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Soil-plant-animal Continuum Concerning the Certain Micro-mineral Status of Indigenous Sheep in Hot Semi-arid Regions

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ABSTRACT

Background: Semi-arid regions have a large livestock population that grazes on nutrient-depleted natural grasses and crop leftovers, resulting in mineral shortages. The goal of this study was to determine the amounts of iron (Fe), copper (Cu), manganese (Mn) and zinc (Zn) in soil, pasture and sheep serum in semi-arid areas with low rainfall (498-522 mm) in order to construct a soil-plant-animal continuum and recommend solutions to increase trace element availability.

Methods: Samples were collected from different parts of the continuum [Soil (n=50), pasture (n=50) and blood samples from sheep (n=50)] from the selected study area and the trace elements were analyzed after digestion using atomic absorption spectrophotometer. Descriptive, correlation and t-test analyses were carried out to categorize the deficiency, their significance and soil-plant-animal continuum.

Result: Although most soil samples were found to be below threshold levels, most straws and stovers in the field had marginal levels of these micro-minerals. Most sheep serum samples had levels of different micro-minerals that were below the threshold level, implying that many indigenous sheep are deficient in micro-minerals. Although there was a substantial association between feed, fodder and sheep serum, there was no such correlation between trace mineral levels in sheep serum and soil. A higher percentage of these micro-minerals in sheep faeces implies that these nutrients are truly recycled through faeces and urine in semi-arid rural areas. To address the sub-clinical micro-mineral insufficiency in semi-arid regions, micro-mineral supplementation, particularly Zn, Cu and Mn, is required.

Key words: Dryland, Fodder, Micro-mineral profile, Nutrient cycling, Small ruminants.

INTRODUCTION

Semi-arid regions have distinct climatic, edaphic, pastoral and eco-social system features in terms of high ambient day temperature, solar radiation, low/ erratic rainfall and long dry days (Bhandari *et al.* 2018). In majority of semi-arid regions of world, livestock production is closely integrated with agriculture production system. Sheep production is important part of livelihoods in India as it harbours third largest population of sheep and engages a large proportion of human population in arid and semi-arid region.

The soil-plant-animal continuum is an important part of the agro-ecosystem where micro-nutrient flow is affected by the negative or positive interactions among them. Fe, Cu, Mn and Zn are the trace minerals playing a pivotal role in many metabolic processes by acting as cofactors, catalysts in enzymes and hormonal system of all animals (Rahman *et al.* 2006). Micro-nutrient deficiencies are one of the main causes for yield decline in semi-arid regions (Katyal and Rattan, 2003) affecting the productivity status of most livestock.

The significance of micro-mineral nutrition relative to the maintenance of productivity and prevention of deficiency in sheep under grazing conditions has been recognized for quite some time (Pankaj *et al.* 2017). The trace mineral requirement for different stages of life, like maintenance, lactation, pregnancy and growth requirements is the true need of tissue, which must be supplied in diet. In semi-arid regions of India, majorities of livestock are maintained under limited input systems (Kumar *et al.* 2021) by resource constraint farmers for their livelihood as they don't have enough feeding resources for their livestock towards optimizing their productivity (Sahoo *et al.* 2013). Under this situation, there is an urgent need to decipher the micro-mineral flow in livestock to suggest suitable mineral supplementation strategies for farmers rearing livestock under semi-arid conditions which are more resource-dependent and vulnerable to environmental stressors. The present investigation was carried out to explain the micro-mineral flow in semi-arid resource-driven areas to suggest suitable measures to prevent its losses as well as alleviate such deficiencies in indigenous sheep.

MATERIALS AND METHODS

Description of the study area

The study was conducted in Anantapuramu district Andhra

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Pradesh, India (Fig 1) Chamuluru (14°27′22.0″N 77° 27′50.0″E) and Ammavari Peta (14°42′08.6″N 77° 39′29.9″E), the most drought-affected district of South India which falls under the scarce rainfall zone (498-522 mm mean annual rainfall) under semi-arid agro-ecologies. The altitude of the study area ranges from 330 to 335 m marked by dry hot summers and mild winters.

Collection of samples

Ten farmers from each village (two villages) rearing Nellore breed of sheep (five sheep from each farmer making a total of 100 sheep) were randomly selected for collection of samples (soil and plant from grazing areas; blood and faecal samples from animals). Samples of soil at a depth of 20 cm were collected along with grass/ forage samples randomly for analysis especially from the grazing areas of sheep (n=50). These grazing areas comprise of naturally growing grasses and crop harvested fields. Samples of cultivated and uncultivated green and dry fodder, concentrate ingredients, *etc.* were collected randomly (n=50) from farmers in each village (Fig 1). Similarly, blood samples (n=50) and faecal samples (n=50) from grazing sheep were also collected.

Sample preparation and analytical methods

The soil samples were air dried, crushed and sieved with a 2 mm sieve. Mehlic-1 extracting solution (0.05 N HCl + 0.025 N H_2SO_4) was used to extract these processed samples. Blood samples were taken using a 20 mL disposable syringe from the jugular vein of sheep. The sample remained unaltered at room temperature. After 2-4 hours, the serum was collected using a micropipette and a micro-centrifuge, labelled properly and stored at -4°C in the refrigerator. Feed samples were dried in an oven at 80°C for 24 hours, pulverized to 1 mm in size and stored in sealed bags until analysis. Microwave digestion was used to digest these samples, which consisted of 5 mL concentrated HNO_3 and 1 mL concentrated HCl. Further, the total volume of mineral extract was made to 25 ml with deionized water. Fecal samples were collected manually after wearing plastic gloves

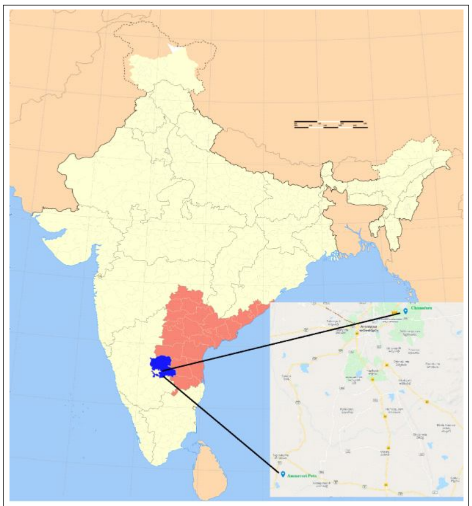


Fig 1: Location of experiment.

directly from the rectum of grazing sheep. These samples were dried in the sun with atmospheric moisture (<30%). All the samples were analyzed for Copper (Cu), Zinc (Zn), Manganese (Mn) and Iron (Fe) using atomic absorption spectrophotometer after proper digestion (Autukaite *et al.* 2021a, Autukaite *et al.* 2021b) at ICAR-Central Research Institute for Dryland Agriculture, Hyderabad.

Validation of area-specific mineral mixture

Twelve Nellore male sheep (from 4 farmers, 2 from each village and 3 sheep from each farmer) of body weight 10.0-12.0 kg and age 4-5 months were selected for the area-specific mineral mixture supplementation (Table 1) @2% of concentrate mixture for 60 days (August-September, 2019). At the start and end of the trial, sheep's weekly body weight (kg) and blood samples (n=12) were taken to track changes in micro-mineral status and average daily gain.

Statistical analysis

The data was analyzed and statistical significance was tested using the SAS 9.2 software. The correlation coefficient for the micro-mineral continuum in soil, plant and animal was estimated from the data collected for soil, fodder, faeces and blood serum where p-values were assessed for the test of significance. Microsoft Excel 2010 software was used to plot the graphs.

RESULTS AND DISCUSSION

Existing feeding practices

The farmers of study area were maintaining their sheep by grazing them on the common property resources/forest/ wastelands and. They were offering cultivated fodders like Hybrid Napier, sorghum green, or local grasses or a mixture of sorghum stovers, paddy straw, rice bran and concentrate mix at their barn after grazing. Majority (87.3%) were not supplementing sheep with common salt or mineral mixture.

Micro-mineral content of the soil

The 23.0, 2.6, 13.3 and 28.3% of soil samples of the study area were falling under the category of below critical level for Zn, Cu, Mn and Fe, respectively (Table 2, Fig 2). The reported critical levels of Zn, Cu, Mn and Fe deficiency in soils fall in the range of 0.2-6.0 ppm (Bünemann *et al.* 2018 and Czekała *et al.* 2020) which may vary with edaphic or agronomic factors (Fenetahun *et al.* 2020 and Bayu 2020). Enough range was available for copper and manganese

Table 1: Composition of area-specific mineral mixture.

Ingredients	Parts (kg/100 kg)
Dicalcium phosphate	60.00
Calcium carbonate	20.00
Magnesium sulphate	12.00
Ferrous sulphate	1.00
Copper sulphate	0.25
Zinc sulphate	5.00
Manganese sulphate	1.60
Cobalt sulphate	0.15

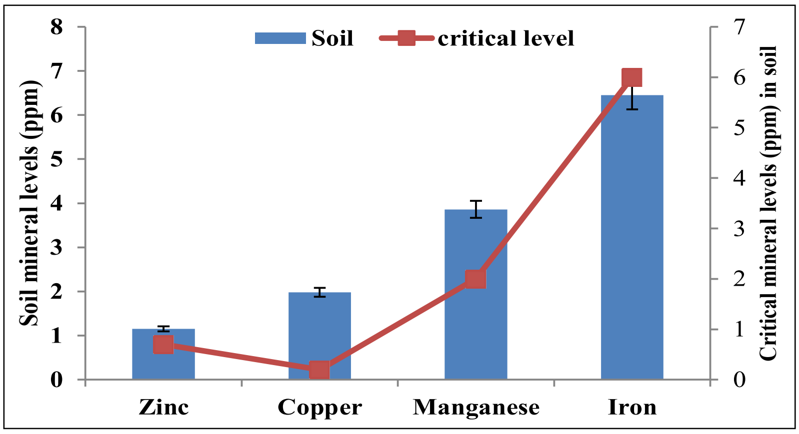


Fig 2: Soil mineral levels (ppm) in the study area.

Table 2: Deficiency status (% of samples below critical level*) of micro-nutrients (ppm) on DM basis in soil, plant and animal continuum.

Samples	Zinc (ppm)	Copper (ppm)	Manganese (ppm)	Iron (ppm)
Soil	23%	2.6%	13.3%	28.3%
Green fodder	83.3%	12.2%	62.3%	Nil
Dry fodder	91.3%	8.9%	Nil	Nil
Concentrate	54.3%	23.4%	2.3%	Nil
Serum	64.3%	52.3%	28.3%	3.4%

*(Pankaj *et al.* 2017; Herdt and Hoff, 2011; Sahoo *et al.* 2017).

minerals in the soil samples, however, the soil was deficient to the extent of 23.0-28.3% for zinc and iron. It revealed that the status of Zn in soil and fodder are falling in a range of marginal to deficient level. Since, the soil is unable to give adequate trace elements to the plants that sheep graze, a shortage is inevitable, which is exacerbated when the ration consists primarily of grazed grass with limited usage of concentrates or minerals in the diet.

Micro-minerals content of forage

In the present study, pasture samples were having higher concentration of Cu, Mn, Fe and Zn than the recommended level (NRC 2007). Zinc content in dry fodder was very poor (8.12-18.28 ppm), however, the level was good in green fodder (21.15-23.15 ppm) (Fig 3a). Concentrate mix (28.26 ppm) and rice bran (63.41 ppm) were found to be a good source of zinc to the sheep unlike rice bran.

The copper content in paddy straw (7.56 ppm), sorghum stove (13.12 ppm), concentrate mix (12.56) was low as compared to other greens (14.95-17.03 ppm). Cu levels were found to be poor in paddy straw and moderate in grasses, stovers and concentrate mix unlike rice bran (21.23 ppm) used in the area (Fig 3b).

The Mn levels in the study area ranged from 33.56-48.25 ppm in green fodder and 91.12-98.22 in rice bran and concentrate mix. However, dry fodders predominantly used had high levels of Mn (paddy straw 164.22 ppm, sorghum stovers 512.23 ppm) which enabled sheep to maintain the level (Fig 3c).

Average Fe content was adequate in all feed samples (536.2-945.4 ppm in green roughages, 168.23 ppm in sorghum stovers, 521.12 ppm in paddy straw, 522.1-654.3 ppm in rice bran and conc. mix (Fig 3d). Unlike Mn, Fe levels were very poor in sorghum stovers as compared to another source of feed ingredients used in the study area.

As reported earlier (Garg *et al.* 2005; Udar *et al.* 2003; Agegnehu and Amede, 2017; Giannoulis *et al.* 2017 and Mutwedu *et al.* 2020), these forages have Zn and Mn levels below the critical level in many of the semi-arid zones of India.

Micro-mineral levels in sheep

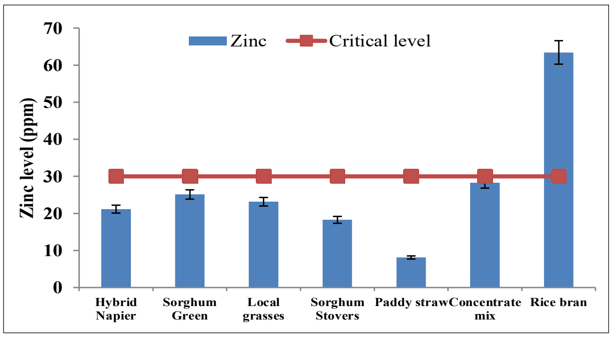
The average serum Zn, Cu, Mn and Fe content in sheep were 0.56, 0.49, 0.62 and 2.23 ppm, respectively (Table 2, Fig 4). More than 50% of the sheep screened showed low levels of Zn and Cu as compared to the critical level of Zn in blood serum (Herdt and Hoff, 2011; Pankaj *et al.* 2017 and Smith and Akinbamijo, 2000). They had enough level of Fe, however, Mn level was slightly deficient in a few of them which matches with earlier reports (Bhandari *et al.* 2018 and Mukangango *et al.* 2020). The critical concentration of these micro-minerals in the forages might have resulted in their lowered levels (Walsh *et al.* 2018 and Sileshi *et al.* 2017) in sheep.

The study area showed low mineral utilization, necessitating the use of area-specific mineral supplementation to help ameliorate these deficiencies. Supplementing them improved their trace element status, as shown by greater serum micro-mineral status and body growth rate (Table 4).

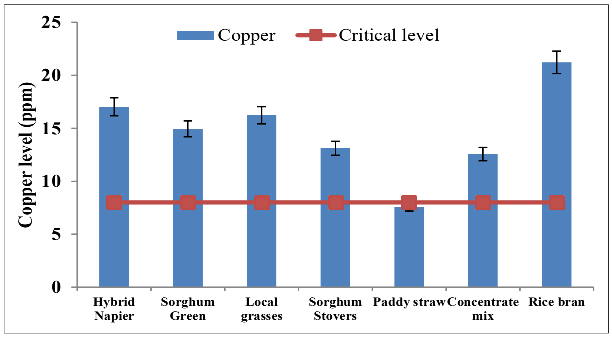
Sheep faecal samples, being highly concentrated form, have high levels of all micro-mineral content (Zn-64.53, Cu-51.36, Mn-153.68 and Fe-783.25 ppm), suggesting that they may be contributing significantly to micro-mineral recycling in nature *via* soil and plants (Fig 5).

Soil-plant-animal relationship

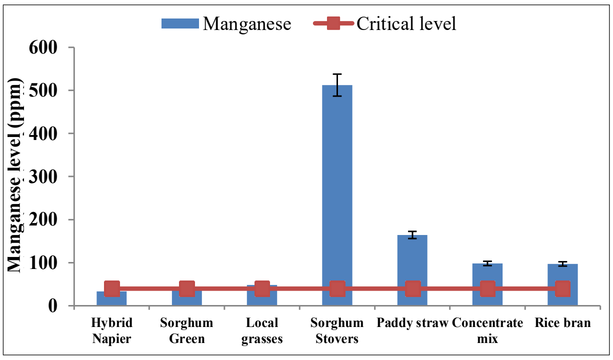
The relationship between faeces and serum concentration was significant for all micronutrients, showing that this is a non-invasive way for assessing micronutrient insufficiency in sheep (Table 3). The soil-plant and soil-animal



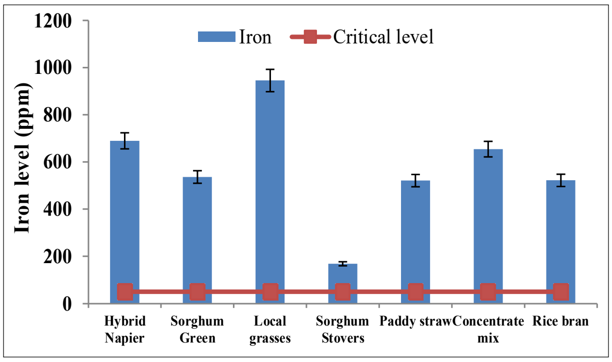
a: Zinc status (ppm) in different feed ingredients.



b: Copper level (ppm) in different feed ingredients.



c: Manganese level (ppm) in different feed ingredients.



d: Iron level (in ppm) in different feed ingredients.

Fig 3: Micro-mineral status in feed ingredients used in the study area with their critical levels.

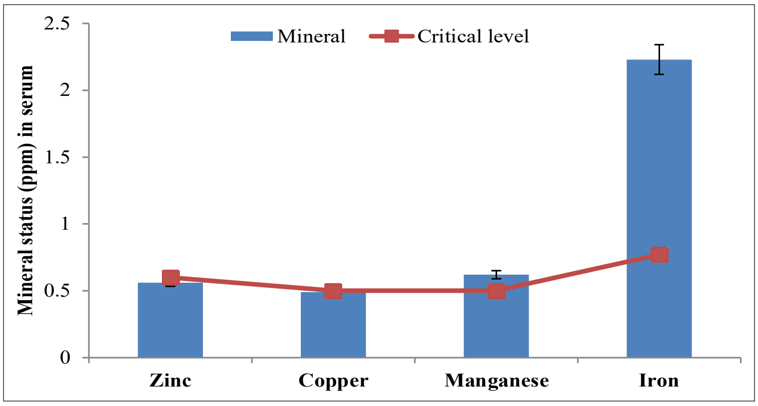


Fig 4: Micro-mineral status (in ppm) in sheep serum.

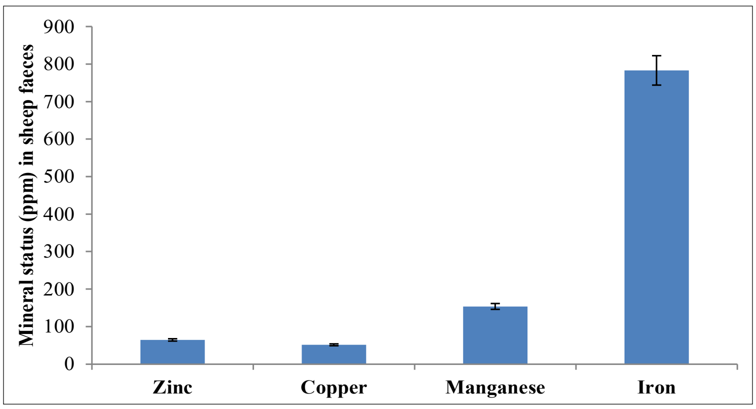


Fig 5: Mineral status in sheep faeces under field conditions.

relationships were both negative for Zn and Mn, but the plant-animal relationship was positive (Table 3). This highlights the importance of several factors in determining the availability of micro-minerals from soil to plants and then to animals. Forage micro-mineral concentration varies depending on soil type (Garg *et al.* 2005) and growing conditions (Agegnehu and Amide, 2017; Giannoulis *et al.* 2017 and Mutwedu *et al.* 2020). Only a small percentage of trace elements are present in the soil utilised by plants, according to the physio-chemical constitution of the soil (Herdt and Hoff, 2011). Low quantities of a trace element in the soil can affect its presence in plants (Pankaj *et al.* 2017); but, due to a regulated absorption process in the roots, higher levels may not be reflected in the plant (Bhandari *et al.* 2018 and Zhou *et al.* 2017).

Although there was a strong association between fodder and sheep serum, no comparable correlations were found between micro-mineral levels in sheep and soil (Table 3). There was no significant correlation between soil micro-mineral content and animal (serum) variables. Plant (feed and fodder) factor was, however, substantially connected with soil micro-mineral concentration in Cu and Fe, but not in Zn and Mn.

Serum micro-mineral content had a highly significant ($p<0.01$) correlation with all faecal micro-mineral content in sheep (Table 2), implying that even if we are not taking blood from sheep, which is difficult in field conditions, faecal samples (non-pricking) can suggest an animal's micro-mineral status as well as in pasture.

Table 3: Soil-plant-animal correlation for micro-nutrient status on DM basis.

Micro-nutrients in components	Soil	Fodder	Concentrate	Serum	Faeces
Zinc					
Soil	1				
Fodder	-0.157	1			
Concentrate	-0.082	0.423**	1		
Serum	-0.089	0.621**	0.612*	1	
Faeces	0.312*	0.462**	0.214	0.812**	1
Copper					
Soil	1				
Fodder	0.572**	1			
Concentrate	0.423**	0.563**	1		
Serum	0.293	0.536**	0.662**	1	
Faeces	0.826**	0.512**	0.414*	0.862**	1
Manganese					
Soil	1				
Fodder	-0.232	1			
Concentrate	-0.145	0.326*	1		
Serum	-0.114	0.311*	0.312*	1	
Faeces	0.512**	0.362*	0.254	0.647**	1
Iron					
Soil	1				
Fodder	0.372*	1			
Concentrate	0.411**	0.614**	1		
Serum	0.232	0.316*	0.352*	1	
Faeces	0.548**	0.566**	0.511**	0.641**	1

**Significant at 1% level, *Significant at 5% level.

Table 4: Effect of mineral mixture supplementation on serum micro-mineral status and body growth rate in Nellore sheep.

Particulars	% of sheep having below critical levels of micro-minerals		Initial	After 60 days
	0-day	60-day		
Zinc (ppm)	16.6%	8.3%	0.63±0.01[a]	0.71±0.03[b]
Manganese (ppm)	8.3%	Nil	0.61±0.02[a]	0.65±0.01[b]
Copper (ppm)	33.3%	Nil	0.53±0.01[a]	0.64±0.02[b]
Iron (ppm)	Nil	Nil	2.63±0.15[a]	2.71±0.17[b]
Average daily gain (g/day)			83.21±5.12[a]	92.23±6.27[b]

[ab]Mean±SE values bearing different superscripts in a row differ significantly at 5% level.

Significant association values between mineral levels in soil, pasture and sheep were not found in this study, which could be due to the development of complexes with organic matter and trace elements (Garg *et al.* 2005). Most trace element concentrations in animals are essentially independent of consumption and can only be changed by dietary intervention at higher concentrations, which was also investigated in this study.

Effect of area-specific mineral mixture supplementation

After 60 days of oral supplementation, area-specific mineral combination was found to be effective in significantly ($p<0.05$) enhancing all micro-minerals under study (Table 4) and improvement in body weight growth rate (10.84%). Mineral supplementation was more effective in animals because they were underfed at the farmer level and couldn't meet their nutrient requirements, particularly micronutrients (Sahoo *et al.* 2017).

Deficiency of these elements in soil or plants, is impacting the mineral status of sheep, which is widespread even under natural grazing conditions in semi-arid locations. A complete image of this continuum, as well as natural solutions such as nutrient recycling *via* faecal (organics) treatment (Maruthi *et al.* 2019) and area-specific mineral mixture supplementation, can provide a feasible answer.

CONCLUSION

Most sheep in India's semi-arid regions are zinc, copper and manganese deficient, as demonstrated by their serum levels. Faecal micro-mineral estimate, which is closely linked with serum micro-mineral content in sheep, can be a promising non-pricking method for monitoring micro-mineral status in animals under field conditions. As a result, supplementation with area-specific minerals may aid in the maintenance of sheep productivity. The bulk of micro-minerals leave the body of sheep through faeces, implying the necessity to tap the run-off micro-minerals from the body of sheep through scientific management of sheep faeces to reintroduce them into the soil-plant-animal continuum.

Conflict of interest: None.

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