

EVALUATION OF DYNAMICS OF IRON AND MANGANESE FROM PASTURE TO BUFFALOES: A CASE STUDY AT RURAL LIVESTOCK FARMS

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Abstract

Trace minerals (Fe and Mn) contents of soil, forages, and blood plasma of dairy buffaloes from five different Rural Livestock Farms in Sargodha, Pakistan were evaluated. Samples of soil, forages, and blood were taken from respective farms and analyzed after wet digestion. The higher mean Fe and Mn content of soil at farm -104, Fe lower at farm-89 and Mn at farm-106 was observed in this investigation. The higher mean forage Fe and Mn concentration at farm-104 and Fe lower at farm-106 and Mn at farm-96 was found. The blood plasma contained high level of Fe at the farm-96 and the lower at farm-104, while higher Mn level at the farm-106 and the lower at the farm-96. Based on these findings, it is evident that soil had higher value of both Fe and Mn than the requirements of forage crops at all farm studied while forage Fe was higher than the requirements of livestock being reared at those farms while reverse was true for forage Mn, pointing to the warranted need of supplementation to the animals to fulfil their Mn requirements. The blood plasma Mn levels were higher and deficient level of Fe was observed at some farms. Therefore supplements with high bioavailability of Fe should be provided to the livestock being reared at these farms to enhance the reproduction potential of the ruminants.

Introduction

Canal system is predominantly holding agriculture in northern Punjab Pakistan with decline in crop productivity and animal husbandry. Day to day expenses of small buffalo holders are maintained by milk selling. Low productivity is due to unawareness of farmers latest information related to feed and nutrition. The mineral content of new sown improved varieties of forages is unknown in Pakistan. The essential part of agriculture is livestock production basically in northern Punjab, Pakistan where the animals are reared in traditional ways. Number of cattle is large and due to poor individual cow productivity milk production is low, which is due to inadequate diet and mineral deficiency like various regions of the world (Sharma *et al.*, 2003). Due to unawareness, farmers faced lost in input in dairy cattle production system that merely depends upon common traditional forages such as oats, barley, rye, wheat, rice and maize (Miles *et al.*, 2001). The forage materials mostly for the buffaloes lack nutrients and farmers are unaware to give them minerals mixture feed. Buffaloes usually do grazing in pastures and feed on crop residues, natural grasses, leaves and shrubs, having very small amounts of minerals (McDowell & Arthington, 2005).

Dairy animals due to their increased requirement for lactation inclined to mineral deficiency (McDowell *et al.*, 1993). Production and reproduction is affected by accessibility of mineral nutrients depends upon environment, nourishing practices and output system (Singh & Bohra, 2005). One of the major factors is soil, as soil contains nutrients taken by plants and then consumed by buffaloes. That is why the role of soil and forage plant is very important in hygiene and production (Rehman *et al.*, 1998). The place where plants are grown affects the mineral composition of forage plants (Beeson & Matrone, 1976).

The association among soil, plant and animal shows a co-ordinate understanding of events that leads to decrease in maintenance of nutrients in buffaloes fodder. The visibility of soil, plant and animal has been determined and reported in other regions of Punjab, Pakistan (Khan *et al.*, 2004; 2005; 2007; 2008; 2009); however, it has not been studied in this area. The purpose of the present study, therefore, was to assess that mineral content of soil, plant and buffaloes and to establish relationship among this system. This information would be useful for farmers and livestock owner of local areas and other areas of Punjab, Pakistan to enhance the production and reproduction potential of buffaloes by supplementation with various mixtures to animals. The present study is conducted to know the soil-plant-animal continuum in relation to Mn and Fe to ascertain the status of these elements in this system.

Materials and Methods

Description of study area: This study was conducted at 5 different farms in Sargodha, Punjab, Pakistan present at a distance of 12 to 20 km in northern side to tehsil Silan Wali Road. Each farm lies at a distance of 7 km. The farm stations are present in different villages. The focus of study lies in chak 102 N.B., which is located in the centre of all farms. In east there is chak 96 N.B. In north chak 106 N.B., in west chak 89 N.B. and in the south there is chak 104 N.B. In summer temperature during the day time ranges from 26 to 51°C, while during winter minimum temperature lowers down up to 8°C. Relative humidity ranges from 30% to 48%, although Sargodha does not experience any snowfall, however, frost in winter is common.

In the study area, buffaloes were reared on local vegetation, forages and wastes of agriculture products. A local analysis showed that a buffalo took 40 to 50 kg green fodders per day. The five composite each of soil, forage and blood samples were collected randomly from each dairy farm. Soil and forage samples were taken from 5 different land areas from five different places within the pasture at a depth of 15 cm to 20 cm. Therefore, the total number of representative samples both soil and forage was 75 collected during winter season. Twenty five forage samples were packed in a polythene bag and labelled to make further analysis. A total number of 25 blood samples (5 from each dairy farm) were taken from where the soil and fodder samples were collected. About 10 mL blood from each buffaloes were taken in a clean sterilized test tube having heparin from the jugular vein (in neck region) using proper 16 gauge needle and permitted for clotting at room temperature and centrifuged for 12 minutes at 75 g. The plasma was separated by centrifugation and frozen at 20°C until analysis.

Processing and digestion of samples: Soil samples were air dried, crushed and sieved through 2 mm sieve samples of forage were dried at 70°C in an oven and ground with the help of mill. Dried soil and forage sample of 1 g each and 1 mL plasma was digested with H₂SO₄ and H₂O₂ and subjected to atomic absorption Spectrophotometry. Samples of

forage (1 g) soil (2 g) and blood plasma (1 mL) were taken in digesting tubes and 6 mL H_2SO_4 was added in each tube. Samples were digested by using hot digestion block and adding H_2O_2 simultaneously until the solution become colourless and volume reduced to a level of 3-5 mL. These digested samples were coded for some time at room temperature and then transferred in a volumetric flask and using de-ionized water made volume of 100 mL. These solutions were filtered by using Whatman # 42 filter paper and transferred in labelled clean plastic bottles before estimation of different minerals.

Statistical analysis: The mineral concentration in soil, forage and blood plasma for different farms was analyzed by ANOVA by using general linear model. Data analysis of mineral levels of soil, forage and blood plasma was used to determine co-relation coefficient of mineral content. Linear regression model of regression equation used to determine relationship among soil-plant, plant-animal and soil-plant-animal. All statistical analyses were carried out by using SPSS statistical analysis software as per the standard procedure (Snedocer & Cochran, 1989).

Results and Discussion

Mineral contents in soil: Soil analysis results showed variations in mineral content among different dairy farms. The mean value of soil minerals are shown in Table 1. The soil Mn ranged from 122.17 to 136.77 mg/kg across different livestock farms. These values were higher than the critical level of 5 mg/kg as established by Viets & Lindsey (1973). The highest values of soil Mn was observed at farm-104 and the lowest level of soil Mn was found at farm-106. These values were sufficiently higher than the requirements of forage plants. These findings are in agreement with that of Aregheore *et al.*, (2007) and lower than those values reported by Miles *et al.*, (2001). Mean soil Fe concentration were highly variable among farms and ranged from 664.57 to 749.97 mg/kg at different farms. Its concentration was higher at farm-104 and lower at farm-89. Mean Fe values were generally high compared to the critical level of 2.5 mg/kg as suggested by Viets & Lindsey (1973). These results may support the report of McDowell (1984) who reported that Fe deficiency is rare in grazing livestock due to generally higher concentrations in soil and forage. These results corroborate with the findings of Aregheore *et al.*, (2007) who found similar fluctuation in Apia Samoa and higher than those reported by Miles *et al.*, (2001) in India and Khan *et al.*, (2007; 2008; 2009) in Pakistan. Results of the present study indicated several variations in soil Mn and Fe and these can be attributed to the alkaline nature of the soil of these farms. Mineral soil concentration depends upon many environmental factors such as moisture level, temperature, pH, nutrient supply, aeration *etc.* These factors may explain the variations found among the different farms (Khan *et al.*, 2007).

Mineral contents in forage: Forage Mn contents ranged from 23.26 to 24.18 mg/kg among different farms of Livestock during this investigation (Table 2). Forage Mn concentrations were higher in farm-104 and lower in farm-96 with inconsistent pattern of fluctuation. Forage Mn concentrations found in this study were lower than the critical values of 40 mg/kg established by Anon. (1984). These results agree closely with previous studies of Khan *et al.*, (2007; 2008; 2009) and in Florida by McDowell *et al.*, (1993). Forage Mn values were higher than those already reported by Espinoza *et al.*, (1991) in Florida and lower than those found by Khan *et al.*, (2004; 2005; 2007; 2009) in Pakistan. Similar livestock experimentation while working in south-western Punjab, Pakistan was obtained.

Table 1. Manganese and iron concentrations (mg/kg) in soil (Mean \pm SE; n=25).

Mineral (mg/kg)	Critical level	Rural livestock farms				
		Farm-104	Farm-89	Farm-102	Farm-96	Farm-106
Mn	5	136.77 \pm 1.73	133.20 \pm 8.51	125.415 \pm 8.30	131.94 \pm 5.90	122.17 \pm 4.49
Fe	2.5	749.97 \pm 7.85	664.58 \pm 8.8	744.97 \pm 3.18	743.92 \pm 8.4	745.18 \pm 4.78

Table 2. Manganese and iron concentrations in composite fodder samples (Mean \pm SE; n=25).

Mineral (mg/kg)	Critical level	Rural livestock farms				
		Farm-104	Farm-89	Farm-102	Farm-96	Farm-106
Mn	40	24.18 \pm 5.20	23.67 \pm 4.64	23.26 \pm 4.88	21.31 \pm 5.89	21.53 \pm 6.11
Fe	50	619.51 \pm 74.88	588.48 \pm 86.33	543.02 \pm 70.99	593.77 \pm 59.33	469.85 \pm 43.58

Iron content of forages varied from 469.85 to 619.51 mg/kg across all sampling farms with consistent pattern of decrease from farm-104 to farm-106, respectively. Higher Fe was found in farm-104 and lower one in farm-106. Forage Fe values were all above the critical value of 50 mg as suggested by Anon., (1984). Lower values for forage Fe were already reported from Pakistan by Khan *et al.*, (2009) while working in another animal ranch in south-western, Punjab of this province and Ahmad *et al.*, (2008) in north-western areas of this province. These forage Fe values were sufficiently higher than those required by the ruminants but were below the tolerance levels as established by McDowell & Arthington, (2005).

Mineral contents in Blood plasma: All the buffaloes included in the present study being reared at these farms received only common salt minerals and for them fodder is the only source of all nutrients. Fodder mineral content depends upon type of soil and environmental conditions (Beeson & Matrone, 1976; McDowell *et al.*, 1993). Geophysical and chemical composition determined mineral content taken by plants (Reid & Horvath, 1980). Uptake mechanism of roots guide the plants in such a way that low content in soil, lower the mineral content in plant but high content in soil, are not accumulated in higher amounts in plants (McDowell *et al.*, 1993).

Mean blood plasma Fe concentration ranges from 0.97 mg/L to 1.17 mg/L across all sampling farms. Higher Fe values were found at farm-96 and lower at farm-104 with consistent pattern of increase up to farm-96 followed by decrease at farm-106 during this investigation. All mean serum Fe values were lower than the critical level of 1.0-2 mg/L as established by Miles *et al.*, (2001) in some instances. Higher values of blood plasma Fe have been reported from Pakistan (Khan *et al.*, 2008) and similar plasma Fe values in Aregheore *et al.*, (2007) in Samoa. It has been known that mostly animals have very low capacity for the excretion of Fe, therefore, its retention in the body of animals is mainly controlled by the process of absorption in the gastrointestinal tract. Often the soil Fe concentration is many times higher than those of plants used in forages for ruminants. Fe deficiency is very rare in ruminants grazing the pastures due to adequate levels in forages and soils and contamination of forage plants in soil particles (Aregheore *et al.*, 2007; Khan *et al.*, 2005).

Table 3. Manganese and iron concentrations in blood plasma of dairy buffaloes (Mean \pm SE; N=25).

Mineral	Rural livestock farms				
	Farm-104	Farm-89	Farm-102	Farm-96	Farm-106
Mn (mg/L)	0.65 \pm 0.176	0.72 \pm 0.16	0.82 \pm 0.18	0.64 \pm 0.27	0.94 \pm 0.18
Fe (mg/L)	0.97 \pm 0.21	1.07 \pm 0.18	1.14 \pm 0.25	1.17 \pm 0.22	1.02 \pm 0.15

Table 4. Soil-plant-buffaloes relationship (correlation) regarding manganese and iron status.

Mineral	Pearson correlation values		
	Soil-fodder	Forage-buffaloes	Soil-blood plasma
Mn	0.104280193	-0.114347016	-0.253857545
Fe	0.100357096	-0.072653886	0.108033311

Table 5. Regression equation on soil-plant-buffaloes continuum in relation to mineral status

Mineral	Regression equation to predict mineral contents in		
	Plant based on the mineral status of soil	Buffaloes based on the mineral status of plant	Buffaloes based on the mineral status of soil and plant
Mn	Y1=12.10489+0.08227 X1	Y2=0.85370-0.00440 X2	Y2=1.79508-0.00342X1-0.00742X2
R2	0.011429	0.071552	0.151587
Fe	Y1=438.11073+0.17109 X1	Y2=102.16748-0.00278 X2	Y2=96.86672-0.00323X1+0.00761X2
R2	0.128458	0.217821	0.142770

Y1 denotes Mn in plants, X1 denotes Mn in Soil, Y2 denotes Mn in Forage and X2 denotes Mn in Forage

The buffaloes contained insufficient amount of plasma Fe and this point out that these animals were affected with blood sucking parasites or worms, which lower the blood plasma Fe level. The lower level of blood plasma Fe might have been occurred by low absorption of Fe through the intestinal tract of animals (Aregheore *et al.*, 2007). In addition to this, metabolism of Fe is also interrelated with those of other metals (McDowell, 1984). However, the serum level found in present study is lower than the limit of Fe tolerance in livestock (McDowell & Arthington, 2005).

Iron deficiency is very rare in different forms of livestock unless there is considerable blood loss by the attack of parasites or any disease. It has been reported that for adult animals the requirement of Fe is estimated to vary from 30-60 mg/kg but in our investigation, the concentration of Fe in forages have been found many times higher than that level but there is no threat of toxicity of Fe as it is considered to be less toxic of essential elements and its maximum tolerable level for livestock is 1000 mg/kg in the dietary sources (McDowell & Arthington, 2005).

Mean blood plasma Mn varied from 0.64 mg/L to 0.94 mg/L respectively and higher value was found at farm-106 and lower at farm-96 with consistent pattern of decrease or increase during this investigation. Of all plasma samples analyzed, none were deficient; all were above the normal range of blood serum Mn as established by Miles *et al.* (2001). Similar values for blood over Mn content were reported previously (Khan *et al.*, 2006) and Aregheore *et al.* (2007). Blood Mn values were highly variable and suggested the optimum levels for buffaloes (Underwood, 1981). These levels are extremely higher than the critical levels of 0.015 to 0.05 mg/L for growing and adult animals (Anon., 1985;

McDowell *et al.*, 1993). The higher serum Mn concentrations seems to indicate that there was no interference from the concentration of Zn and p in the forage and feed on the higher supply of P, which badly affect the utilization of Mn (Aregheore *et al.*, 2007).

In our investigation Mn deficiency has been found in forage and Fe at some farms in animal blood plasma, but higher Mn levels have been found in blood plasma of buffaloes. It has been reported that Mn is among the least toxic metals and it may cause toxic effect if it is taken into the gastrointestinal tract in a considerable concentration. Its maximum tolerable levels of 1000 mg/kg and its higher levels in forages and other dietary sources results in very low reproductive potential and have been observed in many regions of the world (McDowell & Arthington, 2005).

Soil-plant-buffaloes: Results obtained have significant co-relation by keeping the soil and fodder mineral content as independent variable. An attempt was made to predict the mineral content in buffaloes by keeping the soil and fodder mineral content as independent variable.

Regression equation of Mn showed that there is a positive relation in the soil and plant contents whereas it is reverse in the buffaloes and plant relationship. In forage and soil equation it is 0.08227 and in blood and forage equation it is -0.00440. Beta Coefficients in both plant and buffaloes cases are non-significant. Similarly both coefficients are small and non significant and have negative relationship with buffaloes. Regression equation of Fe showed that there is a positive relation in the soil and plant contents whereas it is reverse in the buffaloes and plant relationship. In forage and soil equation its value is 0.17109 and in blood plasma and forage equation its value is -0.00278. Beta Coefficients in both plant and buffaloes cases are non-significant. Similarly both coefficients are small and non significant and have negative relationship with buffaloes.

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