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# SOME BIOCHEMICAL AND HAEMATOLOGICAL ALTERATIONS IN HYPOCUPREMIC EWES AND A TRIAL FOR TREATMENT

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## SUMMARY

A total number of forty two ewes (local breed) 2 - 5 years old , were selected from a flock contains 250 head , were used in this study . The animals grazed in free pasture at El-Tall El-Kebir region, Al-Ismailia Governorate . The pastures were improved by using azotic urea and lime super phosphate as fertilizers .After clinical and parasitological examination of the blood , faeces and skin , ewes were divided into two groups : (A) Healthy Control (20 ewes) and (B) Hypocupremic group manifested by alopecia and wool abnormalities (22 ewes) .

Blood and serum samples were collected for haematological and biochemical studies. Samples of wool , soil , drinking water and ration were analysed for major and minor elements .

Biochemical data revealed a significant decrease of serum copper,zinc,iron, magnesium, ceruloplasmin, total protein and albumin in addition to a significant increase of serum molybdenum and Beta globulins in group B . Moreover , a significant decrease in Hb content, R.B.Cs, PCV, MCHC and MCV was recorded in hypocupremic ewes. Furthermore, wool contents of copper, zinc, iron and manganese in affected ewes were significantly decreased .The general health condition of affected ewes and the a forementioned parameters regained their normal values within one month post treatment with cuperic glycinate subcutaneously beside supplementation of mineral blocks .

It is concluded that biochemical analysis of biological samples of sheep is a reliable tool for early diagnosis of some nutritional deficiency disorders and cuperic glycinate is an effective treatment for hypocupremic sheep.

## INTRODUCTION

Copper plays an important role in the processes of pigmentation, keratinization of hair and wool, bone formation, synthesis of myoglobin, reproduction, myelination of the spinal cord and iron mobilization in haemoglobin formation (Underwood , 1977 and John and Bauer , 1982) .

Copper deficiency has a direct effect on animal's immune competence and consequently incidence of infectious diseases (Cerone et al. 1998).Copper deficiency can occur when ration is inadequate in copper or contain excess amounts of interfering substances particularly sulphate , molybdenum , Zinc , Calcium and iron (Radostits et al. , 1995). Clinical findings associated with copper deficiency in sheep vary from location to another (Lewis , 1976) . These signs include wool abnormalities , alopecia and/or diarrhoea (Tanner et al., 1988) . Several studies referred to some haematological and serum biochemical changes in sheep suffering from copper deficiency (Metwalli et al., 1997 , El.Sangary , 1999 and Mohga 2000) . However a further studies are required to clarify the effect of hypocuprosis on different haematological and serum biochemical values in addition to levels of other trace elements. Hence, the present study was conducted to gain more information on some biochemical and haematological alterations in hypocupremic sheep . Moreover, evaluation of copper glycinate injection beside mineral blocks as therapeutic agents for control of the deficiency and also as a mean of diagnosis was intended .

## MATERIALS AND METHODS

### I - Animals :

A number of 42 ewes were selected from a flock containing 250 local breed of ewes (pregnant and non pregnant) .Their ages ranged from 2 to 5 years. Ewes grazed and watered in free pasture near El-Tall El-Kebir, Al-Ismaelia Governorate and stabled at night. The pasture has improved by application of azotic urea and lime superphosphate fertilizers . All ewes were tested by rose Bengal test for exclusion of brucellosis. After clinical and parasitological examination of the blood , faeces and skin , ewes were divided into two groups :

(A) Healthy control group (20 ewes).

(B) Hypocupremic group manifested by alopecia (22 ewes).

### II- Drugs:

A- Copperplan S®: an injectable solution, each ml contains 20 mg copper in the form of cuperic glcinate produced by Bomac Laboratories LTD. (New zealand).

B- Dextrose saline solution, El-Nasr Co. (Egypt).

C- Biomix 333: mineral blocks manufactured by Biochemical Co. (Egypt) and supplemented free choice.

**II- Sampling:**

A) Blood smears from the ear vein of all animals were stained with Gemesa and examined for excluding or detecting blood parasites (Kelly, 1984).

B) Blood samples were collected from the juglar vein into tubes containing anticoagulant (EDTA) for haematological studies and another samples without anticoagulant for clotting and separation of serum for biochemical investigation.

Determination of blood haemoglobin (Hb) content and total leucocytic count (W.B.Cs) were conducted according to Benjamine (1979) . Total erythrocytic Count (R.B.Cs) and packed cell volume (PCV) were estimated after Schalm et al. (1975 ) . Mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were calculated according to Wintrobe et al. (1974) .

Serum alkaline phosphatase (ALP), ceruloplasmin and Glutathioneperoxidase (GSH-Px) were determined according to Belfield and Goldberry (1971), Mancini et al., (1969) and Paynter et al.(1985) respectively . Serum total protein was estimated after Doumas et al.(1981) and protein electrophoresis was conducted as described by Davis (1964) and Orustein (1964). Determination of serum calcium, inorganic phosphorus and magnesium was carried out according to Ray-Sarkar and Chanhan (1976), Goldenberg (1966) and Gindler (1971) respectively. Estimation of serum copper, zinc (Meret and Henken, 1971) , iron (Allain and Mauros, 1979) , selenium (Little et al.,1979) , manganese , molybdenum and cobalt were also determined according to AOAC (1990).

C) Analysis of soil , ration and water for some macro and micro elements was conducted as described by Olson (1969) and AOAC (1990) using atomic absorption spectrophotometer .

D) Ashed wool samples (O' Mary et al. , 1969) were analysed for its content of copper , zinc , iron and manganese according to AOAC (1990) , before and one month post treatment.

**Treatment :**

All ewes suffered from alopecia due to nutritional deficiency of copper were treated with cuperic glycinate 40 mg for each ewe (about 50 Kg. B.Wt.) .

Ewes suffered from dehydration due to diarrhoea caused by copper deficiency were intravenously injected with Dextrose saline solution (about 250 - 500 cc.) . Moreover, mineral blocks were supplied for all animals ad libitum.

Blood and serum samples were investigated for all mentioned parameters before and one month post treatment.

Statistical analysis was conducted according to Snedecor and Cochran (1976).

## RESULTS

The present study revealed that ewes suffered from nutritional deficiency of copper showed signs of moderate to severe emaciation, depraved appetite, and persistent diarrdoea beside patches of alopecia.

Biochemical and haematological alterations were demonstrated in tables 1 , 2 and 3.

Highly significant ($P < 0.001$) decrease in the wool contents of copper, Zinc, iron and manganese were recorded in hypocupremic group (Table, 4). Table (5) showed the values of major and minor elements of the soil, water and ration.

Treatment of diseased ewes resulted in disappearance of clinical signs within one month and ewes regained its normal health condition.

Table (1): Mean values of some trace elements and minerals in serum of ewes sufered from hypocupremia before and after treatment.

| Parameter | Unit | Control Group | Hypocupremic gorup | |
|---|---|---|---|---|
| | | | Before treatment | After treatment |
| Copper | Ug/dl | 139.36 ± 3.31 | 116 ± 1.58*** | 132.20 ± 5.60 |
| Zinc | Ug/dl | 101.28 ± 1.96 | 92.71 ± 1.16*** | 96 ± 3.26 |
| Iron | Ug/dl | 120.78 ± 1.67 | 81.70 ± 0.96*** | 112.80 ± 4.15 |
| Cobalt | Ppm | 0.047 ± 0.006 | 0.040 ± 0.007 | 0.045 ± 0.005 |
| Selenium | Ppm | 0.31 ± 0.01 | 0.29 ± 0.01 | 0.31 ± 0.01 |
| Manganese | Ppm | 0.25 ± 0.01 | 0.25 ± 0.01 | 0.25 ± 0.01 |
| Molybdenum | Ppm | 0.77 ± 0.07 | 1.61 ± 0.05*** | 0.86 ± 0.08 |
| Calcium | mg/dl. | 9.74 ± 0.22 | 8.41 ± 1.06 | 9.05 ± 1.18 |
| Inorg. Phosphatous | mg/dl. | 4.47 ± 0.23 | 4.15 ± 0.21 | 4.25 ± 0.25 |
| Magnesium | mg/dl. | 2.52 ± 0.1 | 2.18 ± 0.08* | 2.34 ± 0.07 |

Mean ± S.E

* Significant at ($P < 0.05$)

*** Very highly significant at ($P < 0.001$)

- Note the significant decrease of serum copper, zinc, iron and manganese in ewes suffering from hypocupremia and manifested as alopecia.

Table (2): Mean values of some Alkaline phosphatase, Ceruloplasmin activities, total protein and electrophoretic pattern in hypocupremic ewes before and after treatment.

| Parameter | | Unit | Control Group | Hypocupremic gorup | |
|---|---|---|---|---|---|
| | | | | Before treatment | After treatment |
| Alkaline Phosphatase | | Iu/l. | 93.78 ± 2.82 | 97.81 ± 2.43 | 94 ± 6.20 |
| Ceruloplasmin | | mg/l | 165.10 ± 9.73 | 123.10 ± 2.33*** | 154.30 ± 8.10 |
| Total protein | | gm/dl | 6.87 ± 0.17 | 6.00 ± 0.15** | 6.59 ± 0.42 |
| Albumin | | % | 48.55 ± 0.36 | 40.10 ± 1.05** | 46.20 ± 4.10 |
| Globulins | α | % | 18.33 ± 0.53 | 18.52 ± 0.57 | 18.10 ± 0.42 |
| | β | % | 9.46 ± 0.64 | 15.68 ± 0.60*** | 10.80 ± 0.82 |
| | γ | % | 23.65 ± 0.52 | 25.70 ± 0.43 | 24.90 ± 1.06 |

Mean ± S.E

** Highly Significant at ($P < 0.01$)

*** Very highly significant at ($P < 0.001$)

- Note the significant decrease of serum Ceruloplasmin, total serum protein and albumin with the significant increase of β - globulins in hypocupremic ewes.

Table (3): Mean values of some haematological parameters in hypocupremic ewes.

| Parameter | Unit | Control Group | Hypocupremic gorup | |
|---|---|---|---|---|
| | | | Before treatment | After treatment |
| Hb | gm% | 10.12 ± 0.39 | 5.08 ± 0.64*** | 9.48 ± 60 |
| R.B. Cs. | $x10^6$/ cumm | 9.42 ± 0.31 | 6,81 ± 0.60*** | 8.93 ± 0.36 |
| W.B.Cs. | $x10^3$/ cumm | 4.94 ± 0.14 | 5.10 ± 0.48 | 4.89 ± 0.16 |
| PCV | % | 31.74 ± 0.73 | 19.22 ± 0.50*** | 29.97 ± 1.02 |
| MCHC | % | 31.84 ± 0.55 | 25.21 ± 0.45*** | 31.65 ± 0.56 |
| MCV | fl. | 33.39 ± 1.40 | 28.21 ± 0.71* | 33.56 ± 1.03 |

Mean ± S.E

* Significant at ($P < 0.05$)

*** Very highly significant at ($P < 0.001$)

- Note the significant decrease in haemoglobin content, total erythrocytic count, haematocrit value, mean corpuscular haemoglobin concentration and mean corpuscular volume in hypocupremic ewes.

Table (4): Mean values of some trace elements in the wool of hypocupremic ewes before and after treatment.

| Parameter (ppm) | Control Group | Hypocupremic gorup | |
|---|---|---|---|
| | | Before treatment | After treatment |
| Copper | 4.02 ± 0.320 | 2.70 ± 0.151*** | 3.80 ± 0.190 |
| Zinc | 88.34 ± 1.965 | 76.62 ± 2.006*** | 82.40 ± 2.210 |
| Iron | 48.16 ± 1.400 | 37.38 ± 0.873*** | 45.09 ± 1.890 |
| Manganese | 7.50 ± 0.312 | 6.03 ± 0.077*** | 7.12 ± 4.20 |

Mean ± S.E

*** Very highly significant at ($P < 0.001$)

- Note the significant decrease in the wool contents of copper, zinc, iron and mangaese of hypocupremic ewes.

Table (5): Values of major elements and minor elements of the soil, water and ration (ppm).

| Parameter (ppm) | Soil | Water | Raion |
|---|---|---|---|
| Calcium | 591.790 | 47.990 | 66.45 |
| Phosphorus | 298.10 | 10.470 | 20.36 |
| Magnesium | 40.600 | 28.800 | 41.20 |
| Copper | 55.150 | 0.400 | 11.11 |
| Zinc | 107.840 | 16.380 | 45.68 |
| Iron | 89.092 | 0.025 | 59.64 |
| Cobalt | 7.970 | 0.007 | 00.89 |
| Selenium | 0.110 | 0.090 | 00.10 |
| Manganese | 18.450 | 0.050 | 09.10 |
| Molybdenum | 3.490 | 0.280 | 18.00 |

## DISCUSSION

The obtained data revealed a very highly significant ($P < 0.001$) decrease of serum copper , zinc and iron accompanied by an increase of serum molybdenum in ewes suffered from clinical signs in the form of pale mucous membranes , depigmentation or discolouration of the black and brown wool which had a steely appearance, patches of alopecia and diarrhoea . The decrease of serum copper level in this study was in harmony with the recorded clinical signs that aroused suspecion of copper deficiency in affected ewes . The pale colour of the mucous membranes of diseased ewes was a reflection of anaemia induced by copper and iron deficiencies which are necessary for haemoglobin formation (Radostits et al., 1995) .

As copper is essential for the synthesis of tyrosinase enzyme which is involved in the conversion of tyrosine to melanine , depigmentation of the wool was noticed in copper deficient ewes (Underwood, 1977). Moreover , decrease of lysyl oxidase activities due to copper deficiency resulted in adverse effect on the formation of collagen and elastin in the skin (Mills et al., 1976) . The decrease of serum copper level in this study may be attributed to the recorded significant ($P < 0.001$) increase of serum molybdenum level (Dick et al., 1975). The authors referred to the interference of high molybdenum level with copper retention in the body . Lime top dressing raises the soil pH and increase the availability of molybdenum and application of nitrogenous fertilizers raises molybdenum absorption by plants relulting in reduction of pasture copper content (Cunningham, 1950). These suggestions were confirmed by the present finding where pastures on which ewes grazed were fertilized by using lime phosphate and azotic urea fertilizers.

On the other hand, ration rich in calcium may reduce the accumulation of copper in sheep tissues (Dick, 1954). Supporting this concept, the obtained data indicated that total ration content of calcium exceeded the adequate requirement for sheep as reported by Robert (1983) . In addition , as the soil in the present study is sandy and calcareous , therefore , it is expected to be rich in calcium salts which interfere with availability of zinc absorption in plants (Miller, 1967) . Therefore a significant ($P < 0.001$) decrease in serum zinc level of ewes was recorded due to nutritional deficiency as confirmed by analysis of the ration .

The reduction of serum iron level in this study may be attributed to the role of copper deficiency, that previously demonstrated , in impairment of iron transfer to the plasma from reticulo - endothelial system in the liver (Ismail et al., 1988).

Moreover , Standish et al., (1971) observed that excess of calcium in the ration may impair the assimilation of iron. A highly significant ($P < 0.001$) decrease in total protein and Albumin

beside increase of Beta globulins were noticed in hypocupraemic ewes in this study . Similar results were recorded by Metwalli et al. (1997) and Nasser et al., (2000). The latter authors referred to hypoalbuminaemia in animals showing alopecia due to inadequate protein synthesis . In addition , El-Sangary (1999) recorded a highly significant increase in total serum globulins in ewes suffered from hypocupremia and this result was coincided with the present finding in this study .

Ceruloplasmin is the principal cupro- protein in plasma , so it is a good indicator for copper deficiency (Suttle , 1986) . The coupled deficiency of Cerulopalsmin activity and copper which was proved in this study is considered as an important finding in hypocupraemic ewes and offers a reliable tool for detection of copper deficiency (Todd, 1970 and Ismail et al., 1988). Moreover , depressed Ceruloplasmin activity may indicate excess of molybdenum supplement as postulated by Suttle and Angus (1976) .

Biochemical analysis of wool is a useful tool for reliable diagnosis of trace elements deficiencies in ewes (Fahmy et al., 1980 and Essa ,1987) . The present work revealed a very highly significant ($P < 0.001$) decrease in wool contents of copper , zinc , iron and manganese of alopecia affected group . These results were in agreement with the results obtained by the forementioned authors .

Haematological studies revealed that hypocupraemic ewes had a very highly significant ($P < 0.001$) decrease in the mean values of haemoglobin content , total erythrocytic count , packed cell volume and mean corpuscular haemoglobin concentration beside significant ($P < 0.05$) decrease of mean corpuscular volume . Similar results were recorded by Whitelaw et al. (1982) and El-sayed (2000) .The decrease in haemoglobin concentration and packed cell volume may be due to deficiency of copper and iron which had been proved in serum biochemical analysis . Radostits et al., (1995) attributed the occurrence of anaemia in hypocupraemic sheep to the role of copper in the production of haemoglobin through the re-utilization of iron liberated from normal breakdown of haemoglobin .

Treatment of the primary causes of trace elements deficiency in this work by supplying these elements through the oral route using blocks of mineral mixture and parenteral administration of cuperic glycinate had been proved to be an effective tool for restoring serum trace elements levels in treated ewes . Butler (1963) recorded a marked rises in blood and plasma copper levels toward the normal range after subcutaneous injection of 45 mg of copper glycinate . In addition , Hemingway et al. (1970) mentioned that a single injection of 40 - 50 mg cuperic glycinate in ewes at mid pregnancy is effective in preventing sway back disease in lambs and maintains satisfactory blood and liver copper levels in ewes and lambs .

Based on the obtained data, it could be concluded that biochemical analysis of biological samples of sheep , is a reliable tool for early diagnosis of some nutritional deficiency disorders . Furthermore , the relation between the levels of soil contents from major and minor elements and that of plant must be put in consideration . Moreover , cuperic glycinate injection was an effective treatment for the control of copper deficiency in sheep , specially that graze on improved pastures.

## REFERENCES


Allain P. and Mauros V. (1979) : Microdetermination of lead , Cadmium , Copper , Zinc and Iron in blood and urine by graphite furnace atomic absorption spectrophotometer . Clin. Chem . Acta , 91 : 41

Association of Official Analytic Chemists (AOAC) , (1990) : Official Methods of analysis ( 12 th Ed. ) Washington D.C.

Belfield A. and Goldberry D.M. (1971) : A colorimetric determination of alkaline Phosphatase activity . Clin. Chem. , 12 : 561 .

Benjamine M.M. (1979) : Outline of veterinary clinical pathology. 3 rd Ed. Iowa University Press Ames . Iowa , U.S.A .

Butler , E.J. (1963) : Factors influencing the blood and plasma copper levels of sheep in sway back flocks. J. Comp. Path. , 73 : 107 - 118.

Cerone , S.I. ; Sansinanea A. S ; Strettern-berger S.A. ; Garcia M.C. and Auza , N.J. (1998) : The effect of copper deficiency on the peripheral blood cells of cattle . Vet. Res. Communications ( 1998 ) 22 (1) 47 -57

Cunningham , I. J. (1950) : Symposium on copper metabolism ( W.D. Mc Elory and B.Glass Eds.) ,P 246. Johnes Hopkins press , Baltimore , Maryland , U.S.A.

Davis B. J. (1964) : Disc electrophoresis . II- method and application to human serum proteins . Ann. N.Y. Acad. Sci., 121 : 404 .

Dick A.T. (1954) : Studies on the assimilation and storage of copper in cross breed sheep . Aust. J. Agric. Res., 5 : 511 .

Dick A.T. ; DW. Dewey , and J.M. Gawthorne (1975) : An investigation into copper deficiency in young lambs on an improved hill pasture . J. Agric. Sci. Camb., 85: 567.

Doumas , B.T. ; Bayso, D.D.; Carter , R.J. leters , T. and Schaffer R. (1981) : Determination of serum total protein . Clin. Chem. 27 : 1642 .

El-Sayed S.A.K. (2000): Micro-elements status of sheep with special reference to some nutritional deficiency problems and its treatment . M.V. Sc Thesis , Fac. Vet. Med. Zagazig University .

El-Sangary F.H.M (1999) : Studies on causes , diagnosis , biochemical changes and treatment of unthriftiness in sheep . Ph.D. Thesis Fac. Vet. Med. Zagazig University.

Eassa , M. Aziza (1987) : Wool and Hair as an effective tool for diagnosis of some deficiency diseases and environmental pollutions. Ph.D. Thesis of Vet. Med. And clinical diagnosis . Fac. Vet. Med. Zagazig University .

Fahmy , F.A. ; A.A. Amer ; H.M. Abdel-Aziz , and M. abdel-Raoof (1980) : Wool as an effective tool for diagnosis of some deficiency diseases . Assiut. Vet. Med. J., 7: 265 .

Gindler E. (1971) : Colormitric method for the determination of magnesium Clin. Chem. 17 : 662 .

Goldenberg H. (1966) : Determination of inorganic phosphorus Clin. Chem. , 12 : 871 .

Hemingway R.G.; Macpherson A. and Ritchie N.S. (1970) : In : Proc. 1st Int. Symp. Trace elements metabolism in animals : 264 .

Ismail , M. ; el-Magawry , S.; Ibrahim , I.A. and El-Ghanam, A.A. (1988) : Some biochemical alterations in unthrifty sheep suffering from copper deficiency. Assuit Vet. Sci. , 10 : 303 - 305 .

John D., and M.D. Bauer (1982) : Clinical laboratory methods . 9th Ed. ., 516

Kelly , W.R. (1984) : Veterinary clinical diagnosis.3rd Ed. Printed in great Britain by William Clawes Limited , Beccles and London .

Lewis, G. (1976) : C.D.A copper in farming symposium (Cited after Abed-All,1983) : Haematological and some trace elements variations in sheep blood under the influence of some physiological and pathological factors . Ph.D. thesis , Vet. Med., Assiut University.

Little , W.; Vogg, M.J. Collis, K.A. and Gleed , P.T. (1979) : Concentration of selenium by atomic absorption , Res. Vet. Sci. , 26 : 193 - 197.

Mancini , G. ; Carbonara, A. O. and Hermans , J.F. (1969) : Immunochemical quantitation of antigens by singal immunodiffusion Immunochemistry ; 2 : 235 - 254 .

Meret S. and Henken R.I. (1971) : Simultaneous direct estimation by atomic absorption spectrohpotometrey of copper and zinc in serum, urine and C.S.F. Clin. Chem., 17 (5) : 367 - 373 .

Metwalli , A.M. ; Nasser , M.H. and El-Gharib , S.A. (1997): An investigation on some clinico-biochemical changes in Hypocopperemic sheep with trials for treatment . J. Egypt . Vet. Med. Ass. 57 , No. 1 : 495 - 508 .

Miller J.K. (1967) : J. Nutr. , 93 : 386 . Cited In Roy J.H (1980) : Studies in the agricultural and food science (The Calf ) , 4th Ed., Butterworths , London - Boston .

Mills C.F. ; Dalagrano A.C. and Wnham ,G. (1976) : Biochemical and Pathological changes in tissues of friesian cattle during experimental induction of copper deficiency . Br. J. Nutr., 35 : 309 - 335 .

Mohga , S.Z. Abdel-Razik (2000) : An investigation on clinical and some biochemical alterations in unthrifty sheep suffering from copper deficiency and therapy . Zag. Vet. J. 28 (3) : 115 - 125.

Nasser , M.H.; Nassif , M. ; Nasr , M.Y. and Naima , A. Afifi (2000) : Some biochemical alterations associating achromotrichia and alopecia in buffaloe calves with a trial of treatment .J. Egypt Vet. Med. Ass. 60 (3): 115-122

Olson O.E. (1969) : Fluorometric analysis of selenium in plants . J. Assoc . Official Anal . Chem. 52 : 622 .

O'Mary , C.C. ; M.C. Bell ; N.N. Sneed and W.T. Butts (1969) : Effects of irradiation , age , seasons , and colour on mineral composition of Herford Cattle hair . J. Anim. Sci., 28 : 268 .

Orustein L.C. (1964) : Disc electrophoresis. I-backgroup and theory. Ann. N.Y. Acad. Sci. 121 : 321 .

Paynter , D.I ; Halpin , C.G. Caple , I.W. (1985) : Measurement of blood glutathione peroxidase activity for assessment of selenium nutrition in livestock . In Australian Agricultural council : Standing Committee on agricultural , Aust. J. Agric. Res. , 30 : 307 - 309 .

Radostits , O.M. ; Blood , D.C. and Gay, CC. (1995) : Veterinary Medicine 8th Ed. Bailliere Tindal , London .

Ray-Sarkar B.C. and Chanhan U.P (1967): A new method of determination micro quantities of Calcium in biological materials. Annal. Biochem., 20 : 155 .

Robert P. (1983) : Mineral levels in animal health. Published by Sherpa International Clearbook , British Columbia , V2T 4 x 2 , Canada .

Schalm O.W.; Jain, N.C. and Carrol , L. (1975) : Veterinary haematology . 3rd Ed. Lea and Fibiger, Philadephia , U.S.A.

Snedecor , G.W. and Cochran , W.G. (1976) : Statistical methods. 6th Ed., Iowa State . AMES , Iowa , U.S.A.

Standish , J.F. ; C.B. Ammerman ; A.Z. Palmer and C.F. Simpson (1971) : Influence of graded levels of dietary iron , as ferrous sulphate , on performance and tissue mineral composition of steers J.Anim.Sci., 29 : 496.

Suttle , N.F. ( 1986 ): Copper deficiency in ruminants , recent developments. Vet. Record , 119 (21) : 519 ñ 522

Suttle , N.F. And Angus, K.W. (1976) : Experimental copper deficiency in the calf. J.Comp. Pathol., 86 (4) : 595 - 608 .

Tanner, D.Q. ; Stendnic , J.D. and leininger , W.C. (1988) : Mineral herd sample size for determination of blood copper status of cattle . J. Amer. Vet. Med. Associ. , 192 (8) : 1074 - 1076 .

Todd , J.R. (1970) : A survey of the copper status of cattle using copper oxidase (Ceruloplasmin) activity of blood serum . Trace elements metabolism in animals . Edinburgh , Livingstone , P. 448 .

Underwood , E.J. (1977): Trace elements in human and animal nutrition . 4th Ed. Academic Press , New York , San Francisco and London .

Whitelaw A., Fawcett A.R. and Mac Donald A.J. (1982) : Cupric oxide needless in the prevention of sway back . Vet. Record, 104 : 8 - 11 .

Wintrobe , M.M. ; Lee, G.R. ; Boggs, D.R. ; Bithell, T.C. ; Athens , J.W. and forester, J. (1974) : Clinical haematology . Seventh Ed. Indian Ed. K.M. Varghese Company , Bombay .