Indian Journal of Animal Sciences **91** (4): 299–304, April 2021/Article
https://doi.org/10.56093/ijans.v91i4.114338

Feeding zinc biofortified sorghum stover decreases zinc deficiency in sheep

K GIRIDHAR[1], N K S GOWDA[1]✉, D T PAL[1], P KRISHNAMOORTHY[2], R F JOSEPH[1], D K DEY[1] and A K SHUKLA[3]

ICAR-National Institute of Animal Nutrition and Physiology, Bengaluru, Karnataka 560 030 India

Received: 19 October 2020; Accepted: 18 July 2021

ABSTRACT

Study was conducted to evaluate feeding zinc (Zn) biofortifed sorghum stover to ameliorate Zn deficiency in sheep. The sorghum stover grown on zinc fortified soil was used for experimental feeding to Zn deficient sheep and compared with feeding unfortified stover as control for 120 days period. Application of zinc sulfate to soil @ 25 kg/ha increased the sorghum stover yield by 4.20 tonnes/hectare and enhanced the Zn content of stover by 14.4 ppm. Higher Zn intake in sheep fed biofortified stover resulted in significantly higher apparent gut absorption of Zn (37.2 vs 30.1%) and higher bioavailable Zn (6.12 vs 3.20 mg). Feeding Zn biofortified sorghum stover resulted in increase in plasma Zn content and the level was significantly higher after 3rd month of feeding the biofortified stover. The average plasma Zn content was significantly higher in group fed biofortified sorghum stover (1.14 vs 0.90 ppm). This resulted in higher Zn content in liver (150 vs 130 ppm, DM), enhanced activity of plasma superoxide dismutase (15.5 vs 10.3 Units/min) and better immune response to *Peste des petits ruminants* (PPR) vaccination (76.8 vs 59.5% inhibition). The results of this study prove that Zn fertilization of deficient soils is a practical method to increase the Zn content of stover and feeding of such biofortified stover can ameliorate the Zn deficiency in sheep.

Keywords: Biofortification, Sheep, Soil, Sorghum stover, Zinc

Micronutrients play a major role in plant growth and among them, zinc (Zn) is one of the most essential minerals for soil and plant health. Zinc deficiency in soils and field crops is widespread across the world, including India, resulting in severe reduction in crop yield (Shukla *et al.* 2016). Hence, soil application of Zn fertilizers is recommended for ameliorating Zn deficiency and for obtaining higher crop yield and better crop quality (Behera *et al*. 2015, Bhatt *et al*. 2020). Studies have indicated a close relationship between soil Zn content with that of plants and animals (Gowda *et al*. 2000, Yadav and Khirwar 2005). According to the report of All India Coordinated Research Project (AICRP) on soil micronutrients, 40% of samples collected across India are deficient in available Zn (Shukla *et al*. 2016). Zinc deficiencies in crops, green fodders and in livestock are concurrently reported (Rakesh *et al*. 2017) and essentiality of Zn in farm animal health and production has been proved beyond doubt (Prasad and Gowda 2005).

Many approaches to overcome the problem of Zn deficiency both in plants and livestock have been recommended. Of late, the concept of one health comprising soil, plant, livestock and humans has gained importance. To assess and validate this concept, under the AICRP on soil micronutrients, a study was conducted at ICAR-National Institute of Animal Nutrition and Physiology (NIANP), Bengaluru to biofortify sorghum fodder through Zn enrichment of soil. Such biofortified fodder was used to feed Zn deficient sheep for possible amelioration.

Present address: [1]ICAR-National Institute of Animal Nutrition and Physiology, Bengaluru, India. [2]ICAR-National Institute of Veterinary Epidemiology and Disease Informatics, Bengaluru, Karnataka, India. [3]ICAR-Indian Institute of Soil Science, Bhopal, Madhya Pradesh, India. ✉Corresponding author email: nksgowda@rediffmail.com

MATERIALS AND METHODS

Soil fortification with zinc and fodder cultivation: At the Institute fodder farm (ICAR, NIANP), a plot of dimension 15 × 8 square meter with low available zinc (0.45 ppm) in the soil was selected and zinc sulfate was applied @ 25 kg per hectare as basal dressing at the time of sowing fodder sorghum. Another plot of similar dimension with low soil zinc was used as the control to grow unfortified sorghum. In both the plots, CoFS-29 variety of fodder sorghum was cultivated and harvested 65 days after sowing. Harvested sorghum was weighed, chaffed, shade dried and the resultant stover was fed to the sheep as per the treatments.

Feeding trial in sheep: This experiment was conducted in the Experimental Livestock Unit of ICAR-NIANP, Bengaluru. Animal experiment protocol was approved by Committee for the Purpose of Control and Supervision of Experiments in Animals, Ministry of Environment, Forests and Climate Change, Government of India (No.4/2017).

Animal distribution, housing and management: Fourteen crossbred (Bannur × Mandya) ram lambs (3–4 months age) with an average body weight of 12.5±1.05 kg were purchased from an organized sheep farm. The lambs were maintained under uniform conditions by housing them in a well-ventilated barn with individual feeding facility. All lambs were dewormed using Albomar (Albendazole oral suspension; Virbac Animal Health Pvt. Ltd., Mumbai, India) as per the recommended dosage at the beginning of the experiment and protected against enterotoxaemia and *Peste des petits ruminants* (PPR) with vaccines (Institute of Biological Production, Animal Health and Veterinary Biologicals, Bengaluru, India).

Pre-experimental feeding: Pre-experimental feeding was followed to achieve lower Zn content in blood of all the 14 lambs. The lambs were individually fed dry matter (DM) @ 3% of body weight (concentrate mixture 25% and sorghum stover with a zinc content of 20.4 ppm at 75% of DM requirement for a period of 3 months (February–April 2018). Concentrate mixture (Table 1) was formulated with selected feed ingredients to achieve minimum Zn content. Mineral mixture devoid of added source of Zn was included in concentrate mixture. Further, calcite (1.5%) was added to concentrate mixture with an objective to reduce the gut absorption of Zn. After 3 months of feeding, blood sample was collected from all the 14 lambs and Zn was estimated in the plasma. The plasma Zn values of all 14 sheep were arranged in descending order. First seven lambs in the descending order of plasma Zn content were allotted to Group A (Av. Zn content 0.79±0.06 ppm) and remaining 7 sheep were allotted to Group B (Av. Zn content 0.40±0.03 ppm).

Experimental feeding: The composition of concentrate mixture was modified by withdrawing calcite and adjusting the same with wheat bran. All the lambs were individually fed DM @ 3% of body weight with concentrate mixture @ 25% proportion in the offered DM. Lambs in group A were fed unfortified sorghum stover (mean zinc content of 20.4 ppm) @ 75% proportion in the offered DM, whereas the lambs in group B were fed similar proportion of Zn biofortified sorghum stover (mean zinc content of 34.8 ppm). The feeding was continued for 120 days (May–August 2018). Weighed quantities of respective diets were offered to each lambs of both the groups. The concentrate mixture was offered daily at 9:00 AM and sorghum stover was offered at 1:00 PM. The feed residue was weighed next day morning and composited at weekly intervals to estimate DM content. Body weight of each animal was recorded at fortnightly interval. Drinking water was made available to each animal all the time.

Table 1. Composition of concentrate mixture

Ingredient	Level (kg/100 kg)
Crushed maize grain	48.0
Wheat bran	27.0
Groundnut cake (deoiled)	20.0
Urea	1.5
Calcite	1.5
Mineral mixture without added zinc	2.0
Nutritive value (*Calculated*)	
Crude protein (%)	20.0
Total digestible nutrients (%)	68.0
Zinc (ppm, DM basis)	25.0

Blood collection: The blood from jugular vein of each lamb was collected at monthly interval in heparinised tubes in ice cold condition and centrifuged at 2,000 rpm for 10 min. to separate plasma and preserved at –20°C till analyzed for Zn content.

Digestibility trial: After 80 days of experimental feeding, a digestion trial of 7 days inclusive of 2 days of adaptation and 5 days of collection period was conducted to study the DM and Zn utilization. During the collection period, daily feed offered and left over by individual lamb was recorded. Also quantitative collection of faeces voided for 24 h by each lamb was recorded for all the five days. Faecal collection was made by using faecal collection bags free from metal contamination harnessed to hind quarter of each lamb. Percent digestibility was calculated as difference in intake of the nutrient and fecal outgo divided by total intake and expressed in percentage.

Collection of samples

Feed and residues: During the digestibility trial, representative samples of experimental feeds and residues from each animal were collected daily and composited. Dry matter of the feed offered and residue was estimated by drying the samples overnight in a hot air oven maintained at 100°C. The dried composited samples were preserved in air tight polythene bags. At the end of collection period, samples were ground to 1 mm fineness and stored in self-sealed covers for analysis of Zn content.

Faeces: Faecal sample from each animal was collected using faecal collection bags. The faeces voided during 24 h was collected in separate self-sealed polythene bags to prevent moisture loss. The total faeces voided by each lamb during 24 h was weighed at 9:00 AM and thoroughly mixed. Representative aliquot ($1/25^{th}$) from each faecal void was taken for DM estimation in pre-weighed petridishes and dried overnight in hot air oven maintained at 100°C. The dried faecal samples were pooled in a polythene bag for 5 days, then ground to 1 mm fineness and stored in sample containers for further analysis.

Zinc analysis of feeds and faecal samples: The ground samples of feed and faeces were taken in pre-weighed silica crucibles and oven dried at 80°C for 24 h, decarbonized and ashed at 600°C in a muffle furnace for 3 h. The total ash was digested with 5N HCl over a hot plate for 15 min, cooled and filtered through Whatman filter paper (No. 41) into volumetric flasks (AOAC 2000). The Zn content in these samples was estimated using inductively coupled plasma optical emission spectrophotometer (ICP-OES; Optima 8000, Perkin Elmer, Shelton, USA) adopting the standard operating conditions. Zinc standard was prepared

from ICP standard solution (1000 mg/L, Merck Millipore, Germany) to arrive at a concentration of 1000 mg/L (stock solution). Gut absorption of Zn was calculated as the difference of intake and faecal outgo divided by intake and expressed in percentage.

Zinc analysis in soil and plant samples: Representative surface (0–15 cm) soil samples from each plot were collected, air-dried, debris was removed and then ground to pass a 2 mm sieve. The available fraction of Zn in soils was extracted by 0.05 M diethylene triamine penta acetic acid (DTPA) solution as outlined by Lindsay and Novell (1978). The plant samples of sorghum stover were analyzed for their zinc content using the ICP-OES (Perkin Elmer, Optima 8000). Corn-flour mineral extract was used as a standard reference material during the analysis.

Zinc analysis in blood plasma: Plasma sample was digested as per procedure described by Kolmer *et al.* (1951). One ml of serum sample with equal volume of concentrated nitric acid (HNO_3) was mixed in the digestion tube. The samples were kept overnight at room temperature followed by digestion on low heat (70–80°C) using heating bench (digestion bench), until the volume of samples was reduced to about 1 ml. To this, 3 ml of double acid mixture (3 parts concentrated HNO_3 and 1 part 70% H_2SO_4) was added and low heat digestion continued until the digested samples became watery clear and emitted white fumes. As per need, the addition of 3 ml double acid mixture followed by low heat digestion was repeated couple of times. Final volume of filtrate was made up to 10 ml with Millipore water. While digesting of serum samples, simultaneous digestion of reagent blank was undertaken and the final volume was similarly made up to 10 ml. The Zn content was estimated as mentioned above.

Zinc content in liver and muscle: After 120 days of experimental feeding, five sheep from each dietary group were sacrificed by Halal method (jugular vein incision) to collect sample of liver and muscle (*Biceps femoris*) for Zn estimation. The tissues were washed with distilled water and stored in refrigerated condition (–20°C) till further analysis. The liver and muscle samples were oven dried at 80°C for 24 h, decarbonized and ashed at 600°C in a muffle furnace for 3 h. The ash was digested with 5N HCl over a hot plate for 15 min, cooled and filtered through Whatman filter paper (No. 41) into volumetric flasks. The Zn content was estimated as mentioned above.

Humoral immune response: After 100 days of experimental feeding, six sheep from each dietary group were antigenically challenged with subcutaneous injection of a commercial PPR vaccine. Following 14 days of injection, blood samples were withdrawn from jugular vein of the sheep and collected into dry, sterilized centrifuge tubes and allowed for clotting. After clotting, the tubes were centrifuged to collect serum. The collected serum samples were stored in refrigerated condition (–20°C) till further analysis. Antibody titer against PPR vaccine was determined using competitive enzyme linked immunosorbent assay kit procured from Indian Veterinary Research Institute, Izatnagar, India and expressed as percent of inhibition (Singh *et al*. 2004).

Superoxide dismutase (SOD) activity: On 120 day of the experimental feeding, blood (2 ml) was collected from jugular vein of all sheep into vials with anticoagulant (acid citrate dextrose; 300 µl/2 ml blood), and centrifuged at 2000 rpm for 15 min at 4°C for separation of plasma and buffy coat. Haemolysate (1:20 dilution) was prepared by mixing 0.5 ml RBC suspension with 4.5 ml of stabilizing solution (EDTA, 2.7 mM and 0.7 mM, 2-mercaptoethanol). The SOD activity in haemolysate was measured using nitro blue tetrazolium as substrate after suitable dilution as per the method of Marklund and Marklund (1974) with certain modifications as suggested by Minami and Yoshikawa (1979). One unit of SOD activity was defined as the amount of enzyme which inhibited the auto-oxidation of pyrogallol by 50% under the given experimental condition. The values were expressed as units per mg of haemoglobin.

Statistical analysis: The data pertaining to the various parameters were subjected to independent T test (SPSS Version 16.0, SPSS Inc, Chicago, USA) and significance at $P<0.05$.

RESULTS AND DISCUSSION

Stover yield, zinc content in sorghum stover and feed: Application of zinc sulfate to soil @ 25 kg/ha increased the sorghum stover yield to 36.6 t/ha as compared to 32.4 t/ha in the control (no zinc application). The average Zn content of biofortified sorghum stover was 34.8 ppm, an increase of 14.4 ppm over the stover from control plot. Zinc deficient soils are known to produce crops with less Zn content (Shukla *et al*. 2016). Zinc fertilization of soil has increased the zinc content of wheat and also, its bioavailability (Cakmak 2008). Similar results of increase in Zn content of fodder due to soil enrichment has been reported earlier (Rathod *et al.* 2012). Singh (2009) reported increased Zn content in grain and fodder due to Zinc fertilization of soil. The maximum increase in grain yield was achieved when 25 kg/ha of $ZnSO_4$ was applied to soil and 0.5% $ZnSO_4$ was used as a foliar spray (Narwal *et al*. 2010). Zinc biofortification either as a foliar or soil application is an effective method to alleviate the zinc deficiency in humans (Bhatt *et al*. 2020). The findings of present study as confirmed by similar reports suggest that agronomic intervention in the form of Zn application to soil is a practical way to improve soil fertility as well as crop yield. The lambs were fed total DM @ 3% of their body weight with concentrate and fodder at 25 and 75% proportion. The average Zn content in concentrate mixture was 25 ppm. Taking into consideration the Zn content in concentrate mixture and stover, the net Zn content of diet A and B was arrived at 21.6 and 32.3 ppm, respectively. Hence, Zn content was 10.7 ppm higher in diet B due to inclusion of Zn biofortified sorghum stover.

Feed intake, dry matter and zinc utilization: The average daily total intake of DM in both the groups during the experimental period (472 g and 484 g) did not differ

(P>0.05) and was similar to the recommended level of 3% of body weight (ICAR 2013) (Table 2). Feeding Zn biofortified sorghum stover did not influence the voluntary intake of either the stover or the concentrate mixture (Table 2). Data of digestibility trial indicated no significant variation in dry mater intake and its digestibility (Table 3). The total intake of Zn was more (P<0.05) in sheep fed Zn biofortified sorghum stover due to higher Zn content in the latter. This resulted in significantly (P<0.05) higher apparent gut absorption of Zn (37.2 vs 30.1%) and higher bioavailable Zn (6.12 vs 3.20 mg) in sheep fed biofortified sorghum stover (Table 3).

The Zn utilization and retention in animals depends on factors like level of intake, source of supplementation, duration of supplementation and accompanying feed. Hence, reports on effect of Zn supplementation on its utilization in farm animals are varied. Zinc utilization was better in sheep supplemented Zn in the form of Zn-methionine as compared to Zinc sulfate (Pal *et al*. 2009). Additional Zn supplementation to basal diet had no influence on digestibility of DM, Crude protein, ether extract and neutral detergent fibre in goats (Wenbin *et al*. 2009), however higher level of Zn supplementation (40–100 ppm) resulted in greater DM digestibility in deer (Kun *et al*. 2015). Ether extract and fibre digestibility were enhanced in buffalo calves with 80 ppm Zn supplementation to the basal diet having 29.7 ppm Zn (Nagalakshmi *et al*. 2018).

Zinc level in blood plasma: The experimental protocol has comprised of inducing Zn deficiency in the beginning and allotting the sheep to two groups based on the plasma Zn content, ensuring that the second group (B) of sheep had lower and deficient level of plasma Zn (0.40 vs 0.79 ppm). On feeding of Zn biofortified sorghum stover to this group resulted in gradual increase in plasma Zn content and the level was significantly (P<0.05) higher after 3rd month of feeding (Table 4). The additional Zn content in DM due to feeding of biofortified sorghum fodder was 10.7 ppm. The average plasma Zn content was significantly (P<0.05) higher (27%) in group B due to feeding of biofortified sorghum stover (1.14 vs 0.90 ppm). This is attributed to higher Zn intake coupled with better gut absorption in sheep fed Zn biofortified sorghum stover. Feeding of micronutrient dense fodder to livestock has shown increase in mineral content in serum and milk (Shukla *et al*. 2016). Plasma Zn concentrations were increased in deers with Zn supplementation (15, 40 and 100 ppm) than for the control and 5 ppm (Kun *et al*. 2015). Plasma Zn level was increased in goats supplemented with 20 ppm Zn to a basal diet with 22.3 ppm Zn through Zinc sulfate or zinc methionine for 60 days period (Wenbin *et al*. 2009).

Zinc content in visceral organs, plasma SOD activity and immune response: The Zn content in *Biceps femoris* muscle did not differ between the groups but was higher (P<0.05) in liver of sheep fed Zn biofortified sorghum fodder (150 vs 130 ppm, DM) (Table 5). This is supported by the digestibility trial data of increased Zn absorption in gut and also indicate that liver is a natural storage organ for Zn. Higher plasma Zn level in sheep fed biofortified sorghum fodder has resulted in higher (P<0.05) activity of Zn dependent enzyme like SOD in plasma (15.5 vs 10.3 units/min). As a result of this, the humoral immune response to PPR vaccination was significantly better (76.8 vs 59.5% inhibition) in sheep with higher Zn intake through biofortified sorghum fodder (Table 5). The role of Zn in critical biochemical functions is undisputed. Supplementation of Zn in the form of Zinc-methionine to sheep resulted in enhanced activity of Zn dependent enzymes like SOD, alkaline phosphatase and immune status, besides increased in Zn content in liver and kidney (Pal *et*

Table 2. Average daily feed intake in sheep during feeding trial

Group	Concentrate mixture (g/sheep/day)	Sorghum stover (g/sheep/day)	Total feed intake (g/sheep/day)	DM intake (% of body weight)
A	112.5±10.61	360±20	472±36	2.98±0.19
B	118.6±9.98	365±17	484±28	2.99±0.14
P	NS	NS	NS	NS

Table 3. Dry matter and zinc utilization in sheep during digestion trial

Group	Dry matter intake (g/sheep/day)	Dry matter digestibility (%)	Total Zn intake (mg/sheep/day)	Apparent Zn gut absorption (%)	Net bioavailable Zn (mg/sheep/day)
A	490±20	66.7±3.60	10.6±0.84	30.1±1.99	3.20±0.11
B	505±25	67.1±3.31	16.5±1.10	37.2±2.06	6.12±0.19
Significance	NS	NS	<0.05	<0.01	<0.01

Table 4. Blood plasma zinc content in sheep (ppm)

Group	Month					
	0	1st	2nd	3rd	4th	Average
A	0.79±0.06	0.73±0.05	0.91±0.07	1.15±0.08	0.91±0.07	0.90±0.07
B	0.40±0.03	0.87±0.06	1.34±0.09	1.41±0.07	1.72±0.09	1.14±0.08
Significance	<0.01	NS	<0.05	<0.05	<0.05	<0.05

Table 5. Zinc content in visceral organs, plasma superoxide dismutase activity and humoral immune response

Group	Zn (ppm, DM)		SOD (Units/min)	Immunity	
	Liver	Muscle	Plasma	% Inhibition, 14th day	% Inhibition, 0 day
A	130±10	107±6.1	10.3±0.95	24.4±3.99	59.5±4.67
B	150±12	115±9.5	15.5±1.10	16.7±2.88	76.8±4.51
Significance	0.05	NS	0.05	NS	0.05

Table 6. Growth performance in sheep

Group	Initial body weight (kg)	Final body weight (kg)	Average daily gain (g)
A	14.1±1.36	16.6±1.51	20.8±1.80
B	14.2±1.40	17.7±1.56	29.2±1.60
Significance	NS	0.05	0.05

al. 2009, 2010). Higher level of Zn supplementation (140 ppm) to a basal diet containing 29.72 ppm Zn resulted in higher antioxidant activities and immune responses in buffalo calves (Parashuramulu *et al*. 2015).

Growth performance: At the start of feeding experiment, the initial body weights in both the groups were similar (14.1 vs 14.2 kg) (Table 6). The Zn levels in dietary DM were 21.6 and 32.3 ppm, respectively for normal and Zn biofortified stover fed groups. During the experimental feeding period of 120 days, the sheep fed Zn biofortified sorghum stover showed better average daily weight gain ($P<0.05$) as compared to sheep fed normal sorghum stover (29.2 vs 20.8 gm/day/sheep). This shows that additional supply of Zn for second group of sheep through biofortifed stover has helped in better performance in terms of growth. Supplementation of 20 ppm Zn either as $ZnSO_4$ or Zn-methionine to the basal diet containing 22.3 ppm Zn improved the growth performance in goats, and effectiveness of two sources was similar on performance measurements (Webin *et al*. 2009). The basal level of Zn (29.7 ppm) supplied through practical feed ingredients in diet was sufficient for buffalo calves for an average daily gain of 500 g (Nagalakshmi *et al*. 2018). This suggests that the growth response to Zn supplementation is varied as it depends on species of animal, physiological status and basal level in the diet.

In conclusion, the results of this study demonstrate that Zn fertilization of deficient soils is a practical method to increase the Zn content of fodder. Feeding of such biofortified fodder/ stover for sufficiently longer period can decrease the Zn deficiency in sheep. This approach has the dual advantage of improving soil fertility, thereby increasing fodder yield and also correcting the Zn deficiency in farm animals.

ACKNOWLEDGEMENTS

The support and facilities provided by Director, ICAR-NIANP, Bengaluru for conducting this study are thankfully acknowledged. The authors thank Project Coordinator, All India Coordinated Research Project on 'Micro and Secondary Nutrients and Pollutant Elements in Soils and Plants', ICAR-IISS, Bhopal for his support.

REFERENCES

AOAC. 2000. *Ofûcial Methods of Analysis*, 17th edn. Association of Official Analytical Chemists, Washington, DC, USA.

Behera S K, Shukla A K, Singh M V, Wanjari R H and Singh P. 2015. Yield and zinc, copper, manganese and iron concentration in maize (*Zea mays* L.) grown on vertisol as influenced by zinc application from various zinc fertilizers. *Journal of Plant Nutrition* **38**(10): 1544–57.

Bhatt R, Hossain A and Sharma P. 2020. Zinc biofortification as an innovative technology to alleviate the zinc deficiency in human health: A review. *Open Agriculture* **5**: 176–87.

Cakmak I. 2008. Enrichment of cereal grains with zinc: Agronomic or genetic biofortification ? *Plant Soil* **302**: 1–17.

Gowda N K S, Prasad C S, Ramana J V and Ramachandra K S. 2000. Micronutrient content of soil, feeds, fodders and blood samples of animals in southern dry and eastern dry zones of Karnataka. *Indian Journal of Animal Sciences* **71**(2): 150–54.

ICAR. 2013. *Nutrient Requirements of Sheep, Goat and Rabbit*. Indian Council of Agricultural Research, New Delhi. India.

Kolmer J A, Spanbling E H and Robinson H W. 1951. *Approved Laboratory Techniques*. Appleton Century Crofts, New York.

Kun B, Weili S, Chunyi L, Kaiying W, Zhipeng L, Shidan B and Guangyu L. 2015. Effects of dietary zinc supplementation on nutrient digestibility, haematological, biochemical parameters and production performance in male sika deer (*Cervus nippon*). *Animal Production Science* **56**(6): 997–1001.

Lindsay W L and Norvell W A. 1978. Development of DTPA soil test for zinc, iron, manganese and copper. *Soil Science Society of America Journal* **42**: 421–28.

Marklund S and Marklund G. 1974. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *European Journal of Biochemistry* **47**: 469–74.

Minami M and Yoshikawa H. 1979. A simplified assay method of superoxide dismutase activity for clinical use. *Clinica Chimica Acta* **92**: 337–42.

Nagalakshmi D, Sridhar K, Satyanarayana M, Parashu Ramulu S, Narwade V S and Vikram L. 2018. Effect of replacing inorganic zinc with a lower level of organic zinc (zinc propionate) on performance, biochemical constituents, antioxidant, immune and mineral status in buffalo calves. *Indian Journal of Animal Research* **52**: 1292–97.

Narwal R P, Malik R S and Dahiya R R. 2010. Addressing variations in a few nutritionally important micro-nutrients in wheat crop. Proceedings of 19th World congress of soil science. published on DVD,1-6 A, Brisbane, Australia.

Pal D T, Gowda N K S, Prasad C S, Amarnath R, Bellur S R and Sampath K T. 2009. Micronutrient utilization, antioxidant enzyme and immunoglobulin level in sheep supplemented inorganic and organic sources of copper and zinc. *Indian*

Journal of Animal Sciences **79**(6): 615–21.

Pal D T, Gowda N K S, Prasad C S, Amarnath R, Bellur S R and Sampath K T. 2010. Effect of copper and zinc–methionine supplementation on bioavailability, mineral status and tissue concentrations of copper and zinc in ewes. *Journal of Trace Elements in Medicine and Biology* **24**: 89–94.

Parashuramulu S, Nagalakshmi D, Srinivasa Rao D, Kishan Kumar M and Swain P S. 2015. Effect of zinc supplementation on antioxidant status and immune response in buffalo calves. *Animal Nutrition and Feed Technology* **15**: 179–88.

Prasad C S and Gowda N K S. 2005. Importance of trace minerals and relevance of their supplementation in tropical animal feeding system. A review. *Indian Journal of Animal Sciences* **75**(1): 92–100.

Rakesh K, Singh M and Meena B S. 2017. Zinc management effects on quality and nutrient yield of fodder maize. *Indian Journal of Agricultural Sciences* **87**(8): 1013–17.

Rathod D D, Meena M C and Patel K P. 2012. Evaluation of different zinc-enriched organics as source of zinc under wheat-maize (fodder) cropping sequence on zinc-deficient typic haplustepts. *Journal of the Indian Society of Soil Science* **60**(1): 50–55.

Shukla A K, Tiwari P K, Pakhare A and Prakash C. 2016. Zinc and iron in soil, plant, animal and human health. *Indian Journal of Fertilisers* **12**(11): 133–49.

Singh M V. 2009. Micronutrient nutritional problems in soils of India and improvement for human and animal health. *Indian Journal of Fertilisers* **5(4)**: 11–26.

Singh R P, Sreenivasa B P, Dhar P, Shah L C and Bandyopadhyay S K. 2004. Development of monoclonal antibody based competitive ELISA for detection and titration based competitive ELISA for detection and titration of antibodies to *pestedes petits ruminants* (PPR) virus. *Veterinary Microbiology* **98**: 3–15.

Wenbin Jia, Xiaoping Zhu, Wei Zhang, Jianbo Cheng, Cuihua Guo and Zhihai Jia. 2009. Effects of source of supplemental zinc on performance, nutrient digestibility and plasma mineral profile in Cashmere goats. *Asian Australasian Journal of Animal Sciences* **22**(12): 1648–53.

Yadav S and Khirwar S S. 2005. Inter-relationship of soil micro-nutrient with feed stuffs in Jind district of Haryana. *Indian Journal of Animal Sciences* **75**(5): 531–33.