INFLUENCE OF MOLYBDENUM AND SULFATE SUPPLEMENTATION AND WITHDRAWAL OF DIETS CONTAINING HIGH COPPER BROILER LITTER ON TISSUE COPPER LEVELS

by

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INTRODUCTION

The disposal of poultry waste is a problem since soil application of the waste is not always economical. Animal wastes have been shown to be valuable sources of protein and energy for ruminants and many successful feeding trials have been conducted. One of the potential hazards associated with feeding wastes is the excess mineral content they frequently contain. For example, feeding $CuSO_4$ to growing chickens yields a waste product high in Cu.

Sheep are extremely susceptible to copper toxicity. Numerous invesiagators have shown that Cu accumulation in the liver is directly related to the amount of dietary Cu. Feeding high Cu broiler litter to gestating and lactating ewes resulted in many fatalities from Cu poisoning. Some researchers have found that feeding high Cu broiler litter has resulted in hepatic accumulation of Cu but without overt Cu toxicity symptoms.

Molybdenum and SO_4 have been shown to be antagonistic to Cu utilization. Research has indicated excess Mo and SO_4 may induce Cu deficiency on otherwise Cu adequate diets. Some work has also been completed on deterring Cu toxicity from high Cu concentrates fed to growing lambs by supplementing Mo and SO_4.

This study had two objectives. One trial was conducted to determine the effect of Mo and SO_4 supplementation to

diets containing high Cu broiler litter on ovine tissue Cu levels. The second trial investigated whether withdrawal of the broiler litter from the diet would be accompanied by reduction of excessive liver Cu.

REVIEW OF LITERATURE

Two billion metric tons of animal waste are produced per year in the U.S.A., 50% of which is in confinement housing, and therefore collectible (Fontenot, 1981). In the past, poultry wastes have been spread on pastures and crops as a potential nutrient source and means of disposal. With the high degree of urbanization, fewer fields are available near poultry houses. Furthermore, economic studies indicate the plant nutrient content does not always justify the cost of handling and spreading (Fontenot and Webb, 1975). Livestock producers are continually searching for low cost alternative feeds. Therefore, a potentially more feasible means of disposal is feeding the waste.

Broiler Litter

Broiler litter is the material in broiler houses after as long as a year of growing many crops of broiler chickens. It is composed of excreta, bedding, wasted feed, and feathers.

Processing by Deepstacking

Numerous processing methods have been developed to remove pathogenic organisms from animal waste. The simplest and least expensive is deep stacking the waste. This

involves removing the waste from the broiler house and stacking it (approximately 1.2 m deep) in an open, but roofed structure. Hovatter et al. (1979) conducted experiments comparing deep stacked broiler litter to ensiled broiler litter. They found that after 1 wk the temperature of the deepstack litter was sufficient to kill all coliforms.

Nutritional Value

Poultry litter is very high in crude protein. Bhattacharya and Taylor (1975), in a survey of waste research, reported an average crude protein value for broiler litter of 31.3%, dry basis. True protein constitutes about 50% of the nitrogen, and the majority of nonprotein nitrogen is uric acid, a NPN source efficiently utilized by ruminants (Fontenot, 1981). Crude protein digestibility for broiler litter ranges from 75 to 82% (Bhattacharya and Taylor, 1975), indicating that the litter serves as a highly available nitrogen source. Broiler litter is also a valuable energy source, being equivalent to good quality alfalfa hay (Bhattacharya and Taylor, 1975). The digestible energy content is about 2,440 kcal/kg.

Ash content comprises about 15% of the broiler litter (Bhattacharya and Taylor, 1975). They reported an average calcium content of 2.37% and a phosphorous content of 1.8%. However, the remaining 10% includes many microminerals,

often in concentrations in excess of need.

Since sheep are highly sensitive to Cu (Bremner, 1979) the excessive levels of Cu in broiler litter cause concern when feeding broiler litter to sheep (Fontenot and Webb, 1975). The Cu content of broiler litter is normally excessive considering the Cu requirement for sheep is 5 ppm with a maximum tolerable level of 25 ppm Cu although toxicity has been observed at 8 to 11 ppm Cu (NRC, 1980). Copper content in broiler litter has been quantified as 30.7, 79.5, 195, 451 and 593 mg/kg DM (Caper et al., 1978; Suttle et al., 1978; Fontenot et al., 1972; El-Sabban et al., 1969; and Westing et al., 1979, respectively). The high variability in Cu content of broiler waste is due to the frequency and level of $CuSO_4$ administered to the birds. Webb and Fontenot (1975) reported an average of 255 ppm Cu, dry basis, if $CuSO_4$ was supplemented to the birds and 50.8 ppm Cu when no supplemental $CuSO_4$ was used.

Copper Toxicity in Sheep

Poultry Litter

Fontenot et al. (1972) observed sheep fatalities which were directly related to Cu toxicity when feeding broiler litter. In their study, 64% of the ewes fed 50% poultry litter died by 254 d (litter contained 195 ppm Cu). Fifty-five percent of the ewes fed a 25% poultry litter died. These ewes were placed on the broiler litter diets

approximately 6 wk prior to lambing and were kept on litter throughout lactation. Feeding litter did not affect the number of lambs born or their weight at birth. Liver Cu levels were significantly elevated in the litter-fed ewes (control, 572 ppm; 25% litter, 1993 ppm; 50% litter, 2671 ppm, dry basis).

Van Ryssen and Jagoe (1981) conducted a study in which yearling rams were fed 0, 11.6, 23.5 or 35.4% dried battery waste (46 ppm Cu). The Cu and Zn content of the liver after 60 d on the diets did not increase,even at the highest level of battery waste. They postulated high levels of minerals antagonistic to Cu and Zn metabolism decreased the availability of Cu and Zn with the increased battery waste consumption.

Suttle et al. (1978) fed newly weaned lambs diets containing 15, 30, 45 and 60% battery or broiler waste (50.5 or 79.5 ppm Cu, respectively) for 16 wk. The liver Cu concentrations increased with inclusion of the poultry waste, although not in proportion to the inclusion rate, due to a linear decrease in Cu retention as the broiler waste increased. The experiment was curtailed because of health problems. Although the problems included subclinical copper toxicity, more pressing were problems of ascites (accumulation of serous fluids in peritoneal cavity) and anorexia, described in detail by Angus et al. (1978). Extensive lesions were observed in the liver and kidney of

the lambs. The incidence was proportional to inclusion rate of battery waste, but not related to liver Cu level. Lesions were not observed in the broiler litter fed animals. No evidence of mycotoxicosis was found. Hepatic clinico-pathological symptoms suggested involvement of dimethylnitrosamines, although only limited quantities of the volatile nitrosamines were found in battery waste. They suggeted kidney damage was indicative of excretion of an abnormal product, which would increase susceptibility to kidney infection. They suggested there are pathogenic mechanisms inherent to feeding of battery wastes which are not related to Cu toxicity and are not present in broiler litter.

In contrast to the problem encountered in sheep high Cu broiler wastes have been sucessfully fed to beef cows for seven consecutive wintering periods with no adverse effects (Webb et al., 1980). Biopsy sampling of the cattle indicated that liver Cu levels were reduced dramatically each summer after a period of low Cu intake.

Etiology

Sheep are the mammals most susceptible to high dietary Cu (Owen, 1981a). Acute toxicity occasionally occurs in the field in sheep drinking $CuSO_4$ foot baths or inadvertent drenching, although chronic Cu toxicity is more common. Chronic Cu poisoning appears to be a two-step process.

First, there is passive accumulation of Cu over an extended period, then the sudden release of Cu stores termed hemolytic crisis (Bremner, 1974). In most breeds death ensues in a few days, although the Merino breed may survive the crisis. Copper poisoning normally occurs when liver Cu levels exceed 500 ppm and kidney cortex exceeds 80 to 100 ppm Cu, dry basis. Signs of toxicity include hemolysis, jaundice, and hemoglobinuria which results in dark colored liver and kidneys. Serum Cu levels will exceed the normal concentration of .7 to 1.3 ppm (NRC, 1980).

The accumulation of Cu is dependent on dietary Cu content but also influenced by other dietary factors (Weber et al., 1980). Suttle (1973) reported Cu deficiency or toxicity have occurred at similar Cu intake. Dietary factors which influence Cu utilization include antagonistic minerals such as S, Mo, Zn, and Ca and other chemical compounds such as phytate (Weber et al., 1980).

Blood Parameters

Buckley and Tait (1981) completed a survey of 18 blood parameters to determine accurate indicators of high Cu status in lambs. The study was conducted with newly weaned lambs placed on high Cu diets for 10 wk. Analyses included plasma Cu, superoxide dismutase, ferroxidase, allantoin, catalase, Mo, hemoglobin, hematocrit and cholesterol. The only blood constituent which was indicative of excessive

liver Cu levels was plasma aspartate aminotransferase. Although liver Cu of the lambs exceeded 1000 ppm Cu, dry basis, none of the lambs reached the point of hemolytic crisis.

Todd and Thompson (1963) followed blood parameters indicative of Cu toxicity in ovine. Their investigation was conducted through hemolytic crisis. A sharp increase in blood Cu was observed 24 to 48 h prior to the onset of hemolytic crisis. During the occurance of clinical symptoms the blood Cu ranged from 5 to 20 mg/liter. It was determined by in vitro work that a concentration of .40 to .50 mg/liter Cu caused rupture of erythrocytes. If the cells were suspended in serum, hemolysis did not occur until the Cu content reached 50 mg/liter. This observation suggests that circulating Cu levels in vivo may not be the primary cause of hemolysis during Cu toxicity since only a very small precentage of the Cu is not bound to circulating proteins. Therefore an ionic imbalance may not be the cause of hemolysis occurring during hemolytic crisis.

An increase in hematocrit and hemoglobin was noted prior to hemolytic crisis indicated either an elevation in erythrocyte number or a decrease in blood volume. At the onset of the crisis packed cell volume and hemoglobin suddenly decreased. Serum glutamic oxalacetic transaminase (SGOT) activity was increased 6 to 8 wk prior to hemolytic crisis and then fluctuated until the onset of hemolysis at

which time it rose sharply. These results suggest that liver damage preceded hemolytic crisis by 6 to 8 wk with the greatest severity at hemolytic crisis. They also suggested that hemolysis was not precipitated by the increase in circulating Cu but perhaps was more readily correlated to the lower hemoglobin levels and depressed blood glutathione (Todd and Thompson, 1963).

Physiology and Biochemistry

The ovine susceptibility to Cu poisoning is due to an inability to excrete the excess Cu accumulated in the liver. Weber et al. (1980) proposed that the sheep liver has a multifunctional role including biliary Cu excretion, incorporation of Cu into ceruloplasmin and storage of Cu. Biliary excretion was found to be .13 mg Cu/d (Weber et al., 1980), regardless of liver Cu concentration (Saylor and Leach, 1980). Thus, sheep do not have the capacity to regulate Cu homeostasis through biliary excretion. They are similar to humans suffering from Wilson's disease in which there is no biliary excretion of Cu (Owen, 1981b).

Corbett et al. (1978) have studied control and Cu-stressed animals, comparing sheep and rats. Most of the Cu in subcellular fractionation ofhepatocytes was found in the cellular debris fraction, mostly nuclei, in the non Cu-stressed ovine. In the rat over 50% of the Cu was located in the cytosol fraction, whereas in sheep only 15% of the Cu

was located in the cytosol. Further purification of the debris fraction from the sheep liver revealed that only 31% of the Cu was associated with nuclei. Copper stressed rats had an increase in the Cu content of the large granule (lysosome containing) fraction. Sheep had an increase in cytosol Cu content and a decrease in large granule Cu content. The authors suggested that the ovine defect in Cu homeostasis was due to lysosomal inability to sequester and excrete excess hepatic Cu.

Further work by that research group (Saylor et al., 1980) has resulted in modification of this hypothesis. Dietary Zn levels did not affect the level of hepatic Cu or Zn. Liver Cu concentration was directly related to dietary Cu level (Saylor and Leach, 1980). Copper-MT levels were not related to hepatic Cu level, but directly related to levels of dietary zinc. This observation suggests that Zn, but not Cu, will induce MT synthesis in sheep, although Cu will displace Zn yielding Cu-thionein. Elevation of the dietary Cu level without a concommitant increase in Zn resulted in increase in Cu binding in high molecular weight materials (molecular weight in excess of 70,000). Therefore, sheep appear to have a limited capacity to detoxify accumulated hepatic Cu by MT synthesis (Saylor et al., 1980).

Evans (1973) proposed that biliary excretion of Cu is accomplished by lysosomal uptake of protein-bound Cu. If

the ovine has only limited ability to induce synthesis of MT, this may explain the susceptibility of sheep to Cu toxicity. Once the high molecular weight moieties become "saturated" with Cu, excess metal may be found bound to an "overflow" fraction of small proteins and peptides, which are not taken up by the lysosomes for biliary excretion (Saylor et al., 1980).

Lal and Sourkes (1971) found that once the lysosomal fraction of rat liver accumulates excessive levels of Cu the debris fraction begins to increase such that proportionately it contains 50% of the cellular Cu. Therefore, this would seem to indicate that sheep are continually in a state that mimics Cu toxicity in rats.

Gooneratne et al. (1979) in their work with Cu toxicity in sheep, found proportional increases in the nuclear fraction in the Cu-loaded sheep. Although electron microscopy revealed an increase in size and number of lysosomes/cell in the Cu-loaded liver, they suggested the excess Cu in the nuclei was responsible for liver cell lesions due to Cu binding to the DNA.

Copper-Molybdenum-Sulfur Interaction

Dick and Bull (1945) first reported that dietary Mo limited Cu accumulation in ruminant tissue. In 1953 Dick further illucidated the interaction and found inorganic SO_4 altered the control of Mo on Cu accumulation and in fact

regulated Mo excretion. Since then many researchers have directed their efforts toward further understanding of the Cu-Mo-S interaction.

Induced Copper Deficiency

Much research has been done concerning Mo and S induced Cu deficiency. The use of dietary supplements of Mo and SO_4 to reduce potential Cu toxicity has received less attention. Teart pastures (high Mo level) were first recognized as a source of Cu deficiency in 1943 (Ferguson et al., 1943) and still present a problem in some areas of England, California, Florida, and Manitoba, Canada (Ensminger, 1977).

There are two major proposals for the mechanism of Mo induced Cu deficiency. One is that the Mo, S, Cu interaction occurs primarily in the gastrointestinal tract. Highly insoluble complexes of Cu sulfide, Cu molybdate, and Cu thiomolybdates are formed and unaccessible for absorption. Non-complexed Mo and SO_4 are absorbed and subsequently interact with body stores of Cu to form thiomolybdates complexes (probably Cu-tetrathiomolybdate). When taken up into tissues, such complexes and reduces intracellular Cu availability for function (Huisingh et al., 1969; Dick et al., 1975; Dowdy et al., 1969; and Marcilese et al, 1970). The other proposal recognizes the interaction within the gastrointestinal tract but further suggests that the excess Mo and S are taken up by the body and the high

levels induce the de novo synthesis of a protein, which is similar to albumin. The protein contains Mo and binds Cu rendering it inaccessible for utilization and is sequesterd in the kidney for excretion. (Bremner and Young, 1978; Smith and Wright, 1975; and Suttle, 1975).

Decreasing the Possibility of Copper Toxicity

Harker (1976) reported that supplementing a concentrate diet (24.4 ppm Cu) fed to housed sheep with 7.7 ppm Mo was effective in reducing the hepatic accumulation of Cu by 40%. The additional molydbenum also reduced SGOT activity and ceruloplasmin concentration in the serum. He concluded that supplementing Mo to high concentrate diets which often are high in Cu was an effective method of reducing net uptake of Cu.

Suttle (1977) worked with two breeds of lambs and four levels of Mo (2,4,8 or 16 mg Mo/kg DM) supplemented to a high Cu diet (45.3 ppm Cu). Sulfur was supplemented at 2 g S/kg diet in all treatments. Six of the nine lambs given no additional Mo died; histological evidence of subclinical hepato-toxicity was found. He observed a linear decrease in Cu retention as the dietary levels of Mo increased. Plasma aspartate aminotransferase (PAAT) activity increased as the trial progressed in sheep fed the two lowest levels of Mo, but remained stable in the two highest Mo levels. Finnish Landrace lambs retained less Cu, had lowered PAAT levels,

and showed less histological evidence of liver damage than the Scottish Blackface. Male Scottish Blackface lambs showed more evidence of Cu toxicity than their female counterparts. Suttle concluded that adding 4 ppm Mo to a high concentrate diet was adequate for decreasing Cu accumulation, as long as the diet was relatively high in S.

Suttle's observaton of the higher tolerance to Cu toxicity of the females is supported by the research Russanov et al. (1981) which demonstrated Cu metabolism is affected by estrogen surges. They indicated that estrogens induce ceruloplasmin synthesis, therefore increasing serum Cu and lowering liver Cu levels. They suggested other factors of Cu metabolism may also be affected by circulating estrogens.

Van Ryssen and Stielau (1981) conducted an experiment in which varying levels of Mo were fed (.6, 20.8, 38.4, or 58.5 mg/sheep/d). The yearling lambs were first placed on a diet high in Cu (82 ppm Cu/sheep/d) and S (3.77 g S/sheep/d) but low in Mo for 42 d. Animals were then placed on the Mo treatments. They found that supplementation with 38.4 mg Mo/d decreased liver Cu levels, although all other treatments were not effective. They explained the relatively high liver Cu levels of the sheep fed the highest Mo treatment was a systemic affect of the Cu-Mo-S interaction. They suggested that the increase in liver Cu was due to a Cu and Mo containing compound which accumulates

in the tissue, but is unavailable for utilization. They suggested that this situation resulted from the extremely low Cu:Mo ratios (in this case 1.4) and the lengthy trial. They concluded that liver Cu levels may not be an accurate indicator of Cu status at very high Mo intakes.

Molybdenum supplementation was also ineffective when varying levels of Mo and Cu (Cu:Mo ratio ranged from .6 to 3.0) and a constant level of S (2.0 g S/kg DM) were supplemented (Van Ryssen and Stielau, 1980a). No influence of Mo on Cu retention was observed. Circulating levels of Cu were also not affected. They suggested liver Cu accumulation was not as high as expected for the dietary Cu level fed. Therefore, there was an influence on the availability of Cu for absorption. The amount of Mo and SO_4 did not inhibit retention.

Van Ryssen and Stileau (1980b) examined varying levels of supplemental S (2.9, 4.0, 5.3 g S/sheep/d) to a high Cu (55 mg/sheep/d) diet. A linear reduction in Cu retention was observed with increasing S levels. At the 5.3 g S level there was a negative (-.044g) retention of Cu. At the two highest levels of S kidney cortex Cu was elevated. Plasma Cu was depressed by day 154 in the lowest S treatment, but remained constant at the two highest levels. They concluded that Cu:Mo ratio of less than 2.0 presents a danger of Cu deficiency when S intake is great.

Broiler Litter

Van Ryssen (1979) previously investigated the influence of supplementing Mo alone or with S when feeding broiler litter. Earlier work indicated high levels of Cu in broiler litter were indigenous to South Africa (Van Ryssen et al., 1977). The litter used for this work contained only 23.5 ppm Cu, dry basis. Therefore, they added $CuSO_4$ to increase dietary Cu level to 58 ppm Cu. The Cu:Mo ratio was 1.2. The addition of Mo significantly reduced liver Cu accumulation whereas the addition of Mo and S reduced the hepatic Cu level to control levels. Kidney and serum Cu levels and serum Mo were elevated in Mo supplemented lambs. Increases in Mo were not as high when S was supplemented ($P<.01$). They suggested the limited effect of the additional S was probably due to the normally excessive complement of S in the litter. Therefore, they concluded supplemental Mo was an amenable solution to feeding high Cu broiler litter to sheep provided the level of Cu was sufficient to prevent the risk of Cu deficiency.

INFLUENCE OF MOLYBDENUM AND SULFATE SUPPLEMENTATION AND WITHDRAWAL OF DIETS CONTAINING HIGH COPPER BROILER LITTER ON TISSUE COPPER LEVELS IN EWES

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Summary

Thirty-six mature, nongestating, crossbred ewes were randomly allotted to three diets: basal (6 ewes), 50% basal and 50% broiler litter (24 ewes), and 50% basal and 50% broiler litter with the addition of 25 ppm molybdenum (Mo) and 5 g sulfate/kg feed. The 24 litter fed ewes were allotted to withdrawal times of 0-, 30-, 60- and 120-d. The control (basal) diet contained and the deepstacked broiler litter contained 8 and 257 ppm Cu, dry basis, respectively. The ewes were fed the diets for 140-d at which time the control, the six litter-fed ewes with 0-d withdrawal and the Mo and SO_4 supplemented ewes were slaughtered. Liver samples were obtained from these ewes by biopsy initially and at 40-, 80-, and 120-d of the trial. At slaughter liver, kidney, muscle and duodenum samples were taken. Liver Cu content was increased ($P<.01$) by feeding broiler litter (404 vs 1543 ppm, dry basis). Liver Cu (962 ppm) accumulation and serum Cu were depressed ($P<.05$) by supplementing litter containing diets with Mo and SO_4. Zinc

levels of the liver and Zn and Cu levels of the kidney and muscle were not affected by additional Mo and SO_4. Duodenum Cu and Zn levels reflected the dietary metal content. During the withdrawal period liver and duodenum Cu levels were not lowered ($P>.05$). A linear increase ($P<.001$) in SGOT activity was observed with time throughout the withdrawal period, suggesting possible liver damage. Serum Cu decreased at 30-d and increased thereafter (quadratic effect, $P<.05$). This observation coupled with increases in muscle and kidney Cu ($P<.05$) indicated an apparent redistribution of Cu stores. Metallothionein (MT) increased 12-fold above control values in the litter-fed ewes ($P<.05$) and tended to decrease during the withdrawal period. Therefore, it appears that supplementing Mo and SO_4 to a diet containing high Cu broiler litter may reduce the accumulation of hepatic Cu. Liver Cu stores will not be dissipated upon withdrawal of the high Cu litter from the diet.

(Key words: Broiler Litter, Cu Toxicity, Sheep, Mo, SO_4, Cu Withdrawal)

Introduction

Livestock producers are continually searching for high-nutrient, low-cost feeds. Broiler litter has been shown to be a valuable source of protein and energy for ruminants

(Bhattacharya and Taylor, 1975). However, concern has been expressed over the potentially toxic effects related to long term ingestion of of the mineral laden waste (Fontenot and Webb, 1975). Broiler waste often contains high levels of Cu due to dietary supplementation of $CuSO_4$ to growing chicks.

Various investigators have demonstrated that sheep lack the ability to homeostatically regulate dietary Cu absorption and may accumulate toxic quantities of Cu in the liver in response to dietary Cu levels greater than minimum requirements (Bremner and Davies, 1980). Weber et al. (1980) noted other dietary factors, such as Zn, Mo, SO_4 and phytate influence Cu utilization in sheep. Fontenot et al. (1972) reported fatalities after 137 d of feeding high Cu broiler litter to ewes during gestation and lactation. Van Ryssen (1979) and Suttle et al. (1978) reported liver Cu accumulation when feeding broiler litter, but no overt Cu toxicity symptoms.

The antagonistic effect of Mo and SO_4 on Cu utilization became apparent with the work of Dick (1952). Copper deficiency was induced in sheep fed a Cu-adequate diet containing Mo rich alfalfa hay. Supplementation of high Cu concentrates with Mo and SO_4 reduced the incidence of Cu toxicity in growing lambs (Harker, 1976; Suttle, 1977). The objective of this study was to determine if supplementation of Mo and SO_4 would mitigate deleterious effects of feeding high Cu broiler litter. Moreover, the influence of time of

litter withdrawal on depletion of accumulated Cu stores in sheep fed high Cu broiler litter was studied.

Experimental Procedure

Thirty six mature (4 to 13 yr), crossbred, non-gestating ewes were used. They appeared to be in good health and were placed on the basal diet 2 to 4 wk prior to starting the trial. Ewes were allotted at random from blocks formed according to initial liver Cu, breeding and weight to the following three treatments: 1) control (six ewes), 2) broiler litter (24 ewes), and 3) broiler litter plus Mo and SO_4 (six ewes). The litter-fed ewes were concommittently allotted to withdrawal times of 0, 30, 60 and 120-d. The control group received a hay-corn basal diet formulated to meet NRC (1973) requirements for maintenance. The litter-fed group received a diet composed of 50% basal and 50% broiler litter. The Mo supplemented group received the litter diet plus 25 ppm Mo as ammonium molybdate and 5 g SO_4/kg diet as $NaSO_4$ (table 1). The broiler litter, which had been deep stacked contained 257 ppm Cu (dry basis). All diets were mixed in a vertical mixer after being ground through a 2.5 cm screen. The three diets were similar in available energy (TDN).

Ewes were housed and fed in individual 1 m x 1.6 m pens in an open shed, with fresh water available at all times. Animals were fed and refusals were collected once daily.

TABLE 1. COMPOSITION OF DIETS

Item	Diet		
	Basal	Litter	Litter+ Mo+SO_4
Ingredient composition[a],%			
Orchardgrass hay (1-03-438)	49.2	22.0	21.2
Corn (4-02-935)	19.6	11.7	12.0
Soybean meal (5-04-600)	5.6	2.7	2.4
Peanut hulls (1-03-629)	16.0	5.8	5.8
Molasses (4-04-696)	8.6	6.8	6.8
Iodized salt	.52	.47	.47
Dicalcium phosphate	.48	0	0
Litter	0	50.6	50.7
Ammonium molybdate	0	0	+[b]
Sodium sulfate	0	0	.74
Vitamin A and D	+[c]	+[c]	+[c]
Chemical composition			
Dry matter, %[d]	91.0	90.2	90.3
Crude protein[d], %	12.9	23.3	22.0
Acid detergent fiber[d], %	35.8	28.5	26.8
Ash[d], %	6.4	10.3	11.6
Copper[d], ppm	8.0	135.5	133.9
Zinc[d], ppm	28.1	190.8	204.6

[a]As fed basis.

[b]Ammonium molybdate added at 4.6 g/100 kg.

[c]Vitamin A added at 1.5×10^5 IU/100 kg.
Vitamin D added at 3.25×10^4 IU/100 kg.

[d]Dry matter basis.

The animals were weighed every 14 d and feed intake was adjusted accordingly (.017 kg dry matter/ kg body weight) during the first 140 d and every 30 d during the withdrawal period.

Feed consumption was approximately 1 kg of dry matter/day, and similar for all treatment groups. During the sixth week diets were modified to increase palatability by increasing the corn content from 10 to 20% in the basal diet and reducing the hay. Table 1 reflects the average composition of the diets. Litter was maintained at 50% of the diet. The average amount of Cu consumed during the trial by the litter-fed and Mo and SO_4 supplemented ewes was 15.5 times that of the control group.

Liver samples were obtained prior to the start of the trial from all ewes by biopsy using a modified procedure of Dick (1944). The control, litter-fed (0-d withdrawal) and the Mo and SO_4 supplemented ewes were biopsied every 40-d after the begining of the trial to monitor hepatic Cu status. At 140 d the control, 0-d withdrawal and Mo and SO_4 supplemented ewes were slaughtered. The remaining litter-fed ewes were fed the basal diet and groups of six ewes were sacrificed at 30, 60, or 120 d. At sacrifice the following tissue samples were taken from each ewe: the right lobe of the liver, the right kidney, 1 meter of duodenum distal to the bile duct and the section of the longissimus muscle spanning the 11th to 13th ribs. Samples were taken

immediately after slaughter, rinsed with ice cold metal-free physiological saline (.9% W/V) and placed on dry ice. The luminal contents of the duodenum were removed by flushing with saline. Tissue samples were stored at -20C and freeze-dried prior to wet ashing. Tissues, liver cytosol, feed and refusal samples were wet ashed by the method of Sandel (1950) prior to analysis for Cu and Zn. The Cu and Zn analyses were performed with a Perkin Elmer Model 403 atomic absorption spectrophotometer (AAS).

The liver sample was randomly cored (6g) for further analysis. Cores were homogenized in three volumes of buffer (250mM sucrose, 20mM Tris-acetate, 1mM 2-mercaptoethanol, 1mM sodium azide, pH 8.6). Cytosol was prepared and the distribution of Cu and Zn in this fraction was determined by Sephadex G-75 gel filtration chromatography. Details of the procedure have been described by Failla and Kiser (1981). The Sephadex G-75 resin was pretreated with sodium borohydride to decrease the non-specific absorption of trace metals (Lonnerdahl and Hoffman, 1981). An antioxidant (2-mercaptoethanol) was added to the homogenizing and eluting buffers. The levels of Cu and Zn in each fraction were analyzed by AAS. Absorbance at 280 nm of each fration was monitored. Metallothionein (MT) was eluted at 1.8 to 2.2X the void volume. The relative quantity of Cu and Zn associated with MT in the cytosol was determined by summing the amounts of these metals eluted from the column the

indicated volumes. Data from different samples were compared by expressing the MT content as ug (Cu+Zn)/mg cytosol protein applied to the resin. Protein was quantified by the microbiuret procedure (Bailey, 1962), using bovine serum albumin as a standard.

Jugular blood samples were taken 18 hr after feeding at 14-d intervalas during the first 140 d of the trial and the day before sacrificing the ewes throughout the withdrawal period. Total serum Cu was measured after diluting the serum (1:3) with deionized-distilled water. Trichloroacetic acid (TCA)-soluble serum Cu was determined by diluting the sample 1:1 with TCA, heating at 39C for 20 min, and separating the protein percipitate by centrifugation (modified from Bremner and Young, 1978).

Serum glutamic oxalacetic transaminase (SGOT) was determined by a colorimetric enzymatic analysis.[1] Whole blood packed cell volume was determined by microhematocrit.

The data were statistically anaylzed using the general linear models procedure of SAS (1979). Duncan's (1955) multiple range test was used to determine differences between the control, litter and Mo and SO_4 treatments. Linear and quadratic orthagonal polynomials were utilized to to determine significant differences of treatment means due to withdrawal time. The statistical model included effects

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[1]Sigma Chemical Company, St. Louis, Missouri.

of treatment and block for tissue analysis. Serum and biopsy analyses throughout the initial period included effects of treatment, block, time and a all two-way interactions. Interactions that were not significant ($P>.10$) were deleted from the final model. Effects of treatment and time were tested with the block * treatment and block * time interactions if these interactions were significant. All other effects were tested with the error mean square.

Results

Overt symptoms of Cu toxicity were not observed during the trial. Similarly, there were no indications of any health problems that could be related to feeding broiler litter or to Mo and SO_4 supplementation. The packed cell volume was elevated at the 28-d sampling, but remained relatively constant (figure 1) thereafter. Molybdenum and SO_4 supplementation depressed the PCV ($P<.05$). Serum glutamic oxalacetic transaminase activity, an indicator of heart or liver damage, was higher ($P<.05$) for the litter-fed then the other ewes during the trial (figure 1). During the withdrawal segment the SGOT activity increased ($P<.005$, figure 2).

Ewes fed the basal diet had a relatively constant hepatic Cu content throughout the study, while the Cu level in the liver of animals fed the litter increased with time

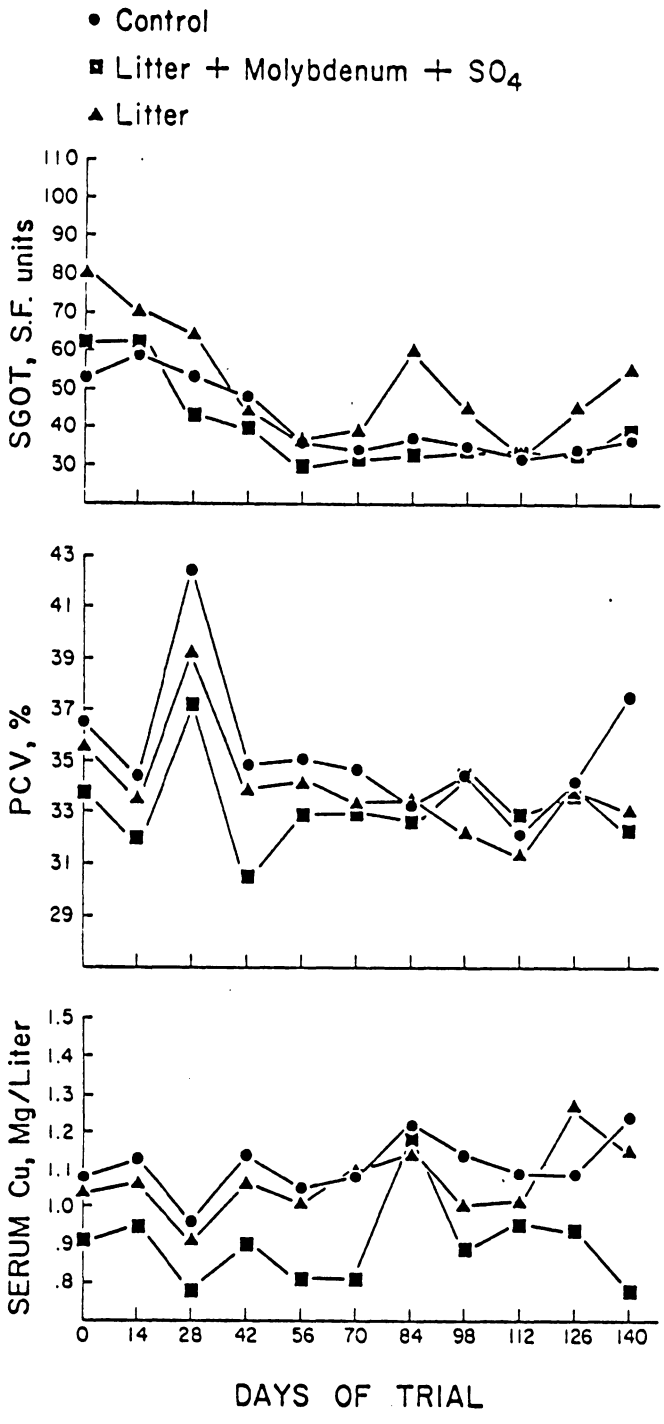

Figure 1. Blood parameters during the initial period. SGOT was highest for the litter-fed ewes ($P<.05$). PCV was effected by time ($P<.05$). Mo and SO_4 supplementation depressed PCV ($P<.05$). A treatment by time interaction was present for serum Cu levels.

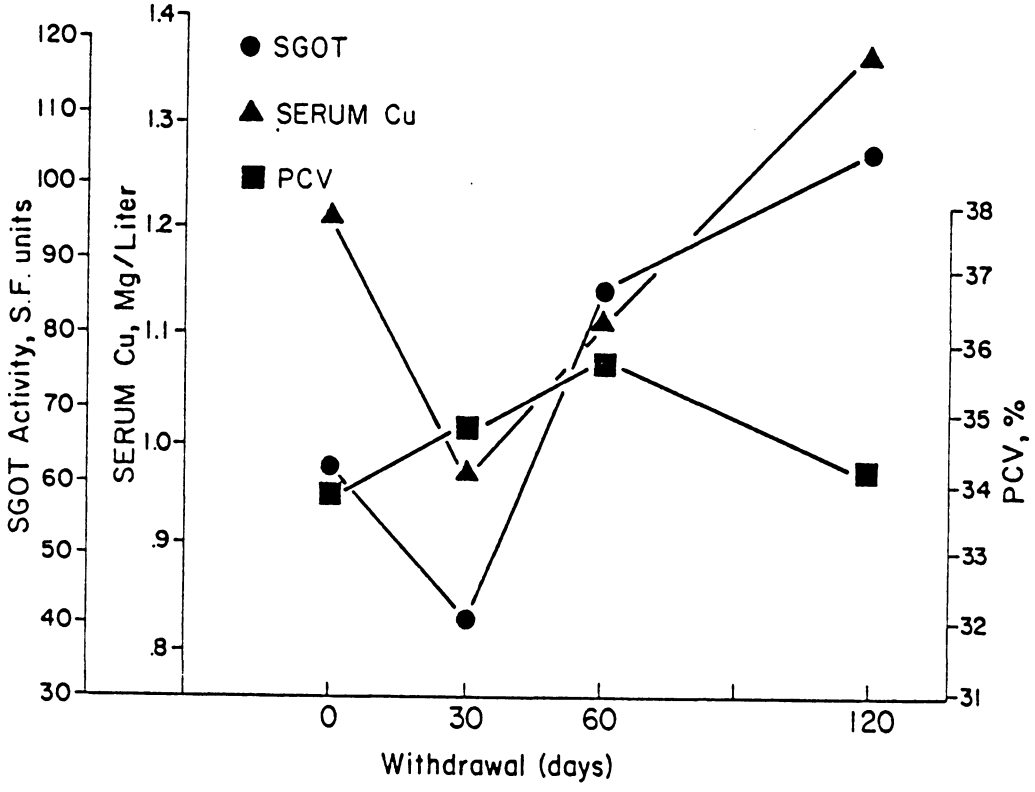

Figure 2. Blood parameters during the withdrawal period. SGOT activity and serum Cu levels decreased at d 30 then increased. PCV increased up to d 60, then decreased. PCV, Serum Cu and SGOT showed a quadratic response ($P<.05$).

(figure 3). By day 80 liver Cu levels were different ($P<.01$) among all three treatments. The final liver Cu content in the litter-fed increased 4-times that of the control ewes. The liver Cu of the Mo and SO_4 supplemented were intermediate between those of the control and litter-fed ewes. Thus, supplementation of the litter diet with Mo and SO_4 effectively diminished the rate of hepatic Cu accumulation.

Liver Cu levels remained elevated throughout the 30-, 60- and 120-d withdrawal of the high Cu broiler litter (figure 4). Withdrawal time had no effect on the Cu level ($P>.05$).

The specificity for Cu accumulation in the liver of sheep fed high Cu litter was demonstrated by mineral anyalysis of the other tissues (table 2). Neither Cu or Zn contents of the kidney or muscle differed significantly among control, litter or Mo and SO_4 supplemented ewes. While the mean copper content of the kidney appears to be elevated in the litter-fed group, this was due to one ewe having a markedly increased concentration of renal Cu (84.5 ppm). Its serum Cu (2.0 mg/liter) and SGOT activity (124 s.f. units) suggest Cu-associated liver dysfunction. The elevated content of the metal in the kidney was probably a result of this condition. In contrast, Cu and Zn levels of the duodenum were elevated when litter was fed alone or with additional Mo and SO_4 ($P<.05$). Liver Zn content tended to

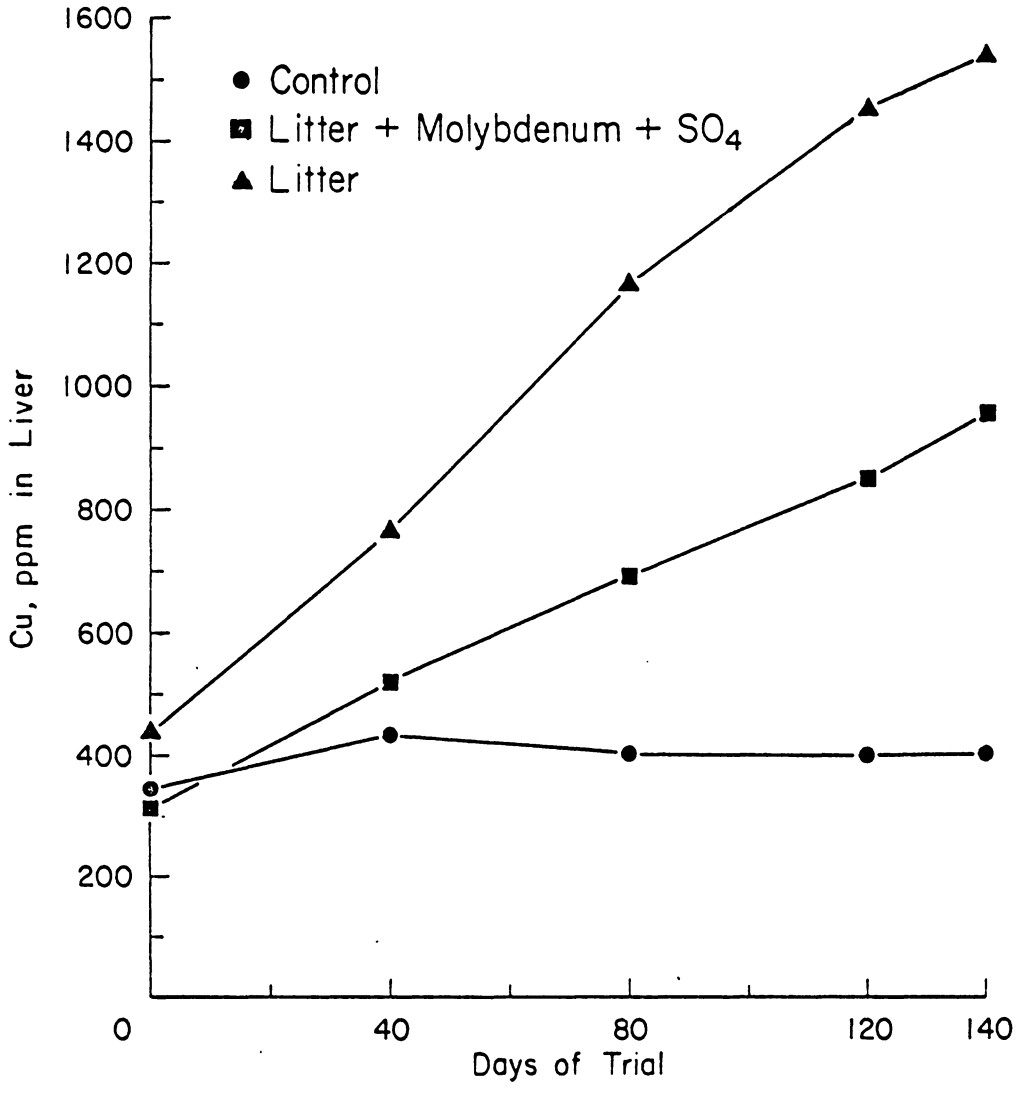

Figure 3. Effect of diet on liver Cu levels. Levels for all treatments differ ($P<.05$). An effect of time and a time by treatment interaction was present ($P<.05$)

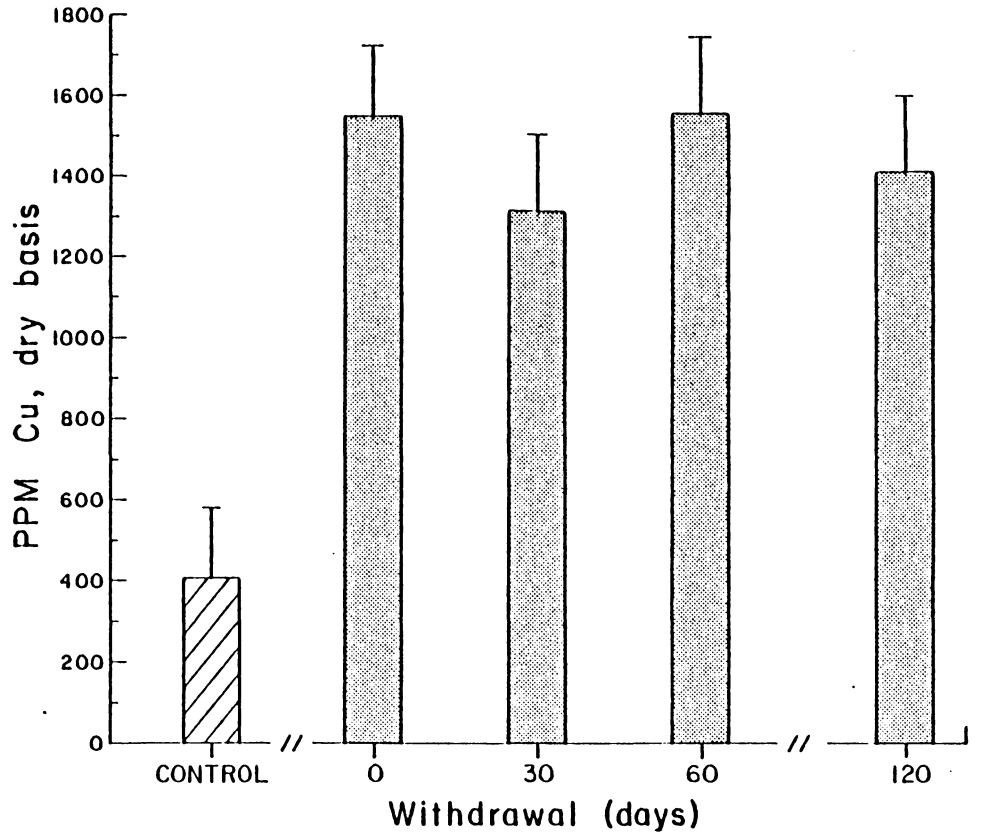

Figure 4. Effect of withdrawal time on liver copper levels

TABLE 2. TISSUE METAL CONTENT[a]

Tissue	Metal	Diet: Control	Diet: Litter + Mo+SO_4	Withdrawal time, days: Litter 0	30	60	120	S.E.
Liver								
	Cu	403.8[b]	961.6[c]	1543.6[d]	1312.6	1546.0	1404.7	195.6
	Zn[e]	193.5	220.7	184.8	111.6	111.8	122.3	33.5
Kidney								
	Cu[f]	14.1	14.5	24.9	18.3	31.1	38.2	7.43
	Zn	118.3	122.2	124.9	109.6	108.1	108.7	6.37
M. longissimus								
	Cu[e]	3.34	3.10	3.60	5.46	5.55	4.85	.40
	Zn	86.5	103.6	80.0	85.1	67.4	82.6	14.0
Duodenum								
	Cu	7.30[b]	9.61[c]	9.48[c]	10.0	10.25	11.13	.71
	Zn	78.1[b]	96.8[b,c]	100.7[c]	106.2	99.5	86.6	7.7

[a]Dry basis, expressed a µg metal/g tissue.
[b,c,d]Means for control, litter + Mo+SO_4, and litter treatments with different superscripts differ ($P<.05$).
[e]Quadratic effect of withdrawal time ($P<.05$).
[f]Linear effect of withdrawal time ($P<.05$).

be elevated when Mo and SO_4 were supplemented (table 2).

During the withdrawal period muscle Cu content increased (quadratic effect, $P<.05$). Kidney Cu levels increased after 60- and 120-d withdrawal (linear effect, $P<.05$). Duodenum Cu levels remained elevated throughout the withdrawal period.

Supplementation of the litter with Mo and SO_4 depressed serum Cu levels (figure 1). During the withdrawal period circulating levels of Cu decreased at day 30, then increased ($P<.05$). Serum Cu was effected by time and a time by treatment interaction was observed ($P>.05$). There was no difference in serum when analysis was performed following with either water or TCA dilution (see methods).

The quantity of Cu-associated with MT increased 15-fold in the litter-fed animals, and sixfold in the Mo and SO_4 supplemented ewes; these increases are proportional with the seven and threefold elevation in cytosol Cu, respectively (table 3). The level of Cu-MT was well correlated with hepatic Cu content ($r=.98$) in both the litter-fed and the Mo and SO_4 supplemented. The Cu-MT, cytosol Cu, and the peak II Cu:Zn ratio were higher ($P<.05$) in the litter-fed then the control ewes. The peak II Cu:Zn ratio is the second metal peak of the elution profile of the liver homogenate off the Sephadex G-75 column (appendix figure 2). By 120-d of the withdrawal period the Cu-MT levels tended to decrease. The relatively constant increase in liver Cu and

TABLE 3. HEPATIC CYTOSOL METALS[a]

Parameter	Diet		Withdrawal time, days		
	Control	Litter + Mo+SO_4	Litter 0	60	120
No. of ewes	4	5	4	6	6
Cytosol Cu	.22[b]	.74[c]	1.50[d]	.99	.87
Cu-metallothionein	.04[b]	.23[c]	.61[c]	.71	.31
Zn-metallothionein	.02	.02	.02	.05	.01
Cu:Zn in peak II	1.06[b]	2.80[b,c]	4.92[c]	6.70	4.06

[a]Values expressed as μg metal/mg protein.
[b,c,d]Means of the control, litter + Mo+SO_4 and litter treatments with different superscripts differ.

the tendency for an decrease in the cytosol Cu levels suggests an increse in the particulate fraction of the hepatocyte. Minimal amounts of Zn-MT were detected in all treatments.

Discussion

The PCV for all treatments remained relatively constant suggesting the ewes suffered from neither Cu-mediated hemolysis (Todd and Thompson, 1963) or Mo toxicosis (Ward, 1978). Similarly, none of the ewes exhibited diarrhea, an initial symptom of molydenosis.

The reduction of hepatic Cu accumulation by supplementation of the litter diet with Mo and SO_4 is in general agreement with the results of Van Ryssen (1979). When sheep were fed diets containing broiler litter and additional $CuSO_4$ (dietary Cu level, 65 ppm) and Mo (Cu:Mo ratio to 1.3) liver Cu levels were maintained at the control levels. More recently, Van Ryssen and Stielau (1981) investigated the influence of Cu:Mo ratios on reduction of hepatic Cu levels in sheep previously fed high levels of dietary Cu. When the dietary Cu:Mo ratio was either 4.0 or 2.2 significant decreases in liver Cu were observed. However, an increase in tissue (liver, kidney and muscle) Cu was found when the lambs were supplemented with the highest quantity of Mo (Cu:Mo ratio of 1.3). The authors suggested the increase tissue Cu was caused by a non-available Cu-molybdate complex in the tissues.

In this study the dietary Cu level was twice that of Van Ryssen (1979). The Cu:Mo ratic was 6.0 in the Mo supplemented diet and the diet contained 50% broiler litter. Which may account for the reduction although in Cu accumulation but not thhe block of Cu absorption.

Maintenance of the high liver Cu levels throughout the withdrawal periods (figure 4) demonstrates the inability of ovine to excrete the excessive amounts of accumulated hepatic Cu. Barden and Robertson (1962) observed that Cu toxicity symptoms appeared after the high Cu diet was withdrawn, which emphasizes the importance of stress and the longterm inability of sheep to detoxify accumulated Cu.

Serum glutamic oxalacetic transaminase activity is an indicator of liver and cardiac damage. Todd and Thompson (1963), in their study of Cu toxicity, found ovine SGOT activity markedly varied 4 to 8 wk prior to the sharp rise in activity, which occurred simultaneously with hemolytic crisis. Increased SGOT activity in the litter-fed ewes observed during the withdrawal period is suggested Cu-related liver dysfunction or damage. Hemolytic crisis is precipitated by a sudden release of Cu from the liver, indicating that liver function is disturbed. By day 70 of the initial period SGOT activity of the litter-fed ewes began to vary considerably with time (figure 1). Hence the litter-fed ewes in this study appeared to be approaching their maximum liver Cu load associated with pathological

changes in tissue function. Circulating Cu levels remained relatively constant throughout the first 140 d period (figure 1); the level increased linearly ($P<.05$) during the withdrawal period which may be due in part to release of Cu from stores.

Bremner and Young (1978) found a TCA-insoluble form of plasma Cu in the presence of excessive Mo and SO_4 . In the present study no difference between total and TCA-soluble levels of serum Cu was detected. The significant decrease in total serum and TCA-soluble serum Cu in the Mo supplemented ewes observed in our experiment is contradictory to some previous findings where the Cu content of the serum increased in Mo treated animals (Bremner and Young, 1978; Van Ryssen, 1979; Van Ryssen and Stielau, 1981). This was attributed to an induced TCA-insoluble protein or to an inorganic Mo-S-Cu complex. The similarity of the serum Cu levels determined by the two methods in the present study supports the suggestion by Dick et al. (1975) that the Mo-S-Cu interaction occurs primarily in the rumen. In the present study Mo and SO_4 supplementation significantly depressed ($P<.05$). circulating Cu levels. This may be related to Mo inhibition of ceruloplasmin synthesis (Ward, 1978). Since ceruloplasmin is th principle carrier of Cu in plasma, Mo-induced depression of ceruloplasmin synthesis and secretion would be expected to cause a reduction in Plasma Cu.

Bremner and Young (1978) found elevated kidney Cu and urinary Cu excretion when Mo was supplemented to the diet which they attributed the TCA-insoluble, Cu binding, low molecular weight protein. They suggested this protein was taken up by the kidneys for excretion.

In contrast Suttle (1977) reported that when varying levels of Mo were supplemented to a high Cu (45.3 ppm) diet (Cu:Mo ratio varied from 22.3 to 2.8) no difference in kidney Cu levels were noted in Finnish Landrace lambs and a slight decrease was found in Scottish Blackface with increasing Mo supplementation. Such results have lead to the conclusion that the Cu-Mo-S interaction takes place within the gastrointestinal tract, presumably the rumen (Dick et al., 1975). It is likely, as Bremner and Davies (1980) suggested that the effect of Mo and SO_4 on Cu metabolism is dependent on the dietary levels of the metals. The effect of Mo and SO_4 seems to be antagonistic to Cu absorption when the Cu:Mo ratio is greater than 2.0, whereas during molybdenosis the interaction occurs within the body complexing the Cu for excretion.

The abscence of increased kidney Cu levels in the litter-fed ewes indicates that the hepatic Cu levels did not reach the acutely toxic level in which Cu is released into circulation and taken up by the kidneys for excretion (Todd and Thompson, 1963). However, the increase in kidney Cu levels during the withdrawal period and the linear increase

in muscle Cu levels with time of the withdrawl period indicates redistribution of Cu stores. The increase in SGOT activity indicates liver damage which may be responsible for the release and redistribution of Cu.

The constant kidney and muscle Zn levels throughout the trial indicate that there is fine regulation in Zn absorption and excretion even in animals fed diets containing greatly elevated Zn levels. Metallothionein synthesis in sheep is not induced by Cu, but by Zn (Saylor et al., 1980), indicating a more effective homeostatic mechanism.

The elevated duodenal Cu levels throughout the withdrawal period suggests Cu is sequestered in the smooth muscle of the intestine and not readily released.

Metallothionein is a cysteine-rich, small intracellular protein with a high affinity for Cu and Zn. It has been isolated from the liver, intestine, and kidney in many animals, including sheep (Bremner and Young, 1978; Saylor et al., 1980). The 12-fold increase in the quanity of Cu bound to hepatic MT found in the litter-fed ewes indicates that MT is serving in a detoxifying capacity for the additional complement of liver Cu. Saylor et al. (1980) suggested that in Cu toxicity results when excessive tissue Cu is not associated with MT, thereby limiting the lysosomal Cu accumulation.

The elevated Cu:Zn ratio (table 3) in the second metal

peak of the cytosol fraction profile gives an indication that MT is not capable of binding all the excess hepatic Cu. This peak which contains superoxide dismutase normally has a 1:1 Cu:Zn ratio. In the ewes with elevated liver Cu the ratio ranged from 2.2:1 to 22.6:1 The increase in Cu in this peak may be due to many factors including polymer formation of MT which may be related to oxidative conditions present during preparation of the sample (Minkel et al., 1980) or to Cu replacement of the Zn molecule on the SOD protein as has been observed in vitro (Valentine and Pantoliano, 1981).

Copper present in broiler litter does not appear to be as toxic as when much lower levels of Cu salts are added to diets (NRC, 1980) We did not observe any ewes with overt symptoms of Cu toxicity and only one ewe with blood and tissue parameters which suggested it was nearing hemolytic crisis. Van Ryssen et al. (1977) reported excessive levels for many minerals in broiler litter (Ca, 1.83 %; Zn, 261 ppm; and S, .6%). Perhaps the decreased susceptibility of sheep to the high Cu levels in poultry litter is related to the Cu-mineral interactions within the gastrointestinal tract. Cu absorption has been shown to be decreased by elevated dietary levels of Ca, Zn and phytate (Weber, 1980).

It appears that feeding broiler litter containing high levels of Cu for a period as long as 140-d is not acutely detrimental to the health of the sheep, probably due to interference of Cu absorption by factors inherent to the

litter. However, accumulated hepatic Cu is not readily dissipated, so feeding high Cu broiler litter for extended periods is probably not safe, even when interrupted by periods of low Cu intake. Furthermore, since the hepatic Cu will not be dissipated the addition of Mo in a 4:1 to 2:1 Cu:Mo ratio may be needed to inhibit or reduce the accumulation of hepatic Cu when feeding for extended periods. In contrast to the problem in sheep, high levels of Cu in poultry litter have been shown to be of little consequence in cattle (Webb et al., 1980). In a 7-yr study in which broiler litter was supplemented to the winter feeding regimen, cows maintained body weight and production. Although liver Cu contents were elevated as a result of the high Cu consumption the tissue levels were markedly reduced after each summer grazing period.

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Epiloque

Molybdenum and SO_4 supplementation to the high Cu broiler litter diet reduced the amount of hepatic Cu accumulation, whereas, withdrawal of the high Cu feed did not dissipate excessive tissue Cu stores. The ewes appeared to maintain good health throughout the trial, although there were a few cases of pneumonia. The litter-fed ewes had a slight weight loss during the trial (appendix figure 1).

Serum Cu level was not a good indicator of liver Cu status. The increased serum Cu level by the end of the trial, is an indication that Cu was released from Cu stores. The increase in muscle Cu and kidney Cu levels, supports a theory for Cu redistributin by 120 d of withdrawal. Molybdenum and SO_4 supplementation reduced serum Cu levels, which may be related to the ability of Mo to inhibit ceruloplasmin synthesis (Ward, 1978). Saylor et al. (1980) suggested cations may replace the Cu in ceruloplasmin, thereby reducing circulating Cu content. Packed cell volume remained relatively stable throughout the initial period with the exception of the sampling at 28 d into the withdrawal period. The PCV's of all treatment groups were elevated, indicating that that an environmental factor may be tha cause. It has been reported that an increase in circulating Cu is not a sufficient ionic disruption to damage erythrocytes. Perhaps release of degradative enzymes

from the damaged liver is responsible for hemolysis. Therefore, the elevated serum Cu levels and the constant PCV indicates that liver damage was not profound enough to cause hemolysis.

Elevated SGOT activity has been reported as the only accurate blood analysis that is an indicator of chronic Cu toxicity (Buckly and Tait, 1981; Todd and Thompson, 1963). There was substantial fluctuation in enzyme activity in the litter-fed ewes after 70 d of high Cu intake. Todd and Thompson (1963) observed fluctuation in SGOT activity 2 to 6 wk prior to the onset of hemolytic crisis. The linear increase in SGOT activity throughout the withdrawal period was indicative of tissue damage, probably liver dysfunction. This suggests that the liver was losing the capacity to detoxify the entire complement of accumulated hepatic Cu.

The trend for a depression of hepatic MT by 120 d of the withdrawal period may have contributed to the increase in the toxicity of Cu stores. The excess Cu stores may bind to many cellular proteins causing aberrant conformation and adversely affecting function. The reduction may be attributed to the relatively fast turnover rate of MT (Bremner et al., 1979) and the minimal amount of Cu induction of MT synthesis (Saylor et al., 1980).

There was an increase in the SOD containing Peak II (appendix figure 2) Cu:Zn ratio in the withdrawal animals in contrast to that observed in the control animals, an

indication that MT was not able to detoxify all the soluble complement of Cu. The increase of Cu content in peak II may be related to Cu binding to the Zn site. Valentine and Pantoliano (1981) reported that Cu replacement for Zn does not impair SOD activity in vitro. However, they cite examples in which the apoenzyme of SOD was reconstituted with Cu, and Cu was bound not only to both metal sites but also complexed to other positions on the protein molecule, probably altering the activity. Another thought on the increase in Peak II Cu content may be the formation of Cu-MT dimers, which may elute at the 20,000 to 40,000 molecular weight range (peak II) because of the eliptical structure of MT (Kojima and Nordberg, 1979). Minkel et al. (1980) observed polymer MT as a result of oxidation during preperation of the the homogenate for gel filtration when an antioxidant was not added to the buffers. To all the buffers used in this study 2-mercaptoethanol was added in a 1mM concentration, however because of the high level of Cu-MT found the level of the antioxidant may have been needed to be greater.

During the withdrawal period there was a tendency for the Cu content of the soluble fraction to decrease even though the total metal content of the liver remained stable. This would suggest more Cu was being accumulated in the particulate fraction, although metal analysis of this fraction was not conducted. Researchers who have employed

differential centrifugation with Cu stressed ovine hepatocytes have observed increased Cu content in all fractions. Cu accumulated in the debris fraction (nuclear containing) in the highest amount whether the animals were Cu stressed or not (Corbett et al., 1979; Gooneratne et al., 1979). However, during chronic Cu toxicity the large granule fraction (lysosome containing) accumulated a greater percentage of Cu than during non-Cu-stress (Corbett et al., 1979). The research of Gooneratne et al. (1979) also indicated that Cu is sequestered in the lysosome as determined by subcellular fractionation, and further confirmed by electron microscopy, which revealed both the number and size of lysosomes increased just prior to Cu-induced hemolysis. Saylor et al. (1980) suggested the ovine's sensitivity to Cu may be partially explained by the decrease in Cu available for lysosomal uptake due to the reduced capability for Cu induction of MT, therefore limiting Cu excretion. However, in the present study, in which dietary Zn levels were elevated, induction of MT synthesis occured, suggesting that there was not a lack of protein bound Cu for lysosomal accumulation.

In a review of heavy metal toxicity and the lysosome, Sternlieb and Goldfischer (1976) suggested uptake of Cu may be detrimental to lysosomal function. Lysosomes will sequester toxic metals in the rat although the uptake may inhibit lysosomal enzyme activity and alter the stability of

lysosomal membranes. Lindquist's (1968) work with Cu induced lysosomal rupture suggest that osmotic fragility was not a factor in lysosomal rupture, but rather that Cu initiates lipid peroxidation of the membrane, hence releasing hydrolytic enzyme. Work with Wilson's diseased patients indicate there is a lapse in lysosomal accumulation of cytosol Cu, although once the Cu is taken up it is less toxic to other cellular organelles.

Further work is needed to understand Cu metabolism when feeding mineral laden feeds such as poultry waste. It is suggested bile cannulae be placed to monitor Cu excretion, to determine if the Zn induced MT will increase Cu excretion. Liver samples need to be procured for histochemical staining to determine patterns of Cu accumulation patterns. Electron microscopy may reflect lysosomal and nuclear aberations during toxicity. Utilization of an electron microprobe may further ellucidate sites of metal accumulation.

The ovine's sensitivity to Cu may be a result of many factors. The inability of Cu to inducte MT, hence impaired lysosomal uptake may be one factor. However, Cu excretion is minimal regardless of hepatic Cu and dietary Zn content. Also, studies with hepatocyte subcellular fractionation of sheep liver and Wilson's disease samples tends to indicate that lysosome is sequestering the Cu, but the aberrant mechanism is in lysosomal excretion of the Cu. Sternlieb

and Goldfischer (1976) indicated a lapse of Cu excretion in Wilson's disease was due to a slow maturation of lysosomes, therefore, a reduced ability to sequester soluble Cu. Perhaps sheep also have a retarded maturation or extremely slow turnover in lysosome; therefore, Cu accumulates until the lysosomal membrane ruptures and release of hydrolytic enzymes causes liver dysfunction. Subcellular fractionation, purification of the large granule fraction and characterization of the protein bound Cu may further illucidate the aberrant mechanism of Cu excretion.

Another factor which needs to be examined is the role of Zn. In the present study a Zn peak was observed in the last fractions of the gel filtration profile. This peak would suggest a very small molecular weight Zn complex or perhaps ionic Zn. This peak was also apparent when liver samples were quickly frozen in a dry ice-acetone mixture, suggesting the observed peak was not from Zn-MT degradation. Saylor et al. (1980) also reported low molecular weight Zn binding peaks. Further isolation found the Zn bound to low molecular weight proteins or polypeptides. They suggested the Zn was displaced from Zn-MT, then bound to cellular polypeptides. Upon characterization of bile samples by gel filtration chromatography they found the only Zn peak was eluted in the same fractions suggesting Zn is sequestered for excretion by these low molecular weight complexes. No

major Cu peaks were found. Perhaps this small molecular weight fraction is the key for excretion.

On an applied basis the next step of the project would be to supplement the broiler litter diet with Mo at a 3:1 or 2:1, Cu:Mo ratio. Supplemental SO_4 is not likely required because of the elevated level of sulfur inherent to broiler litter. The purpose would be to totally inhibit Cu accumulation but also to avoid Cu deficiency. Also, it is important to determine the level of Mo supplementation needed to complex the Cu from the liver stores. Again, precaution must be taken as not to impair Cu utilization. Studies similar to these have been completed in trials utilizing Cu salt toxicity (Van Ryssen and Stileau, 1981 and Van Ryssen, 1979). However, results must be verified with a litter diet because of complications with excessive content of other minerals. Since the FDA does not recognize feeding ammonium molybdate, work with materials high in Mo should be pursued. These materials include alfalfa hay, feeds grown in heavily Mo fertilized land and peat moss.

In summary these trials demostrated that feeding high Cu broiler litter for extented periods of time (such as during gestation and lactation) when nutrient requirements are elevated is not adviseable, since accumulated hepatic Cu levels will not be readily dissipated. Work previously conducted (Van Ryssen and Stielau, 1981) has shown liver Cu stores may be depleted by supplementing excessive quantities

of Mo and SO_4 to the diet. This approach to detoxifying broiler litter does not seem advisable since at the level of Mo needed molybdenosis is actually being induced and may be interfering with many Cu dependent enzymes. A more preferable method of safely feeding high Cu wastes would be by supplementing Mo at the time litter is being fed and at levels which reduce liver Cu retention but induce Cu deficiency.

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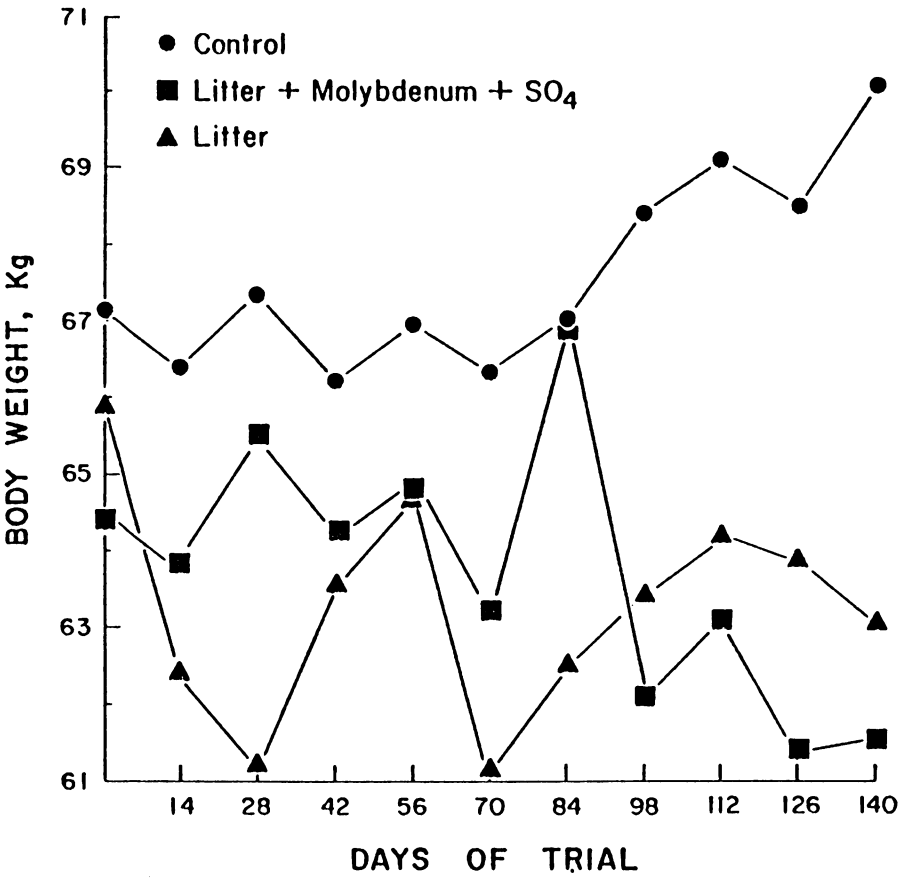
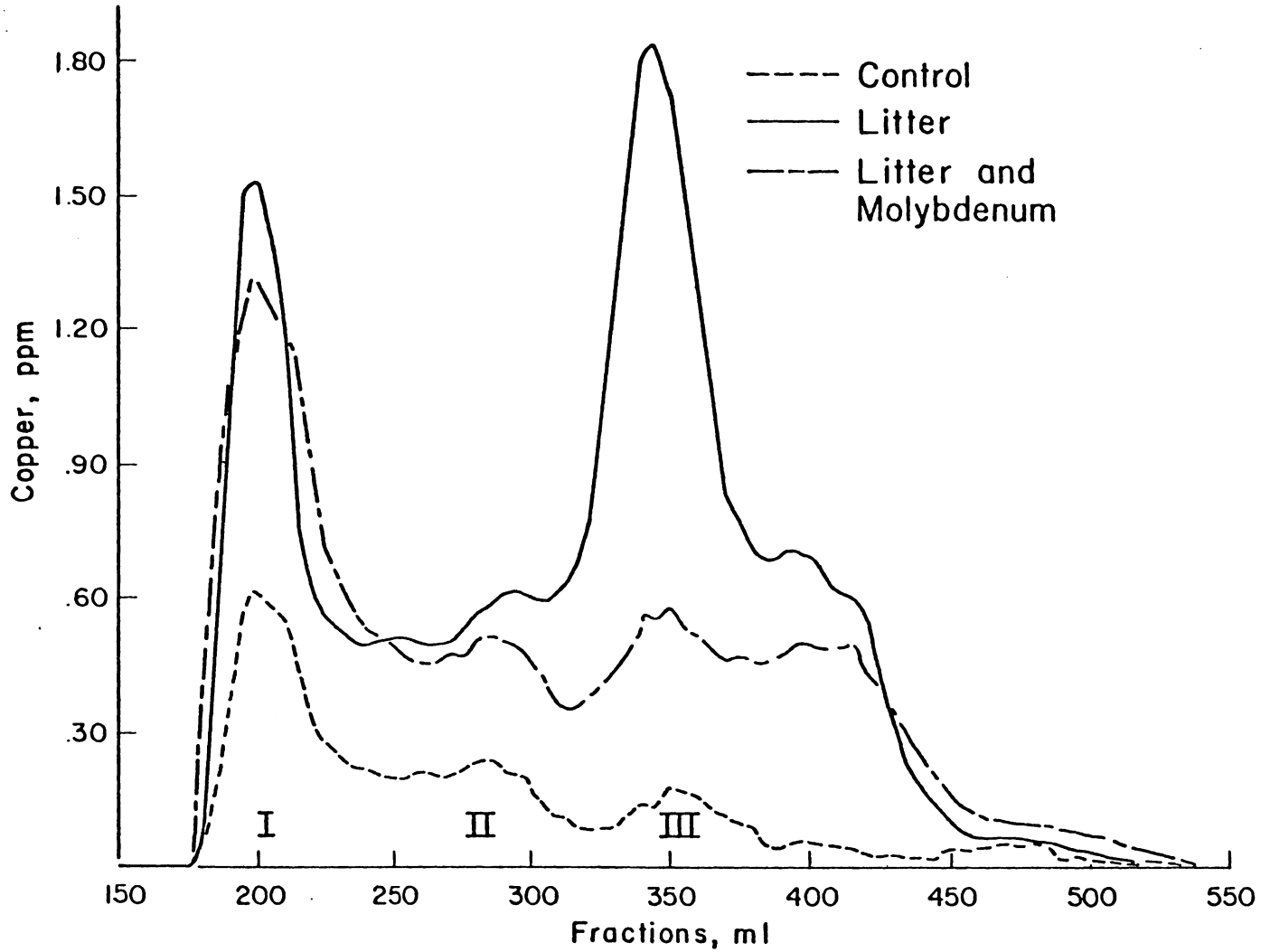


Appendix figure 1. Effect of treatment on body weight



Appendix figure 2. Example of a Sephadex G-75 profile

Appendix table 1. AVERAGE DRY MATTER AND COPPER INTAKE PER EWE

	Diet		
	Control	Litter	Litter + Mo + SO_4
Dry matter, kg/d	1.03	.96	1.02
Copper, mg/d	8.2	122.76	131.80
Copper, g during trial	1.15	171.86	184.53

APPENDIX TABLE 2. EXAMPLE OF ANALYSIS OF VARIANCE FOR INITIAL PERIOD - TOTAL SERUM CU

General Linear Models Procedure

Dependent variable: TCU

Source	DF	Sum of Squares	Mean Square	F value	PR>F	R-square	C.V.
Model	109	10.36349	.09508	2.23	.0001	.653516	19.6640
Error	129	5.49456	.04259				
Corrected total	238	15.85805			STD DEV .206382	TCU Mean 1.04954	

Source[a]	DF	Type III SS	F values	PR>F
Trt[b]	2	1.681370	9.34	.005
Block	6	.485654	1.90	.0855
Time[c]	10	1.422267	2.56	.0250
Block*trt	12	1,131781	2.21	.0145
Block*time	60	3.379079	1.32	.0955
Trt*time	19	1.306574	1.61	.0616

[a]Components under "Model".

[b]Effects of treatment were tested with block*treatment interaction.

[c]Effects of time were tested with block*time interaction.

APPENDIX TABLE 3. EXAMPLE OF ANALYSIS OF VARIANCE FOR INITIAL PERIOD - DUODENUM COPPER

General Linear Models Procedure

Dependent variable: SICU

Source	DF	Sum of Squares	Mean Square	F value
Model	8	75.54289753	9.44286219	6.25
Error	12	18.11799770	1.50983314	PR>F .0026
Corrected total	20	93.66089524		

R-square	C.V.	STD DEV	SICU Mean
..806558	13.2558	1.22875268	9.26952381

Source[a]	DF	Type III SS	F value	PR>F
Block	6	28.70768801	3.17	.0422
Trt	2	48.32972230	16.00	.0004

[a]Components under "Model".

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INFLUENCE OF MOLYBDENUM AND SULFATE SUPPLEMENTATION AND WITHDRAWAL OF DIETS CONTAINING HIGH COPPER BROILER LITTER ON TISSUE COPPER LEVELS

by

Kristi Jo Olson

(ABSTRACT)

Thirty-six mature, nongestating, crossbred ewes were randomly allotted to three diets: basal (6 ewes); 50% basal and 50% broiler litter (24 ewes), and 50% basal and 50% broiler litter with the addition of 25 ppm molybdenum (Mo) and 5 g sulfate/kg feed. The 24 litter fed ewes were allotted to withdrawal times of 0-, 30-, 60- and 120-d. The control (basal) diet contained and the deepstacked broiler litter contained 8 and 257 ppm Cu, dry basis, respectively. The ewes were fed the diets for 140-d at which time the control, the six litter-fed ewes with 0-d withdrawal and the Mo and SO_4 supplemented ewes were slaughtered. Liver samples were obtained from these ewes by biopsy initially and at 40-, 80-, and 120-d of the trial. At slaughter liver, kidney, muscle and duodenum samples were taken. Liver Cu content was increased ($P<.01$) by feeding broiler litter (404 vs 1543 ppm, dry basis). Liver Cu (962 ppm) accumulation and serum Cu were depressed ($P<.05$) by supplementing litter containing diets with Mo and SO_4. Zinc levels of the liver and Zn and Cu levels of the kidney and

muscle were not affected by additional Mo and SO_4. Duodenum Cu and Zn levels reflected the dietary metal content. During the withdrawal period liver and duodenum Cu levels were not lowered ($P>.05$). A linear increase ($P<.001$) in SGOT activity was observed with time throughout the withdrawal period, suggesting possible liver damage. Serum Cu decreased at 30-d and increased thereafter (quadratic effect, $P<.05$). This observation coupled with increases in muscle and kidney Cu ($P<.05$) indicated an apparent redistribution of Cu stores. Metallothionein (MT) increased 12-fold above control values in the litter-fed ewes ($P<.05$) and tended to decrease during the withdrawal period. Therefore, it appears that supplementing Mo and SO_4 to a diet containing high Cu broiler litter may reduce the accumulation of hepatic Cu. Liver Cu stores will not be dissipated upon withdrawal of the high Cu litter from the diet.