Effect of using different levels of nano-zinc oxide with date by products on *in vitro* digestibility, fermentation characteristics and gas production

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Abstract

The experiment was conducted to study the effect of adding different levels of nano zinc oxide (0, 15 and 30 mg) / kg DM to mixture ration consisting of nano-zinc oxide with date by products either 30% date kernel or date peels or mixture (15 date kernel + 15 date peels) to 70% concentrate ration on digestibility, rumen characteristics, total gas and methane gas production. the results showed significant decrease as a result of adding nano-zinc oxide N zno in methane gas production for the treatment 15 and 30 mg/kg, and it reached (16.88 and 15.55 ml/200 mg), and (Date kernel produced significantly more total gas and methane, metabolizable energy (ME), in vitro organic matter digestibility (IVOMD%), and fatty acid short-chain SCFA (45.55, 23.77, 64.14, 11.25, and 1.02), respectively)..(Date kernel produced significantly more total gas and methane, metabolizable energy (ME), in vitro organic matter digestibility (IVOMD%), and fatty acid short-chain SCFA (45.55, 23.77, 64.14, 11.25, and 1.02), respectively. Also, the treatment of adding nano-zinc oxide at the level of 15 mg/kg dry matter significantly was superior to the digestibility factor of dry matter and organic, and it reached 62.29 and 63.96%, respectively, compared to the levels of 0 and 30 mg/kg dry matter and a significant decrease at levels of 0 and 15 mg/kg dry matter, which was 5.31 and 5.15 for pH 26.44 and 30.95 (mg/100ml) for ammonia nitrogen, respectively. whereas significantly increased for the level of 15 mg/kg dry matter, in the total volatile fatty acids and it reached 3.23 (mmol/100ml) after a 24-hour incubation period for the rumen fluid in vitro.

Keywords: Zinc oxide nanoparticles, date kernel, date peels, rumen fermentation

Introduction

Zinc is an essential nutrient for animals, functioning largely or entirely in enzyme systems and being involved in protein synthesis, carbohydrate metabolism, and many other biochemical reactions [1]. Also, Zinc is widely used in agriculture, and deficiency also causes alterations in the activities of some enzymes such as in animals, zinc deficiency causes a disturbance in the formation of the cornea limited limb bone growth, copper/Zn superoxide dismutase (Cu-Zn SOD), carboxypeptidases, DNA and RNA polymerases, and lactate dehydrogenase [2]. Currently the use of Nano Zn at lower doses in conventional livestock feed provides better results than zinc sources and also indirectly prevent contamination [3]. zinc causes loss of appetite and Vision disturbance, reduced metabolism, energy and protein [4]. nano zinc oxide (nZnO) has shown to reduce CH4 and hydrogen sulfide (H2S) production from liquid manure under anaerobic conditions. Four different levels of nZnO and two types of feed were mixed with rumen fluid the efficacy of nZnO in mitigating gaseous production ([5]. In *in vitro* experiment, it was found that the addition of different levels of nano-zinc oxide (0, 50, 100, 200& 400) improved the growth of microorganisms and an increase in the production of microbial protein, crude protein, volatile fatty acids and energy utilization during an incubation period of 6-12 hours. 100 and 200 mg/kg dry matter negative in ammonia nitrogen concentration and acetate to propionate ratio [6]. The purpose was to investigate the effect of using different percentages of Nano zinc oxide (Nzo) at 0, 15, and 30 mg/kg of the forage mixture consisting of date pits or pits or their mixture on in vitro digestibility of dry matter and organic matter, total gas, methane gas, ammonia nitrogen concentration, and total volatile fatty acids

Materials and Methods

Nano zinc oxide which was used by experiment is made in the USA (CAS number 1314-13-2) with a purity of 99.5% and a nano size of 50 nm. Then, the Nano size was confirmed after dissolving 2 g of Nano zinc oxide in 50 ml of distilled water with 50 ml of ethanol., According to [7], after mixing for two hours with a magnetic mixing device at a temperature of 40) [8] calculated the metabolic energy (ME) MJ/kg dry matter, in vitro organic matter digestion factor (IVOMD)%, and short chain fatty acids (SCFA) mmol/100 ml from total gas production after a 24-hour incubation period). In vitro estimation of total gas and methane production: The production of total gas and methane gas was estimated in the laboratory according to the method [8] by adding different percentages of Nzo 0, 15 and 30 mg / kg dry matter to the rations of dates or dates and their mixture. It was taken 0.2 g for each of the experimental diets were placed inside a glass syringe with a capacity of 100 ml with the addition of 10 ml of the filtered rumen fluid from a newly slaughtered ram, then 20 ml of the prepared artificial saliva was added at the same time with the addition of carbon dioxide gas to equalize the pH with pushing the piston up to get rid of the air completely and closing it. The injection needle has a flexible rubber stopper to prevent liquid from

escaping from the syringe when incubating. All syringes were placed in water at a temperature of 39º C for 24 hours.

Table (1): The composition of experimental diets and content from energy and crud protein

Matter	Date kernel	Date Peels	Date kernel+date peels
Date pit	30.00	0.00	15.00
date juicer	0.00	30.00	15.00
Barley	50.00	50.00	50.00
Yalow corn	8.00	8.00	8.00
Soya mail	10.00	10.00	10.00
Salt	1.00	1.00	1.00
Limestone	1.00	1.00	1.00
Total	100%	100%	100%
Crud protein	12.25	12.30	12.28
ME /MJ/KG/DM	11.02	11.48	11.25

*Metabolic energy (MJ/kg dry matter) = 0.012 x crude protein + 0.031 x crude fat + 0.005 x crude fiber + 0.014 x nitrogen free extract(NFE) MAFF[22]. Calculated organic matter = dry matter - ash, nitrogen-free extract = Organic matter - (crude protein + ether extract + crude fiber)

Characteristics of rumen fluid

pH measurement: The pH was measured immediately after the end of the laboratory incubation period using an electronic device of type PH-0091. Rumen ammonia nitrogen concentration (mg/100ml) Under the method [9] 10 ml of the filtered rumen fluid was taken after a period of laboratory incubation and placed in a 50 ml plastic tube with a tight-fitting cap, then 5 ml of 0.1% hydrochloric acid was added to it, and the tube was placed at a level 3 measuring the concentration of total volatile fatty acids (mmol/100 ml). The concentration of total volatile fatty acids was measured after thawing the frozen rumen liquid and purifying it from sediments and impurities according to [10].

The Digestibility factor of dry matter and organic matter (%): The laboratory digestion coefficient of dry matter and organic matter was estimated according to the method of [11], whereby was weighed at 0.5 gm and placed inside the digestion tubes with the addition of 50 ml of the mixture consisting of 10 ml of the filtered rumen fluid taken from a newly slaughtered ram and 40 ml of the prepared artificial saliva at the same time. Carbon dioxide gas twice daily to create anaerobic conditions during the laboratory incubation period and pH adjustment. The tubes were placed in a water bath at a temperature of 39º C for 48 hours with the water bath agitated twice a day. The tubes were immersed in a 39 o C water bath for 48 hours, with the water bath being agitated twice a day. At the end of the incubation perio The tubes are shaken well to break up the undigested parts and to kill the microorganisms and incubate

them again at the same temperature for 48 hours. Then the samples are filtered and the sample is filtered. Only which represents the products of the two stages of microbial digestion and For enzyme, the precipitate is dried at a temperature of 105 ° C for 24 hours to calculate the percentage of the dry matter digestibility factor, then the samples are placed inside the incinerator at a temperature of 600 ° C for 3 hours in order to estimate the percentage of ash and the freezing temperature until the chemical analysis is carried out. Statistical analysis :The data were analyzed in a 3 × 3 factorial experiment using [12], which calculated the statistics for general linear models. Both of the feed types and three levels of nZnO were used as fixed effects models. Means were declared statistically significant at $P \leq 0.05$ using multiple range test.

Results and Discussion

The result showed (Table 2) that there are highly significant differences ($P \leq 0.01$) of adding nano-zinc oxide N ZnO to the characteristic of produced methane, which decreased with the treatment 15 and 30 mg/kg compared to the control treatment 0 mg/kg, but it reached (16.88, 15.55 and 18.11). ml / 200 mg), respectively, and this may be due to the fact that nano-zinc has led to a decrease in the production of methane, which is one of the sources of energy loss in the rumen, which may be a result of a decrease in the number of bacteria methane-producing [13], or it may be a result for the effect of nano-zinc by reducing methane production and redirecting hydrogen flow towards other electron acceptors such as propionate [14] , also agrees with (Sarker,et.al.2018) when adding four levels of nano-zinc to two feed sources led to a reduction in the number of microorganisms by 0.47-22.21% and a decrease in (H_2S +CH_4 and CO_2) gas after 72 hours of *in vitro* incubation, the percentage ranged between 4.89-53.65% compared to control treatment [15] reduction of CH_4 on centration from rumen fluid at the highest application level of nZnO was likely due to the impact of excessive nZnO application rate specifically on methanogens .[16] found that adding different sources of ZnO or nano-ZnO with three levels of 20, 40 or 60 mg/kg dry matter was low in methane production with zinc oxide and nano-Zn compared to the control. While in table 2 type of the ration appeared significantly ($P \leq 0.05$) with all treatments of 30% date nucle or date peels and a mixture of 15% to each for all the characteristics production of total gas, methane and metabolic energy (ME), and in vitro digestibility of organic matter (IVOMD%) and short chain fatty acids (SCFA), which were (45.55 and 23). 77a, 64.14, 11.25, and 1.02) each, respectively. This result may be attributed to fermentation Carbohydrates dissolved with uncle and peels of date caused an increase in the production of propionate, on the other hand the date nucle and peels rich in fiber lead to increased fermentation in rumen liquid. This is rustle corresponds to [17].In another study [18] found that date residues, increases total gas production for different in vitro in cubation periods . Dates are one of the fatty acids that encourage the growth of microorganisms in the rumen fluid [19]. The interaction between the type of ration and the different additives (date nucle 30% or date peels 30% and their mixture 15% for each one) supplemented with three levels

of nano zinc oxide N zno (0, 15 and 30 mg/kg dry matter, the table2 shows significantly decreased (P≤0.05) between treatment for all traits compared to the control . Table (2) showed Significantly differences (P≤0.05) in the in vitro digestibility of organic matter (IVOMD%) by the gas method, and the highest percentage of treatment T3 T2 and T1 of date nucle ration with all levels of N zno 64.54, 62.76 and 65.13%, respectively. There were also significantly differences (P≤0.05) in the level of metabolizable energy (MJ/kg dry matter) and fatty acids. Short-chain SCFA (mmol/100ml) measured from gas production after 24 hours of incubation were 11.32, 11.01 and 11.43 MJ/kg dry matter and 1.03, 0.99 and 1.05 (mmol/100ml), respectively.

Table (2): Effect of adding different levels of Nzo on the rate of total gas production, methane, metabolic energy (ME), digestibility factor of organic matter (IVOMD%) and short chain fatty acids (SCFA)

Effect	total gas TG	Methane CH_4	Metabolic energy (ME)	(IVOMD%)	(SCFA)
levels of Nano zinc oxide					
0	37.44 ±2.41	18.11 ±1.69 a	10.02 ±0.37	57.49 ±2.01	0.83 ±0.05
15	37.11 ±2.03	16.88 ±2.10ab	9.97 ±0.31	57.19 ±1.67	0.82 ±0.04
30	38.22 ±2.41	15.55 ±1.7 b	10.14 ±0.37	58.18 ±2.01	0.85 ±0.05
Significant	NS	*	NS	NS	NS
Type of ration					
Date uncle	45.55± 1.36a	23.77 ±0.79 a	11.25 ±0.21 a	64.14 ±1.21 a	1.02 ±0.03 a
Date peels	35.44 ±0.92b	14.66 ±0.72 b	9.81 ±0.14 b	55.83 ±0.82 b	0.78 ±0.02 b
Date (uncle + peels)	31.77 ±0.61c	12.11 ±0.48 c	9.07 ±0.09 c	52.89 ±0.54 b	0.69 ±0.01 c
Significant	*	*	*	*	*
Interaction between levels of nano zinc oxide and ration					
T1	46.00 ±2.30 a	24.33 ±0.88ab	11.32 ±0.36a	64.54 a ±2.05	1.03 ±0.05a
T2	44.00 ±3.05a	25.00 ±1.73a	11.01 ±0.47a	62.76 a ±2.71	0.99 ±0.07a
T3	46.66 ±2.40a	22.00 ±1.15b	11.43 ±0.37a	65.13 a±2.13	1.05 ±0.05a
T4	35.00 ±2.08b	17.00 ±0.57c	9.74 ±0.32b	55.43 b ±1.85	0.77 ±0.04b
T5	35.33 ±0.66b	13.00 ±0.57ed	9.79 ±0.10b	55.73 b±0.59	0.78 ±0.01b
T6	36.00 ±2.30b	14.00 ±1.15d	9.89 ±0.36b	56.32 b±2.05	0.79 ±0.05b
T7	31.33 ±1.33b	13.00 ±0.57ed	9.00 b±0.20	52.49 ±1.18b	0.68 ±0.03b
T8	32.00 ±1.15b	12.66 ±0.66ed	9.11b±0.18	53.08 ±1.02b	0.70 ±0.02b
T9	32.00 ±1.15b	10.66 ±0.66e	9.11 b±0.18	53.08 ±1.02b	0.70 ±0.02b
Significant	*	*	*	*	*

Different letters within the column mean the presence of significant differences, NS = no significant differences within the same column * there is a significant difference at the level (0.05) ** there is a

significant difference at the level (0.01) T1= 30% date seeds +0 mg (Nzno) T2= 30% date seeds +15 mg (Nzno) T3= 30% date seeds +30 mg (Nzno) T4= 30% Date juicer +0 mg (Nzo) T5= 30% Date juicer + 15 mg (Nzo) T6 = 30% Date juicer + 30 mg (Nzno) T7= 15% date seeds +15% Date juicer + 0 mg Nzno T8 15% date seeds +15% Date juicer + 15 mg Nzno ,T9= 15% date seeds +15% Date juicer + 30 mg Nzno .

Digestibility of dry and organic matter %

The finding (Table 3) displayed a significant differences (P≤0.05) when adding nano-zinc oxide at the level of 15 mg/kg DM to the digestibility of dry and organic matter 62.29 and 63.96% respectively compared to levels of 0 and 30 mg/kg DM this result indicates that the addition of 30 mg/kg DM of nano-zinc had a negative effect on the coefficient of digestion of dry and organic matter, which may be a result of the effect on the total numbers of bacteria and thus led to a decrease in the coefficient of in vitro digestion, [20]. Also the type of ration significantly (P≤0.05) digestibility with (15% nucle + 15% peels) mixture treatment of dry and organic matter to 63.91 and 65.86%, respectively (table 3). This result may be due to higher carbohydrate content and subsequent higher ferment ability resulted to in increases of digestibility .The nutritional compounds in this mixture of fatty acids and dietary fibers, which encouraged the growth of rumen bacteria and increased the coefficient of digestion [19]. The interaction between the type of ration and the levels of nano-zinc oxide showed increased significantly (P≤0.05) T4 and T8 in dry and organic matter digestibility, reached 68.44 and 69.93%, 69.83and 72.60% respectively compared with other treatments (table 3). This improvement may be due to the interaction between nano zinc oxide particles and date residues.

Table (3): Effect of adding different levels of (Nzo) at (0, 15 and 30) mg/kg dry matter in digestibility of dry matter and organic matter (%) after an incubation for 48 hours

Effect	digestibility of dry matter %	digestibility of organic matter%
levels of nano zinc oxide		
0	59.68 ±2.95a	60.95 ±2.98b
15	62.29 ±2.66a	63.96 ±2.86a
30	54.17 ±2.74b	56.07 ±2.64c
Significant	*	*
Type of ration		
Date nucle	52.29 ±3.34c	53.79 ±3.21c
Date peels	59.94 ±2.32b	61.33 ±2.35b
Nucle +peels	63.91 ±1.54a	65.86 ±1.70a
Significant	*	*
Interaction levels of nano zinc oxide and ration		
T1	49.35 ±3.36e	50.51 ±3.34ed

T2	64.04 ±2.86bc	65.20 ±2.97bc
T3	43.47 ±1.48f	45.66 ±0.52e
T4	68.44 ±0.56ab	69.83 ±0.52ab
T5	52.92 ±1.46e	54.09 ±1.57d
T6	58.48 ±0.59d	60.07 ±0.61c
T7	61.24 ±0.35cd	62.50 ±0.40c
T8	69.93 ±0.66a	72.60 ±0.21a
T9	60.56 ±0.98cd	62.48 ±0.78c
Significant	*	*

Different letters within the column mean the presence of significant differences, NS = no significant differences within the same column * there is a significant difference at the level (0.05) ** there is a significant difference at the level (0.01) T1= 30% date nucle +0 mg (Nzno) T2= 30% date nucle +15 mg (Nzno) T3= 30% date nucle+30 mg (Nzno) T4= 30% reached+0 mg (Nzo) T5= 30% Date peels + 15 mg (Nzo) T6 = 30% Date peels + 30 mg (Nzno) T7= 15% date peels +15% Datepeels + 0 mg Nzno T8 15% date nucle +15% Date peels + 15 mg Nzno ,T9= 15% date peels +15% Datenucle + 30 mg Nzno

Effect of adding different levels of (Nzo) on pH, ammonia nitrogen and total volatile fatty acids after incubation period of 24 hours.

Table 4 shows when adding different levels of Nzo (0, 15 and 30) mg/kg DM a significant increase (P≤0.05) for the level of 30 mg of pH and ammonia nitrogen, which reached 5.37 and 37.06 (mg/100ml), respectively, compared to the level of 0 and 15 mg/kg dry matter, but within normal limits, and a significant increase (P≤0.05) for the level 15 mg/kg DM in the total volatile fatty acids, and it reached 3.23 (mmol/100ml) after a 24-hour in *in vitro* incubation period for the rumen fluid, This increase may be due to the high level of fermentation with zinc oxide nanoparticles, which is different from what [5] found when adding different levels of zinc oxide to *in vitro* treatment, where the addition led to a decrease in the total volatile acids. The results of Table 4 also showed a significant effect (P≤0.05) of the type of ration on the rumen fermentation of the date kernel treatments on the rest of the treatments, which amounted to 5.49 and 76.34 mg/100 ml for each of the pH and nitrogen ammonia, respectively. The date pulp also outperformed the mixture diets for the characteristics of pH and ammonia nitrogen, which amounted to 5.23, 5.10, 30.86 and 28.83 mg/100ml, respectively. While the date pulp diets outperformed the kernel and mixture in the total volatile fatty acids, which amounted to 3.27 mmol/100ml. The interaction between the type of ration and the levels of nano-zinc significant different (P≤0.05) of T3 (kernel + nano-zinc), which reached to pH 5.65, while T 2 (kernel + 15 nano-zinc) outperformed the treatments in the concentration of ammonia nitrogen, which amounted to 38.90 mg/100 ml, and the lowest value of T7 (15% kernel +15% Bethel + 0 mg Nzno), which amounted to 19.10 mg/100 ml, while the superiority was for the treatment T5 (dates strawberry + 15 mg Nzno) and it was 3.90 mmol / 100 ml of total volatile fatty acids and this indicates that the addition of nano-zinc oxide by 15 mg Nzno led to the maintenance of the pH from a decrease and a significant

increase in the production of volatile fatty acids for the T5 treatment (a date with 15 mg Nzno), which shows the importance of nano-zinc improving rumen fermentation in *in vitro*. This was indicated by [5] when adding different levels of nano zinc oxide 0, 100, 200, 500, and 1000 µg / g improved the fermentation of the rumen in *in vitro* of total volatile fatty acid for the control treatment and the treatment 1000 µg / g. [21]pointed that increasing the TVFA with the applied higher nZnO levels increased energy utilization by ruminal microbial protein synthesis by the microbes in the early stages of fermentation .

Table (4): Effect of adding different levels of (Nzo) at (0, 15 and 30) mg/kg dry matter on pH, ammonia nitrogen concentration (mg/100ml) and the concentration of volatile fatty acids (mmol/100ml) after incubation for 24 hours

Effect	pH	NH3-N mg/100ml	VFA (mmol/100ml)
levels of nano zinc oxide			
0	5.31 ± 0. 05 ab	26.44 ±3.13c	2.92 ±0.19b
15	5.15 ± 0.04 b	30.95 ±0.91b	3.23 ±0.21a
30	5.37 ± 0.09 a	37.06 ±0.07a	2.62 ±0.26c
Significant	*	*	*
Type of ration			
Date kernel	5.49 ± 0.05a	34.76 ±1.63a	2.85 ±0.31b
Date peels	5.23 ± 0.05 ab	30.86 ±2.42b	3.27 ±0.21a
kernel+peels	5.10 ± 0.03 b	28.83 ±2.63c	2.64 ±0.06c
Significant	*	*	*
Interaction levels of nano zinc oxide and ration			
T1	5.53 ± 0.03 b	28.33 ±0.33 e	3.63 ±0.20ab
T2	5.30 ± 0.03 c	38.90 ±0.49a	3.30 ±0.15b
T3	5.65 ±0.02a	37.06 ±0.17b	1.63 ±0.08d
T4	5.16 ±0.03ed	21.33 ±0.33f	2.50 ±0.05c
T5	5.10 ±0.05ef	34.33 ±0.66c	3.90 ±0.15a
T6	5.45 ±0.02ab	36.93 ±0.06b	3.43 ±0.12b
T7	5.25 ±0.02cd	19.10 ±0.05g	2.63 ±0.08c
T8	5.05 ±0.02f	30.20 ±0.11d	2.50 ±0.05c
T9	5.01 ±0.01f	37.20 ±0.11b	2.80 ±0.11c
Significant	*	*	*

Different letters within the column mean the presence of significant differences, NS = no significant differences within the same column * there is a significant difference at the level (0.05) ** there is a significant difference at the level (0.01) T1= 30% date seeds +0 mg (Nzno) T2= 30% date seeds +15 mg (Nzno) T3= 30% date seeds +30 mg (Nzno) T4= 30% Date juicer +0 mg (Nzo) T5= 30% Date juicer + 15 mg (Nzo) T6 = 30% Date juicer + 30 mg (Nzno) T7= 15% date seeds +15% Date juicer + 0 mg Nzno T8 15% date seeds +15% Date juicer + 15 mg Nzno ,T9= 15% date seeds +15% Date juicer + 30 mg Nzno

Reference

1) Mishra, P. K.; Mishra, H.; Ekielski, A; Talegaonkar, S. and Vaidya, B. (2017). Zinc oxide nanoparticles: a promising nanomaterial for biomedical applications. Drug Discovery Today, 22 (12):1825–1834.
2) Sun, J.Y; Wang J.F; Zi, N.T; Jing, M.Y. and Weng, X.Y. (2011). Effects of zinc supple mentation and deficiency on bone metabolism and related gene expression in rat. Biological Trace Element Research, 143: 394–402.
3) Partha, S.; Swaina, S.; Raob, N.; Rajendr , D. and Selvarajub, J. (2016). Nano zinc, an alternative to conventional zinc as animal feed supplement: A review Animal Nutrition, 2 (3):134-141.
4) Hosnedlová, B.; Trávníček, J. and Šoch, M. (2007). Current view of the significance of zinc for ruminants: a review. Agricultural Tropica et Subtropica, 40: 57–64.
5) Sarke , N. C.; Faithe, K.; Borhan, S. and Rahman, K. S. (2018). In vitro evaluation of nano zinc oxide on mitigation of gaseous. Journal of Animal Science Technology, 60:27eI.SSN: 2055-0391.
6) Chen, J.; Wang, W. and Wang, Z. (2011). Effect of nano-zinc oxide supplementation on rumen fermentation in vitro. Chinese Journal of Animal Nutrition, 8: 023.
7) Sabir, S.; Arshad, M. and Chaudhari, S. K. (2014). Zinc Oxide Nanoparticles for Revolutionizing Agriculture: Synthesis and Applications. The scientific world Journal, 92:54-64.
8) Menke, K.H. and Steingass, A. (1988). Estimation of the energy of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. Animal research and development, 28: 1-7.
9) Association of Official Analytical Chemists (A.O.A.C.). (2005). Official of analysis18 thEd. Gaithersburg, Maryland, USA.
10) Warner, A.C.I. (1964). Production of volatile fatty acid in the rumen methods of measurements. Nutrition Abstracts and Reviews 34:339-343.
11) Tilley, J.M. A. and Terry, R. A. (1963). A two-stage technique for in- vitro digestion of forage crops. Journal of Grassland and Forage Science, 18: 104-111.
12) SAS. (2010). Statistical Analysis System for Windows version 6.13.
13) Morgavi, D.P.; Forano, E.; Martin, C. and Newbold, C.J. (2010). Microbial ecosystem and meth anagenesis in ruminants. Animal, 4 (7): 1024-1036.
14) McAllister, T.A. and Newbold, C.J. (2008). Redirecting rumen fermentation to reduce methanogenesis. Australian Journal of Experimental Agriculture, 48 (2):7- 13.

15) Swain, P.S.; Rao, S.B.; Rajendran, D.; Dominic, G. and Selvaraju, S. (2016). Nano zinc, an alternative to conventional zinc as animal feed supplement: A review. Animal Nutrition, 2(3):134–141.

16) Riazi ، H. ; Javad ,R.; and Yousef, R. (2019). Effects of supplementary ZnO in vitro ruminal fermentation, methane release antioxidants, and microbial biomass. Turkish Journal of Veterinary and Animal Sciences, 43: 737-746.

17) McDonald, P.; Edwards, R. A. ; Greenhalgh, J. F. ; Morgan, C. A. ; Sinclair, L. A. and Wilkinson, R. G. (2011). Animal Nutrition, 7th edition. Prentice Hall، Pearson, Harlow, UK.

18) Boufennara, S.; Boufennara, L. B.; Antonio de, V., M.; Fondevila, Z. A.; Secundi, L. (2016). In vitro assessment of nutritive value of date palm by-products as feed for ruminants, Emirates Journal of Food and Agriculture, 28 (10): 695-703.

19) Hamza, H.; Walid. E. and Kameleddine, N. (2021). Date Palm Seed Oil (*Phoenix dactylifera* L.) Green Extraction: Physicochemical Properties, Antioxidant Activities, and Phenolic and Fatty Acid Profiles Journal of Food Quality, ID 2394220: 9.

20) Starke, I.C.; Pieper, R.; Neumann, K.; Zentek, J. and Vahjen, W. (2014).The impact of high dietary zinc oxide on the development of the intestinal microbiota in weaned piglets. FEMS Microbiology Ecology, 87: 416–427.

21) Zhisheng, C. (2011). Effect of nano-zinc oxide supplementation on rumen fermentation in vitro. Chinese Journal of Animal Nutrition, 8:023.

22) MAFF. (1975). Ministry of Agric., Fisheries and Food Department of Agriculture and Fisheries for Scotland Energy allowances and Feed systems for ruminants, Technology Bull., 33. First published.

23) Duncan, D.B. (1955). Multiple range and multiple F test. Biometrics, 11:142.