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A Review of the Role of Molybdenum in Animals Nutritional Physiology and Pathophysiologic Aspects

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Abstract: Molybdenosis, the syndrome produced by excess Molybdenum, is characterized by debilitating diarrhea, emaciation, weight loss, reproductive alterations, immunosupression and occasionally, death. The clinical signs of molybdenosis were primarily attributed to a conditioned or secondary copper deficiency and increased levels of sulfates in the diet. The pathogenesis of the syndrome includes chemical reactions between molybdates and sulfates, principally in the digestive tract, resulting in the synthesis of thiomolybdate. These compounds affect digestion, absorption, tissue distribution and bioavalability of Cu. This review presents a description of molybdenosis, with emphasis on nutritional physiology and pathophysiologic aspects of Mo in ruminant and non-ruminant animals.

Key words: Molybdenum, nutritional physiology, pathophysiologic

Introduction

Molybdenum (Mo) plays important biological roles in plants as well as in animals. Molybdenosis, the syndrome produced by excess of Mo was first described by Ferguson *et al.*, in 1943. Since then it has been reported in several different countries (Blood and Henderson, 1974; Underwoor, 1977). It is characterized by debilitating diarrhea, emaciation, weight loss, and sometimes death. The clinical signs of molybdenosis were primarily attributed to a conditioned or secondary Cu deficiency and were worsen by increased level of sulfate ($SO_4=$) in the diet. The pathogenesis of the syndrome includes chemical reactions between molybdates and $SO_4=$, principally in the digestive tract, resulting in the synthesis of thiomolybdates. These compounds affect digestion, absorption, tissue distribution and bioavalability of Cu. (Hidiroglou and Ivan, 1990). Although molybdenosis continues to be studied, some of the more important aspects that need investigation are the nutritional functions of Mo, its relation with Cu, and strategies in its supplementation to animals.

Nutritional physiology

Requirements and absorption: Mo requirements are extremely low. The amounts of Cu and S compounds in the diet of ruminants determine whether the Mo intake will be well tolerated, cause depletion of the animal's Cu reserves, or result in molybdenosis (Fell *et al.*, 1979). Ideally, the Cu-Mo ratio should be 5 to 10 ppm Cu: 1 ppm Mo. (Corah, 1992). The Mo concentrations in plants vary from 0.1 to 100 mg/kg of DM. (Barshar, 1948) Legumes and cereals a re the most important sources of Mo.(Penumarthy and Oheme, 1978).

In cattle diets, levels of 0.5 to 3 ppm are considered to be elevated (Corah, 1992). The absorption mechanism of Mo in monogastric and ruminant species is not well understood. Organic forms of Mo are rapidly absorbed from the digestive tract. Dick, *et al.* 1975, described progressive chemical reactions of molybdate with hydrogen and S producing a series of thiomolybdates. ^{99}Mo, molybdate, di, tri and tetrathiomolybdate were detected in blood after introduction of Mo into the rumen in sheep, cows and deer. (Mason, *et al.*, 1982; Kelleher, *et al.*, 1983; Hynes, *et al.*, 1984; Mason, *et al.*, 1984) Tetra, di and tri-thiomolybdate are also absorbed in the small intestine. (Mason *et al.*, 1980)

Transport: The normal blood concentrations of Mo in ruminants reach levels of 1 g/100 ml (Blood and Henderson, 1974; Kselikova *et al.*, 1974). Mo is bound to α_2 globulin in rat and human blood.(Kselikova *et al.*, 1977; Smith and Wright, 1975). In sheep, according to Norheim *et al.*, 1980, practically all of the Mo in plasma and red cells is present in a single protein fraction. This fraction had a molecular weight of less than 1,500. Small amounts of the plasma Mo are found in a high molecular weight fraction. (Mason *et al.*, 1980)

Distribution: Macroscopic autoradiographic studies using ^{99}Mo in rats and sheep demonstrated that Mo is distributed in the whole organism. Major activities in decreasing order were found in renal, hepatic, and spleenic tissues, respectively, (Auza, 1983). Intermediate activities appeared in muscle, fat, bone marrow, lung and adrenal gland. The nervous system showed low activity, with the exception of the hypophysis which demonstrated a higher concentration of the mineral. (Auza, 1983) ^{99}Mo was found in fetal organs 24 hours after an intravenous injection of ^{99}Mo to pregnant rats and sheep (Auza, 1983).

In the majority of domestic species, Mo concentrations in the liver fluctuate between 2 and 4 g/g of DM. (Underwood, 1977; Froslie *et al.*, 1980) In liver and renal cortex, Mo is present in two cytosolic protein fractions, one of high molecular weigh, and other one of low molecular weigh. A small part of the Mo present in the high molecular weight fraction was bound to a protein with an approximate molecular weight, similar to bovine albumin. Variable concentrations of these two proteins are also found in other organs (Norheim *et al.*, 1980).

Biological Role: The important biological role of Mo is as the prosthetic group of the specific enzymatic systems that catalyzed oxide reduction reactions (Rajagopalan, 1987). Mo is also required for the activities of different enzymes such as xanthine dehydrogenase/oxidase, aldehyde oxidase and sulfite oxidase in human and animals (De Renzo, *et al.*, 1953; Richert and Wasterfeld, 1953; Mahler *et al.*, 1954; De Renzo, 1956; Cohen *et al.*, 1971). Xanthine dehydrogenase and aldehyde oxidase have been suggested to be involved in detoxification of xenobiotic compounds (Richert, 1953). Xanthine oxidase plays an important role in the metabolism of uric acid and alantoine. (Rajagopalan, 1987; Richert, 1953). A deficit in the activity of this enzyme produces hyperxanthinuria. (Rajagopalan, 1987; Rajagopalan, 1980). In humans with congenital Mo-enzyme dysfunction, specific anomalies in the central nervous system are related to reduced activity of xanthine oxidase and sulfite oxidase. (Rajagpalan, 1980). Sulfite oxidase catalyzes the terminal step of sulfur amino acid metabolism involving oxidation of sulfite into sulfate (Heimberg *et al.*, 1953). Signs of deficiency of this enzyme

include occular dislocation and profound mental retardation in humans (Beemer *et al.*, 1985).

Molybdenum metabolism:
In Rumen: Several investigators have studied the action of Mo on rumen fermentation in cows and sheep. The addition of Mo to feed increased the digestion of the cellulose and increased the utilization of sulfites and sulfates by the rumen microflora (Beemer *et al.*, 1985). Mo increased the synthesis of sulfites from methionine and inhibited the chemical reduction of sulfates but not that of sulfites. The decrease in sulfate reduction is related to inhibition of ATP-sulfurilase (Huising *et al.*, 1975; Gawthorne and Nader, 1976). Progressive chemical reactions of hydrogen and sulfur with molybdates produce thiomolybdates. On the other hand, high levels of Mo in feed increased the requirements of S by the rumen microorganism (Cardin and Mason, 1975).

In Intestine: In the rat ileum, there is an active transport system carrying molybdate (Mo04=), sulfate (S04=) and other group VI oxyanions. (Cardin and Mason, 1975). The uptake of Mo04= and S04= display characteristics of an active transport system, saturability, concentration by the cells and competitive inhibition. (Mason and Cardin, 1977). Mo04= and S04= have the same charges and stereochemistry and are similar in size, so are likely to be able to interact electrostatically with similar binding sites. (Masonand Cardin, 1977). According to these authors, the general pattern for molybdate and S uptake by ovine intestine resembles that described for rat intestine.(Cardin and Mason, 1975). Similarly to rats, the site of maximal uptake in sheep is the lower ileum, with evidence for a similar uptake gradient along the length of the small intestine. It is thus possible that one of the areas of Mo/S interactions in the ruminant is the competition of these oxyanions for a common membrane transport system.

Excretion: Mo is eliminated from the organism by the gastrointestinal, renal and mammary routes. The importance of these routes depends on the species. The gastrointestinal is the most relevant route in ruminants, followed by urinary and finally mammary excretion (Tolgyesi *et al.*, 1982). However, 99Mo04= administered duodenally in sheep was rapidly absorbed and mostly excreted by the urinary route (Mason *et al.*, 1978). These authors suggested that S obstructs the Mo re-absorption in the renal tubules in sheep. The concentrations of Mo in cow and sheep milk fluctuated from 18 to 120 g/g, these levels are in direct relation with the concentration of Mo in the diet.3 In equine and porcine the renal route is the most important route of excretion (Cymbaluk *et al.*, 1981).

Pathophysiology
Effects of molybdenum deficiency: Animals have a very low but irreplaceable requirement for Mo. Under normal conditions, the mineral is present in the feed at low concentrations. A secondary deficiency of Mo by excess of tungsten characterized by decreased activity of xanthine oxidase and aldehyde oxidase has been described. (Rajagopalan KW, 1987). In New Zealand, Ferrando38 described clinical cases of renal xanthine urolitiasis in cows on forage from Mo deficient soils. This deficiency has been also described in hens with low growth, low plasma Mo levels and reduced oxidation of xanthine to uric acid (Nell *et al.*, 1980).

Toxicosis
In non-ruminants: At present, the majority of studies relate the excess of Mo and S with Cu metabolism.2, 3, 40 In general, clinical symptoms of molybdenosis are specific to the animal species. However, weight loss, growth retardation and loss of appetite are common findings in different species. Rabbits, guinea pigs, pigs and chickens are very resistant to Mo intoxication. (Underwood EJ., 1977). Several studies have reported the action of excess Mo on the Cu metabolism in rats and rabbits. (Mills, 1960; Arrington *et al.*, 1965; Mason *et al.*, 1978; Nederbragt, 1982). Arrington *et al.* (1965) demonstrated differences in the susceptibility to Mo toxicity between rats and rabbits. Rats treated with Mo and low diet Cu concentration, showed symptoms similar to ruminants (Mills *et al.*, 1978). In rats, concentrations of 1000 ppm of Na2Mo4 in water decreased food intake and digestion and body weight lost while the same doses of Mo in drinking water did not produce significant effects in rabbits. In another study, severe bone effects were reported in rats treated with low doses of ammonium tetra-thiomolybdate (6mg of Mo/kg DM) and normal levels of Cu.46 Valli *et al.* (1969) described low growth, anemia and hypothyroid function in rabbits dosed with 0.4 per cent Na2 Mo4.

Thiomolybdate is highly toxic to rats. This toxicity is attributed to a depression of the hepatic sulfide oxidase activity. The enzyme is also depressed in rats fed sodium molybdate (Van Reen, 1959). Nederbrag (Nederbragt, 1982) described the formation of cupric-thiomolybdate in the gastrointestinal tract, plasma and other tissues in rats. This compound reduced the intestinal absorption of dietary Cu.14, 49, 50 Horses fed Mo did not show evidence of the plasma tri-thiomolybdates compounds (Strickland *et al.*, 1987).

In Ruminants: Although different mechanisms of intoxication for Mo have been proposed in monogastric and polygastric animals, some common findings are also described. According to a large list of authors, bovines are the most susceptible to molybdenosis, followed by ovine and deer, respectively (Blood and Henderson, 1974; Underwood, 1977; Fisher *et al.*, 1976).
Molybdates can induce copper deficiency (Gooneratne *et al.*, 1989) by different mechanisms:
1. Decreased Cu absorption in the intestine;
2. Increased albumin bound Cu and reduced Cu uptake by the liver;
3. Depletion of hepatic Cu concentration;
4. Increased Cu elimination by biliary and urinary routes.

Toxic problems appear in ruminants under the following conditions:
a. High concentrations of Mo in the feed (20 or more PPM);
b. Low Cu:Mo ratio (2:1 or less);
c. Absolute Cu deficiency (5 PPM/kg DM);
d. Normal Cu and Mo concentrations but high levels of soluble proteins. (Ward, 1978; Sas, 1989)

Different processes in the rumen create the conditions to enhance the ability of Mo to reduce the availability of Cu. Some studies have indicated that rumen function may not be the only explanation for species differences, because mule deer Ward *et al.*, 1976 and goats Anke, 1991, tolerate up to 1000 ppm of Mo in the diet, the same tolerances that was described in rabbits, rats and chickens. (Ward, 1991)
Extensive reduction of S compounds in the rumen is probably the most important factor contributing to the much greater sensitivity of ruminants to Mo (Suttle, 1974). Studies to elucidate the Cu-Mo-S interaction in ruminants have shown that the concentration of sulfide in the rumen affects the availability of dietary Cu to the animal is itself affected by dietary level of molybdate (Bird, 1970). Rumen microorganisms produce sulfide from both inorganic S, through S reduction (Lewis, 1954) and sulfur amino acids, possibly through desulphydration (Halverston, 1968). In relation to Mo toxicity, the predominant role of dietary sulfur compounds, such as S or as sulfur amino acids, is reduction to sulfide under the

reducing conditions of anaerobic fermentation in the rumen (Nukksm 1987) In addition, sulfites combine with Cu to produce an insoluble and unavailable Cu-sulfide (Suttle, 1986). Sulfide, however, is rapidly absorbed from the rumen (Mills, 1987) and thus Cu-S complexes would not explain the relation of Mo-Cu deficiency. Sulfides also react with molybdate salts in the reducing medium of the rumen to remove oxygen and produce thiomolybdates. Current theories suggest that the principal relation between Mo and Cu is that thiomolybdates react with copper to produce soluble (but unavailable) forms of Cu tetra-thiomolybdate, which has been identified as the key compound (Allen and Gawthorne, 1986; Kincaid and White, 1988; Mason, 1988). Dietary molybdate increase plasma Cu levels in sheep. (Auza, 1983; Mason *et al.*, 1988). The most significant change is the appearance of a new fraction containing both Cu and Mo. This fraction, in contrast to the major Cu fraction of normal plasma contained in ceruloplasmin, is not released during protein precipitation by 5 per cent trichloroacetic acid. It represents a more tightly bound and possibly metabolically unavailable form of Cu. The fact that unavailable tetra-thiomolybdate-bound Cu is found in plasma and liver probably explains many observations where Cu levels in these tissues were not closely related to intakes of Cu, Mo or S or to the clinical conditions of the animal. (Ward, 1978).

Effects of the intoxication in different parts of the organism: The majority of the biological functions of Cu are believed to be associated with Cu as a ligand in the active site of metalloenzymes. Mo exerts antagonistic effect on different Cu-dependent metalloenzymes in the organism (Cerone *et al.*, 1998).

Impacts of Mo toxicosis on organs systems

Body weight: Loss of body weigh and growth retardation are two of the principal characteristics of the syndrome (Auza N, 1983). The origin of these findings are not very clear; however, (Fell *et al.*, 1985) described a low Cu dependant enzymatic activity and modification of pancreatic, renal and intestinal structure as a central cause of a systemic syndrome of depleted enzymatic activity.

Nervous system: Howell and Davison (Howell and Davison, 1959) showed a diminution in cytochrome oxidase activity in lambs with swayback or ataxia. This enzyme participates in synthesis of compounds of high-energy necessary in lipid biosynthesis. Mills and Fell, 1960 demonstrated that lambs feed high levels of sodium sulfates plus ammonium molybdate suffered medullar damage by demyelinization. The principal site of action was on glial cells, which are responsible for the synthesis of myelin (Mills and Williams, 1962). High concentrations of Cu and Mo were found in the brains of sheep that had been dosed with tetra-thiomolybdate for the treatment of Cu poisoning three years before (Haywood *et al.*, 1998). The authors speculated that tetra-thiomolybdate had an effect on the redistribution of Cu-tetrathiomolybdate to the brain.

Cardiovascular system: Cu deficiency increases the fragility of tissues rich in elastine, such as blood vessel, resulting in spontaneous ruptures of arteries. Cu participates in the synthesis of cardiovascular tissues through monoamine oxidase enzyme. This enzyme oxidizes residues of lysine in desmosine and isodesmosine to form elastine (Hill *et al.*, 1967). Cardiovascular Cu deficient tissues maintain normal composition in amine acids but they have absence of desmosine and isodesmosine with the consequent lack of resistance.

Reproductive system: Cu deficiency has been implicated in infertility of cattle and sheep. Delayed onset of puberty, alteration of estrus cycle length (occasionally leading to anestrus), cystic ovarian disease, impaired ovulation, and reduced conception rates have been observed in Cu-deficient animals (Phillippo, 1987). According to Phillippo *et al.*, 1982, reproductive alterations in cows, while appearing during secondary Cu deficiency, are not always Cu dependent, because the response to the Cu treatment can be negative. These authors suggested that Mo itself could be playing a role in this pathology. Mo supplementation by oral route, retards puberty, decreased fertility, and affected the estrus cycle, with a significant number of animals not ovulating in an-estrous Phillippo *et al.*, 1987. Bovines fed diets with high concentration of Mo had lower plasma concentration of luteinizing hormone, possibly associated with a lower pulsatile release during key times of the estrous cycle, or causing lower levels of this hormone concentration at the time of ovulation (Phillippo, 1987). Phillippo also suggested that the secretion of the pineal gland and prolactin liberation was affected and that a modification of the ovarian estradiol production could also be occurring (Phillippo, 1987). Benard and Auza, 1986, demonstrated a highly selective affinity of the hypophysis for 99Mo in rats and sheep. Irregular prolonged estrus cycles had been also described in rats (Winston, 1981). Igarza, 1993, described similar results plus modification of levels of LH, FSH and estrogens. Yang *et al.*, 1991, described longer estrus cycles, fewer and smaller fetuses and more reabsorbed fetuses than the control in rats receiving high concentration of Mo in the diet.

Bone system: Swayback is a neuro-muscular-bone disorder seen in young lambs grazing high Mo pastures (Blood, 1974). The bone pathology may be associated with a disturbance of phosphorus metabolism that results in osteoporosis (spontaneous fractures) and abnormalities.(Mills *et al.*, 1978; D'Dell, 1981). Several studies in rabbits (Valli, 1969) rats (Spence et al., 1983) ovine Pitt *et al.*, 1980 and bovine Cerone *et al.*, 1998; Irwin *et al.*, 1974) demonstrated that Cu deficiency was associated with an alteration in bone structure and integrity. Low dietary phosphorus, high magnesium and Mo have been observed to induce hypophosphatemia in sheep and cattle. (Shirley *et al.*, 1950; Jaswant *et al.*, 1994). A competitive antagonism exists between Mo and phosphorus. High dietary Mo resulted in decreased deposition and increased mobilization of phosphorus from bones Arrington and Davis, 1953 and a probable defect in the metabolism of vitamin D.

Erythropoietic system: Molybdenosis can affect the synthesis and activity of ceruloplasmin oxidase or ferroxidase. Anemia due to deficiency of ceruloplasmin oxidase is found in some cases.Cerone *et al.*, 1998. Ceruloplasmin has oxidase activity on a large number of substrates such as ascorbic acid, adrenaline, nor-adrenaline, serotonin and dopamine (Szpiech *et al.*, 1983; Chidambaran *et al.*, 1984; Lamon and Mason, 1986). Ceruloplasmin is essential for the normal flux of iron from the intestinal, reticulo-endothelial extrahepatic and hepatic cells to the blood. Decreasing activity of ceruloplasmin produces ferropenic anemia (Seeling, 1972). Brem and Roux (Brem and Roux, 1991) and Brem *et al.*, 1991 described microcytic normochromic anemia in ovine and bovine with molybdenosis. According to Andrewartha and Caple, 1980 and Dameron and Harries, 1987 a diminution in red cells life span due to peroxidation damage by superoxide anions is possible in animals with copper deficiency.

Immune system: Cu deficiency has been associated with reduced responsiveness to antigens and increased susceptibility to bacterial infections Graham, 1991. Calves born to Cu-deficient cows, showed increased susceptibility to scours or diarrhea. These

results suggested an effect of Cu deficiency on the immune system, inducing immunosupression leading to enhanced susceptibility of the calves to bacterial or viral diseases (Szpiech *et al.*, 1983; Graham, 1991; Boyne and Arthur, 1981; Prohaska and Lukasewyz, 1990). Although the specific mechanisms responsible for the immunosuppressed state of Cu-deficient animals are not understood, some evidence indicates that Cu is required for the normal function of lymphoid cells (Lukasewyz *et al.*, 1985; Mulhern and Koller, 1988). Bovines supplemented with molybdate and S had significantly lower levels of Cu in serum than those not supplemented. In these supplemented animals the levels of intracellular Cu decreased notably in the neutrophils reaching values of 0.032 gCu/6x107 cells for deficient bovines and 0.116 gCu/6x107 cells for control group, this represent a decreased of 72% of intracellular metal element content (Cerone *et al.*, 1998).

Also, the microbiocidal activity of bovine peripheral blood neutrophils was decreased by Mo induced Cu deficiency. Cerone *et al.*, 1998) indicated that molybdenosis in cattle produced cellular alterations. These changes, which may contribute to an impaired immune defense, included alterations in monocyte numbers, B-lymphocyte sub-populations, neutrophil and ceruloplasmin activity. The diminished number of B-lymphocytes may impair the production of immunoglobulins. In another trial, Arthington *et al.*, 1996, reported an increase in the neutrophil number, without alterations in chemotactic ability following an inflammatory stressor, during Mo-induced Cu deficiency. In a study to evaluate neutrophil and macrophage function, calves fed marginally Cu-deficient diets and Mo-supplemented diet had higher total leukocyte numbers than iron and Cu-supplemented calves at 7 days of age. At 70 days of age, there were no differences in percentages of lymphocytes or neutrophils in the leukocyte populations on either sampling date (Gengelbach *et al.*, 1997). In bovines and ovine, the diminution of activity of superoxide dismutase modified the leukocyte response (Jones and Suttle, 1981), especially the neutrophil activity (Boyne and Arthur, 1986). The control of oxidative reactions is critical to the maintenance of many aspects of immune function. Although free radicals are required for such immune activities as the killing of infectious organisms, the overproduction of these highly reactive molecules can result in adverse effects. Among the agents that have been documented to neutralize the cytopathic effect of neutrophil oxidants are the free radical scavengers, superoxide dismutase and catalase which act to catalyze O_2 to H_2O_2 and H_2O_2 to H_2O respectibily (Rosen *et al.*, 1995). In molybdenosis, decreased Cu,Zn-SOD activity, impaired the dismutation of O_2^- to H_2O_2 and the neutrophil might release toxic products into the intra and extracellular space (Mulhern *et al.*, 1988). Babu and Faille,1989, 1990 found that the cellular Cu status, respiratory burst and candidacidal activity are impaired in macrophages and neutrophils from marginally and severely Cu-deficient rats.

The cytocrhome-c oxidase activity was lower in splenic lymphoid cells of Cu-deficient ratsDavis *et al.*, 1987 and Cu, Zn-SOD activity was lower in the spleen, thymus and cervical lymph node cells of Cu-deficient rats (Babu and Failla, 1989). A diminution of phospholipid concentration in cytoplasm membrane of lymphocytes has been described in rats with Cu deficiency (Korte and Prohaska, 1987). Blakley and Hamilton, 1987, demonstrated that a lower Cu level is associated with a reduction in T-lymphocytes in mice and rats.

The role of Mo in ruminant nutrition continues to be a critical factor in Cu availability by the animals. With changing diets, possible environmental changes, or when molybdenosis or Cu deficiency signs are exhibited, dietary Mo needs to be assessed periodically. Further work is needed to determine the interactions of Mo-Cu-S, protein/amino acids and the rumen microbial environment. With more information about rumen metabolism of trace minerals, more precise recommendations could be made concerning inorganic and organic trace mineral supplementation.

References

Auza, N., 1983. Contribution a l'etude de la distribution et de la toxicite de cuivre et du molybdene chez le rat, la souris et le mouton. These. Toulouse. France.

Andrewartha, K.A., I. W. Caple, 1980. Effects of changes in nutritional copper on erythrocyte superoxide dismutase activity in sheep. Res. Vet. Sci., 28: 101-104.

Brem, J.J., J. P. Roux, 1991. Aspectos clinicos-hematologicos de la molybdenosis experimental en bovinos con y sin tratamiento parenteral de cobre. Vet. Arg., 8: 38-49.

Brem, J.J., D. O. Pochon, J. P. Roux, *et al.*, 1991. Estudios ferrocineticos en ovinos sometidos experimantalmente a un exceso de molybdeno. Rev. Med. Vet., 72: 229-239.

Boyne, R., J. R. Arthur, 1986. Effects of molybdenum and iron induced copper deficiency on the viability and function of neutrophyls from cattle. Res. Vet. Sci., 41: 417-419.

Babu, U., M. L. Failla, 1989. Superoxide dismutase activity and blastogenic response of lymphocytes from copper deficient rats fed diets containing fructose or corn starch. Nutr. Res., 9: 273-282.

Babu, U., M. L. Failla, 1990. Respiratory burst and candidacidal activity are impaired in peritoneal macrophages and neutrophils from marginally and severely copper deficient rats. FASEB J. 4: A509 (abs 1409).

Blakley, B.R., D. L. Hamilton, 1987. The effect of copper deficiency on the immune response in mice. Drug Nutr. Interact., 5: 103-111.

Blood, D.C., O. M. Henderson,1974. Veterinary Medicine. 4th Ed. Bailliere Tindall, London.

Barshard, P., 1948. Molybdenum content of pasture plants in relation to toxicity to cattle. Soil Sci., 66: 187-195.

Chidambaran, M.V., G. Ramer, E. Frieden, 1984. Inhibition of ceruloplasmin and other copper oxidases by thiomolybdates. J. Inorg. Biochem., 22: 231-237.

Corah, L.R., 1992. Trace minerals in cow herd nutrition programs. Part 1- Copper, manganese and molybdenum. Agri-Pract., 13: 29-33.

Cohen, H.J., I. Fridovich, K. V. Rajagopalan, 1971. Hepatic sulfite oxidase. J. Biol. Chem., 246: 374-382.

Cymbaluk, N.F., H. F. Schryber, H. F. Hintz, *et al.*, 1981. Influence of dietary molybdenum on copper metabolism in ponies. J. Nutr., 111: 96-106.

Davis, G.K., W. Mertz, 1987. In: W.Wertz Ed: Copper. Trace elements in human and animal nutrition. Academic Press, New York.

Dameron, T, E. Harries, 1987. Regulation of aortic Cu-Zn-Superoxide dismutase with copper. Effects in vivo. Biochem. J., 248: 663-668.

Dick, A.T., D. W. Dewey, J. M. Gawthorne 1975. Thiomolybdates and the copper-molybdenum-sulfur interaction in ruminant nutrition. J. Agricul. Sci. Camb., 95: 567-658.

De Renzo, E.C., E. Kaleita, P. Heytler, *et al.*, 1953. Identification of the xanthine oxidase factor as molybdenum. Arch. Biochem. Biophys., 43: 247-253.

De Renzo, E.C., 1956. Chemistry and biochemistry of xanthine oxidase. Advenc Enzymol. 17: 293-328.

Ferguson, W.S., A. H. Lewis, S. J. Watson,1973. The teart pastures of Somerset. J. Agric. Sci., 33: 44-51.

Fell, B.F., T. T. Dinsdale, T. EL-Gallad, 1979. Gut pathology of rats dosed with tetrathiomolybdate. J. Comp. Path., 89: 495-514.

Underwood, E.J., 1977. Trace elements in human and animal nutrition. 4th Ed. Academic Press. New York.

Hidiroglou, M., M. Ivan, 1990. Trace element deficiencies:

diagnosis, treatment, prevention. Copper metabolism and status in cattle. Proceeding World Buiatrics Congress.

Penumarthy, L., 1978. Oheme FW: Molybdenum toxicosis in cattle. Vet Hum Toxicol 1978; 20: 11-12.

Kelleher, C.A., M. Ivan, M. Lamand, *et al.*, 1983. The absorption of labeled molybdenum compounds in sheep fitted with re-entrant cannulae in the ascending duodenum. J. Comp. Path., 93: 83-91.

Hynes, M., M. Lamand, G. Montel, *et al.*, 1984. Some studies on the metabolism and the effect of 99Mo and 35S labeled thiomolybdates after intravenous infusion in sheep. Brit. J. Nutr., 52: 149-158.

Kselikova, M., B. Birr, M. Lener, 1974. Radioisotope 99Mo study of the kinetics of molybdenum clearance from the blood and of the role of blood components. Physical Bohemoslov, 23: 83-88.

Kselikova, M., B. Birr, J. Lener, *et al.*, 1977. Interaction of alpha 2-macroglobulin in human and rat serum. Physiol. Bohemoslov., 26: 573-575.

Smith, B.S.W., H. Wright, 1975. Effect of dietary molybdenum in copper metabolism. Evidence for the involvement of molybdenum in abnormal binding of copper to plasma proteins. Clin. Chem. Acta., 62: 55-63.

Norheim, G., N. E. Soli, A. Froslie, *et al.*, 1980. Fractionation of soluble molybdenum-binding proteins from liver, kidney, plasma and erythrocytes from sheep supplemented with molybdenum. Acta. Vet. Scand. 21: 428-437.

Froslie, A., G. Norheim, E. Wassio, 1980. Copper, zinc and molybdenum in livers of Norwegian cattle at slaugther. Acta. Vet. Scand, 21: 62-70.

Rajagopalan, K.W., 1987. Molybdenum. An essential trace element. Nutr. Rev., 45: 321-328.

Richert, D.A., W. W. Westerfeld, 1953. Isolation and identification of the xanthine oxidase factors as molybdenum. J. Biol. Chem., 203: 915-923.

Mahler, H.R., B. Mackler, D. E. Green, 1954. Studies on metalloflavoproteins. III. Aldehyde oxidase: A molybdoflavoprotein. J. Biol. Chem., 210: 465-488.

Rajagopalan, K.V., 1980. Xanthine and aldehyde oxidase. Enzymatic basis of detoxification. Chapter 14. Academic Press, New York.

Heimberg, M., I. Fridovch, P. Hander, 1953. The enzymatic oxidation of sulfite. J. Biol. Chem., 204: 913-926.

Beemer, F.A., M. Duran, S. K. Wadman, *et al.*, 1985. Absence of hepatic molybdenum cofactor: An inborn error of metabolism associated with lens dislocation. Ophthalmic Pediatr Genet., 5: 191-195.

Spears, J.W., L. P. Bush, D. G. Ely, 1977. Influence of nitrate and molybdenum of sulfur utilization by rumen microorganism. J. Dairy Sci., 60: 1889-1893.

Huising, J., D. C. Milholland, G. Matrone, 1975. Effect of molybdate and sulfide production from methionine and sulfate by ruminal microorganism of sheep. J. Nutr., 105: 1199.

Gawthorne, J.M., C. J. Nader, 1976. The effect of molybdenum on the conversion of sulfate to sulfide and microbic-protein-sulfur in the rumen of sheep. Brit. J. Nutr., 35: 11-16.

Tolgyesi, G., I. A. Elmoty, 1982. Elimination of excess molybdenum by cattle. Acta Vet. Acad. Hung., 17: 39-44.

Ferrando, R., 1971. Les besoins en oligo-elements des animaux domestiques et les consequences de leur carences. Ann. Nutr. Alim., 25: B231-B235.

Nell, J.A., E. F. Annison, D. Balnave,1980. The influence of tungsten on the molybdenum status of poultry. Brit. Poult. Sci., 21: 193-202.

Arrington, L.R., C. B. Ammerman, J. E. Moore, 1965. Molybdenum toxicity in rats and rabbits. Quart J. Florida Acad. Sci., 28: 129-136.

Valli, V.E.O., A. Mc Carter, B. T. Mc Sherry, *et al.*, 1969. Hematopoiesis and epiphyseal growth zones in rabbits with molybdenosis. Am. J. Vet. Res., 30: 435-445.

Fisher, G.L., C. A. Hjerpe, C. W. Qualls, 1976. Effect of dietary molybdenum on the metabolism of copper and molybdenum in young cattle. Bioinorganic Chem. 6: 11-28.

Gooneratne, S.R., W. T. Buckley, 1989. Christensen DA: Review of copper deficiency and metabolism in ruminants. Can. J. Anim. Sci., 69: 819-845.

Ward, G.M., 1978. Molybdenum toxicity and hypocuprosis in ruminant : A review. J. Anim. Sci., 1078-1085.

Sas, B., 1989. Secondary copper deficiency in cattle caused by molybdenum contamination of fodder. A case history. Vet. Hum. Toxicol., 31: 29-33.

Ward, G.M., J. Nagy, 1976. Molybdenum and copper in Colorado forages, molybdenum toxicity in deer and copper supplementation of cattle. W. R. Chappell and K.K. Peterson (Ed.) Molybdenum in the environment. Marcel and Dekker, Inc, New York.

Anke, M., B. Groppel, M. Grun, 1991. Cited by Ward JM: Acceptable limits of molybdenum for ruminants exist. Feedstuffs, 63:15-22.

Ward, G.M., 1991. Acceptable limits of molybdenum for ruminants exist. Feedstuffs, 63:15-22.

Suttle, N.F., 1974. Recent studies of the copper-molybdenum antagonism. Proc. Nutr. Soc., 33: 299-305.

Bird, P.R., 1970. Sulphur metabolism and excretion studies in ruminants. III. The effect of Sulphur intake on the availability of copper in sheep. Proc. Aust. Soc. Anim. Prod., 8: 212-218.

Lewis, D., 1954. The reduction of sulfate in the rumen of the seep. Biochem. J., 56: 391-399.

Halverston, A.W., G. D. Williams, G. P. Paulson, 1968. Aspects of sulfate utilization by the microorganisms of the ovine rumen. J. Nutr., 95: 363-368.

Mills, C.F., 1987. Biochemical and physiological indicators of mineral status in animals: Copper, cobalt and zinc. J. Anim. Sci., 65: 1702-1711.

Suttle, N.F., 1986. Copper deficiency in ruminants: recent developments. Vet. Rec., 22: 519-522.

Mills, C.F., G. K. Davis, 1987. Molybdenum. Trace elements in human and animal nutrition. Vol.1. Academic Press.

Allen, J.D., J. M. Gawthorne, 1986. Involvement of organic molybdenum compounds in the interaction between copper-molybdenum and sulfur. J. Inorg. Biochem., 27: 95-112.

Kincaid, R.L., C. L. White, 1988. The effects of ammonium tetrathiomolybdate intake of tissue copper and molybdenum in pregnant ewes and lambs. J. Anim. Sci., 66: 3252-3258.

Cerone, S., A. Sansinanea, S. Streitenberger, *et al.*, 1998. Bovine neutrophyl functionality in molybdenum-induced copper deficiency. Nutr. Res., 18: 557-566.

Auza, N., 1983. Le cuivre chez les ruminants. Une revue. Ann. Rech. Vet., 14: 21-37.

Fell, B.F., L. Farmer, C. I. Farquharson, *et al.*, 1985. Observations on the pancreas of cattle deficient of copper J. Comp. Path., 95: 573-590.

Howell, J.M., A. N. Davison, 1959. The copper content and cytochrome oxidase activity of tissues from normal and Swayback lambs. Bichem. J., 72: 365-367.

Mills, C.F., B. F. Fell, 1960. Demyelination in lambs born of ewes maintained on high intakes of sulfate and molybdenum. Nature, 185: 20-22.

Mills, C.F., R. B. Williams, 1962. Copper concentration and cytochrome oxidase and ribonuclease activities in the brains of copper-deficient lambs. Biochem. J., 85: 629-632.

Haywood, S., Z. Dincer, J. Holding, *et al.*, 1998. Metal (molybdenum-cooper) accumulation and retention in brain, pituitary and other organs of ammonium tetrathiomolybdate-treated sheep. Brit. J. Nutr., 79: 329-331.

Hill, C.H., B. Starcher, C. H. Kim, 1967. Role of copper in the formation of elastin. Fed Procced 26: 129-133.

Phillippo, M., 1987. The effect of dietary molybdenum and iron on copper status, puberty fertility and estrous cycles in cattle. J. Agric. Sci. Camb., 109: 321.

Phillippo, M., W. R. Humphries, C. B. Lawrence, *et al.*, 1982. Investigation of the effect of copper status and therapy on fertility in beef suckler herds. J. Agric. Sci. Camb., 99: 359-364.

Phillippo, M., W. R. Humphries, P. H. Garthwaite, 1987. The effect of dietary molybdenum and iron on copper status and grown in cattle. J. Agric. Sci. Camb., 109: 315-320.

Benard, P., N. Auza, 1986. Transplacental passage of 99Mo in rats and ewes whole-body autoradiographic studies. Proc. Royal Microsc. Soc., 21: 287-288.

Winston PW: Molybdenum disorders of mineral metabolism. New York Academic Press, 1981.

Igarza, L.M., 1993. Molibdenosis experimental en ratas y ovinos. Thesis. Universidad de Mar del Plata. Argentina.

Yang, M., 1991. Cited by Ward JM: Acceptable limits of molybdenum for ruminants exist. Feedstuffs. 63: 15-22.

O'Dell, B.L., 1981. Roles of iron and copper in the connective tissue biosynthesis. Philos.Trans R Soc Lond (Biol.). 249: 91-104.

Lalich, J.L., K. Groupner, J. Jolin, 1965. The influence of copper and molybdate salts on the production of bone deformities in rats. Lab. Invest., 14: 1482-1493.

Pitt, M., J. Fraser, D. C. Thurley, 1980. Molybdenum toxicity in sheep. Epiphysiolysis exostosis and biochemical changes. J. Comp. Path., 90: 567-575.

Irwin, M.R., P. W. Poulos, G. L. Smith, 1974. Radiology and histopathology of lameness in young cattle with secondary copper deficiency. J. Comp. Path., 84: 611-621.

Shirley, R.L., R. B. Owens, G. K. Davis, 1950. Deposition and alimentary excretion of phosphorus in steers on high molybdenum and copper diets. J. Dairy Sci., 9: 552-559.

Jaswant, S., C. S. Randawa, 1994. Randawa SS *et al*: Role of dietary Mo in production of hypophosphatemia in crossbred calves. Indian J. Dairy Sci., 47: 926-927.

Arrington, C.R., G. K. Davis, 1953. Molybdenum toxicity in rabbits. J. Nutr., 84: 367.

Szpiech, M., Z. Dabrowski, H. Miszta, 1983. The effect of molybdenum upon blood serum enzymatic ceruloplasmin activity in rats. Sci. Total Environ., 30: 213-215.

Lamon, B, J. Mason, 1986. The inhibition of bovine ceruloplasmin oxidase activity by thiomolybdates in vivo an in vitro: a reversible interaction. J. Inorg. Biochem., 26: 107.

Seeling, M.S., 1972. Relationships of copper and molybdenum to iron metabolism. Am. J. Clin. Nutr., 25: 1022-1037.

Graham, T.W., 1991. Trace element deficiencies in cattle. Vet. Clinics N. Am. Food Anim. Pract., 7: 153.

Prohaska, J.R., O. A. Lukasewyz, 1990. Effects of copper deficiency on the immune system. Adv. Exp. Biol. Med., 262: 123-143.

Lukasewyz, O.A., J. R. Prohaska, C. G. Meyer, *et al.*, 1985. Alterations in lymphocyte subpopulations in copper-deficient mice. Inf. Immun., 48: 644-647.

Mulhern, S.A., L. D. Koller, 1988. Severe or marginal copper deficiency results in a graded reduction in immune status in mice. J. Nutr., 118: 1041-1047.

Arthington, J.D., A. R. Spell, L. R. Corah, *et al.*, 1996. Effect of molybdenum-induced Cu deficiency on in vivo and in vitro measures of neutrophil chemotaxis both before and following an inflammatory stressor. J. Anim. Sci., 74: 2759-2764.

Gengelbach, G.P., J. D. Ward, J. W. Spears, *et al.*, 1997. Effects of copper deficiency coupled with high dietary iron or molybdenum on phagocytic cell function and response of calves to a respiratory disease challenge J. Anim. Sci., 75: 1112-1118.

Jones, D.G., N. F. Suttle, 1981. Some effects of copper deficiency on leucocyte function in sheep and cattle. Res. Vet. Sci., 1981; 31: 151-156.

Rosen, G.M., S. Pou, C. L. Ramos, *et al.*, 1995. Free radicals and phagocytic cells. FASEB J. 9: 200-209.

Korte, J.J., J. R. Prohaska, 1987. Dietary copper deficiency alters protein and lipid composition of murine lymphocyte plasma membranes. J. Nutr., 117: 1076-1084.