

Assessment of Iron Status of Different Classes of Goats Grazing the Natural Pasture During Different Seasons

Zafar Iqbal Khan, Altaf Hussain, Muhammad Ashraf, Muhammad Yasin Ashraf¹ and Zia-ur-Rahman²
Department of Botany, University of Agriculture, Faisalabad – Pakistan

¹Nuclear Institute for Agriculture and Biology (NIAB) Faisalabad – Pakistan

²Department of Physiology & Pharmacology University of Agriculture, Faisalabad – Pakistan

Abstract

An investigation was conducted in the central region of Punjab, Pakistan to study the relationship of Fe in soil, plant, and animal systems to enable prediction of iron status of three different classes of goats grazing the pasture. Soil, forage, feed, water, and animal samples (blood plasma, milk, faeces, and urine) were collected fortnightly during the winter and summer seasons. It was found that seasons affected soil, forage, feed, and milk Fe concentrations. The forage was found to be deficient in Fe concentration for the requirement of animals during the summer season. Although plasma Fe did not show seasonal effect and was affected only by the sampling periods in all classes of goats, its values were above the critical level during both seasons. No relation was found between source of Fe and concentration of Fe in excreta. Therefore, it is concluded that feed supplement should be provided to animals particularly during summer to complement the forage Fe for the requirement of grazing livestock in this region.

Key words: Goats, Grazing, Iron, Status, Plasma, Milk, Soil, Forage, Pasture.

Introduction

Animals mainly depend on forage for their mineral requirements. Forage and soil mineral imbalances are common in different regions of the world, typified by sandy, infertile soils, and forages are frequently low in essential minerals. Many naturally occurring deficiencies in grazing livestock can be related to soil and associated forages (McDowell 1997). Mineral availability to plants is highly dependent on soil pH. Like other minerals Fe is also affected by soil pH fluctuations. Kabata-Pendias and Pendias (1992) found that as the soil pH increases, the availability of Fe to plants decreases. Iron in its reduced form (Fe^{2+}) is available to the plant. As the pH decreases, more Fe^{3+} is reduced to Fe^{2+} ; thus Fe becomes more available in acid soils (Velasquez-Pereira *et al.*, 1997).

The major cause of low plant Fe is the insolubility of Fe^{3+} oxides, the form generally present in most soils (Lindsay, 1984). The inorganic source of Fe applied to soils are converted rapidly to ferrous which are less available to plants; ferrous iron is oxidized to the ferric forms in well aerated soils, especially as soil pH increases (Mortvedt, 1991). Consequently, the Fe deficiency most frequently found on calcareous soils is difficult to control (Morris *et al.*, 1999; Gupta, 1992; McDowell, 1993). However, it is known that poor drainage and aeration of soil increases the availability and uptake of Fe by plants (Grace, 1991).

Among other minerals, Fe plays an important role in the physiology of animals. It is an important component of haemoglobin, myoglobin, cytochromes, and certain enzymes. Iron deficiencies seldom occur in adult animals unless there is a considerable blood loss from parasitism or disease (McDowell, 1997). Iron deficiency may be a problem where ruminants are fed on low quality forage, such as straw. Free-choice complete mineral mixture is an insurance for providing Fe and other minerals where dietary concentrations are unknown or highly variable due to season, location, forage species, and animal potential.

It is important to know micromineral concentrations for new varieties of plants since forage in tropical and subtropical regions is the primary source of minerals for grazing livestock (McDowell, 1992). Different studies have shown an increase in Fe concentrations in tropical forages when yields increase (Espinoza *et al.*, 1991; Santana and McDowell, 1994). Different grass species have been reported to meet the Fe requirements for livestock in certain areas (Arizmendi-Maldonado *et al.*, 2002). The level of Fe in some grasses studied varied from 90 to 1473 mg/kg while the requirement for Fe of all classes of ruminant livestock is below this range (NRC, 1985, 1989, 1996). Indeed, very high concentration of Fe found in many forages could be detrimental to livestock. In addition, it is known that the changes in dietary Fe intake can influence Cu metabolism and cause Cu deficiency in ruminants under practical conditions (Humphries *et al.*, 1985; Youssef *et al.*, 1999).

It is important to determine mineral concentration of soils, forages, feed, water, and animal fluids to estimate the mineral needs of grazing ruminants as well as the time of the year when they are most required. Thus, the purpose of study was to assess the Fe status of selected goat producing region of Pakistan during different seasons of the year to have the knowledge of the correct time for supplementation of this mineral.

Corresponding author: Zafar Iqbal Khan
Department of Botany, University of Agriculture,
Faisalabad – Pakistan
E.Mail: ashfarm@fsd.paknet.com.pk

Materials and Methods

The study was conducted, during 2000-2002, at the Livestock Experimental unit, located in the southern part of the province of Punjab, Pakistan.

Samples from the animals were collected which had been in the pastures for not less than 1-3 years prior to sample collection. All the animals at the farm had access to feed containing mixture of different minerals, in addition to grazing the improved varieties of forages throughout the year.

Composite forage and soil samples were collected at three sites on goat ranch during two different seasons of the year. Each composite soil sample was derived from five sub-samples taken at a depth of 20 cm as described by Sanchez (1976). Each composite forage sample consisted of five sub-samples of the same forage predominating species that was most frequently grazed by the goats on the farm.

Likewise, feed and drinking water samples being offered to the animals were also collected in five replicates. Blood, milk, faeces, and urine samples were collected from 30 animals of different physiological conditions and gender. Soil samples were processed according to technique set forth by Rhue and Kidder (1983) while the preparation of all the other samples was carried out as described by Fick *et al.*, (1979). The solutions were then analyzed for Fe by atomic absorption spectrophotometry (Pye Unicam Ltd. York street, Cambridge UK).

The data were analysed using a split-plot completely randomized design (Steel and Torrie, 1980). Differences between means were worked out using Duncan's New Multiple Range Test (Duncan, 1955).

Results and Discussion

Pasture Samples

Soil

Significant seasonal and non-significant sampling period effect was found on soil Fe²⁺ concentration (Table-1). The reduction in soil Fe²⁺ at all fortnights during winter progressed with time. In contrast, during summer the reduction in soil Fe²⁺ was observed at only the last fortnight. (Fig-1a). Overall, soil Fe²⁺ was significantly higher in winter than that in summer.

Forage plants

Seasonal and fortnights effects were found to be significant on forage Fe²⁺ level (Table-1). Forage Fe²⁺ concentration was markedly higher in winter than that in summer. A gradual slight reduction in forage Fe²⁺ level was observed with time during both seasons (Fig-1b).

Water

There was a marked significant effect of fortnights on water Fe²⁺ concentration, whereas the seasons remained ineffective in changing the Fe²⁺ level in water (Table-1). However, the Fe²⁺ in water was found to be higher in winter than that in summer at all four fortnights. A consistent decline in water Fe²⁺ was observed up to

fortnight 3 during winter, such consistent decrease in Fe²⁺ level was found from fortnight 2 to onwards (Fig-1c).

Feed

Mean squares from the analysis of variance of the data for feed Fe²⁺ showed significant influence of seasons and fortnights (Table-1). Although feed Fe²⁺ remained unchanged at the first three fortnights during winter, there was a sharp decrease at the 4th fortnight. A similar pattern in feed Fe²⁺ was observed during summer. Overall, feed Fe²⁺ was found to be higher in winter than that in summer (Fig-1d).

Animal Samples

Lactating Goats

Plasma

A non-significant effect of seasons and significant of sampling intervals was observed on plasma Fe²⁺ concentration (Table-2a). During winter, Fe²⁺ level increased from 1st to 2nd fortnight but thereafter it had a decreasing trend up to fortnight 4 (Fig-2a). In contrast, during summer, the plasma Fe²⁺ consistently increased up to 3rd fortnight followed by a sharp decrease at fortnight 4. Overall, the lactating goats contained higher concentration of Fe²⁺ in the blood plasma during winter than that during summer.

Faeces

Non-significant seasonal effect, but significant that of fortnights was observed on fecal Fe²⁺ (Table-2a). During both seasons, a consistent fall in fecal Fe²⁺ level was found with time (Fig-2b). The animals excreted higher amount of Fe²⁺ through faeces during summer than that during winter.

Urine

Urine Fe²⁺ was found below the detection limit.

Milk

Milk Fe²⁺ availability was affected significantly by the seasonal change, while the sampling intervals did not affect Fe²⁺ level (Table-2a). The amount of Fe²⁺ in milk was higher in winter than that in summer (Fig-2d). In contrast, in summer no consistent pattern of increase or decrease in milk Fe²⁺ was found

Non-Lactating Goats

Plasma

There was a marked sampling interval effect on plasma Fe²⁺ level, but seasons had no significant effect on it (Table-2b). In winter, the plasma Fe²⁺ level progressively decreased with time (Fig-2e). In contrast, in summer, the plasma Fe²⁺ level was found to be statistically uniform at 1st, 2nd and 4th fortnights. At the 3rd fortnight, the Fe²⁺ level was slightly higher than that at the other three fortnights. Generally, the plasma Fe²⁺ was higher during winter than that during summer.

Faeces

Seasons had no significant effect on fecal Fe²⁺ concentration, but the effect of sampling periods was found to be significant (Table-2b). In winter, a sharp rise in fecal Fe²⁺ at the 2nd fortnight followed by a sharp

decrease at fortnights 3 and 4 was observed (Fig-2f). Conversely, in summer, almost the same amount of fecal Fe^{2+} was found to be present at the first two fortnights, and the same was true at the last two fortnights.

Urine

Urine Fe^{2+} was below the detection limit.

Male Goats

Plasma

Plasma Fe^{2+} concentration was affected significantly by the sampling periods, but the effect of seasons was non-significant (Table-2b). In winter, there was a consistent decrease in Fe^{2+} level in plasma with time of sampling (Fig-2h). In summer, a pattern of consistent increase in Fe^{2+} was found up to fortnight 3 followed by a sharp decrease at the 4th fortnight.

Faeces

Significant fortnight effect but non-significant that of seasons was found on fecal Fe^{2+} concentration (Table-2b). A consistent trend of decrease in fecal Fe^{2+} level was found with the increase of sampling time during both seasons (Fig-2i). Generally, excretion of Fe^{2+} through faeces was higher in summer than that in winter.

Discussion

Iron requirements of ruminants are not well established, however, it is known that young animals have high requirements than those in adults. Deficiency is likely to occur in young animals that are fed milk diets and in animals with excessive blood loss or through a rate of degradation of blood cells. However, excess Fe^{2+} can cause Cu^{2+} deficiency (McDowell *et al.*, 1993)

Fe^{2+} deficiency is considered rare for grazing livestock due to generally adequate pasture concentrations together with contamination of plants by Fe^{2+} rich soil. Fe^{2+} absorption occurs throughout the gastrointestinal tract including the stomach and colon, but major sites of active absorption are located in the duodenum and jejunum. High dietary levels of phosphorus reduce Fe^{2+} absorption, presumably by the formation of insoluble ferric phosphate and phytate, and higher dietary levels of several other divalent metals, including Cu, Mn, Lead (Pb) and Cd increase Fe^{2+} requirements by competing for absorption sites in the intestinal mucosa (Underwood, 1981). Acidic conditions in the gastrointestinal tract help the absorption. Ascorbic acid also favours iron absorption. Iron is excreted in very less quantities from faeces and urine after absorption. In this study, mean soil Fe^{2+} concentration fluctuated during both seasons. In both seasons, Fe^{2+} values were generally high, compared to the critical level (Viets and Lindsay, 1973). Adequate soil Fe^{2+} values have already been reported by Mooso (1982) and Merkel *et al.* (1990) from Florida. These results may support the report of McDowell *et al.* (1984) in which it was indicated that Fe^{2+} deficiency is rare in grazing animals due to generally adequate content in soils and forages. However, Becker *et al.* (1965) reported Fe^{2+} deficiency in animals grazing on sandy soils in Florida. Similar seasonal trends in soil Fe^{2+} fluctuation were also

reported else where (Tejada *et al.*, 1987; Prabowo *et al.*, 1990).

Forage Fe^{2+} concentration varied and it was significantly affected by seasonal influence. It was greater than the requirement of ruminants in winter and deficient in summer. Percentage of forage samples, deficient in Fe^{2+} for ruminants requirement (NRC, 1984), was higher during summer and lower during winter. Espinoza *et al.* (1991), found variation in forage Fe^{2+} concentration and higher percentage of Fe^{2+} deficient samples in a study conducted in Florida. Vargas *et al.* (1984) and Tejada *et al.* (1987) in Colombia and Guatemala, respectively did not find Fe^{2+} deficient forage samples. The absorption of Fe^{2+} by plants is not always consistent and is affected by the physiological state of the plant, as well as changing condition of soil and climate (Kabata-Pendias and Pendias, 1992). The generally low forage Fe^{2+} found in summer is in disagreement with the normal soil value found. It may be due to the type of forages deficient in iron below the requirements of animals. Feed Fe^{2+} concentrations showed seasonal effect, being higher in winter than that in summer as reported in the results. Water Fe^{2+} level was almost same during both seasons and both feed and water Fe^{2+} concentration seemed to complement the forage Fe^{2+} level required by the ruminants. The high feed Fe^{2+} did not raise the plasma level. The amount of Fe^{2+} absorbed is related to its need by the animal body, the capacity of the body to excrete Fe^{2+} is very low, therefore, its absorption is controlled by the body requirement. Fe^{2+} is absorbed by mucosal cells of gastrointestinal tract. When these become physiologically saturated, the Fe^{2+} absorption is checked. Therefore, supplementation of Fe^{2+} is much less important than for most other trace minerals. It is most warranted for grazing livestock when forages contain less than 100 mg/kg Fe^{2+} and for if insects or parasites are causing substantial blood loss (McDowell, 1997).

Plasma Fe^{2+} concentrations did not differ in different seasons and animal class. The Fe^{2+} was slightly higher in winter than that in summer in all classes of animals (goats). The slight variation in plasma Fe^{2+} may have been due to age effects, gender of the animals, and other factors affecting requirements (Judson and McFarlane, 1998).

The levels of plasma Fe^{2+} were above the critical level suggested by McDowell (1976). These values were much lower than those previously reported by Mpofu *et al.* (1999) and higher than recorded by Merkel *et al.* (1992) and Pastrana *et al.* (1991), who observed similar fluctuations in plasma Fe^{2+} with respect to season.

Fecal Fe^{2+} level did not vary significantly during both seasons in all classes of goats. However, it was higher in summer than that in winter, which was not related to source of Fe^{2+} used. Therefore, fecal Fe^{2+} cannot be considered a good component of pasture status. Urine Fe^{2+} concentrations were found below the detection limit of the instrument used. Milk Fe^{2+} level was significantly higher in winter than that in summer reflecting the dietary intake. These values during both seasons were above the critical level (Pamela *et al.*, 2001). Higher value of milk Fe^{2+} has already been reported by Salih *et al.* (1987).

Table-1 Analysis of variance of data for Fe²⁺ concentration in soil, forage plants, water, and feed at different fortnights during winter and summer seasons at goat ranch.

Source of variation S.O.V	Degree of freedom df	M e a n s q u a r e s			
		Soil	Forage plants	Water	Feed
Season (S)	1	577.60 **	114490.00 ***	0.0002 ^{ns}	47403.23***
Error	8	29.24	39.69	0.0002	561.60
Fortnight (FN)	3	45.90 ^{ns}	424.37***	0.00006*	1656.29*
Sx FN	3	17.40 ^{ns}	8.27 ^{ns}	0.00002	403.03 ^{ns}
Error	24	26.67	8.92	0.00002	364.62

*, *** = Significant at 0.05 and 0.001 levels, respectively.

ns = non-significant.

Table-2a Analysis of variance of data for Fe²⁺ concentration in blood plasma, faeces, urine, and milk of lactating goats at different fortnights during winter and summer seasons.

Source of variation S.O.V	Degree of freedom df	M e a n s q u a r e s			
		Plasma	Faeces	Urine	Milk
Season (S)	1	1.66 ^{ns}	2247.20 ^{ns}	Below detection limit	0.071**
Error	18	3.54	3763.34		0.006
Fortnight (FN)	3	0.36**	3495.55***		0.005 ^{ns}
S x FN	3	0.08 ^{ns}	162.83 ^{ns}		0.001 ^{ns}
Error	54	0.08	154.04		0.005

Table- 2b Analysis of variance of data for Fe²⁺ concentration in blood plasma, faeces, and urine, of non-lactating goats and that of plasma and faeces of male goats at different fortnights during winter and summer seasons.

Source of variation S.O.V	Degree of freedom df	M e a n s q u a r e s				
		Non-lactating goats			Male goats	
		Plasma	Faeces	Urine	Plasma	Faeces
Season (S)	1	2.15 ^{ns}	1692.80 ^{ns}	Below detection limit	0.01 ^{ns}	1336.61 ^{ns}
Error	18	2.90	10680.86		5.23	5042.50
Fortnight (FN)	3	0.45*	14656.63**		1.50***	2422.71***
S x FN	3	0.43*	3972.10 ^{ns}		0.74*	23.95 ^{ns}
Error	54	0.14	2939.27		0.18	18.93

*, **, *** = Significant at 0.05, 0.01, and 0.001 levels, respectively.

ns = non-significant.

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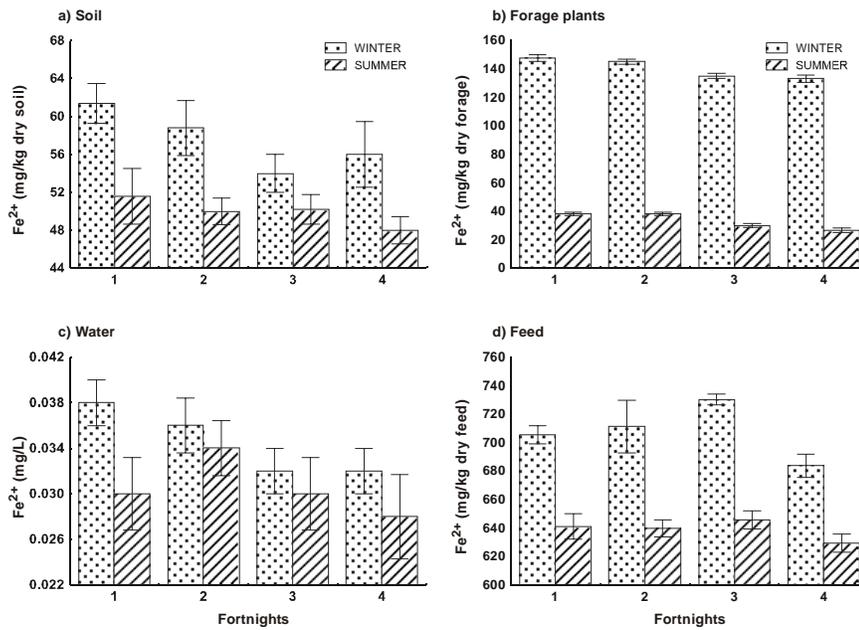


Fig.1: Fe^{2+} concentration in (a) soil, (b) forage plants, (c) water, and (d) feed at different fortnights during winter and summer seasons

(Means with the same letters do not differ significantly at $P < 0.05$)

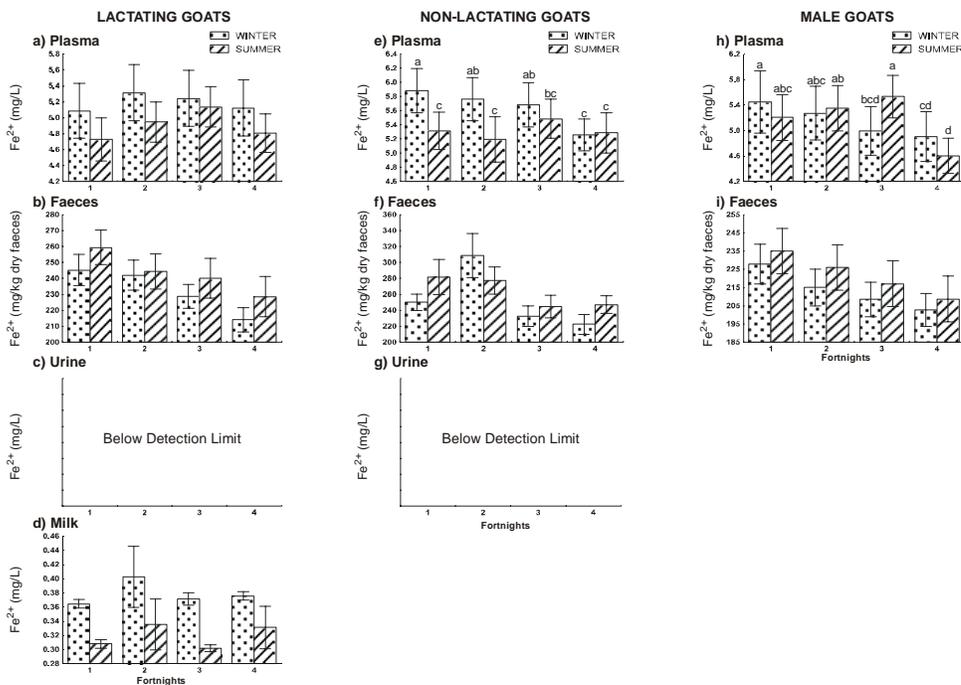


Fig.2: Fe^{2+} concentration in different sample types of lactating, non-lactating, and male goats at different fortnights during winter and summer seasons.

(Means with the same letters do not differ significantly at $P < 0.05$)

The Fe²⁺ absorption is more efficient when body store is low. The amount of Fe²⁺ absorbed is usually a small portion of that ingested, and many dietary factors influence the amount absorbed. The Fe²⁺ absorption is known to occur directly into blood (Thomas, 1970). These may be possible explanations of the adequate level of plasma Fe²⁺ in the present investigation.

The present study has shown that forage Fe²⁺ concentrations were higher in winter than those in summer, but were below the required value for ruminants' need in summer. Based on these findings there are no apparent seasonal or animal class differences in plasma and fecal Fe²⁺ levels, but only milk Fe²⁺ level reflected seasonal influence.

References

- Arizmendi-Maldonado, D., McDowell, L.R., Sinclair, T.R., Mislevy, P., Martin, F.G. and Wilkinson, N.S. Mineral concentrations in four tropical forages as affected by increasing day length. II. Microminerals. *Commun. Soil Sci. Plant Anal.*, 2002. 33:2001-2009.
- Becker, R.B., Henderson, J.R. and Leighty, R.B. Mineral malnutrition in cattle. *Bulletin*, 699-Fla. Agric. Exp. Stn., Gainesville. 1965.
- Duncan, D.B. Multiple range and multiple F-test. *Biometrics*, 1955. 11:1-42.
- Espinoza, J.E., McDowell, L.R., Wilkinson, N.S., Conrad, J.H. and Martin, F.G. Monthly variation of forage and soil minerals in Central Florida. II. Trace Minerals. *Commun. Soil Sci. Plant Anal.*, 1991. 22:1137-1149.
- Fick, K.R., McDowell, L.R., Miles, P.H., Wilkinson, N.S., Funk, J.D. and Conrad, J.H. Methods of mineral analysis for plant and animal tissues (2nd ed.). Dept. Anim. Sci., Univ. Florida, Gainesville. 1979.
- Grace, N.D. and Clark, R.G. Trace element requirements, diagnosis and prevention of deficiencies in sheep and cattle. In: *Physiological Aspects of Digestion and Metabolism in Ruminants*. (Tsuda, T., Sasaki, Y and Kawashima, R. eds.), Academic Press, Inc. London. pp. 321-134. 1991.
- Gupta, U.C. Characterization of the iron status in plants parts and its relation to soil pH and acid soils. *J. Plant Nutr.* 1992. 15:1531-1540.
- Humphries, W.R., Bremner, I., and Phillipps, M. The influence of dietary iron on copper metabolism in the Calf. In: *Trace Elements in Man and Animals-TEMA5* (Eds Mills. C.F., Bremner, I and Chester, J.K.), Farnham Royal, Commonwealth Bureaux, pp. 371-373. 1985.
- Judson, G.J. and McFarlane, J.D. Mineral disorders in grazing livestock and the usefulness of soil and plant analysis in the assessment of these disorders. *Aust. J. Exp. Agric.*, 1998. 38:707-723.
- Kabata-Pendias, A. and Pendias, H. *Trace Elements in Soils and Plants*. CRC Press Inc., Boca Raton, FL. 1992.
- Lindsay, W.L. Soil and plant relationships associated with iron deficiency with emphasis on nutrient interactions. *J. Plant Nutr.* 1984. 7:489-500.
- McDowell, L.R. Mineral deficiencies and toxicities and their effects on beef production in developing countries. In: *Proceedings on Beef Cattle Production in Developing Countries*, p. 216-241. Centre for Trop. Vet. Med. Univ. of Edinburgh, Edinburgh. 1976.
- McDowell, L.R. *Minerals in Animal and Human Nutrition*. Academic Press, San Diego, Calif. 1992.
- McDowell, L.R. Minerals for Grazing Ruminants in Tropical Regions. *Extension Bulletin, Anim. Sci. Dept. Centre for Trop. Agric., Univ. Florida*. pp-81. 1997.
- McDowell, L.R., Conrad, J.H. and Hembry, F.G. Mineral for Grazing Ruminants in Tropical Regions. Univ. of Florida, Gainesville. 1993.
- McDowell, L.R., Conrad, J.H. and Ellis, G.L. Mineral deficiencies and imbalances, and their diagnosis. In: *Symposium on Herbivore Nutrition in Sub-Tropics and Tropics-Problems and Prospects* (F.M.C. Gilchrist and R.I. Mackie, eds.), pp. 67-88. Craighall, South Africa. 1984.
- Merkel, R.C., McDowell, L.R., Popenoe, H.L. and Wilkinson, N.S. Mineral status comparisons between water buffalo and Charolais cattle in Florida. *Buffalo J.*, 1990. 6:33-41.
- Merkel, R.C., McDowell, L.R., Popenoe, H.L. and Wilkinson, N.S. Comparison of the mineral content of milk and calf serum from water buffalo and Charolais cattle. *Buffalo J.*, 1992. 8:9-15.
- Mooso, G.D. Warm Seasons Grass and Nutritive Uptake with liquid and solid N.P.K. Fertilizers. M.S. Thesis. Brigham Young Univ., Provo. UT. 1982.
- Mortvedt, J.J. Correcting iron deficiencies in annual and perennial plants: Present technologies and future prospects. *Plant soil*. 1991. 130:273-279.
- Morris, D.R., Loeppert, R.H. and Moore, T.J. Indigenous soil factors influencing iron chlorosis of soyabean in calcereous soils. *Soil Sci. Soc. Am. J.* 1990. 54:1329-1336.
- Mpofu, I.D.T., Ndlova, L.R. and Casey, N.H. The copper, cobalt, iron, selenium and zinc status of cattle in the Sanyati and Chinamhora small holder grazing area of Zimbabwe. *Asian-Aust. J. Anim. Sci.*, 1999. 12:579-584.

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- National Research Council. Nutrient Requirements of Domestic Animals. Nutrient Requirements of Beef Cattle (6th rev. ed.). Natl. Acad. Sci., Washington, D.C. 1984.
- National Research Council. Nutrient Requirements of Domestic Animals: Nutrient Requirement of sheep, 5th ed. Natl. Acad. Sci., Washington D.C. 1985.
- National Research Council. Nutrient Requirements of Domestic Animals: Nutrient Requirements of Dairy Cattle 6th ed. Natl. Acad. Sci., Washington D.C. 1989.
- National Research Council. Nutrient Requirements of Domestic Animals: Nutrient Requirements of Beef Cattle 7th ed. Natl. Acad. Sci., Washington D.C. 1996.
- Pamela, H.M., Wilkinson, N.S. and McDowell, L.R. Analysis of Minerals for Animal Nutrition Research. Dept. Anim. Sci., Univ. Florida. P-117. 2001.
- Pastrana, R., McDowell, L.R., Conrad, J.H. and Wilkinson, N.S. Mineral status of sheep in the Paramo region of Colombia. II. Trace minerals. Small Rumin. Res. 1991. 5:23-34.
- Prabowo, A., McDowell, L.R., Wilkinson, N.S., Wilcox, C. J. and Cornad, J. H. Mineral status of grazing cattle in South Sulawesi, Indonesia; I. Macrominerals. Am. J. Anim. Sci., 1990. 4:111-120.
- Rhue, R.D. and Kidder, G. Analytical procedures used by the IFAS extension soil laboratory and the interpretation of results. Soil Sci. Dept., Univ. Florida, Gainesville. 1983.
- Salih, Y., McDowell, L.R., Hentges, J.J., Mason Jr., R.M. and Wilcox, C.J. Mineral content of milk, colostrums, and serum as affected by physiological state and mineral supplementation. J. Dairy Sci., 1987. 70:608-612.
- Sanchez, P.A. Properties and management of soils in tropics. John Willey and Sons, NY). 1976.
- Santana, R.R. and McDowell, L.R. Yield *in vitro* digestibility, Crude protein, and mineral concentration of eight Digiteria introduction in Puerto Rico. Commun. Soil. Sci. Plant. Anal. 1994. 25:2019-2022.
- Steel, R.G.D. and Torrie, J.H. Principles and procedures of statistics. A Biometrical approach (2nd Ed.). McGraw Hill Book Co. New York. 1980.
- Tejada, R., McDowell, L.R., Martin, F.G. and Conrad, J.H. Evaluation of cattle trace mineral status in specific regions of Guatemala. Trop. Agric., 1987. 64:55-60
- Thomas, J.W. Metabolism of iron and manganese. J. Dairy Sci., 1970. 53:1107-1123.
- Underwood, E.J. The Mineral Nutrition of Livestock. Commonwealth Agricultural Bureaux, London. 1981.
- Vargas, D.R., McDowell, L.R., Conrad, J.H., Martin, F.G., Buergelt, C. and Ellis, G.L. The mineral status of cattle in Colombia as related to a wasting disease "secadera". Trop. Anim. Proc., 1984. 9:103-113.
- Velasquez-Pereira, J.B., McDowell, L.R., Conrad, J.H., Wilkinson, N.S. and Martin, F.G. Mineral status of soils, forages, and cattle in Nicaragua. I. Micro-minerals. Rev. Fac. Agron., 1997. 14:73-89.
- Viets, F.G. and Lindsay, W.L. Testing soil for zinc, copper, manganese and iron. In: Soil Testing and Plant Analysis. (L.M. Walsh and J. Beaton. eds.), pp. 153-172. Soil. Sci. Soc. Am. Inc., Madison, WI. 1973.
- Youssef, F.G., McDowell, L. R. and Brathwaite, R.A.I. The status of certain trace minerals and sulphur of some tropical grasses in Trinidad. Trop. Agric. 1999. 76:75-62.