THE EFFECT OF SUPPLEMENTAL MOLYBDENUM ON COPPER BALANCE IN MATURE GELDINGS

By

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CHAPTER I

INTRODUCTION

Copper is an essential mineral that is well understood in its nutritional requirements. Copper is required in several physiological functions. These functions include bone formation, pigmentation of hair and wool, cellular respiration, proper cardiac function, normal reproduction, integrity of the central nervous system, and a healthy immune system. Adverse effects due to an excess in dietary molybdenum intake have been established in the grazing ruminant. Research data from other species show that excess molybdenum in a diet can cause copper deficiencies. These copper deficiencies led to swayback of lambs, falling disease in cattle, loss of pigmentation in hair of guinea pigs and goats or abnormalities in sheep wool, bone disorders in rabbits and sheep, and aortic ruptures in rabbits, swine, guinea pigs, and chickens.

Little has been done to establish if there is an interaction between copper and different levels of molybdenum in the horse which may interfere with copper absorption resulting in a deficiency. If copper is influenced by molybdenum in the equine diet, there is a potential for bone abnormalities, achromotrichia, a weakened immune system, abnormal reproduction, and abnormal myelin formation.

Research on the effects of molybdenum on copper balance is needed if owners are to minimize health risks to horses. Thus, the object of this trial was to investigate the effects of supplementary molybdenum on fecal, urine, and serum copper levels.

CHAPTER II

LITERATURE REVIEW

Evolution of Dietary Copper and Molybdenum Interaction

The necessity of copper in ruminant nutrition was first established in 1931 when a copper deficiency was demonstrated in Florida (Becker et al., 1965). A Jersey heifer was supplemented with iron and copper while two herd mates received only iron. The two herd mates died of anemia while the copper supplemented heifer was fully recovered in three months, thus confirming the necessity for copper in ruminants. In 1933, characteristics such as diarrhea, loss of appetite, and anemia were linked with a copper deficiency in Northern Europe (McDowell, 1992). Interest in molybdenum arose in 1938 from the study of a cattle disease spread over 20,000 acres in S.W. England (Underwood, 1981 and Ferguson et al., 1943). Cattle introduced to pasture in this area developed severe diarrhea, experienced significant weight loss, and had lowered milk yield. The stock suffered a high mortality unless removed from pasture in that area. Although pasture copper levels in that area were not regarded as deficient, it was recognized that the symptoms were analogous with a copper deficiency. Analysis of the pasture revealed a high level of molybdenum, 14 to 108 ppm, with no other toxic compounds.

Supplemental copper was found to control the disease among cattle on the high molybdenum pasture. In New Zealand, lower levels of copper and molybdenum revealed the same symptoms in cattle (Cunningham, 1950). Once again, supplemental copper allowed cattle to graze the pasture with no adverse effects. Once copper supplementation was established as a curative as well as a preventative measure against the high levels of molybdenum found in pastures, the copper-molybdenum interaction was established.

Copper and Molybdenum Levels in Animal Feeds and Forages

The very close relationship between copper and molybdenum in nutrition, primarily grazing animals, requires us to understand the large copper and molybdenum fluctuations in animal feeds and forages. In New Zealand, the use of molybdenum as a fertilizer began around 1960 due to its essential requirement for plant growth (Smith, 1973). With many soils having low copper levels due to leaching, adding molybdenum to the soil only increased the instances of a molybdenum induced copper deficiency due to high molybdenum, low copper pasture (Smith, 1973). Many fertilizers have side effects on one another as well as current mineral soil levels. For example, limestone is known to depress copper levels while liberating increased amounts of molybdenum from the soil (Smith, 1973). Further, molybdenum does not leach out of the soil as fast as copper and may not need reapplication for several years (Smith, 1973).

Molybdenum content in many forages increases throughout the growing season which makes it difficult to establish the molybdenum content in feeds (Mill et al., 1987). Legumes and their seeds have the highest molybdenum level among animal feeds (Mill et

al., 1987). For both grasses and legumes, the uptake of molybdenum increases with soil pH (Mill et al., 1987). Mill et al. (1987) report a study that examined edible grasses and clovers for molybdenum and copper content as soil pH changed. The findings associate a high level of molybdenum with soil that had not been yet regarded as over limed. A corresponding increase in molybdenum was also noted with higher soil pH. A corresponding change in copper content was not observed. Thus, increases in soil pH widens the molybdenum-copper ratio of herbage. This in turn increases the chance of a molybdenum induced copper deficiency. With soil pH, fertilization, and maturation of forages playing a role in levels in feeds and forages, the incidence of a molybdenum-copper imbalance is a substantial concern for grazing animals.

OTHER SPECIES

Ruminants

With ruminants being exceptionally sensitive to the molybdenum-copper interaction, an abundance of research has been done to clarify the effect molybdenum has on copper utilization. Several studies were done to locate the site of interaction within the rumen and gut. Through studies done by Mason et al. (1978) and Suttle (1975), it seems that molybdenum must pass through the rumen in order to impair copper absorption as well as its own absorption. Dick et al. (1975) began investigations based on the hypothesis that molybdates are converted to a series of thiomolybdate compounds in the rumen. The rumen is rich in sulfate which is needed for the synthesis of the

thiomolybdate compound. Gawthorne (1985) performed in vitro studies which supported the thiomolybdate hypothesis. Thiomolybdate compounds absorbed from the rumen bind with copper in the albumin and delay the transfer of copper to tissues (Kelleher et al., 1983; Mason et al., 1982; and Gooneratne et al., 1989).

Cattle

Reductions in milk yield and body condition were observed in cows with high molybdenum intakes during certain months (Ferguson et al., 1938). Although not yet associated with copper, molybdenum was held responsible for decreased milk production (Ferguson et al., 1938). Vanderveen and Keener (1964) found added dietary molybdenum (100 or 200 ppm) combined with 0.3% sulfate sulfur caused alopecia, achromatrichia, emaciation, and the loss of nervous control in the hind limbs within three weeks. Liver copper and blood serum copper levels were not altered. Alopecia and achromatrichia were corrected with supplemental copper.

In vitro studies conducted by Boyne and Arthur (1986) suggested that neutrophils from cattle with a molybdenum induced copper deficiency were less viable and had an impaired ability to kill ingested Candida albicans. Ward et al. (1997) found no effect of supplemental molybdenum on the dam's ability to produce antibodies or passively transfer antibodies to the calf. A copper deficiency did appear through visual signs, yet specific immune function seemed to be maintained.

Plasma copper status was monitored by Gengelbach et al. (1994) in order to evaluate deficiency states during gestation, lactation, and parturition. Deficiency levels

were determined very quickly during gestation where copper is required at a higher level than at lactation. Post parturition molybdenum supplemented heifers were unable to regain adequate plasma copper status. This suggests that their body reserves were depleted during gestation. After parturition, molybdenum interfered with copper metabolism and the heifers were unable to replenish adequate copper levels. There was also a reduced rate of gain for the molybdenum supplemented calves. As feed intake was not monitored in this study, it is unclear if reduced gain was due to decreased milk consumption by the calf and/or decreased milk produced by the heifer. It was concluded that molybdenum induced copper deficiency in heifers produced copper deficient calves based on decreased copper levels found in the calves.

Sheep

Marston (1950) and Dick (1956a,b) found adequate hepatic and blood copper concentrations would maintain normal metabolic functions in sheep while clinical signs of a copper deficiency developed due to relatively high supplementation of molybdenum. This reveals an influence on the distribution of copper between tissue stores and the sites at which it has a functional role. However when lower dietary molybdenum was fed, a progressive depletion of blood and tissue copper was found before clinical signs of a copper deficiency occurred (Dick, 1956 and Wynne and McClymont, 1956).

Dowdy and Matrone (1968) found that low copper feeds supplemented with 2 ppm molybdenum maintained a normal level of hemoglobin in sheep. However, sulfate added to the diet caused severe anemia. At 4 ppm supplemented molybdenum, the

degree of the sulfate induced anemia was less. Therefore, at the higher levels of molybdenum, the main depressing factor on hemoglobin was molybdenum.

Wooliams et al. (1986) suggested that a copper deficiency caused a decreased resistance to infection in sheep due to the role of copper in superoxide dismutase. However, Suttle and Jones (1989) found controversy between in vitro and in vivo tests regarding lymphocyte activity. In vivo results in the copper deficient lambs showed a transient increase in peripheral blood lymphocytes which was not predicted from the in vitro tests.

Goats

Sharma and Parihar (1994) looked at the effects of 0, 50, and 100 ppm supplemental molybdenum on young goats. While feed intake was not affected in any of the goats, the molybdenum supplemented goats had moderate to marked reduction in body weights and diarrhea of varying intensity. The 50 and 100 ppm diets showed clinical signs faster than the 0 ppm diet. The goats that exhibited diarrhea had no significant gross or microscopic alterations in their intestines. Hair coats among the molybdenum supplemented goats changed from black to greyish white or brown to yellowish brown. The visible change in hair color occurred first in the 100 ppm supplemented group. Skin biopsies obtained from black-haired goats indicated that a gradual loss of melanin pigment occurs. Melanin is formed via the influence of tyrosinase which is a copper containing enzyme (Mason, 1966). The decrease in serum

copper levels due to the supplemental molybdenum indicates that decreasing copper levels play a role in the loss of melanin pigment in hair.

Mills (1983) suggested in goats that the copper containing enzyme lysyl oxidase was influenced under excess molybdenum intake resulting in an abnormal gait. Lysyl oxidase is responsible for the integrity of tendons and ligaments; therefore, if molybdenum intake interfered with the copper containing enzyme, an abnormal gait may result.

Sharma and Parihar also found reduced serum copper and ceruloplasmin levels which influenced iron metabolism. Anemia and a depressed immune system were also indicative of the molybdenum supplemented diets.

Chicks

Thiomolybdates, a group of compounds of molybdenum and sulfur, administered intravenously have been shown to be extremely efficient in liver copper depletion (Gooneratne et al., 1989). Gooneratne et al. conducted a study to determine the effects of thiomolybdates on the developing embryo. Fertilized eggs at 3 days of incubation were injected with doses of thiomolybdates ranging from 1 microgram to 500 micrograms. All doses of thiomolybdates above 1 microgram were lethal to the embryo. A dose of 1 microgram did not cause mortality or malformations; however, it did cause growth retardation. It was postulated that copper still entered the embryo but it was in the form of the copper-thiomolybdate complex. This complex reduced the availability of copper

to the embryo which, in turn, caused decreased growth in the embryo (Gooneratne et al., 1989).

Guinea Pig

A series of five experiments were conducted to evaluate the effect of molybdenum on the metabolism of molybdenum and copper in the guinea pig (Arthur, 1965). Excess molybdenum in the diet resulted in poor growth, achromotrichia, and mortality. Supplemental copper was found to alleviate achromotrichia but only partially alleviated a depressed growth rate. With the ingestion of molybdenum, an increase in molybdenum was found in blood, liver, kidney, and hair while copper content decreased in the hair. The addition of copper to the molybdenum supplemented diets showed an increase in the copper content of hair and kidney. In contrast to Arthur (1965), Smith and Wright (1975) found that supplementing molybdenum for six weeks at 100 ppm led only to increased liver molybdenum. No other effects were seen in the molybdenum supplemented guinea pig.

Equine Studies

Copper has been established as a necessary mineral in bone and cartilage development and integrity (Carbery 1978, Knight et al. 1988, Bridges et al. 1988, and Hurtig et al. 1991). Carbery (1978) evaluated a thoroughbred foal that had developed

painful swelling of the fetlocks and excess fluid in the joint capsule. The farmland where the foal was pastured caused a copper deficiency in cattle not supplemented with copper. The foal was treated with copper which relieved all pain and greatly reduced the swelling within three months.

Knight et al. (1988) conducted a trial which evaluated the effects of supplemental copper in mares on the incidence of cartilage lesions in their foals at 90 and 180 days of age. Twenty-one mares formed two groups with the first group receiving 130 mg of copper a daily. The second group of mares received 350 mg of copper daily. The copper was supplemented throughout the last trimester and lactation. Foals born to the first group of mares received a concentration with 15 ppm copper while foals born to the second group received 55 ppm supplemental copper. All foals appeared healthy and showed no outward sign of lameness while on the study. Overall, foals receiving the lower copper concentration had three times the number of cartilage lesions. Lesions were not observed in the 90-day lower copper supplemented foals. However, four of the six 180-day old foals had cartilage lesions. The study reveals a decline in cartilage lesions with supplemental copper.

Bridges et al. (1988) observed four foals that were weaned from colostrum at 1 day of age. The foals were fed a low copper diet throughout the four to seven month study. The low copper supplemented foals were compared to foals of a similar age that remained with their dams. All of the treatment foals developed lameness and were removed from the study when lameness became debilitating. The articular cartilage of

several joints in each treatment foal had multiple fractures. These findings confirm that dissecting lesions in cartilage can have their origins in copper deficient tissues.

Bone and cartilage development, based on a copper deficiency, was analyzed by Hurtig et al. (1991). A control group of foals received 30 ppm copper while a treatment group only received 7 ppm copper. After five months, foals in the control group had an average number of 0.3 lesions per foal while the treatment foals had an average of 3.1 lesions per foal. Data from this study also indicated that the copper deficient foals had reduced cross-linking collagen in cartilage and bone. With copper being an established dietary mineral needed for bone and cartilage to function properly, any interference with copper utilization needs to be recognized.

The National Research Council (NRC,1989) does not currently list a requirement for molybdenum. The NRC does report that intakes of 1 to 3 ppm molybdenum have been reported to interfere with copper utilization in ruminants; however, horses have shown a higher tolerance for molybdenum. It is stressed that higher levels of molybdenum may be tolerated as long as dietary copper is adequate. Siegmund et al. (1973) and Bridges et al. (1984) proposed that excessive levels of molybdenum may interfere with copper utilization. Bridges et al. (1984) looked at 8 thoroughbred foals in which osteochondrosis developed before weaning. Seven of these foals had serum copper and ceruloplasmin concentrations below normal. Three of the foals had serum zinc levels high enough to suggests a zinc toxicity which in turn caused a copper deficiency in those three foals. The other four horses had a normal zinc serum content paired with an extremely low serum copper content. Evidence of environmental

exposure to zinc was not found in the four foals with normal zinc serum levels. The lesions that were found by Bridges et al. (1984) in the zones of endochondral ossification of the afflicted foals were similar to those found in molybdenum-induced copper deficient rabbits (McCarter et al., 1972) and sheep (Pitt et al., 1980). Both the rabbits and the sheep in the previous studies experienced degeneration and splitting of growth-plate cartilage which was also evident in the four foals. The lesions were a result of the inhibition of the function of copper-dependent lysyl oxidase which is thought to be attributed to a molybdenum induced copper deficiency (Bridges et al., 1984).

Walsh et al. (1953) investigated 12 farms where one or more of the foals or yearlings exhibited clinical signs of rachitis. Molybdenum levels of the pasture were found to be between 5 and 25 ppm with the soils being mainly derived of limestone. Walsh et al. concluded that the foals on these pastures received an excess of molybdenum from grazing the pasture as well as from the mare's milk.

Stanier et al. (1983) collected blood samples from 39 stabled Thoroughbreds, 28 ponies on grass pasture, and 30 Arabians. The results revealed a lower molybdenum serum concentration in the ponies on grass compared to the Thoroughbreds and Arabians. Within the Thoroughbreds, differences were found in serum molybdenum concentrations among stables where the horses were boarded. Molybdenum serum concentrations obtained by Stanier et al. (1983) were similar to values from other species. The differences found between breeds and boarding facilities were attributed to different management and feeding practices between facilities used in obtaining the samples of blood. The study concluded that the differences were unlikely to affect health and fitness of the animals.

Cymbaluk et al. (1981) fed diets containing 1.01ppm (low), 27.4ppm (medium), and 107.3ppm (high) supplemental molybdenum to ponies. Feces, bile and urine were anlyzed for copper content. Horses readily absorb molybdenum from feeds containing 25 to 100 ppm, but urinary excretion effectively eliminates most of the molybdenum from the body (Cymbaluk, 1981). Fecal consistency was not affected by the addition of molybdenum to the diet (Cymbaluk, 1981). The major route for excretion of copper was through the feces which in turn reduced copper detected in the bile. Urinary copper excretion was increased with the addition of molybdenum to the diet, but was only a small portion of the copper excreted. The absorption and retention of copper was inversely related to molybdenum intake. There was not a correlation seen between plasma copper and molybdenum concentrations. However, Moore (1958) showed an accumulation of molybdenum in the equine liver.

Cape et al. (1982) conducted a study in order to draw attention to any factors other than nutrition that can cause problems when analyzing hair as an indicator of nutritional status. The study was designed to evaluate mineral content of monthly hair samples from mature young ponies fed supplemental molybdenum. Age and month did not influence molybdenum concentrations but month did alter copper concentrations of hair in the young ponies. Hair copper content decreased in young ponies during the study period. Excess molybdenum in the diet did increase hair content of molybdenum. Copper content of hair samples as well as serum copper values remained constant regardless of the supplemental molybdenum added to the diet. Many factors influence mineral content in hair; therefore, this study does not define the factors that influence

mineral levels in the hair. It does however bring to attention the susceptibility of change in mineral content within hair samples.

Short term studies showed that molybdenum in excess caused an increased copper excretion and a slight decrease in plasma copper and ceruloplasmin concentration (Schryver,1990). However, a longer term study by Strickland et al. (1987) showed little effect on plasma copper levels when 20 ppm molybdenum were added to the diet.

Ladefoged et al. (1995) evaluated a case report where fly ash, which is high in molybdenum, polluted pastures where three herds were exposed. One herd had 12 horses along with cattle and sheep and a second herd had strictly horses. The third herd had only cattle. Fly ash was blown onto the pasture due to 20,000 tons being placed near the pasture. After rainfall, parts of the pasture were submerged in water. Channels were constructed to channel the water from the ash pile and the road where it was dumped. Some animals consumed the water from these channels. A few days after the herds were placed on the contaminated pasture diarrhea, impaction and colic appeared in the horses. In a period of a couple months, 5 horses died while 4 horses aborted their foals. All animals in contact with the fly ash contaminated pasture were affected while all animals not in contact with the fly ash were healthy. Blood samples from all of the affected horses showed low hemoglobin values and high lymphocyte percentages. The absorption of molybdenum from the fly ash may be considerably higher than the absorption of molybdenum from plants. This may explain why the horses became intoxicated when normally they can graze pasture that causes a copper deficiency in ruminants. Fly ash has a pH of 10 and molybdenum availability is higher as pH increases for both plants and animals. Furthermore, the horses drank the water contaminated with fly ash. It is known

that molybdenum salts are highly soluble in water where they could be present in very high amounts. There is no measurement of molybdenum in the pasture until 6 months after the contamination and at that time the levels of molybdenum were high for that area.

The main objective of this experiment was to evaluate the effect of molybdenum on copper absorption and balance in the mature gelding. This was accomplished by analyzing the intake of copper and molybdenum as well as measuring the fecal and urinary output of copper and molybdenum. Serum copper levels were also analyzed in order to establish copper content in the blood.

CHAPTER III

MATERIALS AND METHODS

Experimental Design

Four geldings of primarily Quarter Horse breeding were randomly assigned within a 4 x 4 Latin square design experiment to study the effects of supplemental dietary molybdenum (Mo) on dietary copper (Cu) status. Horses were preconditioned for 6 weeks to equalize body condition. The 16 week trial consisted of four experimental periods each with a 21 days adaptation culminating in 3 days of collection. All horses were weighed prior to feeding on the first, eighth, and sixteenth day of each period using a standard livestock scale.

Horses were stalled individually and allowed ad libitum access to water. Horses were fed at 7 am and 7 pm. The geldings were turned out daily, weather permitting, in a dry lot for 3 hours. All horses were immunized and dewormed prior to the initiation of the trial and received standard animal health care throughout the experiment.

Treatments

Diets consisting of a pelleted base concentrate of corn, soybean meal, and cottonseed hulls prepared at the Oklahoma State University Feedmill (Table I). The concentrate was fed with native Prairie grass hay grown at the Oklahoma State University Beef Research Center. The concentrate and hay was fed at a 50:50 ratio at levels necessary to maintain constant individual body weights during the 16 week experiment. Diets were calculated to be isocaloric and isonitrogenous. Digestible energy was calculated at 2.40 (Mcal/kg) and crude protein (%) was calculated at 8.97. Treatments were formulated by supplementing the control diet with molybdenum at 5 (L), 10 (M), and 20 (H) ppm.

Venous Blood Collection

On the last morning of the 3 day collection period, the four geldings were fitted with 18-gauge jugular catheters. An area approximately four inches by 2 inches covering the jugular was shaved with #40 blades 10 to 12 inches below the throat latch. The area was then cleaned with betadine scrub. Lidocaine was used as a local anesthetic prior to the insertion of the catheter. The catheter was inserted in the jugular and taped in a fixed position with the injection cap exposed. Heparin was injected in the catheter to prevent clotting. To prevent damage to the catheter, each horse was tied in its stall in a fashion

TABLE I. COMPOSITION OF TREATMENT DIETS, AS FED BASIS

	Treatment			
Ingredient (%)	Control	Low (5ppm)	Medium (10ppm)	High (20ppm)
Shelled Corn, Ground	29.65	29.65	29.65	29.65
Soybean meal	4.75	4.75	4.75	4.75
Cottonseed Hulls	12.62	12.62	12.62	12.62
Limestone	.31	.28	.25	.20
Dicalcium Phosphate	.41	.41	.41	.41
Trace Mineral Salt[a]	.41	.41	.41	.41
Chromic Oxide	.175	.175	.175	.175
Liquid Cane Molasses	1.65	1.65	1.65	1.65
Molybdenum[b]	-----	.03	.06	.12
Prairie Grass Hay	50	50	50	50
Total	100	100	100	100

[a] Na (97%), Zn (3500 ppm), Mn (2000 ppm), Fe (2000 ppm), and Cu (300 ppm)
[b]In the form of Mighty Mo™, AmeriPac. Ca (33.8%), P (.06%), Mg (.22%), K (.04%), Na (.964%), Fe (2930 ppm), Zn (83 ppm), and Mn (324 ppm)

that allowed access to feed and water. Samples were collected hourly from seven to noon with a last collection as three p.m. Five ml of blood was drawn from each catheter to remove the heparin saline immediately prior to the morning feeding. Twenty ml of sample were drawn off and the catheter was rehaparinized. This sample was then used to fill a seven ml blood collection tube. Samples were allowed to clot at room temperature and then centrifuged for 20 minutes at 2,500 rpm after which the serum was removed and frozen for subsequent analysis of copper. The horses were fed after the time zero sample was drawn. Sampling times were spaced at intervals of five minutes between horses to ensure adequate time for sampling.

Urine Collection

Urine collection harnesses were used to measure total urine output as well as collect samples for analysis. Harnesses were designed according to Wall et al. (1992).

Beginning on the 22nd day of each experimental period, a total urine collection was taken 72 hours each gelding. The harnesses were emptied every 4 hours over the 72 hour collection period. The volume from each four-hour collection was measured and recorded for each gelding. A representative sample (10% of collected volume) was composited over time for each horse within period and treatment intervals. Each sample was labeled by horse, period, treatment, and date. The composite samples were stored frozen for later mineral analysis.

Fecal Collection

Chromic oxide was added to each diet as an indigestible marker at the rate of 3.18 kg per ton of concentrate before pelleting. Six fecal samples were collected randomly over 72 hours such that every two hours post-feeding for 12 hours were represented. Each sample was labeled by horse, date, time, period, and treatment. After labeling, the samples were frozen in freezer safe zip lock bags.

Laboratory Analysis

Feed and Fecal Mineral

Fecal samples were dried in a forced air oven at 60 degrees Celsius for 72 hours. These samples were then composited by weight, four grams per individual sample, for each horse/period/treatment interval. The feed and composite fecal samples were then ground and stored in whirl pacs.

Chromium Analysis

Oven-dried 100 ml beakers were weighed and then approximately 1.0 gram of sample (feed or fecal) was added. The beaker plus air-dried sample weight was then recorded. The beakers were placed in drying ovens at 60° C for 24 hours. The beakers were then removed and placed in a dessicator to cool. The beaker and sample was

reweighed in order to determine oven-dried sample weight. The samples were then ashed in a muffle furnace at 500° C for 6 hours. When the ashing oven cooled to 120° C, the samples were removed and placed in dessicators to cool. Once cooled, the samples were reweighed and 3.00 ml of solution A (30 mls $MnS0_4$ + 1 liter Phosphoric acid 85%) and 4.00 ml of $KBrO_3$ were added to the sample within the beaker. The beaker was covered with a watch glass and the contents digested on a hot plate. The digest was allowed to cool once effervescence ceased and a deep purple color appeared. The watch glass was then rinsed with ddH_20 back into the beaker. The beaker contents were then rinsed 3 times with ddH_20 through a funnel into a 200 ml volumetric flask. Twenty five ml of $CaCl_2$ solution were then added to the volumetric flask. The contents of the flask were brought up to 200 ml with ddH_20. The flask was then covered with parafilm, inverted 3 times, and left to settle for one hour. After settling, each sample was diluted with ddH_20 at a rate of 1:5 and then vortexed. Standards and unknown were then analyzed for chromium concentrations on an atomic absorption spectrophotometer (Model 4000, Perkin-Elmer Corp., Norwalk, CT).

Feed, Fecal and Urinary Mineral Analysis

Fecal and urine samples were analyzed for calcium, phosphorus, magnesium, potassium, sodium, iron, zinc, copper, manganese, and molybdenum. Samples were ashed in a muffle furnace at 500 degrees Celsius for 4 hours. Three mls of 6N HCL were added to the ash residue and evaporated to dryness on a 100 degree to 120 degree Celsius

hot plate. Minerals were extracted with an acid solution (1.5N HNO_3 + 0.5N HCL) and determined using an ICAP 61 (Thermo Jarrell Ash, 1994).

Blood Serum Copper Analysis

Serum was prepared for analysis by diluting with water (1:20). Copper levels were determined on an atomic absorption spectrophotometer (Model 4110ZL, Perkin-Elmer Corp., Norwalk, CT). The samples were read at a wavelength of 324.8 nm.

Statistical Analysis

Serum copper values were analyzed using the general linear model procedure for repeated measures (Table II). All other data were analyzed using a general linear model procedure with horse, period and treatment as main effects (Table III). Least squares means were calculated and differences between treatment means were detected using the pdiff procedure of SAS (1985). A minimum significance level was declared at $P<.05$.

TABLE II. ANALYSIS OF VARIANCE TABLE USED TO TEST THE MAIN EFFECTS OF HORSE, PERIOD, TREATMENT, AND TIME ON COPPER SERUM LEVELS

Source	Degrees of Freedom
Horse	3
Period	3
Treatment	3
Treatment x Time	24

TABLE III. ANALYSIS OF VARIANCE TABLE USED TO TEST THE MAIN EFFECTS OF HORSE, TREATMENT, AND PERIOD ON FECAL AND URINARY PARAMETERS.

Source	Degrees of Freedom
Horse	3
Period	3
Treatment	3

CHAPTER IV

RESULTS AND DISCUSSION

Composition of Treatment Diets

Analysis of the diet revealed a molybdenum concentration of <1 ppm molybdenum for the control with 8.8 ppm, 12.0 ppm, and 30.7 ppm molybdenum for the L, M, and H diets, respectively (Table II). Copper levels were formulated (Table I) and analyzed (Table IV) between 7 and 8 ppm for all of the experimental diets. Furthermore, diets were analyzed to contain approximately equal amounts of calcium, phosphorus, magnesium, potassium, sodium, iron, zinc, manganese, and copper (Table IV).

The National Research Council's (1989) nutrient requirements for horses at maintenance are shown on Table V. Analysis of experimental diets revealed calcium, phosphorus, magnesium, potassium, sodium, iron, and manganese were at or above NRC requirements. Diet analysis revealed a lower level of copper and zinc than is recommended by the NRC.

Dry Matter Digestibility

Fecal dry matter output, expressed in g/d, was calculated by the equation (g Cr fed/d x 100)/% Chromium in the feces (Table VI). Dry matter digestibility was

TABLE IV. FEED ANALYSIS (DRY MATTER BASIS)

	Treatment			
Item	Control	Low (5ppm)	Medium (10ppm)	High (20ppm)
Calcium %				
Concentrate	.54	.61	.54	.59
Hay	.51	.51	.51	.51
Total diet	.52	.56	.52	.55
Phosphorus %				
Concentrate	.41	.47	.39	.45
Hay	.09	.09	.09	.09
Total diet	.25	.28	.24	.27
Magnesium %				
Concentrate	.16	.17	.15	.17
Hay	.17	.17	.17	.17
Total diet	.16	.17	.16	.17
Potassium %				
Concentrate	.81	.89	.86	.92
Hay	.89	.89	.89	.89
Total diet	.85	.89	.87	.90
Sodium %				
Concentrate	.39	.43	.37	.37
Hay	.06	.06	.06	.06
Total Diet	.22	.24	.21	.21
Iron ppm				
Concentrate	362	248	224	238
Hay	204	204	204	204
Total diet	283	226	214	221
Zinc ppm				
Concentrate	29.0	36.0	28.0	30.0
Hay	31.0	31.0	31.0	31.0
Total diet	30.0	33.5	29.5	30.5
Copper ppm				
Concentrate	8.0	7.5	7.0	7.0
Hay	8.0	8.0	8.0	8.0
Total diet	8.0	7.7	7.5	7.5
Manganese ppm				
Concentrate	37.0	31.0	29.0	28.0
Hay	54.0	54.0	54.0	54.0
Total diet	45.5	42.5	41.5	41.0
Molybdenum ppm				
Concentrate	1.0	17.6	24.1	61.4
Hay	<1	<1	<1	<1
Total diet	0.5	8.8	12.0	30.7

TABLE V. ESTIMATED MINERAL REQUIREMENTS FOR HORSES*.

	Estimated Requirement for horse at maintenance (dry matter basis)
Ca %	.24
P %	.17
Mg %	.09
K %	.30
Na %	.10
Fe PPM	40
Zn PPM	40
Cu PPM	10
Mn PPM	40
Mo PPM	---

*Adapted from National Research Council, Nutrient Requirements of Horses (1989)

calculated by dividing grams of DM fecal output by the grams of DM intake per day. Horses were fed at a 1.7 percent body weight throughout the trial. No refusals of concentrate or hay were noted on any diet. Intakes for individual horses ranged from 7.7 to 8.8 kg per day (Appendix Tables I through IV).

Fecal output for the control, L, M and H diets were 2479, 2368, 2257, and 2092 grams per day, respectively. The control diet had a higher ($p<.05$) fecal output than the H diet. The decrease in the fecal output for the H diet was due to a large decrease in fecal output for horse number three on the H feed versus all other feeds (Appendix Tables I through IV). This tendency was not seen among all other horses. The decrease in fecal output for horse three could be an artifact of laboratory and experimental error.

Dry matter digestibility for the control, L, M, and H diets were 70, 71, 72, and 74 percent, respectively. There was an increase ($p<.05$) in dry matter digestibility between the control and the H diet. This decrease in dry matter digestibility was due to the large decrease in fecal output calculated for horse number three while on the high feed. This tendency was not seen among all other horses and could be due to laboratory and experimental error. This increase in dry matter digestibility is contradictory to other results obtained where increase digestibility would have increased growth rate in cattle. No increase in weight gain was seen in any of the horses with constant intakes (Appendix Table V).

Serum Copper

Serum copper levels are shown on Table VII. There was no difference ($p>.05$) in serum copper levels over time or time by treatment due to supplemental molybdenum fed. This

is in agreement with Arthur (1965) and Smith and Wright (1975) who found no effect on copper serum levels when molybdenum was added to the diet of guinea pigs. Cymbaluk et al. (1981) also found no decrease in copper serum levels in ponies when 1.01 ppm to 107.3 ppm molybdenum was added to the diet. Cape et al. (1982) and Strickland et al. (1987) also obtained results showing no correlation between the amounts of molybdenum added to the diet and a decrease in plasma copper concentrations in the equine. In contrast, Shryver (1990) reported that short term studies have revealed a slight decrease in plasma copper and ceruloplasmin concentrations due to excess molybdenum. Gengelbach et al. (1994) found in heifers that molybdenum interfered with copper metabolism after parturition. Heifers were unable to become copper replete and the serum copper deficiency was also passed to the calves.

Absorbed copper becomes loosely bound to serum albumin and amino acids and is thereby transported throughout the body and stored in the liver. Newly absorbed copper disappears rapidly from the plasma into the liver. Copper is released from the cellular and subcellular fractions of the liver primarily for hepatic synthesis of ceruloplasmin (McDowell, 1992). Nearly 90 percent of the copper in plasma is in the form of the copper metalloprotein, ceruloplasmin. Ceruloplasmin is the carrier for the tissue-specific export of copper from the liver to the target organ (McDowell, 1992). It has been noted from experiments with ruminants that a relatively high dietary content of molybdenum frequently increases serum copper content while it ultimately decreases liver copper and provokes clinical signs of a copper deficiency (McDowell, 1992). The increased copper content in serum is due to the formation of a thiomolybdate. The reduction of sulfate to sulfide occurs in the rumen (McDowell, 1992). Molybdenum

reacts with this sulfide to form thiomolybdate. Thiomolybdate (MoS_4^{2-}) is more readily absorbed than the oxygen analog, molybdate (MoO_4^{2-}). Thiomolybdate forms the highly insoluble and therefore non-utilizable copper thiomolybdate, $CuMoS_4$ McDowell, 1992). Plasma copper increases in the ruminant because of the formation of the copper thiomolybdate complex. Allen and Gawthorne (1987) discussed a mechanism where tetrathiomolybdate prolongs the retention of copper by plasma, limits its incorporation into the liver and modifies its distribution among proteins of the kidney. The marked difference in species plasma copper content in response to molybdenum may be attributed to the probability that the digestive tract of the ruminant particularly favors thiomolybdate and or tetrathiomolybdate synthesis. The rumen is rich in sulfate needed for the synthesis of the thiomolybdate compound. Since absorption of minerals occurs mainly in the small intestine of the ruminant, the thiomolybdate compound is formed prior to absorption of minerals. This allows the binding of copper by the thiomolybdate compound before the copper is absorbed in the blood. In the horse, copper is absorbed in the small intestine. The small intestine precedes the cecum, where sulfate is present. Therefore, the horse absorbs copper into the blood before it can be bound and made unavailable.

TABLE VI. THE EFFECT OF SUPPLEMENTAL MOLYBDENUM ON DRY MATTER DIGESTIBILITY IN THE MATURE GELDING [a]

	Treatment				
	Control	5 ppm	10 ppm	20 ppm	S.E.
DM Digestibility %	70[b]	71[b,c]	72[b,c]	74[c]	1.19
Fecal Output g/d	2479[b]	2368[b,c]	2257[b,c]	2092[c]	97.74

[a] Values are least squares means ± SE.
[b,c] Means in rows with different superscripts differ ($p<.05$)

TABLE VII. THE EFFECTS OF SUPPLEMENTAL MOLYBDENUM ON SERUM COPPER LEVELS IN THE MATURE GELDING[a,b]

	Treatment				
Time	Control	5 ppm	10 ppm	20 ppm	S.E.
7am	.8825	.8075	.8000	.8000	.0797
8am	.8225	.5800	.7200	.8525	.0797
9am	.7575	.8225	.8950	.8300	.0797
10am	.7875	.6525	.7825	.6525	.0797
11am	.6575	.8050	.7825	.8400	.0797
12pm	.6875	.8500	.6725	.7125	.0797
3pm	.7150	.7700	.7000	.8250	.0797

[a] Values are least squares means $\pm$ SE.
[b] Means within a row did not differ ($p>.05$)

Urinary Excretion of Minerals

Urinary excretion of calcium, phosphorus, magnesium, potassium, sodium, iron, zinc, and manganese did not differ ($p>.05$) between diets. Urinary excretion of copper also did not differ ($p>.05$) among diets. Intermediate quantities of copper consumed in the diet are excreted in the urine (McDowell, 1992). In contrast, Cymbaluk et al. (1981) found a slight increase in copper excretion following the addition of molybdenum to the diet. Urinary excretion of molybdenum was higher ($p<.05$) in horses fed the high molybdenum diet versus the control, low and medium molybdenum diets (Table VIII). This is in agreement with Cymbaluk et al. (1981) who found that horses readily absorb molybdenum from feeds containing 25 to 100 ppm. Cymbaluk et al. (1981) found that urinary excretion was effective in eliminating most of the absorbed molybdenum from the body. McDowell (1992)found that there is little storage of molybdenum, with the element being present in low levels in all tissues. Most of the element is stored in the liver and bones. Molybdenum is not only rapidly absorbed, but also rapidly excreted. Urinary excretion is the an efficient route of elimination of excess molybdenum. ppm molybdenum, even much higher amounts of molybdenum don't appear to have any detrimental effect in horses.

Fecal Excretion of Minerals

Fecal excretion of calcium, phosphorus, magnesium, potassium, sodium, iron, zinc, and manganese did not differ ($p>.05$) among diets (Table IX). Molybdenum also

did not effect ($p>.05$) fecal excretion of copper based on diets fed. Cymbaluk et al. (1981) found that molybdenum caused an increase in the excretion of the isotope ^{64}Cu in feces; and consequently, a reduction in ^{64}Cu was detected in the bile. However, it was found that the percentage of absorbed isotope (^{64}Cu) excreted in the bile did not change with molybdenum ingestion. Copper that is absorbed in the diet is absorbed into the body through the intestinal mucosa, transported by the portal blood to the liver where it is incorporated into ceruloplasmin. Most of the endogenous copper is excreted into the intestinal tract and excreted along with the unabsorbed copper in the feces. Gooneratne et al. (1989) reported that a high proportion of ingested copper appears in the feces. Most of this copper is unabsorbed with active excretion occurring via the bile.

Molybdenum fecal excretion increased ($p<.05$) with increasing amounts of molybdenum fed. The increase in molybdenum excreted in the feces implies that molybdenum is not being absorbed from the horse as it is consumed in excess.

Mineral Balance in the Mature Gelding

Molybdenum was the only mineral that revealed a difference ($p<.05$) in mineral balance when looking at the various diets (Table X). The control diet (0 ppm) had the lowest molybdenum balance ($p<.05$) with the high diet (20 ppm) having the highest balance ($p<.05$). There was no difference ($p>.05$) in molybdenum balance between the 5 ppm and 10 ppm molybdenum diets (Table X). Although molybdenum had a higher balance on the high (20 ppm) diet, the excess molybdenum consumed by the horse was not a sufficient amount to cause a decrease in the copper balance of the horse. This is in

agreement with Lewis (1995) who states that although copper absorption and balance in ruminants is decreased by as little as 1 to 3 ppm molybdenum, much higher amounts of molybdenum don't appear to have any detrimental effect in horses.

TABLE VIII. THE EFFECTS OF SUPPLEMENTAL MOLYBDENUM ON URINARY EXCRETION OF MINERALS IN THE MATURE GELDING[a]

	Treatment				
Mineral g/d	Control	5 ppm	10 ppm	20 ppm	S.E.
Calcium	13.3058	11.0408	12.3552	14.4742	1.5828
Phosphorus	.0637	.071	.0766	.0558	.0072
Magnesium	2.8456	208156	2.9972	2.603	.2467
Potassium	50.8124	38.5234	52.4582	43.1166	5.4656
Sodium	4.8968	4.7669	4.8172	3.3931	1.2323
Iron	.0109	.0097	.0225	.0088	.0043
Zinc	.0052	.0044	.0052	.004	.0007
Copper	.0005	.0005	.0009	.0006	.0001
Manganese	.0008	.0007	.0012	.0007	.0002
Molybdenum	.0019[b]	.0023[b]	.0027[b]	.0047[c]	.0006

[a] Values are least squares means $\pm$ SE.
[b,c] Means in rows with different superscripts differ ($p<.05$)

TABLE IX. THE EFFECTS OF SUPPLEMENTAL MOLYBDENUM ON FECAL EXCRETION OF MINERALS IN THE Mature Gelding[a]

	Treatment				
Mineral g/d	Control	5 ppm	10 ppm	20 ppm	S.E.
Calcium	14.719	13.444	12.042	13.645	.768
Phosphorus	14.276	14.151	12.001	12.971	.795
Magnesium	6.368	5.909	5.642	5.730	.283
Potassium	10.708	10.582	10.810	11.463	1.038
Sodium	11.559	12.527	9.449	9.071	.981
Iron	1.844	1.433	1.549	1.495	.108
Zinc	.137	.110	.117	.115	.012
Copper	.043	.029	.027	.024	.005
Manganese	.275	.219	.225	.241	.011
Molybdenum	.009[b]	.033[c]	.046[d]	.088[e]	.002

[a] Values are least squares means $\pm$ SE.
[b,c,d,e] Means in rows with different superscripts differ ($p<.05$)

TABLE X. THE EFFECTS OF SUPPLEMENTAL MOLYBDENUM ON MINERAL BALANCE IN THE MATURE GELDING[a]

	Treatment				
Mineral g/d	Control	5 ppm	10 ppm	20 ppm	S.E.
Calcium	15.8024	22.2041	19.4314	17.7523	1.9199
Phosphorus	24.1779	26.7463	25.6215	27.1250	.8399
Magnesium	19.0869	19.9859	19.2542	20.3774	.3635
Potassium	-6.657	9.0242	-6.3645	4.7757	4.8411
Sodium	21.2850	22.1222	22.7389	24.7045	1.5842
Iron	.4956	.4407	.2134	.3384	.1109
Zinc	.8467	.9027	.8619	.8738	.0120
Copper	.8604	.8753	.8707	.8745	.0056
Manganese	.7468	.7773	.7621	.7432	.0110
Molybdenum	.8641[b]	.9064[c]	.9198[c]	1.0281[d]	.0056

[a] Values are least squares means $\pm$ SE.
[b,c,d] Means in rows with different superscripts differ ($p<.05$)

SUMMARY AND CONCLUSIONS

In summary, previous research reveals an interaction between molybdenum and copper in the ruminant causing a copper deficient state. A copper: molybdenum ratio of less than 2:1 causes a decrease in copper absorption in the ruminant. These levels of molybdenum do not appear to have a detrimental effect on the horse. High molybdenum pastures, which result in copper deficiency in cattle, do not effect horses grazing these pastures (Schryver and Hintz, 1983). A molybdenum level of 20 ppm dry matter did not decrease horses plasma copper concentration or appear to interfere with the utilization of copper in the present study. Much of the excess molybdenum ingested by horses is absorbed and rapidly excreted in the urine, with a half life of only 7 to 10 hours (Lewis, 1995). The excess molybdenum ingested by horses doesn't remain in the intestinal tract to interfere with the absorption of other minerals. The persistent protein-bound thiomolybdate, which forms in the rumen of animals consuming excess molybdenum, does not appear to form in horses when excess molybdenum is consumed (Strickland et al., 1987). However, Cymbaluk et al. (1981) found that a copper: molybdenum ratio of 1:8 with 107 ppm molybdenum fed to ponies did decrease the absorption and retention of copper.

The copper content of horse feeds ranges from 0.5 to 35 ppm (Lewis, 1995). The amount of copper recommended by the National Research Council (1989) is 10 mg/kg (ppm) in the diet dry matter for all classes of horses. However, it has been found that mature ponies maintained copper homeostasis when 3.5 mg/kg were fed (Cymbaluk et al. 1981b). Baucus et al. (1989) found that 4 ppm copper was sufficient to maintain copper status in serum and milk in lactating mares as well as the serum copper level of their foals. The foals had no detected effects due to the lower amount of copper fed. A copper deficiency is not common in the mature horse even though horse feeds commonly contain 4 to 10 ppm copper (Lewis, 1995). It appears the NRC may have a high recommendation for the amount of copper needed in the diet. With a possibly high recommendation of copper being fed to mature horses, there may already be an excess of copper to combat any effects that high levels (over 20 ppm) of molybdenum may have on the utilization of copper.

The experiment was designed in a manner which has become evident to allow for potential error in copper and molybdenum intakes as well as fecal output and dry matter digestibility. Copper and molybdenum intakes may have been altered in several ways. First, the water was not analyzed for mineral content. Second, the soil in the turn-out area was not tested for minerals. There is potential contamination of the soil in the turn-out area by the fecal matter of other horses allowed access to that area. Third, the horses were bedded in shavings which may have been consumed. The mineral content of the shavings was also not analyzed. Last, horses will practice coprophagy which was not accounted for in respect to mineral intakes. The mineral content that was actually

ingested by the horses can be altered by the above mentioned events. This could have had an impact on the outcome of the experiment.

The dry matter digestibility is of concern in this experiment. Fecal output was measured by the addition of chromic oxide to the feed. It is unknown if chromic oxide can alter the absorption or detection of copper and molybdenum. Collecting and weighing all fecal material would give a more exact fecal output. Dry matter digestibility would also increase in accuracy with the fecal output. A liver biopsy would be an accurate analyses of the retention of copper.

The differences in dry matter digestibility and fecal output seen between the control and high diet are due to an unduplicated decrease in fecal output for horse three on the high diet. Cymbaluk et al. (1981) found a 27.4 ppm molybdenum diet to have a dry matter digestibility of 74 percent. This is in agreement with the findings of this experiment. However, the dry matter digestibility for the basal ration of Cymbaluk et al. (1981) was 75 percent. Addition of molybdenum to the diet decreased their dry matter digestibility. The findings of this study show an increase in dry matter digestibility with the addition of molybdenum to the diet. A more accurate collection of fecal output is necessary to explain the significant increase in dry matter digestibility coupled with the decrease in fecal output.

LITERATURE CITED

Allen, J.D., and J.M. Gawthorne. 1987. In "Trace Element Metabolism in Man and Animals. Plenum Press, New York.

Arthur, D. 1965. Interrelationships of molybdenum and copper in the diet of the guinea pig. J. Nutr. 87:69-76.

Baucus, K.L., S.L. Ralston, G.A. Rich and E.L. Squires. 1989. The effect of copper and zinc supplementation on mineral content of mares milk. Eq. Vet. Sci. 9: 4, 206-209.

Becker, R.B., J.R.Henderson, and R.B. Leighty. 1965. Mineral malnutrition in cattle. Bull. Fla. Agr. Exp. Stn. 699.

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

Bridges, C.H. and E.D. Harris. 1988. Experimentally induced cartilage fractures (osteochondritis dissecans) in foals fed low copper diets. JAVMA. 193: 2, 215-221.

Bridges, C.H., J.E. Womack, E.D. Harris, et al. 1984. Considerations of copper metabolism in osteochondrosis of suckling foals. JAVMA. 185:173-178.

Cape, L., and H.F. Hintz. 1982. The influence of month, color, age, corticosteroids, and dietary molybdenum on mineral concentration of equine hair. Am. J. Vet. Res. 43: 7, 1132-1136.

Carberry, J.T. 1978. Osteodysgenesis in a foal associated with copper deficiency. N.Z. Vet. J. 26, 279.

Cunningham, I.J. 1950. Copper and molybdenum in relation to disease of cattle and sheep in New Zealand. In Copper Metabolism [Edited by McElroy W.D. and Glass, B.] Baltimore, MD: Johns Hopkins.

Cymbaluk, N.F., H.F. Schryver, H.F. Hintz, et al. 1981. Influence of dietary molybdenum on copper metabolism in ponies. J. Nutr. 3(1):96-106.

Cymbaluk, N.F., H.F. Schryver, H.F. Hintz. 1981b. Copper metabolism and requirement in mature ponies. J. Nutr. 111:87-95.

Dowdy, R.P. and G. Matrone. 1968. Copper-molybdenum interaction in sheep and chicks. J. Nutr. 95: 191-196.

Dick, A.T. 1956a. Molybdenum and coper relationships in animal nutrition. In Inorganic Nitrogen Metabolism. [Edited by McElroy, W.D. and Galss, B.] 230-245: Baltimore: Johns Hopkins.

Dick, A.T. 1956b. Preliminary observations on the effect of high intakes of molybdenum and of inorganic sulfates and blood copper in sheep. Aust. Vet. J. 7, 197-202.

Dick, A.T., D.W. Dewey and J.M. Gawthorne, 1975. Thiomolybdates and the coppper-molybdenum-sulphur interaction in ruminant nutrition. J. Agric. Sci. (Camb.) 85: 567-568.

Furguson, W.S., A.H. Lewis, and S.J. Watson. 1938. Action of molybdenum in nutrition of milking cattle. Nature. 141(1)553.

Furguson, W.S., A.H. Lewis, and S.J. Watson. 1943. The teart pasture of Somerset. J. Agric. Sci. Camb. 33:41-51.

Gawthorne, J.M. 1985. Trace Element interactions, with special reference to copper. In Ruminant Physiology-Concepts and Consequences. University of Western Australia, Perth, Australia.

Gengelbach, G.P., J.D. Ward, and J.W. Spears. 1994. Effects of dietary copper, iron, and molybdenum on growth and copper status of beef cows and calves. J. Anim. Sci. 72: 2722-2727.

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

Gooneratne, S.R., D.A. Christensen, and B.S. Wenger. 1989. Effects of thiomolybdates in chick embryos. Nutrition Dep. Int. 40(1):85-93.

Hurtig, M.B., S.L. Green, H. Dobson, and J. Burton. 1991. Defective bone and cartilage in foals fed low copper diet. Am. Assoc. Equine. Pract. Proc. 637-643.

Kelleher, C.A., M. Evan, M. Lamand and J. Mason. 1983. The absorption of labelled Molybdenum compounds in sheep fitted with reentrant cannulae in the ascending duodenum. J. Comp. Pathol. 93:83-92.

Knight, D.A., S.E. Weisbrode, L.M. Schmall, and A.A. Gabel. 1988. Copper Supplementation and cartilage lesions in foals. Am. Assoc. Equine Pract. Proc. 191-194.

Ladefoged, O. and S. Sturup. 1995. Copper deficiency in cattle, sheep, and horses caused by excess molybdenum from fly ash: a case report. Vet. Hum. Toxicol. 37: 1. 63-65.

Lewis, L.D. 1995. Equine Clinical Nutrition: Feeding and Care. Williams and Wilkins, Media, PA.

Marston, H.R. 1950. Problems associated with copper deficiency in ruminants. In Copper Metabolism. Baltimore: Johns Hopkins. 230-245.

Mason, J., C. A. Kelleher, and J. Letters. 1982. The demonstration of protein-bound 99Molybdenum-di and trithiomolybdates in sheep plasma after the infusion of 99molybdenum labeled molybdate into the rumen. Br. J. Nutr. 48:391-397.

Mason, H.S. 1966. Preliminary remarks on polyphenoloxidase. The Biochemistry of Copper.339-341.

Mason, J., M. Lamand, J.C. Tressol, and C. Lab. 1978. The influence of dietary sulfur, molybdate, and copper on the absorption, excretion, and plasma fraction levels of ^{99}Mo in sheep. Ann. Vet. Res. 9:577-586.

McCarter, A., P.E. Riddell, G.A. Robinson. 1972. Molybdenosis induced in laboratory rabbits. Can. J. Biochem. 40:1415.

McDowell, L.R. 1992. Minerals in Animal and Human Nutrition. Academic Press, Inc., San Diego.

Mills, C.F. 1983. Trace elements in animal and veterinary practice. British Society for Animal Production, Occasional Publication No. 7.

Mills, C.F. and G.K. Davis, 1987. Molybdenum in Trace Elements in Human and Animal Nutrition-Fifth Edition. 429-463.

Moore, P.A. 1958. Molybdenum content of equine liver tissue. Nature. 182, 1175-1176.

National Resource Council: Mineral Tolerance of Domestic Animals. Washington DC, National Academy of Sciences, 1989.

Pitt, M., J. Frasier, D.C. Thurley. 1980. Molybdenum toxicity in sheep: epiphysiolysis exostoses and biochemical changes. J. Comp. Pathol. 90:567-576.

Schryver, H.F. 1990. Mineral and vitamin intoxication in horses. Clin. Nutr. 6: 2, 295-318.

Schryver, H.F. and H.F. Hintz. 1983. Minerals. In " Current Therapy in Equine Medicine. Philedelphia. 71-84.

Sharma, A.K. and N.S. Parihar. 1994. Clinicopathology of induced molybdenum toxicity in young goats. Indian J. of Anim. Sci. 64 (2): 120-125.

Shatz, J. 1990. Rewritten and modified Perkins-Elmer methodology handbook. Oklahoma State University.

Seigmund, O.H., C.M. Frasier eds. 1973. The Merck Veterinary Manuel, 4th Ed. Merck and Co Inc., Rahway NJ.

Smith, B. 1973. Copper and molybdenum- a delicate balance. N.Z. J. of Agr. 126(3)

Smith, S.W. and H. Wright. 1975. Effect of dietary molybdenum on copper metabolism. Evidence of the involvement of molybdenum in abnormal binding of copper to plasma protein. Clin. Chem. Actu. 62:55.

Stanier, P., and D.J. Blackmore. 1983. Molybdenum concentrations in equine serum. Vet. Rec. 113, 518.

Strickland, K., F. Smith, M. Woods, and J. Mason. 1987. Dietary molybdenum as a putative copper antagonist in the horse. Equine Vet. J. 19:1, 50-54.

Suttle, N.F. 1975. Changes in the availability of dietary copper to young lambs with age and weaning. J. Agric. Sci. (Camb.) 84; 225-229.

Suttle, N.F. and D.G. Jones. 1989. J. Nutr. 119, 1055.

Thermo Jarrell Ash. 1994. The Spectroscopist. 3(1).

Underwood, E.J. 1981. The mineral nutrition of livestock. Commonwealth Agricultural Bureaux, London, England.

Vanderveen, J.E. and H.A. Keener. 1964. Effects of molybdenum and sulfate sulfur on metabolism of copper in dairy cattle. J. Dairy Sci. 40:1224-1230.

Walsh, T., and L.B. Moore. 1953. Excess of molybdenum in herbage as a possible contributing factor in equine osteodystrophies. Nature. 171, 166.

Ward, J.D., G.P. Gengelback, and J.W. Spears. 1997. The effects of copper deficiency with or without high dietary iron or molybdenum on immune function of cattle. J. Anim. Sci. 75:1400-1408.

Wooliams, C., N.F. Suttle, J.A. Williams, D.G. Jones, and G. Wiener. 1986. Anim. Prod. 43, 293.

Wynne, K.N. and G.L. McClymont. 1956. Copper-molybdenum-sulphate interactions in induction of ovine hypocupraemia and hypocuprosis. Aust. J. Agr. Res. 7, 45-56.

APPENDIX

TABLE I: INTAKE, FECAL OUTPUT, AND DRY MATTER DIGESTIBILITY FOR MATURE GELDINGS CONSUMING THE CONTROL DIET

Horse	Intake (g/d)	Fecal Output (g/d)	Dry Matter Digestibility (%)
1	8096	2537	68.7
2	7738	2892	62.6
3	8832	2398	72.8
4	8758	2090	76.1
Average	8356	2479	70.1

TABLE II: INTAKE, FECAL OUTPUT, AND DRY MATTER DIGESTIBILITY FOR MATURE GELDINGS CONSUMING THE LOW DIET

Horse	Intake (g/d)	Fecal Output (g/d)	Dry Matter Digestibility
1	8096	2487	69.3
2	7738	2714	64.9
3	8832	2203	75
4	8758	2066	76.4
Average	8356	2367	71.4

TABLE III: INTAKE, FECAL OUTPUT, AND DRY MATTER DIGESTIBILITY FOR MATURE GELDINGS CONSUMING THE MEDIUM DIET

Horse	Intake (g/d)	Fecal Output (g/d)	Dry Matter Digestibility
1	8096	2442	69.8
2	7738	2104	72.8
3	8832	2554	71.1
4	8758	1930	78
Average	8356	2257	72.9

TABLE IV: INTAKE, FECAL OUTPUT, AND DRY MATTER DIGESTIBILITY FOR MATURE GELDINGS CONSUMING THE HIGH DIET

Horse	Intake (g/d)	Fecal Output (g/d)	Dry Matter Digestibility
1	8096	2300	71.6
2	7738	2404	68.9
3	8832	1813	79.5
4	8758	1850	78.9
Average	8356	2257	74.4

VITA

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Master of Science

Thesis: THE EFFECT OF SUPPLMENTAL MOLYBDENUM ON COPPER BALANCE IN MATURE GELDINGS

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