



## Effect of nano zinc oxide on zinc bioavailability and blood biochemical changes in pre-ruminant lambs

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Zinc (Zn) is an essential component of a number of metallo-enzymes and transcription factors (O'Dell 2000), which plays significant roles in the metabolism of essential nutrients in animals (Jia *et al.* 2008). As Zn is not stored in the body, a continuous dietary supply is essential for proper physiological functions (Zalewski *et al.* 2005). In spite of the low solubility of zinc oxide, it is predominant source of Zn used by the animal feed industry (Wedekind and Baker 1990). Recently, the use of nano zinc oxide (nZnO) for supplementation of ruminant diets has begun but whether nano forms are more effective than normal zinc oxide (ZnO) remains unclear. The transition from micro particles to nanoparticles (< 100 nm in diameter) involves an increment of the surface area, among other changes in properties. A larger surface area of the nanoparticles allows greater solubility which may lead to better utilization in animals. Limited knowledge of the effects of this substance highlights the need to ascertain their possible use as a nutritional supplement in ruminants. Hence an experiment was conducted to see the effect of nZnO on zinc availability and its toxicity, if any, in pre-ruminant lambs.

Twenty pre-ruminant *Jalauni* male lambs (body weight 4.5 kg, age 25–35 days) were randomly divided into 2 groups each having 10 animals. All the lambs were maintained on similar basal ration consisting of maize grain, mustard cake, mineral mixture and common salt in the ratio of 64:34:1:1, respectively. The lambs of G<sub>1</sub> served as control and were supplemented with 60 ppm zinc from ZnO while the lambs of G<sub>2</sub> were given 60 ppm zinc from nZnO (30 nm) in the concentrate mixture over and above available from basal ration (30.06 ppm) for a period of 45 days (ICAR 2013).

Live weight of each animal was recorded weekly. At the end of the experimental period, a digestibility cum metabolism trial of 5 days collection was conducted to evaluate the effect of nZnO on zinc availability as well as

blood metabolites in lambs. The chemical composition of biological samples, viz. feed, faeces and urine nitrogen were determined as per AOAC (2000) and their fibre fractions as per Van Soest *et al.* (1991).

For zinc determination, feed, faeces and urine samples were digested in tri-acid mixture while plasma samples were diluted to 1/5 with deionized water. The analysis was performed using VARIAN AA240 Atomic Absorption Spectrophotometer.

Blood samples were collected in heparinized test tubes before and at the end of experimental feeding to study various plasma metabolites, plasma urea (Rahmatullah and Boyde 1980), creatinine (Wootton 1964) and superoxide dismutase (Sun *et al.* 1988). Data were analyzed to test the significant differences between means using 't' test as described by Snedecor and Cochran (1994).

The chemical composition of feed ingredients and basal ration are presented in Table 1. The dry matter intake (DMI) was 215.28 and 218.60 g/d in G<sub>1</sub> and G<sub>2</sub>, respectively, which was similar between groups (Table 2). Jaboli *et al.* (2013) also observed no effect of supplementary zinc oxide on dry matter intake among groups supplemented with different levels (e.g., 0, 20 and 40 ppm) of zinc oxide and nano zinc oxide in Iranian Angora goat kids. Similarly, supplementation of Zn to a basal diet containing more than 25 mg Zn/kg DM had no effect on DMI in growing lambs (Garg *et al.* 2008). Intake of CP and TDN followed the trend exhibited by DM intake.

The apparent digestibility coefficient of DM, OM, CP, NDF and ADF was almost similar between the groups (Table 2). Jadhav *et al.* (2008) reported that supplementation of zinc ranging from 0, 35 and 70 ppm in buffalo calves did not affect the nutrient digestibility. Similarly, there was no difference in the digestibility of CP, EE, NDF, ADF and cellulose in crossbred calves supplemented with 0, 35 and 70 ppm zinc in basal diet containing more than 25 ppm zinc (Mandal *et al.* 2008). All the animals were in positive N balance, which did not differ between the treatments.

There was no difference in zinc intake (19.50 vs 19.72 mg/d) between the groups (G<sub>1</sub> and G<sub>2</sub>). However, the faecal excretion was significantly (P<0.05) higher in G<sub>1</sub> (82.77%)

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Table 1. Chemical composition of feed ingredients

Feed ingredient	CP	CF	EE	Ash	NFE	NDF	ADF	Zinc
Mustard cake	30.02	6.60	7.90	6.90	48.58	25.25	18.62	42.02
Maize	10.00	2.20	4.30	2.10	81.30	12.45	4.32	24.01
Basal ration	16.90	3.72	5.90	3.76	69.04	16.80	9.28	30.06

Table 2. Effect of nano zinc oxide on intake and digestibility of nutrients in lambs

Parameter	G <sub>1</sub>	G <sub>2</sub>
Body wt. (kg)	6.24±0.16	6.21±0.22
DM intake (g/d)	215.28±5.10	218.60±6.42
CP intake (g/d)	36.38±0.35	36.94±0.44
TDN intake (g/d)	143.16±3.14	145.36±4.21
<i>Digestibility coefficient (%)</i>		
DM	66.84±0.64	68.54±0.78
OM	68.24±0.79	69.50±1.16
CP	64.64±0.68	65.45±0.35
CF	65.82±3.60	66.42±4.20
NFE	70.42±1.50	71.20±1.80
EE	80.45±2.64	80.20±2.65
NDF	62.11±1.64	60.24±1.84
ADF	54.60±1.56	52.80±1.66
<i>Nitrogen retention (g/d)</i>		
N intake	5.82±0.15	5.91±0.17
Faecal N	2.04±0.21	2.06±0.22
Urinary N	1.54±0.12	1.63±0.11
N balance	2.22±0.14	2.24±0.14
N retention (% N intake)	38.14±2.12	37.9±02.08
<i>Zinc availability (mg/d)</i>		
Zn intake	19.50±0.36	19.72±0.55
Faecal Zn	16.14±0.52 <sup>a</sup>	14.01±0.74 <sup>b</sup>
Urinary Zn	1.05±0.12	1.16±0.14
Zn balance	2.31±0.08 <sup>a</sup>	4.55±0.09 <sup>b</sup>
Absorption coefficient (%)	11.85±1.16 <sup>a</sup>	23.07±1.02 <sup>b</sup>

Means bearing different superscript in a row differ significantly (P<0.05).

as compared to G<sub>2</sub> (71.04%) without affecting the urinary excretion, which was almost similar (Table 2). This might led to significantly higher zinc retention (4.55 mg/d) in G<sub>2</sub> as compared to G<sub>1</sub> (2.31 mg/d) and so the absorption coefficient (23.07 vs 11.85%). A similar excretion pattern of zinc had been reported earlier by Singh *et al.* (2009) in kids. There has been variable effect of different zinc sources on its retention. Jadhav *et al.* (2008) observed higher zinc retention in buffalo calves supplemented with 0, 35 and 70 ppm zinc from zinc sulphate. Ziva *et al.* (2010) used three different types of Zn sources (ZnO, ZnCl<sub>2</sub> and nZnO) at levels of 2,000 and 5,000 µg/g DM in the diet of invertebrate animals (*Porcellio scaber*). They showed that the potential of these compounds for accumulation were similar.

Initial plasma level of zinc was almost similar (0.84 vs 0.79 µg/dl) between the groups (Table 3). However, after 35 days of supplementation there was a significant increase in the plasma zinc level of lambs in both the groups. But the increase was more prominent in G<sub>2</sub> (1.85 µg/dl) as

Table 3. Effect of nano zinc oxide on blood metabolites in lambs

Parameter	G <sub>1</sub>	G <sub>2</sub>
Plasma Zn initial (µg/dl)	0.84±0.16 <sup>a</sup>	0.79±0.18 <sup>a</sup>
Plasma Zn final (µg/dl)	1.25±0.06 <sup>a</sup>	1.85±0.10 <sup>b</sup>
Plasma urea initial (mg/dl)	32.54±2.44	32.10±2.65
Plasma urea final (mg/dl)	34.10±2.14	31.32±2.44
Creatinine (mg/dl)	0.95±0.09	0.91±0.10
Creatinine (mg/dl)	1.02±0.46	1.12±0.88
Superoxide dismutase initial (U/dl)	230.65±8.65	242.54±10.88
Superoxide dismutase final (U/dl)	250.40±11.45	265.50±14.66

Means bearing different superscript in a row differ significantly (P<0.05).

compared to G<sub>1</sub> (1.25 µg/dl). The higher plasma zinc levels in nZnO (G<sub>2</sub>) might be due to the greater absorption of nZnO. It has been shown that nano particles are absorbed in duodenum by active transport and nano-elemental forms can cross the small intestine and further distribute into the blood (Hillyer and Albrecht 2001). The findings were in agreement with those of Najafzadeh *et al.* (2013) who also observed increased serum zinc levels after oral administration of nano zinc oxide in lambs.

The blood biochemical tests e.g. creatinine and superoxide dismutase (SOD) are good indicator of kidney and liver function. If kidney function fails, the creatinine levels will rise and when liver is in dysfunction, the levels of the SOD enzymes will rise. Results (Table 3) clearly indicated that initial and final plasma creatinine and SOD levels were almost similar and within the normal physiological range in both groups and there was no sign of nZnO toxicity in pre-ruminant lambs. Although, no information is available on the topic as such to date. Contrary to our study, Najafzadeh *et al.* (2013) observed significantly increased creatinine level in the serum of lambs supplemented orally with nano zinc particle at the rate of 20 mg/kg body weight. High serum creatinine levels in lambs may be due to the exposure of high doses (360–400 mg/d) of nZnO. Further, the toxicity of nZnO is reported to be associated with dose and duration of exposure to nano particles (Swain *et al.* 2016). Whereas, in the present study, the nZnO intake was very low (13 mg/d) as compared to the former (360–400 mg/d), hence no apparent toxicity was observed.

The average daily gain (Table 4) was almost similar (42–43 g/d) between the groups. Consistent with our observation, Jaboli *et al.* (2013) also did not find any effect of 20 or 40

Table 4. Effect of nano zinc oxide on growth performance in lambs

Particular	G <sub>1</sub>	G <sub>2</sub>
Initial body weight (kg)	4.50±0.68	4.51±0.55
Final body weight (kg)	6.24±0.34	6.21±0.32
Weight gain (kg)	1.74±0.22	1.70±0.18
Average daily gain (g/d)	43.50±2.48	42.50±2.32

ppm nZnO on average daily gain in Iranian Angora goat kids fed control diet containing 22 ppm zinc. However, nZnO had been reported to enhance growth performance in poultry (Mishra *et al.* 2014). As the zinc level (30 ppm) in the basal ration was adequate (Mc Dowell 1985) for normal growth of *Jalauni* lambs, that might be the reason for non-significant effect on growth performance.

Based on results, in spite of similar effect of both sources of zinc supplementation on nutrient utilization and growth performance, then nZnO significantly improved the Zn availability and plasma Zn levels in pre-ruminant lambs without causing toxicity. Thus, nZnO opens a window for better bio-available zinc source and if possible, to reduce the supplementation cost in future.

#### SUMMARY

Twenty pre-ruminant *Jalauni* male lambs (body weight 4.5 kg, age 25–35 days) were randomly divided into 2 groups each having 10 animals. All the lambs were maintained on similar basal ration consisting of maize grain, mustard cake, mineral mixture and common salt in the ratio of 64:34:1:1, respectively. The lambs of G<sub>1</sub> served as control and were supplemented with 60 ppm zinc from ZnO while the lambs of G<sub>2</sub> were given 60 ppm zinc from nZnO (30 nm) in the concentrate mixture over and above available from basal ration (30.06 ppm) for a period of 45 days. Results indicated that there was no difference in the intake and apparent digestibility coefficient of nutrients between groups. However, the faecal excretion of Zn was significantly ( $P<0.05$ ) higher in G<sub>1</sub> (82.77%) as compared to G<sub>2</sub> (71.04%) in spite similar urinary excretion. This led to significantly higher zinc retention (4.55 mg/d) in G<sub>2</sub> as compared to G<sub>1</sub> (2.31 mg/d) and so the absorption coefficient (23.07 vs 11.85%). All the blood metabolites were within the normal physiological range except zinc which was significantly ( $P<0.05$ ) higher in G<sub>2</sub> (1.85 µg/dl) as compared to G<sub>1</sub> (1.25 µg/dl). Hence, it is concluded that the nZnO significantly improve the Zn availability and plasma Zn levels in pre-ruminant lambs without causing toxicity. Thus, nZnO opens a window for better bio-available zinc source and if possible, to reduce the supplementation cost in future.

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