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Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

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Manganese Deficiency in Bovines: Connection Between Manganese Metalloenzyme Dependent in Gestation and Congenital Defects in Newborn Calves

Paulo Reis de Carvalho[1], Maria Carolina Gonçalves Pita[2], José Eduardo Loureiro[3], Helena Reiko Tanaka[3] and José Carlos Soares Ribeiro[3]
[1]APTA-Secretary of Agriculture and Food Supply of São Paulo State, Av. Rodrigues Alves, 40-40, Horto Florestal, CEP: 17030-000, Bauru, São Paulo, Brazil
[2]University of Guarulhos, Course of Veterinary Medicine, Av. Anton Philips, 01, Vila Hermínia, Guarulhos, São Paulo, Brazil
[3]Coordination of Agricultural Livestock Defense, Secretary of Agriculture and Food Supply of São Paulo State, Brazil

Abstract: In order to determine the relationship between diet low in mineral manganese and birth of calves with bone and joint abnormalities of all complexion in the skeleton develop congenital ataxia offspring, study was conducted in nine regions of the center western of São Paulo State. Were determined the mineral composition of forage plants used for beef cattle in nine regions between the years 1984-1992 and clinical observations of animals were carried out to date in the of year 2009. The forage *Brachiaria decumbens* used in cutting farm was studied during the four seasons of the year writing the collection of sampling the twelve months of the year. We used a completely randomized design with three replicates per season and each of the nine municipalities studied, resulting in the total sample of 108 samples of forage grasses to evaluate the behavior of the macrominerals: Ca, P and Mg and trace elements Cu, Zn, Fe, Mn and Co from the analysis by atomic absorption spectrophotometry and P by molecular absorption of forage available for grazing cattle during the seasons spring, summer, autumn and winter. Still, we sampled animal tissue (liver) and soil to determine the mineral profile. The variation in averages were: Macro 0.36 g to 0.40 g% Ca, 0.05 g to 0.10 g% P, 0.14 g to 0.20 g% Mg. Microminerals: 30 ppm to 40 ppm Zn, 3 ppm to 8 ppm Cu, 0.06 ppm to 0.09 ppm Co and 40 ppm to 80 ppm Mn. Adjusting the requirements supplementation, through dry matter, all small macro: Ca, P and Mg and trace elements Cu, Co and Zn must also be supplemented second animal growth, reproduction and lactation. Trace minerals concentration in liver varied: 152.38 ppm Cu, 219.67 ppm Zn, 6.36 ppm Mn, 260.18 ppm Fe and 0.132 ppm Co. The results found here suggested that the matrix of epiphiseal growth plate cartilage was affected during embryogenesis by manganese deficiency in the diet of the animals causing reproductive malformation and birth of calves with congenital defects in the skeletal tissues manly and articulate.

Key words: Congenital disorder, teratogenic defects, malformation, joint bony abnormality, bovine

INTRODUCTION

Early workers used the adjective "trace" for those elements present in such small amounts in living tissues that they could not be measured with the methods available (Anonymous, 1969). Thus, the term "trace element" was born. Deficiency of an essential trace element results in a characteristic deficiency syndrome in a manner analogous to a specific vitamin or hormone deficiency. The deficiency syndrome is associated with specific structural, functional, biochemical, or physiological abnormalities. These abnormalities, in turn, are prevented or reversed after administration of the deficient element (Mcdowell, 1985, 1992; Hurley and Keen, 1987).

Manganese (Mn) is known for its strong causal relationship with birth congenital and teratogenic defects in animal species rabbits, guinea pigs, pigs, cattle, mice and rats (Hurley *et al*., 1958; Huan-Chang and Everson, 1967; Hurley, 1968, 1976a,b). Experimentally, researchers have promoted the birth of fetuses with abnormalities seen since the internal organs and external joints and bone structure that was extremely affected by Mn deficiency. The defect linked mainly to manganese deficiency in the diet of the parents and teratogenic changes in the structure of DNA or RNA molecule binding Mn has been diagnosed in many animal species newborn (Erway *et al*., 1970; Erway *et al*., 1971; Shrader *et al*., 1973).

Corresponding Author: Paulo Reis de Carvalho, APTA-Secretary of Agriculture and Food Supply of São Paulo State. Av. Rodrigues Alves, 40-40, Horto Florestal, CEP: 17030-000, Bauru, São Paulo, Brazil

Has been possible to prove a link between the deficiency of Mn in the conception and gestation with birth of calves, mice, rabbits, mice, guinea pigs and pigs with congenital ataxia. Lawrence *et al.* (1971) studied strain of mice predisposed to pale birth of progeny with neonatal ataxia prevent the birth of newborns ataxia Mn supplementation to the mothers' diets. However, the relationship between teratogenicity and deficiency of trace elements in specific disability manganese is attributed to the effect of Mn metaloenzyme linking DNA and RNA polymerase for protein synthesis and amino sugars that will form structures mucopolysaccharides, key components of tissue chondrocyte of supply for the calcification and bone therefore stretching the bones of animals (Bell and Hurley, 1973; Shrader *et al.*, 1973; Song and Hunt, 1988). The growth plate shows flattened and there may be abnormalities in the formation of joint tissues connective (cartilage and tendons) and calcification of bone tissue can result in stunting and ataxia. If anomaly on DNA is irreversible will then be installed on the teratogenic effect of Mn on the tissues in the following generations (Hurley, 1976a,b).

The only now-proven manganese metalloenzyme is pyruvate carboxylase (Baly *et al.*, 1985a; Baly *et al.*, 1985b). Various metal ions are exchanged at the active site of enzymes and because manganese may function as an enzyme activator it is probable that it, too, interchanges with other divalent elements and consequently a manganese deficiency may be associated with other trace-element effects. These effects include increased or decreased concentrations of an element or actual exchange of the element with manganese in an enzyme. This possibility has not been thoroughly investigated. Manganese seems to be intimately involved in synthesis of protein, DNA and RNA. The DNA-manganese complex was first reported by Wiberg and Neuman (1957). They concluded from its dissociation constant that manganese binds to DNA more strongly than do other metals. Since minute quantities of manganese were detected during the isolation of RNA and DNA, it was suggested that manganese may bear a functional relationship to protein synthesis and the transmission of genetic information.

Manganese and other cations stabilize the secondary structure of DNA by their electrostatic interaction with the negatively charged phosphate group. Extensive studies relating conformation and reactivity to DNA interaction with manganese ion have recently been reported (Luck and Zimmer, 1972). The results of these investigations indicate that manganese has an important role in initiating protein synthesis by stimulating RNA polymerase and DNA polymerase activities in the mammalian system (Wiberg and Neuman, 1957; Song and Hunt, 1988).

In kwashiorkor, there was a definite correlation between decreased hepatic manganese content and decreased hepatic protein content in the protein-calorie malnutrition (Scrutton *et al.*, 1966). Thus, manganese is a specific nutrient that affects expression of the mutant gene without altering subsequent transmission of the mutation to future generations of mice (Erway *et al.*, 1971).

Effects of large proportions of the animal organism to a particular trace element such as manganese which at first seems so insignificant, often goes unnoticed in its importance on fetal development and after birth. Teratogenic effects due to disorders in the metabolism of Mn may be linked to the synthesis of RNA or DNA (Dyer and Rojas, 1965; Hurley *et al.*, 1963; Hurley, 1969; Hurley, 1976a; Hurley, 1976b; Petukhova, 1971; Hurley, 1981a; Hurley, 1981b; Shrader *et al.*, 1973; De Rosa *et al.*, 1980; Leipold *et al.*, 1983; Hurley *et al.*, 1982; Hidiroglou *et al.*, 1990; Cave *et al.*, 2008).

In the syndrome deficiency of Mn, the congenital chondrodystrophy neonatal calves, attributed to a maternal deficiency manganese. The epiphyses growth plate chondrocytes has improperly aligned and short columns and reducing the width of the zone of hypertrophy when compared to a normal calf. Examinations of numerous calves were performed and pathology focused on the musculoskeletal and joint system (Leach, 1968; Leach and Muenster, 1962; Leach *et al.*, 1969).

The manganese with atomic mass equal to 55, transition metal, in animal tissue actively participates in the formation and activation of enzymes in the body system. As co-factor in catalize enzyme or strongly linked to enzymes metalloenzymes. Enzymes avimanganin, 1 Mn atom, pyruvate carboxylase, 4 atoms of Mn and superoxidismutase, 2 atoms of Mn /mole of protein and the first two liver bird. The Mn catalyzes the formation of structures mucopolysaccharides allow the connection between carbohydrates and amino acids to form complex structures responsible for the functionality of any type of tissue where there is the composition including amino sugars noble fabrics feature in the annexes of the joints and terminal ends of the joint members in the sensory cells responsible for auditory perception present in the mastoid of the hearing aid and a host of other functional cells present in the animal organism. Chondroitin sulfate is the most affected mucopolysaccharide in Mn deficiency (Leach, 1969; Howes and Dyer, 1971). The chondrogenesis rather than osteogenesis is affected by the dysfunction of the synthesis of mucopolysaccharides.

According to Underwood (1976, 1977), the mineral in number of twenty-two elements representados por macrominerals: calcium, phosphorus, sulphur, potassium, sodium, chlorine, magnesium and trace minerals: iron, iodine, copper, manganese, zinc, cobalt, molybdenum, selenium, chromium, tin, vanadium, fluorine, silicon, nickel and arsenic are essential animal

life. Dietary imbalances of many of these elements and their interactions with other minerals are described and their functions and requirements by farm animals are outlined. For some species is included arsenic, boron, silicon and vanadium, have been essential to one or more but there is no evidence that these minerals are of practical importance in beef cattle. The function, signs of deficiency, factors affecting requirements, sources and toxicity of each essential mineral and the many interrelationships between minerals determine complex responses in the animal organism (Underwood, 1983). Mineral nutrients are either positively charged (cations) or negatively charged (anions). Cations are derived from metals, including calcium, cobalt, chromium, copper, iron, magnesium, manganese, molybdenum (as molybdate), potassium, selenium (as selenate), sodium and zinc. Non-metallic elements yield anions: iodine as iodide; sulfur as sulfate; phosphorus as phosphate; chlorine as chloride and fluorine as fluoride. Combinations of anions and cations yield salts such as sodium chloride, calcium phosphate and sodium iodide. The cation manganese is mineral nutrients with positively electric charge (Underwood and Mertz, 1987). Such “borderline” deficiencies are both the most costly and the most difficult to manage, often go unnoticed and unrectified, and yet they may result in poor and expensive gains, impaired reproduction, or depressed production (Conrad *et al*., 1985; Underwood, 1981).

The NRC requirements often do not take into account that in disease, certain minerals are needed at higher-than-recommended levels for response. Minerals play a major role in the immune response, the body's defense system against infectious disease, mineral supplementation above requirements is required for optimal immune responses (Mcdowell, 1994). Mg, P, Na, Cl, Zn, Cu, Fe and Se have been shown to improve an animal's ability to cope with infections. Metabolic interactions among the mineral elements influenced by stress, interactions between minerals and productive stage can determine changes in the minimum requirement of minerals (Underwood, 1966).

Manganese functions as a component of the metalloenzymes pyruvate carboxylase, arginase and superoxide dismutase and as an metal activator for a number of enzymes (Hurley and Keen, 1987). Enzymes activated by manganese include a number of hydrolases, kinases, transferases and decarboxylases. Of the many enzymes that can be activated by manganese, only the glycosyltransferases are known to specifically require manganese (Scrutton *et al*., 1966).

The general pattern of the deformities observed in the manganese deficient guinea pig at birth suggested a defect in cartilagenous tissue and stimulated an interest in the relationship of manganese deficiency to disorders of the tissues of mesenchymal origin hexosamine content occurred primarily in the galactosamine fraction (Huan-Chang and Everson, 1967; Shrader and Everson, 1967; Hurley *et al*., 1958; Hurley and Everson, 1959).

There is a shortening of the long bones, enlargement and malformations of the joints and deviations in the shape of the skull. A defective development of the rib cage is also observed with anterior posterior flattening of the chest. The composition of the ground substance was therefore investigated, beginning with the Acidic Mucopolysaccharides (AMPS) present. The principal compounds in the AMPS group which have been identified in connective tissue are: hyaluronic acid; chondroitin 4-sulfate (chondroitin sulfate A); chondroitin 6-sulfate (chondroitin sulfate C); dermatan sulfate (chondroitin sulfate B) and heparin (Hurley and Everson, 1963; Hurley *et al*., 1960; Hurley, 1967).

At one moment of conception or birth defects in which there is deficiency of Mn can be impaired chondrogenesis in the formation of the fetus. As a result, experimental animals, rats, mice and tested experimental calves under natural breeding, born with various deformities and some are clinically visible, and were specifically described by Dyer *et al*. (1964) in calves because they have curves or joints twisted inward members and or forelegs twisted and rear pasterns in permanent flexion. Slight enlargement of knees and twisted rear legs also has been observed. Rojas *et al*. (1965) observed enlarged joints, stiffness, twisted legs and a general physical weakness were observed in calves from cows fed the low manganese rations. Deficiencies marginal or borderline deficiencies that may go unnoticed because they are asymptomatic.

Dyer and Rojas (1965) and Rojas *et al*. (1965) reported a positive relationship between a low manganese intake of gestating cows and the incidence of neonatal deformities in their calves. The manganese content of different bovine tissues (including liver, kidney, gonads, and blood) was determined. Leg deformities with "overknuckling" and dwarfism in calves, infertility and frequent abortions in cows grazing manganese-depleted pastures were reported for Rojas *et al*. (1965).

Manganese is essential to all known living organisms; it activates numerous enzyme systems including those involved with glucose metabolism, energy production and superoxide dismutase; it is a major constituent of several metalloenzymes, hormones and proteins of animals and humans (Shrader and Everson, 1967).

Manganese is part of the developmental process and the structure of the fragile ear bones and joint cartilage. Deficiency diseases of Mn are very striking ranging from severe birth defects (congenital ataxia, deafness, chondrodystrophy), asthma, convulsions, retarded growth, skeletal defects, disruption of fat and carbohydrate metabolism to joint problems in new born Leach, Muenster and Wien (1969), Leach (1969) and Leach and Nesheim (1972).

Overall Mn deficiency, animals may have diseases such defects of chondroitin sulfate metabolism with poor cartilage formation, shortened long bones, chondrodystrophy, congenital ataxia, slipped tendon, deafness for malformation of otolithes, asthma, chondromalacia, convulsions, infertility for failure to ovulate or testicular atrophy, stillbirths or spontaneous abortions, loss of libido in males and females, glucose intolerance and retarded growth rate (Leach and Muenster, 1962).

Manganese is an essential trace mineral nutrient. Manganese is needed for normal brain and muscle function, building bones, blood clotting, cholesterol synthesis, fat synthesis and DNA and RNA synthesis. Manganese activates the enzyme responsible for the formation of urea, the waste product of protein degradation. In carbohydrate metabolism manganese is required for the synthesis of glucose from non-carbohydrate substances (gluconeogenesis). Manganese assists the action of superoxide dismutase, which degrades superoxide, a free radical and a highly damaging form of oxygen. In addition, manganese is required to synthesize components of mucopolysaccharides (glycosaminoglycans), components of connective tissue (Hurley, 1968). A manganese-dependent enzyme of the brain synthesizes the amino acid, glutamine, as a way of removing ammonia, a toxic product of nitrogen metabolism. Conditions possibly associated with manganese deficiency include osteoporosis, rheumatoid arthritis, lupus erythrematosis, allergies, alcoholism and diabetes (Burch *et al*., 1975).

Biochemical function, manganese is both an activator, and a constituent of several enzymes. Those activated by manganese are numerous and include hydrolases, kinases, decarboxylases and transferases, but most of these enzymes can also be activated by other metals, especially magnesium. This does not apply, however, to the activation of glycosyltransferases or possibly to that of xylosyltransferase. Manganese metalloenzymes include arginase, pyruvate carboxylase, glutamine synthetase and manganese superoxide dismutase (Shrader *et al*., 1973).

The enzymatic activity is the main function of the manganese binding proteins-metalloenzymes -, or activating enzymes. Like other essential trace elements, Mn can function both as an enzyme activator and as a constituent of metalloenzymes. Manganese-containing enzymes include arginase, pyruvate carboxylase and Mn-superoxide dismutase. While the number of Mn metalloenzymes is limited, the enzymes that can be activated by Mn are numerous. They include hydrolases, kinases, decarboxylases, and transferases (Groppel and Anke, 1971). Whether an activator or a component of the enzyme proper, Mn is often the priority cation, but another cation, especially Magnesium (Mg), can partially substitute for Mn with little or no loss in enzymatic activity. Thus, biotin-dependent enzymes such as pyruvate carboxylase continue to fix CO, during Mn deficiency because Mg substitutes for Mn in the enzyme.

Bone growth, Mn-deficient bones are considerably shortened and thickened. Manganese is essential for development of the organic matrix of the bone, which is composed, largely of mucopolysaccharide. Impairment in mucopolysaccharide synthesis associated with Mn deficiency has been related to the activation of glycosyltransferases (Leach, 1971). These enzymes are important to polysaccharide and glycoprotein synthesis, and Mn is usually the most effective of the metal ions required.

Effects on reproduction were among the first signs of Mn deficiency to be observed. The deficiency can cause an irreversible congenital defect in young calves, chicks, rats and guinea pigs characterized by ataxia and loss of equilibrium. Shils and McCollum (1943) found several stages of Mn deficiency in female rodents: (1) birth of viable young with ataxia; (2) nonviable young that die shortly after birth and (3) disturbance of estrus, with no reproduction. Impaired or irregular estrus has also been observed in cattle and swine. Hidiroglou (1975), on the basis of Mn tissue-distribution studies of the reproductive tract of normal and anestrus ewes, has suggested that Mn has a role in corpus luteum functioning. In laying hens, Mn deficiency has resulted in a decreased rate of egg production, poor shell quality, reduced hatchability and an embryonic deficiency called chondrodystrophy. Testicular degeneration has been reported in Mn-deficient rats, mice and rabbits (Leach and Lilburn, 1978).

The main manifestations of manganese deficiency include a high neonatal death rate, impaired growth, abnormal skeletal development, congenital ataxia, disturbed or depressed reproductive function and defects in lipid and in carbohydrate metabolism. Many of these gross manifestations of manganese deficiency are now believed to be due to a defect in the synthesis of mucopolysaccharides. Although available information on manganese deficiency in man is limited, these findings suggest that manganese may play a role as one potential factor in the development of intrauterine malformations.

Lipid metabolism, association between Mn and choline has been known for some time. Fatty liver in rats induced by Mn deficiency is alleviated by either Mn or choline. Also, Mn deficiency increases fat deposition and backfat thickness in pigs. Both Mn and choline are needed for prevention of perosis in poultry. Manganese is involved in the biosynthesis of choline. Furthermore, the changes in liver ultrastructure that arise in choline deficiency are very similar to those in Mn deficiency (Bruni and Hegsted, 1970). Deficiencies of Mn and choline both appear to affect membrane integrity.

Manganese also has a role in cholesterol biogenesis (Davis *et al*., 1990). We may postulate a number of ways in which manganese may play a role in lipid and lipoprotein metabolism, which may be ultimately related to the development of atherosclerosis. Furthermore, manganese, by being a cofactor of MnSOD, protects membranes from free radical formation and preserves the integrity of its lipid components. Glycoproteins are integral components of the arterial extracellular matrix and play an important role in maintaining structural integrity and normal function of the arterial wall including regulating permeability and retention of plasma components, controlling vascular cell growth and interacting with lipoproteins. Manganese, as a specific activator of glycosyltransferases, may also affect glycosylation of glycoproteins on cell membranes including receptors. This would alter receptor composition and structural properties and affect lipoprotein binding and their ultimate metabolic fate (Hurley, 1981a,b).

Manganese may also affect lipoprotein composition and metabolism by its role in stabilizing lipoprotein structure due to its high affinity in complexing with the polar heads of lipoprotein phospholipids and amino acid residues. Furthermore, manganese may modify intramolecular interaction of the lipoprotein particle with its receptor by bridging the anionic groups of cell membrane glycosaminoglycans with certain amino acid residues and phospholipids on the surface of the lipoprotein. Finally, manganese may play a crucial role in the glycosylation of plasma apolipoproteins in the liver Golgi apparatus by specifically activating glycosyl transferases and manganese deficiency may result in abnormal lipoprotein formation and impairment of lipoprotein secretion from the liver, thus resulting in fatty liver formation observed in many studies (Stock and Latshaw, 1981; Hurley *et al*., 1982; Hurley, 1976a,b).

Carbohydrate metabolism, glucose utilization is impaired by Mn deficiency. Necropsy has revealed gross abnormalities in the pancreas such as aplasia or marked hypoplasia of all cellular components, so Mn may in some way be involved in insulin formation or activity (Leach, 1967). Rats deficient in Mn had fewer insulin receptors per cell compared to controls (Baly *et al*., 1988). Biosynthesis of glycoproteins may be impaired in Mn-deficient animals. Prothrombin is a glycoprotein whose synthesis has long been known to be controlled by vitamin K. Manganese is also required, and a Mn deficiency reduces the vitamin K-induced clotting response (Doisey, 1974).

Manganese deficiency in experimental animals results in a diabetic-like glucose intolerance. This may result in part from alterations in processes comprising glucose homeostasis including pancreatic insulin synthesis, secretion and degradation, as well as peripheral insulin action on target tissues. Interestingly, diabetes itself may result in marked changes in manganese metabolism. Abnormalities in cell function and ultrastructure, particularly involving the mitochondria, occur in Mn deficiency (Hurley and Keen, 1987). Manganese deficiency caused alterations in cell membrane integrity in the liver, pancreas, kidney, and heart in aged mice (Bell and Hurley, 1973). Chondrodystrophy and dwarfism is abnormality is characterized by a disproportionate shortening of the long bones.

MATERIALS AND METHODS

To study the seasonal behavior of the forage in *Brachiaria decumbens* used for grazing cattle in the center-west of the state were randomly selected nine counties represented by Arealva, Duartina, Bauru, Lucianopólis, Cabralia Paulista, Avaí, Piratininga and Ubirajara. Research project to rural sampling were performed at the Pathology Laboratory Animal Unit of Research and Development of Bauru. The mineral analysis were carried out in the Section of Metabolic and Deficiency Diseases of Instituto Biologico of São Paulo. Samples of the grass *Brachiaria decumbens* were harvested within twelve months of the year to analyze the macrominerals: Ca, P and Mg and microminerals: Cu, Zn, Co, Fe, Mn (AOAC, 1990). The Dry Matter (DM) of the standardization sample was obtained after drying in the oven at 65°C for 24 h with standardization of DM in 90%. After grinding in Wiley mill type knife with stainless steel and free from contamination by minerals, the samples were transferred to Erlenmeyer flask and subjected to acid digestion in a hot plate with the aid of digestor wrapped inside chapel of exhaust gases. The eleven liver samples after crushing and treated with solvents to extract the fat has been kiln dried and mineralized by acid hot digestion. The extract obtained by wet digestion was transferred to volumetric flask and measured the volume with distilled and demineralized water obtained in deionized and distiller apparatus, both with internal circuit entirely of glass. Then the samples were injected into the atomic absorption spectrophotometer Varian® mark and properly calibrated with standards in reading and hollow cathode lamp specifies the mineral to be analyzed. O Phosphorus (P) was read by molecular absorption spectrophotometry. The reading obtained for the mineral in question was applied the formula to calculate the concentration according to the volume used in the dilution of concentrated extract obtained. The farms with animals with birth defects were registered and systematically analyzed forage, total diet and animal tissue of the newborn for the minerals studied. Were selected nine regions with the birth of animals with deformities in joint and musculoskeletal to sampling of soil, forage and total ration in the farms with cows pregnant and lactating. They were accompanied the birth and calves born with deformities were registered and monitored in their development. Animals born with

deformities and problems worsened during growth were sacrificed and preceded the macroscopic evaluations and mineral analysis of hepatic tissue.

Statistical analysis: A completely randomized design were used for sampling 108 the total samples taken in nine municipalities and four distinct seasons, namely: spring, summer, autumn and winter to determine the three main macro: Ca, Mg and P and five trace minerals: Cu, Zn, Co, Fe and Mn in grass *B. decumbens* in the region studied. Were applied the model of analysis of variance for difference of mean seasons and regions for each mineral analyzed and ingredients to rations by SAS software (SAS, 1994). The Tukey test to compare means at 5% level of significance was used.

RESULTS

The figures above illustrate the use of diet deficient Mn progenitors during conception or pregnancy can lead to end the birth of progeny of calves with teratogenic defects.

The levels of trace manganese in grazing *B.decumbens* showed high standard deviations and of coefficients of variation for the mean that ranged widely according to the times of the seasons of autumn, winter, spring and summer. However, the highest mean of 89.15 mg of Mn (winter) did not differ ($p>0.05$) the lowest average equal to 79.41 mg Mn (winter), 81 mg Mn (autumn) and 83.48 mg Mn/kg. In contrast, the lowest average (winter) had the highest coefficient of variation - 30.78% - and the largest deviation from the average - 20.01 - (Table 1).

The levels of trace manganese in animal tissue, dry liver newborn calves, ranged from 4 ppm to 8 ppm, with an average 6 ppm and a standard deviation 1.79 (Table 2).

The final composition of the diet for cows' feed consisted of forage base of napier grass (*Pennisetum purpureum*) and sugar cane (*Saccharum officinarum*) crushed a mean equal to 36.67 ppm Mn (consumption, sd±2.31). The animals consumed an average of 7.50 kilograms DM/day (sd±1.31). After receiving the roughage in the time between 7-10 am, the animals were managed for grazing *B. decumbens* where they remained until the following day. The average was 56 ppm Mn in the pasture and 36.67 ppm Mn in the final diet of the animals (Table 3). After weaning, calves, cows and bulls, were transferred to pasture, where were offered in troughs sodium chloride additive of limestone. No other mineral supplement was offered to the reproduction herd.

A group of newborn calves were monitored during the first months of development and another group, after autopsy, was used for the macroscopic and microscopic observations. We observed numerous skeletal and joint deformities in newborn calves (Fig. 1-8). Many newborn animals had permanent flexion of the forelimbs, angular deformities and limb shortening. With the growth and weight gain in some animals showed lack of support at the tip of the hull of the front and support on the tarsus articulation radius. Macroscopically, in addition to joint deformities, the legs showed marked shortening of the bones of the forelimbs, curvature and thickening.

DISCUSSION

The birth of calves with severe skeletal deformities, musculoskeletal and knuckle joint in this research are similar in physical conditions equal those described by Dyer *et al.* (1964) in studies conducted in the area Texana the United States and by and in subsequent studies by Dyer and Rojas (1965) and Rojas *et al.* (1965) on manganese deficiency in the etiology of deformed calves. In subsequent research the authors mentioned above reported that after careful study of soil, forage and total ration, levels of 15.8 ppm Mn in the total diet, were increased to 25 ppm in the diet of cows, corrected the problem of the birth of calves with abnormalities. The deficiency Mn in this study, the authors above were associated with the birth of calves with birth defects, confirming the clinical suspicion of nutrients that are in inadequate amounts in the diet deficient in Mn.

The final composition of the diet of the animals showed insufficient levels of the nutrient mineral manganese (Table 3). Although the data of leaf analysis in *B. decumbens* reveal average levels adequate Mn, it is unbalanced or deficient for the other nutrients for the daily needs of cattle grazing. Still, it was observed that all nutrients including high Mn showed high coefficients of variation and standard deviation for the different seasons (Table 1). According Underwood (1983, 1977) in mineral nutrition is a common occurrence of interaction between different nutrients in the gastrointestinal tract and often antagonistic interrelations may occur in the animal organism and result in worsening of the deficiencies of certain trace element that can determine framework pathognomonic of a particular nutrient deficiency.

In this study were observed that the mean of 36 mg Mn/kg DM - "bordeline" deficiency - showed up near the levels of Mn indicated in the NRC (1996) of 40 ppm for adult cattle. However, in some specific conditions such as dependence on the true availability of the element in the diet, several authors considered that the requirements can reach up to 70 ppm Mn (Bentley and Phillips, 1951; Rojas *et al.*, 1965; Grace, 1983; NRC, 1996). In these research should also take into account that the properties that had calves born with birth defects coincidentally registered the addition of significant amounts of Ca as limestone of the total ration of dairy cattle. Breeding bulls and cows in gestation and lactation were above 4 g Ca/kg DM in the total ration added directly to the basal diet of the animals.

Table 1: Means of the mineral content in the pasture *Brachiaria decumbens* by season of year in the regions studied

Variables[1]	Ca (g%)	P (g%)	Mg (g%)	Cu (ppm)	Zn (ppm)	Mn (ppm)	Fe (ppm)	Co (ppm)
Autumn								
µ	0.36[A]	0.070[A]	0.15[A]	4.11[A]	41.18[A]	81.00[A]	132.89[A]	0.096[A]
sd	0.03	0.013	0.02	0.39	7.73	11.94	16.63	0.006
cv	20.51	42.90	21.09	21.42	30.96	23.25	26.95	14.37
Winter								
µ	0.37[A]	0.077[A]	0.15[A]	4.11[A]	42.93[A]	89.15[A]	143.15[A]	0.101[A]
sd	0.05	0.024	0.02	0.65	8.75	20.01	37.04	0.004
cv	20.47	44.07	20.54	28.89	32.28	32.67	47.92	10.65
Spring								
µ	0.39[A]	0.090[A]	0.16[A]	4.78[A]	45.15[A]	79.41[A]	144.00[A]	0.090[a]
sd	0.03	0.020	0.02	0.74	6.06	13.92	29.06	0.006
cv	23.91	47.55	18.00	27.89	22.20	30.78	35.45	13.28
Summer								
µ	0.36[A]	0.080[A]	0.16[A]	4.93[A]	48.82[A]	83.48[A]	129.67[A]	0.084[A]
sd	0.03	0.010	0.01	1.05	7.82	12.54	19.18	0.005
cv	18.24	44.96	18.52	29.75	24.08	26.34	28.13	10.84

*Means in the column with the same superscript denote no significant (p<0,05) difference.

[1]µ = means of the analysis of three months of collection of forage in grazing beef cattle in the regions studied; sd: standart deviation; cv: coefficient of variation

Table 2: Concentrations of Mn and other minerals in the animal tissue of newborn calves with defects in farms of various regions

	Dry matter of liver (mg/kg)							
Region	Ca	P	Mg	Cu	Zn	Mn	Fe	Co
Arealva	160	8000	400	42	140	4	102	0.150
Avaí	280	10500	200	65	365	4	148	0.150
Bauru	400	9000	400	187	160	8	272	0.150
Bauru	800	7000	600	8.20	90	6	246	0.090
Duartina	600	8600	200	12	94	6	340	0.100
Cab Paulista	700	11000	600	335	125	8	232	0.095
Iacanga	120	9000	200	75	200	8	22	0.165
Iacanga	250	11000	200	272	114	8	640	0.130
Lucianopólis	800	6000	200	290	600	4	117	0.090
Piratininga	800	6000	200	290	600	4	128	0.115
Ubirajara	120	11000	106	70	78	6	172	0.170
µ	457.27	8827.27	300.55	149.65	233.27	6.00	219.91	0.128
dp	287.27	1925.66	172.52	126.39	197.86	1.79	165.53	0.031
	Reference value in the liver[1]							
	200	12000	600	100-400[2]	100	10-14	150.00	0.150
	Mineral analysis of soil[3]							
Soil	-	-	-	0.30-0.50	0.70-1.30	41-70	112-125	-

[1,2]Underwood (1969) and NRC (1984); [3]pH = 4.2

Table 3: Composition of the ration of the herd bull and cow in milk production on farms with calves with birth teratogenic malformations

Ingredients in ration	Consumption DM (kg/animal/day)	Mn (ppm/kg DM)	Ca (g/animal/day)	Ca (g/kg DM)
Pennisetum purpureum	4.50	11±6.25		
Saccharum officinarum	2.00	6±2.52		
Pasture[1]	1.00	56±9.51		
	Consumption DM (total/kg/day)	Mn (µ)* (ppm in DM)		
µ±sd	7.50±1.31	36.67±2.31		
Limestone (38% Ca)			30.40-38.00	4.05-5.07
Sodium chloride			45	

*µ = means of Mn in the DM (mg x kg^{-1}); 1- estimated value of consumption of 1 kg DM/day

The additional excess calcium in the diet in some circumstances can intervene with the absorption of trace elements in the small intestine. Absorption of manganese from ^{54}MnCl in lactating dairy cows was less than 1% (Van Bruwaene *et al*., 1984) and little is known concerning dietary factors that may influence manganese absorption. Some evidence suggests that high dietary calcium and phosphorus may increase manganese requirements (Hawkins *et al*., 1955; Dyer *et al*., 1964; Lassiter *et al*., 1972; Alfaro *et al*., 1988). Biliary excretion of manganese plays an important role in manganese homeostasis but little excretion of manganese occurs by the route of urine (Hidiroglou, 1979). From the clinical point of view is a well

Fig. 1: Calf of a month with three supporting members after birth deformities in the joints of the limbs and stiffness and twisted legs of the left foreleg

Fig. 2: Calf of Figure 1 after sacrificed and necropsy showed bone deformities of the skeleton and joints of the forelimbs and hindlimbs

known fact over the table pathognomonic of osteopathy and many other references in the literature since the scientific publications of Hurley *et al.* (1963), Dyer and Rojas (1965) and Hurley (1981a,b) with proven experimental studies in guinea pigs, rats and mice.

The results of this study were similar to those found in the vast literature diponível to deficiency of the trace element manganese which were analyzed retrospectively to the results of research and the NRCs published in different years and which are reported

Fig. 3: Calf in Figure 1 after showing deformities of the feet, permanent flexion and limb shortening of bone of the left foreleg

Fig. 4: Calf of the one month with deformities of the forelimbs and hindlimbs by manganese deficiency

below. Thus, the NRC (1984) mentions requirements Mn of 40 ppm in animal reproduction and the levels in the diet should be increased to 40-70 ppm in stressed animals. Agreeing with these values reported the increased need for 70 ppm and recommended 100-150 ppm referring to the Mn deficiency is to be installed when the soil pH is above 6.0 (Groenewald, 1960). The study of soils can provide indications of possible deficiencies. As the pH increases, the availability and use by plants of iron, manganese, zinc, copper and cobalt decreases, while concentrations of phosphorus, calcium, magnesium, molybdenum and selenium rise (Howes *et al.*, 1973; McDowell and Conrad, 1977).

Fig. 5: Observation of the posterior calf of months with joint deformities grossly of the forelimbs radius carpus metacarpus joint and hindlimbs tibio tarsusmetatarsus articulation enlargement in the manganese deficiency

Fig. 6: Observation of the anterior calf of months with joint deformities of the forelimbs radius carpus metacarpus joint and hindlimbs tibio metatarsus articulation thickening in the manganese deficiency showing the difficulty of balancing the locomotor apparatus

Analysis of forage is limited by the difficulty of representative samples of animal diet, the possibility of contamination of soil and lack of quantity consumed by the animal.

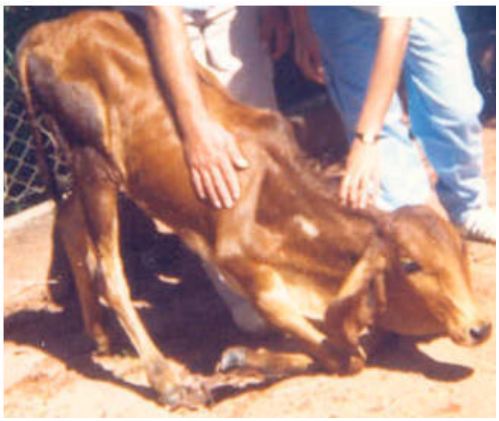

Fig. 7: Persistent flexed forelegs twisted in calf two months age with no support on foreleg and support on the radius carpus metacarpus joint (enlargement of knees) by manganese deficiency

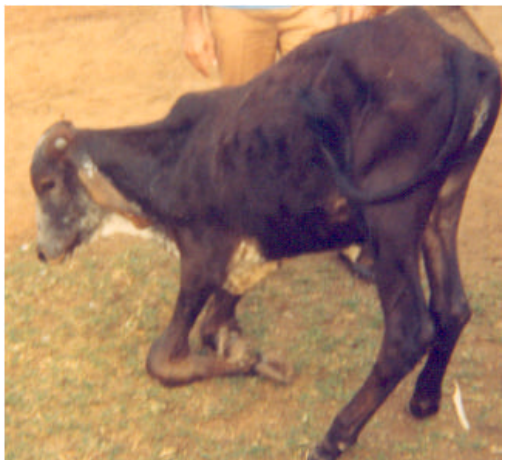

Fig. 8: Angulation deformity of the forelimbs of calf three months of age with lack of support from former members and support on the radius carpus metacarpus joint (enlargement of knees) in the manganese deficiency

The analysis of the concentrations of minerals in tissues may ultimately provide an indication of the environment as a whole and the state (s) element (s) deficient (s) in the diet. However, it is desirable to confirm the diagnosis of mineral deficiency by adequate supplementation of mineral or minerals found deficient in the diet, aiming to observe the response in animal health or performance. The main sources of supplemental are: manganese carbonate: 47.80%, manganese chloride: 27.76%; manganese sulfate: 32.50% and manganese oxide: 77.45% of Mn.

The manganese requirement for growing and finishing cattle is approximately 20 mg Mn/kg diet. Skeletal abnormalities were noted in calves from cows fed diets containing 15.8 mg Mn/kg but were not present when diets were supplemented to contain 25 mg Mn/kg (Rojas *et al*., 1965). The quantity of manganese needed for maximum growth is less than that required for normal skeletal development. Manganese requirements for reproduction are higher than for growth and skeletal development, and the recommended concentration for breeding cattle is 40 mg/kg. Cows fed a diet containing 15.8 mg Mn/kg had lower conception rates than cows fed 25 mg Mn/kg (Rojas *et al*., 1965; Howes *et al*., 1969; Howes and Dyer, 1971). Heifers fed 10 mg Mn/kg exhibited impaired reproduction (delayed cycling and reduced conception rate) compared to those fed 30 mg Mn/kg, but growth was similar for the two groups (Bentley and Phillips, 1951).

The manganese has a direct effect on reproduction, influencing conception rate (Hidiroglou, 1975; Hidiroglou *et al*., 1978). Experimentally, DiCostanzo *et al*. (1986) with the addition of 14 mg Mn/kg on a diet of corn silage containing 32 mg Mn/kg, reduced conception services ratio from 1.6 to 1.1.

Deformations of the skeleton similar to those found in this research (Fig. 1-8) were mentioned in various considerations addressed by several authors in the literature. The calves born to mothers with diet containing manganese bordeline deficiency showed pathognomonic signs of Mn deficiency equal to guinea pig, mice, pig and rat.

In this study, calves born with skeletal abnormalities showed low Mn content in the liver (Table 2 and Fig. 1-8). These results agreed with com Rojas *et al*. (1965) when determined experimentally that mothers receiving 21 ppm manganese born calves content of 11.8 ppm Mn in the liver versus low 6.60 ppm (low) Mn or 7.28 ppm Mn. Cave *et al*. (2008) reported congenital chondrody-strophy in forty-seven holstein calves with a dwarf-like appearance, born in South East Australia. They pointed to manganese deficiency during fetal development as the most likely cause against the evidence prognosis.

Deficiency of an essential trace element results in a characteristic deficiency syndrome in a manner analogous to a specific vitamin or hormone deficiency. The deficiency syndrome is associated with specific structural, functional, biochemical, or physiological abnormalities. These abnormalities, in turn, are prevented or reversed after administration of the deficient element (Leach and Gay, 1987).

The evidence of skeletal abnormalities in calves found in this study and in several studies mentioned in the literature shown to have biochemical and genetic mechanisms in common which are described below. Thus, Hurley and Keen (1987) after numerous experimental researchs were convinced, that manganese functions as a component of the enzymes pyruvate carboxylase, arginase and superoxide dismutase and as an activator for a number of enzymes. Enzymes activated by manganese include a number of hydrolases, kinases, transferases and decarboxylases. Of the many enzymes that can be activated by manganese, only the glycosyltransferases are known to specifically require manganese.

Manganese seems to be intimately involved in synthesis of protein, DNA, and RNA. The DNA-manganese complex was first reported by Wiberg and Neuman (1957). These authors concluded from its dissociation constant that manganese binds to DNA more strongly than do other metals. Since minute quantities of manganese were detected during the isolation of RNA and DNA, it was suggested that manganese may bear a functional relationship to protein synthesis and the transmission of genetic information. Studies with zinc induced Sever and Emanuel (1973) to believe in the connection between the metal effect on DNA and RNA and birth defect in humans similar to that described for Mn. Mammalian cells exhibit two types of RNA polymerase activity, one of which requires manganese. Manganese also affects the DNA polymerase system. The RNA-dependent DNA polymerase activities in human placenta and rat liver nuclei are stimulated predominantly by manganese. Manganese and other cations stabilize the secondary structure of DNA by their electrostatic interaction with the negatively charged phosphate group. Extensive studies relating conformation and reactivity to DNA interaction with manganese ion have recently been reported (Luck and Zimmer, 1972). The results of these investigations indicate that manganese has an important role in initiating protein synthesis by stimulating RNA polymerase and DNA polymerase activities in the mammalian system. A study comparing the effect of manganese to that of other ions showed a slight but significant increase in protein biosynthesis, attributable to manganese, in isolated rat liver nuclei. In kwashiorkor, there was a definite correlation between decreased hepatic manganese content and decreased hepatic protein content in the protein-calorie malnutrition (Underwood, 1981).

Manganese is necessary for optimal growth in mice, rats, and other species. Swine, guinea pigs, and calves do not show impaired growth with manganese deficiency (Asling and Hurley, 1963).

Perhaps the most remarkable discovery was made by Erway *et al*. (1966), who demonstrated that a manganese dietary supplement fed to pregnant mutant mice who develop congenital ataxia would result in ataxia-free offspring. This study demonstrated for the first time that supplementation with an essential nutrient, manganese, can prevent development of a genetically predetermined phenotype. The mutation had not been abolished, because offspring of the ataxia-free mice

developed ataxia. Ataxia in mice, whether attributable to the mutant gene "pallid" or to a maternal dietary manganese deficiency, is identical; however, a high manganese concentration (1 mg) in the diet of mutant mice during pregnancy completely prevented the congenital defect and altered the mutant expression without changing the genetic constitution (Hurley, 1968). Thus, manganese is a specific nutrient that affects expression of the mutant gene without altering subsequent transmission of the mutation to future generations of mice according Erway *et al.* (1971). These offspring exhibited shortening of the radius, ulna, tibia and fibula from birth to maturity. Results similar to this research can be found in several experimental studies developed by Leach (1967, 1968, 1969, 1971), Leach and Muenster (1962), Leach *et al.* (1969), Leach and Nesheim (1972), Leach and Liburn (1978), Leach and Gay (1987) and Leach and Harris (1997).

Thereby, these researchers mentioned that perosis, or "slipped tendon," may also occur in manganese-deficient chicks. It is characterized by enlargement of the hocks, short and twisted tibiae and slipping of the gastrocnemius tendon from its condyles. The discovery that perosis is related to changes in the mucopolysaccharide content of the epiphyseal cartilage focused attention on the chemical composition of the organic matrix of cartilage and bone (Leach and Muenster, 1962). The Mn deficiency can leads to abnormalities in glucose utilization with hypoglycemic and gluconeogenesis effect.

The decrease in chondroitin sulfate in manganese deficiency is the result of impaired mucopolysaccharide synthesis, manganese being a necessary cofactor for the enzymes involved in chondroitin sulfate synthesis. The above studies have demonstrated that manganese affects the primary sites of chondroitin sulfate synthesis. Because the chondroitin sulfate-protein complex is necessary to maintain the rigidity of connective tissue, these findings provide a biochemical explanation for the skeletal abnormalities observed in manganese deficiency. The association of manganese deficiency in experimental animals and the bony abnormalities were find resulting from the inhibition of mucopolysaccharides synthesis extremely fascinating in relation to the mucopolysaccharidoses occurring in humans. In humans, these diseases are characterized by bony abnormalities, mental retardation, and accumulation of tissue mucopolysaccharides. Even if manganese deficiency or an abnormality of manganese metabolism is not subsequently shown to be involved in the human mucopolysaccharidoses, the manganese-deficient animal seems to be an ideal model for the elucidation of the biosynthetic pathways involved in mucopolysaccharide synthesis, pathways that are ill-defined and poorly understood at present (Shrader *et al.*, 1973; Hurley and Keen, 1987).

About teratogenicity, studies confirming manganese as an essential nutrient were reported simultaneously by several investigators Orent and McCollum (1931). Animals on manganese-deficient diets develop numerous biological and physical symptoms, such as decreased manganese concentrations in tissues and milk, suboptimal growth, decreased testicular and ovarian function, accumulation of fat and diabetic-like glucosetolerance curves (Everson and Shrader, 1968). Moreover, the risk of intoxication by Mn this is of 25 times or around (NRC, 1980) the maximum tolerable concentration of manganese was set at 1.000 mg/kg, at least on a short-term basis (Jenkins and Hidiroglou, 1991).

Manganese is needed for the glycosyltransferases that function in the synthesis of the glycosaminoglycan side chains of the proteoglycan (Leach *et al.*, 1969). Other nutrient deficiencies such as deficiencies in choline, niacin, zinc, biotin and folic acid also resultin skeletal deformities grossly similar to manganese deficiency. Although these deficiencies result in a narrow growth plate, they apparently do not share a common mode of action with manganese. The alterations in cartilage proteoglycans are specific for manganese deficiency and are not observed with other deficiencies (Leach, 1969; Stock and Latshaw, 1981).

Manganese deficiency, this research, affect the reproductive performance of animals (or delay cycling estrus, silent estrus and reduced rates of conception), bone deformities and contractures (shortening) of tendons in newborn calves, enlargement of joints and reduced birth weight. There is some evidence that excessively high calcium in the diet predisposes to manganese deficiency. The positive interaction of manganese and choline has been identified in preventing of the "fat cow syndrome".

Thus manganese deficiency can cause reproductive failure, it is recommended to provide the supplemental manganese at levels recommended for the animals. The analysis of the chemical composition of the diet will determine the levels of manganese in the diet. The requeriment for cow and calf is above 20 ppm (70 ppm for stressed calves) and 40 ppm, respectively, and maximum tolerable of 1000 ppm for the two categories (Table 4).

The concentration of manganese in forages varies greatly depending on plant species, soil pH and soil drainage (Minson, 1990). This element is not usually be needed in mineral mixtures of the savannas, where the concentrations are high. Analysis of native pastures and cultivated in this region has always shown concentrations above the maximum level of demand for cattle (Underwood, 1981).

Thus, the above authors, just as this research, showed that experimental diets for calves born of dams receiving 15.8 ppm Mn had forelegs, knees, twisted inward,

Table 4: Mineral requirements and maximum tolerable concentrations

		Requeriment			
			Cows		Maximum tolerable
Mineral	Unit	Growing and finishing cattle	Gestating	Early lactation	concentration
Manganese	mg/kg	20	40	40	1000

NRC (1996)

forelegs twisted and rear pasterns in permanent flexion, twisted forelegs, weak, slight enlargement of knees versus calves born normal of mothers given a diet of 25,1 ppm of Mn. In this study, cows fed diet deficient in Mn (Table 1-3) originated calves with deformities of the limbs, twisting of the legs, standing flexion of the forelimb and hindlimb deformities (Fig. 1-8), similar to those described for animals born in the research Dyer *et al*. (1964).

Likewise, in studies of Dyer *et al*. (1964) the liver manganese content of the control calf was 11.84 ppm compared with 6.94 ppm for two deficient calves, show that the cow's requirement is manganese in excess of 16 ppm rather than 6-10 ppm as suggested by NRC (1966). The requirement is probably about 20 ppm, since all calves from cows on the ration containing 15.8 and 16.9 ppm manganese were deformed. Agreeing with the above authors, this study, averaging 6 ppm in liver of newborn calves and media ranging from 4-8 reflected low levels of Mn in the diet of cows in all seasons of the year (Table 2).

Howes and Dyer (1971) utilized Mn^{54} marked and found that the newborn calf preferentially stores manganese in the liver. With 13 ppm Mn in the diet of mothers, calves had 4.48 ppm in the liver and 6.36 ppm to the seventh day versus 7.96 (the birth) and 9.43 (seventh day) ppm in the liver of calves from mothers with diet of 21 ppm Mn.

Supplementing the newborn calf with manganese resulted increase in liver manganese concentrations when compared to calves given no supplemental manganese. Authors also found that calves responded directly to the administration of Mn. Thus, addition of 1.5% Ca plus manganes in milk from calves raised the level in the liver to 942.67 (with 14 ppm Mn in milk) and 410.87 ppm (21 ppm Mn in milk) Mn in the liver of calves to the seventh day.

Hansen *et al*. (2006) observed signs of Mn deficiency in calves born to heifers fed 16.6 mg of Mn/kg of DM: disproportionate dwarfism (three in each seven), unsteadiness/weakness at birth (three in each seven) and superior brachygnathism (five in each seven). The heifers supplemented with 50 ppm Mn, the calves were born normal ($p \leq 0,03$).

The most recent beef cattle NRC (1996) recommends 40 mg of Mn/kg of DM for reproducing beef cattle. Recently, Weiss and Socha (2005) estimated, based on Mn intake and fecal excretion, that dry and lactating dairy cows, respectively, require approximately 2.7 and 1.6 times more Mn for their maintenance requirements than values calculated using the current dairy cattle NRC model. The present study was designed to observe the effects of long term low-Mn diets on gestating heifers and their offspring. As an essential trace element, Mn plays a crucial role in several enzymes in the body, such as glycosyltransferases. Glycosyltransferases are a group of enzymes that are involved in the metabolism of cartilage proteoglycans, affecting the biosynthesis of glycosaminoglycan and oligosaccharide sidewere randomly selected at the conclusion of the previous side chains (Leach and Harris, 1997). The role of Mn in cartilage formation makes it essential to the formation of the epiphyseal growth plate, which directly affects longitudinal bone growth. Thus, the most frequently observed sign of Mn deficiency in young animals is skeletal malformation (Leach and Muenster, 1962). This signs may indicate that low dietary Mn decreased glycosyltransferase activity.

Huan-Chang and Everson (1967) administered experimentally purified diet containing less than 3 ppm Mn. Defects in biosynthesis of the organic matrix of cartilage during fetal development were observed. When the adult female were fed with less than 3 ppm of Mn throughout gestation there was a significant reduction in the concentration of all Acid Mucopolysaccharides (AMPS) tested in rib and epiphyseal cartilage. The mixture of chondroitin sulfates which makes up the major constituent of the total AMPS present was significantly reduced, with chondroitin 4-sulfate and chondroitin 6-sulfate being affected about equally in epiphyseal cartilage.

According to Leach (1971) and Leach and Harris (1997) the main compounds in the AMPS group which have been identified in connective tissue are: hyaluronic acid; chondroitin 4-sulfate (chondroitin sulfate A); chondroitin 6- sulfate (chondroitin sulfate C); dermatan sulfate (chondroitin sulfate B) and heparin. The precursors of these AMPS compounds are mainly hexosamine and hexuronic acid which have been studied in manganese-deficient poultry.

Rib cartilage and epiphyseal cartilage of mice born to mothers with Mn deficient diet: hyaluronic acid, chondroitin sulfate, chondroitin sulfate A e C (ribs cartilage) and heparin to normal guinea pigs and Mn deficient diet.

The data suggest that manganese is involved in a more general step in metabolism essential for all AMPS. The skeletal abnormalities observed in the manganese-

deficient guinea pig at birth are believed to be related to flaws in the metabolism of cartilage matrix.

Compared to the results of this research, several authors working with other species experimentally reported that the consequences of deficiencies of Mn were also reasons of extensive research conducted by Shrader and Everson (1967) using guinea pigs as an experimental model. Analyzed 13 puppies born of 25 guinea pigs in reproduction were subjected to the control diet with normal levels of Mn and 14 puppies from mothers with Mn deficient diet. We observed a high incidence of postural defects, types of otolith abnormalities and of deformities of the semicircular canals and ampullae. Postural defects were observed in none of the control animals. Of the unsupplemented deficient animals, 60% had observable postural abnormalities, whereas 71% of the deficient animals that had received postnatal supplementation with manganese showed head tilting or retraction. These structural abnormalities of the ear are linked to prejudice the synthesis of sulpho-mucopolysaccharides. According to the authors above, were the numerous complications in the labyrinth and labyrinthitis changing postural balance. According to Leach and Gay (1987) the epiphyseal growth plate plays a key role in skeletal development and factors that influence the metabolism of this tissue can lead to abnormal skeletal development. Abnormalities of epiphyseal plate may be in cellular differentiation may exist where Mn deficiency or shortly after cell differentiation begins when the development of hypertrophic growth area where demand for mucopolysaccharide synthesis catalyzed by Mn metalloenzymes is dependent on intense training for the epiphyseal plate normal. At this stage the board is also sensitive to deficiency of other nutrients such as Ca, P and vitamin D. After the zone chondrocytes formed the board this should receive the minerals responsible for ossification mainly Ca and P (Leach *et al*., 1969).

The bone growth will be normal if the support structure ossification, zone chondrocytes, is fully formed. Abnormal cartilage development is associated with chondrodystrophy, tibial dyschondroplasia and rickets. Manynutrient deficiencies result in chondrodystrophy, which is characterized by shortened, thickened bones and a narrowing of the epiphyseal growth plate. Tibial dyschondroplasia is a condition in which the prehypertrophic cells fail to hypertrophy and vascularization is aborted. This abnormality is found in genetically predisposed animals and its occurrence is altered by subtle changes in calcium, phosphorus and electrolyte content of the diet. Calcium and vitamin D deficiencies cause rickets, which is characterized by an increase in the width of the prehypertrophic zone of the epiphyseal growth plate (Leach, 1969).

The differential diagnostic of rickets is that the anomaly by Mn deficiency in the formation of growth epiphyseal plate chondrocytes region presents malformed and there is little or no available tissue calcification, whereas in rickets that can by no region available calcification or malnutrition of Ca and P in some phase of growth. It is observed flattening of the epiphyses weakening of the bones that can demonstrate it fragile and brittle. Manganese is needed for the glycosyltransferases that function in the synthesis of the glycosaminoglycan side chains of the proteoglycan. Burch *et al*. (1975) mentioned that the evidence of trials indicated that manganese has an important role in initiating protein synthesis by stimulating RNA polymerase and DNA polymerase activities in the mammalian system. Experimentally induced manganese deficiency has been produced in various domestic animals. The pathogenesis of symptoms in this deficiency is unknown and all attempts to explain their etiology have been unsuccessful.

Manganese is necessary for optimal growth in mice, rats, swine, guinea pigs and calves and other species. Skeletal abnormalities, but subsequent experiments demonstrated that manganese deficiency retarded endochondral bone growth per se, not osteogenesis. The discovery that perosis is related to changes in the mucopolysaccharide content of the epiphyseal cartilage focused attention on the chemical composition of the organic matrix of cartilage and bone (Leach and Muenster, 1962). The galactosamine-containing polysaccharides were drastically diminished by manganese deficiency in the chick.

Skeletal abnormalities have been studied extensively in manganese deficiency. In rats, mice and rabbits the primary skeletal effects are shortening and bowing of the forelegs. In rats, these effects are seen only in the offspring of manganese-deficient mothers. These offspring exhibited shortening of the radius, ulna, tibia, and fibula from birth to maturity.

In addition, as observed in this study, poor development of the tibial epiphysis resulted in abnormalities of the knee joint and other skeletal abnormalities in calves and other species have been extensively discussed by Rojas *et al*. (1965), Leach (1969), Dyer *et al*. (1964). The above studies have demonstrated that manganese affects the primary sites of chondroitin sulfate synthesis. Because the chondroitin sulfate-protein complex is necessary to maintain the rigidity of connective tissue, these findings provide a biochemical explanation for the skeletal abnormalities observed in manganese deficiency. Tibial dyschondroplasia is a condition in which the prehypertrophic cells fail to hypertrophy and vascularization is aborted. Leach and Muenster (1962) showed that the concentration of amino sugars hexoseamine, glucoxamine plus galactosamine, components of mucopolysaccharides were found in the amount of 1.54 mg/kg in the diet deficient in Mn (0 ppm Mn) versus 3.93 mg/kg tissue (100 ppm Mn) forming mucopolysaccharides. In the research, showed that choline deficiency did not result in any substantial

change in the mucopolysaccharide content of the epiphyseal cartilage that are observed with manganese deficiency. According to Cave *et al*. (2008), the same way in this research, the histopathology of affected skeletal samples showed chondrodysplasia, strengthening the evidence that the cause of the deformities could be a manganese deficiency during foetal development (Hurley, 1976a,b).

Conclusion: The deficiencies of macro and trace elements in cattle grazing in the region subjected to the nine counties studied supplemented shall at all times and in all seasons. The study revealed a worsening of disability in the dry season of the year (autumn, winter and transition to spring) when the deficiencies highlighted by the low quality and quantity of dry matter and consequently of all nutrients and therefore imposing the need for supplementation of dry matter and correction of nutrient for the provision of adequate energy, protein, minerals and vitamins.

Manganese deficiency was found in pastures and the addition of sugar cane and napier grass in the trough does not replace that with the requirements.

Adding mineral supplementation balanced mainly manganese, discontinued the birth of calves with abnormalities of the skeleton.

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