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Effect of Feeding Various Sources of Zinc on Nutrient Digestibility and Rumen Parameters of Male Crossbred Calves

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ABSTRACT

This study was conducted to investigate the effect of replacing inorganic zinc with lower level of organic and nanozinc on digestibility of nutrients and rumen parameters of growing crossbred calves. For this experiment, 15 male crossbred calves weighing 93.3±3.19 kg (8-10 months) were divided into three groups consisting of five animals in each. TMR having 50:50 roughage to concentrate ratiowith 2 kg of green fodder was provided to animals as a basal diet. Along with basal diet experimental groups were provided with additional Zn sources *i.e.,* ZnSO4 (@40 ppm) in T1, Zn-glycinate (@20 ppm) in T2 and nanoZnO (@10 ppm) in T3 as a toppings over the TMR, for the period of 98 days.Zn content in basal diet and green fodder was 58.6 and 40.5 ppm, respectively.Digestibility of nutrients like DM, OM, CP, EE, CF, NFE, NDF and ADF was comparable (P>0.05) among the groups. Various rumen parameters like ruminal pH, TVFA, ammonia-N, total-N, non-protein and soluble nitrogen concentration was also comparable (P>0.05) among the groups..It may be concluded that, supplementation of nanoZnO (@10 ppm), Zn-glycinate (@20 ppm) and ZnSO4 (@40 ppm) gave comparable (P>0.05) results in terms of nutrient digestibility and rumen parameters on growing crossbred calves without any adverse effect.

KEYWORDS: Crossbred calves, Digestibility, Nano Zn, Rumen parameters

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INTRODUCTION

Although minerals do not provide energy, they play an important role in the intermediary metabolism of the major nutrients, immunity, health, production and reproduction as the components of the various enzyme systems. Zinc is one of the most important trace minerals for the health and productivity of young calves (Wu,2018). Zn is required for structural and functional integrity of more than 2000 transcription factors and 300 enzymes; hence, almost all metabolic pathways are in some way dependent on at least one Zn requiring protein (Ranasinghe et al.,2015). Zn is also essential for proper physiological functioning of the body like growth (Keçeci and Keskin, 2002), reproduction (Berrie et al.,1995, Campbell et al.,1999; Uchida et al.,2001), DNA synthesis, cell division, gene expression (Kincaid et al.,1997), photochemical processes of vision, wound healing (Koracevic et al.,2001, Kietzmann and Braun, 2006), ossification (Walsh et al.,1994), augmenting the immune system (Mandal et al., 2007) through energy production, protein synthesis, protection of membranes from bacterial endotoxins and lymphocyte replication and antibody production (Zhao et al.,2014).Due to the vast role in enzyme systems supplementation of Zn in ruminant diet is essential for maintaining normal growth, reproduction, development and function of the immune system and metabolism (NRC, 2000).

Dietary requirement of Zn is 40 mg/kg feed for cattle and buffalo (ICAR, 2013). Feeds and fodder are main sources of minerals for livestock; however, crop residues forms the bulk of rations in India that are deficient in most minerals mainly Zn (Datt and Chhabra, 2005). Generally requirement of Zn is provided by inorganic sources (i.e. ZnO and ZnSO4) of Zn. However, bioavailability of inorganic sources of Zn is around 6-12% only (Spears et al., 2004;

Singh et al., 2018). Compared to that,the availability of Zn is higher in organic sources (i.e. Zn-amino acid complexes) but is still poorly available as compared to nano forms of the Zn (Schell and Kornegay 1996; Cao et al.,2000) also high cost of chelated minerals limits its use in animal feed (Zhao et al.,2014). Currently, use of nanoZnO has been a topic of interest in many industries, including livestock mineral nutrition (Swain et al.,2016). It is noteworthy that these minerals in nano form come with the characteristic property of large surface area to volume ratio which leads to a great surface activity, high catalytic efficiency and high absorbency, with the particle size in the range of 1-100 nm which accounts for its own kind of better bioavailability in biological systems (Bunglavan et al.,2014). The major advantage with this property of nano Zn is that it could reduce the quantity of inclusion in animal diet thus reduces the environmental pollution.

Considering this background and above facts, the present study was conducted on growing crossbred calves to evaluate the possible effects of the dietary supplementation with high-surface ZnO (i.e. nanoZnO) at lower dosage, as an alternative to ZnSO4 (inorganic source) and Zn glycinate (organic source) on digestibility of nutrients and rumen parameters of growing crossbred calves.

MATERIALSAND METHODS

The experiment was conducted at Animal Nutrition Research Station (ANRS), College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand, Gujarat, India with the approval of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi. Fifteen male crossbred calves (average body weight of 93.39±3.19 kg, 8-10 months old) were divided into three treatment groups of five animals in each with completely randomised design. Total mixed ration (Table 1) having 50:50 roughage to concentrate ratio was provided (mineral mixture except any zinc source was added in TMR) along with fixed quantity of 2 kg green fodder as a basal diet (Table 2) to all the calves for 98 days of experimental period (excluding the adaptation period and pre-experimental feeding of 21 days) as per ICAR (2013) feeding standards.In addition to this, toppings of various sources of Zn like ZnSO4 (@40 ppm) as an inorganic source, Zn-glycinate (@20 ppm) as an organic source and nanoZnO (@10 ppm) as nano particle form were addedover the TMR individually to all the animals as per their body weightin T1, T2 and T3, respectively. Thus, the level of Zn in T2 and T3 was adjusted to 50 and 25% of T1, respectively. Zn content in basal diet and green fodder was 58.60 and 40.50 ppm, respectively (Table 4). ZnSO4 and Zn-glycinatefor the experiment was obtained from Anubhav Mineral Mixture Plant, ANRS, AAU, Anand. Whereas, Nano ZnO for the experiment was prepared by methodas described by Kumar et al. (2013) at Department of Nanotechnology, AAU, Anand.

Table 1. Ingredient composition of total mixed ration

Ingredients	Quantity (kg /100kg)
Jowar straw	50
Soybean meal	20
Maize	10
De-oiled rice bran	10
Molasses	9
Mineral mixture	1

Composition of Mineral Mixture: Calcium 20%, Phosphorus 12%, Sulphur 2%, Manganese 0.12%, Cobalt 0.014%, Copper 0.20%, Iodine 0.030%

Table 2. Chemical composition of TMR and green fodder

Parameter (%)	TMR	Green Fodder
Moisture	11.8	77.8
Organic matter	87.9	80.2
Crude protein	12.0	10.0
Ether extract	2.20	1.23
Crude fibre	21.8	27.9
Nitrogen-free extract	51.9	39.8
Total ash	12.0	20.1
Neutral detergent fibre	51.2	67.8
Acid detergent fibre	26.3	39.5
Calcium	1.81	0.41
Phosphorus	0.47	0.13
Zinc (ppm)	58.6	40.5

Except moisture all the values are on dry matter basis, TMR, Total Mixed Ration

Animals were kept in well ventilated barn having individual feeding facilities. Fresh water was offered *ad libitum* twice daily to all the animals.Quantity of ration provided was adjusted individually on the basis of body weight changes recorded biweekly.Deworming of all the animals was carried out using broad spectrum anthelmintic, before initiation of the experiment.After 75 days of experimental feeding, digestion trial of 7 days was conducted to study the digestibility of nutrients. Feed offered, leftover feed and faeces voided during 24 hours was collected, weighted. Later, uniform representative samples were collected daily in labelled polythene bags for further proximate and fibre fractions analysis. Samples of TMR offered, leftover and faeces were analyzed for proximate principles as per AOAC (1995) and for fibre fractions as per Van-Soest et al (1991).

After digestion trial, rumen fluid samples were collected from all the animals before feeding and after 3- and 6-hours post-feeding using stomach tube attached to a vacuum pump. Ruminal pH was determined immediately after collection using digital portable pH meter. The samples of SRL (Strained rumen liquor) were analyzed for ammonia-N (Pearson and Smith, 1943) and total-N by Kjeldahl's method. Estimation of soluble-N in supernatant of SRL after centrifuging and non-protein nitrogen (NPN) by trichloro-acetic acid precipitation of SRL and nitrogen estimation was carried out by Kjeldahl's method from the supernatant after precipitation. The concentration of TVFA was determined in SRL by the steam distillation method (Barnett and Reid, 1957), using Markham micro-distillation apparatus.

Statistical analysis

The experimental data were subjected to one-way analysis of variance (ANOVA) as per the methods of Snedecor and Cochran (1994). The Completely Randomized Designwas followed. Treatment means were compared using the standard error of the difference between means for significance ($P<0.05$).

RESULTS AND DISCUSSION

Digestibility of nutrients

Average dry matter digestibility (%)was 60.60±1.33, 60.84±0.85 and 61.32±1.10 in groups T1, T2 and T3, respectively, which was comparable ($P>0.05$) among the groups (Table 3).

Table 3. Effect of feeding various sources of Zn on apparent digestibility (%) of nutrients

Parameters	Treatments			SEM*
	ZnSO4	Zn-Gly	Nano ZnO	
Dry Matter	60.6	60.8	61.3	1.112
Organic Matter	61.7	62.1	62.1	1.184
Crude Protein	53.3	53.3	53.5	2.140
Crude Fibre	49.6	50.8	54.1	1.857
Ether Extract	55.3	60.6	59.9	2.510
Nitrogen Free Extract	68.7	68.7	67.3	1.235
Neutral Detergent Fibre	49.8	49.9	53.0	2.294
Acid Detergent Fibre	40.2	41.2	45.2	3.192

*Standard Error of Mean

Likewise, digestibility obtained for other nutrientslike OM, CP, EE, CF, NFE, NDF and ADF were also found to be similar($P>0.05$) among the groups. Numerical improvement in CF, NDF and ADF digestibility was observed. These results indicated that reducing the level of Zn-glycinate (T2) and nano-Zn (T3) by 50 and 75% of ZnSO4 (T1) did not have any effect on digestibility of nutrients.Similar to our study,Garg et al. (2008) found no difference among lambs fed supplements of Zn methionine, Zn sulphate, and control group in digestibility of organic matter, dry matter, crude protein, ether extract, neutral detergent fiber, and total digestible nutrients, but the digestibility of ADF and cellulose were significantly higher in Zn-meth group as compared with the control group. Similar to this, Mandal et al.(2008) also reported that digestibility of nutrients was not affected by supplementation of ZnSO4 and Zn-propionate at 35mg/kg compared to control. Mishra (2017) also observedcomparable digestibility (P>0.05) by supplementation of ZnSO4 (@40 ppm) or nano Zn (@10 and 20 ppm) toMurrah buffalo calves. However, Shakweer et al. (2010), Gaafar et al. (2011) and Hassan et al. (2016) reported higher digestibility on supplementation of ZnSO4 and Zn-methioninecompared to basal diet (no additional Zn). It appears that the basal diet in the present experiment contained sufficient amount of Zn for nutrient utilization and rumen functions. Numerical improvement in fibre digestion indicates further studies in this direction.

Rumen parameters

The mean pH values were similar (P>0.05) among the groups with the values 7.01±0.05, 7.04±0.06 and 7.05±0.05 in the groups T1, T2 and T3, respectively (Table 4).

Table 4. Rumen fermentation parameters of experimental crossbred calves

Parameters	T1			T2			T3			SEM
	0 hr	3 hr	6 hr	0 hr	3 hr	6 hr	0 hr	3 hr	6 hr	
pH	7.11	7.03	6.89	7.24	6.97	6.91	7.21	6.90	7.03	0.049
TVFA (mM/dl)	16.8	17.6	20.2	14.8	18.4	19.2	16.1	18.1	18.4	0.912
TN (mg/dl)	128.8	141.6	130.4	114.8	147.8	131.6	124.8	151.7	119.8	7.120
NH_3-N (mg/dl)	17.0	22.4	18.4	16.5	25.7	16.2	15.6	24.9	16.8	0.849
NPN (mg/dl)	39.2	42.5	41.4	35.8	52.6	47.0	40.3	44.8	48.1	2.769
SN (mg/dl)	38.0	36.9	39.2	43.1	58.8	46.4	32.4	51.5	43.6	3.425

TVFA, total volatile fatty acids, TN, total nitrogen, NH_3-N, ammonical nitrogen, NPN, non-protein nitrogen, SN, Soluble nitrogen, SEM, Standard Error of Mean

However, periodically pH values declined significantly (P<0.05) at 3 and 6hours interval post-feeding compared to the ruminal pH recorded before feeding. Reverse pattern was observed in the values of total volatile fatty acids (TVFA) and total nitrogen(TN)concentration where, average of three groups at different time intervals were similar (P>0.05) but, periodical increase in concentration of TVFA and TN were observed after feeding. Highest concentration for TVFA and TN concentration was recorded at 6 and 3hour post feeding interval, respectively. NH_3-N concentration among the treatment groups also remained similar (P>0.05) at different time intervals. However, periodically concentration of NH_3-N was recorded significantly higher (P<0.05) at 3 hours post-feeding interval and later again reduced to pre-feeding level at 6 hours post-feeding. Mean concentration of NPN and SN also remained similar (P>0.05) among the treatment groups and within the group at different time intervals.

Likewise, Zeedan et al. (2008), Shakweer et al. (2010), Gaafar et al. (2011), El Ashry et al. (2012) and Abdollahi et al. (2020) did not observe any significant (P>0.05) effect on ruminal pH of Zn supplemented groups compared to control however, TVFA concentration was significantly (P<0.05) higher in the groups supplemented with Zn. Zeedan et al. (2008), Shakweer et al.(2010), Gaafar et al.(2011) and Abdollahi et al.(2020) also reported significant decrease (P<0.05) in NH_3-N concentration in Zn supplemented groups irrespective of the sources compared control. However, decreased (P<0.05) NH_3-N concentration was observed in Zn-methionine supplemented group compared to ZnSO4 by El Ashry et al.(2012).

CONCLUSIONS

From the results it was concluded that average DM digestibility along with other nutrients digestibility and rumen fermentation parameters remained similar between the three treatment groups. Numerical improvement in fibre digestibility was observed in the nanoZnO supplemented group. Therefore, supplementation of nanoZnO (@10 ppm), Zn-glycinate (@20 ppm) and ZnSO4 (@40 ppm) gave comparable (P>0.05) results in terms of nutrient digestibility and rumen parameters on growing crossbred calves without any adverse effect.

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