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SHORT COMMUNICATION

Supplementary Effect of Different Levels of Nano Zinc Oxide on Zinc Bioavailability and Blood Metabolites in Lambs

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ABSTRACT

Twenty five *Jalauni* male lambs (average body weight = 4.5 kg, age 35 d) were randomly divided into 5 groups of 5 animals each. All the lambs were maintained on basal ration comprising grass hay and concentrate mixture (Maize grain, mustard cake and common salt in the ratio of 64: 35: 1). The group G_1 served as control supplemented with 40 ppm zinc from ZnO while the lambs in groups G_2, G_3, G_4 and G_5 were supplemented with 40, 30, 20, 10 ppm Zn from nano ZnO (nZnO 30 nm) in the concentrate mixture over and above the basal ration (24.12 ppm) for a period of 150 d. Results indicated no difference in the intake and digestibility of nutrients amongst groups. The Zn intake was higher (P<0.05) in group G_1 and G_2 as compared to groups G_3 to G_5. A similar trend was also observed for faecal and urinary Zn excretion which was higher (P<0.05) in group G_1 as compared to groups G_2, G_3, G_4 and G_5 that led to higher Zn retention (2.01 and 1.88 mg/d) in groups G_2 and G_3 as compared to groups G_1, G_4 and G_5 (1.50, 1.40 and 1.33 mg/d) and absorption was higher (P<0.05) in nZnO supplemented lambs in comparison to ZnO supplemented lambs (G_1). The blood metabolites were within the normal physiological range except that plasma Zn concentration was higher (P<0.05) in nZnO supplemented lambs. Hence, nZnO improved (P<0.05) Zn availability in lambs. Thus, nZnO could be a better source of Zn supplementation at a supplementary level of 20 ppm.

Key words: Blood metabolites, Lamb growth, Nano zinc oxide, Zinc bioavailability

Zinc (Zn) is an important part of a number of enzymes which play a important role in the metabolism of nutrients in animals (Jia *et al.,* 2008). As Zn is not stored in the body, a continuous dietary supply is necessary for proper physiological functions (Zalewski *et al.,* 2005). Inspite of the poor solubility of zinc oxide, it is the main source of Zn used by the animal feed industry (Wedekind and Baker, 1990). Recently, the use of nano zinc oxide (nZnO) for supplementation in ruminant diets has begun but whether nano forms are more effective than normal zinc oxide (ZnO) remains unclear. The changeover from micro particles to nanoparticles (<100 nm in diameter) involves an increase of the surface area among other changes in properties. A larger surface area of the nanoparticles allows greater solubility which might 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 find out the effect of different levels of nZnO on Zn availability in lambs.

For this study, twenty five *Jalauni* male lambs (BW= 4.5 kg; age 35 d) were randomly divided into 5 groups of 5 animals each. All the lambs were maintained on basal ration comprising grass hay and concentrate mixture (Maize grain, mustard cake and common salt in the ratio of 64: 35: 1). The group G_1 served as control supplemented with 40 ppm Zn from ZnO while the lambs in groups G_2, G_3, G_4 and G_5 were supplemented with 40, 30, 20, 10 ppm Zn from nano ZnO (nZnO 30 nm) in the concentrate mixture over and above the basal ration (24.12 ppm) for a period of 150 d (ICAR, 2013). Live weight of each animal was recorded weekly. In the middle of feeding trial, a metabolism trial of 5 days duration was conducted to evaluate the effect of nZnO on zinc availability as well as blood metabolites in lambs. The proximate composition (AOAC, 2000) and fibre fractions (Van Soest *et al.,* 1991) in biological samples were determined. For estimation of Zn, feed, faeces and urine samples were digested in tri-acid

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Table 1. Chemical composition (% DM basis) of feed ingredients

Feed ingredient	CP	CF	EE	Ash	NFE	NDF	ADF	Zinc
Grass hay	6.20	32.20	1.10	9.88	52.80	70.44	35.45	10.20
Mustard cake	30.02	6.60	7.90	6.90	48.58	25.25	18.62	40.02
Maize	10.00	2.20	4.30	2.10	81.30	12.45	4.32	22.01

mixture while plasma samples were diluted to 1/5 with deionized water and analysed using VARIAN AA240 Atomic Absorption Spectrophotometer. Blood samples were collected in heparinized test tubes before (0 d) and at the end of experimental feeding (90 d) to estimate creatinine (Wootton, 1964) and superoxide dismutase (Sun *et al.,* 1988). Data were analyzed statistically (Snedecor and Cochran, 1994).

The chemical composition of feed ingredients has been presented in Table 1. The DM intake ranged from 350.95 to 374.22 g/d with the corresponding values of 3.60 to 3.79 kg/100 kg body weight which was similar in all the groups (Table 2). Zaboli *et al.* (2013) also observed no effect of supplementary zinc oxide on DM

Table 2. Effect of different levels of nano zinc oxide on intake and utilization of nutrients in lambs

Parameter	Group				
	I	II	III	IV	V
Body wt. (kg)	9.75±0.29	9.83±0.32	9.82±0.22	9.82±0.18	9.88±0.16
DM intake (g/d)	350.95±8.20	355.85±7.62	360.48±10.45	355.50±8.42	374.22±14.65
DM intake (% BW)	3.60±0.08	3.62±0.07	3.67±0.09	3.62±0.09	3.79±0.10
CP intake (g/d)	54.34±0.25	54.84±0.40	55.31±0.15	54.80±0.22	55.79±0.20
TDN intake (g/d)	200.52±4.16	204.72±5.11	208.76±4.55	207.53±4.62	210.45±6.42
Digestibility (%)					
DM	64.22±0.52	66.42±0.81	67.36±0.88	65.22±0.74	66.88±0.78
OM	66.12±0.66	67.88±1.24	69.42±1.32	67.65±1.40	68.45±1.00
CP	66.22±0.52	65.96±0.27	66.40±0.30	65.98±0.25	65.48±0.29
CF	58.70±3.10	60.40±3.10	60.45±2.68	57.99±3.12	60.45±2.30
NFE	69.24±1.70	71.00±1.60	72.05±2.45	68.88±2.32	70.12±2.13
EE	78.22±2.41	80.00±2.65	80.55±2.44	78.65±2.66	76.55±3.46
NDF	60.14±1.47	60.24±2.64	58.44±2.14	61.65±1.88	60.21±2.15
ADF	54.60±1.56	52.80±1.66	55.45±1.99	54.68±2.15	52.45±2.10
Nitrogen balance (g/d)					
N intake	8.69±0.12	8.77±0.14	8.85±0.22	8.74±0.16	8.93±0.22
Faecal N	3.05±0.18	3.15±0.20	3.20±0.16	3.24±0.10	3.34±0.12
Urinary N	3.54±0.14	3.57±0.16	3.45±0.11	3.41±0.12	3.43±0.16
N balance	2.10±0.11	2.05±0.12	2.20±0.11	2.12±0.10	2.16±0.14
Zinc retention (mg/d)					
Zn intake	22.99^{a} ±0.32	23.09^{a} ±0.45	19.69^{b} ±0.25	16.09^{c} ±0.18	12.84^{d}±0.15
Faecal Zn	20.2^{a} 8±0.41	19.76^{a} ±0.62	16.92^{b} ±0.19	13.81^{c} ±0.12	10.81^{d} ±0.17
Urinary Zn	1.19^{a} ±0.10	1.33^{a} ±0.16	1.01^{b} ±0.14	0.87^{bc} ±0.09	0.70^{c}±0.11
Zn retained	1.50^{c}±0.11	2.01^{a} ±0.09	1.77^{b} ±0.10	1.40^{c} ±0.09	1.33^{c}±0.08
Absorption (%)	6.55±0.37	8.67±0.43	9.01±0.58	8.70±0.11	10.34±0.32

^{a,b,c,d}Means bearing different superscripts in a row differ significantly (P<0.05)

Table 3. Growth performance of lambs on different feeding regime

Parameter	Group				
	I	II	III	IV	V
Initial body wt. (kg)	3.64±0.04	3.55±0.08	3.60±0.09	3.50±0.07	3.72±0.22
Final body wt. (kg)	11.75±0.10	12.36±0.11	12.22±0.08	11.82±0.07	12.44±0.12
Weight gain (kg)	8.11±0.10	8.81±0.13	8.62±0.11	8.32±0.09	8.72±0.10
ADG (g)	54.06±3.12	58.73±2.26	57.47±1.88	55.46±2.11	58.13±3.22

intake in 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 of DM, OM, CP, NDF and ADFwas similar in all the groups (Table 2). Jadhav *et al.* (2008) reported that supplementation of Zn at 0, 35 and 70 ppm level in buffalo calves did not affect nutrient digestibility. Similarly, there was no difference in digestibility of CP, EE, NDF, ADF and cellulose in crossbred cattle supplemented with 0, 35 and 70 ppm Zn in basal diet containing more than 25 ppm Zn (Mandal *et al.,* 2007). All the animals were in positive N balance.

The average daily gain (Table 3) was similar (54.06 to 58.73 g/d) in all the groups. Zaboli *et al.* (2013) also did not find any effect due to supplementation of 20 or 40 ppm nZnO on average daily gain in Iranian Angora goat kids fed control diet containing 22 ppm Zn. However, nZnO has been reported to enhance growth performance in poultry (Mishra *et al.,* 2014). As the Zn level (30 ppm) in the basal ration was adequate (Mc Dowell, 1985) for normal growth of *Jalauni* lambs which could be the reason for non-significant effect on growth performance.

Zn intake was higher in groups G_1 and G_2 as compared to groups G_3 to G_5 owing to higher supplementation levels. A similar trend was also observed with the faecal and urinary Zn excretion which was higher (P<0.05) in groups $G_1$and G_2as compared to groups G_3, G_4 and G_5 (Table 2) which might have led to higher Zn retention (2.01and 1.88 mg/d) in groups $G_2$and G_3, respectively as compared to groups G_1, G_4 and G_5 (1.50, 1.40 and 1.33 mg/d) and the absorption was higher (P<0.05) in nZnO supplemented lambs (G_2 to G_5) in comparison to ZnO supplemented lambs (G_1). A similar excretion pattern of Zn has been reported earlier by Singh *et al.* (2009) in kids. There has been variable effects of different Zn sources on its retention. Jadhav *et al.* (2008) observed higher Zn retention in buffalo calves supplemented with 0, 35 and 70 ppm Zn from zinc sulphate. Singh *et al.* (2018) supplemented 60 ppm nZnO in the diet of pre-ruminant *Jalauni* lambs over and above the level in basal ration (30 ppm Zn) and

Table 4. Effect of different levels of nano zinc oxide on plasma metabolites in lambs

Parameter	Group				
	I	II	III	IV	V
Zn initial (μg/dL)	0.98±0.16	0.86±0.18	0.82±0.22	0.92±0.16	0.85±0.14
Zn final (μg/dL)	1.22^{d} ±0.06	1.66^{a} ±0.10	1.55^{ab} ±0.09	1.42^{bc} ±0.11	1.16^{d} ±0.08
Creatinine (0 d mg/dL)	0.95±0.09	0.91±0.10	0.94±0.12	0.92±0.11	0.98±0.14
Creatinine (90 d mg//dL)	0.99±0.06	1.02±0.11	1.01±0.14	1.06±0.16	1.10±0.15
SOD (0 d, U/dL)	225.12±6.88	228.12±7.41	227.98±7.62	232.15±6.55	230.32±7.22
SOD (90 d, U/dL)	236.14±8.45	242.65±8.56	240.45±6.55	244.52±5.68	245.85±7.55

^{a,b,c,d}Means bearing different superscripts in a row differ significantly (P<0.05) reported improved Zn availability and plasma Zn levels in pre-ruminant lambs.

Initial plasma level of Zn was similar in different groups (Table 3). However, after 90 d, there was an increase ($P<0.05$) in the plasma Zn levels in both groups. But the increase was more prominent in groups G_2, G_3 and G_4 (1.66, 1.55 and 1.42 µg/dL) as compared to groups G_1 and G_5 (1.22 and 1.16 µg/dL). The higher plasma Zn levels in nZnO supplemented lambs (G_2 to G_4) might be due to the greater retention of Zn than in group G_5. Lower plasma Zn levels in group G_1 might be due to the lower Zn retention due to low solubility of ZnO in GI tract (Wedekind and Baker, 1990; Singh *et al.,* 2018) and in group G_5 due to the comparatively lower Zn intake. 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). Najafzadeh *et al.* (2013) also observed increased serum Zn levels after oral administration of nano zinc oxide in lambs.

The initial and final plasma creatinine and SOD levels were similar and within the normal physiological range in lambs (Table 4). Najafzadeh *et al.* (2013) observed increased creatinine level in the serum of lambs supplemented orally with nano zinc particle at the rate of 20 mg/kg BW. High serum creatinine levels in lambs might 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 study (360-400 mg/d).

Therefore, nutrient utilization and growth performance was similar in groups supplemented with both sources of Zn supplementation, however, nZnO significantly improved Zn bioavailability. Thus, nZnO could be a better source of Zn supplementation at 20 ppm level of supplementation.

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