sigma Aldrich.

1. Preparation of fig leaves extract

The extract of fig leaf was prepared as follows: the fig leaves were cleaned with distillated water and sun-dried at room temperature for 10 days before being cut into small pieces and crushed to a powder with an electric grinder (Moulinex). Approximately 50g of leaf powder was extracted using 250 ml of ethanol to water (90:10, v/v) via cold extraction, and the mixture was left at room temperature for 5 days before being filtered using filter paper. The fig leaf extract mixture was vaporized under reduced pressure to remove all solvents and dryness using a rotary evaporator at 50 °C. The fig leaf extract was stored at 4°C in dark sterilized screw-capped flasks [13].

2. Green synthesis of zinc oxide nanoparticles (nano-ZnOs)

The green synthesis was carried out for nano-ZnO production; the zinc acetate solution (0.2 M) was prepared using distillated water. The prepared solution was stirred using a magnetic stirrer to dissolve the zinc acetate powder in the distillated water. Subsequently, the aqueous solution of fig leaf extract was added drop by drop to the solution of zinc acetate and kept on a magnetic stirrer. Then the mixture of zinc acetate and fig leaf extract was heated on a hot plate and stirred for 6 h at 70 °C until the mixture turned into gelatin form. The gelatin was taken and placed into a crucible. The crucible was reserved in the electrical oven for calcination at 450 °C for 3 h. The biosynthesized nano-ZnOs were obtained in white coloured powder after the calcination process [14, 15].

3. *In vitro* rumen fermentation study

The batch fermentation culture technique using 3 incubation vessels for each treatment was used to evaluate the impact of the control diet supplementation with the produced nano-ZnOs at 30, 60, 90, 120, 150, and 180 mg/kg DM on *in vitro* ruminal feed degradation, ruminal fermentation, and total gas production. Each vessel contained 400 mg of the tested control diet and was filled with 40 ml of a mixture of 1:3 (v/v) rumen fluids: buffer solution as described by Ismail et al. [16]. Diet composition was as follows: 35% corn grain, 30% Berseem hay, 14% wheat bran, 14% cottonseed meal, and 7% soybean meal. The diet was analyzed according to the AOAC [17] methods to determine crude protein (CP), crude fiber (CF), ether extract (EE), and ash content. Nitrogen free extract (NFE) and OM were obtained by difference (100 - Ash %). For the determination of the diet content of Zn, Agilent 5100 Synchronous Vertical Dual View (SVDV) ICP-OES, with Agilent Vapor Generation Accessory VGA 77, was used according to the analytical method of APHA [18]. The chemical composition of the control diet was shown in (Table 1).

After 24 h of incubation at 39° C, all vessels were filtered in fiber filter bags with a 25 micron porosity (ANKOM-USA). The residues in the bags were dried at 60 °C in an oven for 48 h to analyze the DM, OM, NDF, and ADF digestibility. The chemical composition of the experimental diet residual was determined according to the methods of AOAC [17]. While the NDF and ADF were analyzed according to the method of Van Soest [19], rumen fluid pH was measured; the produced gas volume was determined by Hohenheim Syringes (100 ml) as described by Navarro-Villa et al. [20]. The ammonia concentration was analyzed by a spectrophotometer as described in the kit pamphlet of Biodiagnostic Company-Egypt. Lactic, acetic, propionic, and butyric acid concentrations were analyzed by HPLC on the Agilent 1260 series. The separation was carried out using an Eclipse C18 column (4.6 mm x 250 mm i.d., 5 μm) at 30 °C.

4. Statistical analysis:

The following equation was used to analyze data statistically using IBM SPSS [21] Statistics for Windows (2011):

$Yij = \mu+Ti+eij$

Where, Yij is the factor under investigation ij, μ is the general mean, Ti is the influence of the treatment

under analysis, eij is the experimental error for ij on the observation,

The Duncan's multiple range test was achieved to examine the significance between means with a probability level less than 0.05 ($p<0.05$) for significance expression (Duncan, [22]).

Results and discussion

1. Characterization of the biosynthesized ZnO-nanoparticles

1.1. XRD patterns

The XRD pattern of biosynthesized nano-ZnOs in powder form is shown in Figure 1A. A Philips diffractometer was used to study the nano-ZnO (PW 1820 goniometer, PW 1930 generator). The nano-ZnOs sample was examined at two different theta ranges of angles, from 30° to 80°. The XRD peaks for nano-ZnOs were visible at 2θ = 31.78° for (100), 34.50° for (002), 36.35° for (101), 47.68° for (102), 56.65° for (110), 63.12° for (103), and 67.98° for (200). Also, the XRD pattern displayed the alignment and crystalline nature of nano-ZnO. Moreover, the XRD pattern has also been linked to the JCPDS data sheet/ICDD no. 36-1451. This evidently proves that nano-ZnOs have been effectively manufactured by biosynthesis technique, confirming the fabrication of crystalline nano-ZnOs as well as wurtzite hexagonal construction.

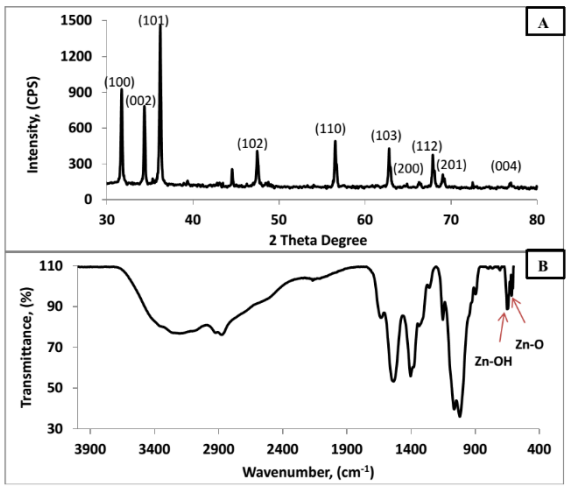

Fig. 1: A) XRD of the biosynthesized nano-ZnOs, B) FTIR spectrum of biosynthesized nano-ZnOs

1.2. Fourier transforms infrared (FT-IR)

The typical FT-IR spectrum of biosynthesized nano-ZnOs generated by the hydrothermal technique is shown in Figure 1B. The following were the main peaks and their matching assignments in all of the prepared nano-ZnOs: 2955 cm-1 plus 2924 cm-1 (CH3 stretching vibration), 1653 cm-1 (C=O stretching vibration equivalent to the amide 1 group), 1560 cm-1 (C=C stretch in the aromatic ring), 1410 cm-1 (C-N stretch of amide-I), 1025 cm-1 (C-O stretching in amino acid), and a band at 532 cm-1 (O-H stretching vibration) (C-O stretching in amino acid). The absorption at 500 cm-1 to 600 cm-1 in the FT-IR spectrum also differentiates the presence of biosynthesized nano-ZnOs.

1. 3. Dynamic Light Scattering (DLS)

The particle size of the nano-ZnOs produced by the eco-friendly technique using zinc acetate in the presence of fig leaf extract (Fig. 2A) was investigated via a DLS instrument, the NICOMP 380 ZLS, from the United States. DLS is a popular method for determining the particle size in a colloidal solution. This tool was used to illustrate the average particle size as well as the size distribution of biosynthesized nano-ZnOs. In addition, the results are shown in Figure 2A. The size distribution of biosynthesized nano-ZnOs is revealed by the particle size distribution, which ranges from 100 to 700 nm. The obtained results show that the produced nano-ZnOs have an average particle size of roughly 215 nm.

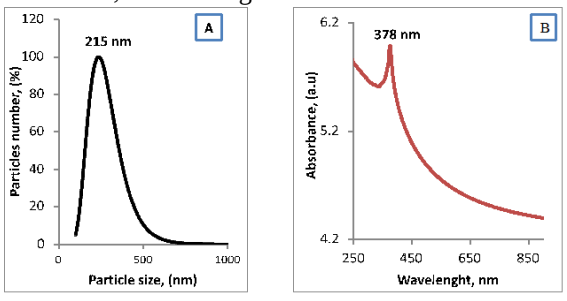

Fig. 2: A) The DLS of the biosynthesized nano-ZnOs, B) UV–Visible spectroscopy of the biosynthesized nano-ZnOs

1.4. UV–Visible spectroscopy

The UV-Visible absorption spectra of the biosynthesized nano-ZnOs by the green approach utilizing fig extract were recognized in the wavelength range of 340 to 350 nm, which is the characteristic wavelength range of nano-ZnOs. The surface Plasmon resonance (SPR) band at 378 nm was validated by biosynthesized nano-ZnOs, as shown in Fig. 2B. In comparison to bulk ZnO, the generated nano-ZnOs have strong blue shift absorption, which may be as a result of a large decline in particle size as well as the effect of their high excitation binding energy at ambient temperature.

1.5. The morphological investigations

The surface morphology of the synthesized nano-ZnOs is shown in Figure 3A. The image of SEM revealed that the nano-ZnOs molecules grow slowly, besides producing small spherical structures similar to bullets, and that the majority of the manufactured nano-ZnOs are spherical as well. Furthermore, as shown in Fig. 3B, The TEM micrographs demonstrate that the biosynthesized nano-ZnOs were cubic structures, which is also supported by XRD and

TEM pictures. For biosynthesized nano-ZnOs, TEM micrographs revealed that the generated ZnO-NPs had an average mean size of 25 nm.

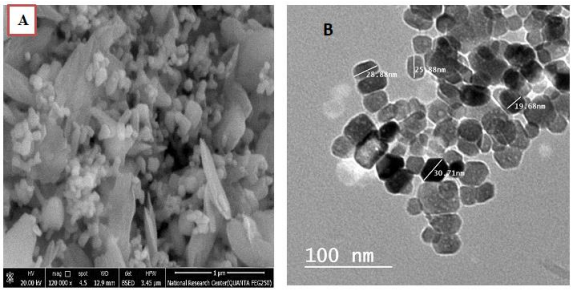

Fig. 3: A) SEM image of the biosynthesized nano-ZnOs, B) TEM image of the biosynthesized nano-ZnOs.

2. In vitro rumen fermentation study

2.1. Effects of nano-ZnOs supplementation levels on the diet ruminal degradability parameters

The effects of nano-ZnOs supplementation levels on diet degradability by rumen microorganisms were shown in (Table 2). The diet supplemented with nano-ZnOs at 30 mg/kg showed the highest degradability values (P<0.05) for DM, OM, NDF, and ADF compared to the control diet. Increasing levels of nano-ZnOs supplementation up to 60 mg/kg caused slight improvement in all diet degradability parameters compared to the control diet. The positive impact of nano-ZnOs on diet degradability parameters (DM, OM, NDF, and ADF) at the lowest level of supplementation (30 mg nano-ZnOs/Kg DM) may be attributed to increased ruminal microbial biomass production (RMBP). Riazi et al. [23] stated that RMBP increased to its maximum due to dietary nano-ZnOs supplementation. It's thought that nano-ZnOs supplementation improves the adhesion ability of ruminal microbes to the feed particles (as Zn is a bivalent cation) and consequently improves their ability to grow and colonize, which leads to more biomass production [24].

Furthermore, Zn makes up many enzymes as a cofactor [2]. Therefore, the improvement of diet digestibility parameters due to improved digestive enzyme activity after nano-ZnOs addition is reasonable. Consonantly with this idea, Adegbeye et al. [25] reported that Zn nanoparticles addition improves protease, amylase, and lipase digestive enzyme activity, resulting in higher diet digestibility. Similarly, diet digestibility was positively affected by nano-ZnOs supplementation in the previous *in vitro* studies [26,23]. The beneficial effect of nano-ZnOs supplementation on diet nutrient digestion may indicate that the ruminal microbes' Zn, energy, and protein requirements have been met efficiently.

In the present study, no significant differences were detected in all degradability parameters (DM, OM, NDF, and ADF) due to the addition of nano-ZnOs at a level of 90 mg/kg when compared to the control. All of the diet degradability parameters decreased significantly as the level of nano-ZnOs supplementation increased above 90 mg/kg. The gradual decline in the diet's nutrient degradability with increasing levels of nano-ZnOs supplementation may be due to the antibacterial activity of zinc oxide nanoparticles, which may cause suppression of ruminal bacterial growth. In agreement with this thought, Arabi et al. [27] reported that nano-ZnOs have bactericidal effects on both Gram-positive and Gram-negative bacteria, and this antibacterial effect depends mainly on the nano-ZnOs dose or concentration.

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

Dry matter (DM %)	Organic matter (OM %)	Crude protein (CP %)	Crude fiber (CF %)	Ether extract (EE %)	Ash (%)	Nitrogen Free Extract (NFE %)	Zn (mg/kg)
89.5	95	15.5	15.67	4.9	5	58.93	25.5

Table (2): Effect of nano-ZnOs supplementation level on ruminal diet degradability parameters

level of nano-ZnOs	DMD%	OMD%	NDFD%	ADFD%
Control	50.84bc	56.16bc	40.29bc	30.66bc
30 mg nano-ZnOs /Kg DM	53.56a	58.88a	42.37a	32.74a
60 mg nano-ZnOs /Kg DM	51.40b	56.72b	40.52b	30.89b
90 mg nano-ZnOs /Kg DM	50.54c	55.86c	39.91bc	30.28bc
120 mg nano-ZnOs /Kg DM	49.15d	54.47d	38.97c	29.34c
150 mg nano-ZnOs /Kg DM	47.68e	53.00e	36.79d	27.16d
180 mg nano-ZnOs /Kg DM	45.32f	50.64f	35.32e	25.69e
MSE±	0.53	0.52	0.49	0.50

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. ±MSE: Mean standard error

2. 2. Effect of nano-ZnOs supplementation on ruminal fermentation parameters

The effect of nano-ZnOs supplementation level on ruminal fermentation parameters was illustrated in Table 3. Diet supplementation with nano-ZnOs at 30 mg/kg DM gave the highest (P<0.05) values for TGP, lactic, acetic, propionic, butyric acid, and NH3-N concentrations. nano-ZnOs supplementation at 60 mg/kg increased the volume of TGP, lactic acid, and

NH3-N concentrations numerically compared to the control. The significantly higher ruminal total gas and organic acid (lactic, acetic, propionic, and butyric) production after the tested diet supplementation with 30 mg/kg DM of nano-ZnOs may be related to the improvement of nutrient digestibility, especially the NDF and ADF, with higher microbial protein synthesis [28].

In line with the current result, Riazi et al. [23] attributed the slightly higher ruminal gas and volatile fatty acids (ex: acetic, propionic, and butyric) production after diet supplementation with nano-ZnOs up to 60 mg/kg DM to the higher truly degraded substrate for this diet compared with the control. In the current study, the gradual decline of the TGP, NH3-N, lactic, acetic, propionic, and butyric acid concentrations with the gradual increase of the level of nano-ZnOs supplementation above 30mg/kg DM may be due to the inhibitory effect of zinc oxide nanoparticles on both Gram-positive and Gram-negative bacteria as reported by Arabi et al. [27].

No significant differences were detected in values of total gas production, pH, acetic, propionic, butyric acid, and NH3-N concentrations due to nano-ZnOs supplementation at 90 mg/kg when compared with the control. However, supplementation of nano-ZnOs above 90 mg/kg has a serious negative impact on most of all rumen basic parameters. No significant change was detected in the pH values due to nano-ZnOs supplementation up to 120 mg/Kg compared with the control, but above this level the pH values increased significantly. Concerning the impact of nano-ZnOs supplementation on ruminal fermentation parameters, there is a slight decrease in the ruminal pH with nano-ZnOs supplementation at a level of 30 mg/Kg DM, with no significant difference when compared with the pH of the control. The *in vitro* ruminal pH (6.42 to 6.51) of the treatments was within the typical range (5.5 to 6.8) in a normal ruminal fermentation circumstance [1].

Table (3): Effect of Nano-ZnOs supplementation level on ruminal basic parameters

level of nano-ZnOs	TGP (ml)	pH	Lactic acid (mg/ml)	Acetic acid (mg/ml)	Propionic acid (mg/ml)	Butyric acid (mg/ml)	NH3-N (mg/dl)
Control	**134.33^{b}**	**6.20^{bc}**	**0.78^{b}**	**0.87^{bc}**	**0.29^{c}**	**0.23^{c}**	**13.15^{a}**
30 mg nano-ZnOs /Kg DM	**139.67^{a}**	**6.13^{c}**	**1.08^{a}**	**1.07^{a}**	**0.43^{a}**	**0.30^{a}**	**13.54^{a}**
60 mg nano-ZnOs /Kg DM	**134.67^{b}**	**6.17^{bc}**	**0.81^{b}**	**0.88^{b}**	**0.29^{c}**	**0.23^{c}**	**13.53^{a}**
90 mg nano-ZnOs /Kg DM	**133.67^{b}**	**6.20^{bc}**	**0.63^{c}**	**0.75^{c}**	**0.28^{c}**	**0.20^{cd}**	**13.03^{a}**
120 mg nano-ZnOs /Kg DM	**130.67^{c}**	**6.22^{bc}**	**0.55d^{c}**	**0.68^{d}**	**0.27^{c}**	**0.19^{d}**	**12.99^{a}**
150 mg nano-ZnOs /Kg DM	**123.33^{d}**	**6.25^{b}**	**0.53d^{c}**	**0.52^{e}**	**0.24^{c}**	**0.13^{e}**	**10.63^{b}**
180 mg nano-ZnOs /Kg DM	**121.00^{d}**	**6.32^{a}**	**0.44^{d}**	**0.46^{e}**	**0.19^{d}**	**0.12^{e}**	**9.02^{c}**
MSE±	**1.34**	**0.02**	**0.05**	**0.04**	**0.02**	**0.01**	**0.34**

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), ±MSE: Mean standard error

More production of the organic acids (lactic, acetic, propionic, and butyric) due to the improvement of feed nutrient digestibility as a result of nano-ZnOs supplementation may be the reason for this reduction in the ruminal pH.

In the current study, there was a slight impact of nano-ZnOs supplementation on ruminal pH. This finding is supported by the findings of Riazi et al. [23], who stated that ruminal pH is unaffected by dietary nano-ZnO supplementation. The ruminal NH3-N concentration was slightly affected by the dietary nano-ZnO supplementation. This may be due to the low degradability of corn protein (zein) of the tested diet [28], or due to better utilization of ammonia by ruminal microbes for more microbial biomass production [23]. Similarly, it has been reported that diet supplementation with nano-ZnOs up to 60 mg/kg DM did not affect the ruminal ammonia nitrogen concentration *in vitro* [26, 23].

Conclusion

In the current study, the zinc oxide nanoparticle was successfully prepared using fig leaf extract and confirmed using XRD, TEM, and DLS. The maximum feed nutrient digestibility and enhancement of fermentation efficiency in the rumen were guaranteed by dietary supplementation with nano-ZnO at a level of 30 mg/kg DM. In light of the foregoing results, it can be concluded that the use of nanotechnology in the production of nano-minerals for feed supplements may offer a viable substitute for the traditional mineral sources in animal diets.

Data Availability

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

Author's contribution

Youssef, A. M., Azzaz, H. H., Noha A. Hassaan, EL-Nomeery, Y.A., Shakweer, W. M. E: Conceptualization, Formal analysis, Investigation, Methodology, Resources, Supervision, Validation, Writing - original draft, Writing - review & editing.

Conflict of interest:

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

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References

[1] Wu G. Principles of Animal Nutrition. 1st ed. Boca Raton, FL, USA: Taylor & Francis Group; (2018).

[2] Cortinhas C.S., Botaro B.G. and Sucupira M.C.A., Antioxidant enzymes and somatic cell count in dairy cows fed with organic source of zinc, copper and selenium. *J. Live st. Sci.* 127, 84-87 (2012).

[3] Bakhshizadeh S., Aghjehgheshlagh M.F., Taghizadeh A., Seifdavati J. and Navidshad B. Effect of zinc sources on milk yield, milk composition and plasma concentration of metabolites in dairy cows. *South African Journal of Animal Science*, 49, 884-891 (2019).

[4] Anil T.S.V., Venkata Seshaiah Ch., Ashalatha P. and Sudhakar K., Effect of dietary supplementation of nano zinc oxide (nano-ZnO) on growth performance in crossbred calves. *Int. J. Curr. Microbiol. App. Sci* , 8(12): 1852-1856 (2019).

[5] Kuzma J. and VerHage P. Nanotechnology in agriculture and food production: anticipated applications: project on *emerging nanotechnologies*, (2006).

[6] Wang C., Zhang L., Su W., Ying Z. and He J et al., Zinc oxide nanoparticles as a substitute for zinc oxide or colistin sulfate: effects on growth, serum enzymes, zinc deposition, intestinal morphology and epithelial barrier in weaned piglets. *PLoS One*; 12 (7): e0181136 (2017).

[7] Raje K., Ojha S., Mishra A., Munde V.K. and Rawat C. et al., Impact of supplementation of mineral nano particles on growth performance and health status of animals: a review. *Journal of Entomology and Zoology Studies*; 6 (3): 1690-1694 (2018).

[8] Tatli S.P., Seven I., Gul Baykalir B., Iflazoglu Mutlu S. and Salem A.Z., Nanotechnology and nano-propolis in animal production and health: an overview. *Italian Journal of Animal Science*; 17 (4): 921-930 (2018). doi: 10.1080/1828051X.2018.1448726.

[9] Gopi M., Pearlin B., Kumar R.D., Shanmathy M., and Prabakar G., Role of nanoparticles in animal and poultry nutrition: modes of action and applications in formulating feed additives and food processing. *International Journal of Pharmacology*; 13 (7): 724-731 (2017). doi: 10.3923/ijp.2017.724.731

[10] Najafzadeh H., Ghoreishi S.M., Mohammadian B., Rahimi E., Afzalzadeh M.R., Kazemivarnamkhasti M., and Ganjealidarani H., Serum biochemical and histopathological changes in liver and kidney in lambs after zinc oxide nanoparticles administration. *Vet. World*, 6 (8), 534–537 (2013).

[11] Pandurangan M., Veerappan M. and Kim D.H., Cytotoxicity of zinc oxide nanoparticles on antioxidant enzyme activities and mRNA expression in the cocultured C2 C12 and 3T3-L1 cells. *Appl. Biochem. Biotechnol.* 175, 1270–1280 (2015).

[12] Swain P.S., Rao S.B., Rajendran D., Dominic G., Selvaraju S., Nano zinc, an alternative to conventional zinc as animal feed supplement: A review. *Animal Nutrition.* 2(3): 134-141 (2016).

[13] El-Naggar M.E., Hussein J., El-sayed S.M. and Youssef A.M., El Bana M., Latif Y.A., Medhat D., Protective effect of the functional yogurt based on Malva parviflora leaves extract nanoemulsion on acetic acid-induced ulcerative colitis in rats. *Journal of Materials Research and Technology*, 9(6), 14500-14508 (2020).

[14] El-Sayed S.M., El-Sayed H.S., Ibrahim O.A. and Youssef A.M., Rational design of chitosan/guar gum/zinc oxide bionanocomposites based on Roselle calyx extract for Ras cheese coating. *Carbohydrate polymers*, 239, 116234 (2020).

[15] El-Sayed H.S., El-Sayed S.M. and Youssef A.M., Novel approach for biosynthesizing of zinc oxide nanoparticles using Lactobacillus gasseri and their influence on microbiological, chemical, sensory properties of integrated yogurt. *Food Chemistry*, 365: 130513 (2021).

[16] 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).

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

[18] APHA, (American Public Health Association), AWWA (American Water Works Association), and WEF (Water Environment Federation), *Standard Methods for the Examination of Water and Wastewater*, 23rd ed. (Rice, E.W., Baird, R.B., Eaton, A.D., Clesceri, L.S. eds.) Washington DC (2017).

[19] 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).

[20] 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).
[21] IBM, Corp Released IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: *IBM Corp* (2011).
[22] Duncan D.B. (1955) Multiple range and multiple F-test. Biometrics. 11:1-42 (2012).
[23] Riazi H., Rezaei J. and Rouzbehany Y., Effects of supplementary nano-ZnO on in vitro ruminal fermentation, methane release, antioxidants, and microbial biomass. *Turk J Vet Anim Sci.,* 43: 737-746 (2019).
[24]Vázquez-Armijo J.F., Martinez-Tinajero J.J., Lopez D., Salem A.Z.M. and Rojo R., In vitro gas production and dry matter degradability of diets consumed by goats with or without copper and zinc supplementation. *Biological Trace Element Research;* 144 (1-3): 580-587 (2011). doi: 10.1007/s12011-011- 9113-y.
[25] Adegbeye M.J., Elghandour M.M., Barbabosa-Pliego A., Monroy J.C. and Mellado M et al., Nanoparticles in equine .: mechanism of action and application as feed additives. *Journal of Equine Veterinary Science*, 78 (1): 29-37(2019). doi: 10.1016/j.jevs.2019.04.001
[26] JunCai C., Wei W., and ZhiSheng W., Effect of nano-zinc oxide supplementation on rumen fermentation *in vitro*. *Chinese Journal of Animal Nutrition*, 23 (8): 1415-1421 (2011).
[27] Arabi F., Imandar M., Negahdary M., Imandar M., Noughabi M.T. and Akbari-dastjerdi H et al Investigation anti-bacterial effect of zinc oxide nanoparticles upon life of Listeria monocytogenes. *Ann Biol Res*; 3:3679-85 (2012).
[28] Azzaz H.H., Eman S.A., Farahat and Hossam, Ebeid 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).