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Original Article

Role of dietary nano-zinc oxide on growth performance and blood levels of mineral: A study on in Iranian Angora (Markhoz) goat kids

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Abstract

This study was conducted to assess the possible effects of zinc oxide (ZnO) and nano zinc oxide (nZnO) on growth performance as well as the level of Ca, P, Fe, Cu and Zn in Markhoz goat kids blood samples. Thirty 5-6 months male Markhoz goat kids were supplemented with 22.12 mg of Zn/ kg DM as basal diet for 70 days. Zinc was administered at daily doses of zero, 20 and 40 ppm in ZnO group , and 20 and 40 ppm in nZnO group by adding to their basal diet. Animals were weighed fortnightly to obtain average daily gain (ADG). Blood samples were taken for analyzing blood mineral level at baseline and days 35 and 70. No significant difference in food intake and ADG was identified between Zn supplemented and control groups. Zn supplementation did not affect the blood mineral levels in kids except for plasma Zn concentration on day 35 ($P < 0.05$). In conclusion, results show that ZnO and nZnO at applied concentrations does not affect growth performance and composition of blood minerals in Markhoz goat kids.

Keywords: Nano zinc oxide, Markhoz goats, Blood minerals, ZnO , nZnO

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Introduction

Zinc (Zn) is a component of numerous metaloenzymes and transcription factors (O'Dell, 2000), which plays significant roles in the metabolism of essential nutrients in ruminants (Jia et al., 2008). This metal is the second most abundant trace element in the body and as it is not stored in the body, a continuous dietary intake is essential for body's appropriate physiological functions (Zalewski et al., 2005). The two predominant sources of Zn used by the animal feed industry are ZnO and ZnSO4. H2O (Wedekind and Baker, 1990). Nanozinc oxide (nZnO) is a new substance that has been produced and marketed using nanotechnologies. This substance has found many applications in the pigments, food and electronics industries as well as in medicine (Song et al., 2010). 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 higher interactions with other organic and inorganic molecules. Many properties of the metals in nano scale are not yet determined (Francisco et al., 2008). Limited knowledge of the toxic effects of these substances on ruminants highlights the need for immediate research to identify their possible adverse effects when used as a nutritional supplement in livestock and poultry feeding. Several studies have investigated physiological effects of nZnO in animals. While some studies have reported toxic effects for nZnO on biological systems (Sharma etal., 2009), there are also studies supporting an inverse conclusion (Song et al., 2010). Hongbu et al. (2009) studied toxic effects of nZnO and zinc chloride in a nematode. They did not find significant difference between theirs toxicity profiles in nematodes. Ziva et al. (2010) used three different types of zn (nZnO, zinc oxide and zinc chloride) at levels of 2000 and 5000 μg/g dry weight in the diets of invertebrate animals (Porcellio scaber). They showed that the potential of these compounds for accumulation were similar. Other investigators (Wang et al., 2006) used powder of Zn in the diet of rats at the level of 5 g/kg of body weight as micro-particles (M-Zn) and nanoparticles (N-Zn) and measured activity of some enzymes in plasma and liver. The results showed that the effect of micro-particles in hepatocellular damage is more severe than that of nanoparticles.

While a number of researches have investigated the effect of zinc oxide on the growth rate when used as food supplement in livestock (Kincaid et al., 1997; Puchala et al., 1999 and Phiri et al., 2009), similar studies on nZnO are limited. Lina et al. (2009) used 40 ppm Zn as nZnO in the diet of broiler and observed an increased growth performance of poultry. Since there is limited information on the adverse effects of nZnO when used as a dietary supplement, we decided to investigate its possible effects on growth performance and blood mineral levels of Markhoz goat kids.

Materials and methods

Thirty male Markhoz goat kids (approximately 5-6 month of age, 14.72 ± 2.72 kg body weight) were stratified by weight, and randomly assigned (n = 6 goats per treatment) for 70 days to one of the following treatments: I) basal diet containing 22.12 mg Zn/kg DM with no Zn supplementation (control); II) basal diet+20 mg Zn/kg DM as zinc oxide (ZnO 20); III) basal diet+40 mg Zn/kg DM as zinc oxide (ZnO 40) ; IV) basal diet+20 mg Zn/kg DM as nano zinc oxide (nZnO 20) and V) basal diet+40 mg Zn/kg DM as nano zinc oxide (nZnO 40). The basal diet was formulated to meet or exceed the entire nutrients requirement for goats with the exception of Zn (NRC, 2007) (Table 1). Zinc was added to the premix using finely barley flour as a carrier.

Daily feed offerings and refusals were recorded prior to the morning feeding to obtain feed intake for each goat. Body weights were obtained

before that goats were fed in the morning for two consecutive days at the beginning of the experiment and fortnight intervals. Blood samples were collected on days 0, 35 and 70 before the morning feeding via the jugular vein. One of the blood samples were added heparin to obtain plasma and the other samples were heparin free to obtain serum. Plasma and serum samples were obtained by centrifuging (3000 rpm; 20 min; 4° C) whole blood. Plasma samples were analyzed for Zn, Fe and Cu, and serum samples were used to determine Ca and P levels.

Feeds were analyzed for dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), ash and non fiber carbohydrates (NFC) using standard procedures (AOAC, 2000). Neutral detergent fiber (NDF) was analyzed according to the method developed by van Soest et al. (1991). The Zn, Fe and Cu contents of feed and plasma samples were estimated in an air-acetylene flame on an atomic absorption spectrophotometer (Varian spectra AA220, Australia) as described by Salama Ahmed et al. (2003) and Rimbach et al. (1998) respectively.

Calcium and phosphorus concentrations of feed samples were determined by dry ash method (AOAC, 2000). Calcium and phosphorus concentrations of serum were determined using commercially available kits (Pars Azmoon, Iran) with an Auto Chemistry Analyzer) Dirui CS 400). The analysis was carried out according to the manufacturer's recommendations. The data were analyzed according to a completely randomized design using the GLM procedure (SAS, 2001). The following model was used: $Yij = \mu + Ti + \varepsilon ij$, where Yij is the dependent variable; μ is the overall mean; Ti is the effect of Zn supplementation (i = 1, 5); εij is the random error. Duncan's multiple range tests was used for comparison of means, considering $P \leq 0.05$ as the significance level. Initial body weight (BW) was considered as a covariate for analysis of final BW, and ADG.

Table 1: Ingredients and nutrient composition of the basal diet

Nutrients	Alfalfa (43%)	Barley grain (40%)	Wheat straw (17%)	Basal diet
Dry matter (%)	93.42	93.36	95.92	93.82
Organic matter (%DM)	90.25	91.50	92.38	91.11
Crude protein (%DM)	15.06	10.35	5.62	11.57
Ether extract (%DM)	3.01	1.40	1.23	2.06
Neutral detergent fiber (%DM)	43.35	31.28	67.35	42.60
Non fiber carbohydrate (%DM)	28.83	48.47	18.18	34.87
Ash (%DM)	9.75	8.50	7.62	8.89
Calcium (%DM)	1.69	0.09	0.04	0.77
Phosphorus (%DM)	0.23	0.32	0.05	0.24
Zinc (mg/kg DM)	23.01	27.79	6.48	22.12
Cupper (mg/kg DM)	11.47	8	3.84	8.79
Iron (mg/kg DM)	377	95.34	156.6	226.87
Metabolizable Energy [1] (Mcal/kg)	2.1	3	1.5	2.36

1- Metabolizable energy was calculated based on NRC (2007)

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Results

Food intake and growth performance

Table 2 reports the effect of dietary Zn supplementation on food intake (FI), average daily gain (ADG) and feeding efficiency. FI, ADG and feeding efficiency increased with Zn supplementation. However, no significant difference among test groups was observed.

Table 2: Effect of dietary Zn supplementation on food intake and growth performance in Markhoz goats in different groups

Item	Treatment [1]					p-value	SEM [2]
	Control	ZnO (20)	ZnO (40)	nZnO (20)	nZnO (40)		
Food intake (g/day)	449.97	500.87	527.20	540.85	552.82	0.783	19.638
Initial body weight (kg)	14.06	14.80	14.59	14.89	15.29	0.984	1.510
Final body weight (kg)	16.11	17.84	17.79	18.09	18.52	0.934	2.052
Average Daily gain (g/day)	32.33	43.25	46.35	44.88	43.56	0.697	9.688
Gain : feed	0.065	0.085	0.085	0.080	0.080	0.774	0.012

Food intake calculated based on 100% dry matter.
1- Control : basal diet (Zn = 22.12 mg/kg DM), ZnO (20) : basal diet + Zn oxide (added Zn = 20 mg/kg DM), ZnO (40) : basal diet + Zn oxide (added Zn = 40 mg/kg DM), nZnO (20) : basal diet + Zn nano oxide (added Zn = 20 mg/kg DM), nZnO (40) : basal diet + Zn nano oxide (added Zn = 40 mg/kg DM).
2- Standard error of mean.

Blood levels of minerals

Table 3 shows the blood levels of minerals at 1st , 35th and 70th days of supplementation. Zn supplementation did not affect the blood profile of minerals. Except for plasma Zn concentration in animals supplemented with 40 ppm Zn from ZnO source on day 35; the Zn level was found significantly higher than that in controls and the groups supplemented with 20 ppm Zn. No significant differences were observed for blood concentrations of Ca, P, Fe and Cu in the blood of kids on the first day and days 35 and day 70 of the experiment.

Table 3: Comparison of effect of dietary Zn supplementation on concentration of minerals in blood of Markhoz goats in different groups

Minerals*	Treatment [1]					p-value	SEM [2]
	Control	ZnO (20)	ZnO (40)	nZnO (20)	nZnO (40)		
Zinc (mg/l)							
Day 0	0.64	0.59	0.65	0.68	0.66	0.633	0.042
Day 35	0.96 [b]	1.00 [b]	1.23 [a]	1.04 [b]	1.13 [ab]	0.019	0.054
Day 70	1.02	1.01	1.06	1.16	1.12	0.205	0.050
Calcium(mg/dl)							
Day 0	9.86	9.80	9.88	9.90	10.00	0.953	0.180
Day 35	9.18	9.00	9.25	9.22	9.58	0.376	0.201
Day 70	9.65	9.56	9.97	9.08	9.23	0.309	0.264

Minerals*	Treatment [1]					p-value	SEM [2]
	Control	ZnO (20)	ZnO (40)	nZnO (20)	nZnO (40)		
Phosphorus (mg/dl)							
Day 0	7.52	7.57	7.22	8.18	7.53	2.716	0.686
Day 35	7.03	7.35	6.68	6.82	6.50	2.755	0.678
Day 70	7.08	7.04	6.73	7.23	7.13	2.839	0.702
Iron (mg/l)							
Day 0	1.23	1.21	1.09	1.32	1.05	0.512	0.117
Day 35	1.21	1.51	1.43	1.34	1.38	0.262	0.093
Day 70	1.28	1.08	1.10	1.07	1.01	0.568	0.112
Copper (mg/l)							
Day 0	1.05	1.135	1.06	0.97	1.10	0.904	0.115
Day 35	1.05	1.16	1.19	1.09	1.237	0.916	0.164
Day 70	1.19	1.31	1.26	1.17	1.38	0.387	0.083

* Ca , P and Fe concentrations were evaluated in serum, Zn and Cu concentrations were evaluated in plasma.
Means with different superscript letters in rows are significantly different ($p<0.05$).
1- Control: basal diet (Zn = 22.12 mg/kg DM), ZnO (20): basal diet+Zn oxide (added Zn = 20 mg/kg DM), ZnO (40): basal diet+Zn oxide (added Zn = 40 mg/kg DM), nZnO (20): basal diet+Zn nano oxide (added Zn = 20 mg/kg DM), nZnO (40): basal diet+Zn nano oxide (added Zn = 40 mg/kg DM).
2- Standard error of mean.

Discussion

Food intake and growth performance

Consistent with our observation, Puchala et al. (1999) did not find any effect for zinc oxide on FI and ADG of Angora goat. In addition, Kincaid et al. (1997) did not identify any effect for 300 ppm Zn (as zinc oxide) on FI and ADG in weaning calves. In other studies, supplementation of Zn had no effect on FI in dairy goats (Salama et al., 2003), growing lambs (Fadayifar et al., 2012) and beef steers (Mandal et al., 2007). However, contrary to our results, Jia et al. (2008) identified an increase in ADG and feed efficiency when 15-45 mg Zn/kg DM was supplemented to a basal diet containing 22 mg Zn/kg DM in Liaoning Cashmere goats.

However, it has been reported that a low level of dietary Zn could lead to the reduced food intake and growth performance (Jia et al., 2008). Our results indicated that goat kids fed the control diet containing 22.12mg Zn/kg DM had a growth similar to that of the Zn supplemented kids. In addition our data suggest that this level of Zn in the basal diet was adequate for normal growth of Markhoz goat kids and supplementation of 20 and 40 ppm zinc from both zinc oxide and nano zinc oxide sources had no effect on the FI and ADG.

Blood levels of minerals

An increasing trend for plasma Zn concentration was observed from the first day to day 70, showing a lower level of Zn in the intake by kids before starting this study as compared to the basal diets. To our knowledge, no report is available regarding the effect of nano zinc oxide on blood mineral concentrations of livestock and poultry.

Some studies have reported no effect on Zn level of blood for supplementation of Zn in the diet of Angora goats (Puchala et al., 1999; Eryavuz et al., 2002), steers (Spears et al., 2004), growing lambs (Droke et al.,1998) and dairy goats (Salama et al., 2003). In contrast, Jia et al. (2008) who supplemented inorganic Zn in the diet of Cashmere goats and found the concentration of Zn in plasma was significantly higher in supplemented groups as compared with the control. Also Phiri et al. (2009) reported an increased plasma Zn concentration

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in goats supplemented with Zn in the form of zinc oxide.

Based on results reported by Haenlein and Anke (2011), serum Zn concentrations of goat were 1.16 ± 0.58 mg/l. Also, Puchala et al. (1999) reported that the amount of Zn in plasma of Angora goats was 0.72 mg/l. Plasma zinc concentration in our study veried within the range of 0.59 to 1.23 mg/l, which shows that zinc concentration in the basal diet was adequate for normal blood Zn concentration, leading to normal growth of goats.

Our results are supported by other studies in different species of ruminants; Zn supplementation had no effect on level of blood calcium in Cashmere goats (Jia et al., 2009) and growing lambs (Fadayifar et al., 2012; Garg et al., 2008), inorganic phosphorus in growing lambs (Garg et al., 2008), Copper in Angora goats (Puchala et al., 1999) and growing lambs (Fadayifar et al., 2012; Garg et al., 2008) and Iron in Cashmere goats (Jia et al., 2009).

On the contrary, Dagash and Mousa (1999) observed a decreased serum Ca concentration in buffaloes supplemented with 50 and 100 mg Zn/kg DM in their diets. Bedi (1976) and Khan (1978) have reported an increase in the serum Ca level after supplementation of Zn in growing calves. Phiri et al. (2009) reported that supplementation of basal diet with Zn from zinc oxide source, decreased the plasma calcium and inorganic phosphate concentrations in goats. Attia et al. (1987) reported that supplementation of 250 and 1000 mg Zn of zinc oxide in the basal diet of male buffalo calves decreased serum Cu levels. The inconsistency between the observations may be due to very high levels of Zn supplementation used by these investigators, which might have an antagonistic effect on Cu absorption.

Garg et al. (2008) supplemented the basal diet of lambs with 20 mg Zn/kg DM from organic and inorganic source and found an adverse effect for Zn supplementation on serum with Fe content. In addition, Wiering et al. (2007) showed that Zn supplementation negatively affect Fe level in serum of human infants.

However, Zn is a bivalent metal and excessive concentration of zinc in the diet may be competed with the absorption of other bivalent metals such as Ca, Fe and Cu as antagonists. Zn interferes with the uptake and absorption of these elements and as a result, the amount of these elements in blood would change (Phiri et al., 2009). In addition, the high intake of Zn interferes with the absorption of phosphorous by forming insoluble phosphates, thereby reducing the amount of plasma phosphorus (Phiri et al., 2009). However, in our study, the levels of Zn intake in control and supplemented groups were not so high as to affect the levels of this element in blood of goat kids.

Based on our results, growth performance, plasma and mohair Zn level of Markhoz goat kids fed a basal diet containing 22.12 mg Zn/kg DM were not affected by supplementation of the basal diet by 20 and 40 ppm Zn as zinc oxide or nano zinc oxide. Further research is needed to determine the Zn requirement of this animal.

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