EFFECTS OF DIETARY MOLYBDENUM ON PRODUCTION PERFORMANCE AND ON PARAMETERS REFLECTING BODY COPPER AND MOLYBDENUM STATUS OF LACTATING COWS AND EWES AND THEIR OFFSPRING

BY

MARIE KATHERINA WITTENBERG

A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

DOCTOR OF PHILOSOPHY

EFFECTS OF DIETARY MOLYBDENUM ON PRODUCTION PERFORMANCE AND ON PARAMETERS REFLECTING BODY COPPER AND MOLYBDENUM STATUS OF LACTATING COWS AND EWES AND THEIR OFFSPRING

by

Marie Katherina Wittenberg

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the Requirements for the Degree of DOCTOR OF PHILOSOPHY

Department of Animal Science
Faculty of Graduate Studies
University of Manitoba

April, 1985

ABSTRACT

Effects of dietary molybdenum on production performance and on parameters reflecting body copper and molybdenum status of lactating cows and ewes and their offspring.

by

Marie Katherina Wittenberg

Two trials were conducted to study the effects of supplementary (0, 20 and 40 mg kg^{-1} D.M.) molybdenum (Mo) in corn silage - barley based diets on lactation performance of early lactation beef cows and fresh ewes (with twins lambs) and on growth performance of their suckling offspring. Various plasma and liver parameters were monitored to determine copper (Cu) and Mo status of lactating animals and their offspring. Calves and lambs were not given feed to supplement their milk intake.

Mo content of diets, referred to as 0Mo, 20Mo and 40Mo, fed to beef cows was 0.6, 19.3 and 34.8 mg kg^{-1} D.M. Cu and sulfur (S) content of diets ranged from 5.8-6.0 mg and 1.3-1.4 g kg^{-1} D.M., respectively. Rate of decline for cow plasma Cu ($P < 0.001$), trichloroacetic acid (TCA) soluble Cu ($P < 0.01$) and ceruloplasmin oxidase (Cp) activity ($P < 0.001$) were significantly lower for 0Mo and 20Mo than for 40Mo. Milk yield dropped more rapidly ($P < 0.01$) for cows fed 40Mo than those fed 0Mo and 20Mo. Diet influenced ($P < 0.01$) milk Mo content, which was 0.10, 0.51 and 1.19 $\pm$ 0.04 mg L^{-1} for cows fed 0Mo, 20Mo and 40Mo, respectively. Calf weight gains, plasma Cu, TCA-soluble Cu and Cp activity were not influenced ($P > 0.05$) by treatment.

Mo content of 0Mo, 20Mo and 40Mo diets fed to ewes was 0.9, 18.4 and 40.7 mg kg^{-1} D.M. Cu and S content of diets ranged from 4.5-4.9 mg and 1.4-1.5 mg kg^{-1} D.M. Three ewes fed Mo supplemented diets developed apparent thiamine deficiencies. Lamb ADG and ewe D.M. intake, weight change and milk yield were not influenced ($P > 0.05$) by diet. Mo supplementation did not influence ($P > 0.05$) plasma Cu concentration or distribution but did increase ($P < 0.001$) plasma Mo concentrations for ewes and their lambs. Least square means $\pm$ SE for ewe milk Mo were 0.14 $\pm$ 0.24, 1.53 $\pm$ 0.27 and 2.69 $\pm$ 0.27 mg L^{-1} for 0Mo, 20Mo and 40Mo, respectively.

Supplementing 20 mg Mo kg^{-1} D.M. to the diet of midlactation Holstein-Friesian cows did not influence ($P > 0.05$) D.M. intake, weight change and milk yield, but did result in increased plasma Cu ($P < 0.05$), TCA-insoluble Cu ($P < 0.001$), plasma Mo ($P < 0.001$) and milk Mo ($P < 0.05$) levels.

ACKNOWLEDGEMENTS

To my friends and colleagues in the Department of Animal Science, my thanks. I have learned from you and deeply appreciate the relationship.

In particular, I would like to thank Dr. T. Devlin whose confidence (of which there was much) and encouragement were important to me in deciding to undertake this Ph.D. program. Also, Dr. G. Phillips for his assistance and advice during my supervisor's sabbatical leave and for critical reading which helped in the preparation of this manuscript.

Appreciation also goes to Mr. Cliff Batenchuk and Peter Mills for their technical assistance and for help with lab analyses; to personnel in the Beef, Sheep and Dairy Units for their help in data collection and care of the experimental animals; Dr.'s Phillips and Stanger for their time and skills required to obtain the liver biopsy samples, and to Dr.'s Crow, Parker and McKay for their advice in statistical analysis of the data.

To Eric, a special thank you for the patient and loving support you have provided me during the course of this study and for the inspiration and friendship you have always provided.

Financial assistance by the Federal Provincial Agro-Man Agreement and the University of Manitoba is acknowledged.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDIX TABLES

LIST OF ABBREVIATIONS

α	-	alpha, significance level
A	-	absorbance, A is the absorbance change
ad lib	-	Ad libitum
ANOVA	-	analysis of variance
ARC	-	Agriculture Research Council
C	-	centigrade
cm	-	centimeter
Cp	-	ceruloplasmin
Cu	-	copper
d	-	day
D.M.	-	dry matter
E.C. (followed by numbers)	-	Enzyme Commission (numbers indicate formal classification of the enzyme)
FCM	-	fat-corrected milk
g	-	gram
x g	-	times the gravitational force
G	-	gauge
GLM	-	General Linear Model
>	-	greater than
h	-	hour
ha	-	hectare
H_2S, S^{2-}	-	sulfide
I.U.	-	international units
J	-	joule
kg	-	kilogram
L	-	liter
<	-	less than
m	-	meter
μg	-	microgram
min	-	minute
mg	-	milligram
MJ	-	megajoule
ml	-	milliliter
Mo	-	molybdenum
MO_4^{2-}	-	molybdate
NRC	-	National Research Council
%	-	percent
P	-	probability value, under the null hypothesis
pH	-	-log of H^+ concentration
PPD	-	p-phenylenediamine dihydrochloride
R^2	-	R-square measures amount of variation in the dependent variable that is accounted for by the model
S	-	sulfur
SAS	-	Statistical Analysis System
SE	-	standard error
SE(B)	-	standard error of the regression coefficient or slope

SNK	-	Student-Newman-Keuls
SO_3^{2-}	-	sulfite
SO_4^{2-}	-	sulfate
STP	-	standard temperature and pressure
t	-	tonne
TCA	-	trichloroacetic acid
VFA	-	volatile fatty acid(s)
v/v	-	volume/volume
W	-	tungsten
WO_4^{2-}	-	tungstate
w/v	-	weight/volume

INTRODUCTION

The current interest in trace element supplementation by researchers and the livestock industry has been brought about by a number of factors. Animal productivity has been limited by shortages of available dietary energy or protein, infectious or parasitic diseases and genetic inadequacies of the animal. As these limitations are increasingly rectified, local mineral deficiencies and/or imbalances are likely to become more apparent and more critical. Along with increasing rates of animal performance; decreasing availability of animal by-products in feed formulations and the increased use of industrial products and by-products have increased the need for a better understanding of trace element requirements and tolerances. Although minerals (especially trace elements) are a small part of the total cost of a feed, a considerable reduction in net income can occur when performance is affected adversely by deficiencies, excesses or imbalances.

As is the case with all of the essential trace elements, the role played by molybdenum is extremely complex. There are places around the globe where plants will not grow optimally because of deficiencies of molybdenum and other places where the levels of molybdenum in plants can be toxic to livestock grazing upon these plants. The latter have been identified in Canada (Fletcher and Brink, 1969) and more specifically in the Northwestern agricultural region of Manitoba (Cunningham et al, 1953; Boila et al,

1984a). More detailed information is provided in the literature review.

Evidence of the biological function of molybdenum was obtained in 1930 when Bortels, cited by Underwood (1971), showed that this element is an essential nutrient for the growth of Azotobacter. The first indication of the function of this element in animal production appeared in 1953 when two groups of researchers (Renzo et al., 1953 and Richert and Westerfeld, 1953) discovered that the flavoprotein enzyme, xanthine oxidase, is a molybdenum containing metalloenzyme. Since that time efforts have been made to substantiate the essentiality of this element through the use of highly purified diet and/or diets containing molybdenum antagonists fed to chicks, rats, lambs and goats. The essentiality and function of molybdenum is further outlined in the literature review. It should be noted, however, that under naturally occurring conditions, uncomplicated molybdenum deficiency has never been reported in animals.

The effect on animals of excessive molybdenum intake is a complex problem dependent upon a number of variables including: age and species of animal, the animal's previous dietary history, and relative amounts of molybdenum, copper, sulfur and other nutrients (i.e., sulfur containing amino acids) in the diet. Ruminants are much more sensitive to high dietary molybdenum levels than monogastrics (Underwood, 1977). Most interest in the metabolism of molybdenum in ruminants has centered on its pronounced antagonistic effect on copper availability. Remarkably little attention has been directed to the adverse effects of the

element that are independent of changes in copper metabolism, particularly if the sulfur intake is low. The symptomatology of excess dietary molybdenum varies among species but may include: failure to thrive represented by poor weight gains, poor growth or loss of body weight or condition; anemia; dermatological changes; anorexia and diarrhoea (Underwood, 1977; Ward, 1978; Hainline and Rajagopalan, 1983). Data available on the effects of excess dietary molybdenum on milk production are limited. Much of the work that was done involved dietary molybdenum concentrations well in excess of those found in feedstuffs. As the only food of the young preruminant is milk, its capacity to deal with potential changes in milk production and composition (i.e., increased molybdenum levels) is also crucial.

Experimental procedure, results and discussion are provided for three trials. The effects of excess dietary molybdenum on milk production and composition were studied for early lactation beef cows and ewes fed corn silage and barley rations. Also, parameters evaluating body copper and molybdenum stores and growth performance were monitored, for sucking beef calves and lambs, to evaluate "carry-over" effects of the dams' diets.

A trial was conducted with sixteen mid-lactation Holstein-Friesians to determine whether the detrimental effects of excess dietary Mo observed in the two previous trials could be overcome with Cu supplementation.

REVIEW OF LITERATURE

Molybdenum--An Essential Trace Element?

In a 1970 report discussing essentiality of trace elements, Mertz provided two definitions. The first definition, now widely accepted (Underwood, 1977) states that an element is essential if its deficiency reproducibly results in impairment of function from optimal to suboptimal. A very low dietary requirement of molybdenum (Mo) by poultry and mammals and the presence of these amounts of the metal in unsupplemented and semipurified diets has precluded the induction of a simple Mo deficiency by withholding Mo from the diet. A possible exception was shown in a study on female goats (Anke et al., 1978).

Researchers have administered high levels of tungsten (W) as a means of preventing the uptake and utilization of Mo, thereby creating a state of Mo deficiency. Tungsten inhibits the absorption of Mo from the digestive tract and may also act directly on molybdoenzymes (Johnson et al., 1974). The existence of a common active transport system carrying tungstate (WO_4^{2-}), molybdate (MoO_4^{2-}) and sulfate (SO_4^{2-}) in rat and sheep ileal epithelium has been demonstrated by Cardin and Mason (1975, 1976). As will be demonstrated in the following paragraphs, W administration has been shown to produce a variety of symptoms associated with low Mo intake, but a recent study by Nell et al. (1980) indicated that W may act by exerting a direct toxic effect on growth rather than by causing a Mo deficiency.

A slight reduction in growth rate of chicks fed low Mo diets was reported when W was used as a Mo inhibitor (Higgins, et al.,

1956). Leach and Norris (1957) found no growth response when Mo was added to semipurified, low Mo (0.5-0.8 mg Mo kg^{-1}) diets fed to chicks. However, supplemental Mo was effective in restoring growth and liver Mo and xanthine oxidase (a Mo containing metalloenzyme) concentrations which had been depressed by the addition of W to the rations. Efforts to produce a Mo deficiency in rats through the administration of low Mo diets (less than 0.6 mg kg^{-1}) reduced liver xanthine oxidase concentrations to 10% of normal without affecting animal health or changing the excretion of uric acid and allantoin (Richert et al., 1957).

Reports of a poor hatchability syndrome, characterised by a high incidence of weak, sickly chicks with altered feather maturation (Payne, 1977) and femoral epiphyseal changes (scabby hip syndrome) in chicks (Payne and Bains, 1975) have indicated that Mo supplementation allowed improvement. However, a recent report suggests that a deficiency of additional dietary components besides Mo may be required to produce the symptoms described (Nell and Annison, 1980).

Only one study with ruminants has shown reproducible adverse effects to low Mo diets. Anke et al. (1978) reported the following deficiency symptoms in quadruply repeated experiments with growing, pregnant and milking goats fed a high cellulose diet containing less than 0.06 mg Mo kg^{-1} DM: (1) does on test from 13 weeks of age to kidding gained 75% the weight gained by control does receiving a 1.0 mg Mo kg^{-1} diet. Body weight gains of the kids from Mo deficient does were 75% of the gains reported for animals on the control ration for the first 90 days of age;

(2) Feed intake by Mo deficient does was 29% lower than that of control does; (3) does fed Mo deficient diets had 45% the number of kids produced by control does at first kidding; (4) liver and cerebrum copper levels were significantly higher in kids from Mo deficient does than those from control does. This study concluded that Mo is an essential element and is required in excess of 0.06 mg kg^{-1} in ruminant diets.

There are some concerns regarding the validity of the report by Anke et al. (1978) in demonstrating Mo deficiency in the goats. First, no description of the synthetic diets or of nutrient analysis are provided to determine whether any other dietary components besides Mo were altered in developing the low Mo ration. Second, data from lambs provided by Ellis et al. (1958), and from in vitro studies provided by Martinez and Church (1970) suggest that Mo is required by rumen microorganisms for optimum cellulose digestion. Ellis et al. suggested that Mo supplementation of a semipurified diet containing 0.36 mg Mo kg^{-1} improved lamb weight gains by increasing cellulose degradation in the rumen. In vitro studies showed that concentrations of 10 to 100 mg kg^{-1} added Mo significantly increased cellulose digestion. The maximum stimulation (27%) occurred with additions of 30 mg kg^{-1} to a washed suspension of rumen microorganisms. Possible interactions of Mo with other elements normally present in rumen fluid but not present in the media used for these in vitro studies may nullify or alter the effects of Mo demonstrated by Martinez and Church (1970). However, these studies do bring to light the possibility that adverse response of the goats to the

semipurified diet, containing less than 0.06 mg Mo kg^{-1}, was a result of an altered or potentially less active rumen microflora.

The second definition of an essential trace element given by Mertz (1970) states that the constant occurrence of an element in an enzyme in stoichiometric amounts and correlation of enzyme activity with the degree of metal saturation of the active sites is positive proof for essentiality of the metal involved, even if a deficiency *in vivo* is difficult to attain.

In animals, Mo is known to be functional in three enzymes; xanthine oxidase/dehydrogenase (E.C. 1.2.3.2, 1.2.1.37), aldehyde oxidase (E.C. 1.2.3.1) and sulphite oxidase (E.C. 1.8.2.1). Xanthine oxidase catalyses the oxidative hydroxylation of a number of purines, pteridines, pyrimidines and other heterocyclic nitrogenous aromatic molecules (Bray, 1975; Coughlan, 1980). The principle site of enzyme activity in animals is the liver, followed by lung, kidney and intestinal mucosa (Al-Khalidi and Chaglassian, 1965). Molybdenum was identified to be a component of xanthine oxidase and therefore potentially, an essential element by Renzo et al. (1953) and Richert and Westerfeld (1953). They showed that Mo was the dietary constituent which had until then been called the 'xanthine oxidase factor'. This factor had been suggested to explain why some diets of equal protein content were better than others in enabling rats to maintain the level of xanthine oxidase in the liver. The molecular weight of the enzyme isolated from bovine milk has been estimated at 300,000 and it contains one flavin adenine dinucleotide, two non-haem Fe_2S_2 centers with one Mo center per subunit (Hainline and

Rajagopalan, 1983). The oxidation-hydroxylation of the substrates occurs at the Mo center of these enzymes. The exact biological function of xanthine dehydrogenase is poorly understood. Animals rendered functionally deficient in xanthine dehydrogenase, by treatment with Mo antagonists, grow and reproduce normally demonstrating the lack of long-term effects from the loss of enzyme activity despite a change in their excretion of oxidized purine (Johnson et al., 1974).

Aldehyde oxidase is very similar to xanthine oxidase/dehydrogenase having a molecular weight of 270,000 and one Mo, one flavin adenine dinucleotide and two Fe_2S_2 centers per subunit. The enzyme reacts with purines to yield 8-hydroxy purine, catalysing the in vivo conversion of N-methylnicotinamide to 2- and 4- pyridones (Hainline and Rajagopalan, 1983). A review of the literature lead to the suggestion that aldehyde oxidase activity is under genetic and hormonal control in the mouse compared to nutritional control demonstrated for xanthine oxygenase/dehydrogenase (Hainline and Rajagopalan, 1983).

Sulfite oxidase is involved in the metabolism of sulfur amino acids, oxidizing the sulfite (SO_3^{2-}) derived from cysteine and methionine to sulfate (SO_4^{2-}). Molecular weight of the enzyme ranges from 110,000 to 122,000, depending on the source. The enzyme consists of two identical subunits, each containing one Mo center and one cytochrome b_2-type haem (Rajagopalan, 1980). Cohen et al. (1973) found that W-treated rats deficient in sulfite oxidase remained healthy for lengthy periods of time. However, they could be differentiated from normal animals when

stressed by injected sodium bisulfite or by atmospheric sulfur dioxide.

The presence of the above three enzymes in animals argue for an essential role of dietary Mo in animal nutrition. However it is not yet established that these Mo-containing enzymes are necessary for the well-being or normal physiology of the animal.

The most direct evidence for the requirement of Mo in animals has been the discovery of a genetic defect in humans resulting in undetectable amounts of the Mo cofactor (see p. 26), sulfite oxidase, xanthine oxidase and Mo in the patient's blood and liver and resulting in early death (Johnson et al., 1980). Oral administration of Mo as molybdate did not alleviate the clinical condition of the patient or the abnormal S metabolism as evidenced by high levels of urinary sulfite.

Molybdenum Sources

Potential sources of Mo for ruminants include air, water, soil and plant materials. Although there are areas where optimum growth of crops is not possible because of a deficiency of Mo, there are also many areas where naturally occurring high levels of Mo in forages result in reduced animal performance. This section will deal mainly with the latter. Also, "man-made disturbances" including industrial emissions to the atmosphere, land disposal of wastes and certain agricultural practices which may result in significant increases in plant and soil Mo concentrations and/or availabilities will be discussed.

Air. Air Mo concentrations (mg m^{-3}, STP) have been reported to

be less than 0.2 to 3.2 in Europe and less than 1 to 10 in North America (Bowden, 1979). It was not stated in that review whether the discrepancies between European and North American values were real or because of sensitivity of the analytical techniques used. Dust in air samples is often associated with industrial emissions or soil erosion. Molybdenum levels in dust from air samples taken in London (in 1974) ranged from 0.2 to 12 mg kg^{-1} and in Milan (1975-77) averaged 30 mg kg^{-1} (Bowden, 1979). Probably the more critical factor with regard to Mo associated with airborne dust is potential contamination of soils and crops. Bowden (1979) estimated that the residence time of Mo in air was 10 to 30 days before particles containing Mo were removed by gravity or washed out by rain. No reports were found on the effects of inhalation of Mo however, molybdenosis (a disorder associated with high Mo intake) related to air pollution has been reported in cattle in several areas in the vicinity of industrial plants (Thornton, 1976; Ward, 1978).

Water. Although water is often suggested as a source of excess Mo intake, a review by Ward (1978) found no confirmed cases of molybdenosis related to Mo contaminated drinking water. Mo concentrations (as MoO_4^{2-}) in fresh water average 0.5 mg L^{-1} with a range of 0.03 to 16 mg Mo L^{-1} (Thornton, 1976; Bowden, 1979). Given that cattle consume approximately three times as much water as dry matter (NRC, 1976) the toxic level for Mo in drinking water should be approximately one-third that of feed.

Soil. Chappell (1977) estimated that the average abundance of Mo

in the earth's crust is 1 mg kg^{-1} but emphasized that there are large variations among geographical areas. A study conducted in west-central Manitoba identified a few areas characterized by soils containing up to 20 mg kg^{-1} Mo and bedrock from several river formations containing up to 40 mg kg^{-1} Mo (Doyle and Fletcher, 1977). Throughout most of their study area, however, soils and sediments contained less than 3 mg kg^{-1} Mo. Under normal grazing conditions ingestion of soil constitutes 3 to 14% of total dry matter intake of sheep and cattle (Field and Purves, 1964; Healy et al., 1974; Thornton, 1974) and may be as high as 40% under circumstances associated with overgrazing (Suttle et al., 1975). Therefore if soil Mo concentrations are greater than those of plants and if the bioavailability of this Mo source is high, soil consumption can be a contributing factor to excess Mo intake by the grazing ruminant. Suttle et al. (1975) found that the ingestion of soils containing 32 to 41 mg Mo kg^{-1} at 10% of total D.M. intake caused 4 to 6 fold increases in daily urinary Mo excretions in sheep, indicating that the Mo in soils can be biologically available. Urinary excretion accounted for 25% of Mo ingested from one of these high Mo soils and only 12% of that ingested from the other suggesting that differences in soil properties influence Mo absorption from the gastrointestinal tract. More information on this could be useful in identifying Mo associated problems of grazing ruminants in many parts of Canada. Aside from the effects of biologically available Mo, soil ingestion may affect copper (Cu) metabolism by reducing copper absorption from the gut due to: 1) adsorption of Cu on an

organo-mineral cation exchange complex, 2) sorption by hydrous oxides of iron, manganese and aluminum, and 3) formation of stable complexes with soil organic matter (Suttle et al., 1975).

Plants. The Mo content of plant feedstuffs varies with soil conditions, plant species, plant part and agricultural practices. Underwood (1977) stated that Mo levels in pasture herbage have been recorded from as low as 0.10 mg kg^{-1} to as high as 100 mg kg^{-1} on a dry matter basis. The majority of work done with Canadian forages has been from British Columbia and Manitoba. Fletcher and Brink (1969) reported values between 0.6 and 12.0 mg Mo kg^{-1} D.M. for range forages in south-central B.C. Forage samples taken from west-central Manitoba were found to have a range of 0.4 to 45 mg Mo kg^{-1} D.M. (Doyle and Fletcher, 1977; Boila et al., 1984a).

The uptake of Mo by plants is well correlated with the Mo in soil solution rather than total soil Mo concentration (Evans et al., 1950; West, 1981). Concentrations of Mo in soil solutions have been found to range from 10^{-8} M in low Mo soils (Lavy and Barber, 1964) to 10^{-5} M in soils producing toxic herbage (Kubota et al., 1963). The two major factors influencing the availability of Mo to plants are drainage and acidity. A good review on the association between soil wetness and availability to plants of soil Mo was provided by Allaway (1977). This report concluded that soils with poor drainage and aeration result in increased plant Mo uptake because of an enhanced mass flow (movement of soil solution to root surface) and diffusion (movement of ions in the direction of root surface). Also, leaching losses of soil Mo

appear to be reduced on poorly drained soils so that soluble Mo remains in the plant root zone until removed by cropping. Next to drainage, soil pH is the most important factor in determining availability of Mo to plants. Farm practices which result in increased organic matter in the soil and leaching of calcium, thus tending to increased soil acidity, decrease Mo availability (West, 1981). For example, Gupta (1969, 1970) reported increased contents of Mo in alfalfa and brome grass after liming. Limited information is available on the comparable effects of application of fertilizers containing large amounts of the major plant nutrients, nitrogen, phosphorus, potassium, and sulfur. West (1981) reported that potassium and sulfur fertilizers decreased Mo content in the plant, however the changes were small and not tested for various soil types or growing conditions. Reith et al. (1984) examined the effects of applying nitrogen and phosphorus on the Cu and Mo concentrations in mixed grassland herbage grown on three mineral soils and on a deep peat acid soil. Applying ammonium nitrate (56-190 kg N ha^{-1}) reduced Mo content and had a variable effect on Cu content of herbage. Applying phosphorus, especially at high rates (220 kg P ha^{-1}), resulted in slightly lower Cu and Mo contents in herbage. Whether the changes in plant Mo content were associated with decreased availability of Mo or a dilution effect, because of more rapid plant growth, has not been clearly defined in these fertilizer studies. Application of Mo fertilizers will result in relatively large increases in forage Mo contents (Gupta and McLeod, 1975; Reith et al., 1984), however it should be noted that this practice is

generally restricted to areas where there are plant Mo deficiencies (Allaway, 1977).

Different plant species accumulate different amounts of Mo from the same soil. In general, legumes have higher concentrations of Mo than grasses when grown under similar conditions. However, the reverse also has been found often (Miltimore and Mason, 1971; Reid and Hovarth, 1980; Boila et al., 1984a). Fletcher and Brink (1969) reported Mo concentrations in Kentucky bluegrass of 5.0 $\pm$ 4.0 compared with less than 1.6-1.8 mg kg^{-1} D.M. in other grasses sampled at the same locations, suggesting large differences in Mo uptake among species of grass.

Stage of plant growth and plant part harvested can also influence dietary Mo and Cu of ruminants. Burridge (1970) reported that there was a dilution effect for Cu, related to rapid growth of plants from the two-leaf to the headed stage after which concentrations stabilized. Molybdenum concentrations, on the other hand, steadily declined from the two-leaf stage to that of ripe grain. A study by Davey and Mitchell (1968) on Dactylis glomerata (cocksfoot) at flowering illustrates the differences in Mo concentrations of various plant parts. The Mo content (D.M. basis) in the spikelets (0.35 mg kg^{-1}), sheath (0.20 mg kg^{-1}) and stem (0.20 mg kg^{-1}) were considerably lower than that of the leaves (0.70 mg kg^{-1}). Comparable data on the distribution of Mo in plants containing high levels of Mo have not been found in the literature. A preference for leaf material relative to plant stem material has been demonstrated by ruminants in grazing studies (Langlands, 1967; Hodgson and Jamieson, 1981). Such

selective grazing would increase the potential for excess Mo intake by livestock, if plant Mo is above normal and plant Mo distribution patterns are similar to that shown by Davey and Mitchell (1968). Stage of growth and frequency of cutting could also affect the concentration of Mo in forage dry matter by altering the leaf to stem ratio.

Miltimore and Mason (1971) found that corn silage and grains were low in Mo content, again suggesting that dietary Mo may vary with plant species and/or plant part fed to the animal. The chemical states of Mo in feedstuffs have not been identified to date (Bowden, 1979; Winston, 1981).

Other Sources of Molybdenum. Other dietary sources of Mo include sodium molybdate, ammonium molybdate, calcium molybdate and molybdenum trioxide (hexavalent forms of Mo) which have a high bioavailability (Underwood, 1981) and are generally only used in experimental studies with animals. A case in which dairy cattle developed molybdenosis following ingestion of Mo contaminated magnesium oxide, added as a supplement to the grain mix (Lloyd et al., 1977), illustrates that high levels of dietary Mo may occur in feedstuffs that are not normally considered to be a source of Mo.

Molybdenum Metabolism in Ruminants

The metabolism of Mo in animals will be discussed under the headings: absorption and transport; distribution and storage in the body; intercellular metabolism; and excretion. Where appropriate, the effects of Mo on Cu metabolism will be discussed.

Absorption and Transport of Molybdenum. Molybdenum is most stable in the hexavalent oxidation state where it is normally bound to four oxygens and exists as the oxyanion molybdate (MoO_4^{2-}) (Huisingh and Matrone, 1977). Molybdate and SO_4^{2-} have the same charge and stereochemistry, have a similar size and therefore could be biologically antagonistic (Mason and Cardin, 1977). Since the amount of SO_4^{2-} normally present in ruminant diets is several hundred times that of MoO_4^{2-} (Suttle, 1974), interaction between these two oxyanions may be of major significance in Mo absorption and transport as well as in other aspects of Mo metabolism.

An active transport system carrying MoO_4^{2-}, SO_4^{2-} and other group VI oxyanions was shown to exist in the rat ileum using an in vitro technique of everted intestinal sacs (Cardin and Mason, 1975, 1976). In a subsequent study, Mason and Cardin (1977) established that the general pattern of MoO_4^{2-} and SO_4^{2-} uptake by ovine intestine resembled that of the rat intestine, the site of maximal MoO_4^{2-} uptake being the distal ileum. They also found exclusive competitive behavior for MoO_4^{2-} by SO_4^{2-} but only a partly competitive inhibition of SO_4^{2-} uptake by MoO_4^{2-} in the ovine intestine. This suggests other interactions for MoO_4^{2-} in the gut in addition to competition for the sulfate binding sites. Tungstate (WO_4^{2-}), discussed earlier as a Mo antagonist, and selenate also compete for sites on the MoO_4^{2-} transport system.

Hansard (1983) reported that 20% of dietary Mo is absorbed from the gastrointestinal tract. The availability, however, is

strongly influenced by other nutrients, i.e., sulfur and copper.

Influence of Dietary Sulfur - The most important effect of dietary sulfur (S) on Mo absorption arises as a result of interactions between Mo and S compounds in the ruminal digesta. Reduction of both inorganic and organic S to sulfide (H_2S) by sulfolytic bacteria takes place in the rumen (Bray and Till, 1975). The various pathways by which Mo can affect the equilibrium concentrations of free sulfide in the rumen ultimately also influence the fate of Mo in the animal.

Molybdenum, as sodium molybdate, has been shown to inhibit the rate of SO_4^{2-} reduction in suspensions of washed rumen microorganisms (Huisingh and Matrone, 1972) and in vivo (Gawthorne and Nader, 1975). This inhibition of SO_4^{2-} reduction is thought to be due to competition at the first stage of SO_4^{2-} activation, catalysed by ATP and sulfate adenyltransferase (E.C. 2.7.7.4) (Wilson and Bandurski, 1958). However, ruminal H_2S levels were found to increase when dietary Mo levels were increased (Mills, 1960; Gawthorne and Nader, 1975). Two possible explanations can be found in the literature. First, Huisingh et al. (1975) demonstrated that sheep fed a diet containing methionine as a source of organic S and 50 mg Mo kg^{-1} had increased ruminal H_2S production compared with sheep fed a non-Mo supplemented control diet. In the same study depressed H_2S production was found in the presence of Mo if SO_4^{2-} was used as the dietary source of S. Therefore increased ruminal H_2S levels may be related to enhanced production of H_2S from sulfur amino acids. The second mechanism was described by Gawthorne and Nader (1975) who found that appar-

ent absorption of labelled H_2S from the rumen of sheep was almost entirely inhibited by intraruminal infusion of 50 mg Mo d^{-1}. The resulting increase in the physiological half-life of H_2S in the rumen would result in increased ruminal H_2S concentration despite the decreased rate of SO_4^{2-} reduction. Gawthorne and Nader (1975) speculated that once apparent absorption of H_2S was inhibited, further additions of Mo could reduce concentrations of rumen H_2S by interfering with SO_4^{2-} reduction.

Dick et al. (1975) proposed that the progressive reaction of H_2S with molybdate in the rumen gives rise to a series of new compounds, thiomolybdates: MoO_4^{2-} --> $MoO_2S_2^{2-}$ --> $MoOS_3^{2-}$ --> MoS_4^{2-} referred to as di-, tri- and tetrathiomolybdates, respectively.

Direct evidence demonstrating the synthesis of thiomolybdates in ruminants is limited. Clarke and Laurie (1980) concluded from in vitro studies that the formation of thiomolybdates is critically dependent on the ruminal S:Mo ratio and that under physiological conditions, the production of di- and trithiomolybdates would be favored. Extensive synthesis of tetrathiomolybdate occurred only after a relatively long period of time and at high dietary S:Mo ratios. Bray et al. (1982) found a mixture of thiomolybdates with a predominance of trithiomolybdate in the liquid phase of digesta in a Rusitec artificial rumen system. The fact that Mo was found predominantly in the liquid phase should be recognized as a limitation in interpreting the results of this study because Grace and Suttle (1979) found that in vivo Mo in rumen fluid is predominantly in the solid

phase.

Mason et al. (1978b) and Kelleher et al. (1983) demonstrated *in vivo* that the infusion of ^{99}Mo-labelled molybdate into the duodenum caused the appearance of trichloroacetic acid-(TCA-) soluble ^{99}Mo in plasma but that ruminal administration resulted in the appearance of plasma ^{99}Mo that was protein bound and TCA-insoluble. The TCA-insoluble Mo was increased by increasing dietary S levels.

In vivo studies by Mason et al. (1982), Kelleher et al. (1983), and Hynes et al. (1984) have shown that thiomolybdates bind to albumin to form the protein-bound plasma Mo fraction and as such are relatively stable. The TCA-soluble fraction of plasma Mo was probably molybdate or at least a form of Mo that competes with sulfate ions for a common intestinal transport system (Cardin and Mason, 1976; Mason and Cardin, 1977).

Mason et al. (1982) provided *in vivo* evidence of thiomolybdate synthesis and absorption in ruminants. They found protein bound, TCA-insoluble ^{99}Mo appeared in plasma a few hours after infusion of ^{99}Mo-labelled molybdenum (30 mg Mo) into the rumen of sheep fed a concentrate diet supplemented with (3 g S d^{-1}). When the plasma ^{99}Mo was displaced from its protein carrier *in vitro*, the labelled compounds displaced were identified as di- and trithiomolybdates. Tetrathiomolybdate was not detected in plasma.

The absorption of ^{99}Mo compounds also was studied by Kelleher et al. (1983) by exchanging digesta between pairs of sheep with re-entrant duodenal cannulae. They found that Mo from tri- and tetrathiomolybdate infused into the rumen was rapidly

absorbed from the rumen and was present in plasma in a protein-bound form. Although thiomolybdates are sensitive to acid hydrolysis, in the order di- >tri-> tetrathiomolybdate (Mason, 1981; Clarke and Laurie, 1982), a significant proportion of the intraruminally infused thiomolybdates survived the acid environment of the abomasum and were found in the circulation in the protein-bound form. Molybdenum from thiomolybdates that were degraded by the acid conditions of the post ruminal tract resulted in increased plasma molybdate which is not bound to plasma protein.

Influence of Dietary Copper - In proposing the thiomolybdate theory, Dick et al. (1975) suggested that these compounds could interact with Cu in the gut and reduce both Cu and Mo absorption and/or interact with tissue Cu and interfere with Cu metabolism following absorption. In other words, there are two possible areas of interaction between Mo compounds and Cu; in the gut resulting in decreased Cu availability; and systemically, where several effects are possible. This is the basis for distinguishing between two interrelated syndromes: molybdenum-induced hypocupraemia and molybdenosis (Mason, 1981). The former is predominant if the dietary Mo:Cu ratio is relatively low and the latter, with high Mo levels and a high Mo:Cu ratio. Both syndromes will be discussed in more detail later.

With low dietary Mo concentrations (less than 8 mg kg^{-1}) increasing dietary S levels accelerate the depletion of hepatic Cu reserves and promote a fall in plasma Cu (Mason, 1981). For example, Suttle and McLauchlan (1976) varied dietary Mo between 1

and 5 mg kg^{-1} and S (organic and inorganic) between 1 and 3 g kg^{-1} in feed for sheep and found that Cu absorption, measured by a repletion technique, was reduced by 35% when fed the high Mo and S diets. Mason (1981) cited a study by Mills and Bremner who found an 80% reduction of Cu absorption in cattle subjected to similar dietary regimes. On the other hand, Mason et al. (1978b) found that dietary Cu supplementation reduced TCA-insoluble Mo concentrations in plasma and reduced the absorption of Mo in sheep on a MoO_4^{2-} and SO_4^{2-} supplemented diet. Results from these three studies provide some evidence that Cu and Mo can be bound together in a form that is unabsorbable from the gut.

A second effect of Cu may be on Mo inhibition of the reduction of SO_4^{2-} to H_2S in the rumen. A review of the literature did not reveal any work reported in this area, however, Dowdy and Matrone (1968) observed that Cu of copper sulfate ($CuSO_4$) and sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$) form a complex which precipitates in a near neutral solution (similar to rumen pH). The ratio of Cu to Mo in this complex, identified as lindgrenite ($2CuMoO_4 \cdot Cu(OH)_2$, Dowdy et al., 1969) is 3:2. If such a complex does form <u>in vivo</u>, MoO_4^{2-} concentrations in rumen fluid would be reduced which in turn would reduce both the inhibitory effects of Mo on SO_4^{2-} reduction and the availability of Mo for thiomolybdate formation.

Distribution and Storage of Molybdenum. Under normal dietary conditions, the Mo content in tissues is low, ranging from 0.1 to 5 mg kg^{-1} dry weight (Kienholz, 1977; Hainline and Rajagopalan, 1983) with tissue concentrations being highest in liver, kidney,

bone, muscle and skin. Based on a review of the literature, Underwood (1977) reported that species differences appear to be small and that there is little Mo accumulation in tissues or any one particular organ with age. Blood levels of Mo in animals appear to be dependent upon the animal species and the dietary intake of Mo, Cu and S (Underwood, 1977).

Ingestion of feedstuffs high in Mo, or of Mo supplemented diets, modifies the Mo levels in tissues of both monogastric and ruminant animals. Increasing the Mo content of rat diets from 1 to 30 mg kg^{-1} was found to raise the hepatic Mo from 1 to 11-12 mg kg^{-1} D.M. and the Mo content of bone from 0.2 to 9-12 mg kg^{-1} D.M. (Davis, 1950). Vanderveen and Keener (1964) found that the Mo levels in livers of Holstein cows increased from 5-6 to 29-30 mg kg^{-1} D.M. and serum Mo increased from 0.01-0.03 to 2.07-3.92 mg L^{-1} by adding 50 mg kg^{-1} of Mo to the control diet. Sheep grazing a Mo-fertilized pasture (ranging from 5.5 to 33.5 mg Mo kg^{-1} D.M.) had 80-168, 3 and 16 fold higher Mo contents in the plasma, liver and kidney, respectively, compared to sheep grazing an unfertilized pasture (Pitt et al., 1980). It should also be pointed out that in this study yearling sheep had higher plasma Mo content (2.4 ± 0.6 mg L^{-1}) than did mature sheep (1.7 ± 0.4 mg L^{-1}) reflecting either increased ability to absorb dietary Mo in young compared to mature sheep or a difference in grazing behavior between these two groups of animals. Adult sheep and cattle maintain Mo concentrations of 25-30 mg kg^{-1} D.M. in their livers as long as the element is ingested in large amounts. These high levels rapidly return to normal when intake of the extra Mo stops

(Underwood, 1977).

Underwood (1977) and Hainline and Rajagopalan (1983) both stated that increased dietary inorganic S reduces the tissue Mo content in rats and in cattle and sheep. This decrease was ascribed to increased competition for absorption in the intestine and/or reduced absorption of Mo-S complexes formed in the rumen. The magnitude of this SO_4^{2-} effect on tissue Mo content was well demonstrated by Dick (1956), who found that sheep fed a control ration providing 0.3 mg Mo d^{-1} had a reduction in total body Mo from 92.9 to 16.8 mg when daily sulfate intake was increased from 0.9 to 6.3 g d^{-1}. The response to sulfate was even more dramatic if Mo intakes were high (20.9 mg d^{-1}) as total body Mo dropped from 297.7 to 28.4 mg for the same sulfate intakes.

Dick (1956) found that the kidney does not follow the above pattern as the highest Mo concentrations in the kidney occurred in the sheep fed the high levels of both SO_4^{2-} and Mo. More recently, Bremner and Young (1978) reported that Cu and Mo accumulated in the kidneys of Mo and SO_4^{2-} fed sheep and attributed this to accumulation of a Cu-thiomolybdate complex formed systemically as described by Suttle (1974a) and Dick (1975).

The effect of dietary S on plasma Mo content is more complicated. Under normal dietary conditions plasma Mo exists in the form of molybdate, and is TCA-soluble (p. 19). Normal serum Mo concentrations can range from 0.06-60 mg L^{-1} in sheep and cattle (Hansard, 1983). As mentioned earlier (p. 19) administration of Mo to ruminant diets can result in the appearance of thiomolybdates in the plasma. Increased levels of dietary S can increase

circulating levels of thiomolybdates for animals fed the same concentration of Mo (Bremner, 1976).

There are some inconsistencies in the reported data on the effects of dietary S on tissue and blood Mo content, as exemplified by the results of Ademosun and Munyabuntu (1982). They fed four groups of lambs a basal diet containing 9.1 mg Cu kg^{-1}, 0.66 mg Mo kg^{-1} and 1.6 g S kg^{-1} with either 0 or 10 mg kg^{-1} added Mo ($(NH_4)_6Mo_7O_{24}.4H_2O$) and either 0 or 4 g kg^{-1} added S (1:1 ratio of Na_2SO_4 and K_2SO_4). After twelve weeks liver Mo content was significantly higher in lambs receiving the Mo supplement but the added SO_4^{2-} had no effect. Plasma Mo levels were 1 and 3-4 mg L^{-1} in sheep supplemented with 0 and 10 mg kg^{-1} Mo respectively, but there was no response to added S. It appears from this study that the production of thiomolybdates or Cu-thiomolybdate complexes in the gastrointestinal tract of the sheep either did not affect Mo absorption or interfered with the excretion of absorbed Mo. However, the treatments influenced Cu metabolism as expected. Plasma Cu levels were reduced by supplements of either Mo or S and the combination of both Mo and S supplementation caused a further significant decrease. Dietary Mo caused a greater reduction than dietary S in liver Cu content and plasma ceruloplasmin oxidase activity. Ceruloplasmin, E.C. 1.16.3.1, is a glycoprotein containing six Cu atoms and nine sialic acid residues per molecule (Frieden, 1980). The combination of both Mo and S resulted in an even greater depression of liver Cu content and ceruloplasmin oxidase activity.

The results of this study (Ademosun and Munyabuntu, 1982)

leave several questions unanswered. First, if the decreased plasma Cu observed for the Mo + S treatment was due to the formation of unabsorbable thiomolybdate complexes in the gut, why was there not a corresponding decrease in plasma Mo levels? The answer may be increased production of unabsorbable copper sulfide (Suttle, 1974b) relative to copper-thiomolybdate complexes in the gut. Second, as dietary S had a greater effect on plasma Cu content than did dietary Mo; why were ceruloplasmin oxidase activity and liver Cu content more affected by dietary Mo than by dietary S? Again the results imply that S was more effective than Mo in reducing Cu absorption or in facilitating removal of Cu from the blood. Molybdenum, however, appeared to influence absorbed Cu utilization, as evidenced by plasma ceruloplasmin oxidase activity and liver Cu content. Ishida et al. (1982) outlined two possible ways in which absorbed thiomolybdates may affect Cu utilization. First, by preventing Cu from entering liver cells so that liver Cu storage and Cu utilization for ceruloplasmin synthesis are indirectly affected. Second, by a primary intercellular metabolic effect preventing the synthesis of Cu-storage complexes and ceruloplasmin. The latter suggests a general control of liver Cu metabolism and may also explain situations in which ceruloplasmin oxidase activity responds relatively rapidly (within 1 to 2 weeks) to dietary administrations of Mo and SO_4^{2-} (Ishida et al., 1982).

Copper supplementation can also influence tissue and blood Mo content. Hogan et al. (1971) found that monthly subcutaneous injections of Cu, as cuproxoline (cupric 8-hydroxyquinoline bis-

(diethylamine sulphonate)) significantly increased molybdenum concentrations in the kidney, but did not affect plasma Mo values of pastured sheep. Dietary Cu supplementation exerts a major effect on Mo at the gut level by decreasing Mo absorption (Mason, 1978).

The chemical state of Mo in animal tissues is largely unknown although a part has been presumed to be bound to molybdoenzymes or to unknown storage proteins (Hainline and Rajagopalan, 1983). Johnson et al. (1977) examined the distribution of Mo present in livers of rats fed a control diet containing 0.02-0.03 mg Mo kg^{-1} D.M. Sulfite oxidase and xanthine oxidase accounted for 60 percent of the Mo present and the remainder was associated with a Mo cofactor. Under these controlled conditions liver Mo was quantitatively accounted for, but it is possible that under conditions of more variable exposure to Mo, other forms of Mo, including free molybdate ion, might be present in tissues.

Intracellular Metabolism of Molybdenum. Most current knowledge concerning Mo in animal tissues has been obtained through the discovery and characterization of the molybdoenzymes, xanthine oxidase/dehydrogenase, aldehyde oxidase and sulfite oxidase. Despite advances in knowledge concerning the other prosthetic groups of molybdoenzymes very little was known about the Mo center of these enzymes until Pienkos et al. (1977) isolated the molybdenum cofactor of milk xanthine oxidase. They suggested that this same cofactor was shared by the other molybdoenzymes of animal origin.

Also, studies have shown the existence of storage forms of

this cofactor in combination with Mo, and that these storage forms are distinct from the defined molybdoenzymes present in animal tissues (Johnson et al., 1977). These authors reported that the cofactor in rat tissue is associated with the outer membrane of mitochondria, the major storage site being the liver. A current hypothesis regarding the structure of the active Mo cofactor suggests that it consists of a reduced pterin linked to a molybdenum atom by sulphur bonds (Hainline and Rajagopalan, 1983).

In vitro studies by Cardo et al. (1983) suggested that intracellular molybdate may interact with the glucocorticoid receptor molecule turning it into a form that has a low affinity for the steroid. This receptor, after binding to the steroid, undergoes a process called 'transformation' or 'activation' that makes the steroid-receptor complex able to translocate into the nucleus of the target cell (Chan and O'Malley, 1976). Cardo et al. (1983) also found reduced transformation ability of the glucocorticoid receptor in the presence of molybdate. This suggested that both the reduced affinity and reduced transformation responses were related to weak and reversible association of molybdate with sulphydryl groups of the receptor. Molybdate also was found to directly inhibit the in vitro transformation of uterine estrogen receptors to the DNA binding state (Shyamala and Leonard, 1980). No data were found on intracellular fluid molybdate concentrations for animals receiving varying dietary concentrations of Mo.

Excretion of Molybdenum. The major excretion routes for Mo are in feces, urine and milk. Hansard (1983) cited a study by Bell et al. (1966) in which fecal excretion in steers averaged 92% of oral ^{99}Mo doses and 30% of those given intravenously with 0.5% and 9.5%, respectively, in the urine. Robinson et al. (1966) gave intravenous infusions of ^{99}Mo as $Na_2MoO_4.2H_2O$ to six lactating (15.9 $\pm$ 2.2 L milk d^{-1}) Holstein-Friesian cows. Excretion in urine, milk and feces represented 52.4 $\pm$ 4.9, 26.2 $\pm$ 3.0 and 21.4 $\pm$ 2.0% of total ^{99}Mo losses, respectively during the period 31.5 to 55.5 hours post infusion.

In a similar experiment on six pregnant (1 to 7 mo.), late lactation (4.5 $\pm$ 1.0 L milk d^{-1}) Holstein-Friesian cows, urinary, milk and fecal Mo excretion represented 53.8 $\pm$ 5.8, 2.1 $\pm$ 0.4 and 44.1 $\pm$ 5.2%, respectively of total ^{99}Mo losses (Robinson et al., 1966). The biological half-life of injected ^{99}Mo also differed between these two groups of cows. The total 24 hour Mo losses were 2.24 $\pm$ 0.52 and 11.5 $\pm$ 2.4 mg kg^{-1}, respectively for the high and low lactation cows. Whether the differences observed between these two groups of animals were related to milk production, pregnancy, previous nutritional history or genetics cannot be determined from this study. The two studies (Bell et al., 1966; Robinson et al., 1966) reported here and many earlier studies, failed to report dietary concentrations of nutrients, such as S and Cu, that may affect Mo excretion.

Weber et al. (1983) found that 40 kg sheep fed a lucerne and oaten chaff diet containing 5.5 mg Cu, 0.7 mg Mo and 1.1 g S d^{-1},

excreted less than 10% of ingested Mo, the majority of this being in the urine. This would suggest that the Mo is retained in tissues. Therefore, either Mo excretion as a percent of total intake would be higher for mature animals or tissue Mo content would increase with animal age. The latter is in conflict with statements made by Underwood (1977).

Increasing the dietary Mo intake from 5 to 50 mg d^{-1} resulted in approximately 50 to 60% of the dietary Mo being excreted with approximately 65% of that excreted appearing in the feces (Weber et al., 1983). The systemic effects of the Mo retained by animals fed diets containing Mo in the range used by Weber et al. (1983) merits further study.

Grace and Suttle (1979) used ^{99}Mo to study the effects of dietary S on Mo metabolism in sheep and found increasing dietary S decreased Mo absorption. However, increased S intake also increased body Mo retention as a result of decreased urinary excretion and fecal endogenous losses of Mo. These data contradict results of Dick (1956) who reported reduced whole body Mo retention. An explanation for the contradictory results may be the differences in dietary S content of the rations used in the two studies, as daily S intakes for sheep in the latter study were 6.3 g d^{-1} as compared to 3.3 g d^{-1}, the highest value used by Grace and Suttle (1979).

Plasma thiomolybdates bind to albumin *in vivo* to form relatively stable complexes (Kelleher et al., 1983). However if displacement occurs or if the binding capacity of the albumin carrier is saturated, the free compounds are rapidly hydrolyzed

to MoO_4^{2-} and SO_4^{2-} (Hynes et al., 1984). These compounds may be recycled, but with relatively high dietary S levels the Mo tends to be excreted in urine. This is probably because of molybdate-sulfate competition for transport processes in the renal tubules which blocks Mo reabsorption (Mason, 1981).

Sulfur can also influence Mo recycling via salivary secretions (Suttle and Grace, 1978). For low Mo intakes (0.3 mg d^{-1}), salivary Mo secretions accounted for 0.045 to 0.008 mg d^{-1} or 17.6 to 6.0% of the absorbed dietary Mo. These values were not significantly affected by dietary S intakes, ranging from 0.98 to 3.23 g d^{-1} but there was a trend towards reduced Mo secretion with increased dietary S. With low S intake (0.98 g d^{-1}) and high Mo intake (3.5 mg d^{-1}), 2.4 mg Mo d^{-1}, representing 89 % of absorbed dietary Mo was secreted via saliva into the rumen. The coefficients of absorption for dietary and dietary plus secreted Mo were the same (0.75) indicating that secreted Mo was extensively reabsorbed. However, additions of dietary S resulting in daily intakes from 1.33 up to 3.23 g d^{-1} significantly reduced recycling of Mo, whether determined on the basis of Mo secreted into the rumen (0.109 to 0.009 mg d^{-1}) or as a proportion of absorbed Mo. Therefore it was concluded that S reduced the amount of Mo recycled via saliva by decreasing the amount of Mo available for secretion because of decreased absorption, and by decreasing the "secretability" of circulating Mo.

Copper and Molybdenum Content of Milk

The Cu content of ruminant milk is low and varies with species, stage of lactation and Cu status of the animal (Archi-

bald, 1958; Underwood, 1977; Lönnerdal et al., 1981). Copper content in colostrum ranges from 0.20 to 0.30 mg L^{-1} for cows and from 0.50 to 1.40 mg L^{-1} for ewes. Milk has concentrations around 0.10-0.20 and 0.20-0.40 mg L^{-1} for cows and ewes, respectively (Lönnerdal et al., 1981). Vanderveen and Keener (1964) demonstrated that the Cu concentrations in cows' milk declined from 0.28 mg L^{-1} on day 75 of lactation to 0.06 mg L^{-1} on day 300. Underwood (1977) cited a study by Beck (1941) in which the milk of normal ewes declined from 0.20-0.60 mg L^{-1} in early lactation to 0.40-0.16 mg L^{-1} several months later. Subnormal Cu levels of 0.01 to 0.02 mg L^{-1} in the milk of cows and ewes grazing Cu-deficient pastures were also reported by Beck (1941). Subcutaneous injection of a copper-glycinate suspension increased the Cu levels in cows' milk for at least 4 weeks (Dunkley et al., 1963), a result in contrast with reports of no milk response to dietary Cu supplementation (Underwood, 1977).

Milk is a major excretion route for Mo in the lactating animal and the Mo content of milk is influenced by dietary Mo intakes in cows, ewes and does (goats). Early work reported by Archibald (1951) showed that cows fed 500 mg Mo d^{-1} as ammonium molybdate had 0.37 mg Mo L^{-1} milk compared with 0.07 mg Mo L^{-1} for animals fed the control ration. Cunningham et al. (1953) administered daily drenches of ammonium molybdate containing 340 (days 1 to 10), 1360 (days 11 to 52) and 2721 (days 53 to 65) mg Mo to a lactating cow. He found no change in Mo content of milk in response to the lowest level of dosing, and a gradual increase to 2.05 mg L^{-1} over the first twenty-three days on the interme-

diate dosage level, followed by a decline to initial levels. At the highest Mo dosage the Mo content of milk remained at less than 0.52 mg L^{-1}. A second animal was given either 1360 (days 1 to 20), 2721 (days 21 to 31), or 5442 (days 31 to 37) mg Mo d^{-1} in the same method and the Mo content of milk averaged 0.75, 1.16 and 2.32 mg L^{-1}, respectively. Ten other cows in this herd fed a typical dairy ration produced milk containing 0.12 mg Mo L^{-1}. Unfortunately the Mo, Cu and S contents of the basal diet and daily dry matter intakes were not reported for either study. Also, this study by Cunningham et al. (1953) did not allow for possible carryover effects with progressively increased levels of orally administered Mo.

Kiermeier and Capellari (1958) compared the effects of feeding hay providing 2.6 or 34 mg Mo d^{-1}. The milk from cows fed the high Mo hay had two to three fold higher levels of Mo than that of 0.15 mg L^{-1} milk from cows fed low Mo hay. The fact that two different sources of hay, which may have had different nutrient profiles, were used leave the results of this experiment open to question.

Hart et al. (1967) gave cows daily doses of 0, 45 or 95 mg Mo as ammonium molybdate, representing 0.4, 3.5 and 7.3 mg Mo kg^{-1} diet, and found Mo content of milk to be 0.26, 0.40-0.45 and 0.40-0.60 mg L^{-1}, respectively. The basal ration provided 4.5 to 5 mg Mo d^{-1} depending upon feed intake. These authors also found that increasing the daily Mo intake from 1.1 mg to 13 mg resulted in Mo in milk being increased from 0.12 to 0.20-0.70 mg L^{-1}.

Vanderveen and Keener (1964) found that Holstein cows fed

diets containing from 5 to 50 mg kg^{-1} added Mo, as ammonium molybdate, had milk Mo content of 0.08 to 0.87 mg Mo L^{-1}, respectively compared to 0.03 mg Mo L^{-1} for control animals. Milk Cu content was not affected by dietary Mo. The cows were put on test at parturition and maintained on the respective rations for 300 days. Over this period the concentrations of Mo in milk increased for cows that received the Mo supplemented rations. Addition of 3% S (as sulfate) did not affect Mo content of milk for a second group of cows fed the control diet but reduced the milk Mo levels for cows fed the supplemental levels of Mo. Diets containing from 5 to 50 mg kg^{-1} added Mo and 3.0 g kg^{-1} added S resulted in milk Mo levels of 0.05 to 0.22 mg L^{-1}. Adding Mo and S to the diet depressed milk Cu concentrations. The Cu content of the basal diet was 2 mg kg^{-1} but Mo and S levels were not reported. There were no statistical analyses of the data.

Hogan and Hutchinson (1965) measured the Mo content of milk from ewes grazing three pastures with Mo contents of less than 1, 13 and 25 mg kg^{-1} D.M., and found the Mo concentrations in milk to be less than 0.01, 0.98 and 1.04 mg L^{-1}, respectively. The oral administration of 23 g SO_4^{2-} d^{-1} to ewes grazing the pasture that contained 25 mg kg^{-1} Mo forage caused milk Mo content to decrease to 0.14 mg L^{-1}.

Lactating dairy cows fed a basal diet containing 6.4 mg Cu kg^{-1} to which was added 0, 53 or 173 mg Mo kg^{-1} D.M., as sodium molybdate, produced milk containing 0.03, 1.34 and 2.37 mg Mo L^{-1}, respectively (Huber et al., 1971). Dietary Mo increased the Cu content of milk from 0.10 mg L^{-1} for control animals to 1.08-

1.27 mg L^{-1} for Mo supplemented animals. This result is in contrast to the response observed by Vanderveen and Keener (1964).

The studies mentioned above illustrate that increased Mo intake results in increased milk Mo content. With the exception of the studies done by Vanderveen and Keener (1964) and Hogan and Hutchinson (1965), no literature is available regarding the effects of other dietary nutrients on the secretion of Mo in milk. To date, reports providing milk Mo data have failed to provide information concerning the nutrient profile of the basal diet used, daily feed intakes and feed intake responses to dietary treatments. Also, there is limited information on the stage of lactation and the milk yield of the animals used in these studies. Therefore current knowledge of factors influencing milk Mo content and total daily excretion of Mo in milk is limited. This also presents a question regarding the ability of lactating animals to rid themselves of excess absorbed Mo as compared to non-lactating animals.

Hart et al. (1967) determined both the xanthine oxidase activities and Mo content of cows milk and observed that the xanthine oxidase activity was proportional to the Mo content. They also found that oral administration of ammonium molybdate to the cows resulted in increased Mo content of milk but did not change its xanthine oxidase activity. A similar response was seen in goats given supplemental Mo.

Molybdenum is found in both the cream and skimmilk fractions of milk. Archibald (1951) reported that cream from cows on a

control ration contained 0.26 mg Mo L^{-1} compared with 0.51 mg Mo L^{-1} for animals receiving 500 mg Mo daily. The amounts in the skimmilk were 0.05 and 0.19 mg Mo L^{-1}, respectively. Butterfat content of the milk was not reported. Hogan and Hutchinson (1965) found a high proportion of the Mo associated with the aqueous phase in ewe's milk.

Copper and Molybdenum Metabolism in Preruminants

The literature on body copper stores in newborn calves and lambs was reviewed by Hidiroglou and Knipfel (1981). Copper contents of bovine neonatal tissue were similar to those of mature animals with the exception of significantly higher Cu contents in the liver of neonates compared to their dams. Calves born to Cu deficient cows had lower liver Cu compared to those from normal cows. Copper supplementation by either oral or parenteral administration increased liver Cu concentration of the dams and their calves. Newborn lambs, however, have lower Cu concentrations in liver and in other tissues than their dams. As for bovines, Cu deficiency in or Cu administration to ewes resulted in decreased or increased neonate liver Cu content, respectively.

No information was found on neonate body Mo stores. However, serum Mo levels of 0.16 and 0.30 mg L^{-1} were found in newborn calves from cows fed supplemental Mo and sulfate during pregnancy (Vanderveen and Keener, 1964). This indicates that Mo can cross fetal membranes.

Relatively few studies have been carried out on Cu and Mo metabolism in preruminants. Two studies, Miller et al. (1972)

and Suttle (1975) give some indication of differences between ruminants and preruminants.

In the first study (Miller et al., 1972) ^{99}Mo was administered to calves either in the rumen in gelatin capsules using a balling gun or into the abomasum in milk sucked from nipple pails. The calves were fed alfalfa hay free choice plus a limited grain ration and hence had functional rumens. Eight hours after dosing, plasma and saliva Mo levels were more than 4 times higher in abomasal dosed calves as compared to ruminal dosed animals indicating a more rapid absorption from the lower gut. During the 72 hours post dosing there was a general decline in plasma and saliva levels of Mo for both ruminally and abomasally dosed calves, however, levels remained higher during that time for calves given ^{99}Mo into the abomasum. In a second trial, Miller et al. (1972) found that apparent absorption of ^{99}Mo after seven daily doses averaged 29.8% and 62.2% for ruminal and abomasal dosed calves, respectively. This result is in conformance with the thiomolybdate theory previously discussed. It is of interest to note that the pattern of uptake into the plasma, the disappearance of ^{99}Mo from the plasma, and the route of excretion found for abomasally dosed calves were more similar to those for pigs, which were included in this comparative study, than for rumen dosed calves.

Percentages of the total ^{99}Mo dose retained by calves during the seven days of dosing were calculated from recoveries in urine, feces and in the contents of the digestive tracts at slaughter. Average retentions of the total ^{99}Mo dose were 25.5 $\pm$

4.2% and 30.4 ± 6.3% for ruminal and abomasal dosed calves, respectively.

The fact that abomasal dosed calves had functional rumens may, in part, explain the similarities in ^{99}Mo retention for these two groups of calves, and therefore may not resemble preruminant calves. Any Mo recycled into the rumen via saliva would presumably undergo the same processes as Mo initially dosed into the rumen, thus similar absorption and systemic effects would be expected.

Suttle (1975) used ^{64}Cu and an indigestible ^{103}Ru-labelled marker to measure apparent availability of Cu in lambs bottle fed a milk substitute (preruminants) and for six weeks after weaning. Prior to weaning, the isotopes were administered in 100 ml of the milk substitute just prior to giving the lambs the remainder of the afternoon feed. Weaned lambs were given their doses by intraruminal injection. The lambs were weaned at 38 to 64 days of age.

Mean Cu availabilities decreased with age, from 71.0 ± 3.7 to 47.2 ± 7.8% for 28 and 14 days prior to weaning, respectively. Cu availability further decreased to 10.8 ± 1.4% by 15 days postweaning. Suttle (1975) attributed the latter decrease to some effects of weaning. However, as noted above there was a decrease of 23.8 percentage units during a two week period before weaning. Therefore the postweaning decrease in Cu availability may have been related to either the age of the lambs or to developing rumen function.

Half the original group of lambs were given either abomasal

or ruminal doses of ^{64}Cu and ^{103}Ru at 42 days postweaning. The mean Cu availability was 21.4 $\pm$ 4.0% and 3.7 $\pm$ 2.1% for animals dosed via the abomasum and the rumen, respectively. These data suggest that Cu undergoes processes in the rumen which reduce availability to the animal. A similar high availability of dietary Cu was demonstrated in veal calves on milk substitute diets, since over 50% of the dietary Cu was retained in the liver of these animals (Bremner and Dalgarno, 1973).

Bremner and Davies (1980) cited an unpublished report on calves orally dosed with ^{64}Cu and with $^{141}CeCl_3$ as an indigestible marker. Cu absorption prior to weaning was 68% compared to only 27% after weaning. In this study, the calves were fitted with duodenal and ileal reentrant cannulae, thus enabling the sites of Cu absorption to be determined. Absorption from mouth to duodenum, mouth to ileum and mouth to anus in milk fed animals was 10, 59 and 68%, respectively, compared with 10, 19 and 27% after weaning.

Work done with monogastric animals may shed some light on the differences in Mo and Cu metabolism between simple stomached animals and ruminants. In rats, liver Cu content was increased by supplementary oral Mo, an effect that was alleviated by dietary sulphate S (Miller et al., 1956; Nederbragt, 1982) whereas in ruminants liver Cu content was reduced by dietary Mo and sulphate (Bremner and Young, 1978; Van Ryssen and Stielau, 1981). This difference is related to the complexing of Cu with thiomolybdates synthesized by rumen microorganisms thus reducing dietary Cu absorption.

Molybdenum, however, does appear to have a similar systemic effect in both ruminants and non-ruminants. Smith and Wright (1975) found that Mo supplementation resulted in a plasma Cu fraction that was TCA-insoluble in both sheep and guinea pigs. Diets containing Mo at concentrations of 17 and 100 mg kg^{-1} were fed to sheep and guinea pigs, respectively. Nederbragt and Van dem Hamer (1981) showed that a protein-bound, non-ceruloplasmin Cu complex in the plasma of Mo supplemented rats was part of an albumin bound Cu-Mo-S complex. The complex had properties similar to those of in vitro synthesized Cu-thiomolybdates described by Mills et al. (1978). This suggests that the effect of Mo on systemic Cu is similar in monogastrics and ruminants.

Nederbragt (1982) also found that the administration of 500 mg Mo kg^{-1} in diet to Cu adequate rats resulted in increased liver and plasma Cu but a rapid decrease in plasma ceruloplasmin activity. The experiment was repeated with rats fed on diets with the same composition but given additional Cu for 2 weeks. The added Cu, as $CuSO_4$, was given either by increasing dietary Cu from 6.0 to 25.0 mg kg^{-1} or by intraperitoneal injection of 250 mg Cu every second day. Mode of copper supplementation did not influence the response which was a decrease in plasma and liver Cu concentrations and a rise in ceruloplasmin activity.

Nederbragt (1982) argued that an Mo-S compound was formed in the gastrointestinal tract. The increase in dietary Cu may then have given rise to a higher concentration of a Cu-Mo-S compound in the gastrointestinal tract which cannot be absorbed. Therefore less of the Mo-S compound was available at systemic sites

for Cu binding. Nederbragt (1982) went on to argue that rats given Cu intraperitoneally increased Cu excretion into the bile. Assuming minimal reabsorption of the biliary-Cu, it is then available for binding to the thiomolybdates which would again reduce absorption of the thiomolybdates.

There is no direct evidence for the suggested formation of a Mo-S compound or thiomolybdate in the small intestine of the rat. The presence of protein-bound or TCA-insoluble plasma Cu in Mo supplemented guinea pigs (Smith and Wright, 1975) and rats (Nederbragt and Van dem Hamer, 1981; Nederbragt, 1982) may have been related to coprophagy. The guinea pigs were housed in galvanized metal cages and the rats in steel wire bottom cages. Although wire bottom cages are designed to allow feces to drop out of the cage, rats can recycle part of their feces by direct ingestion from the anus (Barnes et al., 1967). Therefore, it is possible that microbial metabolism in the large intestine, of dietary sulfate or S containing compounds, produces thiomolybdates which upon re-ingestion are absorbed from the gut. Rats and guinea pigs would therefore be poor models for trace element studies of preruminants.

On the other hand, if the above results were not due to coprophagy, and similar responses were observed for preruminants, then milk diets high in Mo could result in depletion of body Cu stores in a similar manner to that described for ruminants. This becomes even more relevant when taking into consideration the low Cu content of cows' milk (0.10 to 0.20 mg L^{-1}) and ewes' milk (0.20 to 0.30 mg L^{-1}) (Lönnerdal et al., 1981).

The effects of excess dietary Mo in preruminants have not been determined, with the exception of some work with thiomolybdates by Suttle and Field (1983). They compared four groups of three lambs reared on a milk substitute diet containing 10.5 mg Cu kg^{-1} D.M., with or without supplements of 5 mg Cu kg^{-1} D.M. or 3 mg Mo kg^{-1} D.M. in a 2 x 2 factorial design. Supplementary ammonium tetrathiomolybdate reduced hepatic Cu retention by one-third but had no effect on the amount or distribution of Cu in plasma or on plasma Mo concentrations. The growth of lambs treated with ammonium tetrathiomolybdate was similar to that of untreated lambs (350 g d^{-1}). The effect of tetrathiomolybdates on hepatic Cu storage occurred in the absence of "systemic effects" on Cu metabolism and was probably because of reduced Cu absorption from the gut.

In attempting to quantitate the reactions, Suttle and Field (1983) found that the effect of adding 3 mg Mo to the diet, as tetrathiomolybdate, was similar to removing 5 mg Cu from the diet. This represents a Mo:Cu ratio close to that required for the formation of Cu_2MoS_4.

Effects of Excess Dietary Molybdenum

Rose (1983) defined toxicity as a chemically induced alteration in structure and/or function, resulting in an adverse or malfunctional response. The syndrome produced by excess dietary Mo in ruminants is complex due to the variety of biochemical and clinical effects produced. The syndrome may have the two interrelated components, that of an induced copper deficiency and of molybdenosis arising at high Mo intakes (Mason, 1981). Numerous

terms have been used to describe this syndrome including, molybdenosis, teart scours (teartness), peat scours, Mo-induced Cu deficiency and hypocuprosis. Case reports describing this disorder are always concerned with ruminants and almost always concern grazing as opposed to confined animals (Hartmans and Bosman, 1970; Ward, 1978).

Conditioned Hypocuprosis. Low dietary Cu intakes have been reported to be responsible for the development of Cu-responsive disorders (Todd, 1976) and are commonly referred to as "simple" or "primary" Cu deficiencies. However, the majority of cases in which Cu deficiencies have been observed in grazing animals occurred where pasture forage analysis indicated adequate Cu supplies (Bingley and Anderson, 1972; Ward, 1978). These "conditioned" or "secondary" Cu deficiencies are caused by antagonistic effects of other dietary constituents on Cu absorption and/or utilization of absorbed Cu. Excess dietary levels of zinc, iron, cadmium and manganese have been associated with reduced Cu availability to animals (Bremner, 1970; Ivan and Grieve, 1976; Mills, 1980). The most important inorganic constituents of diets associated with reduced Cu availability in ruminants are Mo and S (Bremner and Davies, 1980).

Manifestations of Cu deficiency described by McMurray (1980), Fell (1981) and Underwood (1981) include anemia, bone disorders, neonatal ataxia, cardiovascular disorders, achromotrichia, defective keratinization of wool and hair, infertility and scours. Symptoms associated with Cu deficiency differ to some extent between sheep and cattle (Underwood, 1981). The

classic Cu deficiency syndrome associated with sheep is swayback (enzootic ataxia) which results from motor dysfunction caused by defective synthesis of myelin (amyelination) in the neonate or young lamb (Paterson et al., 1971). The condition appears to be related to impaired biosynthesis of catecholamines involving a number of Cu-dependent enzymes (Paterson et al., 1971). The loss of the characteristic crimp in wool of Cu deficient sheep has been associated with the loss of disulfide bridges, normally providing the cross-linkages of keratin, and an increase in free sulphydryl groups (Marston, 1952). The precise biochemical involvement of Cu is not known. Diarrhoea has been observed both experimentally (Mills et al., 1976) and in the field (Cunningham, 1953) in cattle, but not in sheep. Lawrence et al. (1982) found that noradrenaline concentrations in the intestinal musculature of Cu-deficient steers were lower than in those on a Cu adequate diet, but found no change in noradrenaline concentrations in the intestinal musculature of rats because of Cu deficiency. The steers had diarrhoea and the rats did not, which lead to the suggestion that the noradrenaline response was responsible for the diarrhoea, which does not occur in other species. The remaining manifestations of Cu deficiency have been reported in both sheep and cattle.

Molybdenosis. Excessive Mo intake may be detrimental to animals in other ways. That ruminants are particularly susceptible to Mo toxicity, which can occur without concomitant hypocuprosis, was first documented by Allcroft and Lewis (1956), although the diagnostic markers used in that study to rule out hypocuprosis

are not known. Molybdenosis occurred in cattle with normal Cu status placed on high Mo pasture (Farmer et al., 1982) or rations (Ruston, 1982). These animals developed profuse diarrhoea before becoming Cu-deficient.

The possibility that sulfides and/or impairment of sulfide oxidation may play a role in Mo-induced syndromes has received some attention (Suttle, 1974b; Mason, 1978). The number of steps involved in oxidation of sulfide to sulfite, which in the presence of sulfite oxidase forms sulfate, is unknown. Sulfide accumulation in tissues or plasma are used as an indicator of changes in 'sulfide oxidase' activity.

To determine if excessive Mo intake could result in animals becoming susceptible to poisoning by metabolically generated sulfides, Mason et al. (1978a) fed rats diets containing 1000 mg Mo kg^{-1} D.M. They found a decline in liver sulfite oxidase activity and reduced overall sulfide oxidation capacity, but no evidence of a rise in either blood or hepatic sulfide concentrations. They proposed several theories to explain these results: 1) the sulfide oxidation, although impaired, was still sufficient to eliminate the relatively small amounts of endogenous sulfide; 2) if the major defect was at the sulfite oxidation step then the accumulation of other earlier metabolites would be more likely.

Since ruminants may generate high levels of sulfide in the ruminal fermentation process, it is possible that the scouring caused by feeding high Mo rations may be due in part to the failure to oxidize highly toxic endogenous sulfide. Mason et al. (1978a) tested this hypothesis by adding sulfur as either sulfate

(SO_4^{2-}) or sulfide (S^{2-}) to high Mo rat diets. Addition of SO_4^{2-} produced no increase in plasma TCA-insoluble Cu over the levels observed for Mo alone, but added S^{2-} doubled the levels. Ceruloplasmin oxidase activity did not change in any of the treatment animals compared with controls. Unfortunately, data on sulfide metabolism were not collected in this study. However, these above two sets of experiments suggest that excess dietary Mo may be detrimental to the animal in two ways. First, the animals may be exposed to sulfide toxicity per se. Second, the animal may develop conditioned hypocuprosis because of the Cu-Mo-S interactions in the gut and/or systemically. No further work on systemic toxic effects related to inhibition of hepatic sulfite oxidase has been found.

Impairment of sulfide metabolism in the gut was considered by Fell et al. (1979) as a possible cause of the diarrhoea that occurs shortly after cattle are fed high Mo diets. They found that pathological changes occurred in the alimentary canal of rats fed a diet containing 3 mg kg^{-1} Cu and 6 mg kg^{-1} Mo as tetrathiomolybdate. Mitochondrial abnormalities in the duodenum and jejunum and single cell deletion by apoptosis and necrosis in the caecum and colon were present from the first day of treatment until the end of the investigation. In a second trial, rats given thiomolybdate for 11 or more days had a marked increase in the incidence of apoptosis in the small intestine, and gross disorganization and edema of the caecal mucosa. There were also changes in the bacterial populations of the caecal contents.

These changes, with the possible exception of the mitochon-

drial lesions, could not be attributed to a conditoned hypocuprosis. Fell et al. (1979) suggested that S transported into the intestinal cells as thiomolybdate may have cytotoxic effects related to reduced sulfite oxidase activity. In the mucosa of the caecum, the cytological lesions progressed to gross pathological changes similar to those seen in the presence of bacterial endotoxins. These authors suggested that this may be related to unabsorbed thiomolybdate accumulated in the caecum or reformation of thiomolybdates from Mo excreted into the gut lumen. With the possibility of reduced sulfite oxidase activity in the caecal epithelium, sulfide generation in the gut lumen could have deleterious effects and result in the absorption of bacterial endotoxins.

Thus it is clearly difficult to define the effects of excess dietary Mo intake other than that of impaired Cu metabolism in ruminants. Most of the data related to impaired sulfide metabolism have been obtained from rats and extrapolated to ruminants. There may well be other deleterious effects associated with high Mo intake which have not been well defined to date. For example, molybdate has been shown to interact with the thiol group of cysteine and the imidazol group of histidine (Weathers et al., 1979) and thus may interfere with metabolism of amino acids, peptides or proteins.

Excess dietary Mo may also adversely affect rumen microbial activities. Nikolić et al. (1983) assessed the influence of different concentrations of Mo on metabolic processes in rumen contents incubated in vitro. Mo concentrations in the incubation

mixture were 0.041, 0.210 and 0.609 mg kg^{-1}. Increasing Mo levels did not affect the utilization of urea and sulfate for microbial protein synthesis. However, there was a tendency for VFA production to be decreased in two of the three experiments conducted. Although no direct comparisons between the Mo concentrations in the incubation mixtures and dietary Mo levels can be made, Grace and Suttle (1979) observed that rumen Mo concentrations increased from 0.17 to 1.14 mg L^{-1} for sheep when dietary Mo was increased from 0.5 to 4.8 mg kg^{-1} D.M. Studies similar to that of Nikolić et al. (1983) using higher levels of Mo and taking into account S sources and concentrations would be of value in trying to assess possible toxic effects of Mo on rumen fermentation.

Prediction/Diagnosis of Conditioned Hypocuprosis

Analysis of Animal Diets. Attempts have been made to predict a conditioned hypocuprosis from the analysis of animal diets. Many of the early studies attempted to do this by defining the minimum Cu:Mo ratios required by ruminants. Based on animal weight gains and incidence of scouring, Miltimore et al. (1964) suggested that Cu:Mo ratios above 2.0 in feedstuffs seemed "healthy" for cattle and lower ratios were associated with symptoms of Cu deficiency. Alloway (1973) identified Cu:Mo ratios of approximately 4.0 with reduced incidence of swayback in sheep. Use of these ratios proved to be inaccurate in diagnosing or predicting conditioned hypocuprosis because the effects of dietary sulfur were not taken into account.

From the results of sheep trials using semipurified diets

containing levels of Mo ranging from 0.5 to 4.5 mg kg^{-1} and S ranging from 1.0 to 4.0 g kg^{-1} D.M., Suttle and McLauchlan (1976) developed a prediction equation relating true availability of dietary Cu to dietary Mo and S concentrations:

$$\log \text{TA Cu} = -0.0019\ \text{Mo} - 0.0755\ \text{S} - 0.0131\ (\text{Mo} \times \text{S}) - 1.153\ , \qquad \text{Equation 1}$$

where TA Cu is the true availability (%) of Cu and Mo and S are dietary concentrations as mg kg^{-1} and g kg^{-1}, respectively. True availability was determined by monitoring changes in Cu content of liver. Sheep were Cu deficient at the time they were placed on test.

Available Cu in a feedstuff can be determined by multiplying the concentration of Cu with the predicted availability. The prediction equation, with modification, was later adopted by the Agricultural Research Council (1980) in revising Cu requirements for ruminants.

Subsequent studies (Givens et al., 1981; Langlands et al., 1981; Suttle, 1982; Suttle, 1983) have shown that the Cu-Mo-S interactions for natural diets can differ from those observed for semi-purified diets. Suttle (1982) found the effects of the Mo - S antagonism on Cu availability to be greater for fresh herbage and less for hay than for the semi-purified diets.

To the contrary, Langlands et al. (1981) found that the effects of Mo on the liver Cu status of grazing sheep were less than those predicted by Suttle and McLauchlan's equation. Deviations between observed Cu availability in natural feedstuffs and those predicted from the equation derived using semi-purified

diets are largely unexplained. One influencing factor may be the rate or extent to which Mo, S and Cu are solubilized or degraded from a particular feedstuff during fermentation in the rumen. For example, the higher Cu availability observed for brassica crops compared to predictions (Barry et al., 1981; Suttle, 1982) may be related to the presence of non-degradable forms of S as methyl-cysteine sulphoxide and thioglycosides in the plant material. Comparing the differences in Cu absorption between fresh forage and hay, Suttle (1983) suggested that reduced solubility of Cu in the dried forage protected dietary Cu from the harmful effects of Mo in the rumen.

A second factor to consider is the use of this equation for feedstuffs containing Mo, S and Cu contents outside of the ranges used by Suttle and McLauchlan (1976). Langlands et al. (1981) evaluated the effects of pasture forage containing 6.6-13.7 mg Cu kg^{-1} D.M., 2.6-4.7 g S kg^{-1} D.M. and 0.33-28.0 mg Mo kg^{-1} D.M. on hepatic Cu concentrations and found that dietary Mo and S did not affect Cu availability to the extent suggested by equation 1. Langlands et al. (1981) calculated the following multiple regression equation to determine the Cu concentration in the dry matter of green forage necessary to maintain a constant hepatic Cu concentration with forages containing various levels of Mo and S:

$$Cu = 3.58 \div (0.629 - 0.0491\ S \times Mo) \qquad \text{Equation 2}$$

where Cu and Mo are dietary concentrations as mg kg^{-1} D.M. and S is percent of dietary dry matter. Dietary Cu concentrations below the calculated value would result in depletion of hepatic Cu reserves thereby inducing Cu deficiency. A criticism of the

study by Langland et al. (1981) is that pasture forage samples taken to estimate dietary Cu, Mo and S concentrations may not reflect actual dietary intake because the workers did not take into account selective grazing practices of sheep (Langlands, 1967).

Analysis of Animal Tissues and Blood. Indicators of the Cu status of ruminants, on the basis of tissue or blood samples, fall into two general categories. The first is the actual analysis of the tissue and blood samples for the actual element and the second involves determination of concentrations or activities of Cu containing enzymes. There is no definitive method because of the incomplete understanding of the biochemical basis of Mo-induced hypocuprosis relevant to clinical symptoms and therefore to early or accurate diagnosis.

According to guidelines provided by Agriculture Canada (Puls, 1981) for practising veterinarians, cattle are Cu deficient if serum and liver Cu levels range from 0.06-0.70 mg L^{-1} and 1.0-10.0 mg kg^{-1} wet tissue, respectively, and are marginally Cu deficient with the levels of 0.55-0.80 mg L^{-1} and 5.0-25.0 mg kg^{-1} wet tissue, respectively. Calves under 3 months of age are identified as deficient with wet liver Cu concentrations ranging from 2.0-25.0 mg kg^{-1}. Sheep are considered deficient with serum and liver concentrations ranging from 0.10-0.60 mg L^{-1} and 0.5-4.0 mg kg^{-1}, wet basis, respectively. Neither the basis for establishing the above threshold values nor references were provided by Puls (1981). Smith and Coup (1973) suggested that the threshold value used to differentiate deficient from normal

cattle should be 0.5 mg L^{-1} and 5 mg kg^{-1} for plasma and liver dry matter, respectively. Numerous other attempts (Mills et al., 1976) to use tissue and/or blood Cu concentrations as a diagnostic aid for the detection of Cu deficient animals have been used with limited success.

Copper in plasma is normally found in three fractions; the major amount is in ceruloplasmin, with minor amounts in albumin and in complexes with amino acids (Harris, 1983). Absorption of thiomolybdates resulting from ingested Mo and S by ruminants leads to the appearance of a fourth Cu fraction in plasma, characterised by its insolubility in 5% trichloroacetic acid (TCA) (Smith and Wright, 1975). The appearance of TCA-insoluble Cu may result in either no change (Mason et al., 1978b) or an increase in total plasma Cu levels (Bremner and Young, 1978). Therefore plasma Cu content is not necessarily a good index of metabolically available Cu.

Disadvantages of using liver Cu concentrations include difficulties in acquiring representative samples and variations with species, age and reproductive state of the animal (Underwood, 1977). As well, little is known about the physiological roles of hepatic Cu storage proteins (Aspin and Sass-Kortsak, 1981) and their changing proportions with changes in hepatic Cu concentrations.

The copper content of hair has also been suggested as a marker of copper status (Kellaway et al., 1978) but to obtain reliable results potential environmental contamination must be accounted for or eliminated.

Serum or plasma ceruloplasmin (Cp) activity is the most frequently used of the Cu-containing enzymes as an index of Cu deficiency. Cp synthesis takes place in the liver (Owen and Hazelrig, 1966), where Cu is incorporated into the apoprotein to produce holoceruloplasim just prior to its secretion. The physiological roles of Cp have been the subject of much discussion. Frieden and Hsieh (1976) proposed that the major roles of this enzyme are mobilization of plasma iron, transport of Cu and regulation of biogenic amines. All species appear to have low Cp levels at birth, after which the levels rise until adult values are reached (Pojerova and Tovarek, 1960). Both serum and plasma have been used for Cp analysis, and values obtained for one are often directly compared to the other. A recent study by Paynter (1982) showed that Cp activities in serum were 13-40% lower than in plasma, for nine different groups of sheep and cattle, and 10-65% lower for individual animals. It was therefore concluded that the values are not directly interchangeable and that plasma rather than serum should be used for estimating the Cu nutritional status of sheep and cattle.

There are a number of other metalloenzymes in which Cu provides either structural integrity to the protein molecule or is involved in its enzymatic activity (Aspin and Sass-Kortsak, 1981). Many have been studied as potential markers of animal Cu status, including cytochrome c oxidase, superoxide dismutase and amine oxidases (Poole, 1970; Mills et al., 1976; Paynter and Allen, 1982).

Prediction/Diagnosis of Molybdenosis

Difficulties associated with the ability to distinguish between the detrimental effects of dietary Mo associated with a conditioned hypocuprosis and with molybdenosis per se, as discussed in this review, have resulted in few attempts being made to define diet and/or animal tissue and blood indices of molybdenosis. "Teart" pastures containing 20-100 mg kg^{-1} Mo on a dry matter basis can cause profuse scouring in Cu adequate cattle (Underwood, 1977). Compared with pastures, higher Mo concentrations are required in dry forages or mixed feeds to produce similar symptoms (Vanderveen and Keener, 1964; Huber et al., 1971). Liver and blood concentration of Mo have been shown to increase with increased dietary Mo intake (Bremner and Young, 1978; Pitt et al., 1980; Van Ryssen and Stielau, 1981), however this has not been utilized to diagnose molybdenosis.

MATERIALS AND METHODS

Beef Cow-Calf Trial

The objective of this trial was to determine if such dietary Mo levels as are found in high Mo pasture forages of the north-western agricultural region of Manitoba (Boila et al., 1984a) and other parts of the world (Kovalsky, 1970; Hogan et al., 1971; Allaway, 1977) can affect the productivity of cow-calf herds. Milk yield and composition and animal body weight changes were monitored for a nine week period during which cows were fed diets formulated to contain 0, 20 or 40 mg kg^{-1} added Mo. The progressive changes in total plasma Cu and plasma Cu distribution of cows were used as indicators of Cu status.

A second objective was to evaluate calf growth, liver Cu levels and plasma Cu content responses to any changes in their dams' milk yield or composition resulting from the above dietary treatments.

Experimental Animals. Twelve cow-calf pairs from the University beef herd were used for this study. The University herd, identified as Selkirk-Red, is the result of a breeding program that started with Angus x Charolais dams bred to North Devon sires. The sire breeds of the calves were either Selkirk-Red or Simmental.

At the time of this study the cows ranged from two to ten years of age. Calf ages at the start of the trial ranged from 16 to 43 days. All animals were on pasture and receiving supplemen-

tal hay prior to being placed on test (May 18). Efforts were made to use cows showing no evidence of mastitis and with healthy calves. One calf (#59), on the control treatment, was diagnosed to have lymphosarcoma at the end of the trial (week 9). Data for this calf and its dam were used.

Experimental Diets. The three experimental diets, identified as 0Mo, 20Mo and 40Mo, were formulated to contain 0, 20 and 40 mg kg^{-1} D.M. of added Mo, respectively. Supplemental Mo, as ammonium molybdate ($(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, Fisher Scientific Company) was incorporated into a pelleted, barley-based concentrate (Table 1) which provided 23% of the total D.M. intake, the remainder being supplied by corn silage.

The experimental diets were fed at the rate of 11.6 kg D.M. cow^{-1} d^{-1}. The concentrate was fed once a day and corn silage was provided after the concentrate had been consumed. Feed weigh backs were measured once a week.

Urea had been added to fresh corn forage at the time of ensiling at the rate of 5.0 kg t^{-1}. Dry matter content of the corn silage used in this trial was 27.0%.

The diets were formulated to meet the energy, protein, calcium and phosphorus requirements of lactating beef cows with superior milking ability (NRC, 1976). Samples of concentrate and corn silage were taken weekly for nutrient analysis (Table 2). The mean values for chemical composition of diets are shown in Table 3, in which data for the separate analyses of concentrate and corn silage have been combined to give dietary composition. Copper and S concentrations were uniform across dietary treat-

Table 1: Composition of barley based concentrate fed to lactating beef cows

Ingredient	%, air dry basis
Ground barley	91.9
Molasses	2.0
Mono-dibasic calcium phosphate	1.6
Colbalt-iodized salt	0.5
Mo premix†	4.0

† Premix contained 0, 30.16 and 60.28 g ammonium molybdate made up to 10 kg with wheat middlings for the 0Mo, 20Mo and 40Mo concentrate mixes, respectively.

Ammonium molybdate ($(NH_4)_6Mo_7O_{24}\cdot 4H_4O$) contains 54.34% Mo.

Table 2: Proximate and mineral analyses of the concentrates and corn silage fed to lactating beef cows

Analysis, D.M.	Concentrates			Corn Silage
	OMo	20Mo	40Mo	
Crude Protein, %	14.8	14.5	13.8	14.3
Acid detergent fibre, %	6.7	5.7	7.4	37.1
Calcium, g kg^{-1}	3.7	3.6	3.7	5.7
Phosphorus, g kg^{-1}	8.3	8.2	8.5	2.5
Magnesium, g kg^{-1}	1.5	1.5	1.5	4.2
Sulfur, g kg^{-1}	1.8	2.1	1.6	1.2
Iron, mg kg^{-1}	319.8	308.9	325.1	411.9
Zinc, mg kg^{-1}	35.2	35.9	37.7	31.2
Copper, mg kg^{-1}	5.5	6.1	6.2	5.9
Molybdenum, mg kg^{-1}	1.3	82.1	149.1	0.4

Table 3: Proximate and mineral analyses of experimental diets fed to lactating beef cows†

Analysis, D.M.	Diets		
	0Mo	20Mo	40Mo
Crude protein, %	14.4	14.4	14.2
Acid detergent fibre, %	30.1	29.9	30.3
Calcium, g kg^{-1}	5.2	5.2	5.2
Phosphorus, g kg^{-1}	3.8	3.8	3.9
Magnesium, g kg^{-1}	3.6	3.6	3.6
Sulfur, g kg^{-1}	1.3	1.4	1.3
Iron, mg kg^{-1}	390.7	388.1	391.9
Zinc, mg kg^{-1}	32.2	32.3	32.7
Copper, mg kg^{-1}	5.8	6.0	6.0
Molybdenum, mg kg^{-1}	0.6	19.3	34.8

† Corn silage and concentrate made up 77 and 23% of total dry matter intake, respectively.

ments. Molybdenum content for the 40 Mo diet was low compared to the concentration formulated. There was little variation in Mo analysis among the weekly samples. Cows were given an intramuscular injection of vitamins A, D and E (1 ml of Vit A.D. Jex†) prior to the start of the trial. Fresh water and cobalt-iodized salt were available to cows and calves at all times.

Management Procedures. Cow age, calving date and calf sire breed were taken into account when allocating the 12 cow-calf pairs to one of the three dietary treatments (Appendix Table I-1). Each treatment group had one first calf heifer and three mature cows. Mean ages of calves at the start of the experiment were 30, 31 and 33 $\pm$ 3 days. Calf sex was not balanced across treatment groups; two male and two female calves were allocated to 0Mo and three male and one female calves each for the 20Mo and the 40Mo treatments.

Prior to being placed on test, cows and calves were put into pens that had feed bunks allowing no or minimum feed access for calves. The distance from the ground to the ledge of the feed bunk, over which animals were required to reach to gain access to feed, was 65 cm. Therefore, calves had to rely on their dams' milk as the supplier of nutrients for the duration of the study. However, calves were observed consuming straw bedding and some of the corn silage dropped from the feed bunks by the cows.

Each pen, representing one treatment group, held four cow-calf pairs. The feed bunk in each pen was 4.9 m long, which

†Vit.-A.D. Jex, produced by Kvet, contains 500,000 I.U. vitamin A, 75,000 I.U. vitamin D_3 and 50 I.U. vitamin E per ml.

allowed cows ample space during feeding. The north side of the pens was sheltered by a pole barn. Barley straw bedding was provided as required.

Animals were fed corn silage and barley concentrate during a five-day pretrial adjustment period after which cows were fed their respective diets for 63 days.

Collection and Sampling Procedures and Schedules. Cow and calf blood samples, calf body weights, cow milk yields and composition were determined weekly, the first observations were taken after animals had been on test for seven days. On sampling days, cows and calves were separated at 8:00 A.M. and the calves placed in another barn, out of sight of the cows until milking was completed. To avoid variation due to time of day, blood samples were taken and body weights measured for calves at 1:00 P.M.

At 2:00 P.M., six hours after calves and cows were separated, blood sampling and milking of the cows was started. Calves and cows were returned to their respective pens after milking and blood sampling were completed. Cows were restrained in a squeeze chute with a head gate. A blood sample was taken, and then 1 ml oxytocin (Oxcin†) was administered into the jugular vein. Then, the udder and teats were washed with water and dried using paper towels. Cows were hand milked into plastic pails that had been washed with distilled water and rinsed three times with deionized distilled water. The milk was weighed and two representative samples were taken, one for milk fat, protein and

†Oxcin, produced by Agri-vet Pharmaceuticals Limited, contains 20 I.U. USP oxytocin per ml.

lactose analyses and the other for mineral analyses. Samples were placed in a styrofoam cooler containing ice until they were either submitted to the Provincial Dairy Laboratory for milk fat, protein and lactose analysis or frozen for subsequent Mo and Cu analyses.

The procedure for determining milk production was based on a method described by Lamond et al. (1969). The exact time that milking commenced was recorded for each cow and the order in which cows were milked was randomly altered from one milking to the next. Hand milking the twelve cows took approximately two hours.

Blood samples from cows and calves were taken from the jugular vein and collected into two 10 ml sodium heparinized vacutainer tubes using 20 G thin wall vacutainer needles. Samples were placed on ice until returned to the lab. Samples were centrifuged at 2500 x g for 20 minutes. The separated plasma was frozen immediately and stored (-20° C) until analysis for total plasma Cu, plasma TCA-insoluble Cu and ceruloplasmin (Cp) oxidase activity.

Liver samples were taken from calves on days 3, 28 and 57 of the trial. The liver biopsy technique described by Chapman et al. (1963) was used with two modifications. First, animals were given a local anesthetic, Lidocaine HCl 2%†, at the site of incision. Second, a 25 cc plastic syringe was used to create a negative pressure in the cannula of the biopsy instrument while removing the liver sample. Chapman et al. (1963) criticized the

†Lidocaine HCl 2% is produced by Armitage Carroll.

use of negative pressure in the biopsy procedure because it may cause blood and other body fluids to be drawn into the cannula with the liver sample. Contamination of liver samples associated with excess body fluids in this study was not measured. However, it was found that placing the biopsy sample onto a paper towel before transfer into glass vials using forceps, removed the majority of the fluids from the sample. Liver biopsy samples were frozen and stored at -20° C. Liver samples were dried (95° C), under vacuum, to a constant weight immediately prior to analysis.

Cow body weight was measured on two consecutive days at the beginning (days 1 and 2) and end (days 63 and 64) of the trial.

Statistical Analysis. The experiment was conducted in a split-plot design with four cow-calf pairs assigned to each of three diets. The data were analysed using Statistical Analysis System (SAS); the Analysis of Variance (ANOVA) for balanced data and the General Linear Models (GLM) procedures for unbalanced data (Ray, 1982) were used. Animals within diet was used to test the effect of experimental diets; animals by week within diet (error B) was used to test the effect of week and the diet by week interaction (Gill and Hafs, 1971). There were nine observation sets representing nine weeks for all criteria, with the exception of calf liver Cu levels for which there were three observation sets and for plasma TCA-soluble and TCA-insoluble Cu concentrations which were determined for weeks 1, 3, 5, 7 and 9.

Mean comparisons were carried out with the Student-Newman-Keuls (SNK) procedure (Snedecor and Cochran, 1980). Linear and

quadratic equations were used to describe the diet by week interactions that were significant ($P < 0.05$) (Appendix table I-6, I-7.) For figures (2, 3, 4, 5, 7, 8 and 10) in which the equations are illustrated, the dependent variable '$\hat{Y}$' refers to the parameter defined on the y axis and 'X' refers to week. The standard error of the slope, designated as SE(B) and the portion of the variation in the data set explained by the model describing the regression equations, designated as R^2, are included in the description of the diet by week interaction.

A one way analysis of variance was done to determine the effect of dietary Mo on cow weight change using SAS ANOVA procedures (Ray, 1982).

Ewe-Lamb Trial

The objective of this study was to compare the responses of ewe-lamb sets with those found for the cow-calf pairs fed similar diets. Plasma and liver Mo concentrations of ewes and lambs as a response to dietary Mo were also monitored. Ewes with twin lambs were used in this study to encourage high milk yields by the ewes (Doney et al., 1981). Use of twin lambs also allowed some comparisons to be made between lambs receiving the same milk from birth to the end of the trial.

Experimental Animals. Twelve mature Suffolk ewes with twin lambs from the University minimum disease flock were used in this study (Appendix table II-1). All lambs were sired by Suffolk rams. Prior to lambing ewes had been fed ad libitum good quality legume-grass hay.

Lambs were weighed within 24 hours after lambing. Within 48 hours after lambing, the ewes were shorn and given an intramuscular injection (.5 ml) of vitamins A, D and E (Vit. A.D. Jex). The ewes were then weighed and the ewe-lamb sets transferred to individual pens for the duration of the trial. Average weight of the shorn ewes was 79.4 $\pm$ 3.0 kg.

Day 1 of the trial for each ewe-lamb set was considered to be the first Wednesday after being placed in the pens. Lamb age on day 1 of the trial ranged from 1 to 7 days. Lambings occurred from March 12 to April 2, 1984. All lambs received a subcutaneous injection (5 ml) of Covexine 8† and an intramuscular

†Covexine 8 is an anticlostridial vaccine produced by Wellcome Veterinary Division.

injection (.5 ml) Dystosel† on April 8 and 11, respectively.

Experimental Diets. Diets used in this trial were similar to those in the beef cow-calf study. Three experimental diets, identified as 0Mo, 20Mo and 40Mo, were formulated to contain 0, 20 and 40 mg kg^{-1} D.M. added Mo as $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, respectively (Table 4). Nitrogen sources used in the pelleted barley-based concentrate included soybean meal and urea. No urea was added to the corn silage used in this study.

Ewes were fed their experimental diets as soon as they were placed in the pens. They were offered 200 g and 400 g of the pelleted concentrate on the first and second days in the pens, respectively. Corn silage was offered on an ad libitum basis. Thereafter, the amount of concentrate fed to ewes was dependent on the previous day's corn silage intake. Approximately 30 percent of total dry matter intake was provided by the concentrate, which was fed to ewes once a day. The remainder was provided by corn silage (33.2% D.M.) which was fed twice a day; once in the morning after ewes had consumed the concentrate and again in the late afternoon. Animals were not restricted in the amount of corn silage they were allowed to consume. Feed weighbacks were measured daily.

The diets were formulated to meet energy, protein, calcium and phosphorus requirements of ewes in the first eight weeks of lactation with sucking twin lambs (NRC, 1975). For the purposes

†Dystosel, produced by Rogar / STD - Division of BTI Products Inc., contains the following ingredients per ml: selenium as sodium selenite - 3.0 g, d-alpha tocopherol acetate - 136 I.U., Benzyl alcohol (preservative) - 1.5%.

Table 4: Composition of barley based concentrate fed to lactating ewes.

Ingredient	%, air dry basis
Ground barley	79.5
Soybean meal, 44% C.P. as fed	12.0
Urea	1.0
Molasses	2.0
Dicalcium phosphate	2.0
Cobalt-iodized salt	1.0
Mo premix	2.5

† Premix contained 0, 24.13 and 48.26 g $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ made up to 10 kg with wheat middlings for the 0Mo, 20Mo and 40Mo concentrate mixes, respectively.

of diet formulation, daily dry matter intakes were forecast to be 3.0% of body weight, because the effects of high moisture forages (corn silage) on dry matter intake of ewes was not known. The suggested (NRC, 1975) daily dry matter intake is 3.7-4.3% of body weight for ewes in the weight range used in this study.

Analysis of the concentrate diets (Table 5) showed that phosphorus levels were high, resulting in calcium to phosphorus ratios approaching 1:1 in the ewe diets. The high level of phosphorus in the concentrate was eventually traced to improper labelling of the calcium-phosphorus mineral supplement used by the feed mill. The supplement, labelled to be dicalcium phosphate, when analysed, contained 137 and 202 g kg^{-1} calcium and phosphorus, respectively. Dicalcium phosphate should contain approximately 230 and 180-190 g kg^{-1} calcium and phosphorus, respectively. Proximate and mineral analyses of corn silage are shown in Table 5.

Water and cobalt-iodized salt were available for ewes and lambs at all times.

Management Procedures. Lambing date was used to allocate ewe-lamb sets to the experimental diets; the first mature ewe lambing with twins was assigned to 40Mo, the second to 20Mo, the third to 0Mo, and so on until four ewe-lamb sets had been assigned to each of the three dietary treatments. Sex distribution of the twin lambs was not taken into account (Appendix table II-1).

Each ewe-lamb set was placed in a 1.5 by 1.2 m pen. Feed boxes holding plastic pails were attached to the outside of each pen to which access was by a 25.5 x 20.5 cm hole cut into the

Table 5: Proximate and mineral analyses of the concentrates and corn silage fed to lactating ewes

Analysis, D.M.	Concentrates			Corn Silage
	0Mo	20Mo	40Mo	
Crude protein, %	21.6	20.6	20.8	10.3
Acid detergent fibre, %	7.2	6.1	6.7	27.0
Calcium, g kg^{-1}	7.3	6.9	6.4	4.1
Phosphorus, g kg^{-1}	9.5	9.0	9.3	2.8
Magnesium, g kg^{-1}	2.1	2.1	2.4	2.5
Sulfur, g kg^{-1}	2.1	1.9	1.9	1.2
Iron, mg kg^{-1}	377.2	329.5	292.9	263.8
Zinc, mg kg^{-1}	44.4	40.1	37.5	28.3
Copper, mg kg^{-1}	8.4	6.9	6.7	3.6
Molybdenum, mg kg^{-1}	2.5	62.8	139.5	0.3

plywood wall of the pen.

The distance from the ground to the edge over which animals were required to reach to gain access to the food was 53 cm to prevent lambs from consuming any of the concentrate diet and to minimize corn silage consumption by lambs. Lambs were observed consuming corn silage pulled into the pens by the ewes. Also, lambs were occasionally observed standing on ewes to gain access to corn silage in the feeders. Lambs were not given any supplemental feed during the six to seven week test period. Wood shavings were used for bedding.

The concentrate diets were made in one batch, and representative samples were taken for nutrient analysis. Corn silage samples were taken on a weekly basis.

Postmortem examinations were performed on all animals that died in this study.

Collection and Sampling Procedures. Blood samples and body weight measures were taken from ewes and lambs on the first day the animals were placed in the pens and every Wednesday thereafter for a period of six weeks (i.e., 7 observations per ewe-lamb set). With the exception of day 1, the same schedule was followed to estimate daily milk production of ewes and collect samples for milk composition.

The milk yield from each ewe was measured by the oxytocin (.25 ml Oxcin)/hand milking method (McCance, 1959; Doney et al., 1979). Lambs were removed from the pens at 9:00 A.M. and moved to another part of the barn, where they remained until milking was completed, approximately five hours later. Udder and teats

were washed with water and dried using paper towels prior to milking. Plastic pails washed and rinsed three times with deionized, distilled water were used for milk collection. Milk was weighed and representative samples were taken and processed as described for the beef cow-calf study.

Blood samples were taken from the lambs at 1:00 P.M. after which lambs were weighed. Blood samples were taken from the jugular vein and collected into two 10 ml sodium heparinized vacutainer tubes using 20 G thin wall vacutainer needles. The same sampling procedure was followed for the ewes except that blood samples were taken just before the oxytocin was administered. Samples were kept on ice until returned to the lab where they were centrifuged at 2,500 x g for 20 minutes. The resulting plasma samples were immediately frozen and stored at -20^{o} C until analysis for total plasma Cu and Mo, plasma TCA-soluble Cu and Cp oxidase activity.

Liver biopsy samples were taken from all lambs and ewes as they were removed from the test. Liver samples were taken from the dorsal lobe during postmortem of animals that died while on test. The modified procedure of Chapman et al. (1963), as described in the previous study, was used on all ewes and all but the following lambs: 67, 68, 61, 74 and 78. Several unsuccessful attempts had been made to obtain a biopsy sample from these animals. Therefore, lambs were given a general anesthetic and a laparotomy was performed and the liver sample obtained from the dorsal lobe.

Rumen fluid samples were obtained from ewes, with a stomach

tube, three hours after the morning feeding on April 27th. The number of days that ewes had been on their respective diets at the time of sampling ranged from 24 to 44. Volatile fatty acids (VFA), pH and Mo content of the strained rumen fluid samples were analysed.

Statistical Analysis. The experiment was designed as a split plot with four ewe-lamb sets allocated to each of the three diets. Some animals were removed from test (Appendix table II-2) and therefore GLM procedures (Ray, 1982) were used for analysis of unbalanced data as outlined in the cow-calf study. Ewes within diet was used to test the effect of treatment. Lambs within ewes within diet was used to test the effect of ewe on lambs. Linear equations (GLM) were used to describe the diet by week interactions that were significant ($P < 0.10$). For figures (14 and 16) in which the equations are illustrated, the dependent variable '$\hat{Y}$' refers to the parameter defined on the y axis and 'X' refers to week. The standard error of the slope, designated as SE(B) and the portion of the variation in the data set explained by the model describing the regression equations, designated as R^2 are included in the description of the diet by week interaction.

Rumen fluid parameters and liver Cu and Mo concentrations were analysed as a one-way analysis of variance using GLM procedures.

Student-Newman-Keuls procedure was used for mean comparisons.

Dairy Cow Trial

The objectives of the dairy cow trial were two-fold. First, some of the differences in response to experimental diet between the two previous studies may have been related to lactation stresses. Dairy cows, subjected to similar dietary Mo concentrations, provide comparative data for cattle at higher production and, consequently, higher feed intake levels. Second, the effects of Cu supplementation of animals fed either low or high Mo diets were monitored. To do this, dairy cows were fed diets formulated to contain 0 or 20 mg kg^{-1} D.M. added Mo for eight weeks. After being fed their respective diets for four weeks, half of the cows in each of the non and the Mo supplemented groups were fed supplemental Cu (40 mg kg^{-1} D.M.). Parameters measured included those indicative of animal body Cu and Mo status, body weight change, feed intake and milk production and composition.

Experimental Animals. Sixteen Holstein-Friesian cows in midlactation (day 113-217 of lactation) and of varying ages (2 to 8 years) were used in this study (Appendix table III-1). Reproductive status of animals ranged from non-pregnant (seven animals) to cows that were in the fourth month of pregnancy. The calving interval for the herd was approximately 14 months. Therefore, reproductive status of cows selected for the study was normal relative to other animals in the herd.

The cows were housed in a stanchion barn and were fed individually. Chopped straw was used for bedding.

Prior to being placed on this study cows had been fed a diet

formulated to contain 10 mg kg^{-1} added Cu, as copper sulfate, on a dry matter basis. That diet was made up of alfalfa-grass hay, corn silage, fababean silage and a barley-based concentrate.

Animal #14 was removed from test in the fifth week due to a displaced abomasum. Data for the four weeks that this animal was on test were used in the statistical analyses.

Experimental Diets. The four dietary treatments were: a basal diet containing no added Cu or Mo; basal plus 40 mg kg^{-1} added Cu, D.M. basis; basal plus 20 mg kg^{-1} added Mo, D.M. basis and the basal diet plus 40 mg kg^{-1} added Cu and 20 mg kg^{-1} added Mo, D.M. basis. Experimental diets are referred to as basal, +Cu, +Mo and +Mo+Cu, respectively. The Mo and Cu, provided by $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ and cupric sulfate ($CuSO_4\cdot 5H_2O$, Fisher Scientific), respectively, were added to a supplement which was fed to cows once a day (Table 6a, 6b). The supplement supplied approximately 4.2% of the total dry matter intake (Table 7). In addition to the supplement, cows were fed concentrate, corn silage and long hay, representing 46.4, 36.2 and 13.2% of total D.M. intake respectively. The concentrate and corn silage were mixed in a 56:44 ration on a D.M. basis and fed twice a day. Long hay was fed once a day in the morning. Amount of feed offered to cows and feed weighbacks were recorded daily. The amount of supplement offered to cows was based on the previous day's feed intake.

Diets were formulated to meet the nutrient requirements of lactating cows (NRC, 1978). To determine whether dietary

Table 6a: Composition of barley concentrate and supplements fed to midlactation dairy cows

Ingredient	%, air dry basis
Ground barley	46.9
Canola meal	30.0
Soybean meal, 44% as fed	6.0
Dehydrated alfalfa meal	7.0
Tallow	4.0
Molasses	2.0
Limestone	1.0
Dicalcium phosphate	1.0
Urea	0.5
Cobalt-iodized salt	0.5
Vit. min premix†	1.1

† See Table 6b.

Table 6b: Premixes used for concentrate and supplements fed to dairy cows

Ingredients, g kg^{-1} premix†	Concentrate Premixes	Supplement Premixes			
		Basal	+Cu	+Mo	+Mo+Cu
Vitamin A, 500,000 I.U. g^{-1}	2.92	2.92	2.92	2.92	2.92
Vitamin D, 500,000 I.U. g^{-1}	0.25	0.25	0.25	0.25	0.25
Vitamin E, 20,000 I.U. g^{-1}	0.15	0.15	0.15	0.15	0.15
ZnO	9.23	9.23	9.23	9.23	9.23
$MnO_2 \cdot H_2O$	9.46	9.46	9.46	9.46	9.46
$MgO_2 \cdot H_2O$	158.3	158.3	158.3	158.3	158.3
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	-	-	-	51.5	51.5
$CuSO_4 \cdot 5H_2O$	-	-	219.9	-	219.9

† Remainder of premix was wheat middlings.

Table 7: Calculated nutrient content of experimental diets fed to midlactation dairy cows†

	Experimental Diets			
	Basal	+Cu	+Mo	+Mo+Cu
Ingredients, % total D.M. intake				
Corn silage	36.2	36.2	36.2	36.2
Hay	13.2	13.2	13.2	13.2
Concentrate	46.4	46.4	46.4	46.4
Basal supplement	4.2	-	-	-
+Cu supplement	-	4.2	-	-
+Mo supplement	-	-	4.2	-
+Mo+Cu supplement	-	-	-	4.2
Composition, D.M. basis				
Crude protein, %	17.4	17.4	17.4	17.4
Acid detergent fibre, %	21.1	21.1	21.1	21.1
Calcium, g kg^{-1}	6.2	6.3	6.2	6.2
Phosphorus, g kg^{-1}	5.5	5.5	5.5	5.5
Magnesium, g kg^{-1}	3.1	3.1	3.2	3.1
Sulfur, g kg^{-1}	2.1	2.1	2.1	2.1
Iron, mg kg^{-1}	256.5	253.4	258.2	252.9
Manganese, mg kg^{-1}	43.7	44.6	44.3	44.8
Zinc, mg kg^{-1}	57.9	56.4	56.6	56.5
Copper, mg kg^{-1}	6.1	44.0	6.1	51.2
Molybdenum, mg kg^{-1}	2.1	2.1	20.2	20.0

† Based on proximate and mineral analyses of corn silage, hay, concentrate and supplements, as referred to in Table 8.

treatments affected dry matter intake, no restrictions were placed on the amount of feed offered to cows. Therefore, intake of most nutrients were in excess of daily requirements recommended by NRC (1978).

Fresh water was available to animals at all times.

Management Procedures. Animals were allocated to one of four treatment groups; Trt. I--basal diet during periods I and II; Trt. II--basal diet during period I and +Cu diet during period II; Trt. III--+Mo diet during periods I and II; Trt. IV--+Mo diet during period I and +Mo+Cu diet during period II (Appendix table III-1). Each period was four weeks long and there was no time for adjustment between periods.

Cows were allocated to treatment groups on the basis of age and stage of lactation.

Two mixes of basal and +Mo supplements and one mix of +Cu and +Mo+Cu supplements were made. The concentrate component was mixed as required.

Representative samples of the supplements were taken, following mixing, at the feed mill. Also, grab samples of the supplements, concentrate and corn silage were taken every second week. Grab samples for each period were composited. Core samples from twelve bales of hay, from different sections of the hay stack, were taken the first week cows were on test. Proximate and mineral analyses were done on all feed samples. Phosphorus levels in the supplements and concentrate were higher than expected (Table 8). As for the ewe-lamb trial, this was due to improper labelling of the calcium-phosphorus supplement used

Table 8: Proximate and mineral analyses of forage, concentrate and supplements fed to midlactation dairy cows

Analysis, D.M.	Hay	Corn Silage	Concentrate	Supplements			
				Basal	+Cu	+Mo	+Mo+Cu
Crude protein, %	11.2	9.4	24.7	25.8	24.61	26.7	24.7
Acid detergent fibre, %	39.7	28.0	11.5	10.7	12.2	12.3	10.8
Calcium, g kg^{-1}	7.8	2.2	8.7	8.6	9.9	9.4	8.4
Phosphorus, g kg^{-1}	2.1	2.4	8.8	8.7	8.7	8.9	8.4
Magnesium, g kg^{-1}	3.0	2.7	3.4	4.3	4.1	4.6	4.0
Sulfur, g kg^{-1}	1.4	1.3	3.7	4.0	4.6	4.4	4.5
Iron, mg kg^{-1}	68.0	273.2	291.2	322.3	247.3	361.2	236.5
Zinc, mg kg^{-1}	18.5	26.5	85.0	153.0	118.6	121.5	119.9
Manganese, mg kg^{-1}	30.5	19.4	61.8	96.0	115.0	108.2	121.8
Copper, mg kg^{-1}	11.9	3.4	6.2	6.8	910.0	7.1	1081.4
Molybdenum, mg kg^{-1}	3.9	0.6	2.3	2.1	2.2	436.0	431.1

by the feed mill. Consequently calcium to phosphorus ratios of D.M. intake approached 1:1.

The dairy cow rations were formulated to contain 16 percent crude protein. Actual diet protein levels were higher than the formulated levels (Table 7) because crude protein content of the concentrate and supplements were 2 to 3% higher than expected. The concentrate and supplements were formulated to contain 22% crude protein.

Collection and Sampling Procedures. The cows were milked twice daily and production was recorded. A 24-hour composite sample of morning and evening milk was taken weekly for milk fat, protein and lactose determinations. Milk samples, representing two milkings in a 24-hour period, were taken in weeks 2 and 4 of each period for subsequent Cu and Mo determinations.

Liver biopsy samples were taken, as described on p. 61, from cows two days prior to being placed on test and on the last day of the study. It was not possible to obtain the second biopsy sample from cow #16 on the scheduled day. Therefore, she was kept on the experimental diet for an additional week, at the end of which a liver sample was obtained using the standard procedure.

Blood samples were taken from the tail vein, into two 10 ml sodium heparinized vacutainer tubes using 20 G thin wall vacutainer needles. The samples were held at 4° C until centrifuging (2,500 x g for 20 minutes). The resulting separated plasma was stored at -20° C for subsequent analysis.

Body weights were recorded for cows at weeks 0, 4 and 8.

Statistical Analysis. The trial was designed as a split-plot with four cows allocated to each of the four treatment groups. Eight observation sets, representing four weeks in each of periods I and II represent time for feed intake, milk yield and composition. Plasma Cu and Mo parameters were compared for five times representing day 1 (day animals were placed on test) and weeks 2 and 4 for each period.

Effects of Cu and/or Mo supplementation were analysed as a split-plot with four cows allocated to each of four treatment groups: Trt. I, Trt. II, Trt. III and Trt. IV and time representing observations made in periods I and II. Mean comparisons, using orthogonal contrasts, were used when significant differences for treatment by week interactions were identified. Treatment and week mean comparisons were carried out with the SNK procedure. Differences in liver Mo and Cu response to treatment were determined with a one-way analysis of variance.

All analyses were done with GLM procedures for unbalanced data (Ray, 1982).

Analytical Procedures

The same methods and procedures for laboratory analysis were used for all three trials unless otherwise indicated in text.

Dry matter content of feeds was determined by drying to a constant weight in a forced air oven set at 60° C. Samples were ground through a 1 mm stainless steel screen after equilibrating to air humidity at room temperature and then stored in plastic bags for further analysis.

Crude protein content (nitrogen x 6.25) of feeds was determined by the macro-kjeldahl method (A.O.A.C., 1980). Gross energy was determined with a Parr adiabatic oxygen bomb calorimeter. Acid-detergent fiber (ADF) was determined by the method of Goering and Van Soest (1970).

Total S for feed samples collected from the beef cow-calf and the ewe-lamb trials were digested and prepared for analysis according to Boila et al. (1984b). The resulting filtrate was analyzed according to Hamm et al. (1973). Feed samples collected from the dairy cow trial were analyzed for total S according to the procedure of Roe et al. (1966).

Feed samples were prepared for analysis of the remaining minerals; calcium, phosphorus, magnesium, iron, zinc, manganese, copper and molybdenum by charring a 2 g sample at 200° C for 4 hours and then dry-ashing at 500° C for 4 hours. The charring process was required to obtain a yellow to white ash. The upper temperature (500° C) and time (4 hours) used for ashing were not considered to cause significant losses of minerals (they are within guidelines of A.O.A.C., 1980). Phosphorus content was

determined colorimetrically (A.O.A.C., 1980) using a Baush & Lomb Spectronic 20; the remaining elements were determined by flame atomic absorption spectrophotometry (Instrumentation Laboratory AA/AE Spectrophometer Model 551).

Rumen fluid, total plasma and liver Cu and Mo were determined by flame atomic absorption spectrophotometry, after digestion in a nitric (70-71%, Fisher Scientific)-perchloric (70-72%, Fisher Scientific) acid mixture. The ratio of nitric acid to perchloric acid used for this wet ashing procedure was 4:1 (v/v) (Thompson and Blanchflower, 1971).

Determination of the TCA-soluble Cu fraction of plasma was based on the procedure described by Mason et al. (1978). An equal volume of 10% (w/v) trichloroacetic acid (CCl_3COOH, Fisher Scientific) was added to samples of plasma. After mixing with a vortex mixer, the preparation was centrifuged at 2500 x g for 25 min and the supernatant transferred into a weighed tube. The precipitate was resuspended into 5% (w/v) TCA, using 75% of original plasma volume, and the mixing and separation procedure repeated. The resulting supernatant was added to the first fraction removed. Weight of the recovered supernatant was converted to recovered volume, using a factor for the density of 5% TCA. Flame atomic absorption spectrometry was used to determine the resulting TCA-soluble Cu. Plasma TCA-insoluble Cu was taken as the difference between total plasma Cu and TCA-soluble Cu.

Several procedures were attempted for wet ashing of milk samples for Cu and Mo analyses. Contrary to observations made by Clegg et al. (1981), wet ashing procedures, using either 25 ml

glass vials or micro-Kjeldahl flasks for increased refluxing and with nitric-perchloric acid mixtures with either 4:1 or 5:1 ratios (v/v) did not totally digest the milk fat. Therefore the following procedure was employed. Twenty ml of milk was transferred into 25 ml glass vials, freeze-dried and then dry ashed at 500° C for 4 hr. Three ml HCl (37.0-38.0%, Fisher Scientific) and four drops of nitric acid were added to the ash and the residue was dried on a hot plate using low heat. Prior to analysis by flame atomic absorption spectrophotometry, 10 ml of 5% (w/v) HCl solution was added to the ash and the preparation was vortexed. This procedure resulted in a solution containing twice the Cu and Mo concentrations found in milk. Accuracy was verified using bovine liver (1577a) standard reference material (National Bureau of Standards (NBS), Washington, D.C.).

Standards used for mineral analyses were prepared with certified atomic absorption reference standards (Fisher Scientific) diluted with the same solutions used to prepare the samples. For example, standards for TCA-soluble Cu analysis were prepared with 5% (w/v) TCA. Lanthanum was added to standards and samples at 1% for calcium analysis to control interferences from silicon, aluminum, phosphate and sulfate. Similarly, 1000 mg ml^{-1} Na_2SO_4 was added to standards and samples for Mo analysis.

The lower detection limit for Cu and Mo analysis was calculated to be 3 times the standard deviation of the measurement made for blank values (Wolf, 1982). Runs were repeated when blank values suggested contamination or when instrumentation sensitivity was erratic. Samples that had concentrations below

this detection limit were identified as non-detectable. For purposes of statistical analyses these values were assumed to be zero.

All glassware used for trace element analyses was soaked in 10% nitric acid for at least 24 h and then rinsed several times with distilled water, followed by five rinses with deionized, distilled water. Rinsed glassware was dried in a forced air oven and stored in sealed plastic bags until used. The same procedure was used to clean plastic containers used to store milk samples.

Plasma Cp oxidase activity was assayed according to the procedure outlined by Smith and Wright (1974), using purified p-phenylenediamine dihydrochloride (PPD) as a substrate. Purification of PPD ($C_6H_4(NH_2)_2 \cdot 2HCl$, Fisher Scientific) was done by adding 50 g of the substrate and 3.0 g decolorizing charcoal to 150 ml distilled H_2O. The mixture was brought to a boil and immediately filtered. The filtrate was stored under dark conditions at 4° C for 24 h, allowing PPD crystals to form. Crystals were then removed, dried (25° C) and stored under minimum light conditions until used. Plasma samples from four healthy animals were combined and used to test the pH, amount of substrate and temperature conditions of the assay when applied to ovine and bovine plasma. Plasma enzyme activity was determined from the rate of PPD oxidation, Δ A min^{-1}, using a Beckman DU-8 Spectrophotometer.

Milk fat, protein and lactose were determined on a FOSS MS300 Infra-red Analyzer (Milko-SCAN-203B Type 17920) according to procedures outlined by Marth (1978).

Volatile fatty acids in the rumen fluid were determined by the method of Erwin et al. (1961). One ml of 25% (w/v) metaphosphoric acid was added to 5 ml of rumen fluid. The mixture was centrifuged at 1500 x g for 15 min. and the resulting supernatant analyzed on a Tracor Model 500 gas chromatograph.

RESULTS AND DISCUSSION

Beef Cow-Calf Trial

Plasma Cu Parameters in Cows. Plasma Cu levels were significantly greater ($P < 0.05$) for cows assigned to 0Mo and 20Mo than for those on 40Mo; the treatment means being 0.94, 0.84 and 0.52 $\pm$ 0.08 mg L^{-1}, respectively (Figure 1, Appendix table I-2). TCA-soluble Cu concentrations in plasma were influenced ($P < 0.01$) by diet. Although the actual TCA-insoluble Cu concentration was not ($P > 0.05$) influenced by diet, the percent of total plasma Cu that was TCA-insoluble was greater ($P < 0.01$) for animals fed the high Mo diet (Figure 1); the treatment means being 18.9, 20.6 and 24.9 $\pm$ 0.9% for 0Mo, 20Mo and 40Mo, respectively.

TCA-insoluble Cu has been associated with an altered systemic Cu metabolism (Smith and Wright, 1974; Bremner and Young, 1978). The higher percentage of this plasma Cu fraction for 40Mo cows relative to 0Mo and 20Mo cows may be related to a slower turnover rate of TCA-insoluble Cu, as suggested by Weber et al. (1983) and Hynes et al. (1984) or because of an increased rate of formation of a Cu-Mo-S complex. For cows fed the 40 Mo diet in the present study, the increase in percent of TCA-insoluble Cu reflects a decline in TCA-soluble Cu with no concomitant change in the insoluble fraction.

The appearance of TCA-insoluble Cu was identified as an early and characteristic response of sheep to dietary Mo (Lamand, 1977); however, it is usually associated with an increase in

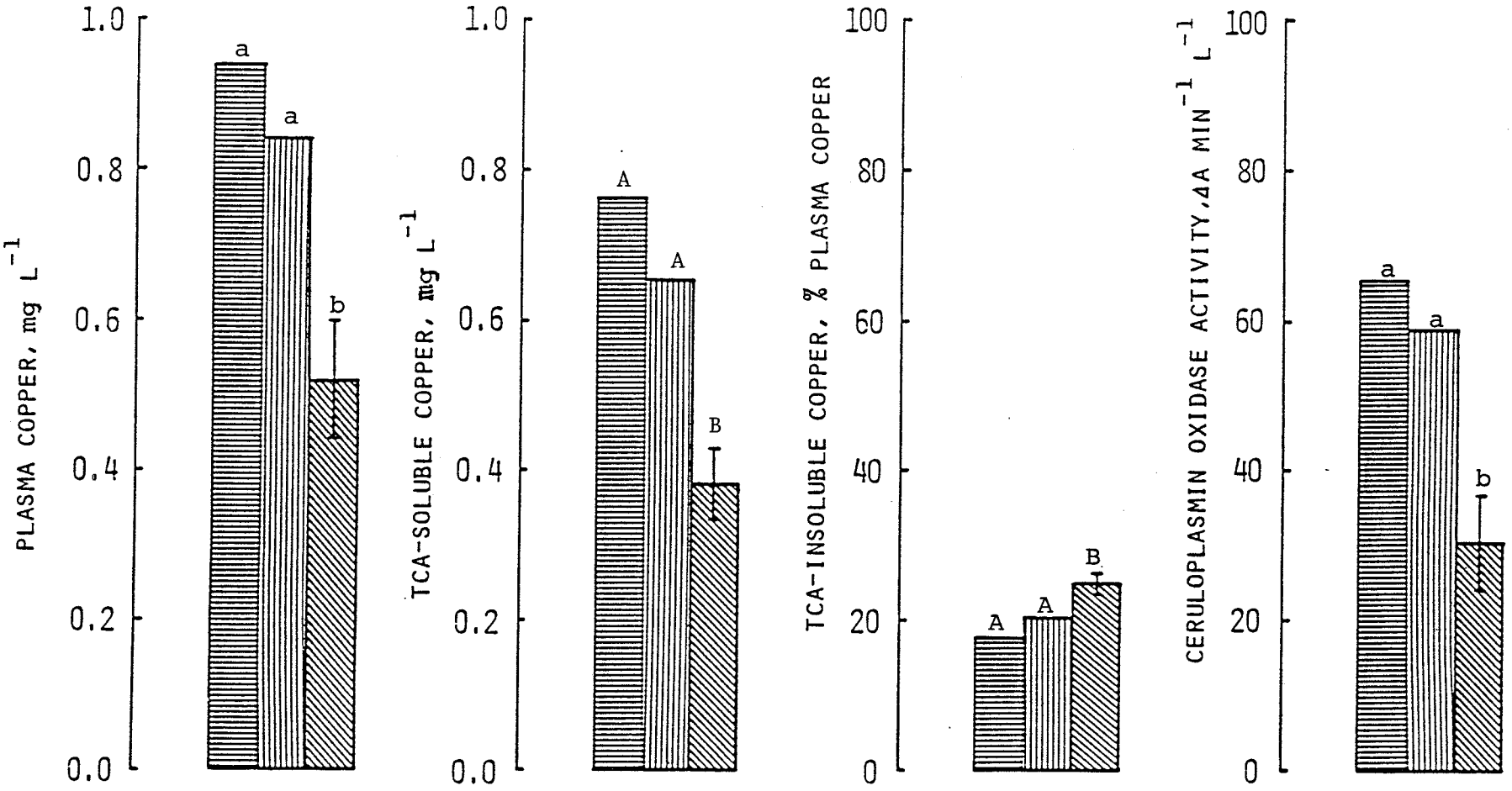

Figure 1: Plasma copper concentration and distribution and ceruloplasmin oxidase activity of cows fed 0Mo (≡), 20Mo (||||) and 40Mo (▨) diets for nine weeks. Mean of four animals sampled weekly ± SE. Means with different letters differ; a, b ($P<0.05$), A, B ($P<0.01$).

total Cu concentration of the plasma (Smith and Wright, 1975; Bremner and Young, 1978; Suttle and Field, 1983). The results observed in the present study suggest that the cows had low liver Cu stores and were dependent upon dietary Cu to try to maintain normal plasma Cu levels. Reduced Cu absorption from the gastro-intestinal tract because of complexing of Cu with thiomolybdates (Suttle, 1974a; Dick et al., 1975) may have been the cause of reduced plasma Cu levels.

Plasma Cp oxidase activity followed the same pattern as total plasma Cu and TCA-soluble Cu (Figure 1). Cows fed 40Mo had significantly lower ($P < 0.05$) enzyme activity (30.89 Δ A min^{-1} L^{-1}) than did animals fed 20Mo (59.13 Δ A min^{-1} L^{-1}) and 0Mo (65.59 Δ A min^{-1} L^{-1}). The standard error (SE) for these means is 9.3.

The significant treatment differences between cows fed 40Mo and those fed 0Mo and 20Mo diets can be partially attributed to differences in initial plasma Cu parameters between the three groups of animals (Figures 2, 3 and 4). However there were also significant interactions between diet and week observed for the blood Cu parameters. Total plasma Cu levels declined more rapidly ($P < 0.01$) for cows fed 40Mo than for the other two diets (Figure 2). Using the critical value for Cu deficiency in cattle (0.50 mg Cu L^{-1} plasma) suggested by Smith and Coup (1973), all four cows assigned to 40Mo diet were Cu deficient at the end of the trial. One of the cows in the 20Mo and all four allocated to 40Mo were Cu deficient according to the criteria of Puls (1981). (Appendix table I-15).

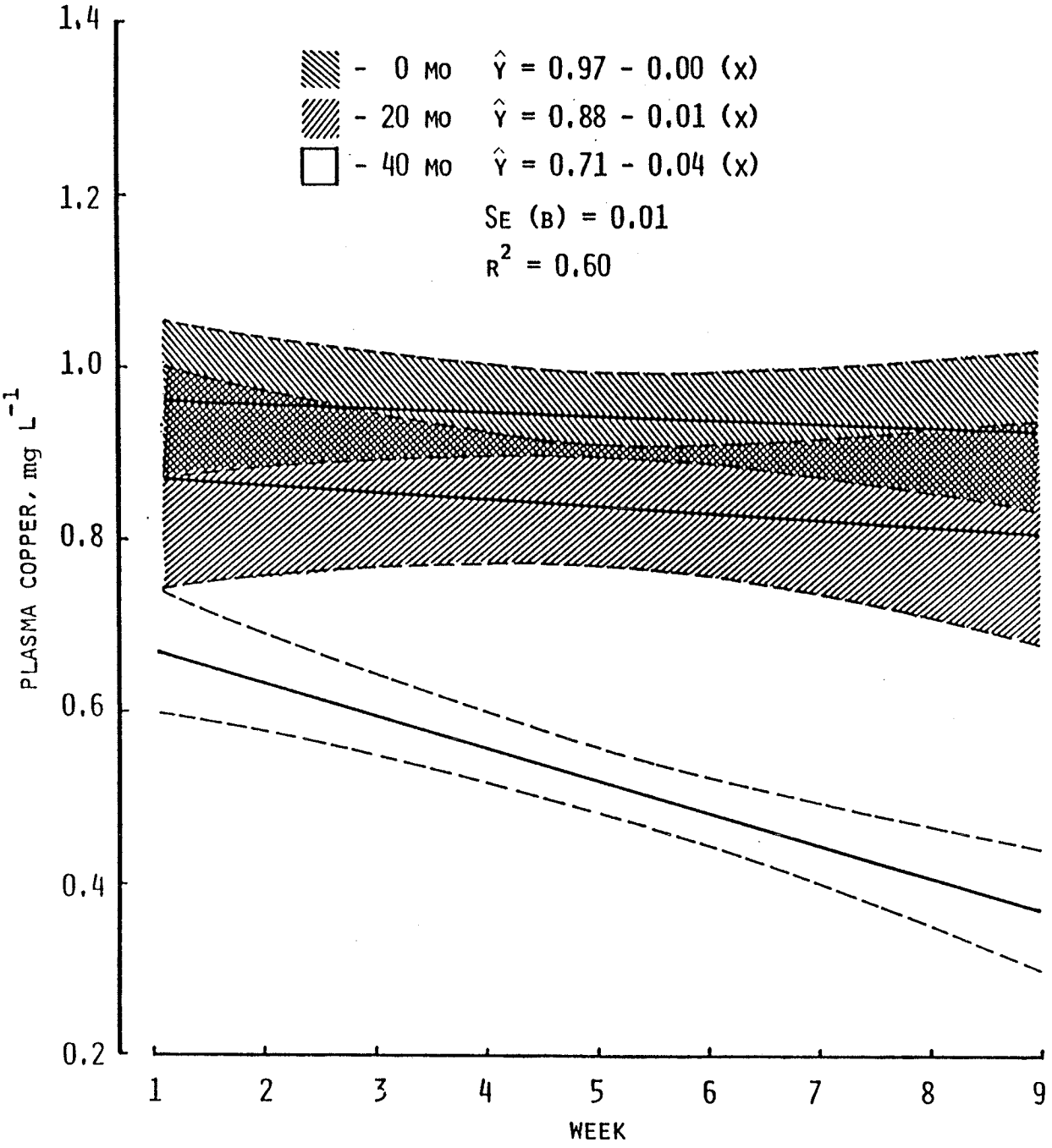

Figure 2: Plasma copper response for beef cows fed varying levels of molybdenum. Linear regression with 95% confidence limits. (For means and mean comparisons see Appendix table I-9.)

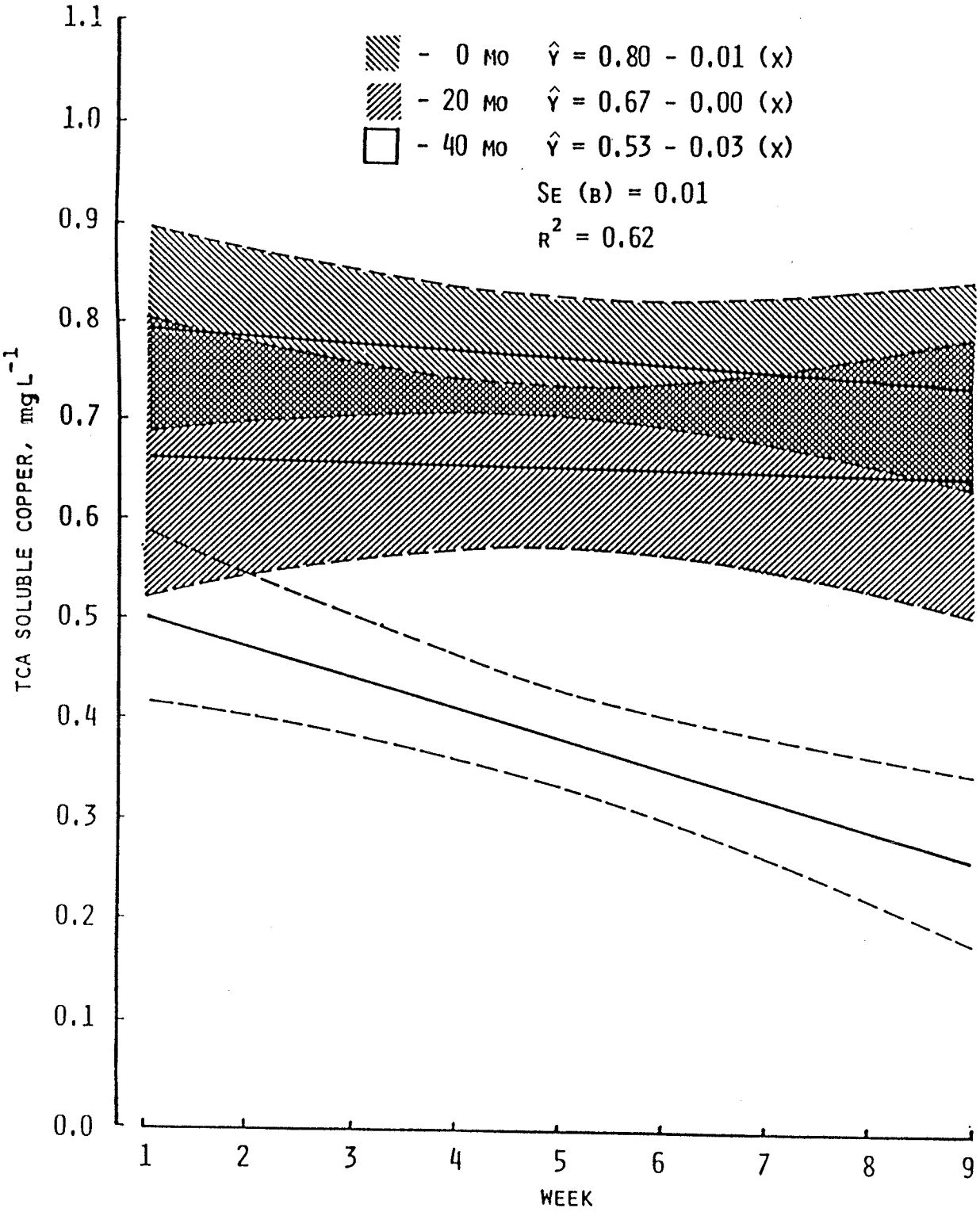

Figure 3: Plasma TCA-soluble copper response for beef cows fed varying levels of molybdenum. Linear regressions with 95% confidence limits. (For means and mean comparisons see Appendix table I-10)

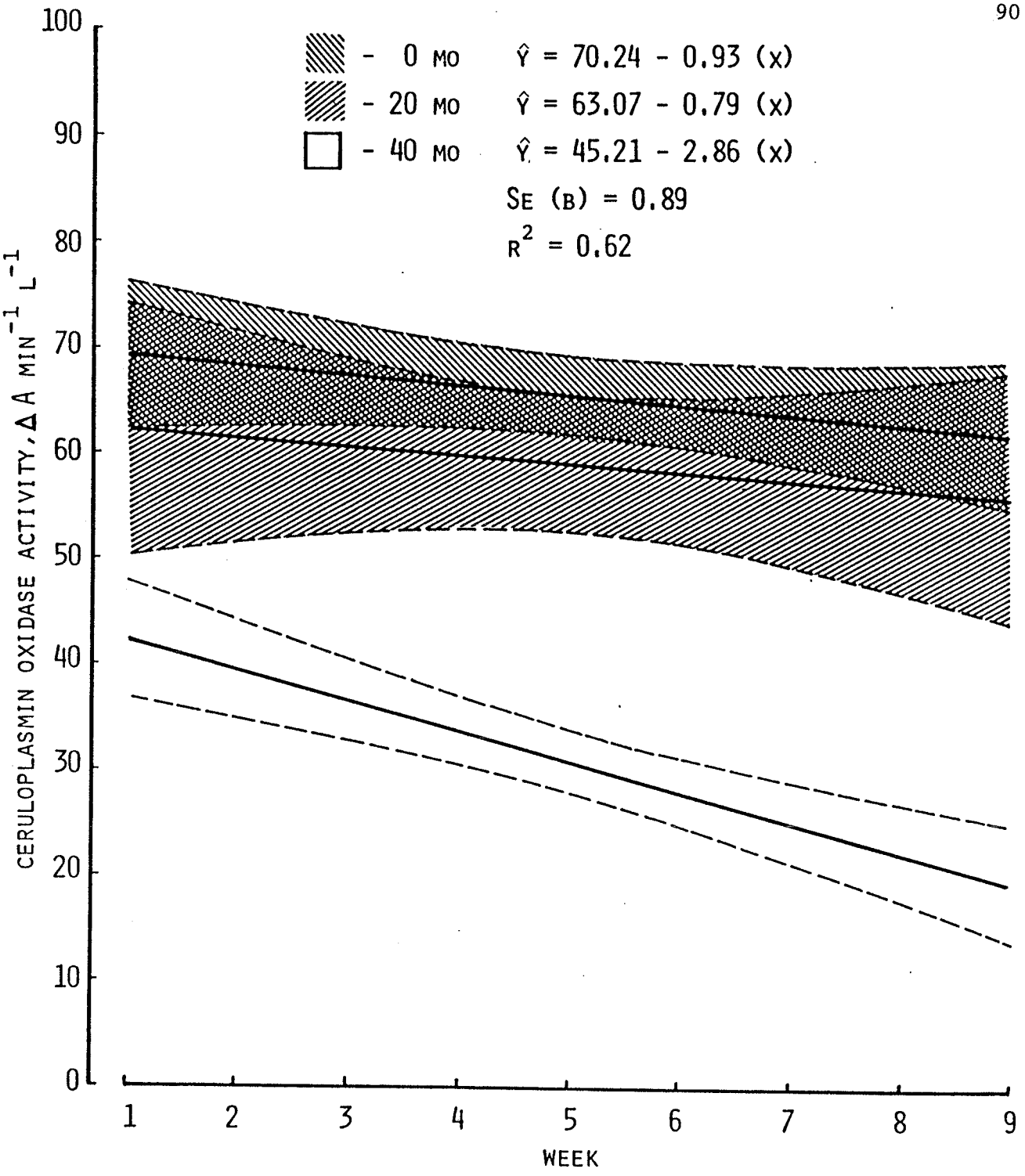

Figure 4: Plasma ceruloplasmin oxidase activity response for cows fed varying levels of dietary molybdenum. Linear regressions with 95% confidence limits. (For means and mean comparisons see Appendix table I-11.)

Similarly, TCA-soluble Cu decreased more rapidly over time ($P < 0.01$) for cows fed the 40Mo diet (Figure 3). There was not a significant ($P > 0.05$) diet by week interaction for the percentage of total plasma Cu that was TCA-soluble or insoluble (Appendix table I-2).

Plasma Cp oxidase activity followed the same pattern as total plasma and TCA-soluble Cu, showing a more rapid ($P < 0.01$) decline for animals fed the high Mo diet (Figure 4).

Past studies with lactating cattle showed a much slower decline in serum (Vanderveen and Keener, 1964) or no decline in whole blood (Huber et al., 1971) Cu concentrations when supplemental Mo was fed. Differences in dietary S may be partially responsible for differences observed in these two studies and in the present study. Unfortunately, plasma Cu fraction distribution and basal dietary S source and content were not provided in the two studies cited.

A study by Bremner and Young (1978), in which supplemental dietary Mo and S levels were fed to growing lambs, suggested that dietary S influences blood Cu parameters in ruminants. A diet containing 25.5 mg Mo kg^{-1} and 0.8 g S kg^{-1} had no effect on plasma Cu, TCA-insoluble Cu or Cp oxidase activity, which is comparable to that observed for the cows fed the 20Mo but not the 40Mo diet. However, adding 5 g SO_4^{2-} kg^{-1} to the diet of the lambs caused the Cu concentration in plasma and the TCA-insoluble Cu fractions to increase with no observable changes in enzyme activity. The ability of the lambs fed high Mo diets to maintain plasma Cu levels may have been related to adequate liver Cu

stores (84 to 180 mg kg^{-1} fresh tissue) at the start of the trial.

A second study with growing lambs (Ademosun and Munyabuntu, 1982) found that plasma Cu and Cp oxidase activity decreased and liver Cu was halved when lambs were fed 10.7 mg Mo and 1.6 g S kg^{-1} D.M. for 12 weeks. Although liver Cu stores of the lambs were adequate, plasma Cu levels declined as a result of dietary Mo supplementation. Plasma TCA-insoluble Cu was not determined by Ademosun and Munyabuntu (1982).

In comparing hypocupremic ewes fed diets containing 4 to 24 mg Mo kg^{-1} D.M., with similar animals fed a low Mo diet, Suttle (1983) found that the Mo decreased total plasma Cu and Cp oxidase activity in conjunction with the appearance of a TCA-insoluble Cu fraction in plasma. The dietary S levels in diets used by Suttle (3.5 mg kg^{-1} D.M.) were higher than that used in the present beef cow-calf trial; however, the data suggested that depleted or unavailable body Cu stores can result in the response observed for the latter.

In summary, results from the cow-calf study indicate that for lactating cows fed diets containing 1.3 to 1.4 g S kg^{-1}, feeding 34.8 Mo mg kg^{-1} D.M. resulted in a more rapid decrease in total plasma Cu and a higher proportion of the total plasma Cu being TCA-insoluble compared to cows fed 0.6 (0Mo) or 19.3 (20Mo) mg Mo kg^{-1} D.M. A general decline over time ($P < 0.01$) was observed across all treatment groups for total plasma Cu, plasma TCA-soluble Cu and Cp oxidase activity; ($P < 0.01$, Appendix table I-2) the rate of decline being greatest for cows fed 40 Mo.

Milk Composition and Yield. Milk fat, protein and lactose content were not influenced ($P > 0.05$) by dietary Mo (Table 9). Milk Cu levels tended to decrease with increased dietary Mo, however the response was not significant ($P > 0.05$). Milk Cu concentrations for all cows during the nine week trial ranged from 0.05 to 0.27 mg L^{-1} which is in the range reported for cattle by Underwood (1977) and Lönnerdal et al. (1981). The influence of dietary Mo on milk Cu levels observed in the present study is similar to that of Vanderveen and Keener (1964) but differs from Huber et al. (1971) who found a ten-fold increase in milk Cu when 53 mg Mo kg^{-1} D.M. was fed. Huber et al. (1971) provided no explanation for the increased milk Cu levels and a similar response has not been reported in other studies with lactating animals fed high levels of Mo.

Milk Mo levels were significantly ($P < 0.01$) higher for each increment in dietary Mo (Figure 5, Appendix table I-12). Individual cows fed 20Mo and 40Mo diets had milk Mo levels ranging from 0.22 to 0.80 mg L^{-1} and from 0.38 to 2.47 mg L^{-1}, respectively. Milk Mo concentrations were not affected by time with the exception of low concentrations ($P < 0.01$) observed in the first and sixth week of the trial for cows fed 20Mo and 40Mo. The initial low levels (week 1) observed for milk Mo are probably related to the recent introduction of animals to their experimental diets. The reason for the lower levels observed in week 6 is not known; however milk Mo levels returned to previous levels thereafter. Laboratory analysis procedures are not considered to be a cause because repeated analysis on these milk samples produced similar results.

Table 9: Influence of dietary molybdenum on milk yield and composition for beef cows.†

Items	DIET 0Mo	20Mo	40Mo	SE
Milk yield, kg d^{-1}	10.7	10.8	8.3	2.5
Fat corrected milk kg d^{-1}	14.6	15.7	9.9	2.4
Butterfat, %	6.00	6.91	5.26	0.53
Protein, %	3.50	3.53	3.55	0.09
Lactose, %	5.25	5.29	5.24	0.08
Copper, mg L^{-1}	0.16	0.15	0.10	0.02
Molybdenum, mg L^{-1}	0.10^{a}	0.51^{b}	1.19^{c}	0.04

† Each value represents the mean of four cows for nine weeks.

a - c Means in the same row with different superscripts are significantly different, $\alpha = 0.01$.

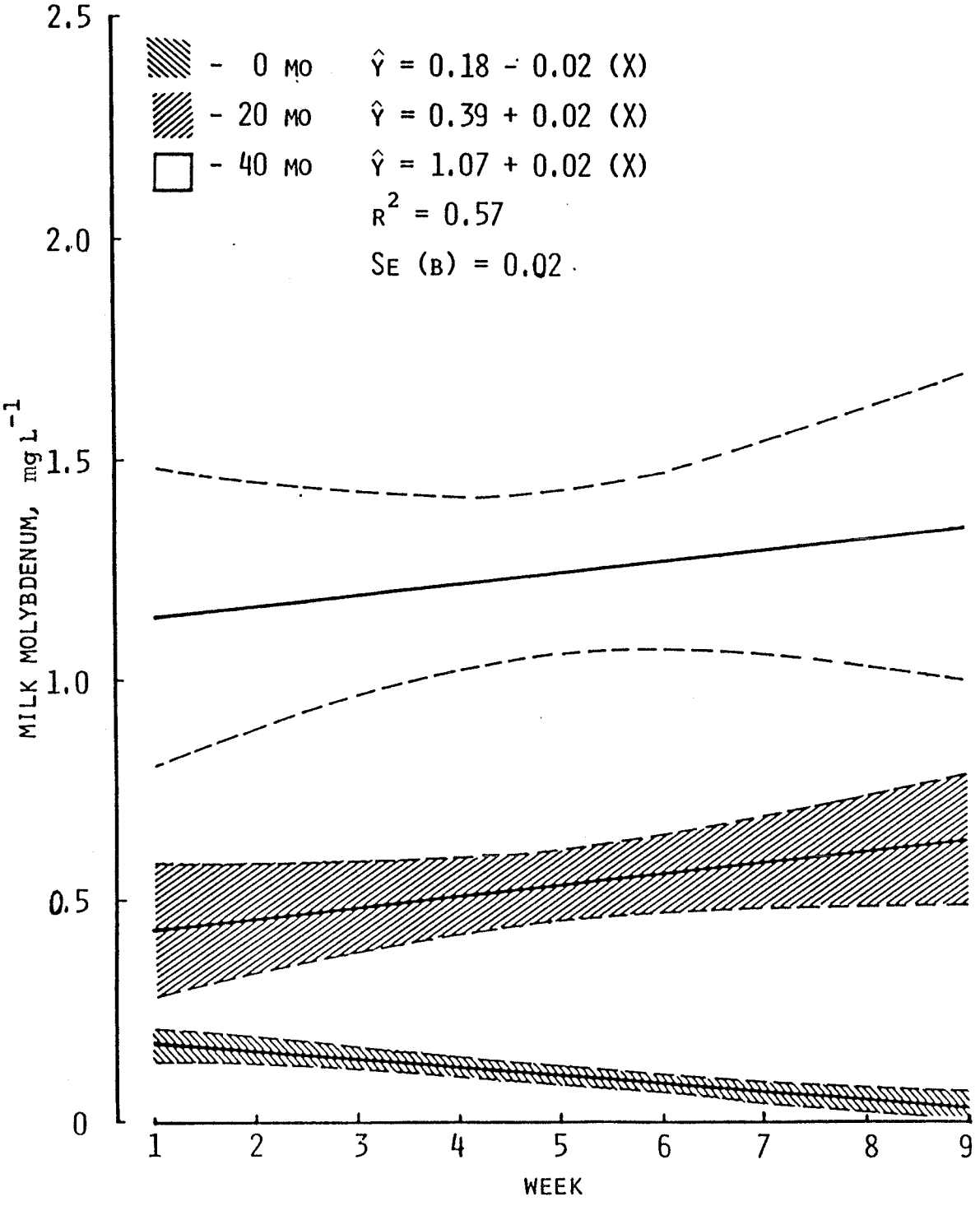

Figure 5: Effect of dietary molybdenum on molybdenum concentrations in milk produced by beef cows. Linear regression with 95% confidence limits. (For means and mean comparisons see Appendix table I-12.)

By comparison, Vanderveen and Keener (1964) found average milk Mo concentrations over a 300 day lactation to range from approximately 0.07 to 0.86 mg L^{-1} for cows fed diets containing 5 to 50 mg Mo kg^{-1} D.M. It is interesting to note that Vanderveen and Keener (1964) reported no milk Mo response to dietary Mo in the first 75 days that animals were fed high Mo diets and that the response was much greater in late lactation than early and midlactation, whereas in the present study there was an immediate response to increased Mo intake. Huber et al. (1971) observed milk Mo levels to average 1.03 mg L^{-1} in the last four months of a six month study in which cows were fed 53 mg Mo kg^{-1} D.M.

In summary, in the present study milk Mo levels were found to be equal to or greater than those observed in studies by Vanderveen and Keener (1964) and Huber et al. (1971), for which higher dietary Mo levels were fed to cows.

Milk yield was not significantly ($P > 0.05$) influenced by diet (main effect); however there was a diet by week interaction effect ($P < 0.01$) (Figure 6). Animals fed 0Mo and 20Mo diets maintained milk yield during the trial, whereas those fed the 40Mo diet had decreasing milk yields. A similar trend was observed for 4% fat corrected milk (FCM) yield; however the difference between 40Mo and the other two diets was not significant ($P > 0.05$).

An attempt was made to use regression lines that best fit the data to explain the interaction effect (Figure 7), however the ability of the model to explain data variability (R^2) was low. Data collected from cows assigned to 0Mo and 20Mo were pooled for this figure.

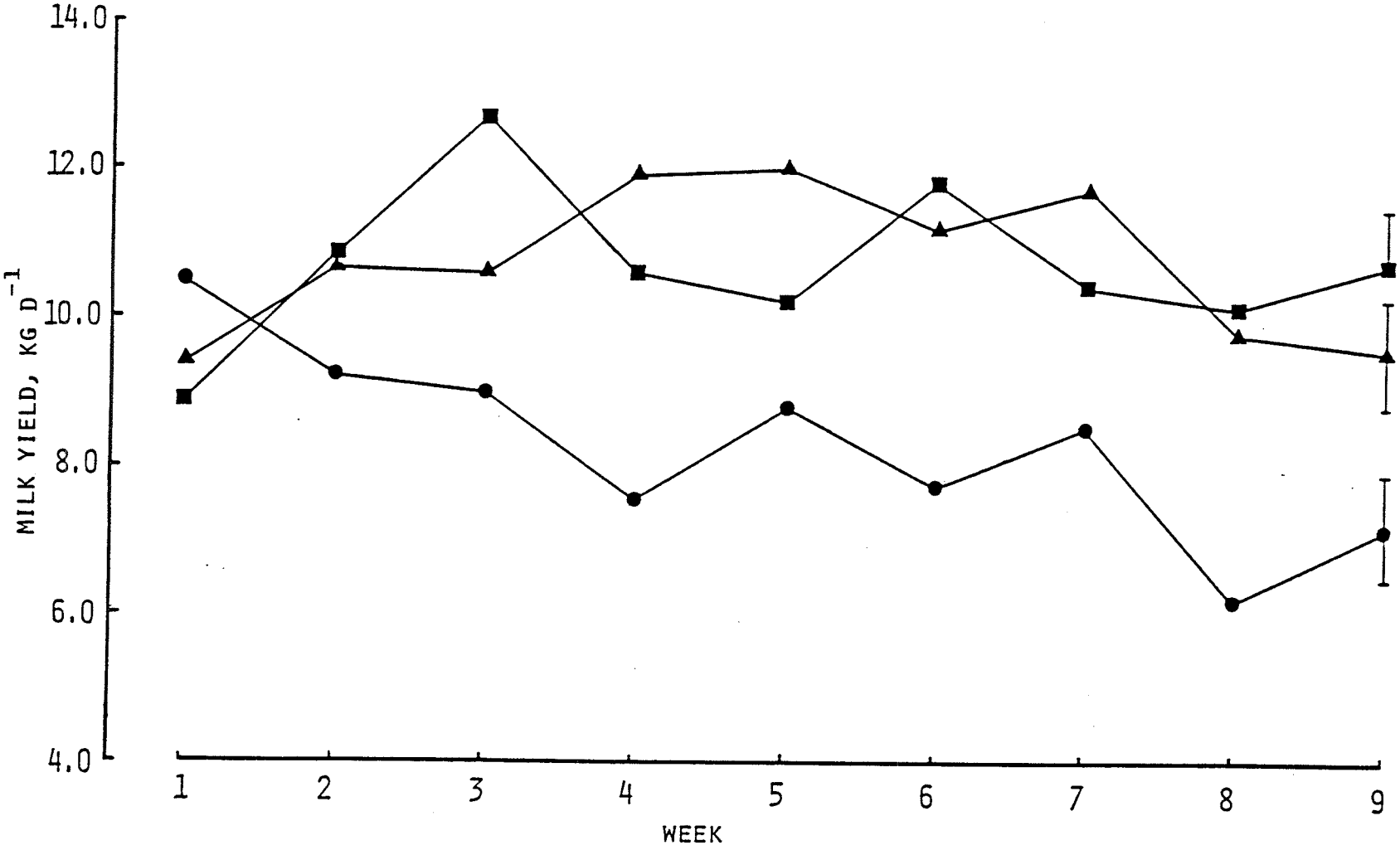

Figure 6: Milk yield response of beef cows fed 0Mo (■), 20Mo (▲) and 40Mo (●) diets. (For means and mean comparisons see Appendix table I-13.)

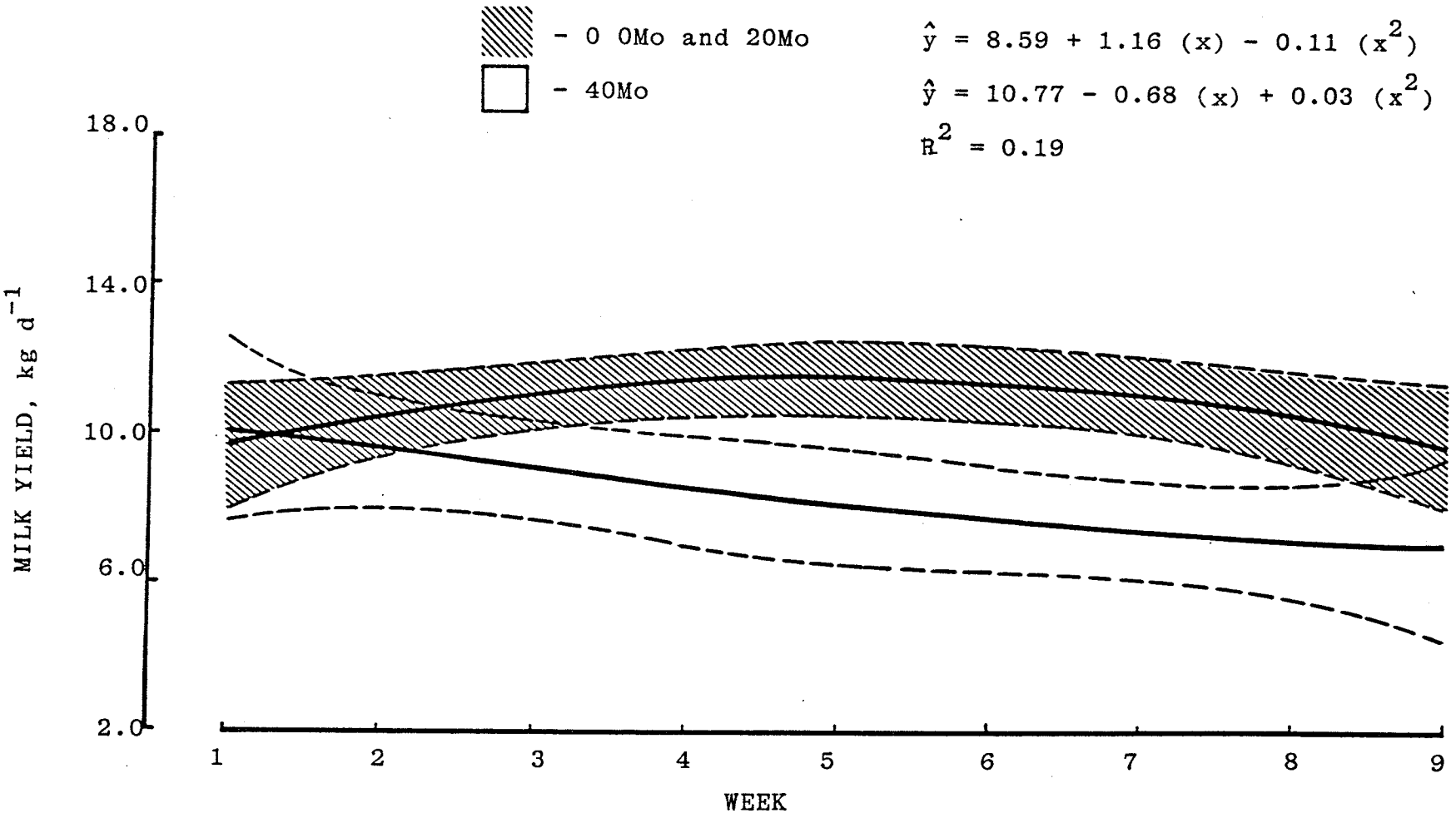

Figure 7: Milk yield response of beef cows fed varying levels of dietary molybdenum. Quadratic regression with 95% confidence limits.

The relationship between Cu parameters used to indicate animal Cu status and milk production of lactating animals has not been well defined to date. Based on records of more than 700 cows, Poole and Walshe (1970) and Rogers and Poole (1977) found Cu supplementation increased blood and liver Cu concentrations but had little effect on milk yield of Cu deficient cows. In the beef cow-calf study milk yield decreased concurrently with declining plasma Cu values and presumably was not a response to plasma Cu level or Cp oxidase activity reaching some lower threshold level. Therefore the high level of Mo may have affected milk yield independently of its effect on Cu parameters.

There was a week effect ($P < 0.001$) and a diet by week interaction ($P < 0.10$) for daily milk Cu excretion (milk yield x milk Cu content, Table 10). Daily excretion levels increased during the first four weeks for cows fed 0Mo and 20Mo. Daily milk Cu excretions decreased from week 5 to the end of the trial for these two groups of cows. By comparison, there was a steady decline in excretion of milk Cu by the 40Mo cows (Table 10). The pattern described for milk Cu excretion reflects the daily milk yield for cows assigned to the three diets. Average daily milk Cu excretions were 2.7, 2.2 and 1.2% of daily total Cu intake and 48.4, 96.3 and 95.6% of the estimated daily available Cu intake (pp. 110-111) for 0Mo, 20Mo and 40Mo, respectively.

Contrary to the response observed for milk Cu excretion, daily milk Mo excretion was significantly influenced by diet ($P < 0.01$) with no interaction effects (Table 11). Mean daily milk Mo excretions were 5 and 10-fold higher for cows fed the 20Mo and 40Mo diets, respectively compared with 0Mo cows (Table 11). The

Table 10: Calculated† daily milk copper excretion (mg d^{-1}) for beef cows fed three levels of molybdenum for nine weeks.

Sampling Time	DIET 0Mo	20Mo	40Mo	Mean ± SE
Week 1	1.42	1.64	1.11	1.39 ± 0.14 B
Week 2	1.79	1.86	1.08	1.58 ± 0.14 B
Week 3	2.56	1.63	1.01	1.73 ± 0.14 A B
Week 4	2.95	2.10	0.93	2.00 ± 0.14 A
Week 5	1.87	1.71	0.94	1.51 ± 0.14 B
Week 6	1.87	1.81	0.67	1.45 ± 0.14 B
Week 7	1.31	1.04	0.69	1.02 ± 0.14 C
Week 8	1.22	0.93	0.64	0.93 ± 0.14 C
Week 9	1.09	1.13	0.65	0.96 ± 0.14 C
Mean ± SE	1.79 ± 0.31	1.54 ± 0.31	0.86 ± 0.31	

† Calculated from values for daily milk production (kg d^{-1}) and milk Cu concentrations (mg L^{-1}). A conversion factor (0.97) was used to convert milk from liters to kilograms.

A - C Values in the same column with different superscripts are significantly different, $\alpha = 0.05$.

Table 11: Calculated† daily milk molybdenum excretion (mg d^{-1}) by beef cows fed three levels of dietary molybdenum for nine weeks.‡

Sampling Time	DIET 0Mo	20Mo	40Mo	Mean ± SE
Week 1	1.93	2.42	7.81	4.05 ± 0.82 B
Week 2	1.24	3.70	10.25	5.06 ± 0.82 B
Week 3	1.51	5.96	11.72	6.40 ± 0.82 A B
Week 4	1.02	6.82	10.71	6.18 ± 0.82 A B
Week 5	1.46	9.80	14.74	8.67 ± 0.82 A
Week 6	0.89	5.48	6.21	4.19 ± 0.82 B
Week 7	0.86	4.82	11.21	5.63 ± 0.82 A B
Week 8	0.36	4.97	10.90	4.93 ± 0.82 B
Week 9	0.53	5.57	6.79	4.30 ± 0.82 B
Mean ± SE	1.09 ± 4.40 a	5.50 ± 4.40 a b	10.02 ± 4.40 b	

† Calculated from values for daily milk production (kg d^{-1}) and milk Mo concentrations (mg L^{-1}). A conversion factor (0.97) was used to convert milk from liters to kilograms.

‡ Each value represents the mean of four animals.

a - b Values in the same row with different superscripts are significantly different, $\alpha = 0.05$.

A - B Values in the same column with different superscripts are significantly different, $\alpha = 0.05$.

excretion levels represent 15.8, 2.5 and 2.5% of total daily Mo intake for 0Mo, 20Mo and 40Mo, respectively. Similar values were observed by Huber et al. (1971) who reported that 2.6 and 1.3% of dietary Mo was accounted for by milk Mo excretions for cows fed diets containing 53 and 173 mg Mo kg^{-1} D.M., respectively.

Gross Effects in Cows. All cows gained weight on test; daily gains averaging 0.4, 0.4 and 0.4 $\pm$ 0.1 kg for animals fed 0Mo, 20Mo and 40Mo, respectively. Dietary Mo did not influence ($P > 0.05$) cow weight change. Reduced milk yield for animals fed the 40Mo diets did not appear to be the result of a change in energy partitioning, which would have resulted in increased body tissue deposition.

Clinical symptoms of hypocuprosis or molybdenosis such as diarrhoea were not observed during the experiment.

Plasma Cu Parameters in Calves. Total plasma Cu of suckling calves was not influenced ($P > 0.05$) by their dams' dietary Mo intake. The treatment means, over the nine week trial, were 0.77, 0.81 and 0.80 $\pm$ 0.06 mg L^{-1} for 0Mo, 20Mo and 40Mo, respectively.

At the beginning of the trial the mean plasma Cu content for the calves was 0.94 $\pm$ 0.37 mg L^{-1} which was similar to that of their dams (0.85 $\pm$ 0.47 mg L^{-1}). Calf plasma Cu levels were significantly ($P < 0.001$) lower in week 2 (Table 12). For the remainder of the trial there was a tendency for plasma Cu concentration to continue declining, however this trend was not significant ($P > 0.05$). This agrees in part with data reported by Bingley and Duffy (1969) showing that, although plasma Cu concen-

Table 12: Changes in calf plasma copper concentration and distribution during the nine week study.

Week	PARAMETER		
	Plasma Cu (mg L^{-1})	TCA-soluble Cu (mg L^{-1})	Cp oxidase activity (Δ A min^{-1} L^{-1})
1	0.94^{A}	0.73^{A}	72.4^{A}
2	0.84^{B}	-	63.8^{B}
3	0.79^{B}	0.69^{A}	$49.9^{C\ D}$
4	0.76^{B}	-	$58.1^{B\ C}$
5	0.78^{B}	0.71^{A}	$55.2^{B\ C}$
6	0.74^{B}	-	$53.6^{C\ D}$
7	0.83^{B}	0.59^{B}	44.1^{D}
8	0.72^{B}	-	$52.5^{C\ D}$
9	0.71^{B}	0.59^{B}	$48.7^{C\ D}$
SE	0.03	0.03	4.1

A - D Means in columns with different superscripts are significantly different, $\alpha = 0.05$.

trations of newborn calves were much lower than those of their dams, adult values were reached by the time calves were one week of age. Plasma Cu concentrations were not monitored for calves over one week of age in that study.

The decreases in plasma Cu observed in the current study coincided with decreases ($P < 0.001$) in TCA-soluble Cu and Cp oxidase activity (Table 12). The TCA-soluble Cu levels for weeks 1, 3 and 5 were significantly higher ($P < 0.05$) than for the latter part of the trial. Period effect for Cp oxidase activity was not as easily defined as for the other two plasma Cu parameters. Although there was a general tendency for values to decrease during the trial, there was also variability from week to week (Table 12). As TCA-soluble Cu values appear to correspond with fluctuations in Cp oxidase activity it is speculated that animal variability (i.e., response to environmental changes) rather than sample handling procedures were responsible for the weekly fluctuations observed.

The general decline observed for all three plasma Cu parameters (Figure 8) may be associated with low dietary Cu intake. Daily Cu requirement for these calves was estimated to be 1.3 mg d^{-1} (ARC, 1980). Estimated total Cu intake was 1.79, 1.54 and 0.86 mg d^{-1} for calves sucking cows assigned to 0Mo, 20Mo and 40Mo diets, respectively (Table 10). Dietary Cu availability is greater prior to than following weaning (Suttle, 1975; Bremner and Davies, 1980); however Suttle (1975) also observed a negative relationship between Cu availability and age prior to weaning. The decline in calf plasma Cu parameters over time observed in the present study (Table 12) may have been a response to decreas-

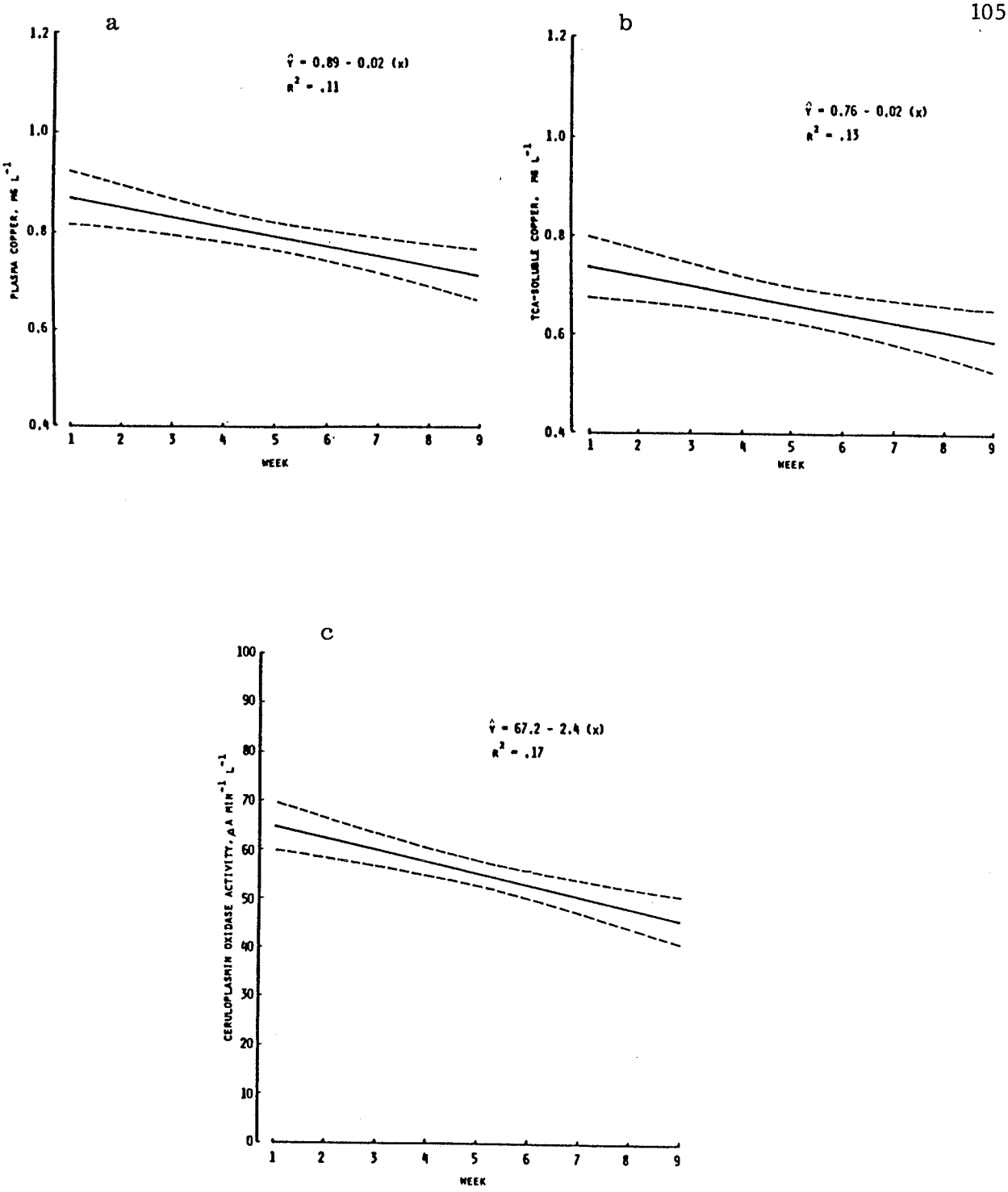

Figure 8: Changes in plasma copper (a) and TCA-soluble copper (b) concentrations and ceruloplasmin oxidase activity (c) of sucking calves.

ing availability of dietary Cu in combination with increased requirement for Cu.

Liver Cu in Calves. Initial liver Cu concentrations ranged from 51.2 to 351.9 mg kg^{-1} D.M. Initial liver Cu concentrations for all calves were above the suggested threshold range of 5 to 25 mg Cu kg^{-1} liver D.M. used to differentiate deficient from normal animals (Ammerman, 1970; Smith and Coup, 1973). Mean hepatic Cu concentrations were affected by experimental diet of dams, as calves in the 40Mo group had significantly ($P < 0.10$) lower concentrations of 87.6 $\pm$ 27.6 mg kg^{-1} D.M. compared to 153.3 $\pm$ 27.6 and 182.7 $\pm$ 27.6 for calves in the 20Mo and 0Mo treatment groups, respectively. However, this difference may in part be related to the lower initial (day 3) hepatic Cu concentrations of the 40Mo calf group (131.8 mg kg^{-1} D.M.) compared to those in the 0Mo (217.0 mg kg^{-1} D.M.) and 20Mo (195.5 mg kg^{-1} D.M.) groups, respectively.

Hepatic Cu concentrations declined at rates of 1.49, 2.08 and 1.58 $\pm$ 0.74 mg kg^{-1} liver D.M. d^{-1} for calves nursing cows fed 0Mo, 20Mo and 40Mo, respectively (Figure 9). The Cu concentration in the last (day 57) sample from one calf in the 20Mo group was 28.6 mg kg^{-1} D.M. Two calves in the 40Mo group had levels of 18.3 and 24.1 mg kg^{-1} D.M. (Appendix table I-17). These calves had liver Cu levels at or approaching threshold levels stated to define Cu deficiency as noted above.

Simpson et al. (1982) found that for calves receiving a low Cu diet, liver Cu concentrations declined at rates that were positively related to initial liver Cu levels despite marked

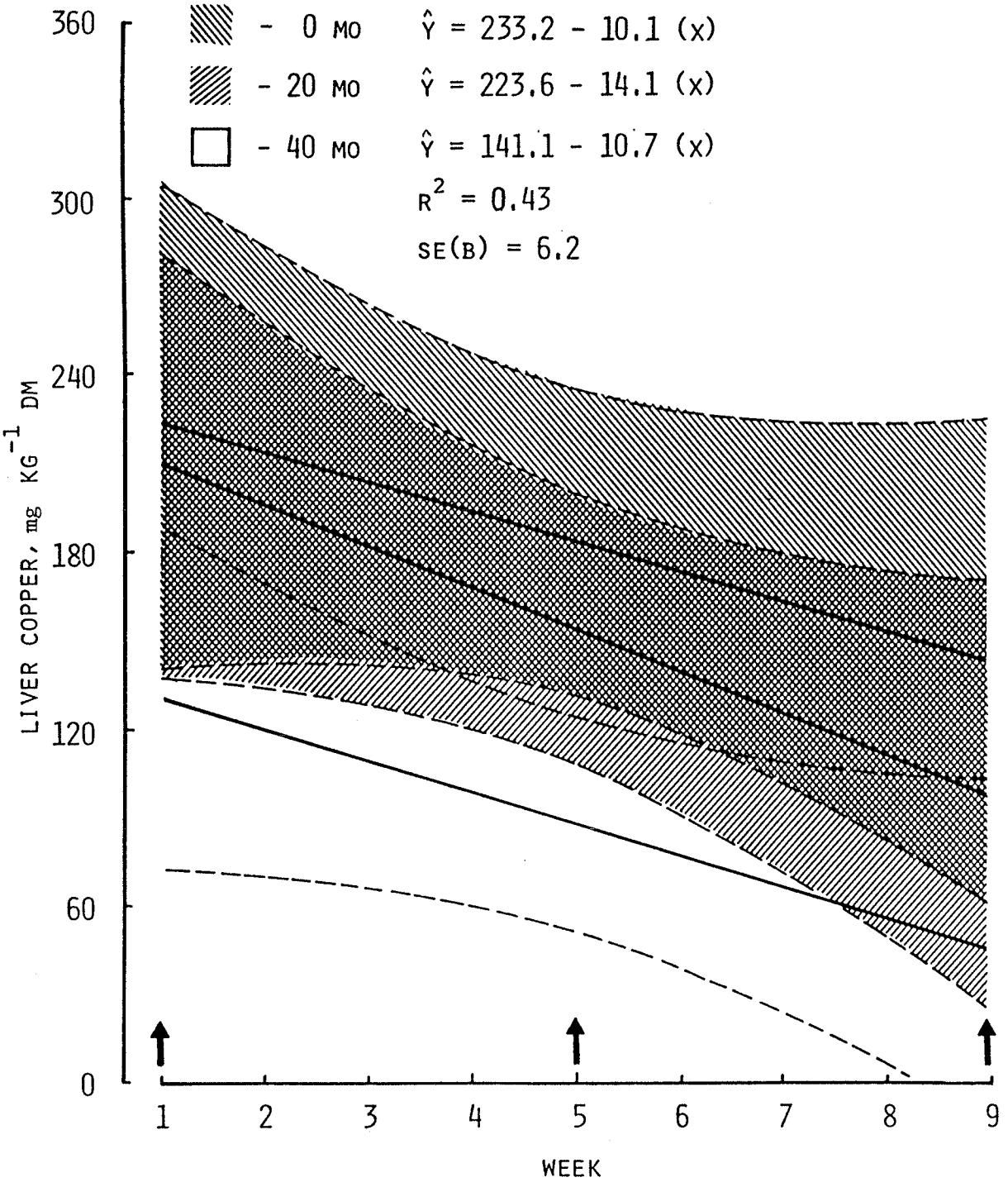

Figure 9 : Changes in liver copper concentrations of calves sucking cows fed varying levels of molybdenum. Linear regressions with 95% confidence limits. Arrows indicate sampling times. (For means and mean comparisons see Appendix table I-14.)

differences in the latter at the start of the treatment. Hepatic Cu changes, calculated as the daily fractional decline (mean change on daily basis (mg kg^{-1}) divided by initial liver Cu concentration (mg kg^{-1}) tended to be lower for calves in the 0Mo group compared with those in the 20Mo and 40Mo groups, with values of 0.006, 0.010 and 0.012 $\pm$ 0.002 respectively. This trend was not significant ($P > 0.05$).

Dietary Mo intakes were estimated to be 1.09, 5.50 and 10.02 mg d^{-1} or 0.91, 4.64 and 10.82 mg kg^{-1} D.M. intake for calves in the 0Mo, 20Mo and 40Mo groups, respectively. Molybdenum intake did not affect blood Cu parameters, however calves consuming high Mo milk (20Mo and 40Mo) tended to have greater decreases in liver Cu concentrations, relative to initial liver Cu levels compared with the 0Mo calves.

Weight Gain in Calves. Diet of the cows did not significantly ($P > 0.05$) affect average daily gains (ADG) of calves which were 0.75, 0.66 and 0.66 $\pm$ 0.08 kg for 0Mo, 20Mo and 40Mo, respectively. However there was a significant ($P < 0.05$) diet by week effect on ADG (Figure 10). Calves in the 0Mo group had higher ($P < 0.05$) daily gains than 20Mo and 40Mo calves in period 3 (week 3-4) and had higher gains than 40Mo calves in period 7 (week 7-8) and 8 (week 8-9).

Food conversion ratios, calculated as average daily FCM yield (kg) divided by average daily gain (kg) of calves, were 19.4, 23.8 and 15.0 for calves in 0Mo, 20Mo and 40Mo groups, respectively. This suggests that poor feed conversion, as opposed to reduced cow milk production may have been associated

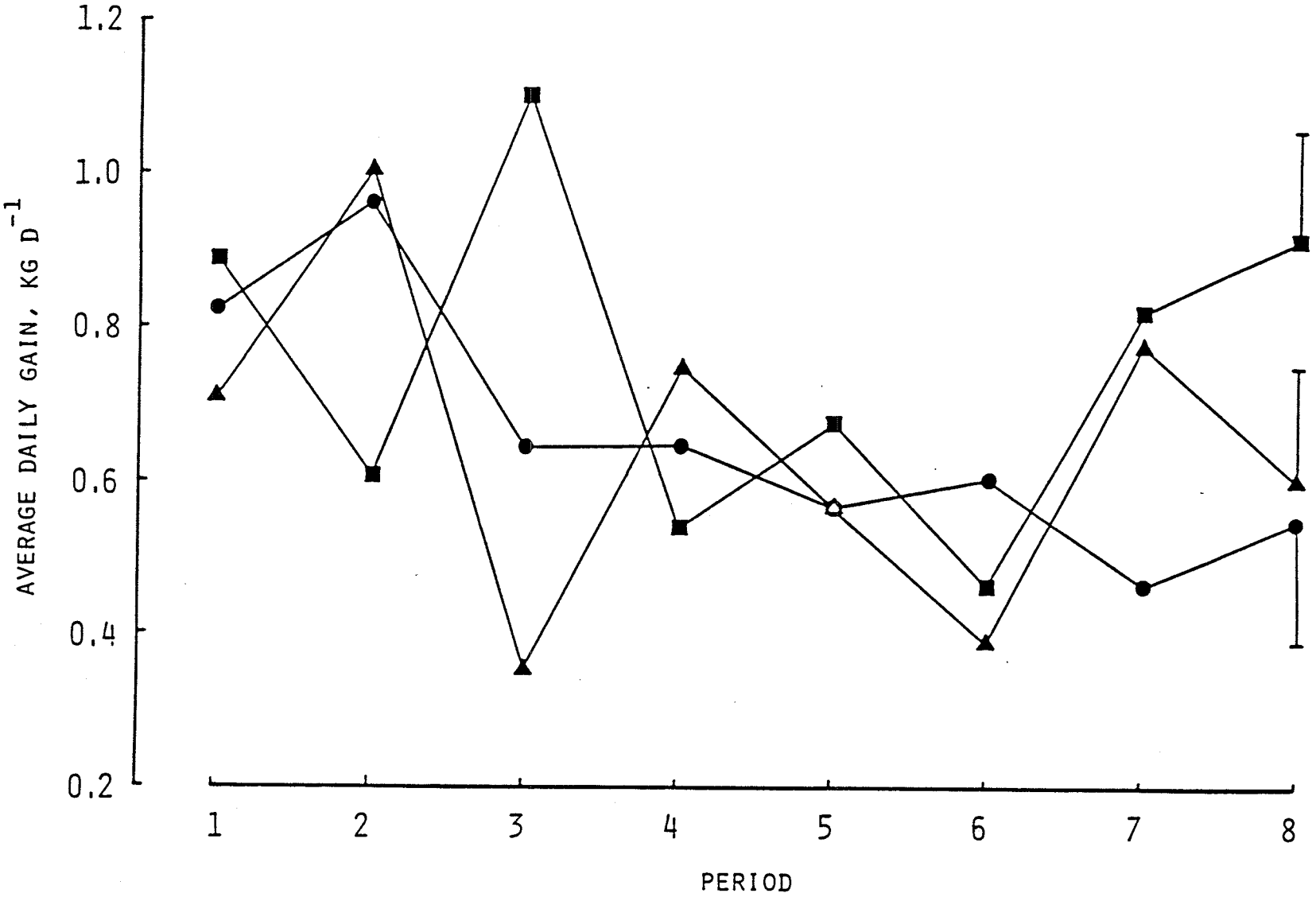

Figure 10: Average daily gains of calves suckling cows fed 0Mo (■), 20Mo (▲) and 40Mo (●) diets. Means ± SE.

with reduced calf growth for the 20Mo group. It is questionable whether high Mo levels in the milk of cows in the 20Mo group resulted in the poor feed conversion ratios because feed conversion of calves in the 40Mo group was much better. The short duration of the trial and the low number of animals per treatment make it difficult to predict whether similar losses may be incurred in the field or the magnitude of the losses. Also, the potential causes for a reduced calf growth rate were not well defined from the results of the present study. Reduced cow milk yield may have contributed to lower ADG in the latter part of the study for calves assigned to the 40Mo group. However, this did not appear to be the cause for any differences in ADG observed between calves in 20Mo relative to those in the 0Mo group.

A further consideration for future work in this area would be to place cows and calves on test immediately post-calving. Bailey and Lawson (1981) stated that calves on native range are observed to drink water and to graze within 30-60 days of birth. However, they estimated that greater than 50% of the calf's digestible energy intake was still supplied by milk for calves at 80 days of age.

Discussion. Estimated requirements of Cu by cattle are 7 $\mu g\ kg^{-1}$ liveweight d^{-1} for maintenance plus 0.1 mg kg^{-1} milk produced (ARC, 1980). The mean body weight and average daily milk yields of cows at the start of the trial were 568 $\pm$ 10.4 and 9.6 $\pm$ 1.5 kg, respectively. Therefore the net or available Cu requirement for this group of cows was estimated to be 4.9 mg d^{-1} (ARC, 1980). Incorporating the actual Cu content of milk (0.14 $\pm$ 0.01

mg kg^{-1}) produced by cows in the present study, the estimated daily requirements would be revised to 5.3 mg d^{-1}. The predicted coefficients of absorption of Cu (equation 1, Suttle and McLauchlan, 1976) for the 0Mo, 20Mo and 40Mo diets were 0.05, 0.02 and 0.01, respectively. Therefore predicted total available Cu for cows fed 11.6 kg of the respective diets was 3.7, 1.6 and 0.9 mg d^{-1}, which suggests that all three groups of animals were in a potentially negative Cu balance. Copper balance in this context refers to the ability of dietary Cu to meet the Cu requirements of the animal for maintenance and production.

The equation (equation 2) developed by Langlands et al. (1981), when applied to the experimental diets, predicts dietary Cu concentrations had to be 5.7, 7.2 and 8.8 mg kg^{-1} D.M. to maintain liver Cu concentrations. From these values it can be deduced that the cows fed 0Mo would have been in a positive Cu balance, but those cows fed the other two diets would have been in negative Cu balance, on the basis of actual Cu intakes.

Plasma Cu levels reflected the low availability of Cu in the 40Mo diet, the levels declined at a rate of 0.04 $\pm$ 0.01 mg L^{-1} $week^{-1}$ (Figure 2). The majority of this decline was attributable to the TCA-soluble Cu fraction which declined at 0.03 $\pm$ 0.01 mg L^{-1} $week^{-1}$ (Figure 3). However, the implied negative Cu balances for 20Mo fed cows, or for 0Mo and 20Mo fed cows, using the equations of Suttle and McLauchlan (1976) and Langlands et al. (1981), respectively, were not supported by the observed plasma Cu parameters or cow production records.

There are several possible reasons for the discrepancies.

First, both equations, used to estimate the ability of a diet to meet an animal's Cu requirements, were derived from studies with sheep and not cattle. Second, the Cu source should be considered when attempting to apply the above equations. Copper in silages and fresh forages may be less available than that derived from dried feeds or synthetic diets (Suttle, 1980; Suttle, 1983). Third, the Cu requirements suggested by ARC (1980) for cattle are based, to a large extent, on unpublished data and therefore cannot be verified.

In the past decade numerous studies (Rogers and Poole, 1977; Givens and Hopkins, 1978; Suttle et al., 1980; Jarvis and Austin, 1983; Boila et al., 1984b) have considered hypocuprosis in cows and their nursing calves and the related low productivity of cow-calf herds. In many cases, despite biochemical responses, there was a lack of animal production response to Cu supplementation in terms of milk yield or growth leading to the suggestion that low Cu status in the cows need not cause production losses (Rogers and Poole, 1977; Givens and Hopkins, 1978; Boila et al., 1984b). In other studies (Bingley and Anderson, 1972; Jarvis and Austin, 1983) calf growth rate has responded to Cu supplementation. It may be that cow milk yield and calf growth rate give variable responses to Cu supplementation under conditions of diagnosed hypocupremia because their production responses may be affected by toxic effects of Mo other than those associated with conditioned Cu deficiency.

Ewe-Lamb Trial

Actual concentrate intake, as a percentage of total D.M. intake, was 28.1, 28.9 and 29.0% for ewes fed 0Mo, 20Mo and 40Mo, respectively. Diet Cu content, based on nutrient analysis of the concentrates and corn silage (Table 5) and the intakes of each, were below the level for which the diets had been formulated (6.0 mg kg^{-1} D.M., Table 13). This was due to the low Cu content of the corn silage. Molybdenum concentrations in the experimental diets were close to formulated levels (Table 13).

Gross Effects in Ewes. Clinical signs typically associated with feeding excess Mo or low levels of available Cu were not observed in the ewes during the trial. However, three ewes, #99, 52 and 119, developed signs of thiamine deficiency after 5, 4 and 5 weeks on trial, respectively. The first ewe (#99) showing such signs was found lying on its side with opisthotonus and with the limbs extended, in a manner typical of polioencephalomalacia (Edwin and Jackman, 1982). The second ewe (#52) had muscle tremors and incoordination of the hind legs the day before she went down. Both ewes were initially treated for hypocalcemia and hypomagnesemia but showed no improvement. Ewe #52 was given 2.0 ml thiamine hydrochloride† and showed dramatic recovery within 24 hours. The third ewe to develop the symptoms (#119), was given a thiamine injection when the initial signs of muscle tremors were observed.

†Thiamine Hydrochloride Injectable (B_1) contains 100 mg thiamine HCl ml^{-1} and is a product of Professional Veterinary Laboratories.

Table 13: Calculated† nutrient composition of experimental diets fed to lactating ewes‡.

Nutrient, D.M.	DIET 0Mo	20Mo	40Mo
Crude protein, %	13.5	13.3	13.3
Acid detergent fiber, %	21.4	21.2	21.1
Calcium, g kg^{-1}	5.0	4.9	4.8
Phosphorus, g kg^{-1}	4.7	4.6	4.7
Magnesium, g kg^{-1}	2.4	2.4	2.5
Sulfur, g kg^{-1}	1.5	1.4	1.4
Iron, mg kg^{-1}	295.7	282.8	272.2
Zinc, mg kg^{-1}	32.8	31.7	31.0
Copper, mg kg^{-1}	4.9	4.5	4.5
Molybdenum, mg kg^{-1}	0.9	18.4	40.7

† Calculated from the results of nutrient analyses of concentrations and corn silage (Table 5) and actual intakes of each.

‡ Concentrate made up 28.1, 28.9 and 29.0% of total D.M. for 0Mo, 20Mo and 40Mo respectively. The remainder was corn silage.

Ewes #99 and 52 were removed from the test but ewe #119 was left on test because she resumed eating and appeared normal following the thiamine injection.

Animal response to the thiamine injections led to the speculation that the syndrome observed was a thiamine deficiency. Further analyses to confirm this were not performed except that an autopsy on ewe #99 provided no further information concerning the syndrome. A blood sample, taken 24 hours after the symptoms were first observed in this animal, had low calcium levels.

Two of these ewes had been consuming the 40Mo diet (#99 and 119) and the other one had been consuming the 20Mo diet (#52). A similar occurrence associated with high Mo intake has not been reported in the literature.

Assuming that the syndrome observed in the three ewes was a thiamine deficiency, one or a combination of the following mechanisms may have been in operation: blocked absorption of thiamine from the gut; inadequate rumen synthesis of thiamine; destruction of formed thiamine in the gut; altered metabolism of absorbed thiamine, which may have been caused by or related to excessive Mo intake.

Acute thiamine deficiencies reported in cattle and sheep have been related to the feeding of rapidly fermentable feeds (Brent, 1976) and to sudden changes in dietary patterns (Edwin and Jackman, 1982). These reports suggested that the maintenance of a stable and well-balanced microbial population helps to prevent the disease.

Nikolić et al (1983), Suttle (1983) and Huisingh et al.

(1975) have investigated the effects of Mo on various parameters related to rumen metabolic processes. There are some indications that changes in the microbial population occur in the presence of increasing concentrations of Mo in rumen fluid. Thus, changes in the numbers of thiamine-producing microorganisms, or more probably (Edwin and Jackman, 1982) changes in the numbers of thiaminase producing organisms could have caused the signs observed for three of the eight ewes assigned to Mo supplemented diets.

Another ewe (#60) assigned to the 20Mo diet, was removed from test when she developed gangrenous mastitis (week 4).

Initial weights of ewes were 76.1, 84.2 and 77.9 $\pm$ 3.03 kg for 0Mo, 20Mo and 40Mo, respectively. Ewes in all treatment groups lost weight during the trial ($P < 0.01$, Figure 11). Weight loss by ewes fed the two Mo supplemented diets tended to be greater than for the control animals, however the differences among diets were not significant ($P > 0.05$). Least square means for average daily weight change were -0.2 $\pm$ 0.1, -0.5 $\pm$ 0.2 and -0.3 $\pm$ 0.1 kg d^{-1} for ewes assigned to 0Mo, 20Mo and 40Mo, respectively.

Feed Intake and Rumen Fluid Parameters in Ewes. Feed intakes, expressed as daily D.M. intake or as a percentage of body weight, tended to be lower with increasing levels of dietary Mo; however the differences were not significant ($P > 0.05$, Table 14). Daily D.M. intake was more irregular for animals assigned to the high Mo diets and may have accounted for the tendency toward reduced intake by these animals.

Rumen fluid samples were taken (see page 71 for details)

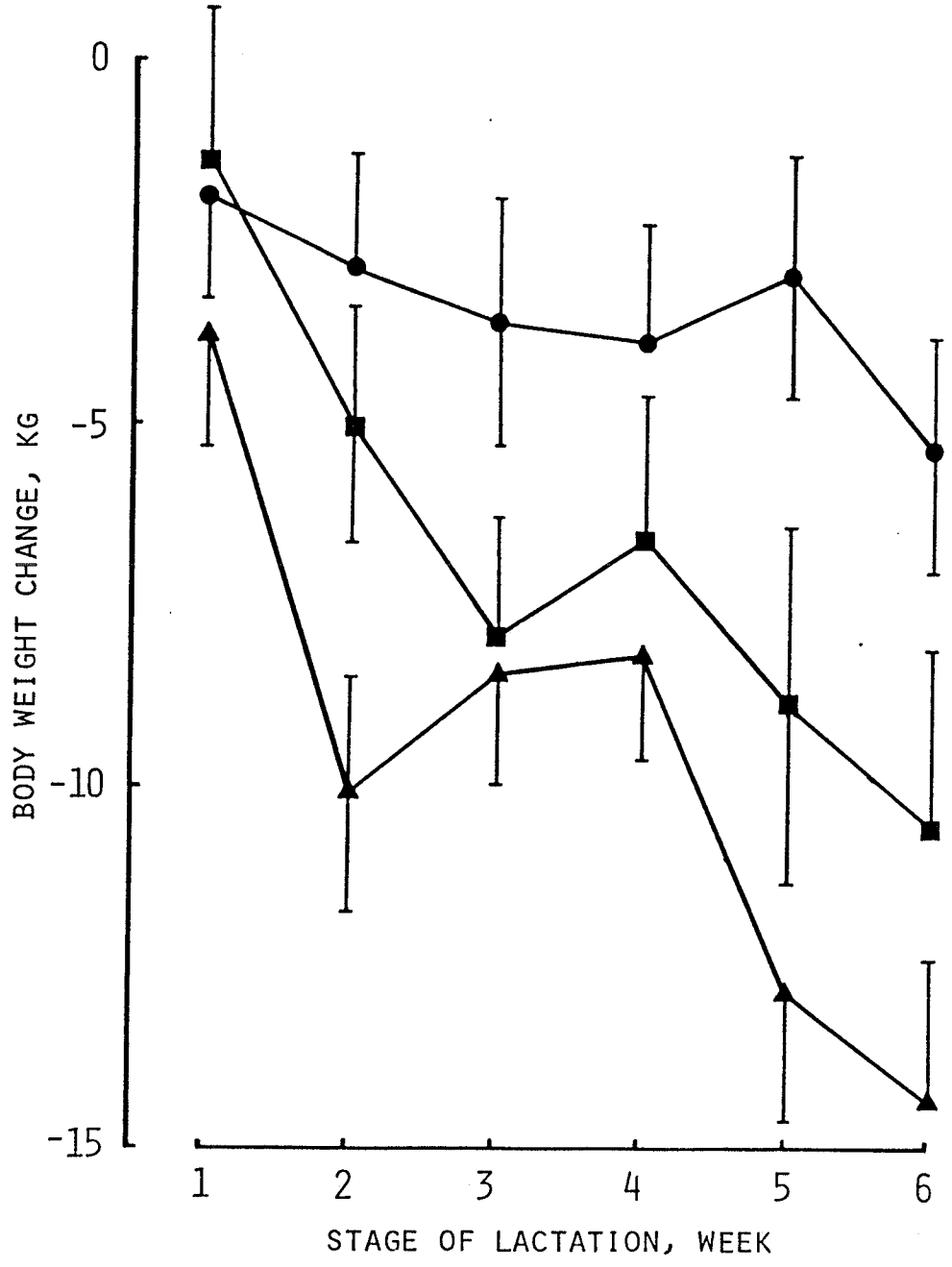

Figure 11: Effect of feeding 0Mo (●), 20Mo (▲) and 40Mo (■) on ewe body weight change relative to initial weight (48 hours postpartum). Least square means ± SE.

Table 14: Influence of dietary molybdenum on dry matter intake and feed efficiency of lactating ewes†.

Items:	DIET		
	0Mo	20Mo	40Mo
D.M. intake, kg d^{-1}	2.1 ± 0.2	2.0 ± 0.2	1.8 ± 0.2
D.M. intake, % body weight	2.9 ± 0.1	2.7 ± 0.1	2.7 ± 0.1
Feed efficiency, kg D.M. kg^{-1} milk	0.7 ± 0.1	0.7 ± 0.1	0.6 ± 0.1

† Least square means ± SE. Based on daily D.M. intake for ewes on test (Appendix Table II-2) which were averaged on a weekly basis.

from the nine ewes that completed the trial. Total ruminal VFA concentrations tended to be lower ($P < 0.10$) for ewes assigned to 40Mo than for the other two diets (Table 15). The molar percentages of acetate, propionate and butyrate were similar among treatment groups.

Dietary Mo concentrations did not have a significant effect on rumen fluid pH ($P > 0.05$). However, ewes fed 40Mo tended to have higher rumen pH values than animals fed the 0Mo and 20Mo. By comparison, Suttle (1983) found no change in rumen pH (5.81) when dietary Mo levels of semipurified diets were increased from 0.5 to 4.5 mg kg^{-1}, however rumen pH increased ($P < 0.01$) to 6.13 when the Mo content of the semipurified ewe diet was increased to 8.5 mg kg^{-1} D.M. A similar response was not observed by Suttle (1983) for hay or pasture forages containing similar levels of Mo. The hay was fed long and pasture forage was grazed. Therefore, differences among animals in selection of plant type and/or plant part may have contributed to the lack of response in rumen pH to dietary Mo. Possible causes for the difference in response between the semipurified diets and forage diets were not suggested by Suttle (1983).

The rumen fluid VFA and pH data of the current study suggest that dietary Mo may affect rumen microbial activity and lend support to the theory proposed previously for the apparent thiamine deficiency. However, the small number of observations and rumen parameters that were evaluated must be considered when making this assessment.

Table 15: Influence of dietary molybdenum on pH, volatile fatty acid and molybdenum concentration in rumen fluid of ewes fed diets containing varying levels of molybdenum†, ‡.

Items:	DIET 0Mo	20Mo	40Mo
No.	4	2	3
pH	6.2 ± 0.2	5.9 ± 0.3	6.7 ± 0.3
Total VFA, mM	115.4 ± 9.0[a]	118.0 ± 12.8[a]	84.9 ± 10.4[b]
VFA, % total VFA:			
acetate	75.3 ± 1.4	76.6 ± 1.9	77.1 ± 1.6
propionate	16.7 ± 2.4	18.1 ± 3.4	13.6 ± 2.8
isobutyrate	0.6 ± 0.4	0.6 ± 0.5	1.1 ± 0.4
n-butyrate	5.8 ± 1.3	3.6 ± 1.8	6.5 ± 1.5
isovalerate	5.5 ± 2.3	5.0 ± 3.3	7.5 ± 2.7
n-valerate	9.2 ± 1.5	6.2 ± 2.2	9.0 ± 1.8
Acetate: propionate ratio	4.78 ± 0.72	4.84 ± 1.02	5.69 ± 0.83
Molybdenum, mg L^{-1}	0.19 ± 0.18 A	0.61 ± 0.25 A B	1.28 ± 0.20 B

† Least square means ± SE.

‡ Means in the same row with different superscripts are significantly different, $^{a} - ^{b}$ $\alpha = 0.10$, $^{A} - ^{B}$ $\alpha = 0.05$.

Rumen fluid Mo concentrations were higher with the higher dietary Mo concentrations ($P < 0.001$). The values found for the 0Mo fed ewes are comparable to those obtained by Suttle and Grace (1978) for animals with similar daily intakes of Mo and S. There was little difference between Mo content in rumen fluid of ewes consuming 36.8 to 73.3 mg Mo d^{-1} (20Mo and 40Mo) in the present study and animals consuming much less Mo (3.5 mg d^{-1}) in the study by Suttle and Grace (1978) at similar daily S intakes. This may reflect differences in rumen fluid sampling or processing. Samples in the current study were strained through three layers of cheesecloth.

Plasma Cu and Mo Parameters in Ewes. Plasma Cu and TCA-soluble Cu concentration were not influenced ($P > 0.05$) by diet; the least square means being 0.97 $\pm$ 0.07, 1.03 $\pm$ 0.08 and 1.10 $\pm$ 0.07 mg L^{-1} and 0.88 $\pm$ 0.06, 0.96 $\pm$ 0.07 and 0.97 $\pm$ 0.06 mg L^{-1}, respectively for 0Mo, 20Mo and 40Mo (Figure 12). The plasma TCA-insoluble fraction was not increased ($P > 0.05$) and plasma Cp oxidase activity was not decreased ($P > 0.05$) by increased Mo intake. Also, there were no diet by week interactions ($P > 0.05$) for plasma Cu parameters. This lack of response to Mo supplementation at low S concentrations is similar to the results of Bremner and Young (1978) but contrasts with that of Ademosun and Munyabuntu (1982).

Total plasma Cu, TCA-soluble Cu and Cp oxidase activity, for ewes in all three treatment groups, increased ($P < 0.001$) during the course of this study (Table 16). Howell et al. (1968) found increased plasma Cu levels in ewes which peaked one week follow-

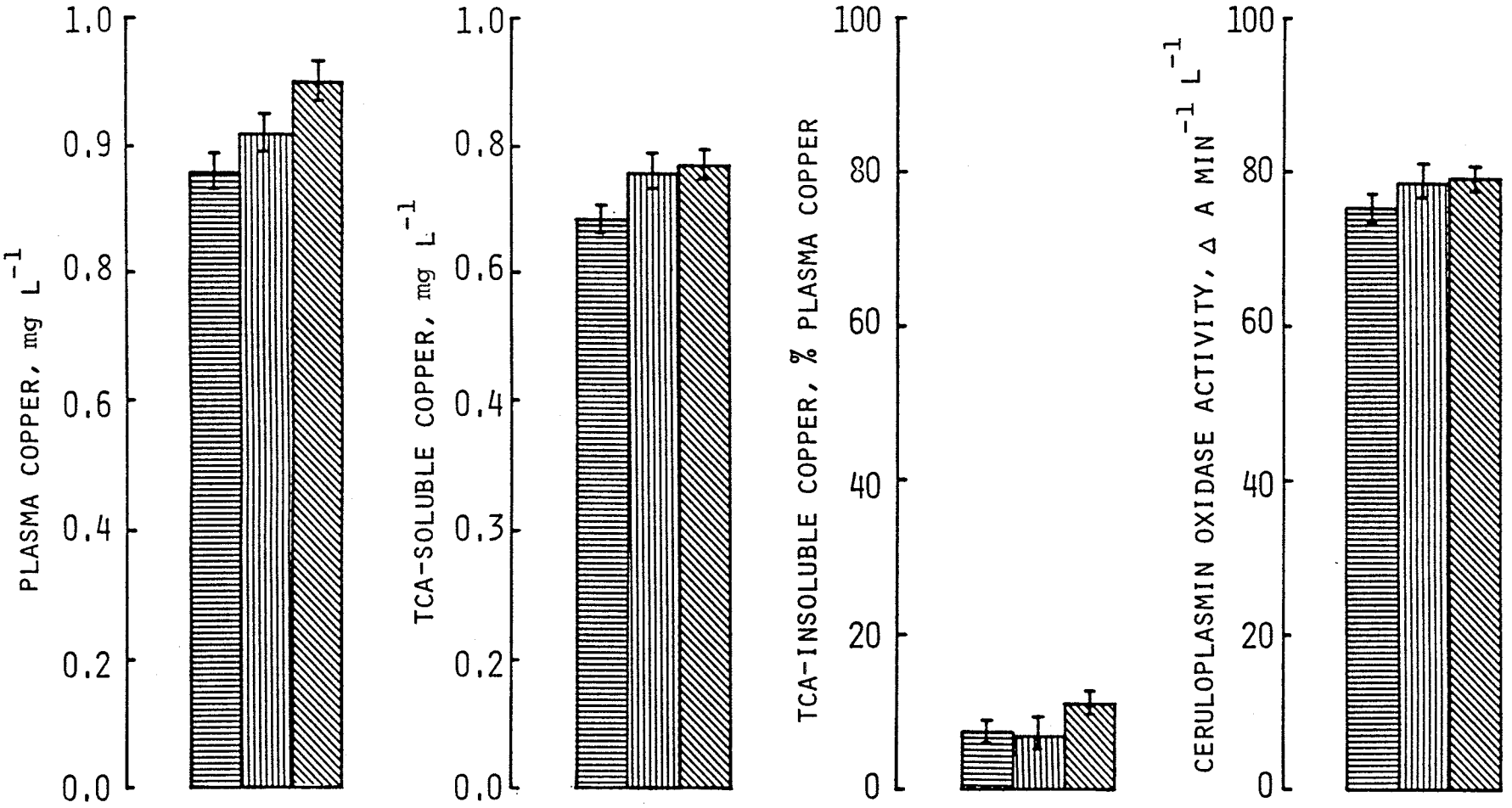

Figure 12: Plasma copper concentration and distribution and ceruloplasmin oxidase activity for ewes fed 0Mo ▤, 20Mo ▥ and 40Mo ▨ diets. Least square means ± SE.

Table 16: Changes in ewe plasma copper concentration and distribution. Least square means ± SE.

Sampling Time†	PARAMETER		
	Plasma Cu (mg L^{-1})	TCA-soluble Cu (mg L^{-1})	Cp oxidase activity (Δ A min^{-1} L^{-1})
Initial	$0.94^{A} \pm 0.04$	$0.86^{A} \pm 0.03$	$71.0^{A} \pm 3.0$
Week 1	$1.01^{A} \pm 0.04$	$0.90^{A} \pm 0.04$	$73.3^{A} \pm 3.2$
Week 2	$1.02^{A} \pm 0.04$	$0.86^{A} \pm 0.04$	$72.2^{A} \pm 2.7$
Week 3	$1.03^{A} \pm 0.04$	$0.92^{A} \pm 0.03$	$71.0^{A} \pm 2.7$
Week 4	$0.98^{A} \pm 0.04$	$0.93^{A} \pm 0.04$	$75.3^{A} \pm 2.9$
Week 5	$1.02^{A} \pm 0.05$	$0.97^{A\ B} \pm 0.04$	$80.4^{A} \pm 3.2$
Week 6	$1.23^{B} \pm 0.09$	$1.13^{B} \pm 0.04$	$97.3^{B} \pm 3.3$

† Initial sample was taken within 48 hours post lambing; weeks 1 to 6 referring to the first to sixth Wednesday thereafter.

A - B Values in the same column with different superscripts are significantly different, $\alpha = 0.05$.

ing lambing. In the present study, a nonsignificant ($P > 0.05$) increase in plasma Cu levels occurred in the first week animals were on test. This level was maintained until week six when a significant ($P < 0.05$) increase was observed. The increased plasma Cu in the present study and that of Howell et al. (1968) appear to be the result of increased Cp levels.

Wiener et al. (1977) found the opposite response; ewe plasma Cu levels declined in the initial weeks of lactation. Animals were fed to appetite on a diet containing 2.0 mg Cu kg^{-1} D.M. during the lactation phase of this study.

The effects of body weight loss on plasma Cu parameters and liver Cu stores have not been considered in past studies. Assuming that 1.15 μg Cu kg^{-1} body weight gain is required (ARC, 1980), a similar amount would be released per kg weight loss. If metabolically available, this would have been an important Cu source for lactating ewes in this study. ARC (1980) values were derived from studies with growing animals and may vary if proportions of fat, muscle and bone deposition vary.

Plasma Mo concentrations were significantly higher ($P < 0.01$) for the Mo supplemented ewes compared with control ewes, the least square means being 0.73 ± 1.26; 8.16 ± 1.42 and 11.54 ± 1.31 mg L^{-1} for 0Mo, 20Mo and 40Mo, respectively. Similar responses in plasma Mo concentration to dietary Mo supplementation were reported by Bremner and Young (1978) and Ademosun and Munyabuntu (1982), the magnitude of response being greater for low S diets than for high S diets. One ewe in the 0Mo group, #11, accidentally received 200 g of the 40Mo concentrate on the first

day she was placed on test. Mean plasma Mo concentration for this ewe during the test period was 1.46 mg L^{-1} compared with a mean value of 0.49 mg L^{-1} for the remaining three ewes in the 0Mo treatment group. Data from ewe #11 were included in all analyses performed.

A diet by week interaction effect ($P < 0.07$) reflected the increased plasma Mo concentrations over time for ewes fed the two Mo supplemented diets in comparison to a relatively constant plasma Mo concentration for ewes receiving the 0Mo diet (Figure 13).

Liver Cu and Mo in Ewes. Liver biopsy samples were taken from all ewes that had completed a minimum of 41 days on test. Copper content of liver from 40Mo fed ewes was higher ($P < 0.07$) than for the 0Mo group (Figure 14). The mean liver Cu concentrations of ewes fed 20Mo were intermediate and did not differ significantly ($P > 0.05$) from the other two diets.

It is well established that dietary Mo in the presence of dietary S reduces Cu concentrations in the livers of sheep (Suttle, 1974b; Bremner and Young, 1978; Weber et al., 1983). Several studies (Dick, 1954; Van Ryssen and Stielau, 1981), however, have reported increased hepatic Cu concentrations in response to increased dietary Mo. Daily Mo and S intakes used in the studies by Dick (1954) and Van Ryssen and Stielau (1981) ranged from 20 to 100 mg and from 0.46 to 3.0 g, respectively, which are similar to intakes of ewes on the high Mo diets in the current study. Van Ryssen and Stielau (1981) fed high Cu diets (84 mg d^{-1}) and observed a high TCA-insoluble fraction in the plasma

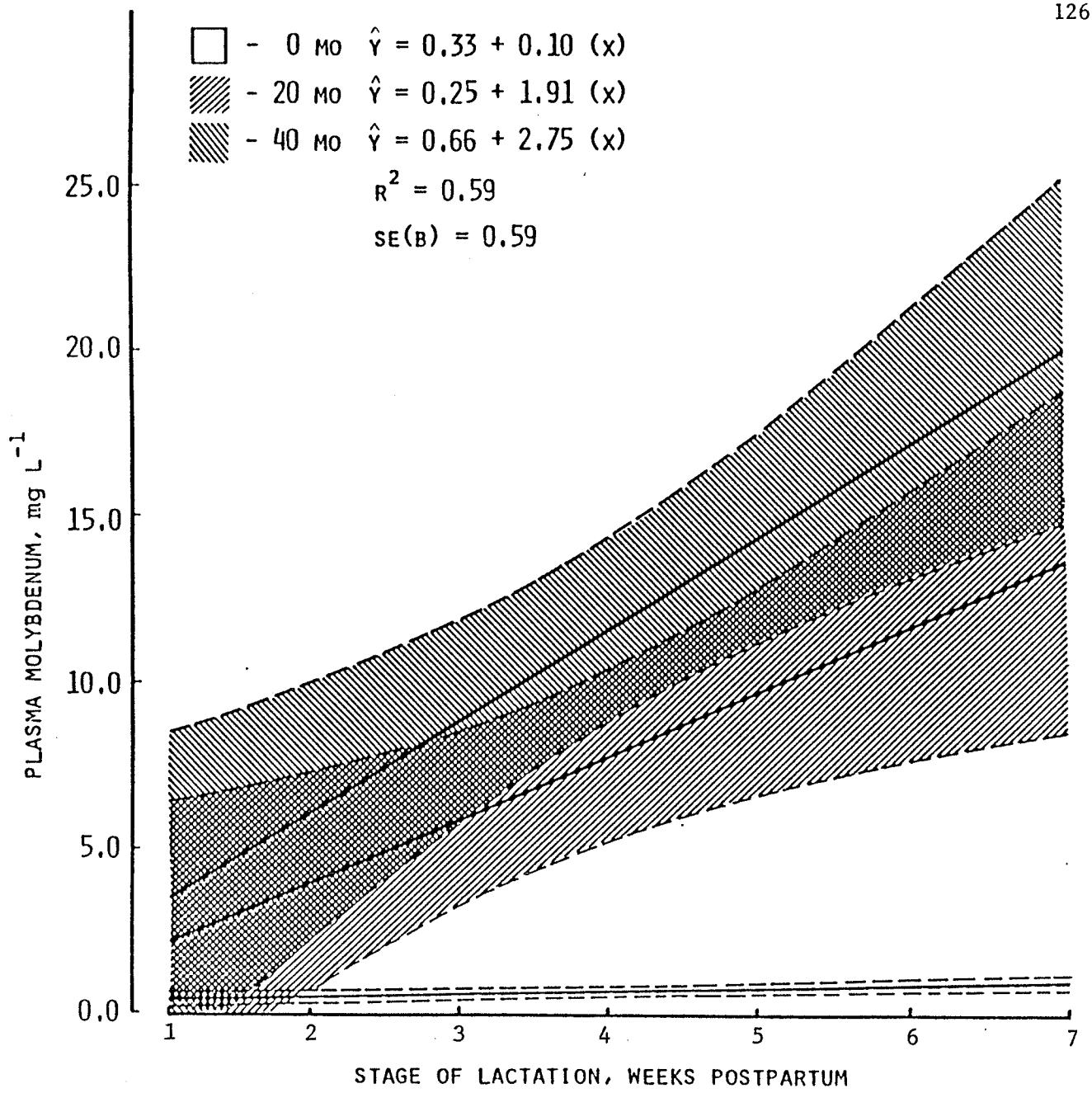

Figure 13: Plasma molybdenum concentrations for ewes fed varying levels of dietary molybdenum. Linear regressions with 95% confidence limits. (For means and mean comparisons see Appendix table II-10.)

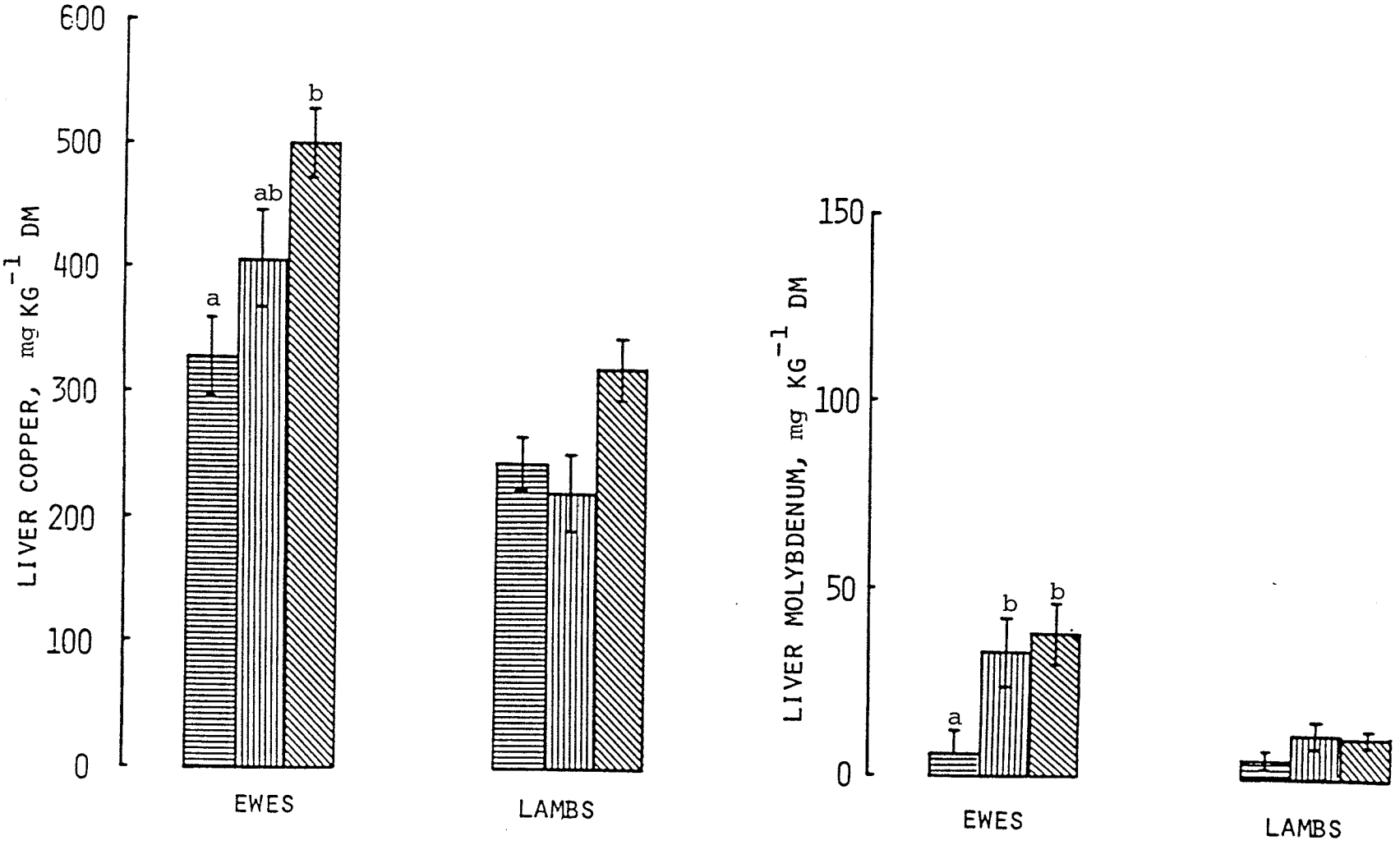

Figure 14: Effect of 0Mo ▤, 20Mo ▥ and 40Mo ▨ diets on copper and molybdenum concentrations in livers of ewes and their suckling lambs. Least square means ± SE. Means with different letters differ; a,b ($\alpha = 0.10$).

of the Mo supplemented sheep, which led to the conclusion that a similar Cu-Mo containing complex was accumulating in the liver of those animals.

For the present study, based on TCA-soluble Cu levels and Cp oxidase activity in plasma of ewes on the 20Mo and 40Mo diets, metabolic availability of absorbed Cu was not affected by levels of dietary Mo. Therefore, the higher liver Cu concentrations for these animals cannot be considered a result of increased dietary Cu absorption or complexing of Cu with Mo-S compounds. Two other factors that may have contributed to the treatment effect observed for Cu concentration in livers at the end of the study are pretest variation of Cu concentration in ewe livers and effect of body weight losses during the trial. Liver biopsy samples were not taken from ewes before they were placed on their respective experimental diets. Therefore, it may be that treatment differences reflect differences in initial liver Cu concentrations rather than differences in rate of liver Cu accumulation or depletion. Body weight losses during the trial averaged 7.1, 12.5 and 19.6% of initial weight for ewes on the 0Mo, 20Mo and 40Mo diets, respectively. As suggested previously (p. 124), the effects of dramatic losses in body weight on liver Cu stores have not been studied and therefore any potential effects on accumulation or mobilization of liver Cu stores are not known.

Average Mo concentrations in liver biopsy samples taken from ewes at the end of the study, were higher for higher levels of Mo intake ($P < 0.07$, Figure 14). This is similar to data reported

by Lesperance and Bohman (1963) and Van Ryssen and Stielau (1981) and reflects the ability of the liver to accumulate Mo when animals are fed excess Mo.

Milk Composition and Yield. Dietary Mo concentrations did not influence ($P > 0.05$) ewe milk yield or fat corrected milk yield during the six week study (Table 12). Milk production for all treatment groups declined ($P < 0.01$) over time (Table 18). Linear regression analysis ($r^2 = 0.16$) showed the rate of decline in milk production to be -0.1 ± 0.1, -0.4 ± 0.2 and -0.3 ± 0.1 kg $week^{-1}$ for ewes fed 0Mo, 20Mo and 40Mo diets, respectively. The rate of decline tended to be greater for ewes fed the two Mo supplemented diets, however this trend was not significant ($P > 0.05$).

The milk yields of the Suffolk ewes in this study were comparable to yields reported for Suffolk-cross ewes, but normal lactation curves for ewes suckling twin lambs show peak production occurring at 3 to 5 weeks following parturition (Doney et al., 1981; Kleemann et al., 1981).

Feed efficiency, calculated as kg D.M. intake ÷ kg milk produced, was not significantly ($P > 0.05$) influenced by level of dietary Mo (Table 14). Ewe feed efficiency declined with time ($P < 0.001$); the rate of decline varying with diet ($P < 0.05$, Table 19). The values for feed efficiencies did not increase as rapidly for ewes fed the 0Mo diet as for ewes fed the two Mo supplemented diets.

The value of a measure such as feed efficiency is reduced when body weight changes of the lactating animals are highly

Table 17: Influence of dietary molybdenum on milk yield and composition for Suffolk ewes. Least square means† ± SE.

Items	DIET					
	0Mo		20Mo		40Mo	
Milk yield, kg d^{-1}	3.4	± 0.5	3.3	± 0.5	3.1	± 0.5
Fat corrected milk, kg d^{-1}	5.8	± 1.2	6.5	± 1.4	5.2	± 1.4
Butterfat, %	8.39	± 0.75	9.51	± 0.84	8.20	± 0.82
Protein, %	4.77	± 0.10	4.88	± 0.11	4.59	± 0.11
Lactose, %	5.83	± 0.85	5.92	± 0.109	5.89	± 0.09
Copper, mg L^{-1}	0.49	± 0.07	0.64	± 0.08	0.61	± 0.08
Copper, mg d^{-1} ‡	1.70	± 0.39	2.17	± 0.43	1.93	± 0.43
Molybdenum, mg L^{-1}	0.14	^{A} ± 0.24	1.53	^{B} ± 0.27	2.69	^{C} ± 0.27
Molybdenum, mg d^{-1} ‡	0.46^{a}	± 0.89	4.30^{b}	± 1.00	7.68^{c}	± 0.97

† Means in the same row with different superscripts are significantly different: $^{a} - ^{c}\alpha = 0.01$, $^{A} - ^{C}\alpha = 0.001$.

‡ Calculated from values for daily milk production (mg d^{-1}) and milk mineral concentrations (mg L^{-1}). A conversion factor (0.97) was used to convert milk from liters to kilograms.

Table 18: Estimated milk yield (kg d^{-1}) for ewes fed varying levels of molybdenum for six weeks. Least square means.

Sampling Time	DIET			Mean ± SE
	0Mo	20Mo	40Mo	
Week 1	3.4	4.0	4.2	3.9 ± 0.2 A
Week 2	3.5	3.4	3.3	3.4 ± 0.2 A B
Week 3	3.5	3.4	3.2	3.4 ± 0.2 A B
Week 4	3.3	3.2	3.1	3.2 ± 0.2 A B
Week 5	3.7	2.7	2.3	2.9 ± 0.3 B
Week 6	2.7	2.7	2.2	2.5 ± 0.3 B
Mean ± SE	3.4 ± 0.5	3.3 ± 0.5	3.1 ± 0.5	

A - B Values in the same column with different superscripts are significantly different, $\alpha = 0.05$.

Table 19: Feed efficiency (kg D.M. intake ÷ kg milk yield) for ewes fed varying levels of molybdenum for six weeks. Least square means.

Sampling Time	DIET 0Mo	20Mo	40Mo	Mean ± SE
Week 1	0.57 A	0.46 A	0.44 A	0.49 ± 0.03 A
Week 2	0.58 A	0.61 A B	0.62 B	0.60 ± 0.03 B
Week 3	0.66 A	0.68 B	0.61 B	0.65 ± 0.03 B
Week 4	0.72 A B	0.78 B	0.75 B	0.75 ± 0.03 C
Week 5	0.63^{a}, A	1.00^{b}, C	0.75^{a}, B	0.80 ± 0.04 C
Week 6	0.85^{b}, B	0.92^{b}, B C	0.65^{a}, B	0.81 ± 0.04 C
Mean ± SE	0.67 ± 0.06	0.74 ± 0.06	0.63 ± 0.06	

A - C Values in the same column with different superscripts are significantly different, $\alpha = 0.05$.

variable, as they were in this trial.

Milk fat, protein and lactose contents were not affected ($P > 0.05$) by level of dietary Mo (Table 17).

Copper concentration and, therefore, daily Cu excretions in the milk of ewes fed Mo supplemented diets tended to be higher than for control animals, however the differences were not statistically significant ($P > 0.05$). Concentrations of Cu in milk were slightly higher than the normal levels (0.2-0.4 mg L^{-1}) as defined by Lonnerdal et al. (1981) but fall within the normal range (0.2-0.6 mg L^{-1}) suggested by Underwood (1977).

Mean Mo concentrations in milk increased ($P < 0.001$) with each increment in dietary Mo (Table 17). The concentration of Mo in milk increased over the six weeks of lactation for ewes fed the 20Mo and 40Mo diets but remained at consistently low levels for the 0Mo fed ewes (Figure 19).

Daily milk Mo excretion was significantly ($P < 0.01$) affected by diet. The mean values were approximately 9 and 17-fold higher for ewes fed 20 and 40Mo, respectively, than for ewes fed 0Mo (Table 17). These excretion levels represent 24.3, 11.7 and 10.5% of total daily Mo intake for ewes fed 0Mo, 20Mo and 40Mo, respectively.

Despite reduced milk production over time (Table 18), daily milk Mo excretions were maintained or increased for ewes fed the Mo supplemented diets (Table 20). This differs from data obtained from the beef cow-calf trial which had a decline in daily milk Mo excretions (Table 11) with declining milk production.

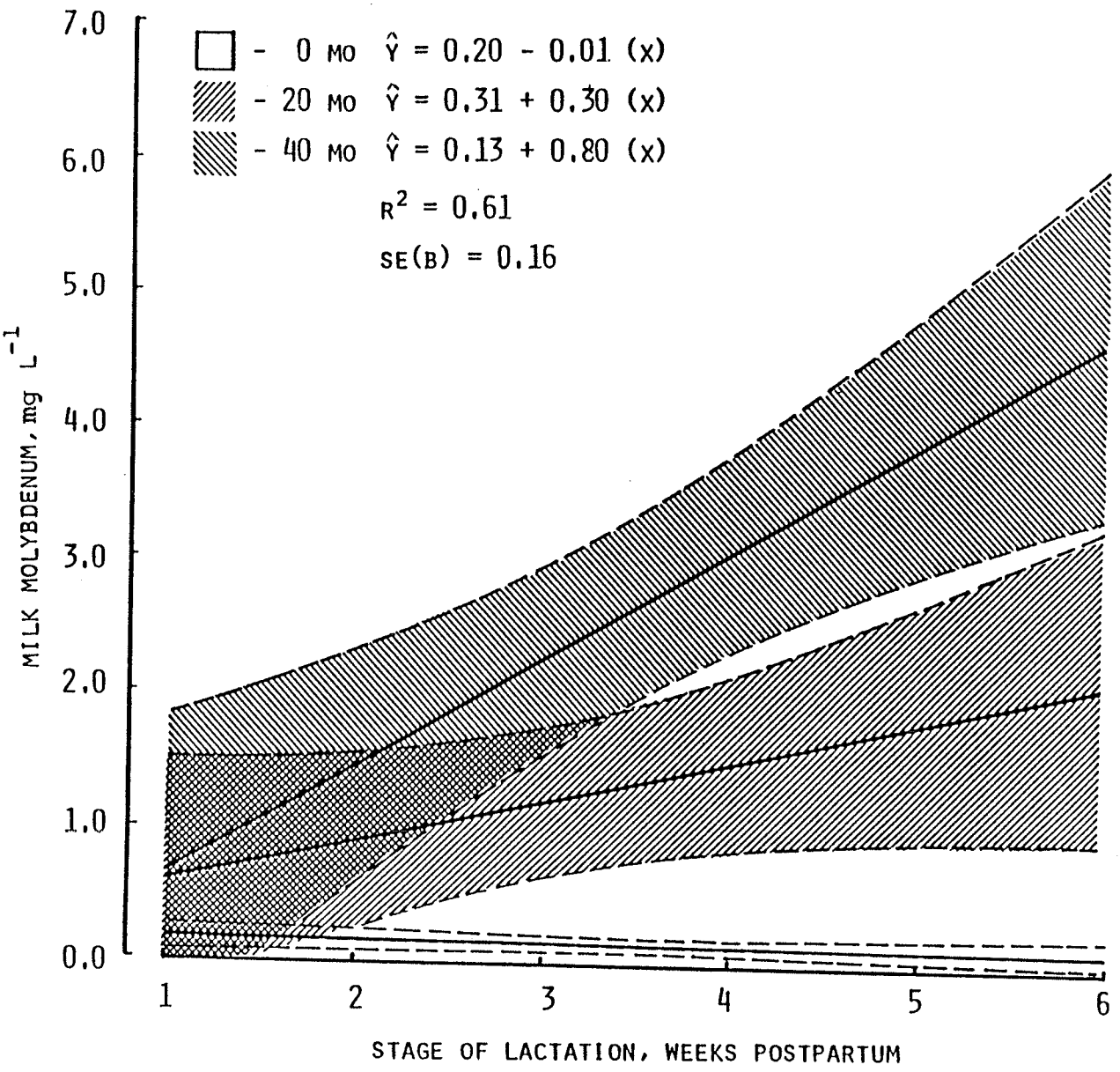

Figure 15: Milk molybdenum concentrations for ewes fed varying levels of dietary molybdenum. Linear regressions with 95% confidence limits. (For means and mean comparisons see Appendix table II-11.)

Table 20: Calculated daily milk molybdenum excretions (mg d^{-1}) by ewes fed varying levels of dietary molybdenum. Least square means.

Sampling Time	DIET			Mean ± SE
	0Mo	20Mo	40Mo	
Week 1	0.54	1.56	2.84	1.65 ± 0.99
Week 2	0.58	3.55	6.78	3.64 ± 0.99
Week 3	0.51	3.62	5.53	3.22 ± 0.99
Week 4	0.36	6.22	7.73	4.77 ± 1.06
Week 5	0.42	5.46	12.01	5.97 ± 1.24
Week 6	0.35	5.41	11.20	5.65 ± 1.24
Mean ± SE	0.46 ± 0.89[a]	4.30 ± 1.00[b]	7.68 ± 0.97[c]	

a - c Values in the same row with different superscripts are significantly different, $\alpha = 0.05$.

Plasma Cu and Mo Parameters in Lambs. Ewes significantly ($P < 0.05$) influenced plasma Cu and Mo parameters of their lambs (Appendix Table II-7).

Molybdenum concentrations in the ewe diets did not influence ($P > 0.05$) plasma Cu, TCA-soluble Cu or Cp oxidase activity of the lambs (Table 21). Plasma TCA-insoluble Cu (% of total plasma Cu) was not different among treatment groups, the least square means were 7.5 $\pm$ 1.5, 11.0 $\pm$ 1.7 and 9.2 $\pm$ 1.5% for lambs sucking ewes assigned to 0Mo, 20Mo and 40Mo diets, respectively.

Blood samples taken from lambs 24 to 48 hours after birth had low plasma Cu levels (0.39 $\pm$ 0.05 mg L^{-1}, Figure 16). Plasma Cu concentrations rose in the initial weeks ($P < 0.05$) and peaked when lambs were four to five weeks of age. Similar observations were made by McC. Howell and Edington (1968) and Weiner et al. (1977). In all three studies the increased plasma Cu levels were related to increased Cp oxidase activity. Means and mean comparisons describing the changes of plasma Cu, TCA-soluble Cu and Cp oxidase activity with lamb age are found in Appendix table II-12.

The variation in Mo concentration in diets fed to ewes influenced lamb plasma Mo levels ($P < 0.05$). The mean plasma Mo level for control lambs was 0.14 $\pm$ 0.21 mg L^{-1} which was significantly lower than for lambs sucking ewes fed the 20Mo (0.88 $\pm$ 0.23 mg L^{-1}) and 40Mo (1.20 $\pm$ 0.21 mg L^{-1}) diets.

Age of lamb also affected ($P < 0.001$) lamb plasma Mo concentrations, levels for lambs aged two to three weeks of age being significantly higher ($P < 0.05$) than for the same lambs at a

Table 21: Concentration and distribution of plasma copper and ceruloplasmin oxidase activity for lambs sucking ewes fed different levels of molybdenum. Least square means ± SE.

Parameter	DIET		
	0Mo	20Mo	40Mo
Plasma Cu, mg L^{-1}	0.89 + 0.09 ($\bar{55}$)†	1.11 + 0.10 ($\bar{44}$)	0.92 + 0.09 ($\bar{54}$)
TCA soluble Cu, mg L^{-1}	0.82 + 0.08 ($\bar{56}$)	1.04 + 0.09 ($\bar{42}$)	0.85 + 0.08 ($\bar{53}$)
Cp oxidase activity, Δ A min^{-1} L^{-1}	80.8 + 8.8 ($\bar{54}$)	103.0 + 9.8 ($\bar{44}$)	77.9 + 8.8 ($\bar{54}$)

† Values in parentheses are the number of observations for each treatment mean.

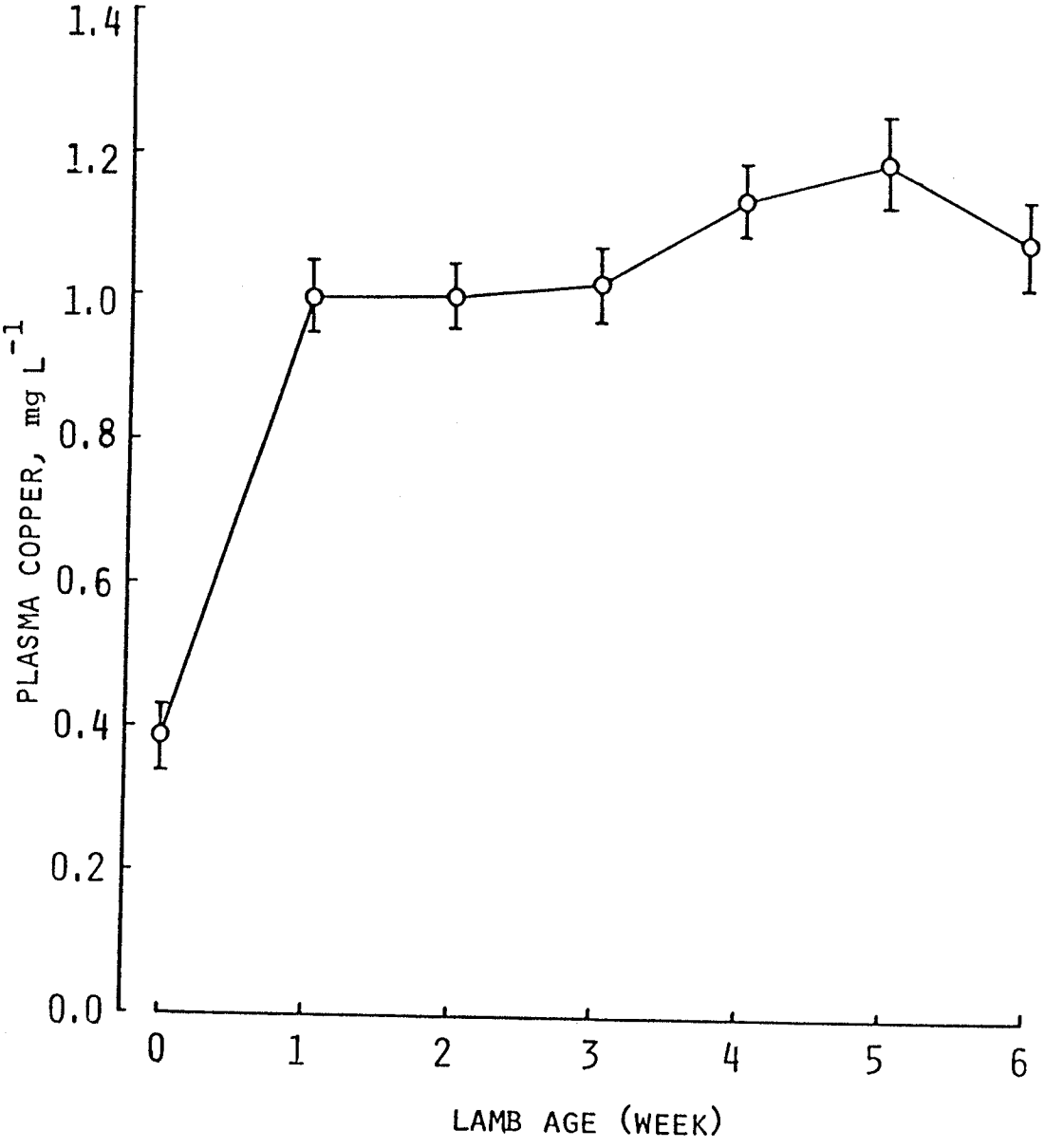

Figure 16: Changes with age for plasma copper concentrations of suckling lambs. Least square means (O) for all lambs on test ± SE.

younger or older age (Figure 17). A diet by week interaction ($P < 0.001$) reflected greater increases in plasma Mo of lambs sucking ewes fed Mo supplemented diets relative to those sucking ewes fed the control diet.

Lambs assigned to the 20Mo and 40Mo groups had plasma Mo concentrations that increased until lambs were 3 weeks of age, following which there was a decrease to concentrations of approximately 32 and 21% of the peak levels, respectively, by the time lambs were six weeks of age. This sudden decrease was not related to changes in daily Mo intake, which was assumed to be the equivalent of daily milk Mo excretion of ewes (Table 20).

It is probable that the sudden drop was because of the development of a functional rumen. Miller et al. (1972) reported that absorption of dietary Mo entering a functional rumen was much lower than Mo which was passed directly to the abomasum. Although it would be expected that Mo in suckled milk passed to the abomasum via the esophageal groove closure, endogenous or recycled Mo may be entering the rumen of lambs via salivary secretions (Suttle and Grace, 1978).

Suttle and Grace (1978) reported that 89% of absorbed dietary Mo may be recycled or secreted into the rumen when S intake is low. Based on a S to nitrogen ratio of 0.06 for ewe's milk (Langlands and Sutherland, 1973), the estimated S intake was 5 to 8 g d^{-1} milk D.M. for lambs in the present experiment which is high compared to the 'low' levels used by Suttle and Grace (1978). However, as with the milk Mo, milk S by-passes the rumen and therefore would have had limited interference with Mo absorp-

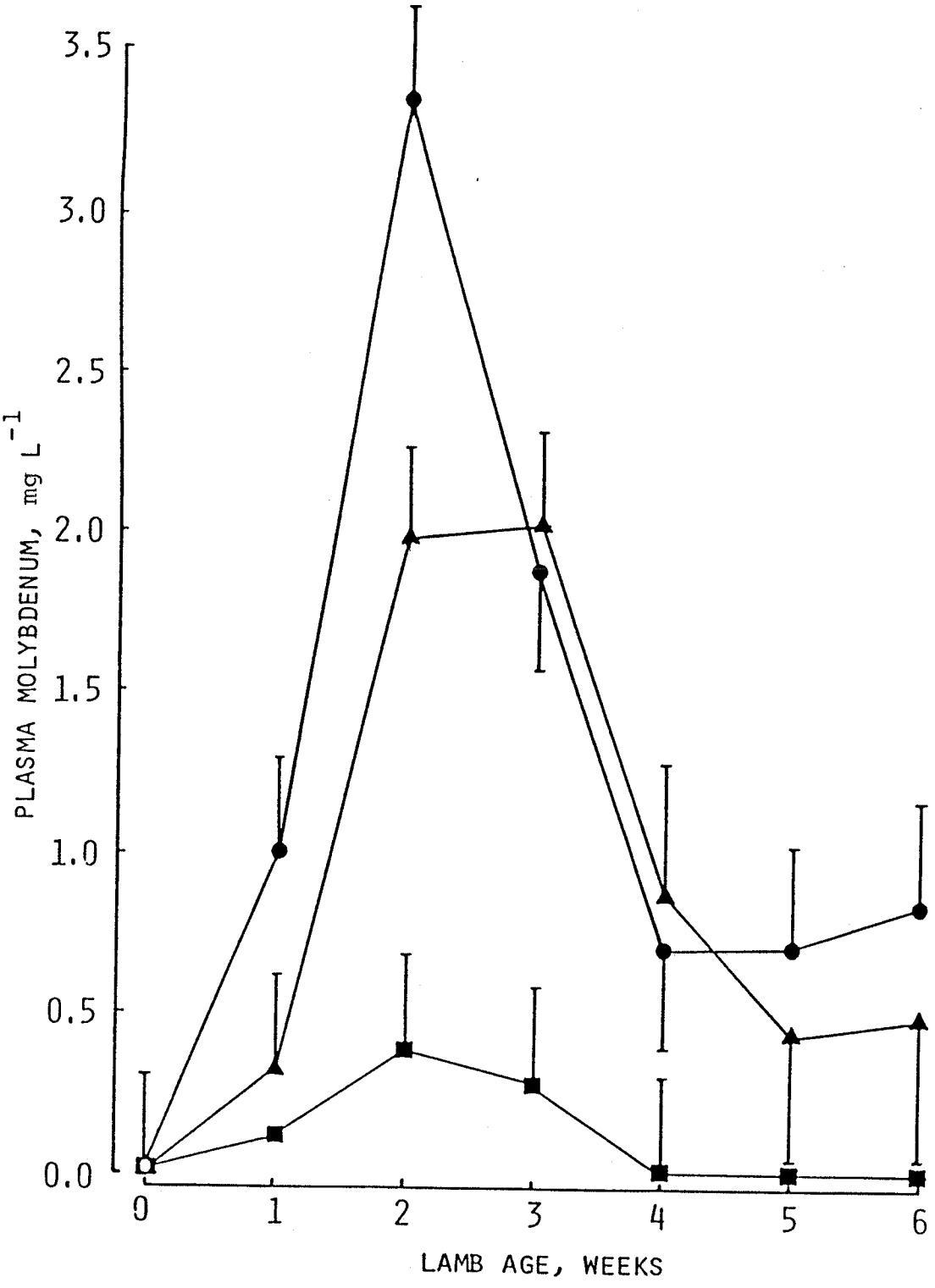

Figure 17: Molybdenum concentrations in plasma of lambs sucking ewes fed 0Mo (■), 20Mo (▲) and 40Mo (●) diets. Least square means ± SE.

tion and recycling. The absorption of dietary Mo and S may not differ too greatly between preruminant lambs and lambs with a developing or developed rumen as long as the feedstuff bypasses the rumen. Therefore, the marked drop in plasma Mo concentration observed (Figure 17) in lambs at approximately one month of age in the present study was probably associated with the formation of thiomolybdates, from recycled Mo and S, in the rumen.

Liver Cu and Mo in Lambs. Mo concentration in the diet of ewes influenced ($P < 0.07$) the Cu concentrations in the livers of their lambs at the end of the study (Figure 14). Lambs in the 40Mo group tended to have higher liver Cu concentrations than lambs in the other two groups. The least square means were 243.5 $\pm$ 22.7, 219.1 $\pm$ 32.1 and 320.4 $\pm$ 26.2 mg Mo kg^{-1} liver D.M. for 0Mo, 20Mo and 40Mo, respectively.

Liver Mo levels in lambs sucking 20Mo and 40Mo ewes were approximately double the levels found in livers of control lambs. Least square means were 4.9 $\pm$ 2.4, 11.2 $\pm$ 3.4 and 10.9 $\pm$ 2.8 mg Mo kg^{-1} liver D.M. for 0Mo, 20Mo and 40Mo, respectively. These differences were not significant ($P > 0.05$).

Ewes did not significantly ($P > 0.05$) influence liver Cu or liver Mo concentrations of their lambs (Appendix table II-7), which contrasts a signficant ewe effect observed for lamb plasma Cu and Mo parameters. This suggests that individual variation between lambs in a twin set is greater for liver Cu stores than is the effect of dam. Whether the individual variation was apparent at birth or developed after birth is not known. However, the significant ewe effect on plasma Cu and Mo parameters

suggests that dietary Cu and Mo uptake from the gut is related to milk Cu and Mo content.

Gross Effects in Lambs. Average daily gain of lambs was not shown to be affected by differences in ewe milk yield ($P > 0.05$) or milk composition ($P > 0.05$). Least square means were 0.24 $\pm$ 0.01, 0.24 $\pm$ 0.02 and 0.22 $\pm$ 0.02 kg d^{-1} for 0Mo, 20Mo and 40Mo, respectively. Lambs gained less weight during the first week on test compared to the remainder of the trial ($P < 0.001$). A diet by week interaction was not observed ($P > 0.05$) for lamb ADG.

Food conversion ratios for lambs, expressed as one-half of daily ewe milk yield $\div$ kg lamb body weight gain, averaged 5.1 $\pm$ 0.7, 4.8 $\pm$ 1.1 and 3.9 $\pm$ 0.7 for 0Mo, 20Mo and 40Mo, respectively. These values suggest that high milk Mo levels did not affect ($P > 0.05$) the efficiency of gain in sucking lambs.

Clinical symptoms indicative of Cu deficiency or molybdenosis were not observed in lambs.

Discussion. Using the mean value for ewe weight, 79.4 $\pm$ 3.0 kg, and for milk production, 3.9 $\pm$ 1.2 kg d^{-1}, in the first week of the trial, the estimated (ARC, 1980) Cu requirement for ewes in this study was 1.4 mg d^{-1}. When the mean Cu concentration of milk (0.59 mg L^{-1}) was incorporated into the calculations, the daily Cu requirement for ewes in the present study was increased to 3.3 mg d^{-1}.

The predicted coefficient of absorption of Cu (equation 1, Suttle and McLauchlan, 1976) for the 0Mo, 20Mo and 40Mo diets were 0.05, 0.02 and 0.01, respectively. Taking into account the

daily D.M. intake for the ewes (Table 14), the available Cu intake was 0.5, 0.2 and < 0.1 mg d^{-1} for animals allotted to 0Mo, 20Mo and 40Mo, respectively. These calculations suggest that all three groups of ewes would have been in a potentially negative Cu balance.

A similar conclusion can be drawn from the application of the equation (equation 2) derived by Langlands et al. (1981). Accordingly, Cu concentrations in the 0Mo, 20Mo and 40Mo diets would have had to be 5.8, 7.3 and 10.6 mg kg^{-1} D.M. to maintain liver Cu concentrations.

The equations derived by Suttle and McLauchlan (1976) and Langlands et al. (1981) were based on studies with growing sheep and may not be directly applicable to lactating ewes. For example, the high dry matter intake associated with lactating as compared with nonlactating sheep (Peart et al., 1972; Arnold, 1975) may affect rate of disappearance of both liquid and particle digesta components from the rumen (Mudgal et al., 1982; McAllan and Smith, 1983). Changes in turnover rate of dietary and microbial components associated with Mo and S may influence both the rate and type of thiomolybdates formed in the rumen (Clarke and Laurie, 1980). Copper absorption from the gastrointestinal tract as well as systemic Cu metabolism could be influenced by these changes.

Several studies have demonstrated a positive correlation between turnover rate and increased microbial growth (Harrison et al., 1976; Kennedy et al., 1976). Sulfur is required by rumen microorganisms for protein synthesis and this may affect S utili-

zation from the rumen H_2S and organic S pools. Increased dilution rates, particularly if associated with high starch intake (Bray and Till, 1975) may increase the population of rumen bacteria capable of reducing SO_4^{2-} to H_2S, thereby reducing the inhibitory effects of MoO_4 on rumen sulfide production from sulfates (Huisingh and Matrone, 1972).

The amounts of dietary S, Mo and Cu that by-pass or are not subjected to rumen microbial actions may also increase as a result of increased turnover rates associated with high feed intakes. These factors should also be taken into consideration when applying the equations to diets for lactating cows. The importance of the above factors relative to parameters known to influence dietary Cu utilization, such as Cu source (Lamand, 1977; Suttle, 1983) and dietary Mo and S concentrations (Dick et al., 1975; Suttle, 1975) have not been evaluated.

Cow-Calf and Ewe-Lamb Trial Comparison

Plasma and Liver Cu Parameters. Cows fed 34.8 mg Mo kg^{-1} D.M. had become Cu deficient by the end of the nine week study (Figure 2). The presence of a TCA-insoluble Cu fraction in the plasma of these animals suggests that thiomolybdates had been formed in the gastrointestinal tract. Formation of thiomolybdates may account for reduced Cu absorption from the gut and altered metabolism of absorbed Cu.

A similar response was not observed for the ewes. Although ewes fed the Mo supplemented diets tended to have higher plasma Cu concentrations, the difference could not be attributed to diet as pretrial plasma Cu concentrations (24 to 48 hours postlambing) for this group of ewes also tended to be higher. Furthermore, liver Cu concentrations at the end of the trial were greater for the Mo supplemented ewes relative to control ewes. As pretrial liver biopsy samples were not taken, it could not be determined whether differences in Cu concentrations of ewe livers reflected pretrial levels or were a response to treatment. The data indicate that liver Cu stores were not being depleted by high Mo intakes in the dietary regime of the sheep.

Differences in response to high dietary Mo intake between cows and ewes may be due to a number of factors. Kennedy et al. (1975) stated that SO_4^{2-} recycling was considerably less for sheep than for cattle. This may have resulted in relatively lower ruminal H_2S concentrations in the ewes. Various studies have demonstrated that dietary S has a predominant effect on the availability of dietary Cu in the presence of dietary Mo (Suttle

and McLauchlan, 1976; Bremner and Young, 1978). Therefore, although experimental diets in the two trials had similar S concentrations higher rumen thiomolybdate production related to higher rumen H_2S levels may have adversely affected availability of dietary and systemic Cu in the cows. Other factors that may have contributed to differences observed include differences between cattle and sheep in rumen metabolism and/or differences in the absorption, metabolism or excretion of Mo.

Cu concentrations in the plasma of sucking calves and lambs were not influenced ($P > 0.05$) by Mo intake of the dams. Depending on the criterion used, calf liver Cu levels may have been affected by milk Mo concentrations. Although actual rate of decline for liver Cu levels in calves was similar across treatment groups (Figure 9), decline relative to initial concentrations tended to be greater for the 20Mo and 40Mo groups.

Change in liver Cu concentration of lambs was not monitored; however, liver Cu concentrations of lambs sucking ewes fed Mo supplemented diets were equal to or greater than those of lambs sucking ewes assigned to control diets.

Production Parameters. Lactating animals may rely heavily on body fat reserves as an energy source for milk production (Moe et al., 1971). Therefore, consideration of milk energy production, alone, may not be a good estimate of the efficiency of feed energy utilization when comparing diets fed to lactating animals. A better assessment would involve some measure of tissue energy changes as well as milk energy production. Unfortunately, an accurate measure of the energy value of body tissue gain or loss

by lactating ruminants is difficult to obtain because the composition or the relative proportions of water, protein and fat for the weight change is difficult to determine (Reid and Robb, 1971). ARC (1980) has suggested that the energy value of 26 MJ kg^{-1} body weight gain or loss be adopted for adult sheep and cattle. Use of a constant value, 26.0 MJ kg^{-1} change in body weight, does not provide accurate quantitative figures but may provide comparative data for animals assigned to the three treatment groups in each of the trials being discussed. The effects of excess dietary Mo on feed energy utilization by lactating animals in the beef cow-calf and ewe-lamb trials were determined by comparing estimates of net energy retention (MJ d^{-1}) for cows and ewes fed 0Mo, 20Mo and 40Mo diets (Table 22). Net energy retention, in this context, refers to the energy provided by feed for milk production and weight gain.

Daily milk energy yields were calculated from the mean daily milk fat, protein and lactose yields of animals during their respective trials, using energy values for each component of 38.5, 23.7 and 16.5 J g^{-1}, respectively (Van Soest, 1982).

Increased Mo concentrations in a concentrate-corn silage diet affected productivity in both cattle and sheep; however, the parameters affected differed between the two species. Cow weight, milk yield and milk composition, with the exception of Mo concentrations, were not affected by increasing dietary Mo concentrations from 0.6 to 19.3 mg kg^{-1} D.M. for nine weeks. Diet Mo concentration, when increased to 34.8 mg kg^{-1} D.M., was associated with a more rapid rate of decline in daily milk yield but

Table 22: Estimated energy retention of cows and ewes fed diets containing varying levels of molybdenum.

Species	Diet	Milk Energy MJ d^{-1} †	Body tissue gain or loss MJ d^{-1} ‡	Net Energy retention MJ d^{-1} §
Bovine				
	0Mo	44.6	11.4	56.1
	20Mo	47.7	10.4	58.1
	40Mo	30.8	11.2	42.0
Ovine				
	0Mo	20.8	-2.6	18.2
	20Mo	20.0	-9.2	10.8
	40Mo	15.9	-9.4	6.5

† Calculated from means (bovine) or least square means (ovine) for daily milk fat, protein and lactose production using energy values for each component of 38.5, 23.7 and 16.5 J g^{-1}, respectively (Van Soest, 1982).

‡ Based on the assumption that the energy value of body weight gain or loss is 26 MJ kg^{-1} (ARC, 1980).

§ Sum of milk energy production and body tissue energy change.

did not affect other production parameters. There was an apparent reduction in energy retention for cows assigned to 40Mo (Table 22).

Diets containing 18.4 and 40.7 mg Mo kg^{-1} D.M. fed to early lactation ewes resulted in a three-fold greater body weight losses and decline in milk production relative to animals fed a low Mo diet. These differences were not statistically significant, which may be partially due to missing observations. Dietary Mo also appeared to affect ewe health; three of the animals assigned to the Mo supplemented diets apparently were thiamine deficient. Removal of two of these animals from the trial accounted for 2 missing observations each of weeks 5 and 6 of this trial.

Although the 20Mo diet did not appear to influence feed energy utilization of beef cows, there was an apparent effect for ewes which was reflected in a tendency toward greater body weight losses (Table 22). High levels of Mo intake tended to reduce milk yields for both cows and ewes; however only ewes responded with increased body tissue losses. Estimated differences in energy retention cannot be accounted for by differences in metabolizable energy intake, which were the same across treatment groups in the cow-calf study and differed by an estimated -1.1 and -3.2 MJ d^{-1} from control ewes for 20Mo and 40Mo, respectively in the ewe-lamb study.

Reduced performance of cows fed the 40Mo diet appeared to be related to an induced Cu deficiency. However, it should be noted that the decline in milk yield was apparent before cows assigned

to 40Mo were actually Cu deficient, based on Cu parameters used in this study. Toxic levels of Mo in the gastrointestinal tract or systemically may have contributed to this adverse response. The ewe data, on the other hand, suggested that reduced animal performance was related to an effect of dietary Mo other than a reduced Cu availability or altered Cu metabolism in the body.

Excessive Mo intake is usually associated with depletion of Cu reserves. However, several studies, Dick et al., 1975; Suttle and McLauchlan, 1976 and Langlands et al., 1981, evaluating this Cu-Mo interaction did not take into account animal productivity. The effect of an increased blood and tissue Mo concentration in association with either an accumulation of Mo compounds over an extended period of time for animals fed high Mo, high S diets (Grace and Suttle, 1977) or rapid increases associated with animals exposed to a high Mo-low S diet may explain the reduced productivity with no apparent changes in Cu metabolism. These increases in tissue Mo concentrations also occur in animals that have a Mo induced Cu deficiency and may contribute, to some extent, to the reduced performance of such animals. If so, this may be the reason that previous efforts (Smith and Coup, 1973; Mills et al., 1976; Puls, 1981) to define critical lower values, for plasma or liver Cu and/or Cu-containing enzyme concentrations, predicting reduced animal performance have not been satisfactory.

Observed Cu concentrations in ewe and cow milk were in agreement with the literature (Underwood, 1977; Hidiroglou and Knipfel, 1981; Lőnnerdal et al., 1981). Copper concentrations in

milk were three to six-fold higher for ewes than for cows. Copper concentrations in milk were much more variable among individual ewes than among individual cows.

Variations in Mo concentrations in milk were greater for ewes than for cows fed similar dietary Mo levels. Expressed as a percent of total Mo intake, ewes excreted a higher percentage of Mo for all three experimental diets. Whether these increased milk Mo excretions by ewes reflected more efficient Mo absorption, less efficient Mo excretion via other routes or were related to reduced S recycling, is not known.

Comparisons of Cu and Mo parameters within twin sets resulted in some confusion regarding the significance of maternal effects. Ewes had a significant effect on lamb plasma Cu, TCA-soluble Cu, Cp oxidase activity and plasma Mo concentrations. However there was not a significant ewe effect on lamb liver Cu and Mo concentrations. It may be that diet influenced the circulating levels of plasma Cu and Mo of the suckled lambs, however it appears that factors such as differences in liver Cu stores at birth (values were not determined) or differences in deposition and/or mobilization of liver Cu reserves between lambs within twin sets had a greater effect on lamb liver Cu content at the end of the trial than did the milk supply of their dams.

Dairy Cow Trial

Gross Effects. Cows assigned to Trt. III and Trt. IV (Mo supplemented diets) tended to have greater weight losses during the eight week study than did cows assigned to non-Mo supplemented diets (Table 23). This trend was not significant ($P > 0.05$). A week effect ($P < 0.01$) reflected greater weight losses for all cows in weeks 5 to 8 (-0.6 ± 0.1 kg d^{-1}) than during the first four weeks (0.3 ± 0.1 kg d^{-1}) animals were on test.

The trend towards greater body weight losses by animals fed Mo supplemented diets was similar to that for the ewes in the ewe-lamb trial. However, in both trials the large individual animal variability precluded significant differences ($P > 0.05$) due to treatment.

No clinical symptoms ascribable to hypocuprosis or molybdenosis were observed in the cows.

Feed Intake. Molybdenum supplementation did not affect ($P > 0.05$) D.M. intake of the cows (Figure 18). Daily D.M. intakes averaged 18.4 and 19.2 ± 0.6 kg d^{-1} for cows fed the basal and +Mo diets, respectively, during the first four weeks of the study. Intake dropped ($P < 0.01$) in the last four weeks for cows in all treatment groups; the average intakes were 17.4, 16.5, 17.8 and 16.8 kg D.M. d^{-1} for the basal, +Cu, +Mo and +Mo+Cu diets, respectively. Higher average intakes by all treatment groups during the initial weeks, followed by lower levels in the last weeks of the study cannot be explained. However, it should be noted that for cows housed in this barn, a reduction in milk production is generally observed in hot weather. Similar envi-

Table 23: Effect of molybdenum and copper supplementation on cow body weight change (kg d^{-1})†,‡.

	TREATMENT				
	I	II	III	IV	
Period 1 diet:	Basal	Basal	+Mo	+Mo	
Period 2 diet:	Basal	+Cu	+Mo	+Mo+Cu	SE
Time					
Period 1	0.6 (4)	0.3 (4)	0.2 (4)	0.0 (4)	± 0.3
Period 2	-0.6 (4)	-0.5 (4)	-0.9 (4)	-0.7 (3)	± 0.3
Overall	0.0 (8)	-0.1 (8)	-0.3 (8)	-0.4 (7)	± 0.2

† Least square means.

‡ Values in parentheses represent the number of observations for the corresponding least square mean.

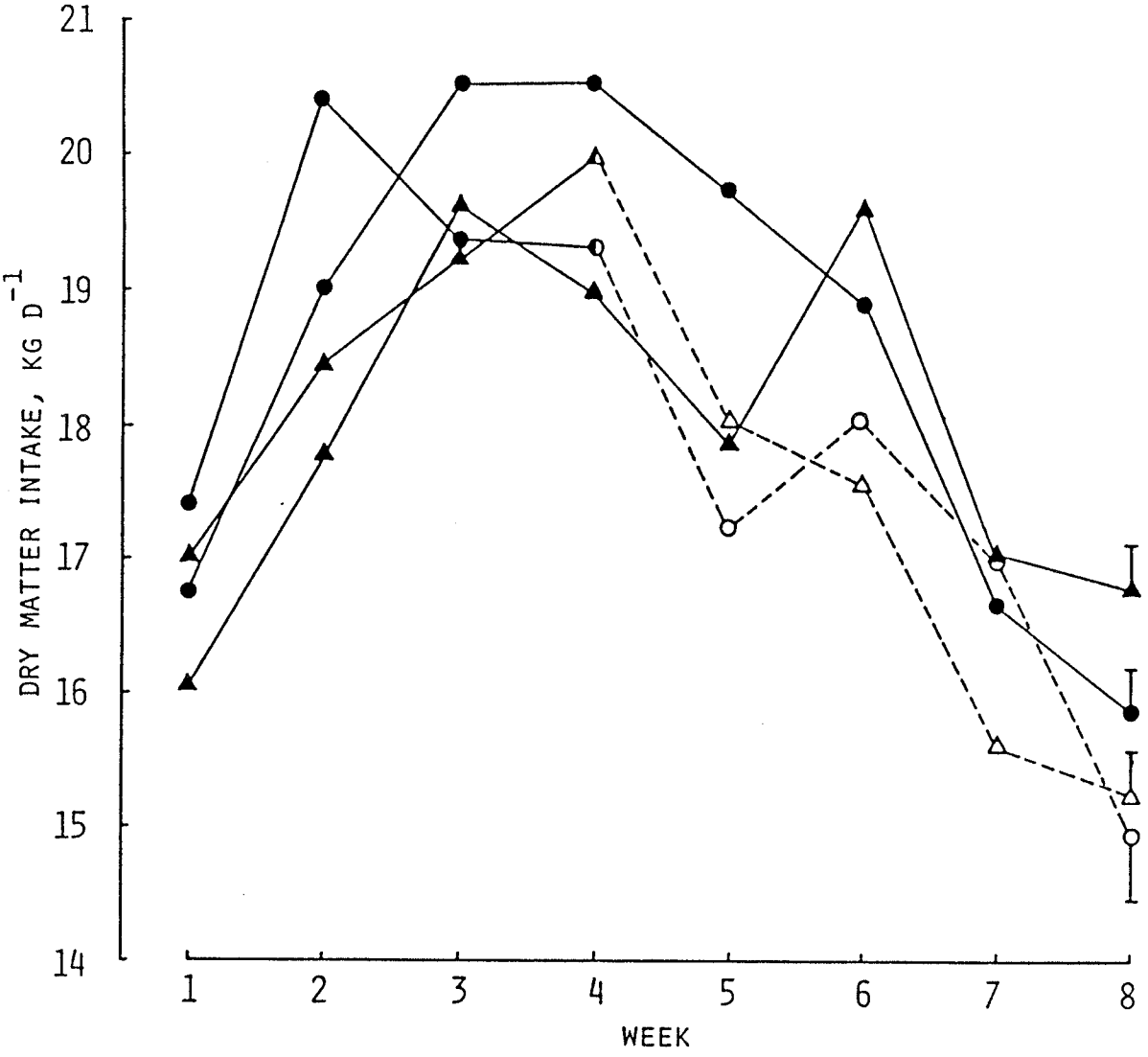

Figure 18: Effect of molybdenum and/or copper supplementation on average daily dry matter intake of midlactation Holstein-Friesian cows. Cows were fed basal (▲——▲), +Cu (△---△), +Mo (●——●) or +Mo+Cu (○--○) diets. Least square means ± SE. (For means and mean comparisons see Appendix table III-8.)

ronmental effects may have influenced daily D.M. intake as the present study was undertaken from June to August. Barn temperatures were not monitored.

Contrary to the ewe-lamb trial, day to day fluctuations in total D.M. intake for animals fed Mo supplemented diets did not differ from animals fed non-Mo supplemented diets. Feed intakes were from 2.8 to 3.0% of body weight which are similar to the level of intake of ewes in the ewe-lamb trial.

Plasma Cu and Mo Parameters. Inclusion of 20 mg Mo kg^{-1} D.M. in the diet resulted in increased plasma Cu levels ($P < 0.07$) in period 1 (Figure 19). The elevated plasma Cu levels were maintained for animals assigned to Trt. III throughout the 8-week feeding trial. The plasma Cu levels of cows that received 20 mg Mo kg^{-1} responded to Cu supplementation (Trt. IV) during weeks 5 to 8 with reduced ($P < 0.05$) plasma Cu levels (within 2 weeks) to those of cows that had received the basal diet throughout (Trt. I) and those that received the basal diet in period 1 and +Cu in period 2 (Trt. II) (Figure 19).

Plasma Cu concentrations for cows assigned to Trt. I decreased ($P < 0.05$) at a rate of 0.01 mg L^{-1} $week^{-1}$. Cows initially fed the basal diet and then fed +Cu in weeks 5 to 8 (Trt. II) did not have a significant ($P > 0.05$) decline in plasma Cu levels over time.

The patterns described for plasma Cu levels were not similar to plasma TCA-soluble Cu concentrations (Figure 19) or Cp oxidase activity (Appendix table III-14) for which no treatment or treatment by week interaction effects were found ($P > 0.05$, Figure

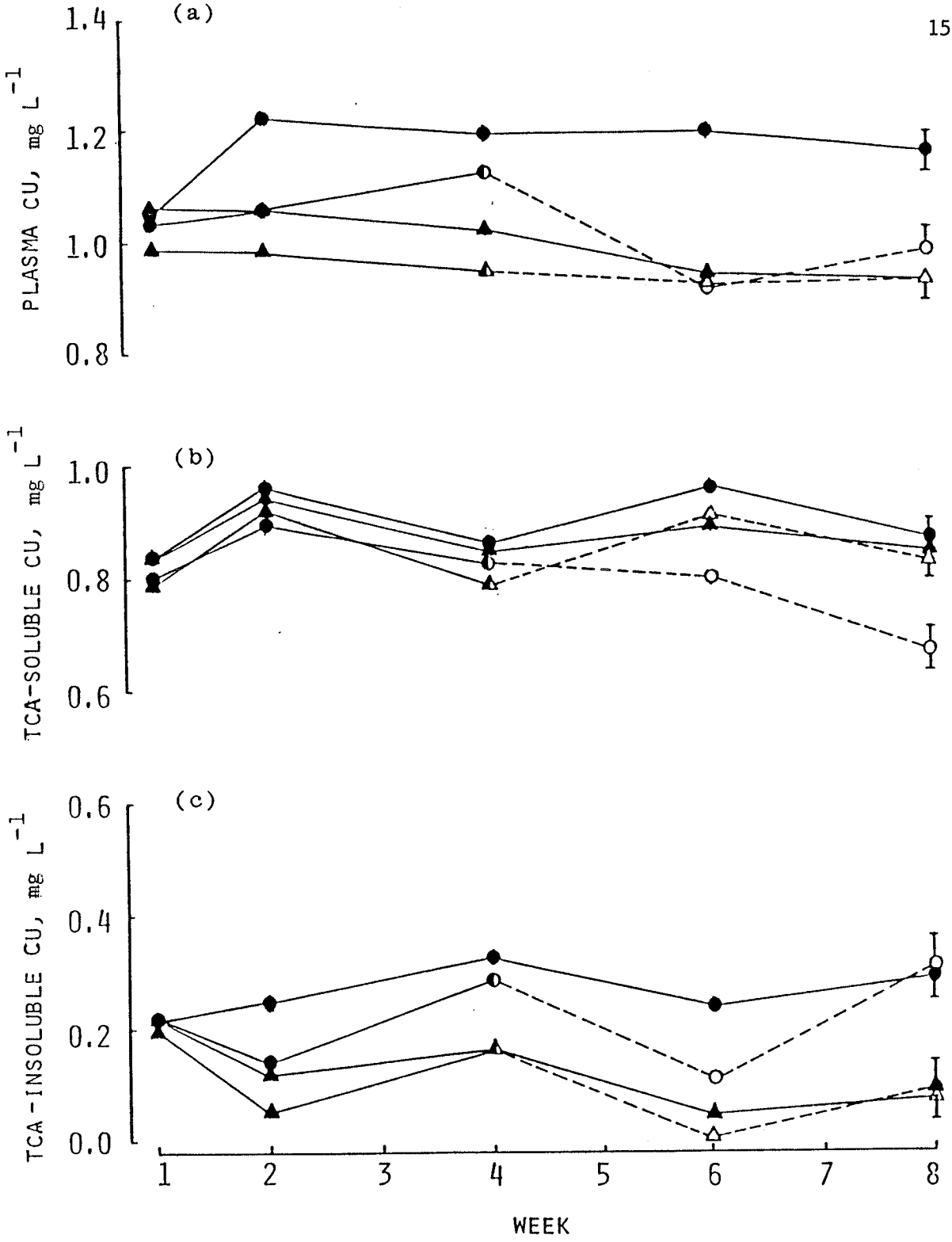

Figure 19: Plasma Cu (a), TCA-soluble Cu (b) and TCA-insoluble Cu (c) concentration response to molybdenum and/or copper supplementation of diets fed to midlactation Holstein-Friesian cows. Cows were fed basal (▲——▲), +Cu (△----△), +Mo (●——●) or +Mo+Cu (O--O) diets. Least square means ± SE.

19). Least square means were 0.87, 0.85, 0.90 and 0.80 $\pm$ 0.04 mg L^{-1} and 77.5, 72.0, 76.1 and 73.7 $\pm$ 4.9 Δ A min^{-1} L^{-1} for TCA-soluble Cu concentrations and Cp oxidase activity for cows assigned to Trt. I, Trt. II, Trt. III and Trt. IV, respectively. There was a week effect ($P < 0.001$), in that mean plasma TCA-soluble Cu levels were higher for the second and sixth weeks compared with means for samples taken in weeks 1, 4 and 8. It is unlikely that this is a result of errors in laboratory analyses for TCA-soluble Cu as samples for weeks 1 and 2 were prepared and analysed at the same time, as were samples from weeks 4, 6 and 8. The variability may reflect animal response to a common stimulus or handling and/or storage of blood samples prior to separation of the plasma.

The previously described higher concentration of plasma Cu for cows fed the +Mo diet was accounted for by an increased ($P < 0.001$) plasma TCA-insoluble Cu fraction (Figure 19). Plasma TCA-insoluble Cu levels for cows assigned to Trt. I and Trt. II were similar during the trial and were significantly lower ($P < 0.05$) than for cows assigned to Trt. III. A treatment by week interaction effect ($P < 0.07$) reflects the response of cows assigned to +Mo+Cu. These cows had plasma TCA-insoluble levels similar to +Mo cows at week 4. The supplementation of Cu appeared to result in an initial decline in plasma TCA-insoluble Cu levels by week 6 to levels that were similar to those for cows not receiving supplemental Mo but lower than for cows fed +Mo. This may have been a transient effect of supplementing the Mo diet with Cu (+Mo+Cu) as by week 8 the mean plasma TCA-insoluble levels were

back to a similar level as for cows fed the +Mo diet (Trt. III), and significantly ($P < 0.05$) greater than levels for cows fed the non-Mo supplemented diets.

The effects of supplementation with Cu of a diet containing a high concentration of Mo are not clearly defined from results of the present study. Total plasma Cu levels were reduced by Cu supplementation. The TCA-soluble Cu fraction tended to decrease during weeks 5 to 8, however this was not significant. Variations in the TCA-insoluble fraction were too great to determine whether or not the net effect was a reduction in Cu complexing with circulating thiomolybdates. These variations may indicate that circulating Cu pools had not stabilized within the four week period that animals were fed the supplemental Cu or that sampling frequency was not adequate to demonstrate possible effects during such a short term.

Plasma Cu levels were above suggested critical values for Cu deficiency (Smith and Coup, 1973; Puls, 1981) for cows in all treatment groups during the trial. As for cows fed the 20Mo diet in the beef cow-calf trial (Figure 3), 20 mg Mo kg^{-1} D.M. did not affect ($P > 0.05$) TCA-soluble Cu; however there were differences in animal response in the two trials for total plasma Cu and TCA-insoluble Cu.

Although the major dietary ingredients in both the cattle trials were barley and corn silage, differences in the remaining feed ingredients, in nutrient composition and, possibly, in feeding regimes, make comparisons between the two studies difficult. A possible reason for the appearance of a TCA-insoluble Cu

plasma fraction in cows fed Mo supplemented diets in the dairy trial is the higher dietary S content compared with the beef cow-calf trial (Bremner and Young, 1978; Lamand et al., 1980; Ishida et al., 1982). However, it should be recognized that the differences in S levels between basal (not measured, 1.0 and 0.8 g S kg^{-1} D.M., respectively) and experimental diets (1.7, 3.0 and 1.7 g added S kg^{-1} C.M., respectively) in these cited studies were much greater than the difference in S content of diets used in the beef cow-calf (1.3-1.4 g kg^{-1}) and the dairy trials (2.1 g kg^{-1}).

Plasma Mo concentrations (mg L^{-1}) were influenced by treatment ($P < 0.001$); cows assigned to Trt. III had significantly higher concentrations (0.50) than cows assigned to Trt. IV (0.27). Cows assigned to Trt. I (0.05) and Trt. II (0.10) treatment groups had lower plasma Mo concentrations than cows fed Mo supplemented diets (Figure 20). The standard error for these treatment means was 0.04. A treatment by week interaction effect ($P < 0.01$) reflected the drop in plasma Mo levels when cows fed +Mo for four weeks were switched to +Mo+Cu. While fed +Mo, this group of cows had higher ($P < 0.05$) plasma Mo levels than the cows fed non-Mo supplemented diets. Addition of Cu (+Mo+Cu) reduced their plasma Mo levels within two weeks to levels found in cows fed non-Mo supplemented diets and less than ($P < 0.05$) levels in cows consuming the +Mo diet. Mason (1978) showed that Cu supplementation can reduce plasma Mo levels by decreasing Mo absorption from the gastrointestinal tract and such a mechanism could explain the present results.

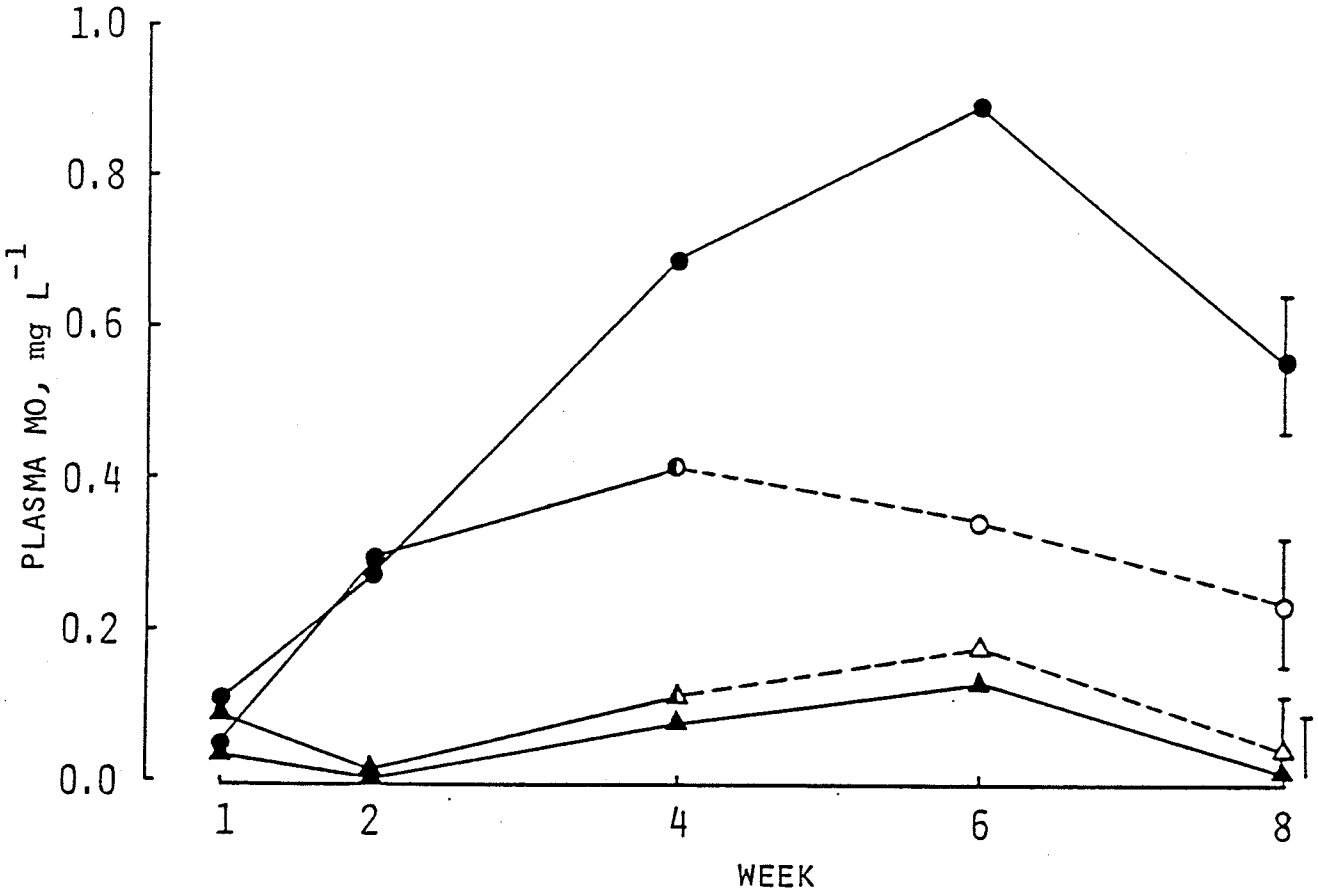

Figure 20: Plasma Mo concentration response to molybdenum and/or copper supplementation of diets fed to midlactation Holstein-Friesian cows. Cows were fed basal (▲——▲), +Cu (△----△), +Mo (●——●) or +Mo+Cu (○---○) diets. Least square means ± SE.

The decrease in plasma Mo levels from week 4 to week 8 was 0.17 mg L^{-1} for cows assigned to +Mo+Cu. The plasma Mo fraction that responded to Cu supplementation was not related to Mo in the TCA-insoluble Cu fraction as no comparable decline in the latter was observed. The decrease in plasma Mo may have been due to reduced circulating molybdates (MoO_4^{2-}).

Liver Cu and Mo. Initial liver Cu concentrations were not significantly different ($P > 0.05$) across treatment groups, the mean values were 413.0, 397.3, 376.6 and 379.0 $\pm$ 24.9 mg kg^{-1} D.M. for cows assigned to the Trt. I, Trt. II, Trt. III and Trt. IV, respectively. Cows had been fed a diet containing 10 mg added Cu kg^{-1} D.M. which may account for the high initial concentrations of Cu in their livers. Cows fed Cu supplemented diets in weeks 5 to 8 had high liver Cu levels by the end of the trial compared with cows fed non-Cu supplemented diets ($P < 0.001$, Table 24). The rate of liver Cu deposition was greater ($P < 0.05$) for cows assigned to Trt. II than for those assigned to Trt. IV.

Rate of decline in liver Cu concentrations for cows that consumed a basal or a 20 mg Mo kg^{-1} D.M. diet were greater in the present study than values calculated from data reported by Vanderveen and Keener (1964) and Huber et al. (1971). In the present experiment initial liver Cu contents were higher than those reported in the earlier studies, therefore, it is possible that differences in the proportions of liver Cu storage forms (Van Ryssen and Stielau, 1981; Weber et al., 1983) may have accounted for differences in rate of decline. Also, basal diet nutrient composition and milk production records were not provided

Table 24: Effect of dietary molybdenum and/or copper supplementation on liver copper and molybdenum concentrations of lactating Holstein-Friesian cows. Least square means ± SE.

	TREATMENT				
	I	II	III	IV	SE
Number of Observations:	4	4	4	3	
Liver Cu, D.M. basis					
initial†, mg kg^{-1}	413.4	397.3	376.6	421.6	51.2
final, mg kg^{-1}	352.8 b	602.4 a	280.4 b	507.2 a b	59.0
change, mg kg^{-1} d^{-1}†	-1.1 a	3.7 c	-1.7 a	1.5 b	0.7
Liver Mo, D.M. basis					
initial†, mg kg^{-1}	6.3	7.6	5.1	5.3	1.3
final, mg kg^{-1}	4.8b	3.8b	6.9a	4.2b	0.6
change, mg kg^{-1} d^{-1}†	-0.03	-0.07	0.03	-0.02	0.03

† Initial liver biopsy samples were taken two days before cows were placed on test.

† Liver Cu or Mo change (mg kg^{-1} d^{-1}) = (final concentration - initial concentration) ÷ total days on test.

a - c Means in the same row with different superscripts are significantly different, $\alpha = 0.05$.

by Vanderveen and Keener (1964) and Huber et al. (1971).

The effect of Cu supplementation on liver Cu concentration can be estimated if the following assumptions are accepted. First, that the rate of change in liver Cu for animals fed the basal or the +Mo diet was constant during the eight week study. Second, cows assigned to Trt. I and Trt. II had similar liver Cu responses in weeks 1 to 4, as did cows assigned to Trt. III and Trt. IV. Using these assumptions, the estimated changes in liver Cu, during weeks 5 to 8, for cows fed +Cu and +Mo+Cu were 4.74 and 3.25 mg kg^{-1} d^{-1} (D.M. basis), respectively (Figure 21).

Simpson et al. (1982) found the relationship between liver (D.M., kg) and live weight (W, kg) in 73 Friesian cattle to be:

$$D.M. = 0.0033\ W + 0.30 \qquad \text{Equation 3}$$

Application of this equation to determine liver D.M. of cows used in the present study resulted in a predicted accumulation of Cu in livers of cows fed +Cu and +Mo+Cu in weeks 5 to 8 to be 10.5 and 7.2 mg d^{-1}, respectively. These rates of Cu deposition in the liver represent approximately 1.6 and 0.8% of daily Cu intake, respectively.

Initial liver Mo levels were not significantly different ($P > 0.05$) across treatment groups (Table 24). There was a significant treatment effect ($P < 0.07$) as liver Mo levels increased for cows assigned to +Mo and decreased for cows assigned to the other three treatments.

Using the same assumptions made for liver Cu, the net effect of feeding Cu supplemented diets to cows in weeks 5 to 8 resulted in a decreased liver Mo concentration (Figure 22).

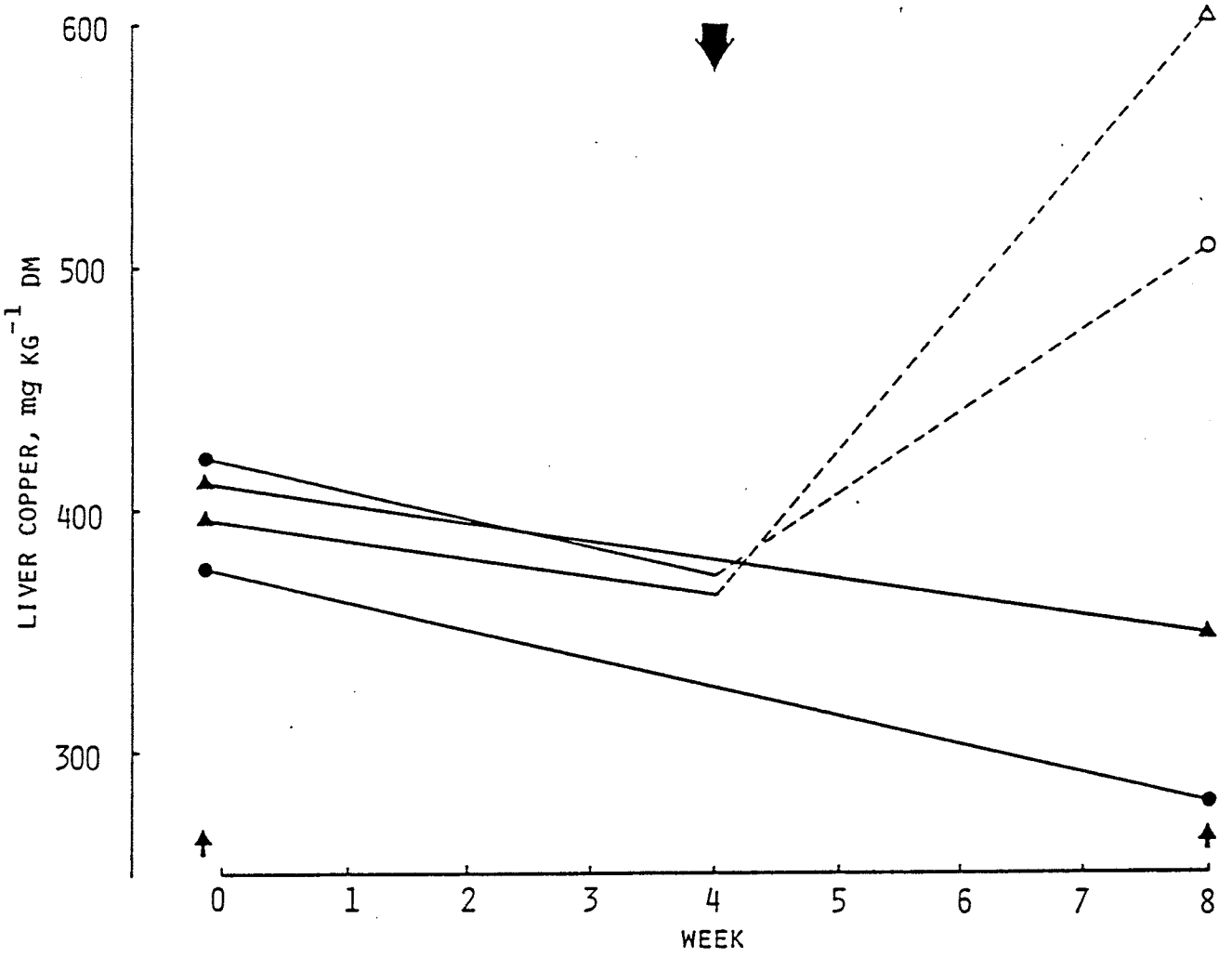

Figure 21: Calculated response of liver copper concentrations of lactating Holstein-Friesian cows assigned to Trt. I (▲——▲), Trt. II (▲——△), Trt. III (●——●) and Trt. IV (●——○). Small arrows indicate liver sampling times. Large arrow indicates the point at which copper was supplemented to diets of cows assigned to Trt. II and Trt. IV. (See p. 163 of text for further explanation.)

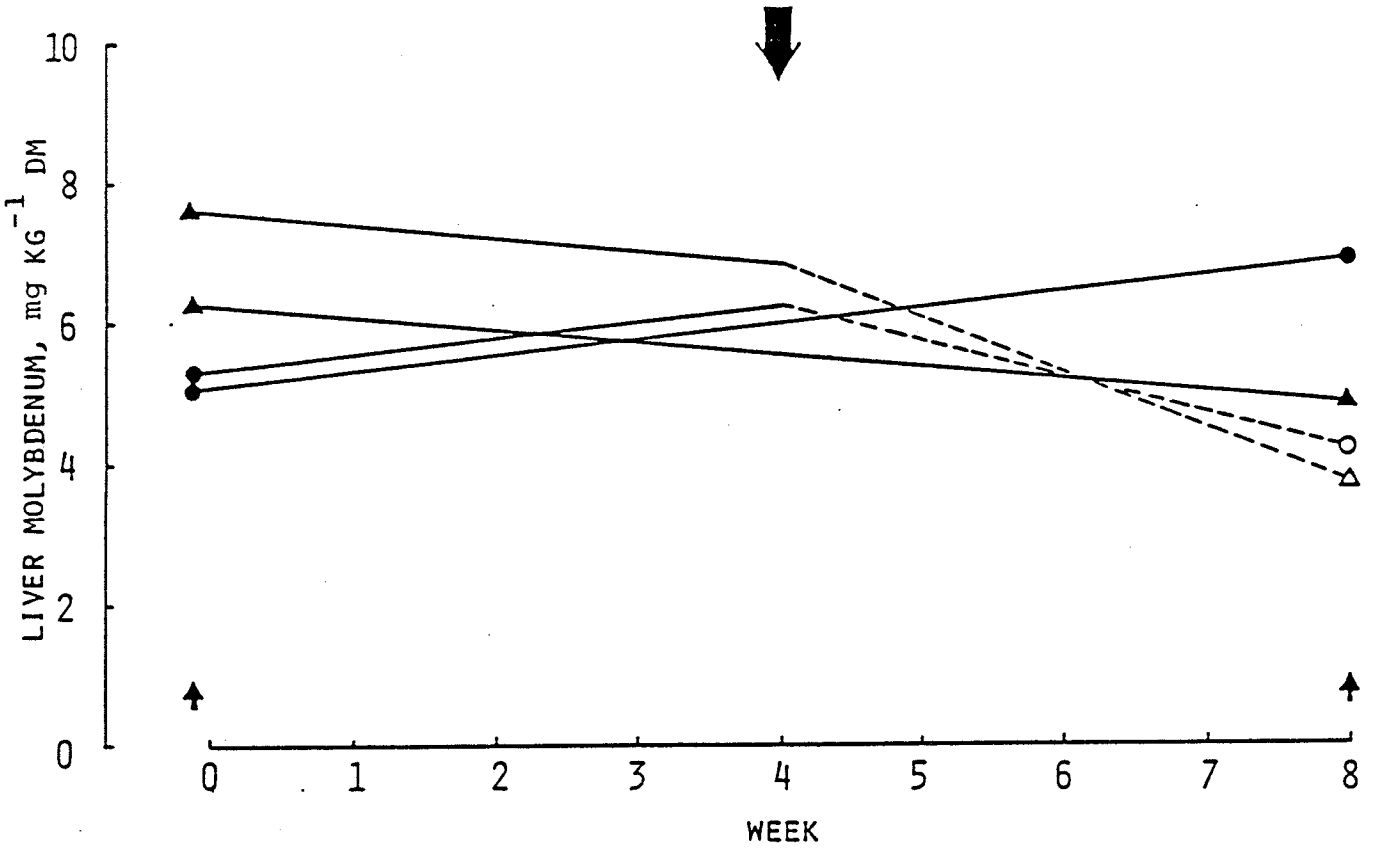

Figure 22: Calculated response of liver molybdenum concentrations to lactating Holstein-Friesian cows assigned to Trt. I (▲——▲), Trt. II (▲---△), Trt. III (●——●) and Trt. IV (●---○). Small arrows indicate liver sampling times. Large arrow indicates the point at which copper was supplemented to diets of cows assigned to Trt. II and Trt. IV. (See p. 163 of text for further explanation.)

Comparisons between the ewe-lamb and present trials show some differences in plasma Mo response for animals fed 20 mg Mo kg^{-1} D.M. relative to animals fed the low Mo control diets. Although increased Mo intake resulted in significant increases for this parameter in the two trials, the magnitude of the response differed. Changes in plasma Mo concentrations were much greater in ewes than in cows when fed Mo supplemented diets. This suggested greater absorption and/or less efficient excretion of Mo by the ewes.

Milk Composition and Yield. Milk fat, protein and lactose concentration in milk were not influenced by Cu and/or Mo supplementation (Table 25). The effects of Mo supplementation in the present trial are in agreement with results of the earlier two trials with respect to milk Cu and Mo content. Milk Cu levels for animals that have adequate body Cu reserves, as determined from liver and plasma Cu parameters, were not affected ($P > 0.05$) by intake of high Mo diets. Milk Mo levels, on the other hand, increased ($P < 0.001$).

Increases in milk Mo levels as a response to increased dietary Mo levels may be controlled by other factors as well as dietary Mo concentrations. For example, animals fed a diet containing approximately 20 mg Mo kg^{-1} D.M. produced milk containing 0.41, 1.39 and 0.06 mg kg^{-1} more Mo than their control counterparts for beef cows, ewes and dairy cows, respectively. Diet ingredients and nutrient composition for the beef cow-calf and ewe-lamb trials were similar but ewe response to dietary Mo was much greater than for the beef cows. Species differences may have

Table 25: Influence of dietary molybdenum and/or copper supplementation on milk yield and composition for midlactation Holstein-Friesian cows. Least square means ± SE.

	TREATMENT				
	I	II	III	IV	SE
Number of Observations	32	32	32	28	
Milk yield, kg d^{-1}	27.7	24.9	25.0	26.5	1.9
Fat corrected milk, kg d^{-1}	25.4	22.3	21.6	23.7	1.7
Butterfat, %	3.40	3.35	3.10	3.36	0.25
Protein, %	2.96	2.99	3.23	3.04	0.12
Lactose, %	4.94	4.74	4.79	4.73	0.13
Copper, mg kg^{-1}	0.03	0.04	0.04	0.04	0.003
Copper, mg d^{-1}	0.95	0.91	0.91	0.99	0.05
Molybdenum, mg kg^{-1}	0.03^a	0.03^a	0.09^b	0.09^b	0.01
Molybdenum, mg d^{-1}	0.84^a	0.72^a	2.31^b	2.41^b	0.31

† Each mean represents data collected on a weekly basis for 8 weeks (4 animals/treatment) with the exception of Cu and Mo data which was collected during the initial week and weeks 2 and 4 of each period (i.e. 5 weeks x 4 animals/treatment = 20 observations).
Cow #14 was removed from Treatment IV in week 5.

a - b Means in same row with different superscripts are significantly different, $\alpha = 0.05$.

contributed to differences in response between sheep and cattle. There are several differences between the dairy cow trial and the beef cow-calf trial, including stage of lactation, breed and diet, that may have caused the lower response to dietary Mo in the former. Data collected by Vanderveen and Keener (1964) suggest that ability to excrete Mo in milk does not change with stage of lactation. Similarly, studies by Vanderveen and Keener (1964) and Huber et al. (1971) demonstrated that Holstein-Friesian cows consuming diets containing 50 mg Mo kg^{-1} diet produced milk containing as much as 1.03 to 1.57 mg L^{-1}. Both of these studies reported plasma Mo levels much higher than those found for cows fed +Mo and +Mo+Cu in the dairy cow trial. Comparable data for other breeds of cattle were not found. However, it may be speculated that breed differences may exist in absorption of Mo from the gastrointestinal tract or ability to excrete circulating Mo in milk.

Dietary S, as mentioned previously, may account for quantitative differences in response of milk Mo level for animals fed high versus low Mo diets. Vanderveen and Keener (1964) demonstrated decreased milk Mo levels in response to S supplementation of Mo containing diets; however the level of S supplementation (3.0 g kg^{-1}) used to elicit this effect was several fold greater than the difference in S content (0.7 g kg^{-1}) of the dairy cow diets relative to diets fed in the beef cow-calf trial.

Milk yield and fat corrected milk yield were not influenced ($P > 0.05$) by supplemental Mo and/or Cu (Table 25). No differences were observed ($P > 0.05$) for constituent production with

the exception of daily milk Mo production ($P < 0.001$). Milk Mo excretions represented approximately 2% of daily Mo intake for cows fed non-Mo supplemented diets and represented 0.7% for cows assigned to +Mo and +Mo+Cu. There was a diet by week interaction, ($P < 0.01$) for daily milk Mo excretions. Milk Mo excretions decreased from 2.62 to 1.79 mg d^{-1} following Cu supplementation of the diet of cows receiving Mo supplemented feed (Appendix table III-10). Milk Mo excretion during this four week period represented 0.5% of Mo intake. The reduced daily Mo excretion in milk was the result of a decline ($P < 0.001$) in milk Mo concentrations (Appendix table III-15).

Feed efficiency for milk production, determined weekly over the eight week study, was not influenced ($P > 0.05$) by Cu and/or Mo supplementation (Table 26). Feed required per unit fat corrected milk produced was influenced ($P < 0.08$) by treatment, cows fed +Mo for eight weeks were less efficient than the other three groups of animals. Cows fed a Cu supplemented, high Mo diet (+Mo+Cu) had similar feed efficiencies to cows fed low Mo diets.

Estimated net energy retention (Table 27) for these midlactation dairy cows were reduced in the last four weeks of the trial. This drop is related to the weight losses recorded ($P < 0.01$) for these cows. Generally, dramatic weight losses are not experienced for midlactation cows. Reduced feed intake ($P < 0.01$) in the last two weeks of the trial may account for some of the losses. Differences in gut fill may have been a factor in the body weight changes recorded. If that was the case then the assumption that body weight changes were associated with fat mo-

Table 26: Influence of molybdenum and/or in copper supplementation on feed intake and feed efficiency of midlactation Holstein-Freisian cows. Least square means ± SE.

	TREATMENT				
	I	II	III	IV	SE
Number of Observations	32	32	32	28	
D.M. intake, kg d^{-1}	17.8	17.6	18.5	17.9	± 5.2
Feed efficiency, kg, D.M. kg^{-1} milk	4.5	5.0	5.2	4.7	± 0.3
Feed efficiency, kg, D.M. kg^{-1} FCM	5.0[a]	5.6[a b]	6.1[b]	5.2[a]	± 0.3

a - b Values in the same row with different superscripts are significantly different, α = 0.10.

Table 27: The effect of molybdenum and/or copper supplementation on estimated energy retention for lactating Holstein-Friesian cows.

Week	Treatment	Diet	Milk energy MJ d^{-1} †	Body tissue gain or loss MJ d^{-1} ‡	Net energy retention MJ d^{-1} §
1 to 4	I	Basal	80.1	15.1	95.2
	II	Basal	70.8	7.0	77.8
	III	+Mo	71.1	5.5	76.6
	IV	+Mo	77.4	-0.5	76.9
5 to 8	I	Basal	77.3	-15.3	62.0
	II	+Cu	66.8	-11.8	55.0
	III	+Mo	65.9	-22.5	43.4
	IV	+Mo+Cu	68.6	-18.7	49.9

† Calculated from least square means for daily milk fat, protein and lactose production using energy values for each component of 38.5, 23.7 and 16.5 J g^{-1}, respectively (Van Soest, 1982).

‡ Based on the assumption on that the energy value of body weight gain or loss is 26 MJ kg^{-1} (ARC, 1980).

§ Sum of milk energy production and body tissue energy change.

bilization is invalid. However, the data presented in Table 27 suggest a tendency for lower net energy retention for cows fed the +Mo diet in weeks 5 to 8 compared with cows in the other three treatment groups. This occurred despite the fact that cows fed +Mo during that time period tended to have a higher daily feed intake (17.8 kg D.M. d^{-1}) than cows fed basal (17.4 kg D.M. d^{-1}), +Cu (16.5 kg D.M. d^{-1}) and +Mo+Cu (16.8 kg D.M. d^{-1}) diets.

Discussion. Actual intakes of corn silage, hay, concentrate and supplement and the nutrient analyses of these dietary constituents were used to determine mineral content of the basal, +Cu, +Mo and +Mo+Cu diets used in this study (Table 6). The level of Mo supplementation used was similar to the 20Mo diets used in the two earlier studies (Tables 3 and 13). The S content (2.1 g kg^{-1} D.M.), however, was higher than that found for the beef cow-calf (1.3 g kg^{-1} D.M.) and the ewe-lamb (1.4 g kg^{-1} D.M.) diets. Therefore the predicted Cu absorption coefficient (equation 1, Suttle and McLauchlan, 1976) for the +Mo and +Mo+Cu diets in the present trial fell between predicted values for the 20Mo diets and the 40Mo diets of the two earlier studies. Based on the dietary S level, predicted Cu absorption coefficients were 0.04 and 0.01 for diets containing no added Mo and 20 mg kg^{-1} D.M. added Mo, respectively.

Copper concentrations in the non-Cu supplemented diets were similar to the concentrations in the diets used for the two earlier studies. The level of Cu supplementation for +Mo+Cu was based on estimated available Cu requirements of cows and the predicted Cu absorption coefficient with the objective that Cu

requirements would be met for cows fed +Mo+Cu. Initial mean body weight and milk production of cows used in this study were 593 $\pm$ 7 kg and 26.8 $\pm$ 0.7 kg d^{-1}, respectively. The estimated available Cu requirement was 6.8 mg Cu d^{-1}. The +Cu diet served as a control for +Mo+Cu. Analysed Cu content in the +Mo+Cu and +Cu diets were higher than levels for which they were formulated (40 mg kg^{-1} D.M.). It is felt that this did not interfere with the objective of this study.

Cows consuming the basal diet in weeks 1 to 4 had an estimated 4.8 mg available Cu per day. Cows consuming +Mo in weeks 1 to 4 consumed an estimated 1.5 mg available Cu per day. These calculations suggest daily requirements for available Cu were not met for cows in all treatment groups during the first four weeks of the trial and that the rate of body Cu depletion was greatest for animals fed the Mo supplemented diets. Estimated available Cu intakes for cows fed basal, +Cu, +Mo and +Mo+Cu diets in weeks 5 to 8 were 4.6, 31.0, 1.4 and 10.8 mg d^{-1}. Therefore cows fed the +Cu and +Mo+Cu diets were predicted to meet daily Cu requirements during weeks 5 to 8.

This calculation of adequate Cu intake for cows fed +Cu and +Mo+Cu was supported by the observed changes in liver Cu concentrations, but not by plasma Cu parameters. The calculated Cu balances for this and the two earlier studies are meant to describe metabolically available Cu. Copper bound to Mo-S complexes can accumulate in the body because the animal's ability to excrete these compounds may not equal their rate of appearance systemically when high Mo and S diets are fed (Bremner and

Young, 1978; Hynes et al., 1984). Plasma TCA-soluble Cu did not reflect the estimated Cu balance. It may be that a plasma TCA-soluble Cu response does not occur until some critical level is reached, which may be associated with rate of liver Cu release for Cp oxidase synthesis. If this is so, studies of a longer duration may be needed to use plasma TCA-soluble Cu as a criterion for Cu balance.

The appearance of a TCA-insoluble fraction in the present study suggests that rumen conditions (rumen retention times, pH, etc., Laurie and Clarke, 1980) were more conducive to metabolic interactions between Cu, Mo and S or the basal level of S in the Mo supplemented diets was sufficiently high to encourage the metabolic interactions of Cu, Mo and S. Thiomolybdate formation was not apparent for the ewe-lamb trial and inconclusive for the beef cow-calf trial.

Thiomolybdate production in the rumen may be suggested to account for the low Mo absorption, as determined from plasma, liver and milk Mo concentrations in the present study relative to the beef cow-calf and ewe-lamb trials.

Dairy cow performance parameters, which included dry matter intake, milk yield and composition and body weight changes were not affected by supplemental Mo and/or Cu.

Animals fed +Mo+Cu (weeks 5 to 8) were similar to cows fed non-Mo supplemented diets in terms of plasma Cu and Mo concentrations but not plasma Cu distribution.

The effect of Cu supplementation in high Mo diets on production performance of lactating cows was not conclusive. High Mo

diets in this trial did not reduce milk production, however it did reduce feed efficiency for 4% FCM production and appeared to result in a lower net energy retention of feed energy relative to cows fed non-Mo supplemented diets. Addition of Cu to the high Mo diet (+Mo+Cu) improved feed efficiency for FCM and resulted in intermediate values for calculated net energy retention (Table 27). As for the beef cow-calf and ewe-lamb trials, the adverse effects of high Mo diets did not appear once animals had become Cu deficient (defined by plasma and liver Cu parameters). Also, despite significant reductions in liver Cu levels ($P < 0.05$) and plasma Cu levels ($P < 0.07$), cows fed the basal diet had a better feed efficiency than the cows fed +Mo.

These data suggest that adverse effects associated with feeding high Mo diets to lactating cows are associated with toxic effects of molybdate and/or Mo complexes rather than a conditioned hypocuprosis.

CONCLUSIONS

Beef Cow-Calf and Ewe-Lamb Trials

The cow-calf trial was designed to determine whether high Mo levels (20 and 40 mg kg^{-1} D.M.) in the diets of lactating beef cows would affect 1) the productivity and 2) the Cu and Mo status of the cows and their suckling calves. The ewe-lamb trial was designed to 1) provide comparative data on the effects of high dietary Mo content between cattle and sheep and 2) further investigate the effects of these diets on lactating ruminants and their offspring. The following conclusions were drawn from the two trials:

1. Plasma Cu parameters of cows responded more readily to increased dietary Mo concentrations than for ewes when fed similar diets.

Lactating beef cows fed a corn silage and barley based diet containing 5.8 mg Cu, 1.3 g S and either 0.6 (0Mo) or 19.3 (20Mo) mg Mo kg^{-1} D.M. had similar rates of decline over time for plasma Cu and TCA-soluble Cu concentrations and for plasma Cp oxidase activities. The rate of decline was significantly greater for these three parameters when dietary Mo levels were increased to 34.8 mg kg^{-1} D.M. (40Mo). The percent of total plasma Cu that was TCA-insoluble was greater for the cows fed 40Mo than for the other two groups.

Ewes fed a similar ration containing 4.9 mg Cu, 1.4-1.5 g S and either 0.9 (0Mo), 18.4 (20Mo) or 40.7 (40Mo) mg Mo kg^{-1} D.M.

had similar changes in plasma Cu parameters during the trial.

The potential effects of body weight loss on plasma and liver Cu concentrations of lactating animals were discussed.

2. Milk production of beef cows was more responsive to dietary Mo levels than for ewes fed similar diets.

Increasing diet Mo content of beef cows to 34.8 mg kg^{-1} resulted in a more rapid decline in daily milk yield than for cows fed the same diet with 0.6 or 19.3 mg Mo kg^{-1} D.M. Diet Mo concentrations up to 40.7 mg kg^{-1} D.M. fed to ewes for six to seven weeks did not affect their milk yield relative to ewes fed 0.9 or 18.4 mg kg^{-1} D.M.

3. Milk fat, protein and lactose concentrations were not influenced by dietary Mo concentrations as high as 34.8 mg kg^{-1} for beef cows and 40.7 mg kg^{-1} for ewes.

Varying levels of dietary Mo did not influence the fat, protein, lactose or copper content of milk produced by cows or ewes. Milk Mo concentrations, however, increased with increased diet Mo concentrations. Cows and ewes fed no supplemental Mo produced milk with similar Mo contents; the means being 0.10 and 0.14 mg L^{-1}, respectively. Cows and ewes produced milk containing 0.51 and 1.53 mg Mo L^{-1}, respectively when fed 20Mo diets and 1.19 and 2.69 mg Mo L^{-1}, respectively when fed 40Mo diets.

4. Milk excretions of Mo were greater for ewes than for cows fed similar diets.

Ewes excreted a greater percentage (15.5 vs. 6.9%) of daily Mo intake via milk than did cows. Also, ewes and cows excreted a

greater percentage (28.0 vs. 6.8%) of daily Mo intake when fed 0Mo diets than when fed Mo supplemented diets.

5. Feeding high Mo diets to lactating ruminants will result in increased plasma Mo concentrations in the suckling offspring.

Increased Mo concentrations in diets of lactating cows and ewes did not influence plasma Cu parameters but did increase plasma Mo concentrations of calves and lambs. Liver Mo levels of lambs were not significantly increased by Mo supplementation to the diet of ewes. The effects of Mo supplementation of the dams' diets did not appear to influence liver Cu concentration of lambs, but tended to increase the daily fractional decline (p. 105) of liver Cu in calves.

6. The effect of Mo content in the diet of lactating cows and ewes on growth performance of suckling calves and lambs were not conclusive.

There was no treatment effect on the daily gains of calves and lambs. However, a diet by week interaction for calf ADG reflected better gains by 0Mo calves than 20Mo and 40Mo calves during 3 of 8 periods monitored (7 days per period).

Similar studies in which calf performance is monitored from parturition through to weaning are required to obtain a better estimate of the effects of Mo in the cow's diet on calf growth performance as well as other factors discussed above.

7. Supplementing Mo in the diets of ewes caused a high incidence of thiamine deficiency.

Three of eight ewes fed Mo supplemented diets developed an apparent thiamine deficiency. Although the mechanism was not

determined, it was suggested that dietary Mo may alter microbial activity in the rumen resulting in either reduced thiamine or increased thiaminase production.

8. Estimated feed energy utilization for production is reduced when lactating cows and ewes are fed high Mo diets.

Cows fed the 40Mo diet and ewes fed 20Mo and 40Mo diets had lower estimated net energy retention based on milk production and body weight changes than cows and ewes fed non-Mo supplemented diets.

Dairy Cow Trial

The objectives of this trial were to 1) determine whether increasing dietary Cu would influence any observed animal responses to high Mo (20 mg kg^{-1} D.M.) diets and 2) to obtain data on the effects of feeding supplemental Mo to cattle at a higher production level than was observed for the beef cow-calf trial. The following conclusions were drawn from the dairy cow trial.

1. Increasing dietary Mo from 2.1 to 20.1 mg kg^{-1} D.M. increased plasma Cu and TCA-insoluble concentrations of midlactation Holstein-Friesian cows. Addition of Cu (40 mg kg^{-1} D.M.) reduced plasma Cu concentrations.

2. Increasing dietary Cu from 6.1 to 44.0-51.2 mg kg^{-1} D.M. resulted in a significant increase in liver Cu and a significant decrease in liver Mo for lactating dairy cows fed either low (2.1 mg kg^{-1} D.M.) or high (20.2 mg kg^{-1} D.M.) Mo diets.

3. Supplementation of Mo (20 mg kg^{-1} D.M.), Cu (40 mg kg^{-1} D.M.) or both in diets did not influence feed intake, milk yield or body weight change of midlactation Holstein-Friesian cows.

However, feed efficiency for fat corrected milk production was lower ($P < 0.07$) for cows fed the Mo supplemented diet than for cows fed low Mo or Cu supplemented diets. It is suggested, however, that the number of animals used and the time span of this study were not adequate to be conclusive regarding the effects of dietary Mo on animal productivity and on the ability of supplementary Cu to alleviate any of the adverse effects.

4. Milk fat, protein and lactose content were not influenced by Mo and/or Cu supplementation of diets fed to midlactation dairy cows.

LITERATURE CITED

A.O.A.C. 1980. Official methods of analysis, 13th ed. Association of Official Analytical Chemists, Washington, D.C.

Ademosun, A. A. and Munyabuntu, C. M. 1982. Sheep: Metabolic interactions of copper with iron, molybdenum and sulphur. Int. Goat and Sheep Res. 2: 13-21.

Agricultural Research Council. 1980. The nutrient requirements of ruminant livestock. Commonwealth Agricultural Bureaux, Slough, England.

Al-Khalidi, U. A. S. and Chaglassian, T. H. 1965. The species distribution of xanthine oxidase. Biochem. J. 97: 318-320.

Allaway, W. H. 1977. Perspectives on molybdenum in soils and plants in Molybdenum in the environment. Vol. 2. The geochemistry, cycling and industrial uses of molybdenum, 1977. W. R. Chappell and K. K. Peterson, eds. Marcel Dekker, Inc., New York.

Allcroft, R. and Lewis, G. 1956. Relationship of copper, molybdenum and inorganic sulphate content of feedingstuffs to the occurence of copper deficiency in sheep and cattle. Landourukundig Aijdshrift 68: 711-722. As cited by Fell et al., 1979.

Alloway, B. J. 1973. Copper and molybdenum in swayback pastures. J. Agric. Sci. (Camb.) 80: 521-524.

Ammerman, C. B. 1970. Recent developments in cobalt and copper in ruminant nutrition--A review. J. Dairy Sci. 53: 1097-1123.

Anke, M., Grun, M., Partschefeld, M. and Groppel, B. 1978. Molybdenum deficiency in ruminants in Trace Element Metabolism in Man and Animals. 3, 1978. M. Kirchgesmer, ed. Arbeitskries fur Tierernahrungsforschung, Weihenstephan.

Archibald, J. G. 1951. Molybdenum in cows' milk. J. Dairy Sci. 34: 1026-1029.

Archibald, J. G. 1958. Trace elements in milk--A review. Part I. Dairy Sci. Abst. 20: 711-725.

Arnold, G. W. 1975. Herbage intake and grazing behavior of ewes in four breeds at different physiological states. Aust. J. Agric. Res. 26: 1017-1024.

Aspin, N. and Sass-Kortsak, A. 1981. Copper in Disorders of Mineral Metabolism. Vol. 1. Trace Minerals, 1981. F. Bronner and J. W. Coburn, eds. Academic Press, Toronto.

Bailey, C. B. and Lawson, J. E. 1981. Estimated water and forage intakes in nursing range calves. Can. J. Anim. Sci. 61: 415-421.

Barnes, R. H., Fiala, G. and Kwong, E. 1963. Decreased growth rate resulting from prevention of coprophagy. Fed. Proc. 22: 125-131.

Barry, T. N., Reid, T. C., Millar, K. R. and Sadler, W. A. 1981. Nutritional evaluation of kale (Brassica oleracea) diets. 2. Copper deficiency, thyroid function, and selenium status in young cattle and sheep fed kale for prolonged periods. J. Agric. Sci. (Camb.) 96: 269-282.

Beck, A. B. 1941. Aust. J. Exp. Biol. Med. Sci. 19: 145 and 249. As cited by Underwood, E. J., 1971.

Bell, M. C., Sneed, N. N. and Hall, R. F. 1966. Effect of ration and route of administration on metabolism of ^{99}Mo by sheep and swine in Proceedings of the Seventh International Congress of Nutrition. Vol. 5, 1966. J. Kuhnau, ed. Permagon Press Ltd. As cited by Hansard, S. L., 1983.

Bingley, J. B. and Duffy, J. H. 1969. Distribution of copper in the blood of the bovine neonate. Clinica Chim. Acta. 24: 316-319.

Bingley, J. B. and Anderson, N. 1972. Clinically silent hypocuprosis and the effect of molybdenum loading on beef calves in Gippsland, Victoria. Aust. J. Agric. Res. 23: 885-904.

Boila, R. J., Devlin, T. J., Drysdale, R. A. and Lillie, L. E. 1984b. Supplementary copper for grazing beef cattle--injectable copper glycinate, and copper sulfate in free-choice mineral supplements. Can. J. Anim. Sci. 64: 675-696.

Boila, R. J., Devlin, T. J., Drysdale, R. A. and Lillie, L. E. 1984a. Geographical variation in the copper and molybdenum content of forages grown in North Western Manitoba. Can. J. Anim. Sci. 64: 899-918.

Bowden, H. J. M., ed. 1979. Environmental Chemistry of Elements. Academic Press Inc. (London) Ltd, New York pp. 333.

Bray, A. C. 1975. Molybdenum iron-sulfur flavin hydroxylases and related enzymes. Enzymes 12: 299-419.

Bray, A. C. and Till, A. R. 1975. Metabolism of sulphur in the gastro-intestinal tract in Digestion and Metabolism in the Ruminant, 1975. I. W. McDonald and A. C. I. Warner, eds. The University of New England Publishing Unit, Australia.

Bray, A. C., Suttle, N. F. and Field, A. C. 1982. The determination of tri-and tetra-thiomolybdates in continuous cultures of rumen microorganisms and their adsorption onto 'fibre.' Proc. Nutr. Soc. 41: 67A.

Bremner, I. 1970. Zinc, copper and manganese in the alimentary tract of sheep. Br. J. Nutr. 24: 769-783.

Bremner, I. 1976. The effect of dietary molybdenum and sulphate on plasma Cu distribution in sheep. Proc. Nutr. Soc. 35: 21A-22A.

Bremner, I. and Dalgarno, A. C. 1973. Iron metabolism in the veal calf. 2*. Iron requirements and the effect of copper supplementation. Br. J. Nutr. 30: 61-76.

Bremner, I. and Young, B. W. 1978. Effects of dietary molybdenum and sulphur on the distribution of copper in plasma and kidneys of sheep. Br. J. Nutr. 39: 325-336.

Bremner, I. and Davies, N. T. 1980. Dietary composition and the absorption of trace elements by ruminants in Digestive Physiology and Metabolism in Ruminants, 1980. Y. Ruckebusch and P. Thivend, eds. MTP Press Ltd.

Brent, B. E. 1976. Relationship of acidosis to other feedlot ailments. J. Anim. Sci. 43: 930-935.

Bull, L. S., Rumpler, W. V., Sweeny, T. E. and Zinn, R. A. 1979. Influence of ruminal turnover on site and extent of digestion. Fed. Proc. 38: 2713-2719.

Burridge, J. C. 1970. Vegetational factors affecting the trace element content of plants in Trace Element Metabolism in Animals, 1970. C. F. Mills, ed. E & S Livingstone, London.

Cardin, C. J. and Mason, J. 1975. Sulphate transport by rat ileum. Effect of molybdate and other anions. Biochimica et Biophysica Acta 394: 46-54.

Cardin, C. J. and Mason, J. 1976. Molybdate and tungstate transfer by rats ileum. Competitive inhibition by sulphate. Biochimica et Biophysica Acta 455: 937-946.

Cardo, P. P., Gambett, M., Vignale, B. and Divano, M. C. 1983. Kinetic and thermodynamic evidence of a molybdate interaction with glucocorticoid receptor in calf thymus. Eur. J. Biochem. 137: 173-178.

Chan, L. and O'Malley, B. W. 1976. Mechanism of action of the sex steroid hormones. New Eng. J. Med. 294: 1322-1328 and 1372-1381 and 1430-1437.

Chapman, H. L. Jr., Cox, D. H., Hains, C. E. and Davis, G. K. 1963. Evaluation of the liver biopsy technique for mineral nutrition studies with beef cattle. J. Anim. Sci. 22: 733-737.

Chappell, W. R. 1977. The Molybdenum Project--An Interdisciplinary program in Molybdenum in the Environment. Vol. 1. The Biology of Molybdenum, 1977. W. R. Chappell and K. Kellogg Petersen, eds. Marcel Dekker, Inc., New York.

Clarke, N. J. and Laurie, S. H. 1980. The copper-molybdenum antagonism in ruminants. The formation of thiomolybdates in animal rumen. J. Inorg. Biochem. 12: 37-45.

Clarke, N. and Laurie, S. H. 1982. The copper-molybdenum antagonism in ruminants. II. Interactions of thiomolybdates in aqueous media. Inorganica Chimica Acta 66: L35-L38.

Clegg, M. S., Keen, C. L., Lönnerdal, B. and Hurley, L. S. 1981. Influence of ashing techniques on the analysis of trace elements in animal tissue. I. Wet Ashing. Biol. Trace Element Res. 3: 107-115.

Cohen, H. J., Drew, R. T., Johnson, J. L. and Rajagopalan, K. V. 1973. Molecular basis of the biological function of molybdenum. The relationship between sulfite oxidase and the acute toxicity of bisulfite and SO_2. Proc. Nat. Acad. Sci. USA 70: 3655-3659.

Coughlan, M. P. 1980. Aldehyde oxidase, xanthine oxidase and xanthine dehydrogenase: hydroxylases containing molybdenum, iron, sulfur and flavin Molybdenum and Molybdenum-containing Enzymes, 1980. M. P. Coughlan, ed. Permagon Press, London.

Cunningham, H. M., Brown, J. M. and Edie, A. E. 1953. Molybdenum poisoning of cattle in the Swan River Valley of Manitoba. Can. J. Agr. Sci. 33: 254-260.

Davey, B. G. and Mitchell, R. L. 1968. The distribution of trace elements in cocksfoot (Dactylis glomerata) at flowering. J. Sci. Fd. Agric. 19: 423-431.

Davis, G. K. 1950. The influence of copper on the metabolism of phosphorus and molybdenum in Symposium on Copper Metabolism, 1950. W. D. McElroy and B. Glass, eds. John Hopkins Press, Maryland. As cited by Underwood, E. J., 1977.

Dick, A. T. 1954. Studies on the assimilation and storage of copper in cross-bred sheep. Aust. J. Agric. Res. 5: 511-544.

Dick, A. T. 1956. Molybdenum in animal nutrition. Soil Sci. 81: 229-258.

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

Doney, J. M., Peart, J. N., Smith, W. F. and Louda, F. 1979. A consideration of the techniques for estimation of milk yield by suckled sheep and a comparison of estimates obtained by two methods in relation to the effect of breed, level of production and stage of lactation. J. Agric. Sci. (Camb.) 92: 123-132.

Doney, J. M., Peart, J. N. and Smith, W. F. 1981. The effect of interaction of ewe and lamb genotype on milk production of ewes and on growth of lambs to weaning. Anim. Prod. 33: 137-142.

Dowdy, R. P. and Matrone, G. 1968. Copper-molybdenum interaction in sheep and chicks. J. Nutr. 95: 191-195.

Dowdy, R. P., Kunz, G. A. and Sauberlich, H. E. 1969. Effect of a copper-molybdenum compound upon copper metabolism in the rat. J. Nutr. 99: 491-496.

Doyle, P. J. and Fletcher, K. 1977. Molybdenum content of bedrock, soils and vegetation and the incidence of copper deficiency in cattle in Western Manitoba in Molybdenum in the Environment. Vol. 2. The Geochemistry, Cycling and Industrial Uses of Molybdenum, 1977. W. R. Chappell and K. K. Peterson, eds. Marcel Dekker, Inc., New York.

Dunkley, W. L., Ronning, M. and Voth, J. 1963. Influence of injection of cows with copper glycinate on blood and milk copper and oxidized flavor in milk. J. Dairy Sci. 46: 1059-1063.

Edwin, E. E. and Jackman, R. 1982. Ruminant thiamine requirement in perspective. Vet. Res. Comm. 5: 237-250.

Ellis, W. C., Pfander, W. H., Muhrer, M. E. and Pickett, E. E. 1958. Molybdenum as a dietary essential for lambs. J. Anim. Sci. 17: 180-188.

Erwin, E. S., Marco, G. J. and Emery, E. M. 1961. Volatile fatty acid analysis of blood and rumen fluid by gas chromatography. J. Dairy Sci. 44: 1768-1771.

Evans, H. J., Purvis, E. R. and Bear, F. E. 1950. Molybdenum nutrition in alfalfa. Plant Physiol. 25: 555-566.

Farmer, P. E., Adams, T. E. and Humphries, W. R. 1982. Copper supplementation of drinking water for cattle grazing molybdenum-rich pasture. Vet. Rec. 111: 193-195.

Fell, B. F. 1981. Pathological consequences of copper deficiency and cobalt deficiency. Phil. Trans. R. Soc. Lond. B294: 153-169.

Fell, B. F., Dinsdale, D. and El-Gallad, T. T. 1979. Gut pathology of rats dosed with tetra-thiomolybdate. J. Comp. Path. 89: 495-514.

Field, A. C. and Purves, D. 1964. The intake of soil by grazing sheep. Proc. Nutr. Soc. 23: 24-25.

Fletcher, K. and Brink, V. C. 1969. Content of certain trace elements in range forages from south central British Columbia. Can. J. Plant Sci. 49: 517-520.

Frieden, E. 1980. Caeruloplasmin: a multi-functional metalloprotein of vertebrate plasma in Biological Roles of Copper. Ciba Foundation Symposium 79 (new series). Excerpta Medica, Oxford.

Frieden, E. and Hsieh, H. S. 1976. Ceruloplasmin: The copper transport protein with essential oxidase activity. Adv. Enzymol. 44: 187-235.

Gawthorne, J. M. and Nader, C. J. 1975. The effect of molybdenum on the conversion of sulphate to sulphide and microbial-protein-sulphur in the rumen of sheep. Br. J. Nutr. 35: 11-23.

Gill, J. L. and Hafs, H. D. 1971. Analysis of repeated measurements of animals. J. Anim. Sci. 33: 331-336.

Givens, D. I. and Hopkins, J. R. 1978. The availability of copper to grazing ruminants in parts of North Yorkshire. J. Agric. Sci., (Camb.) 91: 13-16.

Givens, D. I., Hopkins, J. R., Brown, M. E. and Walsh, W. A. 1981. The effect of copper therapy on the growth rate and blood composition of young growing cattle. J. Agric. Sci. (Camb.) 97: 497-505.

Goering, H. L. and Van Soest, P. J. 1970. Forage fiber analysis (apparatus reagents, procedures and some applications). Agriculture Handbook. No. 379. U.S. Department of Agriculture, Washington, D.C.

Grace, N. D. and Suttle, N. F. 1979. Some effects of sulphur intake on molybdenum metabolism in sheep. Br. J. Nutr. 35: 125-136.

Gupta, V. C. 1969. Effect and interaction of molybdenum and limestone on growth and molybdenum content of cauliflower, alfalfa, and bromegrass on acid soils. Soil Sci. Amer. Proc. 33: 929-932.

Gupta, V. C. 1970. The effect of limestone, molybdenum and incubation upon the extractable molybdenum in soils of eastern Canada. Plant Soil 33: 497-500.

Gupta, V. C. and McLeod, L. B. 1975. Effects of sulfur and molybdenum on the molybdenum, copper and sulfur concentrations of forage crops. Soil Sci. 119: 441-447.

Hainline, B. E. and Rajagopalan, K. V. 1983. Molybdenum in animal and human health in Trace Elements in Health, 1983. T. Rose, ed. Butterworth & Co. (Publishers) Ltd., Toronto.

Hamm, J. W., Bettany, J. R. and Halstead, E. H. 1973. A soil test for sulfur and interpretive criteria for Saskatchewan. Comm. in Soil Sci. and Plant Anal. 4: 219-231.

Hansard, S. L. 1983. Microminerals for ruminant animals. Nutr. Abst. Rev. B. 53: 1-24.

Harris, E. D. 1983. Copper in human and animal health in Trace Elements in Health, 1983. J. Rose, ed. Butterworth & Co. (Publishers) Ltd., Toronto.

Harrison, D. G., Beever, D. E., Thomson, D. J. and Osbourn, D. F. 1976. Manipulation of fermentation in the rumen. J. Sci. Food Agr. 27: 617-620.

Hart, L. J., Owen, E. C. and Proudfoot, R. 1967. The influence of dietary molybdenum on the xanthine oxidase activity of the milk of ruminants. Br. J. Nutr. 21: 617-630.

Hartmans, J. and Bosman, M. S. M. 1970. Differences in the copper status of grazing and housed cattle and their biochemical backgrounds in Trace Element Metabolism in Animals, 1970. C. F. Mills, ed. E & S Livingstone, London.

Healy, W. B., Rankin, P. C. and Watts, H. M. 1974. Effect of soil contamination on the element composition of herbage. New Zealand J. Agric. Res. 17: 59-61.

Hidiroglou, M. and Knipfel, J. E. 1981. Maternal-fetal relationships of copper, manganese, and sulfur in ruminants. A review. J. Dairy Sci. 64: 1637-1647.

Higgins, E. S., Richert, D. A. and Westerfeld, W. W. 1956. Molybdenum deficiency and tungstate inhibition studies. J. Nutr. 59: 539-559.

Hodgson, J. and Jamieson, W. S. 1981. Variations in herbage mass and digestibility, the grazing behavior and herbage intake of adult cattle and weaned calves. Grass & Forage Sci. 36: 39-48.

Hogan, K. G. and Hutchinson, A. J. 1965. N.Z. J. Agric. Res. 8: 625. As cited by Underwood, E. J., 1977.

Hogan, K. G., Money, D. F. L., White, D. A. and Walker, R. 1971. Weight responses of young sheep to copper, and connective tissue lesions associated with the grazing of pastures of high molybdenum content. N.Z. J. Agric. Res. 14: 687-701.

Howell, J. McC., Edington, N. and Ewbank, R. 1968. Observations on copper and caeruloplasmin levels in blood of pregnant ewes and lambs. Res. Vet. Sci. 9: 160-164.

Huber, J. T., Price N. O. and Engel, R. W. 1971. Response of lactating dairy cows to high levels of dietary molybdenum. J. Anim. Sci. 32: 364-367.

Huisingh, J. and Matrone, G. 1972. Copper-molybdenum interactions with the sulfate-reducing system in rumen microorganisms. Proc. Soc. Exp. Biol. Med. 139: 518-521.

Huisingh, J. and Matrone, G. 1977. Interactions of molybdenum in animal nutrition in Molybdenum in the Environment. Vol. I. The biology of molybdenum, 1977. W. R. Chappell and K. K. Peterson, eds. Marcel Dekker Inc., New York.

Huisingh, J., Milholland, D. C. and Matrone, G. 1975. Effect of molybdate on sulfide production from methionine and sulfate by ruminal microorganisms of sheep. J. Nutr. 105: 1199-1205.

Hynes, M., Lamand, M., Montel, G. and Mason, J. 1984. Some studies on the metabolism and the effects of ^{99}Mo- and ^{35}S-labelled thiomolybdates after intravenous infusion of sheep. Br. J. Nutr. 52: 149-158.

Ishida, N., Yoshikawa, T. and Kawashima, R. 1982. Effects of oral and intravenous administrations of molybdenum and sulphate on the distribution of copper in blood of sheep. Memoirs of the College of Agriculture 119: 23-34.

Ivan, M. and Grieve, C. M. 1976. Effects of zinc, copper and manganese supplementation of high-concentrate ration on gastrointestinal absorption of copper and manganese in Holstein calves. J. Dairy Sci. 59: 1764-1768.

Jarvis, S. C. and Austin, A. R. 1983. Soil and plant factors limiting the availability of copper to a beef suckler herd. J. Agric. Sci. (Camb.) 101: 39-46.

Johnson, J. L., Rajagopalan, K. V. and Cohen, H. J. 1974. Molecular basis of the biological function of molybdenum. Effect of tungsten and xanthine oxidase and sulphite oxidase in the rat. J. Biol. Chem. 249: 859-866.

Johnson, J. L., Jones, H. P. and Rajagopalan, K. V. 1977. In vitro reconstitution of demolybdosulphite oxidase by a molybdenum cofactor from rat liver and other sources. J. Biol. Chem. 252: 4994-5003.

Johnson, J. L., Waud, W. R., Rajagopalan, K. V., Duran, M., Beemer, F. A. and Wadman, S. K. 1980. Inborn errors of molybdenum metabolism: combined deficiencies of sulfite oxidase and xanthine oxidase in a patient lacking the molybdenum cofactor. Proc. Natl. Acad. Sci. USA 77: 3715-3719.

Kellaway, R. C., Sitorus, P. and Leibholz, J. M. L. 1978. The use of copper levels in hair to diagnose hypocuprosis. Res. Vet. Sci. 24: 352-357.

Kelleher, C. A., Ivan, M., Lamand, M. and Mason, J. 1983. The absorption of labelled molybdenum compounds in sheep fitted with re-entrant cannulae in the ascending duodenum. J. Comp. Path. 93: 83-92.

Kennedy, P. M., Williams, E. R. and Siebert, B. D. 1975. Sulfate recycling and metabolism in sheep and cattle. Aust. J. Biol. Sci. 28: 31-41.

Kienholz, E. W. 1977. Effects of environmental molybdenum upon wildlife in Molybdenum in the Environment. Vol. 2. The Geochemistry, Cycling and Industrial Uses of Molybdenum, 1977. W. R. Chappell and K. K. Peterson, eds. Marcel Dekker, Inc., New York.

Kiermeier, F. and Capellari, K. 1958. Biochem. Z. 330: 160. As cited by Hart et al., 1967.

Kleeman, D. O., Dolling, C. H. S. and Ponzoni, R. W. 1981. The contribution of maternal environment and lamb genotype to growth of lambs from Merino, Poll Dorset X Merino and Border Leicester X Merino Ewes. Aust. J. Agric. Res. 32: 965-973.

Kovalsky, V. V. 1970. The geochemical ecology of organisms under conditions of varying contents of trace elements in the environment in Trace Element Metabolism in Animals, 1970. C. F. Mills, ed. E & S Livingstone, London.

Kubota, J., Lemon, E. R. and Allaway, W. H. 1963. The effect of soil moisture content upon the uptake of molybdenum, copper and cobalt by alsike clover. Soil Sci. Soc. Amer. Proc. 27: 679-683.

Lamand, M. 1977. Relationship between different criteria of nutritional quality and availability of trace elements of forages in sheep in Trace Element Metabolism in Man and Animals. 3. 1977. M. Kirchgesmer, ed. Arbeitskries fur Tierernahrungsforschung, Weihenstephan.

Lamand, M., Lab, C., Tressol, J. C. and Mason, J. 1980. Biochemical parameters useful for the diagnosis of mild molybdenosis in sheep. Ann. Rech. Vet. 11: 141-145.

Lamond, D. R., Holmes, J. H. G. and Haydock, K. P. 1969. Estimation of milk yield and composition of milk produced by grazing beef cows. J. Anim. Sci. 29: 606-611.

Langlands, J. P. 1967. Studies on the nutritive value of the diet selected by grazing sheep. II. Some sources of error when sampling oesophageally fistulated sheep at pasture. Anim. Prod. 9: 167-175.

Langlands, J. P. and Sutherland, H. A. M. 1973. Sulfur as a nutrient for Merino sheep 1. Storage of sulfur in tissues and wool, and its secretion in milk. Br. J. Nutr. 30: 529-535.

Langlands, J. P., Bowles, J. E., Donald, G. E., Smith, A. J. and Paull, D. R. 1981. Copper status of sheep grazing pastures fertilized with sulphur and molybdenum. Aust. J. Agric. Res. 32: 479-486.

Lavy, T. L. and Barber, S. A. 1964. Movement of molybdenum in the soil and its effect on availability to the plant. Soil Sci. Amer. Proc. 28: 93-97.

Lawrence, C. B., Davies, N. T. and Mills, C. F. 1982. Noradrenaline concentrations in gut muscle and mucosa of the copper-deficient steer and rat. Comp. Biochem. Physiol. "C" 73: 37-40.

Leach, R. M. and Norris, C. L. 1957. Studies on factors affecting the response of chicks to molybdenum. J. Poultry Sci. 36: 1136.

Lesperance, A. L. and Bohman, V. R. 1963. Effect of inorganic molybdenum and type of roughage on the bovine. J. Anim. Sci. 22: 686-694.

Lloyd, W. E., Hill, H. T. and Meerdink, G. L. 1977. Observations of a case of molybdenosis-copper deficiency in a South Dakota Dairy Herd in Molybdenum in the Environment. Vol. 1. The Biology of Molybdenum, 1977. W. R. Chappell and K. K. Peterson, eds. Marcel Dekker, Inc., New York.

Lönnerdal, B., Keen, C. L. and Hurley, L. S. 1981. Iron, copper, zinc and manganese in milk. Ann. Rev. Nutr. 1: 149-174.

McAllan, A. B. and Smith, R. H. 1983. Estimation of flows of organic matter and nitrogen components in postruminal digesta and effects of level of dietary intake and physical form of protein supplement on such estimates. Br. J. Nutr. 49: 119-127.

McCance, J. 1959. The determination of milk yield in the Merino ewe. Aust. J. Agric. Res. 10: 839-853.

McMurray, C. H. 1980. Copper deficiency in ruminants. Pages 183-204 in Biological Roles of Copper. Ciba Foundation Symposium 79 (new series). Excerpta Medica, Amsterdam.

Marston, H. R. 1952. Cobalt, copper and molybdenum in the nutrition of animals and plants. Physiol. Rev. 32: 66-121. As cited by McMurray, C. H., 1980.

Marth, E. H., ed. 1978. Standard Methods for the Examination of Dairy Products, 14th ed. American Public Health Association, Inc., Washington.

Martinez, A. and Church, D. C. 1970. Effect of various mineral elements on in vitro rumen cellulose digestion. J. Anim. Sci. 31: 982-990.

Mason, J. 1978. The relationship between copper, molybdenum and sulphur in ruminant and non-ruminant animals: a preview. Vet. Sci. Communications 2: 85-94.

Mason, J. 1981. Molybdenum-copper antagonism in ruminants: A review of the biochemical basis. Irish Vet. J. 35: 221-229.

Mason, J. and Cardin, C. J. 1977. The competition of molybdate and sulfate ions for a transport system in the ovine small intestine. Res. Vet. Sci. 22: 313-315.

Mason, J., Cardin, C. J. and Dennehy, A. 1978a. The role of sulphide and sulphide oxidation in the copper molybdenum antagonism in rats and guinea pigs. Res. Vet. Sci. 24: 104-108.

Mason, J., Lamand, M., Tressol, J. C. and Lab, C. 1978b. The influence of dietary suphur, molybdate and copper on the absorption, excretion and plasma fraction levels of ^{99}Mo in sheep. Ann. Rech. Vet. 3: 577-586.

Mason, J., Kelleher, C. A. and Letters, J. 1982. The demonstration of protein-bound ^{99}Mo-di- and trithiomolybdate in sheep plasma after the infusion of ^{99}Mo-labelled molybdate into the rumen. Br. J. Nutr. 48: 391-397.

Mertz, W. 1970. Some aspects of nutritional trace element research. Fed. Proc., Fed. Amer. Soc. Exp. Biol. 29: 1482-1489.

Miller, J. F., Price, N. O. and Engel, R. W. 1956. Added dietary inorganic sulfate and its effect upon rats fed molybdenum. J. Nutr. 60: 539-547.

Miller, J. K., Moss, B. R., Bell, M. C. and Sneed, N. N. 1972. Comparison of ^{99}Mo metabolism in young cattle and swine. J. Anim. Sci. 34: 846-850.

Mills, C. F. 1960. Comparative studies of copper, molybdenum and sulphur metabolism in the ruminant and the rat. Proc. Nutr. Soc. 19: 162-169.

Mills, C. F. 1980. Metabolic interactions of copper with other trace elements. Pages 49-69 in Biological Roles of Copper. Ciba Foundation Symposium 79. Experta Medica, New York.

Mills, C. F., Dalgarno, A. C. and Wenham, G. 1976. Biochemical and patholgical changes in tissues of Friesian cattle during the experimental induction of copper deficiency. Br. J. Nutr. 35: 309-331.

Mills, C. F., Bremner, I., El-Gallad, T. T., Dalgarno, A. C. and Young, B. W. 1978. Mechanisms of the molybdenum/sulphur antagonism of copper utilization by ruminants in Trace Element Metabolism in Man and Animals. Vol. 3, 1978. M. Kirchgesmer, ed. Freising. Weihenstephan: Arbeitskries Tierernahrungsforschung.

Miltimore, J. E., Mason, J. L., McArthur, J. M. and Carson, R. B. 1964. Ruminant Mineral Nutrition. The effect of copper injections on weight gains and haemoglobin levels of cattle pastured on ground-water soils in the British Columbia interior. Can. J. Comp. Med. Vet. Sci. 28: 108-112.

Miltimore, J. E. and Mason, J. L. 1971. Copper to molybdenum ratio and molybdenum and copper concentrations in ruminant feeds. Can. J. Anim. Sci. 51: 193-200.

Moe, P. W., Tyrrell, H. F. and Flatt, W. P. 1971. Energetics of body tissue mobilization. J. Dairy Sci. 54: 548-553.

Mudgal, V. D., Dixon, R. M., Kennedy, P. M. and Milligan, L. P. 1982. Effect of two intake levels on retention times of liquid, particle and microbial markers in the rumen of sheep. J. Anim. Sci. 54: 1051-1055.

National Academy of Sciences. National Research Council. 1975. No. 5. Nutrient requirements of sheep, 5th ed. National Academy of Sciences, Washington, D.C.

National Academy of Sciences. National Research Council. 1976. No. 4. Nutrient requirements of beef cattle. National Academy of Sciences, National Research Council, Washington, D.C.

National Academy of Sciences. National Research Council. 1978. No. 3. Nutrient requirements of dairy cattle, 5th ed. National Academy of Sciences, Washington, D.C.

Nederbragt, H. 1982. Changes in the distribution of copper and molybdenum after Mo administration and subsequent additional oral or intraperitoneal Cu administration to rats. Br. J. Nutr. 48: 353-364.

Nederbragt, H. and Van dem Hamer, C. J. A. 1981. Changes in the binding of copper in the plasma of molybdenum supplemented rats. J. Inorg. Biochem. 15: 293-306.

Nell, J. A. and Annison, E. F. 1980. Molybdenum requirements of chickens. Br. J. Poult. Sci. 21: 183-191.

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

Nikolić, J. A., Jovanović, M., Andrić, R., Djordjević, D. and Krsmanović, J. 1983. Examination of the effect of copper and molybdenum on microbial protein synthesis in rumen contents using ^{35}S. Int. J. Appl. Radiat. Isot. 34: 809-812.

Owen, C. A. and Hazelrig, J. B. 1966. Metabolism of Cu^{64} labelled copper in the isolated rat liver. Am. J. Physiol. 210: 1059-1064.

Patterson, D. S. P., Sweasey, D. and Herbert, C. D. J. 1971. Changes occuring in the chemical conformation of the central nervous system during faetal and post-natal development in sheep. J. Neurochem. 18: 2027-2040.

Payne, C. G. 1977. Involvement of molybdenum in feather growth. Br. J. Poult. Sci. 18: 427-432.

Payne, C. G. and Bains, B. S. 1975. Femoral degeneration and scabby hip syndrome in chicks. Vet. Rec. 97: 436-437.

Paynter, D. I. 1982. Differences between serum and plasma ceruloplasmin activities and copper concentrations. Investigations of possible contributing factors. Aust. J. Biol. Sci. 35: 353-361.

Paynter, D. I. and Allen, J. D. 1982. Copper superoxide dismutase and copper deficiency in ruminants in Trace Element Metabolism in Man and Animals, 1982. J. M. Gawthorne, J. McC. Howell and C. L. White, eds. Springer Verlag, New York.

Peart, N. J., Edwards, R. A., and Donaldson, E. 1972. The yield and composition of the milk of Finnish Landrace X Blackface ewes. I. Ewes and lambs maintained indoors. J. Agric. Sci. (Camb.) 79: 303-313.

Pienkos, P. T., Shah, V. K., and Brill, W. J. 1977. Molybdenum cofactors from molybdoenzymes and in vitro reconstitution of nitrogenase and nitrate reductase. Proc. Natl. Acad. Sci. USA 74: 5468-5471.

Pitt, M., Fraser, J., and Thurley, D. C. 1980. Molybdenum toxicity in sheep: epiphysiolysis, exotoses and biochemical changes. J. Comp. Path. 90: 567-576.

Pojerova, A. and Tovarek, J. 1960. Ceruloplasmin in early childhood. Acta Paediat. (Stockholm) 49: 113-120. As cited by Aspin, N. and Sass-Kortsak, A., 1981.

Poole, D. B. R. 1970. Cytochrome oxidase in induced hypocuprosis in Trace Element Metabolism in Animals, 1970. C. F. Mills, ed. E & S Livingstone, London.

Poole, D. B. R. and Walshe, M. J. 1970. Copper status and milk yield of dairy cows in Trace Element Metabolsim in Animals, 1970. C. F. Mills, ed. E & S Livingstone, London.

Puls, R. 1981. Veterinary Trace Mineral Deficiency and Toxicity Information. Publication 5139. Information Services, Agriculture Canada, Ottawa.

Rajagopalan, K. V. 1980. Sulphite oxidase (sulfite: ferricytochrome c oxidoreductase) in Molybdenum and Molybdenum-containing enzymes, 1980. M. P. Coughlan, ed. Permagon Press, London.

Ray, A. A. 1982. SAS User's Guide: Statistics, 1982 Edition. SAS Institute Inc., Cary, North Carolina.

Reid, J. T. and Robb, J. 1971. Relationship of body composition to energy intke and energetic efficiency. J. Dairy Sci. 54: 553-564.

Reid, R. L. and Horvath, D. J. 1980. Soil chemistry and mineral problems in farm livestock. A review. Anim. Feed Sci. Technol. 5: 95-167.

Reith, J. W. S., Burridge, J. C., Berrow, M. L. and Caldwell, K. S. 1984. Effects of fertilizers on the contents of copper and molybdenum in herbage cut for conservation. J. Sci. Food. Agric. 54: 245-256.

Renzo, E. C. de, Kaleita, E., Heytler, P., Oleson, J. J., Hutchings, B. L. and Williams, J. H. 1953. Identification of xanthine oxidase factor as molybdenum. Arch. Biochem. Biophys. 45: 247-253.

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

Richert, D. A., Bloom, R. J. and Westerfeld, W. W. 1957. Purine catabolism in molybdenum deficiency. J. Biol. Chem. 227: 523-531.

Robinson, G. A., McCarter, A., Rowsell, H. C., Meads, E. B., Riddell P. E. and Taylor, P. A. 1966. The biological half-life of molybdenum in normal and nutritionally deficient cows. Am. J. Vet. Res. 25: 1040-1043.

Roe, D. A., Miller, P. S. and Lutwak, L. 1966. Estimation of sulfur in biological materials by atomic absorption spectrometry. Anal. Biochem. 15: 313-322.

Rogers, P. A. M. and Poole, D. B. R. 1977. Effects of copper edate injection on copper and copper enzyme status of blood and liver in cattle and on the milk yield of copper deficient versus treated cows in Proceedings of the Third International Conference on Production Disease in Farm Animals, 1977. P. M. W. Van Adrichem, ed. Centre for Agricultural Publishing and Documentation, Netherlands.

Rose, J., ed. 1983. Trace Elements in Health. Butterworth and Co. (Publishers) Ltd., Toronto.

Ruston, J. D. 1983. Copper metabolism in growing beef bull calves and grazing yearling heifers. M.Sc. Thesis, Univ. of Man.

Shyamala, G. and Leonard, L. 1980. Inhibition of uterine estrogen receptor transformation by sodium molybdate. J. Biol. Chem. 255: 6028-6031.

Simpson, A. M., Mills, C. F. and McDonald, I. 1982. Tissue copper retention or loss in young growing cattle in Trace Element Metabolism in Man and Animals, 1982. J. M. Gawthorne, J. McC. Howell and C. L. White, eds. Springer-Verlag, New York.

Smith, B. and Coup, M. R. 1973. Hypocuprosis: A clinical investigation of dairy herds in Northland. N.Z. Vet. J. 21: 252-258.

Smith, B. S. W. and Wright, H. 1974. Improved manual and automated procedures for estimation of caeruloplasmin oxidase activity. Clin. Chim. Acta. 50: 359-366.

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

Snedecor, G. E. and Cochrane, W. G. 1980. Statistical methods, seventh ed. Iowa State University Press, Ames, Iowa, USA.

Suttle, N. F. 1974a. Effects of organic and inorganic sulphur on the availability of dietary copper to sheep. Br. J. Nutr. 32: 559-568.

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

Suttle, N. F. 1975. Changes in the availability of dietary copper to young lambs associated with age and weaning. J. Agric. Sci. (Camb.) 84: 255-261.

Suttle, N. F. 1980. Some preliminary observations on the absorbability of copper in fresh and conserved grass. Proc. Nutr. Soc. 39: 63A.

Suttle, N. F. 1982. Predicting the effects of molybdenum and sulfur concentrations on the absorbability of copper in grass and forage crops to ruminants in Trace Element Metabolism in Man and Animals, 1982. J. M. Gawthorne, J. McC. Howell and C. L. White, eds. Spanger-Verdag., New York.

Suttle, N. F. 1983. Effects of molybdenum concentration in fresh herbage, hay and semi-purified diets on the copper metabolism of sheep. J. Agric. Sci. (Camb.) 100: 651-656.

Suttle, N. F. and McLauchlan, M. 1976. Predicting the effects of dietary molybdenum and sulphur on the availability of copper in ruminants. Proc. Nutr. Soc. 35: 22A-23A.

Suttle, N. F. and Grace, N. D. 1978. A demonstration of marked recycling of molybdenum via the gastrointestinal tract of sheep at low sulphur intakes. Proc. Nutr. Soc. 37: 68A.

Suttle, N. F. and Field, A. C. 1983. Effects of dietary supplements of thiomolybdates on copper and molybdenum metabolism in sheep. J. Comp. Path. 93: 379-389.

Suttle, N. F., Alloway, B. J. and Thornton, I. 1975. An effect of soil ingestion on the utilization of dietary copper by sheep. J. Agric. Sci. (Camb.) 84: 249-254.

Suttle, N. F., Field, A. C., Nicolson, T. B., Mathieson, A. O., Prescott, J. D. H., Scott, N. and Johnson, W. S. 1980. Some problems in assessing the physiological and economic significance of hypocupraemia in beef suckler herds. The Vet. Rec. 106: 302-304.

Thompson, R. H. and Blanchflower, W. J. 1971. Wet-ashing apparatus to prepare biological materials for atomic absorption spectrophotometry. Lab. Prac. 20: 859-861.

Thornton, I. 1974. Biogeochemical and soil ingestion studies in relation to the trace element nutrition of livestock in Proceedings of Second International Symposium on Trace Element Metabolism in Animals. 2, 1974. W. G. Hoekstra, J. W. Suttie, H. E. Ganther and H. E. Mertz, eds. University Park Press, Baltimore, Maryland.

Thornton, I. 1977. Biochemical studies on molybdenum in the United Kingdom in Molybdenum in the Environment. Vol. 2. The Geochemistry, cycling and industrial uses of molybdenum, 1977. W. R. Chappell and K. Kellogg Peterson, eds. Marcel Dekker, Inc., New York.

Todd, J. R. 1976. Problems of copper-molybdenum imbalance in the nutrition of ruminants in Northern Ireland in Molybdenum in the Environment. Vol. 1, 1976. The biology of molybdenum. W. R. Chappell and K. K. Peterson, eds. Marcel Dekker, Inc., New York.

Underwood, E. J. 1971. Trace Elements in Human and Animal Nutrition, 3rd ed. Academic Press, New York.

Underwood, E. J. 1977. Trace Elements in Human and Animal Nutrition, 4th ed. Academic Press, New York.

Underwood, E. J., ed. 1981. The Mineral Nutrition of Livestock. Commonwealth Agricultural Bureaux, London. pp. 91-108.

Van Ryssen, J. B. J. and Stielau, W. J. 1981. Effect of different levels of dietary molybdenum on copper and molybdenum metabolism in sheep fed on high levels of Cu. Br. J. Nutr. 45: 203-210.

Van Soest, P. J., ed. 1982. Nutritional Ecology of the Ruminant. O & B Books, Inc., Cornwallis, Oregon.

Vanderveen, J. E. and Keener, H. A. 1964. Effects of molybdenum and sulphate sulphur on metabolism of copper in dairy cattle. J. Dairy Sci. 47: 1224-1230.

Ward, G. M. 1978. Molybdenum toxicity and hypocuprosis in ruminants. A review. J. Anim. Sci. 46: 1078-1085.

Weathers, B. J., Grate, J. H. and Schrauzer, G. N. 1979. The chemical evolution of a nitrogenase model. 17. Simulation of steric and of inhibitory effects at the enzymic active site with acetylenes and nitriles as substrates, and "molybdoinsulin" catalysts. J. Amer. Chem. Soc. 101: 917-924.

Weber, K. M., Boston, R. C. and Leaver, D. D. 1983. The effect of molybdenum and sulfur on the kinetics of copper metabolism in sheep. Aust. J. Agric. Res. 34: 295-306.

West, T. S. 1981. Soil as a source of trace elements. Phil. Trans. R. Soc. Lond. B. 294: 19-39.

Wiener, G., Wilmut, I. and Field, A. C. 1977. Maternal and lamb breed interactions in the concentration of copper in tissues and plasma of sheep in Trace Element Metabolism in Man and Animals. 3, 1977. M. Kirchgemner, ed. Arbeitskries fur Tierernahrungsforschung, Weihenstephan.

Wilson, L. G. and Bandurski, R. S. 1958. Enzymatic reactions involving sulfate, sulfite, selenate and molybdate. J. Biol. Chem. 223: 975-981.

Winston, P. W. 1981. Molybdenum in Disorders of Mineral Metabolism. Vol. 1. Trace Minerals, 1981. F. Bronner and J. W. Coburn, eds. Academic Press, Toronto.

Wolf, W. R. 1982. Trace element analysis in food in Clinical, Biochemical and Nutritional Aspects of Trace Elements, 1982. A. E. Prasaad, ed. Alan R. Liss, Inc., New York.

APPENDIX I

BEEF COW-CALF TRIAL

For Appendix tables I-15 to I-18:

Trt 0 refers to 0Mo

Trt 1 refers to 20Mo

Trt 2 refers to 40Mo

Period refers to week

Appendix Table I-1: Experimental diet allocation and background information for cows and calves used in the beef cow-calf trial.

Experimental Diet	Animal Numbers		Cow Age (Years)	Calf		
	Cow	Calf		Age (days)†	Sex‡	Sire breed
0Mo	36	59	2	32	M	Selkirk
	15	32	3	43	F	Selkirk
	156	71	9	28	M	Simmental
	17	86	4	16	F	Simmental
20Mo	166	40	2	39	M	Selkirk
	28	55	4	34	M	Selkirk
	43	54	9	35	M	Simmental
	27	84	5	17	F	Simmental
40Mo	23	69	3	29	M	Selkirk
	19	42	2	39	M	Selkirk
	29	51	10	35	M	Simmental
	18	70	3	23	F	Simmental

† Calf age (days) on day 1 of the trial.

‡ M - male, F - female.

Appendix Table I-2: Analysis of variance for cow plasma copper parameters for the beef cow-calf trial (means squares and degrees of freedom†).

Source of Variation:	Diet	Animal (Diet)	Week	Diet x Week	Error
Item:					
Plasma Cu, mg L^{-1}	1.754*† (2)	0.229 (9)	0.042*** (8)	0.015*** (16)	0.005 (72)
Cp oxidase activity, A min^{-1} .5 ml^{-1} ($x10^3$)	12.261* (2)	1.559 (9)	0.518*** (8)	0.085*** (16)	0.029 (72)
TCA-soluble Cu, mg L^{-1}	0.777** (2)	0.079 (9)	0.055*** (4)	0.013** (8)	0.004 (36)
TCA-insoluble Cu, % total plasma Cu ($x10^2$)	1.193** (2)	0.149 (9)	3.870*** (4)	0.177 (8)	0.425 (36)
TCA-insoluble Cu, mg L^{-1} ($x10^{-1}$)	0.154 (2)	0.051 (9)	0.239*** (4)	0.028 (8)	0.030 (36)

† Figures in parenthesis represent the degrees of freedom for the corresponding mean squares.

† Significant differences at: $*\alpha = 0.05$, $**\alpha = 0.01$, $***\alpha = 0.001$.

Appendix Table I-3: Analysis of variance of calf plasma and liver copper parameters for the beef cow-calf trial (mean squares and degrees of freedom†).

Source of Variation:	Diet	Animal (Diet)	Week	Diet x Week	Error
Item:					
Plasma Cu, mg L^{-1} ($x10^{-1}$)	0.322‡ (2)	1.397 (9)	0.593*** (8)	0.072 (16)	0.108 (71)
Cp oxidase activity, $\Delta A\ min^{-1}\ .5\ ml^{-1}$ ($x10^{2}$)	1.980 (2)	11.480 (9)	8.190*** (8)	0.504 (16)	0.777 (72)
TCA-soluble Cu, mg L^{-1} ($x10^{-2}$)	0.008 (2)	6.543 (9)	5.144** (4)	0.934 (8)	1.130 (35)
TCA-insoluable Cu, % total plasma Cu ($x10^{1}$)	4.967 (2)	6.769 (9)	74.728*** (4)	4.841 (8)	5.397 (35)
Liver Cu, mg kg^{-1} D.M. ($x10^{4}$)	2.844 (2)	0.917 (9)	2.746** (2)	0.101 (4)	0.331 (18)

† Figures in parenthesis represent the degrees of freedom for the corresponding mean squares.

‡ Significant differences at: $^{*}\alpha = 0.05$, $^{**}\alpha = 0.01$, $^{***}\alpha = 0.001$.

Appendix Table I-4: Analysis of variance for milk yield and composition for beef cows fed varying levels of dietary Mo (mean squares).

Source of Variation:	Diet	Animal (Diet)	Week	Diet x Week	Error
Degrees of Freedom:	2	9	8	16	72†
Milk yield	72.168	84.450	5.143***‡	5.136***	1.730
Fat corrected milk yield ($x10^2$)	3.586	2.212	0.492***	0.085	0.931
Butterfat content	24.659	10.064	14.040***	0.508	1.891
Protein content	0.017	0.298	0.084**	0.029	0.025
Lactose content	0.022	0.203	0.383***	0.021	0.0198
Copper content	0.028	0.019	0.010***	0.002	0.001
Copper yield	8.306	3.466	1.611***	0.383	0.219
Molybdenum content	10.756***	0.189	0.241**	0.139*	0.072
Molybdenum yield, ($x10^2$)	7.06**	0.439	0.251**	0.108	0.088

† Degrees of freedom for fat corrected milk yield and butterfat content were 71.

‡ Significant difference at: $*\alpha = 0.05$, $**\alpha = 0.01$, $***\alpha = 0.001$.

Appendix Table I-5: Analysis of variance for body weight and average daily gains of sucking calves (mean squares and degrees of freedom†).

Source of Variation:	Diet	Animal (Diet)	Week	Diet x Week	Error
Item:					
Body Weight, kg ($x10^3$)	2.269 (2)	3.027 (9)	2.027***‡ (8)	0.006 (16)	0.015 (72)
Average daily gain, kg	0.103 (2)	0.259 (9)	0.173 (8)	0.168* (14)	0.085 (62)

† Figures in parenthesis represent the degrees of freedom for the corresponding mean squares.

‡ Significant differences at: * $\alpha = 0.05$, ** $\alpha = 0.01$, *** $\alpha = 0.001$.

Appendix Table I-6: Analysis of variance for cow body weight gain and for calf liver Cu change in relation to initial liver Cu content (mean squares).

Source of Variation:	Diet	Error
Degrees of Freedom:	2	9
Cow ADG, kg (x 10^{-3})	1.825	41.108
Calf liver Cu change (10^{-5})	2.999	1.629

Appendix Table I-7: Analysis of variance for linear regressions describing diet by time interactions shown to be significant ($P < 0.05$) in the split-plot analyses (mean squares and degrees of freedom†).

Source of Variation:	Diet	Diet x Week	Error
Item:			
Cow plasma Cu	0.132***‡ (2)	0.119*** (3)	0.026 (102)
Cow TCA-soluble Cu	0.087** (2)	0.050* (3)	0.019 (54)
Cow Cp oxidase activity ($x10^2$)	7.751*** (2)	12.594*** (3)	1.891 (102)
Cow milk Mo	1.611**** (2)	0.119 (3)	0.106 (101)
Calf liver Cu ($x\ 10^4$)	9.203 (2)	1.766** (3)	0.494 (30)

† Figures in parenthesis represent the degrees of freedom for the corresponding mean squares.

‡ Significant differences at * $\alpha = 0.10$, ** $\alpha = 0.05$, *** $\alpha = 0.01$, **** $\alpha = 0.001$.

Appendix Table I-8: Analysis of variance for quadratic regressions describing diet by time interactions shown to be significant ($P < 0.05$) in the split plot analyses (mean squares and degrees of freedom †).

Source of Variation:	Diet	Diet x Week	$Week^2$	Diet x $Week^2$	Error
Item:					
Milk yield, kg d^{-1}) (0Mo and 20Mo combined)	7.812 (1)	18.391 (2)	6.003 (1)	17.050 (1)	9.196 (102)
Milk yield, kg d^{-1} (0Mo and 20Mo not combined)	4.612 (2)	13.163 (3)	16.474 (1)	10.121 (2)	9.440 (99)

† Figures in parenthesis represent the degrees of freedom for corresponding mean squares.

Appendix Table I-9: Plasma copper concentrations (mg L^{-1}) for beef cows fed varying levels of molybdenum for nine weeks.†

Sampling Time	DIET			SE
	0Mo	20Mo	40Mo	
Week 1	0.98 A	0.84 A	0.73 A	0.02
Week 2	0.94a A B	0.80a b, A B	0.63b, A	0.02
Week 3	0.92a A B	0.83a, A	0.54b, B	0.02
Week 4	0.91a A B	0.86a, A	0.54b, B	0.02
Week 5	1.01a A	0.92a, A	0.49b, B	0.02
Week 6	0.99a A	0.93a, A	0.54b, B	0.02
Week 7	0.95a A B	0.83a, A	0.41b, C	0.02
Week 8	0.93a A B	0.80a, A B	0.42b, C	0.02
Week 9	0.88a B	0.71a, B	0.38b, C	0.02
SE	0.08	0.08	0.08	

† Each value represents the mean of four animals.

a - b Values in the same row with different superscripts are significantly different, $\alpha = 0.05$.

A - C Values in the same column with different superscripts are significantly different, $\alpha = 0.05$.

Appendix Table I-10: Plasma TCA-soluble copper concentrations (mg L^{-1}) for beef cows fed varying levels of molybdenum for nine weeks.†

Sampling Time	DIET			SE
	0Mo	20Mo	40Mo	
Week 1	0.80a, A B	0.65a b, B	0.55b, A	0.02
Week 3	0.72a, B C	0.61a, B	0.38b, B	0.02
Week 5	0.86a, A	0.78a, A	0.41b, B	0.02
Week 7	0.71a, C	0.60a, B	0.28b, C	0.02
Week 9	0.74a, B C	0.64a, B	0.30c, C	0.02
SE	0.06	0.06	0.06	

† Each value represents the mean of four animals.

a - b Values in the same rows with different superscripts are significantly different, $\alpha = 0.05$.

A - C Values in the same columns with different superscripts are significantly different, $\alpha = 0.05$.

Appendix Table I-11: Plasma ceruloplasmin oxidase activity (Δ A min^{-1} L^{-1}) for beef cows fed varying levels of molybdenum for nine weeks.†

Sampling Time	DIET 0Mo	20Mo	40Mo	SE
Week 1	67.3^{a}, B	60.4^{a}, A B	44.9^{b}, A	1.6
Week 2	68.6^{a}, B	56.7$^{a\ b}$, B C	37.2^{b}, A B	1.6
Week 3	56.6^{a}, C	54.9$^{a\ b}$, B C	33.1^{b}, B	1.6
Week 4	76.9^{a}, A	67.5^{a}, A	36.4^{b}, B	1.6
Week 5	71.3^{a}, A B	65.5^{a}, A	31.8^{b}, B	1.6
Week 6	71.0^{a}, A B	68.0^{a}, A	30.2^{b}, B	1.6
Week 7	55.1^{a}, C	53.6^{a}, B C	21.3^{b}, C	1.6
Week 8	67.2^{a}, B	57.2^{a}, B	24.0^{b}, C	1.6
Week 9	56.6^{a}, C	48.8^{a}, C	19.3^{b}, C	1.6
SE	6.6	6.6	6.6	

† Each value represents the means of four animals.

a - b Values in the same row with different superscripts are significantly different, $\alpha = 0.05$.

A - C Values in the same column with different superscripts are significantly different, $\alpha = 0.05$.

Appendix Table I-12: Molybdenum concentrations (mg L^{-1}) in milk produced by beef cows fed varying levels of dietary molybdenum for nine weeks.

Sampling Time	DIET 0Mo	20Mo	40Mo	SE
Week 1	0.21[a]	0.25[a], A	0.73[b], A	0.08
Week 2	0.12[a]	0.36[a], A	1.11[b], B C	0.08
Week 3	0.12[a]	0.58[b], A B	1.29[c], C	0.08
Week 4	0.11[a]	0.62[b], A B	1.41[c], C	0.08
Week 5	0.13[a]	0.79[b], B	1.64[c], C	0.08
Week 6	0.07[a]	0.47[b], A B	0.80[c], A B	0.08
Week 7	0.08[a]	0.42[b], A B	1.34[c], C	0.08
Week 8	0.03[a]	0.53[b], A B	1.52[c], C	0.08
Week 9	0.05[a]	0.63[b], B	0.93[c], A B C	0.08
SE	0.07	0.07	0.07	

† Each value represents the mean of four animals.

a - c Values in the same rows with different superscripts are significantly different, $\alpha = 0.05$.

A - C Values in the same column with different superscripts are significantly different, $\alpha = 0.05$.

Appendix Table I-13: Estimated milk yield (kg d^{-1}) for beef cows fed varying levels of molybdenum for nine weeks.†

Sampling Time	DIET			SE
	0Mo	20Mo	40Mo	
Week 1	8.9 A	9.4	10.5 B	0.7
Week 2	10.9 A B	10.7	9.2 A B	0.7
Week 3	12.7 B	10.6	9.0 A B	0.7
Week 4	10.6 A B	11.9	7.5 A B	0.7
Week 5	10.2 A B	12.0	8.8 A B	0.7
Week 6	11.8 A B	11.2	7.7 A B	0.7
Week 7	10.4 A B	11.7	8.4 A B	0.7
Week 8	10.1 A B	9.8	6.2 A	0.7
Week 9	10.7 A B	9.5	7.2 A	0.7
SE	1.5	1.5	1.5	

† Each value represents the mean of four animals.

A - B Values in the same column with different superscripts are significantly different, $\alpha = 0.05$.

Appendix Table I-14: Liver copper concentrations (mg kg^{-1} D.M.) of calves sucking cows fed varying levels of molybdenum.†

Sampling Time	DIET 0Mo	20Mo	40Mo	SE
Week 1	216.0 A	195.5 A	131.8 A	16.6
Week 5	196.9^{a} A	181.4^{a}, A	84.8^{b}, A B	16.6
Week 9	135.3^{a} B	83.0^{a} b, B	46.3^{b}, B	16.6
SE	27.6	27.6	27.6	

† Each value represents the mean of four animals.

a - b Values in the same row with different superscripts are significantly different, $\alpha = 0.05$.

A - B Values in the same column with different superscripts are significantly different, $\alpha = 0.05$.

Appendix Table I-15: Raw data for plasma copper parameters of cows in the beef cow-calf trial.

COW NUMBER	TRT	PERIOD	TOTAL PLASMA COPPER (MG/L)	TCA-SOLUBLE COPPER (MG/L)	PLASMA CP OXIDASE ACTIVITY (ΔA/MIN/L)
15	0	1	1.01	0.83	65.8
15	0	2	0.94	.	66.8
15	0	3	0.93	0.71	58.0
15	0	4	0.88	.	79.4
15	0	5	0.99	0.87	73.0
15	0	6	0.85	.	80.0
15	0	7	0.98	0.75	53.2
15	0	8	0.93	.	62.2
15	0	9	0.87	0.72	61.2
17	0	1	0.76	0.63	51.8
17	0	2	0.74	.	51.4
17	0	3	0.76	0.56	47.6
17	0	4	0.88	.	64.8
17	0	5	0.79	0.72	65.0
17	0	6	0.86	.	62.6
17	0	7	0.84	0.58	47.4
17	0	8	0.83	.	64.4
17	0	9	0.84	0.68	53.4
36	0	1	1.18	1.02	84.4
36	0	2	1.17	.	87.2
36	0	3	1.11	0.93	63.8
36	0	4	1.06	.	93.6
36	0	5	1.17	0.93	77.0
36	0	6	1.42	.	75.8
36	0	7	1.12	0.85	65.6
36	0	8	1.02	.	77.6
36	0	9	0.95	0.84	61.0
156	0	1	0.96	0.73	67.0
156	0	2	0.90	.	68.8
156	0	3	0.86	0.67	57.0
156	0	4	0.82	.	69.6
156	0	5	1.07	0.92	70.0
156	0	6	0.85	.	65.6
156	0	7	0.84	0.66	54.2
156	0	8	0.95	.	64.4
156	0	9	0.84	0.72	50.6
27	1	1	1.10	0.81	82.8
27	1	2	1.04	.	82.6
27	1	3	1.00	0.75	68.2
27	1	4	1.09	.	89.4
27	1	5	1.04	0.93	78.8
27	1	6	0.98	.	78.6
27	1	7	0.99	0.70	79.0
27	1	8	1.00	.	70.8
27	1	9	0.92	0.78	68.4
28	1	1	0.58	0.46	41.0
28	1	2	0.59	.	31.2
28	1	3	0.56	0.40	35.6
28	1	4	0.61	.	38.6
28	1	5	0.73	0.57	48.8
28	1	6	0.70	.	44.2
28	1	7	0.58	0.43	30.6
28	1	8	0.58	.	35.8

Appendix Table I-15--Continued

COW NUMBER	TRT	PERIOD	TOTAL PLASMA COPPER (MG/L)	TCA-SOLUBLE COPPER (MG/L)	PLASMA CP OXIDASE ACTIVITY (ΔA/MIN/L)
28	1	9	0.54	0.45	35.0
43	1	1	1.04	0.86	77.0
43	1	2	1.02	.	74.2
43	1	3	1.03	0.70	74.8
43	1	4	1.07	.	83.0
43	1	5	1.04	0.79	72.8
43	1	6	1.18	.	87.6
43	1	7	1.03	0.74	63.2
43	1	8	1.00	.	73.2
43	1	9	0.86	0.84	57.0
166	1	1	0.64	0.48	40.8
166	1	2	0.63	.	38.6
166	1	3	0.73	0.59	40.8
166	1	4	0.68	.	58.8
166	1	5	0.88	0.81	61.4
166	1	6	0.86	.	61.4
166	1	7	0.72	0.54	41.4
166	1	8	0.64	.	48.8
166	1	9	0.54	0.48	34.6
18	2	1	0.47	0.38	30.0
18	2	2	0.44	.	26.6
18	2	3	0.37	0.26	22.4
18	2	4	0.42	.	25.6
18	2	5	0.45	0.31	23.6
18	2	6	0.41	.	22.4
18	2	7	0.29	0.20	16.6
18	2	8	0.32	.	16.0
18	2	9	0.28	0.21	16.2
19	2	1	0.72	0.44	33.8
19	2	2	0.64	.	28.4
19	2	3	0.47	0.32	25.4
19	2	4	0.54	.	29.4
19	2	5	0.46	0.45	34.0
19	2	6	0.64	.	29.6
19	2	7	0.41	0.28	21.4
19	2	8	0.38	.	21.6
19	2	9	0.37	0.30	16.0
23	2	1	0.78	0.65	56.8
23	2	2	0.68	.	43.6
23	2	3	0.62	0.45	37.6
23	2	4	0.57	.	43.6
23	2	5	0.54	0.40	30.4
23	2	6	0.48	.	26.8
23	2	7	0.41	0.27	20.4
23	2	8	0.42	.	23.2
23	2	9	0.43	0.36	16.4
29	2	1	0.95	0.72	58.8
29	2	2	0.75	.	50.2
29	2	3	0.68	0.50	47.0
29	2	4	0.63	.	47.0
29	2	5	0.51	0.46	39.0
29	2	6	0.63	.	41.8
29	2	7	0.54	0.36	26.8

Appendix Table I-15--Continued

COW NUMBER	TRT	PERIOD	TOTAL PLASMA COPPER (MG/L)	TCA-SOLUBLE COPPER (MG/L)	PLASMA CP OXIDASE ACTIVITY (ΔA/MIN/L)
29	2	8	0.57	.	35.2
29	2	9	0.45	0.34	28.4

Appendix Table I-16: Raw data for milk yield and composition for cows in the beef cow-calf trial.

COW NUMBER	TRT	PERIOD	DAILY MILK PRODUCTION (KG)	FAT CORRECTED MILK YIELD (KG/DAY)	MILK FAT (%)	MILK PROTEIN (%)	MILK LACTOSE (%)	MILK MOLYBDENUM (MG/L)	MILK COPPER (MG/L)
15	0	1	7.98	9.14	4.97	3.56	5.43	0.24	0.14
15	0	2	11.27	15.36	6.42	3.49	5.04	0.22	0.14
15	0	3	11.74	17.37	7.20	3.42	4.78	0.14	0.19
15	0	4	9.32	13.55	7.03	3.70	4.87	0.12	0.24
15	0	5	10.45	14.99	6.90	3.67	5.01	0.13	0.17
15	0	6	10.65	13.30	5.66	3.55	5.12	0.11	0.13
15	0	7	9.59	11.63	5.42	3.57	5.25	0.10	0.14
15	0	8	10.53	13.24	5.72	3.40	5.07	0.00	0.14
15	0	9	10.12	9.71	3.73	3.55	5.40	0.06	0.10
17	0	1	6.76	.	.	4.44	5.50	0.18	0.23
17	0	2	10.30	16.01	7.70	3.75	5.42	0.12	0.23
17	0	3	13.86	22.38	8.10	3.52	4.90	0.10	0.30
17	0	4	11.03	20.04	9.45	3.64	4.76	0.23	0.45
17	0	5	10.48	18.18	8.90	3.88	4.96	0.15	0.25
17	0	6	12.19	19.46	7.98	3.37	4.89	0.05	0.19
17	0	7	11.64	20.26	8.94	3.40	5.07	0.08	0.19
17	0	8	10.81	17.16	7.92	3.58	5.05	0.04	0.15
17	0	9	11.72	18.71	7.98	3.51	5.28	0.08	0.14
36	0	1	6.83	6.73	3.91	3.44	5.47	0.20	0.07
36	0	2	6.56	6.50	3.94	3.42	5.40	0.07	0.07
36	0	3	7.20	5.50	2.43	3.61	5.23	0.14	0.08
36	0	4	5.81	5.75	3.94	3.84	5.35	0.07	0.12
36	0	5	4.80	5.76	5.34	3.85	5.47	0.04	0.08
36	0	6	6.04	5.86	3.81	3.59	5.44	0.05	0.08
36	0	7	4.93	5.18	4.35	3.50	5.62	0.03	0.09
36	0	8	4.83	4.46	3.50	3.51	5.54	0.00	0.07
36	0	9	6.40	6.43	4.04	3.48	5.60	0.00	0.06
156	0	1	14.04	14.08	4.02	3.50	5.65	0.23	0.18
156	0	2	15.41	18.55	5.36	3.33	5.34	0.05	0.18
156	0	3	18.10	24.53	6.37	3.27	5.11	0.11	0.18
156	0	4	16.24	24.98	7.59	3.22	5.10	0.00	0.24
156	0	5	15.06	24.14	8.02	3.35	5.36	0.18	0.18
156	0	6	18.35	30.13	8.28	2.90	5.08	0.08	0.18
156	0	7	15.50	25.21	8.18	2.99	5.25	0.09	0.08
156	0	8	14.36	17.61	5.51	3.09	5.52	0.07	0.10
156	0	9	14.73	21.13	6.90	3.27	5.63	0.04	0.09
27	1	1	11.64	13.97	5.34	3.60	5.65	0.25	0.19
27	1	2	13.68	19.81	6.99	3.07	5.92	0.31	0.19
27	1	3	15.00	21.95	7.09	3.35	5.23	0.49	0.12
27	1	4	14.78	22.78	7.61	3.27	5.25	0.25	0.07
27	1	5	15.68	27.27	8.93	3.33	5.49	1.21	0.17
27	1	6	15.79	20.17	5.85	3.20	5.23	0.64	0.17
27	1	7	14.65	22.86	7.74	3.14	5.31	0.55	0.09
27	1	8	13.39	15.82	5.21	3.42	5.27	0.77	0.09
27	1	9	9.10	10.81	5.26	3.67	5.47	0.61	0.10
28	1	1	10.24	12.80	5.67	3.59	5.18	0.33	0.10
28	1	2	13.27	21.96	8.37	3.81	5.22	0.32	0.13
28	1	3	12.13	18.53	7.52	3.81	4.93	0.63	0.12
28	1	4	14.24	21.63	7.46	3.68	5.02	0.69	0.18
28	1	5	16.99	29.45	8.89	3.94	5.33	0.63	0.12
28	1	6	11.32	14.25	5.73	3.68	5.13	0.38	0.12
28	1	7	15.68	24.21	7.63	3.60	5.19	0.36	0.07
28	1	8	12.38	17.89	6.97	3.61	5.40	0.26	0.05

Appendix Table I-16--Continued

COW NUMBER	TRT	PERIOD	DAILY MILK PRODUCTION (KG)	FAT CORRECTED MILK YIELD (KG/DAY)	MILK FAT (%)	MILK PROTEIN (%)	MILK LACTOSE (%)	MILK MOLYBDENUM (MG/L)	MILK COPPER (MG/L)
28	1	9	11.90	12.81	4.51	3.71	5.59	0.290	0.07
43	1	1	8.04	8.45	4.34	3.68	5.69	0.220	0.29
43	1	2	8.45	9.80	5.07	3.79	5.43	0.370	0.26
43	1	3	8.53	13.68	8.03	3.61	4.97	0.420	0.26
43	1	4	10.69	19.17	9.29	3.49	4.74	0.570	0.27
43	1	5	8.80	14.41	8.25	3.95	5.09	0.410	0.13
43	1	6	8.84	15.49	9.02	3.38	4.98	0.390	0.21
43	1	7	11.08	18.62	8.54	3.72	5.23	0.260	0.12
43	1	8	7.93	12.93	8.21	3.62	5.39	0.330	0.16
43	1	9	7.66	12.24	7.99	3.69	5.57	0.800	0.20
166	1	1	7.64	9.26	5.42	3.29	5.36	0.210	0.13
166	1	2	7.26	10.61	7.08	3.22	5.75	0.440	0.13
166	1	3	6.91	6.56	3.67	3.50	5.11	0.760	0.15
166	1	4	8.06	12.48	7.66	3.25	5.03	0.950	0.24
166	1	5	6.56	9.39	6.88	3.78	5.15	0.900	0.15
166	1	6	8.88	14.67	8.35	3.09	4.96	0.460	0.15
166	1	7	5.39	7.07	6.09	3.64	5.25	0.500	0.08
166	1	8	5.36	6.29	5.16	3.54	5.27	0.700	0.12
166	1	9	9.51	13.87	7.06	3.43	5.53	0.750	0.13
18	2	1	13.18	9.38	2.08	3.88	5.50	0.560	0.12
18	2	2	9.22	8.07	3.17	3.74	5.39	1.490	0.12
18	2	3	11.55	14.58	5.75	3.84	5.10	1.560	0.14
18	2	4	8.80	11.91	6.36	3.84	5.10	1.720	0.15
18	2	5	9.88	12.74	5.93	3.84	5.21	2.470	0.12
18	2	6	9.60	12.04	5.70	3.55	5.16	0.780	0.10
18	2	7	9.27	11.13	5.34	3.90	5.39	1.700	0.07
18	2	8	8.56	9.58	4.80	3.67	5.25	2.410	0.09
18	2	9	9.27	10.99	5.24	3.78	5.43	1.150	0.07
19	2	1	9.41	10.58	4.83	3.40	5.52	0.380	0.08
19	2	2	9.41	13.61	6.98	3.42	5.58	1.270	0.12
19	2	3	6.06	7.77	5.89	3.42	5.11	1.410	0.10
19	2	4	5.84	9.18	7.82	3.64	5.08	1.090	0.09
19	2	5	8.18	13.51	8.35	3.49	5.35	1.760	0.14
19	2	6	4.71	3.09	1.71	3.67	5.43	0.760	0.09
19	2	7	7.38	13.36	9.41	3.23	5.07	1.350	0.15
19	2	8	5.82	8.08	6.59	3.35	5.24	1.110	0.12
19	2	9	4.96	6.01	5.42	3.48	5.61	0.890	0.16
23	2	1	8.46	5.31	1.52	3.41	5.33	0.480	0.12
23	2	2	9.22	10.21	4.72	3.31	5.41	0.810	0.13
23	2	3	8.79	10.26	5.12	3.28	5.03	0.630	0.13
23	2	4	7.36	8.17	4.74	3.41	5.26	1.890	0.18
23	2	5	6.48	7.08	4.62	3.52	5.46	1.160	0.12
23	2	6	7.29	8.86	5.44	3.48	5.32	0.850	0.10
23	2	7	8.16	9.81	5.35	3.58	4.98	1.760	0.07
23	2	8	4.94	4.76	3.77	3.57	5.57	1.170	0.07
23	2	9	6.46	6.51	4.06	3.44	5.76	0.817	0.07
29	2	1	10.97	11.15	4.11	3.56	5.03	1.480	0.10
29	2	2	9.02	10.50	5.10	3.54	5.08	0.870	0.10
29	2	3	9.54	10.71	4.82	3.58	4.82	1.550	0.07
29	2	4	8.09	13.17	8.19	3.56	4.70	0.920	0.07
29	2	5	10.73	15.49	6.96	3.61	4.97	1.180	0.06
29	2	6	9.33	12.56	6.31	3.28	4.86	0.810	0.06
29	2	7	8.97	13.45	7.33	3.40	4.95	0.530	0.05

Appendix Table I-16--Continued

COW NUMBER	TRT	PERIOD	DAILY MILK PRODUCTION (KG)	FAT CORRECTED MILK YIELD (KG/DAY)	MILK FAT (%)	MILK PROTEIN (%)	MILK LACTOSE (%)	MILK MOLYBDENUM (MG/L)	MILK COPPER (MG/L)
29	2	8	5.44	4.18	2.46	3.53	5.28	.	0.14
29	2	9	7.91	7.19	3.40	3.50	5.29	0.86	0.09

Appendix Table I-17: Raw data for plasma and liver copper parameters of calves in the beef cow-calf trial.

CALF NUMBER	TRT	PERIOD	TOTAL PLASMA COPPER (MG/L)	TCA-SOLUBLE COPPER (MG/L)	PLASMA CP OXIDASE ACTIVITY (ΔA/MIN/L)	FRESH LIVER COPPER CONTENT (MG/KG)	LIVER COPPER CONTENT (DM) (MG/KG)
32	0	1	1.07	0.75	.	84.41	351.92
32	0	2	0.83	.	59.4	.	.
32	0	3	0.73	0.74	52.4	.	.
32	0	4	0.66	.	49.8	.	.
32	0	5	0.66	0.61	47.6	39.94	154.20
32	0	6	0.76	.	47.8	.	.
32	0	7	0.72	0.61	41.4	.	.
32	0	8	0.68	.	48.8	.	.
32	0	9	0.71	0.61	49.6	40.12	155.82
59	0	1	1.04	0.97	82.2	29.72	141.63
59	0	2	1.03	.	77.0	.	.
59	0	3	1.31	1.10	88.0	.	.
59	0	4	1.00	.	72.2	.	.
59	0	5	0.82	0.76	62.0	26.42	90.47
59	0	6	0.66	.	55.8	.	.
59	0	7	0.81	0.53	37.8	.	.
59	0	8	0.68	.	49.4	.	.
59	0	9	0.82	0.70	59.8	14.81	58.29
71	0	1	0.83	0.70	60.8	37.72	146.10
71	0	2	0.62	.	44.0	.	.
71	0	3	0.54	0.51	27.4	.	.
71	0	4	0.51	.	44.0	.	.
71	0	5	0.61	0.56	41.8	71.97	301.13
71	0	6	0.60	.	38.8	.	.
71	0	7	0.60	0.43	29.6	.	.
71	0	8	0.57	.	44.2	.	.
71	0	9	0.47	0.41	32.6	32.77	138.32
86	0	1	0.91	0.59	76.4	57.46	224.32
86	0	2	0.90	.	65.0	.	.
86	0	3	0.75	0.64	49.8	.	.
86	0	4	0.74	.	52.6	.	.
86	0	5	0.82	0.72	56.2	62.68	241.99
86	0	6	0.78	.	48.6	.	.
86	0	7	0.89	0.64	52.8	.	.
86	0	8	0.67	.	55.0	.	.
86	0	9	0.79	0.65	55.0	46.63	188.71
40	1	1	0.67	0.51	46.4	38.52	160.00
40	1	2	0.74	.	55.6	.	.
40	1	3	0.62	0.53	41.6	.	.
40	1	4	0.88	.	70.8	.	.
40	1	5	0.78	0.78	58.4	21.65	88.35
40	1	6	0.73	.	49.8	.	.
40	1	7	0.96	0.68	54.8	.	.
40	1	8	0.71	.	50.8	.	.
40	1	9	0.69	0.61	47.2	9.71	38.57
54	1	1	1.01	0.87	84.4	76.30	342.47
54	1	2	1.07	.	81.8	.	.
54	1	3	0.85	0.61	60.2	.	.
54	1	4	0.87	.	64.6	.	.
54	1	5	0.85	0.73	57.6	58.09	229.59
54	1	6	0.94	.	60.8	.	.
54	1	7	0.93	0.67	53.2	.	.
54	1	8	0.86	.	61.4	.	.

Appendix Table I-17--Continued

CALF NUMBER	TRT	PERIOD	TOTAL PLASMA COPPER (MG/L)	TCA-SOLUBLE COPPER (MG/L)	PLASMA CP OXIDASE ACTIVITY (Δ A/MIN/L)	FRESH LIVER COPPER CONTENT (MG/KG)	LIVER COPPER CONTENT (DM) (MG/KG)
54	1	9	0.78	0.65	46.8	28.51	103.00
55	1	1	0.95	0.68	66.0	34.38	140.08
55	1	2	0.76	.	73.2	.	.
55	1	3	0.68	0.63	45.0	.	.
55	1	4	0.59	.	41.4	.	.
55	1	5	0.61	0.53	43.0	54.92	233.72
55	1	6	0.66	.	53.0	.	.
55	1	7	0.76	0.49	39.4	.	.
55	1	8	0.67	.	50.8	.	.
55	1	9	0.73	0.62	52.0	27.27	105.92
84	1	1	1.14	0.85	93.0	32.63	139.43
84	1	2	0.97	.	74.4	.	.
84	1	3	0.83	0.67	63.4	.	.
84	1	4	0.77	.	64.2	.	.
84	1	5	0.97	0.83	71.2	43.20	174.12
84	1	6	0.75	.	59.6	.	.
84	1	7	0.87	0.59	41.6	.	.
84	1	8	0.77	.	61.4	.	.
84	1	9	0.66	0.58	49.6	20.79	84.45
42	2	1	0.82	0.63	63.4	13.05	60.48
42	2	2	0.70	.	60.4	.	.
42	2	3	0.70	0.61	4.0	.	.
42	2	4	0.61	.	47.2	.	.
42	2	5	0.66	0.54	46.4	8.66	33.58
42	2	6	0.62	.	50.6	.	.
42	2	7	0.54	0.38	29.8	.	.
42	2	8	0.48	.	36.6	.	.
42	2	9	0.65	0.37	29.4	4.66	18.26
51	2	1	0.83	0.68	59.2	44.49	184.34
51	2	2	0.76	.	54.6	.	.
51	2	3	0.75	0.73	47.2	.	.
51	2	4	0.72	.	44.4	.	.
51	2	5	0.66	0.55	42.2	29.42	121.56
51	2	6	0.79	.	50.4	.	.
51	2	7	0.99	0.73	44.2	.	.
51	2	8	0.79	.	58.8	.	.
51	2	9	0.79	0.60	46.0	18.92	75.30
69	2	1	1.07	0.85	94.8	57.02	231.15
69	2	2	0.88	.	73.2	.	.
69	2	3	0.96	0.86	69.6	.	.
69	2	4	1.08	.	87.0	.	.
69	2	5	1.19	1.12	84.8	29.62	118.03
69	2	6	0.89	.	77.0	.	.
69	2	7	1.12	0.79	65.8	.	.
69	2	8	0.89	.	70.2	.	.
69	2	9	0.86	0.70	64.2	17.54	67.51
70	2	1	0.91	0.64	70.0	13.35	51.24
70	2	2	.	.	46.8	.	.
70	2	3	0.75	.	49.8	.	.
70	2	4	0.66	.	58.8	.	.
70	2	5	0.74	0.74	50.8	17.21	66.18
70	2	6	0.72	.	51.4	.	.
70	2	7	0.82	0.57	38.6	.	.

Appendix Table I-17--Continued

CALF NUMBER	TRT	PERIOD	TOTAL PLASMA COPPER (MG/L)	TCA-SOLUBLE COPPER (MG/L)	PLASMA CP OXIDASE ACTIVITY (ΔA/MIN/L)	FRESH LIVER COPPER CONTENT (MG/KG)	LIVER COPPER CONTENT (DM) (MG/KG)
70	2	8	0.86	.	42.4	.	.
70	2	9	0.62	0.57	52.4	8.17	24.11

Appendix Table I-18: Raw data for body weight and average daily gains in the beef cow-calf trial.

CALF NUMBER	TRT	PERIOD	CALF BODY WEIGHT (KG)	AVERAGE DAILY GAIN (KG/DAY)
32	0	1	80	.
32	0	2	87	1.00
32	0	3	91	0.57
32	0	4	102	1.57
32	0	5	107	0.71
32	0	6	112	0.71
32	0	7	116	0.57
32	0	8	123	1.00
32	0	9	131	1.14
59	0	1	48	.
59	0	2	51	0.42
59	0	3	52	0.14
59	0	4	59	1.00
59	0	5	57	-0.28
59	0	6	64	1.00
59	0	7	65	0.14
59	0	8	69	0.57
59	0	9	72	0.42
71	0	1	73	.
71	0	2	81	1.14
71	0	3	87	0.85
71	0	4	94	1.00
71	0	5	100	0.85
71	0	6	104	0.57
71	0	7	110	0.85
71	0	8	117	1.00
71	0	9	123	0.85
86	0	1	55	.
86	0	2	62	1.00
86	0	3	68	0.85
86	0	4	74	0.85
86	0	5	80	0.85
86	0	6	83	0.42
86	0	7	85	0.28
86	0	8	90	0.71
86	0	9	99	1.28
40	1	1	59	.
40	1	2	62	0.42
40	1	3	70	1.14
40	1	4	75	0.71
40	1	5	78	0.42
40	1	6	81	0.42
40	1	7	84	0.42
40	1	8	85	0.14
40	1	9	93	1.14
54	1	1	82	.
54	1	2	87	0.71
54	1	3	94	0.71
54	1	4	98	0.57
54	1	5	104	0.85
54	1	6	106	0.28
54	1	7	112	0.85
54	1	8	116	0.57

Appendix Table I-18--Continued

CALF NUMBER	TRT	PERIOD	CALF BODY WEIGHT (KG)	AVERAGE DAILY GAIN (KG/DAY)
54	1	9	117	0.14
55	1	1	71	.
55	1	2	77	0.85
55	1	3	85	1.14
55	1	4	90	0.71
55	1	5	94	0.57
55	1	6	97	0.42
55	1	7	100	0.42
55	1	8	106	0.85
55	1	9	107	0.14
84	1	1	65	.
84	1	2	74	1.28
84	1	3	80	0.85
84	1	4	84	0.57
84	1	5	89	0.71
84	1	6	97	1.14
84	1	7	102	0.71
84	1	8	104	0.28
84	1	9	109	0.71
42	2	1	44	.
42	2	2	48	0.57
42	2	3	54	0.85
42	2	4	55	0.14
42	2	5	60	0.71
42	2	6	59	-0.14
42	2	7	62	0.42
42	2	8	68	0.85
42	2	9	73	0.71
51	2	1	67	.
51	2	2	71	0.57
51	2	3	82	1.28
51	2	4	84	0.28
51	2	5	91	1.00
51	2	6	99	1.14
51	2	7	102	0.42
51	2	8	107	0.71
51	2	9	112	0.71
69	2	1	39	.
69	2	2	45	0.85
69	2	3	51	0.85
69	2	4	51	0.00
69	2	5	54	0.42
69	2	6	58	0.57
69	2	7	57	-0.14
69	2	8	63	0.85
69	2	9	65	0.28
70	2	1	69	.
70	2	2	75	0.85
70	2	3	84	1.28
70	2	4	91	1.00
70	2	5	97	0.85
70	2	6	102	0.71
70	2	7	108	0.85

Appendix Table I-18--Continued

CALF NUMBER	TRT	PERIOD	CALF BODY WEIGHT (KG)	AVERAGE DAILY GAIN (KG/DAY)
70	2	8	113	0.71
70	2	9	118	0.71

APPENDIX II

EWE-LAMB TRIAL

For Appendix tables II-14 to 22:

Trt 1 refers to 0Mo

Trt 2 refers to 20Mo

Trt 3 refers to 40Mo

Period refers to week

Appendix Table II-1: Experimental diet allocation and background information of ewes and lambs.

Experimental Diet	Ewe	Lambing Date Day/Month	Lamb	Lamb Sex †	Lamb Birthweight (kg)
0Mo	31	15/3	15	M	3.17
			16	M	2.72
	104	23/3	54	F	4.54
			55	M	4.99
	47	25/3	67	F	4.54
			68	F	4.54
	11	31/3	74	F	3.17
			75	F	2.95
20Mo	51	14/3	11	M	4.08
			12	F	2.95
	52	21/3	42	F	4.54
			43	F	3.17
	54	24/3	61	M	4.99
			62	M	4.99
	60	2/4	71	F	4.08
			72	F	4.54
40Mo	99	11/3	9	M	4.99
			10	M	4.54
	56	23/3	52	M	4.54
			53	F	3.85
	119	23/3	56	M	4.54
			57	F	4.08
	53	2/4	77	F	4.08
			78	F	4.54

† M - male, F - female.

Appendix Table II-2: Animals on test at various sampling times in the ewe-lamb trial.

Sampling Time†	Number of Ewes			Number of Lambs		
	0Mo	20Mo	40Mo	0Mo	20Mo	40Mo
Initial	4	4	4	8	8	8
Week 1	4	4	4	8	8	8
Week 2	4	4	4	8	8	8
Week 3	4	4	4	8	8	8
Week 4	4	3	4	8	6	8
Week 5	4	2	3	8	4	6
Week 6	4	2	3	8	4	6

† Initial sample was taken within 48 hours post lambing; weeks 1 to 6 refer to the first to sixth Wednesday thereafter.

Appendix Table II-3: Analysis of variance for body weight, body weight change, feed intake and feed efficiency of ewes in the ewe-lamb trial (mean squares and degrees of freedom†).

Source of Variation	Diet	Ewe (Diet)	Week	Diet x Week	Error
Items					
Body weight, kg ($x10^2$)	4.151 (2)	6.080 (9)	1.210***‡ (6)	0.149 (12)	0.129 (47)
Bodyweight change, kg d^{-1}	0.299 (2)	0.571 (9)	0.484 (5)	0.108 (10)	0.375 (39)
D.M. intake, kg d^{-1}	0.389 (2)	0.582 (9)	0.357 (5)	0.103 (10)	0.087 (41)
D.M. intake, % bodyweight (10^{-4})	0.272 (2)	0.406 (9)	0.100 (5)	0.121 (10)	0.104 (41)
Feed efficiency, kg D.M. intake kg^{-1} milk	0.049 (2)	0.134 (9)	0.151*** (5)	0.027* (10)	0.009 (38)

† Figures in parentheses represent the degrees of freedom for the corresponding mean squares.

‡ Significant differences at * $\alpha = 0.05$, *** $\alpha = 0.001$.

Appendix Table II-4: Analysis of variance for rumen fluid parameters (mean square).†

Source of Variation:	Diet	Ewe(Diet)
Degrees of Freedom:	2	6
Items:		
pH	0.397	0.225
Total volatile fatty acids ($x10^2$)	9.910	3.263
Molar proportions:		
acetate ($x10^{-3}$)	0.292	0.759
propionate ($x10^{-2}$)	0.286	0.229
isobutyric ($x10^{-4}$)	0.228	0.511
n-butyric ($x10^{-3}$)	0.554	0.662
iso-valeric ($x10^{-5}$)	0.944	0.219
valeric ($x10^{-5}$)	0.642	0.925
Acetate:propionate ratio	0.767	2.086
Mo content	1.022*	0.126

† Significant difference at * $\alpha = 0.05$.

Appendix Table II-5: Analysis of variance for plasma and liver parameters describing ewe copper and molybdenum status (mean squares and degrees of freedom†).

Source of Variation:	Diet	Ewe (Diet)	Week	Diet x Week	Error
Items:					
Plasma Cu	0.126 (2)	0.135 (9)	0.078**‡ (6)	0.010 (12)	0.0187 (48)
TCA-soluble Cu	0.062 (2)	0.103 (9)	0.073*** (6)	0.006 (12)	0.014 (46)
TCA-insoluble Cu, % Total Plasma Cu ($x10^2$)	1.010 (2)	0.899 (9)	1.278 (6)	0.394 (12)	0.669 (46)
Cp oxidase activity ($x10^2$)	0.856 (2)	13.429 (9)	7.157*** (6)	0.324 (12)	0.855 (43)
Plasma Mo ($x10^2$)	7.518*** (2)	0.428 (9)	1.327*** (6)	0.455 (12)	0.242 (43)
Liver Cu, D.M. basis ($x10^5$)	2.85 (2)	0.72 (7)	-	-	-
Liver Mo, D.M. basis ($x10^3$)	1.13 (2)	0.52 (7)	-	-	-

† Figures in parentheses represent the degrees of freedom for the corresponding mean squares.

‡ Significant difference at: $*\alpha = 0.05$, $**\alpha = 0.01$, $***\alpha = 0.001$.

Appendix Table II-6: Analysis of variance for milk yield and composition for ewes fed varying levels of dietary Mo (means squares)†.

Source of Variation:	Diet	Ewe (Diet)	Week	Diet x Week	Error
Degrees of Freedom:	2	9	5	10	37
Items:					
Milk yield, kg d^{-1}	0.441	5.561	1.890*	0.492	0.559
FCM yield, kg d^{-1} ($x10^1$)	0.705	3.690	1.545**	0.316	0.298
Butterfat, % ($x10^1$)	0.696	1.344	0.986***	0.271	0.157
Protein, %	0.371	0.247	0.373	0.278	0.228
Lactose, %	0.050	0.187	0.522*	0.164	0.172
Copper, mg kg^{-1}	0.127	0.118	0.217**	0.024	0.054
Copper, mg d^{-1}	1.003	3.630	4.03**	0.474	0.821
Molybdenum, mg kg^{-1} ($x10^1$)	3.637***	0.142	0.552**	0.272*	0.121
Molybdenum, mg d^{-1} ($x10^2$)	2.900**	0.189	0.252	0.120	0.118

† Significant difference at: $*\alpha = 0.05$, $**\alpha = 0.01$, $***\alpha = 0.001$.

Appendix Table II-7: Analysis of variance for plasma and liver parameters describing lamb copper and molybdenum status (mean squares and degrees of freedom†).

Source of Variation:	Diet	Ewe (Diet)	Lamb (Ewe(Diet))	Week	Week x Diet	Week x Ewe(Diet)	Error
Items:							
Plasma Cu	0.60 (2)	0.40*† (9)	0.12 (12)	1.64*** (6)	0.04 (12)	0.07* (48)	0.04 (63)
TCA-souluble Cu	0.62 (2)	0.35* (9)	0.13 (12)	1.77*** (6)	0.06 (12)	0.08*** (49)	0.03 (60)
TCA-insoluble Cu, % plasma Cu ($x10^2$)	1.88 (2)	1.37 (9)	2.28 (12)	9.60*** (6)	2.17 (12)	2.83 (47)	2.06 (59)
Cp oxidase activity ($x10^{-2}$)	2.48 (2)	0.56* (9)	0.74 (12)	2.48** (6)	1.41* (12)	0.47 (48)	0.71 (62)
Plasma Mo	15.59* (2)	2.40** (9)	0.36 (12)	9.26*** (6)	2.17*** (12)	1.37*** (48)	0.26 (63)
Liver Cu, D.M. basis ($x10^5$)	1.52 (2)	0.07 (6)	0.04 (9)	-	-	-	-
Liver Mo, D.M. basis ($x10^1$)	8.29 (2)	2.53 (6)	4.58 (9)	-	-	-	-

† Figures in parentheses represent the degrees of freedom for the corresponding mean squares.

† Significant differences at: * $\alpha = 0.05$, ** $\alpha = 0.01$, *** $\alpha = 0.001$.

Appendix Table II-8: Analysis of variance testing the effects of ewe, ewe diet and time on average daily gain and estimated feed efficiency of lambs (mean squares†).

Source of Variation	Degrees of Freedom	PARAMETER ADG ($x10^{-2}$)	PARAMETER FE ($x10^{1}$)†
diet	2	0.564	0.789
ewe (diet)	9	1.561	3.258
lamb (ewe (diet)	12	1.142	4.266
week	5	5.709***	8.260**
diet x week	10	0.762	0.467
week x ewe (diet)	39	1.077	1.843
error	51	0.642	2.027

† Significant differences at ** α = 0.01, *** α = 0.001.

† FE is calculated as one-half daily ewe milk production divided by average daily gains of lamb for that period.

Appendix Table II-9: Analysis of variance for linear regressions describing diet by week interactions shown to be significant ($P < 0.07$) in split-plot analyses (mean squares and degrees of freedom†).

Source of Variation	Diet	Diet x Week	Error
Item			
Ewe plasma Mo, mg L^{-1} ($x10^{-2}$)	0.003 (2)	3.533***† (3)	0.267 (67)
Milk Mo, mg L^{-1}	0.227 (2)	14.312*** (3)	1.179 (59)

† Figures in parentheses are degrees of freedom for the corresponding mean squares.

† Significant differences at *** $\alpha = 0.001$.

Appendix Table II-10: Plasma molybdenum (mg L^{-1}) for ewes fed varying levels of dietary molybdenum for six weeks. Least square means.

Sampling Time†	DIET			Mean ± SE
	0Mo	20Mo	40Mo	
Initial	0.37	0.21	0.45	0.34 ± 1.42 A
Week 1	0.49	3.54	7.27	3.77 ± 1.60 A B
Week 2	0.73	8.10	14.69	7.84 ± 1.51 B C
Week 3	0.99	10.53	8.91	6.81 ± 1.52 B
Week 4	0.72	12.02	12.10	8.28 ± 1.52 B C
Week 5	0.89	11.75	19.40	10.68 ± 1.69 C
Week 6	0.91	10.97	17.98	9.95 ± 1.84 C
Mean ± SE	0.73 ± 1.31[a]	8.16 ± 1.43[b]	11.54 ± 1.26[b]	

† Initial sample was taken within 48 hours post lambing; weeks 1 to 6 referring to the first to sixth Wednesday thereafter.

a - b Values in the same row with different superscripts are significantly different, = 0.05.

A - C Values in the same column with different superscripts are significantly different, = 0.05.

Appendix Table II-11: Milk molybdenum concentration (mg L^{-1}) for ewes fed varying levels of dietary molybdenum. Least square means.

Sampling Time	DIET			Mean ± SE
	0Mo	20Mo	40M0	
Week 1	0.19	0.35 A	0.63 A	0.39 ± 0.32 A
Week 2	0.18[a]	0.96[a b], A	2.08[b], B	1.07 ± 0.32 A
Week 3	0.15[a]	1.59[b], B	1.70[b], A B	1.14 ± 0.32 A
Week 4	0.11[a]	1.73[b], B	2.30[b], B	1.38 ± 0.34 A B
Week 5	0.12[a]	2.32[b], B	5.05[c], C	2.50 ± 0.40 B
Week 6	0.13[a]	2.21[b], B	4.43[c], C	2.25 ± 0.40 B
Mean ± SE	0.14 ± 0.24[a]	1.53 ± 0.27[b]	2.69 ± 0.27[c]	

a - c Values in the same row with different superscripts are significantly different, $\alpha = 0.05$.

A - C Values in the same column with different superscripts are significantly different, $\alpha = 0.05$.

Appendix Table II-12: Weekly concentration of plasma copper, TCA-soluble copper and ceruloplasmin oxidase activity for lambs sucking ewes fed varying levels of molybdenum. Least square means ± SE.

Sampling Time†	Plasma Cu (mg L^{-1})	TCA-soluble Cu (mg L^{-1})	Cp oxidase activity (Δ A min^{-1} L^{-1})
Initial	0.39 ± 0.05 A	0.31 ± 0.05 A	22.4 ± 5.6 A
Week 1	0.99 ± 0.05 B	0.86 ± 0.05 B	88.6 ± 5.9 B
Week 2	0.99 ± 0.05 B	0.92 ± 0.05 B C	88.1 ± 5.8 B
Week 3	1.02 ± 0.05 B	1.00 ± 0.05 B C	96.8 ± 5.8 B
Week 4	1.14 ± 0.05 B	1.08 ± 0.05 B C	107.8 ± 6.0 B
Week 5	1.20 ± 0.06 B	1.16 ± 0.06 C	114.2 ± 6.7 B
Week 6	1.09 ± 0.06 B	1.02 ± 0.06 B C	92.6 ± 7.0 B

† Initial sample was taken within 48 hours post lambing; weeks 1 to 6 referring to the first to sixth Wednesday thereafter.

A - E Values in the same column with different superscripts are significantly different, α = 0.05.

Appendix Table II-13: Plasma Mo concentrations (mg L^{-1}) for lambs sucking ewes fed diets containing varying levels of molybdenum. Least square mean.

Sampling Time†	DIET 0Mo	20Mo	40Mo	Mean ± SE
Initial	0.07	0.05 A	0.10 A	0.07 ± 0.17 A
Week 1	0.12[a]	0.33[a b], A B	1.03[b], B C	0.50 ± 0.17 A
Week 2	0.39[a]	1.97[b], C	3.38[c], D	1.91 ± 0.17 B
Week 3	0.29[a]	2.04[b], C	1.68[b], C	1.33 ± 0.17 B
Week 4	0.06	0.86 B	0.69 A B	0.54 ± 0.19 A
Week 5	0.03	0.45 A B	0.71 A B	0.40 ± 0.20 A
Week 6	0.05	0.49 A B	0.83 A B	0.46 ± 0.21 A
Mean ± SE	0.14 ± 0.21[a]	0.88 ± 0.23[a b]	1.20 ± 0.21[b]	

† Initial sample was taken within 48 hours post lambing; weeks 1 to 6 referring to the first to sixth Wednesday thereafter.

a - c Values in the same row with different superscripts are significantly different, $\alpha = 0.05$.

A - D Values in the same column with different superscripts are significantly different, $\alpha = 0.05$.

Appendix Table II-14: Raw data for daily silage, concentrate and total dry matter intake of ewes in the ewe-lamb trial.

EWE NUMBER	TRT	PERIOD	GRAIN INTAKE (KG DM/DAY)	CORN SILAGE INTAKE (KG DM/DAY)	TOTAL DM INTAKE (KG/DAY)
11	1	1	0.29	0.81	1.10
11	1	2	0.57	1.17	1.74
11	1	3	0.60	1.45	2.05
11	1	4	0.56	1.52	2.09
11	1	5	0.51	1.27	1.79
11	1	6	0.62	1.62	2.24
31	1	1	0.41	1.34	1.75
31	1	2	0.52	1.42	1.94
31	1	3	0.50	1.46	1.96
31	1	4	0.51	1.18	1.70
31	1	5	0.57	1.47	2.04
31	1	6	0.52	1.37	1.89
47	1	1	0.22	1.57	1.79
47	1	2	0.48	1.40	1.88
47	1	3	0.55	1.53	2.08
47	1	4	0.76	1.66	2.42
47	1	5	0.51	1.51	2.02
47	1	6	0.50	1.21	1.72
51	2	1	0.42	1.09	1.52
51	2	2	0.54	1.20	1.74
51	2	3	0.43	1.15	1.59
51	2	4	0.46	1.17	1.64
51	2	5	0.49	1.24	1.74
51	2	6	0.51	1.34	1.86
52	2	1	0.40	1.36	1.76
52	2	2	0.53	1.66	2.20
52	2	3	0.61	1.40	2.02
52	2	4	0.64	1.54	2.18
52	2	5	0.58	1.39	1.98
52	2	6	.	.	.
53	3	1	0.34	1.29	1.63
53	3	2	0.62	1.50	2.12
53	3	3	0.52	1.23	1.76
53	3	4	0.53	1.27	1.80
53	3	5	0.49	1.37	1.86
53	3	6	0.51	1.31	1.83
54	2	1	0.26	1.57	1.83
54	2	2	0.49	1.65	2.15
54	2	3	0.77	1.76	2.53
54	2	4	0.84	2.14	2.98
54	2	5	0.82	2.12	2.94
54	2	6	0.73	1.80	2.54
56	3	1	0.37	1.10	1.48
56	3	2	0.48	1.47	1.96
56	3	3	0.56	1.41	1.98
56	3	4	0.70	1.58	2.28
56	3	5	0.59	1.54	2.13
56	3	6	0.54	1.32	1.86
60	2	1	0.39	1.41	1.81
60	2	2	0.60	1.30	1.91
60	2	3	0.56	1.03	1.59
60	2	4	0.44	1.07	1.51
60	2	5	.	.	.
60	2	6	.	.	.

Appendix Table II-14--Continued

EWE NUMBER	TRT	PERIOD	GRAIN INTAKE (KG DM/DAY)	CORN SILAGE INTAKE (KG DM/DAY)	TOTAL DM INTAKE (KG/DAY)
99	3	1	0.41	1.43	1.84
99	3	2	0.55	1.00	1.55
99	3	3	0.43	1.12	1.55
99	3	4	0.64	1.44	2.08
99	3	5	0.48	1.11	1.59
99	3	6	.	.	.
104	1	1	0.37	1.52	1.89
104	1	2	0.59	1.69	2.29
104	1	3	0.76	1.86	2.63
104	1	4	0.86	2.25	3.11
104	1	5	0.88	2.24	3.12
104	1	6	0.83	2.11	2.94
119	3	1	0.37	1.38	1.76
119	3	2	0.55	1.67	2.22
119	3	3	0.64	1.58	2.23
119	3	4	0.80	1.88	2.68
119	3	5	0.52	1.07	1.59
119	3	6	0.23	0.69	0.93

Appendix Table II-15 : Raw data for plasma copper and molybdenum parameters of ewes in the ewe-lamb trial

EWE NUMBER	TRT	PERIOD	PLASMA COPPER (MG/L)	TCA-SOLUBLE COPPER (MG/L)	CP OXIDASE ACTIVITY (ΔA/MIN/L)	PLASMA MOLYBDENUM (MG/L)
11	1	1	0.96	0.90	0.0379	0.55
11	1	2	1.01	0.91	0.0382	.
11	1	3	0.95	0.74	0.0410	.
11	1	4	0.98	0.88	0.0420	1.77
11	1	5	0.93	0.85	0.0390	0.98
11	1	6	1.15	1.06	0.0445	0.85
11	1	7	1.20	1.04	0.0450	0.88
31	1	1	0.75	0.70	0.0305	0.04
31	1	2	0.81	.	.	0.26
31	1	3	1.09	1.07	0.0403	0.57
31	1	4	0.92	0.87	0.0381	0.61
31	1	5	0.91	0.82	0.0373	0.56
31	1	6	0.85	0.81	0.0365	0.94
31	1	7	0.91	0.85	0.0366	0.81
47	1	1	0.81	0.76	.	0.33
47	1	2	0.74	0.70	0.0254	0.40
47	1	3	0.85	0.70	0.0322	0.56
47	1	4	0.76	0.80	0.0329	0.68
47	1	5	0.87	0.90	0.0340	0.90
47	1	6	0.81	0.78	0.0367	0.87
47	1	7	1.36	1.31	0.0575	1.04
51	2	1	1.07	1.03	.	0.14
51	2	2	1.22	1.08	.	5.07
51	2	3	1.29	1.19	0.0489	9.91
51	2	4	1.30	1.26	0.0506	12.69
51	2	5	1.30	1.22	0.0586	16.25
51	2	6	1.37	1.42	0.0576	17.67
51	2	7	1.38	1.35	0.0664	16.15
52	2	1	0.92	0.82	0.0284	0.02
52	2	2	1.13	0.92	0.0350	.
52	2	3	0.93	.	0.0238	7.85
52	2	4	0.78	0.64	0.0259	15.72
52	2	5	0.81	0.82	0.0303	17.76
52	2	6	.	.	.	.
52	2	7	.	.	.	.
53	3	1	1.08	1.08	0.0441	0.34
53	3	2	1.04	0.98	0.0385	0.39
53	3	3	1.01	0.85	0.0389	0.78
53	3	4	1.10	1.10	0.0410	6.31
53	3	5	1.10	1.08	0.0383	14.90
53	3	6	1.28	1.19	0.0477	20.79
53	3	7	1.68	1.38	0.0629	26.81
54	2	1	1.05	1.03	0.0312	0.09
54	2	2	0.98	0.87	0.0294	0.69
54	2	3	0.95	0.77	0.0295	5.03
54	2	4	0.89	0.75	0.0279	2.97
54	2	5	0.91	0.89	0.0289	1.84
54	2	6	0.93	0.89	0.0361	3.00
54	2	7	1.09	1.09	0.0411	2.97
56	3	1	0.70	0.56	0.0270	0.13
56	3	2	0.94	0.93	0.0318	7.12
56	3	3	0.94	0.88	0.0275	19.96
56	3	4	0.90	0.80	0.0288	8.40

Table II-15—Continued

EWE NUMBER	TRT	PERIOD	PLASMA COPPER (MG/L)	TCA-SOLUBLE COPPER (MG/L)	CP OXIDASE ACTIVITY (ΔA/MIN/L)	PLASMA MOLYBDENUM (MG/L)
56	3	5	1.00	0.92	0.0325	13.70
56	3	6	0.87	0.71	0.0280	22.38
56	3	7	0.94	1.05	0.0369	20.13
60	2	1	0.88	0.81	0.0307	0.58
60	2	2	0.85	0.76	0.0323	2.24
60	2	3	0.92	0.77	0.0358	9.62
60	2	4	0.93	0.92	0.0374	.
60	2	5	.	.	.	.
60	2	6	.	.	.	.
60	2	7	.	.	.	.
99	3	1	1.24	1.03	0.0484	0.56
99	3	2	1.22	1.04	0.0428	13.67
99	3	3	1.42	1.05	0.0478	18.46
99	3	4	1.32	1.16	0.0439	11.23
99	3	5	1.12	0.95	0.0437	3.44
99	3	6	1.06	1.01	0.0439	4.72
99	3	7	.	.	.	.
104	1	1	0.84	0.75	0.0306	0.55
104	1	2	1.12	0.90	0.0453	0.58
104	1	3	0.98	0.92	0.0343	0.83
104	1	4	1.50	0.94	0.0296	0.89
104	1	5	0.91	0.90	0.0349	0.46
104	1	6	0.91	0.87	0.0387	0.88
104	1	7	1.16	1.14	0.0473	.
119	3	1	0.93	0.80	0.0349	0.78
119	3	2	1.02	0.87	.	7.88
119	3	3	0.90	0.68	0.0332	19.54
119	3	4	0.96	0.96	0.0281	9.68
119	3	5	1.00	0.92	0.0372	16.36
119	3	6	1.07	0.94	0.0381	29.71
119	3	7	1.42	1.00	0.0435	8.80

Appendix Table II-16: Raw data for milk yield and composition of ewes in the ewe-lamb trial.

EWE NUMBER	TRT	PERIOD	MILK YIELD (KG/DAY)	4% FAT CORRECTED MILK YIELD (KG/DAY)	MILK FAT (%)	MILK PROTEIN (%)	MILK LACTOSE (%)	MILK COPPER (MG/KG)	MILK MOLYBDENUM (MG/KG)
11	1	1	2.20	3.34	7.48	4.58	5.71	0.92	0.59
11	1	2	2.90	3.95	6.43	4.32	5.48	0.73	0.44
11	1	3	3.55	7.19	10.84	4.34	6.15	0.69	0.35
11	1	4	2.95	4.50	7.52	4.44	5.88	0.84	0.15
11	1	5	3.07	4.67	7.49	4.40	6.10	0.42	0.13
11	1	6	3.27	5.47	8.50	5.01	5.62	0.38	0.14
31	1	1	2.33	3.27	6.71	4.89	5.73	0.47	0.03
31	1	2	3.02	4.01	6.20	4.14	6.01	0.17	0.04
31	1	3	3.01	4.87	8.12	4.70	6.02	0.30	0.06
31	1	4	2.69	4.00	7.25	4.67	5.56	0.25	0.11
31	1	5	3.12	3.97	5.83	4.85	5.83	0.23	0.16
31	1	6	1.92	2.12	4.72	4.31	6.10	0.24	0.09
47	1	1	2.49	4.08	8.27	5.10	5.23	0.26	0.01
47	1	2	3.17	4.88	7.60	4.74	5.94	0.85	0.12
47	1	3	2.45	4.27	8.96	4.30	5.92	0.66	0.08
47	1	4	2.56	3.64	6.83	5.01	6.20	0.39	0.08
47	1	5	2.74	4.63	8.61	4.77	6.29	0.27	0.10
47	1	6	1.91	3.11	8.21	5.48	5.98	0.21	0.11
51	2	1	4.83	10.95	12.46	4.98	5.68	0.49	0.39
51	2	2	2.80	6.91	13.80	6.44	4.01	0.65	0.62
51	2	3	2.94	5.84	10.58	4.40	6.08	1.28	1.85
51	2	4	2.49	4.03	8.14	4.40	5.87	0.40	1.50
51	2	5	1.99	3.10	7.75	4.63	6.34	0.38	3.45
51	2	6	2.08	3.26	7.81	4.73	6.23	0.30	3.15
52	2	1	4.47	8.67	10.27	4.30	6.83	0.39	0.69
52	2	2	4.88	11.24	12.70	4.83	5.99	1.04	1.29
52	2	3	6.18	12.84	11.19	3.96	6.20	0.52	0.13
52	2	4	5.11	10.46	10.99	5.59	5.76	0.58	2.50
52	2	5	.	.	.	.	.	.	.
52	2	6	.	.	.	.	.	.	.
53	3	1	3.76	9.72	14.57	5.04	5.12	1.19	0.04
53	3	2	3.68	7.30	10.57	3.91	5.99	0.83	0.08
53	3	3	2.79	4.86	8.96	4.40	6.05	0.56	1.43
53	3	4	2.96	5.25	9.18	3.95	6.18	1.05	2.58
53	3	5	2.64	3.91	7.22	4.66	5.95	0.43	4.44
53	3	6	2.93	3.96	6.35	4.94	5.62	0.38	7.64
54	2	1	2.67	4.70	9.08	5.33	5.37	0.25	0.05
54	2	2	2.63	4.67	9.18	5.11	6.02	1.03	0.63
54	2	3	2.59	4.69	9.43	4.43	5.95	0.58	0.38
54	2	4	2.48	4.26	8.80	5.15	6.32	0.38	0.31
54	2	5	2.24	3.53	7.84	4.65	6.44	0.47	0.30
54	2	6	2.23	3.65	8.27	5.63	5.89	0.31	0.39
56	3	1	2.65	3.63	6.47	4.77	5.47	0.38	0.52
56	3	2	2.38	3.89	8.25	4.43	5.99	0.69	2.74
56	3	3	2.65	3.93	7.24	4.12	5.96	0.58	0.86
56	3	4	2.28	2.96	6.01	4.73	6.02	0.65	2.62
56	3	5	2.53	3.27	5.96	4.94	6.17	0.52	3.29
56	3	6	2.25	2.71	5.38	4.91	5.92	0.52	3.51
60	2	1	4.20	8.50	10.84	5.04	5.65	1.20	0.27
60	2	2	3.46	6.20	9.29	4.07	5.80	0.81	1.30
60	2	3	1.82	.	.	5.56	4.66	1.26	3.99
60	2	4	.	.	.	.	.	.	.
60	2	5	.	.	.	.	.	.	.
60	2	6	.	.	.	.	.	.	.

Appendix Table II-16--Continued

EWE NUMBER	TRT	PERIOD	MILK YIELD (KG/DAY)	4% FAT CORRECTED MILK YIELD (KG/DAY	MILK FAT (%)	MILK PROTEIN (%)	MILK LACTOSE (%)	MILK COPPER (MG/KG)	MILK MOLYBDENUM (MG/KG)
99	3	1	3.85	5.91	7.57	4.57	5.97	0.64	1.12
99	3	2	3.02	5.27	8.97	3.78	5.83	0.34	2.10
99	3	3	2.72	4.34	7.99	4.79	5.85	0.80	2.65
99	3	4	2.56	3.42	6.26	4.26	5.91	0.58	0.82
99	3	5	.	.	.	.	.	.	.
99	3	6	.	.	.	.	.	.	.
104	1	1	6.53	14.14	11.77	5.40	4.87	0.43	0.12
104	1	2	4.91	10.30	11.33	5.01	5.66	1.00	0.11
104	1	3	4.89	10.02	11.00	4.70	5.57	0.82	0.09
104	1	4	5.16	11.47	12.16	4.98	5.95	0.45	0.10
104	1	5	5.86	10.92	9.76	4.94	6.12	0.35	0.09
104	1	6	3.61	6.71	9.74	5.40	5.88	0.46	0.16
119	3	1	6.58	14.73	12.26	5.67	4.83	0.70	0.84
119	3	2	4.10	8.17	10.62	4.99	5.96	0.96	3.41
119	3	3	4.65	8.56	9.62	4.59	5.90	0.78	1.86
119	3	4	4.78	8.12	8.67	4.85	6.39	0.38	3.18
119	3	5	2.25	4.30	10.09	4.28	6.21	0.65	7.43
119	3	6	1.89	2.95	7.77	4.68	6.06	0.32	2.14

Appendix Table II-17: Raw data for rumen fluid parameters for the ewe-lamb trial.

EWE NUMBER	TRT	PH	TOTAL VOLATILE FATTY ACIDS	ACETIC ACID (%)	PROPIONIC ACID (%)	ISOBUTYRIC ACID (%)
11	1	6.40	111.200	0.762410	0.134532	0.0052158
31	1	5.61	115.590	0.740375	0.222597	0.0050177
47	1	6.90	106.280	0.791306	0.127023	0.0099737
51	2	5.62	106.268	0.736816	0.242971	0.0052697
53	3	6.48	106.200	0.767420	0.142373	0.0032957
54	2	6.22	129.760	0.794929	0.119451	0.0065506
56	3	6.54	93.250	0.770402	0.135335	0.0048257
99	3	.	.	.	.	.
104	1	5.92	128.700	0.719658	0.184615	0.0052836
119	3	7.07	55.220	0.776168	0.129844	0.0248099

EWE NUMBER	N-BUTYRIC ACID (%)	ISOVALERIC ACID (%)	N-VALERIC ACID (%)	ACETIC : PROPIONIC RATIO
11	0.0794065	0.0096223	0.0088129	5.66711
31	0.0191193	0.0056233	0.0072671	3.32608
47	0.0566428	0.0051750	0.0098796	6.22963
51	0.0125155	0.0010633	0.0013645	3.03253
53	0.0761770	0.0014124	0.0093220	5.39021
54	0.0589550	0.0090166	0.0110974	6.65484
56	0.0731367	0.0081501	0.0081501	5.69255
99	.	.	.	.
104	0.0780886	0.0014763	0.0108780	3.89815
119	0.0469033	0.0128577	0.0094169	5.97768

Appendix Table II-18: Raw data for ewe liver copper and molybdenum concentrations in the ewe-lamb trial.

EWE NUMBER	TRT	LIVER COPPER CONTENT,DM BASIS (MG/KG)	LIVER MOLYDBENUM CONTENT,DM BASIS (MG/KG)
11	1	322.29	5.64
31	1	390.04	5.29
47	1	352.74	10.35
51	2	455.93	57.86
53	3	449.61	74.77
54	2	354.58	7.59
56	3	631.39	44.73
104	1	248.67	2.92
119	3	382.96	23.13

Appendix Table II-19 : Raw data for body weight of ewes in the ewe-lamb trial.

EWE NUMBER	TRT	PERIOD	EWE BODY WEIGHT (KG)
11	1	1	79.3
11	1	2	77.2
11	1	3	79.5
11	1	4	78.5
11	1	5	76.8
11	1	6	74.8
11	1	7	74.0
31	1	1	65.3
31	1	2	65.8
31	1	3	63.0
31	1	4	63.2
31	1	5	59.4
31	1	6	61.8
31	1	7	58.2
47	1	1	69.4
47	1	2	68.6
47	1	3	66.5
47	1	4	63.3
47	1	5	65.7
47	1	6	68.6
47	1	7	63.3
51	2	1	82.5
51	2	2	77.1
51	2	3	71.5
51	2	4	66.9
51	2	5	65.5
51	2	6	62.6
51	2	7	61.6
52	2	1	77.1
52	2	2	77.5
52	2	3	79.7
52	2	4	75.3
52	2	5	74.7
52	2	6	.
52	2	7	.
53	3	1	69.8
53	3	2	70.0
53	3	3	63.7
53	3	4	60.9
53	3	5	61.2
53	3	6	61.0
53	3	7	56.6
54	2	1	92.5
54	2	2	94.5
54	2	3	90.2
54	2	4	92.1
54	2	5	96.0
54	2	6	93.6
54	2	7	91.2
56	3	1	68.7
56	3	2	64.8
56	3	3	64.2
56	3	4	65.9
56	3	5	67.0

Appendix Table II-19--Continued

EWE NUMBER	TRT	PERIOD	EWE BODY WEIGHT (KG)
56	3	6	65.0
56	3	7	61.3
60	2	1	84.6
60	2	2	82.0
60	2	3	75.0
60	2	4	70.7
60	2	5	.
60	2	6	.
60	2	7	.
99	3	1	87.1
99	3	2	77.7
99	3	3	64.1
99	3	4	71.5
99	3	5	71.1
99	3	6	.
99	3	7	.
104	1	1	90.5
104	1	2	85.9
104	1	3	84.0
104	1	4	85.2
104	1	5	87.3
104	1	6	87.5
104	1	7	87.5
119	3	1	86.1
119	3	2	84.0
119	3	3	79.2
119	3	4	80.0
119	3	5	79.7
119	3	6	65.5
119	3	7	69.1

Appendix Table II-20: Raw data for plasma copper and molybdenum parameters of lambs in the ewe-lamb trial.

LAMB NUMBER	TRT	PERIOD	PLASMA COPPER (MG/L)	TCA-SOLUBLE COPPER (MG/L)	CP OXIDASE ACTIVITY (ΔA/MIN/L)	PLASMA MOLYBDENUM (MG/L)
9	3	1	0.47	0.29	11.4	0.10
9	3	2	0.75	0.61	59.4	3.15
9	3	3	0.89	0.72	61.4	3.52
9	3	4	0.73	0.68	42.8	0.84
9	3	5	0.96	0.97	86.8	0.27
9	3	6	1.33	1.12	121.8	0.00
9	3	7	.	.	.	.
10	3	1	0.18	0.28	12.6	0.15
10	3	2	0.79	0.57	74.8	2.83
10	3	3	0.89	0.76	67.8	6.48
10	3	4	0.70	0.66	60.2	1.26
10	3	5	0.86	0.88	81.2	0.48
10	3	6	1.20	1.13	114.0	0.58
10	3	7	.	.	.	.
11	2	1	0.55	0.62	42.6	0.02
11	2	2	1.33	0.88	135.8	0.58
11	2	3	0.87	0.84	78.0	2.25
11	2	4	0.69	0.64	61.6	4.24
11	2	5	0.86	0.87	99.0	1.02
11	2	6	1.23	1.19	131.2	1.11
11	2	7	1.07	1.01	103.0	0.88
12	2	1	0.53	0.43	41.4	0.05
12	2	2	0.80	.	175.2	0.62
12	2	3	0.88	0.81	82.2	2.93
12	2	4	0.79	0.76	74.2	4.04
12	2	5	0.93	0.99	88.8	2.00
12	2	6	0.95	0.93	100.6	1.10
12	2	7	1.22	1.11	127.6	1.03
15	1	1	0.20	0.17	9.0	0.00
15	1	2	0.71	0.72	56.8	0.00
15	1	3	0.64	0.54	40.2	0.12
15	1	4	0.56	0.61	41.0	0.13
15	1	5	0.54	0.59	31.0	0.16
15	1	6	0.60	0.68	44.0	0.00
15	1	7	0.69	0.59	49.0	0.00
16	1	1	0.22	0.21	10.2	0.00
16	1	2	0.79	0.63	57.0	0.00
16	1	3	0.59	0.46	38.6	0.11
16	1	4	0.58	0.52	43.4	0.22
16	1	5	0.68	0.60	55.2	0.19
16	1	6	0.82	0.82	69.8	0.09
16	1	7	0.66	0.64	59.8	0.00
42	2	1	0.26	0.21	9.2	0.09
42	2	2	.	1.19	101.0	0.39
42	2	3	0.81	0.72	64.4	2.29
42	2	4	1.08	.	92.6	0.57
42	2	5	1.12	1.01	101.0	0.62
42	2	6	.	.	.	.
42	2	7	.	1.84	.	.
43	2	1	0.32	0.21	13.4	0.07
43	2	2	1.65	.	.	0.49
43	2	3	1.45	1.35	116.2	1.95
43	2	4	1.49	1.20	142.8	0.80

Appendix Table II-20--Continued

LAMB NUMBER	TRT	PERIOD	PLASMA COPPER (MG/L)	TCA-SOLUBLE COPPER (MG/L)	CP OXIDASE ACTIVITY (ΔA/MIN/L)	PLASMA MOLYBDENUM (MG/L)
43	2	5	1.44	1.38	131.6	0.47
43	2	6	.	.	.	.
43	2	7	.	.	.	.
52	3	1	0.33	0.23	11.6	0.22
52	3	2	0.75	0.78	60.6	0.74
52	3	3	0.90	0.88	47.4	7.13
52	3	4	0.78	0.80	71.2	5.52
52	3	5	1.27	1.23	123.0	0.62
52	3	6	1.11	0.85	106.6	0.43
52	3	7	0.98	0.90	79.8	0.34
53	3	1	0.31	0.22	11.2	0.00
53	3	2	0.53	0.45	37.6	0.87
53	3	3	0.56	.	71.2	4.98
53	3	4	0.67	0.58	54.4	1.54
53	3	5	0.93	0.90	72.4	0.38
53	3	6	1.13	1.25	105.4	0.61
53	3	7	0.86	0.69	72.4	0.89
54	1	1	0.24	0.17	12.0	0.06
54	1	2	1.00	0.88	86.8	0.15
54	1	3	0.66	0.67	64.8	0.02
54	1	4	0.78	0.93	80.2	0.05
54	1	5	1.54	1.38	133.4	0.00
54	1	6	1.48	1.28	142.2	0.00
54	1	7	1.35	1.35	119.6	0.06
55	1	1	0.28	0.17	12.6	0.36
55	1	2	1.09	0.95	91.8	0.10
55	1	3	0.84	0.82	.	0.01
55	1	4	0.89	0.74	85.0	0.00
55	1	5	1.50	1.32	167.8	0.09
55	1	6	1.38	0.96	152.2	0.01
55	1	7	1.05	1.06	94.6	0.07
56	3	1	0.27	0.27	16.6	0.10
56	3	2	1.64	1.65	149.0	0.11
56	3	3	1.77	1.64	160.0	3.13
56	3	4	1.37	1.26	116.0	1.92
56	3	5	1.33	1.17	122.2	0.85
56	3	6	1.42	1.41	127.8	0.93
56	3	7	1.40	1.23	110.8	0.30
57	3	1	0.35	0.25	18.6	0.16
57	3	2	1.12	1.02	73.8	0.50
57	3	3	0.94	0.97	73.6	1.58
57	3	4	0.94	0.92	104.6	0.46
57	3	5	1.18	1.08	109.4	0.76
57	3	6	1.14	1.07	94.0	1.14
57	3	7	1.03	0.97	82.6	0.97
61	2	1	0.94	0.81	71.4	0.01
61	2	2	1.27	1.05	113.8	0.10
61	2	3	1.19	0.95	80.8	1.04
61	2	4	1.09	1.06	97.4	1.23
61	2	5	1.16	0.83	108.2	0.64
61	2	6	1.18	1.13	108.8	0.21
61	2	7	1.03	.	83.6	0.22
62	2	1	0.71	0.65	49.0	0.00

Appendix Table II-20 --Continued

LAMB NUMBER	TRT	PERIOD	PLASMA COPPER (MG/L)	TCA-SOLUBLE COPPER (MG/L)	CP OXIDASE ACTIVITY (ΔA/MIN/L)	PLASMA MOLYBDENUM (MG/L)
62	2	2	0.94	0.82	77.8	0.19
62	2	3	1.06	1.05	102.4	0.89
62	2	4	1.34	1.22	130.2	1.75
62	2	5	1.56	1.53	168.4	.
62	2	6	1.83	2.05	2144.0	0.00
62	2	7	1.88	1.01	174.6	0.45
67	1	1	0.37	0.32	18.6	0.00
67	1	2	0.51	0.48	42.6	0.00
67	1	3	0.83	0.84	89.0	0.00
67	1	4	1.02	0.99	102.4	0.04
67	1	5	1.38	1.30	132.2	0.00
67	1	6	1.05	0.99	90.6	0.00
67	1	7	0.89	0.92	82.4	0.05
68	1	1	0.46	0.25	24.4	0.00
68	1	2	.	0.46	.	0.04
68	1	3	0.99	0.93	93.2	0.00
68	1	4	1.40	1.30	135.4	0.02
68	1	5	1.12	1.11	123.2	0.03
68	1	6	1.19	1.06	117.2	0.00
68	1	7	0.91	0.92	87.4	0.04
71	2	1	0.73	0.63	48.2	0.10
71	2	2	1.41	1.33	141.8	0.00
71	2	3	1.41	1.41	146.6	2.22
71	2	4	.	.	.	.
71	2	5	.	.	.	.
71	2	6	.	.	.	.
71	2	7	.	.	.	.
72	2	1	0.45	0.43	28.4	0.03
72	2	2	0.88	0.77	73.2	0.25
72	2	3	0.80	0.56	70.2	2.18
72	2	4	1.06	1.05	94.8	1.66
72	2	5	.	.	.	.
72	2	6	.	.	.	.
72	2	7	.	.	.	.
74	1	1	0.44	0.35	15.8	0.13
74	1	2	1.35	1.31	130.6	0.39
74	1	3	1.22	1.10	114.6	1.38
74	1	4	1.24	1.17	119.2	1.63
74	1	5	1.05	1.00	93.2	0.00
74	1	6	0.89	1.34	77.2	0.05
74	1	7	1.00	0.81	75.0	0.17
75	1	1	0.25	0.22	27.8	0.00
75	1	2	0.60	0.52	50.2	0.31
75	1	3	1.09	1.04	123.6	1.49
75	1	4	1.59	1.60	174.2	0.19
75	1	5	1.32	1.26	131.0	0.00
75	1	6	1.45	1.24	118.4	0.06
75	1	7	1.23	1.11	107.4	0.01
77	3	1	0.20	0.09	9.4	0.00
77	3	2	0.91	0.81	71.2	0.00
77	3	3	1.39	1.11	126.2	0.00
77	3	4	1.65	1.58	161.8	0.82
77	3	5	1.19	1.07	97.8	1.74

Appendix Table II-20--Continued

LAMB NUMBER	TRT	PERIOD	PLASMA COPPER (MG/L)	TCA-SOLUBLE COPPER (MG/L)	CP OXIDASE ACTIVITY (ΔA/MIN/L)	PLASMA MOLYBDENUM (MG/L)
77	3	6	0.94	0.88	75.4	0.97
77	3	7	0.86	0.77	60.4	0.98
78	3	1	0.27	0.09	11.2	0.04
78	3	2	0.86	0.78	73.0	0.07
78	3	3	1.17	1.04	104.8	0.22
78	3	4	0.96	1.54	137.6	.
78	3	5	1.03	1.11	94.4	0.44
78	3	6	0.89	0.81	86.0	1.05
78	3	7	0.82	0.67	54.6	0.76

Appendix Table II-21: Raw data for lamb liver copper and molybdenum concentrations in the ewe-lamb trial.

LAMB NUMBER	EWE	TRT	LIVER COPPER CONTENT,DM BASIS (MG/KG)	LIVER MOLYDBENUM CONTENT,DM BASIS (MG/KG)
9	99	3	.	.
10	99	3	.	.
11	51	2	226.88	3.84
12	51	2	147.07	7.41
15	31	1	207.13	5.54
16	31	1	126.60	8.00
42	52	2	.	.
43	52	2	.	.
52	56	3	230.61	16.90
53	56	3	260.16	4.45
54	104	1	256.21	8.32
55	104	1	215.81	4.84
56	119	3	264.53	10.90
57	119	3	439.87	12.75
61	54	2	286.60	4.48
62	54	2	215.75	29.00
67	47	1	298.21	3.74
68	47	1	274.12	1.83
71	60	2	.	.
72	60	2	.	.
74	11	1	277.09	3.14
75	11	1	292.75	3.92
77	53	3	437.75	12.97
78	53	3	289.21	7.50

Appendix Table II-22: Raw data for lamb body weight in the ewe-lamb trial.

LAMB NUMBER	EWE	TRT	PERIOD	LAMB BODY WEIGHT (KG)
9	99	3	1	4.80
9	99	3	2	8.20
9	99	3	3	9.80
9	99	3	4	11.90
9	99	3	5	13.80
9	99	3	6	15.20
9	99	3	7	.
10	99	3	1	4.80
10	99	3	2	8.10
10	99	3	3	10.20
10	99	3	4	11.30
10	99	3	5	.
10	99	3	6	14.30
10	99	3	7	.
11	51	2	1	4.10
11	51	2	2	6.10
11	51	2	3	8.45
11	51	2	4	10.50
11	51	2	5	13.10
11	51	2	6	14.10
11	51	2	7	15.40
12	51	2	1	2.90
12	51	2	2	4.60
12	51	2	3	6.50
12	51	2	4	8.00
12	51	2	5	9.50
12	51	2	6	10.20
12	51	2	7	11.20
15	31	1	1	2.90
15	31	1	2	4.70
15	31	1	3	6.70
15	31	1	4	8.40
15	31	1	5	10.20
15	31	1	6	10.20
15	31	1	7	12.30
16	31	1	1	3.60
16	31	1	2	3.90
16	31	1	3	6.00
16	31	1	4	7.70
16	31	1	5	9.40
16	31	1	6	10.40
16	31	1	7	12.00
42	52	2	1	4.30
42	52	2	2	5.20
42	52	2	3	7.50
42	52	2	4	9.60
42	52	2	5	10.90
42	52	2	6	.
42	52	2	7	.
43	52	2	1	3.20
43	52	2	2	3.90
43	52	2	3	5.30
43	52	2	4	7.20
43	52	2	5	8.40

Appendix Table II-22--Continued

LAMB NUMBER	EWE	TRT	PERIOD	LAMB BODY WEIGHT (KG)
43	52	2	6	.
43	52	2	7	.
52	56	3	1	5.0
52	56	3	2	6.5
52	56	3	3	8.7
52	56	3	4	11.0
52	56	3	5	12.2
52	56	3	6	14.0
52	56	3	7	15.0
53	56	3	1	4.1
53	56	3	2	5.3
53	56	3	3	7.3
53	56	3	4	9.6
53	56	3	5	10.5
53	56	3	6	12.0
53	56	3	7	13.4
54	104	1	1	4.9
54	104	1	2	6.0
54	104	1	3	7.9
54	104	1	4	10.5
54	104	1	5	12.0
54	104	1	6	14.2
54	104	1	7	14.7
55	104	1	1	5.3
55	104	1	2	7.1
55	104	1	3	9.3
55	104	1	4	12.1
55	104	1	5	13.3
55	104	1	6	15.4
55	104	1	7	17.3
56	119	3	1	4.5
56	119	3	2	5.2
56	119	3	3	6.5
56	119	3	4	8.4
56	119	3	5	9.0
56	119	3	6	10.7
56	119	3	7	10.5
57	119	3	1	4.4
57	119	3	2	6.0
57	119	3	3	8.8
57	119	3	4	11.5
57	119	3	5	13.4
57	119	3	6	15.1
57	119	3	7	16.1
61	54	2	1	5.4
61	54	2	2	5.9
61	54	2	3	8.4
61	54	2	4	11.3
61	54	2	5	13.0
61	54	2	6	15.3
61	54	2	7	16.6
62	54	2	1	5.0
62	54	2	2	5.8
62	54	2	3	7.9

Appendix Table II-22--Continued

LAMB NUMBER	EWE	TRT	PERIOD	LAMB BODY WEIGHT (KG)
62	54	2	4	10.50
62	54	2	5	11.80
62	54	2	6	13.10
62	54	2	7	14.40
67	47	1	1	4.00
67	47	1	2	4.50
67	47	1	3	6.00
67	47	1	4	8.20
67	47	1	5	9.40
67	47	1	6	10.70
67	47	1	7	11.40
68	47	1	1	4.90
68	47	1	2	5.50
68	47	1	3	7.50
68	47	1	4	9.70
68	47	1	5	11.20
68	47	1	6	12.60
68	47	1	7	14.70
71	60	2	1	3.80
71	60	2	2	3.80
71	60	2	3	6.60
71	60	2	4	.
71	60	2	5	.
71	60	2	6	.
71	60	2	7	.
72	60	2	1	4.60
72	60	2	2	4.90
72	60	2	3	7.70
72	60	2	4	9.60
72	60	2	5	.
72	60	2	6	.
72	60	2	7	.
74	11	1	1	3.95
74	11	1	2	4.40
74	11	1	3	6.70
74	11	1	4	8.20
74	11	1	5	10.90
74	11	1	6	11.40
74	11	1	7	12.80
75	11	1	1	3.75
75	11	1	2	4.00
75	11	1	3	6.60
75	11	1	4	8.20
75	11	1	5	10.80
75	11	1	6	11.30
75	11	1	7	12.60
77	53	3	1	3.90
77	53	3	2	4.10
77	53	3	3	6.50
77	53	3	4	7.80
77	53	3	5	9.00
77	53	3	6	10.20
77	53	3	7	11.00
78	53	3	1	4.90

Appendix Table II-22--Continued

LAMB NUMBER	EWE	TRT	PERIOD	LAMB BODY WEIGHT (KG)
78	53	3	2	4.6
78	53	3	3	7.2
78	53	3	4	8.2
78	53	3	5	9.8
78	53	3	6	10.8
78	53	3	7	12.0

APPENDIX III

DAIRY COW TRIAL

Appendix Table III-1: Experimental diet allocation and background information for Hostein-Friesian cows used in the dairy trial.

Experimental Diet						
Period 1	Period 2	Treatment	Cow	Lactation	Days of Lactation	Last breeding date†
Basal	Basal	I	1	1	200	Feb. 28
			2	1	134	Open
			3	5	171	June 2
			4	2	177	Mar. 13
Basal	+Cu	II	5	2	168	Mar. 27
			6	1	177	May 6
			7	1	132	Open
			8	3	202	Mar. 4
+Mo	+Mo	III	9	2	197	Open
			10	1	113	June 12
			11	3	217	May 5
			12	2	203	Open
+Mo	+Mo+C	IV	13	1	174	May 11
			14	1	117	Open
			15	3	133	Open
			16	6	185	Open

† Trial started June 18. Open refers to cows that had not been successfully bred at the time the trial started.

Appendix Table III-2: Analysis of variance for body weight and weight change of cows in the dairy cow trial (mean squares and degrees of freedom†).

Source of Variation:	Treatment	Animal (Treatment)	Week	Treatment Week	Error
Items:					
Body weight ($x10^3$)	9.037 (3)	9.047 (12)	0.922**‡ (2)	0.066 (6)	0.007 (23)
Average daily gain	0.230 (3)	0.206 (12)	6.235*** (1)	0.108 (3)	0.263 (11)

† Figures in parentheses represent the degrees of freedom for the corresponding mean squares.

‡ Significant difference at: ** $\alpha = 0.01$, *** $\alpha = 0.001$.

Appendix Table III-3: Analysis of variance for feed intake and feed efficiency of cows in the dairy cow trial (mean squares and degrees of freedom).

Source of Variation:	Treatment	Animal (Treatment)	Week	Treatment x Week	Error
Degrees of Freedom:	3	12	7	21	77
Items:					
D.M. intake, kg d^{-1}	2.349	8.747	16.871***†	0.101	0.811
Feed efficiency, kg D.M. kg^{-1} milk	3.455	2.411	0.461***	0.146	0.128
Feed efficiency, kg D.M. kg^{-1} FCM	6.992	2.304	2.421***	0.376	0.408

† Significant differences at: *** α = 0.001.

Appendix Table III-4: Analysis of variance for plasma copper and molybdenum parameters of Holstein-Friesian cows fed supplemental copper and/or molybdenum (mean squares).

Source of Variation:	Treatment	Animal (Treatment)	Week	Treatment x Week	Error‡
Degrees of Freedom:	3	12	4	12	45
Plasma Cu, mg L^{-1} ($x10^{-1}$)	1.683	0.533	0.234**†	0.118**	0.045
TCA-soluble Cu, mg L^{-1} ($x10^{-1}$)	0.289	0.359	0.411***	0.049	0.046
TCA-insoluble, Cu, mg L^{-1}	0.114***	0.008	0.47***	0.010	0.006
Cp oxidase activity Δ A min^{-1} L^{-1} ($x10^{2}$)	1.186	4.714	0.185	0.305	0.449
Plasma Mo	0.803***	0.024	0.250***	0.076**	0.025

† Significant differences at: * α = 0.05, ** α = 0.01, *** α = 0.001.

‡ Degrees of freedom for error were 46 for plasma Cu and plasma Mo.

Appendix Table III-5: Analysis of variance for milk yield and milk fat, protein and lactose content for lactating Holstein-Friesian cows fed supplemental copper and/or molybdenum (mean squares).

Source of Variation:	Treatment	Animal (Treatment)	Week	Treatment x Week	Error
Degrees of Freedom:	3	12	7	21	80
Items:					
Milk yield, kg d^{-1} ($x10^2$)	0.537	1.038	0.565***†	0.027	0.037
FCM yield, kg d^{-1} ($x10^1$)	8.793	8.950	3.774***	0.572	0.653
Butterfat, %	0.610	1.979	0.949*	0.205	0.334
Protein, %	0.466	0.487	0.046**	0.011	0.014
Lactose, %	0.289	0.534	0.137***	0.007	0.018

† Significant differences at: * α = 0.05, ** α = 0.01, *** α = 0.001.

Appendix Table III-6: Analysis of variance for milk copper and molybdenum concentrations (mg L^{-1}) and daily excretions (mg d^{-1}) for lactating Holstein-Friesian cows fed supplemental copper and/or molybdenum (mean squares).

Source of Variation:	Treatment	Animal (Treatment)	Week	Treatment x Week	Error
Degrees of Freedom:	3	12	4	12	44
Items:					
Milk copper, mg L^{-1} ($x10^{-4}$)	0.493	2.229	0.982**†	0.328	0.255
Milk copper, mg d^{-1} ($x10^{-1}$)	0.265	0.566	0.274	0.241	0.169
Milk molybdenum, mg L^{-1} ($x10^{-2}$)	2.338***	0.122	0.045	0.085***	0.024
Milk molybdenum, mg d^{-1} ($x10^{1}$)	1.579***	0.198	0.056	0.067**	0.022

† Significant differences at: ** α = 0.01, *** α = 0.001.

Appendix Table III-7: Analysis of variance for liver copper and molybdenum concentrations of lactating Holstein-Friesian cows fed supplemental molybdenum and/or copper (mean squares).

Source of Variation:	Treatment	Error
Degrees of Freedom:	3	11
Liver Cu change, mg kg^{-1} ($x10^4$)	7.618***†	0.498
Liver Mo change, mg kg^{-1} d^{-1} ($x10^1$)	2.177	0.661
Final liver Cu content, mg kg^{-1} ($x10^4$)	8.338*	1.357
Final liver Mo content, mg kg^{-1}	7.490*	1.597

† Significant differences at: * α = 0.05, *** α = 0.001.

Appendix Table III-8: Dry matter intakes (kg d^{-1}) of lactating Holstein-Friesian cows fed supplemental molybdenum and/or copper (least square means).

	TREATMENT				
	I	II	III	IV	Mean ± SE
Period 1 diet:	Basal	Basal	+Mo	+Mo	
Period 2 diet:	Basal	+Cu	+Mo	+Mo+Cu	
Sampling Time					
Period 1:					
Week 1	16.1	17.0	16.7	17.4	16.8 ± 0.3 C
Week 2	17.8	18.5	19.0	20.4	18.9 ± 0.3 A B
Week 3	19.6	19.3	20.5	19.4	19.8 ± 0.3 A
Week 4	19.0	19.9	20.5	19.3	19.7 ± 0.3 A
Period 2:					
Week 5	17.9	18.1	19.7	17.2	18.1 ± 0.3 B
Week 6	18.1	17.6	18.9	18.0	18.1 ± 0.3 B
Week 7	17.0	15.6	16.6	17.0	16.6 ± 0.3 C
Week 8	16.7	14.7	15.9	15.0	15.5 ± 0.3 D
Mean ± SE	17.8 ± 0.7	17.6 ± 0.7	18.5 ± 0.7	17.9 ± 0.7	

A - D Values in the same column with different superscripts are significantly different, $\alpha = 0.05$.

Appendix Table III-9: Daily milk yield (kg d^{-1}) for Holstein-Friesian cows fed supplemental molybdenum and/or copper (least mean squares).

	TREATMENT				
	I	II	III	IV	Mean ± SE
Period 1 diet:	Basal	Basal	+Mo	+Mo	
Period 2 diet:	Basal	+Cu	+Mo	+Mo+Cu	
Sampling Time					
Period 1:					
Week 1	27.7	24.8	26.3	28.6	26.8 ± 0.5 A
Week 2	28.0	26.7	25.9	29.3	27.5 ± 0.5 A
Week 3	29.3	26.5	25.8	28.9	27.6 ± 0.5 A
Week 4	29.7	27.1	26.8	27.1	27.7 ± 0.5 A
Period 2:					
Week 5	28.7	25.7	25.2	26.9	26.6 ± 0.5 A
Week 6	28.1	24.9	24.9	25.9	26.0 ± 0.5 A
Week 7	24.8	22.6	23.4	24.0	23.7 ± 0.5 B
Week 8	25.0	21.2	22.1	21.3	22.4 ± 0.5 B
Mean ± SE	27.7 ± 1.9	24.9 ± 1.9	25.0 ± 1.9	26.5 ± 1.9	

A - B Values in the same column with different superscripts are significantly different, $\alpha = 0.05$.

Appendix Table III-10: Milk molybdenum excretions (mg d^{-1}) for lactating Holstein-Friesian cows fed supplemental molybdenum and/or copper (least square means).

Sampling Time	TREATMENT I	II	III	IV	Mean ± SE
Initial	0.77[a]	0.81[a]	2.05[b]	2.21[b], B	1.46 ± 0.12
Week 2	0.92[a]	0.73[a]	2.19[b]	3.71[c], A	1.88 ± 0.12
Week 4	0.82[a]	0.68[a]	2.32[b]	2.62[b], B	1.61 ± 0.12
Week 6	0.92[a b]	0.75[a]	2.53[b]	1.71[b], C	1.48 ± 0.12
Week 8	0.76[a]	0.66[a]	2.47[b]	1.79[a b], B C	1.42 ± 0.12
Mean ± SE	0.84 ± 0.31[a]	0.72 ± 0.31[a]	2.31 ± 0.31[b]	2.40 ± 0.31[b]	

a - c Values in the same row with different superscripts are significantly different, $\alpha = 0.05$.

A - B Values in the same column with different superscripts are significantly different, $\alpha = 0.05$.

Appendix Table III-11: Plasma copper concentrations (mg L^{-1}) for lactating Holstein-Friesian cows fed supplemental molybdenum and/or copper (least square means).

Sampling Time	TREATMENT I	II	III	IV	Mean ± SE
Initial	1.07 A	0.99	1.06 B	1.04 B	1.03 ± 0.02 A B
Week 2	1.06[a] b, A	0.98[a]	1.23[b], A	1.06[a] b, A B	1.08 ± 0.02 A
Week 4	1.02[a] b, A	0.95[a]	1.19[b], A	1.12[a] b, A	1.07 ± 0.02 A
Week 6	0.94[a], B	0.92[a]	1.20[b], A	0.91[a], C	0.99 ± 0.02 B
Week 8	0.93[a], B	0.93[a]	1.16[b], A	0.99[a] b, B C	1.00 ± 0.02 B
Mean ± SE	1.00 ± 0.05	0.95 ± 0.05	1.17 ± 0.05	1.02 ± 0.05	

a - b Values in the same row in the different superscripts are significantly different, $\alpha = 0.05$.

A - C Values in the same column with different superscripts are significantly different, $\alpha = 0.05$.

Appendix Table III-12: Plasma TCA-soluble copper concentrations (mg L^{-1}) for lactating Holstein-Friesian cows fed supplemental molybdenum and/or copper (least square means).

Sampling Time	TREATMENT I	II	III	IV	Mean ± SE
Initial	0.85	0.79	0.85	0.81	0.82 ± 0.02 A B
Week 2	0.94	0.92	0.96	0.90	0.93 ± 0.02 B
Week 4	0.85	0.79	0.86	0.93	0.83 ± 0.02 A B
Week 6	0.89	0.91	0.96	0.81	0.89 ± 0.02 B
Week 8	0.84	0.83	0.87	0.68	0.80 ± 0.02 A
Mean ± SE	0.87 ± 0.04	0.84 ± 0.04	0.89 ± 0.04	0.80 ± 0.04	

A - B Values in the same column with different superscripts are significantly different, $\alpha = 0.05$.

Appendix Table III-13: Plasma TCA-insoluble copper concentrations (mg L^{-1}) for lactating Holstein-Friesian cows fed supplemental molybdenum and/or copper (least square means).

Sampling Time	TREATMENT I	II	III	IV	Mean ± SE
Initial	0.22	0.20	0.21	0.22	0.22 ± 0.02 A B
Week 2	0.12	0.06	0.27	0.14	0.15 ± 0.02 B C
Week 4	0.17	0.16	0.33	0.29	0.24 ± 0.02 A
Week 6	0.05	0.01	0.24	0.11	0.10 ± 0.02 C
Week 8	0.09	0.10	0.29	0.31	0.20 ± 0.02 A B
Mean ± SE	0.13 ± 0.02[a]	0.11 ± 0.02[a]	0.29 ± 0.02[b]	0.22 ± 0.02[b]	

a - b Values in the same row with different superscripts are significantly different, $\alpha = 0.05$.

A - C Values in the same column with different superscripts are significantly different, $\alpha = 0.05$.

Appendix Table III-14: Plasma ceruloplasim oxidase activity ($\Delta A\ min^{-1}\ L^{-1}$) for lactating Holstein-Friesian cows fed supplemental molybdenum and/or copper (least square means).

Sampling Time	TREATMENT I	II	III	IV	Mean ± SE
Initial	78.0	70.7	74.7	76.6	75.0 ± 1.7
Week 2	80.5	71.3	76.7	73.3	75.4 ± 1.7
Week 4	81.0	72.5	75.8	74.8	76.0 ± 1.7
Week 6	72.6	72.2	79.4	68.1	73.1 ± 1.7
Week 8	75.4	73.2	74.0	76.0	74.6 ± 1.7
Mean ± SE	77.5 ± 4.9	72.0 ± 4.9	76.1 ± 4.9	73.7 ± 4.9	

Appendix Table III-15: Plasma molybdenum concentrations (mg L^{-1}) for lactating Holstein-Friesian cows fed supplemental molybdenum and/or copper (least square means).

Sampling Time	TREATMENT I	II	III	IV	Mean ± SE
Initial	0.04	0.10	0.11 A B	0.06 A	0.08 ± 0.04 A
Week 2	0.00[a]	0.01[a]	0.28[b], A	0.30[b], B	0.15 ± 0.04 A B
Week 4	0.08[a]	0.12[a]	0.70[b], B	0.41[b], B	0.32 ± 0.04 C D
Week 6	0.14[a]	0.19[a]	0.89[c], B	0.35[b], B	0.39 ± 0.04 D
Week 8	0.01[a]	0.04[a]	0.55[c], B	0.24[b], A B	0.21 ± 0.04 B C
Mean ± SE	0.05 ± 0.04[a]	0.09 ± 0.04[a]	0.50 ± 0.04[c]	0.27 ± 0.04[b]	

a - c Values in the same row with different superscripts are significantly different, α = 0.05.

A - D Values in the same column with different superscripts are significantly different, α = 0.05.

Appendix Table III-16: Raw data for corn silage concentrate, hay, supplement and total dry matter intakes (kg d^{-1}) for cows in the dairy cow trial.

COW NUMBER	TRT	WEEK	CORN SILAGE AND CONCENTRATE (KG DM/DAY)	HAY (KG DM/DAY)	SUPPLEMENT (KG DM/DAY)	DAILY INTAKE (KG DM/DAY)
1	1	1	11.38	3.27	0.75	15.41
1	1	2	11.24	2.37	0.76	14.38
1	1	3	14.11	2.34	0.71	17.16
1	1	4	14.28	2.03	0.72	17.04
1	1	5	13.12	2.21	0.69	16.03
1	1	6	12.77	2.16	0.72	15.66
1	1	7	12.80	2.16	0.68	15.65
1	1	8	11.46	1.89	0.62	13.97
2	1	1	10.71	3.08	0.71	14.50
2	1	2	13.78	2.28	0.72	16.79
2	1	3	13.51	2.26	0.71	16.49
2	1	4	13.26	2.21	0.71	16.19
2	1	5	13.18	2.21	0.71	16.10
2	1	6	13.82	2.31	0.71	16.85
2	1	7	12.73	2.16	0.68	15.58
2	1	8	12.96	2.16	0.71	15.83
3	1	1	12.33	3.49	0.80	16.63
3	1	2	15.87	2.59	0.83	19.31
3	1	3	18.49	3.00	0.99	22.49
3	1	4	17.57	2.89	0.95	21.42
3	1	5	16.41	2.70	0.89	20.00
3	1	6	16.50	2.71	0.90	20.12
3	1	7	15.65	2.57	0.82	19.05
3	1	8	15.19	2.52	0.80	18.51
4	1	1	13.35	3.76	0.86	17.98
4	1	2	16.97	2.77	0.92	20.67
4	1	3	18.33	2.95	0.96	22.25
4	1	4	17.50	2.88	0.94	21.32
4	1	5	15.86	2.57	0.82	19.25
4	1	6	16.23	2.72	0.89	19.85
4	1	7	14.43	2.39	0.73	17.55
4	1	8	15.26	2.52	0.80	18.58
5	2	1	13.42	3.76	0.86	18.05
5	2	2	13.80	2.52	0.81	17.13
5	2	3	16.14	2.62	0.85	19.62
5	2	4	17.04	2.80	0.94	20.78
5	2	5	15.18	2.52	0.80	18.50
5	2	6	14.88	2.46	0.77	18.13
5	2	7	12.45	2.10	0.68	15.25
5	2	8	11.69	1.94	0.61	14.25
6	2	1	12.66	3.60	0.82	17.09
6	2	2	15.78	2.57	0.82	19.17
6	2	3	14.09	2.70	0.89	17.68
6	2	4	15.77	2.59	0.83	19.21
6	2	5	14.93	2.46	0.78	18.19
6	2	6	15.11	2.52	0.80	18.43
6	2	7	13.50	2.26	0.71	16.48
6	2	8	12.84	2.18	0.66	15.68
7	2	1	11.38	3.27	0.75	15.41
7	2	2	15.41	2.31	0.72	18.45
7	2	3	15.83	2.59	0.83	19.27
7	2	4	16.03	2.39	0.85	19.27
7	2	5	13.80	2.34	0.75	16.89
7	2	6	12.79	2.16	0.71	15.67

Appendix Table III-16--Continued

COW NUMBER	TRT	WEEK	CORN SILAGE AND CONCENTRATE (KG DM/DAY)	HAY (KG DM/DAY)	SUPPLEMENT (KG DM/DAY)	DAILY INTAKE (KG DM/DAY)
7	2	7	12.61	2.13	0.69	15.44
7	2	8	11.47	1.92	0.61	14.01
8	2	1	13.22	3.44	0.86	17.53
8	2	2	15.63	2.57	0.82	19.03
8	2	3	16.71	2.72	1.04	20.47
8	2	4	16.73	2.77	0.91	20.42
8	2	5	15.25	2.54	0.81	18.61
8	2	6	14.77	2.44	0.76	17.97
8	2	7	12.56	2.13	0.68	15.39
8	2	8	12.14	2.08	0.66	14.88
9	3	1	12.69	3.34	0.71	16.75
9	3	2	14.25	2.41	0.78	17.46
9	3	3	16.16	2.64	0.86	19.67
9	3	4	14.94	2.49	0.78	18.23
9	3	5	13.42	2.26	0.71	16.39
9	3	6	13.77	2.26	0.71	16.75
9	3	7	11.05	1.85	0.58	13.49
9	3	8	12.36	2.08	0.67	15.12
10	3	1	13.11	3.65	0.85	17.61
10	3	2	16.75	2.34	0.85	19.94
10	3	3	17.56	2.85	0.94	21.35
10	3	4	17.25	2.82	0.95	21.04
10	3	5	18.16	2.95	0.97	22.10
10	3	6	15.87	2.62	0.85	19.35
10	3	7	15.74	2.59	0.83	19.18
10	3	8	11.97	1.89	0.64	14.51
11	3	1	11.20	3.98	0.94	16.12
11	3	2	17.00	2.80	0.91	20.72
11	3	3	16.73	2.70	0.89	20.32
11	3	4	18.17	2.82	0.92	21.92
11	3	5	16.73	2.77	0.91	20.42
11	3	6	16.98	2.77	0.92	20.68
11	3	7	14.41	2.44	0.80	17.65
11	3	8	14.20	2.34	0.73	17.28
12	3	1	12.47	3.18	0.82	16.49
12	3	2	14.77	2.44	0.77	17.99
12	3	3	16.84	2.75	0.91	20.50
12	3	4	17.23	2.82	0.92	20.98
12	3	5	16.27	2.64	0.86	19.78
12	3	6	15.21	2.52	0.92	18.66
12	3	7	13.32	2.23	0.69	16.25
12	3	8	13.54	2.26	0.68	16.49
13	4	1	12.46	3.58	0.81	16.86
13	4	2	14.53	2.41	0.76	17.71
13	4	3	16.19	2.64	0.86	19.70
13	4	4	16.12	2.64	0.86	19.63
13	4	5	14.75	2.44	0.76	17.95
13	4	6	14.78	2.44	0.76	17.98
13	4	7	14.72	2.44	0.76	17.93
13	4	8	11.38	1.98	0.63	13.99
14	4	1	11.00	3.96	0.92	15.89
14	4	2	16.07	2.77	0.90	19.75
14	4	3	10.49	1.85	0.59	12.94
14	4	4	.	.	.	.

Appendix Table III-16--Continued

COW NUMBER	TRT	WEEK	CORN SILAGE AND CONCENTRATE (KG DM/DAY)	HAY (KG DM/DAY)	SUPPLEMENT (KG DM/DAY)	DAILY INTAKE (KG DM/DAY)
14	4	5	.	.	.	.
14	4	6	.	.	.	.
14	4	7	.	.	.	.
14	4	8	.	.	.	.
15	4	1	12.45	3.00	0.82	16.29
15	4	2	17.34	2.82	0.94	21.11
15	4	3	17.64	2.88	0.94	21.46
15	4	4	16.40	2.97	0.97	20.35
15	4	5	15.76	2.70	0.89	19.35
15	4	6	17.85	2.90	0.97	21.74
15	4	7	16.27	2.72	0.90	19.90
15	4	8	14.97	2.49	0.78	18.25
16	4	1	15.31	4.29	0.99	20.60
16	4	2	19.04	3.11	1.00	23.15
16	4	3	19.18	3.12	1.01	23.33
16	4	4	16.95	2.80	0.92	20.68
16	4	5	13.90	2.36	0.76	17.03
16	4	6	14.14	2.36	0.72	17.24
16	4	7	13.21	2.21	0.71	16.13
16	4	8	12.48	2.13	0.68	15.30

Appendix Table III-17: Raw data for body weight of cows in the dairy cow trial.

COW NUMBER	TRT	PERIOD	COW BODYWEIGHT (KG)
1	1	1	533
1	1	2	558
1	1	3	556
2	1	1	458
2	1	2	463
2	1	3	451
3	1	1	640
3	1	2	651
3	1	3	617
4	1	1	662
4	1	2	686
4	1	3	668
5	2	1	578
5	2	2	588
5	2	3	568
6	2	1	586
6	2	2	586
6	2	3	578
7	2	1	540
7	2	2	564
7	2	3	528
8	2	1	612
8	2	2	608
8	2	3	621
9	3	1	632
9	3	2	640
9	3	3	608
10	3	1	616
10	3	2	615
10	3	3	574
11	3	1	682
11	3	2	688
11	3	3	678
12	3	1	615
12	3	2	626
12	3	3	640
13	4	1	548
13	4	2	554
13	4	3	542
14	4	1	578
14	4	2	570
14	4	3	.
15	4	1	616
15	4	2	621
15	4	3	620
16	4	1	599
16	4	2	594
16	4	3	555

Appendix Table III-18: Raw data for plasma copper and molybdenum parameters of cows in the dairy cow trial.

COW NUMBER	TRT	WEEK	PLASMA COPPER (MG/L)	TCA-SOLUBLE COPPER (MG/L)	CP OXIDASE ACTIVITY (ΔA/MIN/L)	PLASMA MOLYBDENUM (MG/L)
1	1	1	1.00	0.75	71.6	0.00
1	1	2	0.96	0.87	68.4	0.00
1	1	3	0.94	0.74	73.0	0.04
1	1	4	0.94	0.74	73.6	0.04
1	1	5	0.94	0.74	75.6	0.04
2	1	1	1.33	1.02	107.6	0.07
2	1	2	1.37	1.16	108.8	0.00
2	1	3	1.25	1.06	104.6	0.00
2	1	4	1.25	1.06	95.6	0.00
2	1	5	1.25	1.06	91.2	0.00
3	1	1	0.92	0.79	69.4	0.10
3	1	2	0.93	0.89	65.2	0.00
3	1	3	0.99	0.78	75.8	0.28
3	1	4	0.99	0.78	57.2	0.28
3	1	5	0.99	0.78	70.4	0.28
4	1	1	1.01	0.82	63.2	0.00
4	1	2	0.98	0.84	79.4	0.00
4	1	3	0.90	0.82	70.6	0.00
4	1	4	0.90	0.82	64.0	0.00
4	1	5	0.90	0.82	64.4	0.00
5	2	1	0.93	0.74	66.6	0.17
5	2	2	0.92	0.83	61.8	0.05
5	2	3	0.84	0.63	60.8	0.00
5	2	4	0.84	0.63	59.4	0.00
5	2	5	0.84	0.63	65.6	0.00
6	2	1	1.11	0.89	87.0	0.05
6	2	2	1.10	1.01	86.2	0.00
6	2	3	1.07	0.93	80.6	0.00
6	2	4	1.07	0.93	77.8	0.00
6	2	5	1.07	0.93	82.0	0.00
7	2	1	0.91	0.71	57.8	0.10
7	2	2	0.91	0.82	63.4	0.00
7	2	3	0.89	0.72	76.0	0.26
7	2	4	0.89	0.72	70.2	0.26
7	2	5	0.89	0.72	57.2	0.26
8	2	1	1.01	0.81	71.2	0.07
8	2	2	0.98	1.03	73.8	0.00
8	2	3	0.98	0.86	72.6	0.21
8	2	4	0.98	0.86	81.4	0.21
8	2	5	0.98	0.86	88.0	0.21
9	3	1	0.97	0.87	72.0	0.12
9	3	2	1.10	0.94	75.6	0.45
9	3	3	1.18	0.83	73.6	0.79
9	3	4	1.18	0.83	86.2	0.79
9	3	5	1.18	0.83	70.0	0.79
10	3	1	0.96	0.76	68.0	0.12
10	3	2	1.27	0.88	66.4	0.00
10	3	3	1.12	0.87	71.6	0.74
10	3	4	1.12	0.87	73.8	0.74
10	3	5	1.12	0.87	77.2	0.74
11	3	1	1.03	0.84	70.8	0.10
11	3	2	1.15	0.95	74.0	0.28
11	3	3	1.26	0.92	83.6	0.34
11	3	4	1.26	0.92	74.6	0.34

Appendix Table III-18--Continued

COW NUMBER	TRT	WEEK	PLASMA COPPER (MG/L)	TCA-SOLUBLE COPPER (MG/L)	CP OXIDASE ACTIVITY (ΔA/MIN/L)	PLASMA MOLYBDENUM (MG/L)
11	3	5	1.26	0.92	84.4	0.34
12	3	1	1.27	0.91	88.0	0.10
12	3	2	1.38	1.05	90.8	0.38
12	3	3	1.21	0.82	74.4	0.91
12	3	4	1.21	0.82	82.8	0.91
12	3	5	1.21	0.82	64.2	0.91
13	4	1	1.13	0.87	75.8	0.00
13	4	2	1.14	.	74.4	0.33
13	4	3	1.14	0.82	71.8	0.46
13	4	4	1.14	0.82	68.2	0.46
13	4	5	1.14	0.82	68.0	0.46
14	4	1	0.91	0.70	67.6	0.00
14	4	2	0.97	0.82	71.6	0.57
14	4	3	1.19	0.83	75.0	0.18
14	4	4	1.19	0.83	.	0.18
14	4	5	1.19	0.83	.	0.18
15	4	1	1.01	0.85	81.6	0.00
15	4	2	1.03	0.90	76.2	0.00
15	4	3	1.05	0.87	74.4	0.68
15	4	4	1.05	0.87	76.4	0.68
15	4	5	1.05	0.87	77.6	0.68
16	4	1	1.09	0.81	81.2	0.23
16	4	2	1.11	0.94	70.8	0.28
16	4	3	1.10	0.79	78.0	0.33
16	4	4	1.10	0.79	63.2	0.33
16	4	5	1.10	0.79	85.8	0.33

Appendix Table III-19: Raw data for liver copper and molybdenum concentrations (mg kg^{-1} D.M.) of cows in the dairy cow trial.

COW NUMBER	TRT	INITIAL LIVER COPPER CONCENTRATION	FINAL LIVER COPPER CONCENTRATION	INITIAL LIVER MO CONCENTRATION	FINAL LIVER MO CONCENTRATION
1	1	401.26	315.97	4.29	5.23
2	1	410.79	332.50	4.06	3.88
3	1	479.88	448.70	6.40	5.14
4	1	361.67	314.15	10.32	4.79
5	2	365.52	478.13	11.12	2.09
6	2	195.61	456.74	4.85	3.80
7	2	495.39	698.70	6.85	4.09
8	2	532.51	776.10	7.77	5.11
9	3	389.74	265.85	4.20	7.18
10	3	335.01	240.95	4.80	7.13
11	3	327.33	321.20	4.86	6.02
12	3	454.38	293.55	6.40	7.18
13	4	314.64	316.00	3.95	1.66
14	4	.	.	.	.
15	4	419.38	636.34	3.36	4.55
16	4	530.65	569.22	8.62	6.24

Appendix Table III-20: Raw data for milk yield and composition of cows in the dairy cow trial.

COW NUMBER	TRT	WEEK	MILK YIELD (KG/DAY)	MILK FAT CONTENT (%)	MILK PROTEIN CONTENT (%)	MILK LACTOSE CONTENT (%)	MILK COPPER CONTENT (MG/L)	MILK MOLYBDENUM CONTENT (MG/L)
1	1	1	26.4658	2.43	3.05	5.17	0.03	0.01
1	1	2	26.7250	2.15	2.96	5.14	0.04	0.03
1	1	3	27.9236	1.90	3.14	5.27	.	.
1	1	4	27.5348	5.26	2.81	4.97	0.04	0.02
1	1	5	27.2757	2.18	3.00	5.01	.	.
1	1	6	25.5912	2.70	3.11	5.28	0.04	0.03
1	1	7	23.9715	2.41	3.06	5.25	.	.
1	1	8	21.2504	2.78	3.01	5.46	0.04	0.03
2	1	1	26.0771	2.78	2.99	5.20	0.04	0.03
2	1	2	25.7532	2.01	2.95	5.20	0.03	0.03
2	1	3	25.7208	1.83	2.95	5.19	.	.
2	1	4	25.0729	2.40	2.90	5.14	0.04	0.03
2	1	5	25.2672	2.77	2.83	4.92	.	.
2	1	6	26.4982	3.42	2.81	5.10	0.03	0.03
2	1	7	22.8053	3.16	2.80	5.18	.	.
2	1	8	23.6476	3.97	2.94	5.05	0.04	0.02
3	1	1	30.1587	3.77	2.86	4.77	0.03	0.04
3	1	2	30.1263	4.61	2.94	4.66	0.04	0.04
3	1	3	33.6249	3.67	2.99	4.89	.	.
3	1	4	35.3094	3.53	3.00	4.80	0.03	0.03
3	1	5	33.1714	4.36	2.94	4.49	.	.
3	1	6	31.6812	4.12	2.96	4.66	0.03	0.04
3	1	7	27.5672	4.64	2.91	4.59	.	.
3	1	8	28.7982	4.42	3.15	4.67	0.03	0.04
4	1	1	28.0207	4.12	2.85	4.89	0.03	0.03
4	1	2	29.4785	4.14	3.06	4.77	0.03	0.03
4	1	3	30.0615	3.63	3.09	4.81	.	.
4	1	4	30.9686	3.56	2.99	4.88	0.03	0.03
4	1	5	28.8954	4.46	2.87	4.68	.	.
4	1	6	28.6362	4.14	2.96	4.80	0.03	0.03
4	1	7	24.6842	3.48	2.79	4.80	.	.
4	1	8	26.4334	4.11	3.13	4.47	0.04	0.03
5	2	1	25.6236	3.94	3.12	5.06	0.04	0.05
5	2	2	25.9799	3.55	3.24	5.14	0.04	0.03
5	2	3	27.0165	3.09	3.24	5.27	.	.
5	2	4	27.9236	2.86	3.23	5.16	0.03	0.03
5	2	5	26.6278	3.75	3.18	4.92	.	.
5	2	6	25.9151	3.16	3.30	5.21	0.05	0.03
5	2	7	22.8053	3.48	3.23	5.18	.	.
5	2	8	20.2786	3.78	3.32	4.98	.	0.04
6	2	1	19.9546	4.17	3.06	4.78	0.04	0.03
6	2	2	20.7645	4.18	3.12	4.87	0.04	0.03
6	2	3	20.9718	3.85	3.09	4.93	.	.
6	2	4	22.9997	3.96	3.02	4.90	0.03	0.02
6	2	5	23.1940	4.07	2.96	4.76	.	.
6	2	6	22.7405	3.59	3.04	4.90	0.04	0.02
6	2	7	21.6715	2.85	2.96	5.02	.	.
6	2	8	19.6307	3.41	3.04	5.03	0.04	0.03
7	2	1	25.6236	3.09	2.75	4.47	0.04	0.02
7	2	2	30.2883	2.29	2.68	4.56	0.03	0.02
7	2	3	30.1263	2.42	2.94	4.75	.	.
7	2	4	30.7742	2.27	2.73	4.57	0.03	0.02
7	2	5	28.8306	3.48	2.55	4.57	.	.
7	2	6	27.5348	3.57	2.62	4.59	0.03	0.03

Appendix Table III-20--Continued

COW NUMBER	TRT	WEEK	MILK YIELD (KG/DAY)	MILK FAT CONTENT (%)	MILK PROTEIN CONTENT (%)	MILK LACTOSE CONTENT (%)	MILK COPPER CONTENT (MG/L)	MILK MOLYBDENUM CONTENT (MG/L)
7	2	7	25.5264	2.90	2.62	4.60	.	.
7	2	8	27.7940	2.57	2.77	4.61	0.03	0.02
8	2	1	28.0531	3.04	2.93	4.73	0.03	0.03
8	2	2	29.7700	2.54	2.97	4.55	0.03	0.03
8	2	3	27.8264	2.85	3.06	4.59	.	.
8	2	4	26.5630	2.82	2.97	4.43	0.03	0.03
8	2	5	24.0363	3.36	3.01	4.17	.	.
8	2	6	23.4532	6.08	2.90	4.22	0.05	0.04
8	2	7	20.2786	3.24	3.04	4.17	.	.
8	2	8	17.0716	3.07	3.13	4.14	0.05	0.04
9	3	1	25.0729	4.07	3.28	4.81	0.04	0.08
9	3	2	26.1743	3.21	3.41	4.79	0.03	0.10
9	3	3	26.2391	2.33	3.49	4.89	.	.
9	3	4	25.2025	2.78	3.24	4.99	0.03	0.08
9	3	5	23.9067	3.31	3.39	4.64	.	.
9	3	6	22.4814	3.35	3.36	4.67	0.03	0.09
9	3	7	20.3758	2.86	3.34	4.64	.	.
9	3	8	21.8659	3.83	3.45	4.65	0.03	0.07
10	3	1	32.5559	3.01	2.97	5.12	0.03	0.08
10	3	2	30.1587	3.46	2.91	5.11	0.03	0.06
10	3	3	30.7094	3.14	2.99	5.13	.	.
10	3	4	33.1714	2.92	1.98	5.20	0.03	0.08
10	3	5	30.3855	2.64	2.87	4.67	.	.
10	3	6	31.0334	2.48	2.96	5.16	0.03	0.09
10	3	7	28.6038	2.90	2.86	5.04	.	.
10	3	8	25.9151	2.96	2.86	5.12	0.03	0.11
11	3	1	27.5996	3.53	3.00	4.74	0.03	0.08
11	3	2	27.5024	2.73	3.08	4.73	0.03	0.10
11	3	3	25.9151	2.71	3.07	4.70	.	.
11	3	4	26.8222	2.88	3.07	4.75	0.03	0.09
11	3	5	25.3320	3.19	3.02	4.32	.	.
11	3	6	23.0645	3.04	3.10	4.60	0.03	0.12
11	3	7	22.8053	2.73	3.03	4.61	.	.
11	3	8	20.3434	3.21	3.18	3.98	0.04	0.16
12	3	1	19.8899	2.88	3.69	4.88	0.06	0.07
12	3	2	19.6307	2.62	3.89	4.86	0.05	0.08
12	3	3	20.2138	2.05	3.75	4.85	.	.
12	3	4	21.8335	3.70	3.68	4.64	.	0.10
12	3	5	21.2504	3.66	3.57	4.61	.	.
12	3	6	22.9997	3.48	3.62	4.87	0.06	0.11
12	3	7	21.7039	3.78	3.67	4.78	.	.
12	3	8	20.4730	3.71	3.64	4.68	0.05	0.11
13	4	1	23.9391	4.22	3.00	4.69	0.04	0.07
13	4	2	23.3884	3.61	3.20	4.81	0.03	0.09
13	4	3	26.1743	3.02	2.94	4.86	.	.
13	4	4	25.9151	4.40	2.99	4.62	0.03	0.08
13	4	5	26.1743	3.93	3.08	4.63	.	.
13	4	6	25.2025	3.33	3.23	4.83	0.04	0.04
13	4	7	24.2306	3.71	3.02	4.73	.	.
13	4	8	18.5617	4.03	2.98	4.95	0.04	0.04
14	4	1	26.4658	4.08	3.11	5.09	0.05	0.07
14	4	2	26.3362	3.12	3.34	5.22	0.05	0.11
14	4	3	21.5743	3.43	3.11	5.27	.	.
14	4	4	16.1970	3.97	2.83	5.29	0.04	0.06

Appendix Table III-20--Continued

COW NUMBER	TRT	WEEK	MILK YIELD (KG/DAY)	MILK FAT CONTENT (%)	MILK PROTEIN CONTENT (%)	MILK LACTOSE CONTENT (%)	MILK COPPER CONTENT (MG/L)	MILK MOLYBDENUM CONTENT (MG/L)
14	4	5	.	.	.	.	.	.
14	4	6	.	.	.	.	0.05	0.03
14	4	7	.	.	.	.	.	.
14	4	8	.	.	.	.	.	.
15	4	1	34.2728	2.64	2.80	4.79	0.03	0.06
15	4	2	34.4023	2.68	2.69	4.48	0.03	0.18
15	4	3	37.0262	2.09	2.75	4.55	.	.
15	4	4	35.8925	2.44	2.76	4.64	0.03	0.14
15	4	5	32.3939	2.64	2.70	4.17	.	.
15	4	6	31.5517	3.53	2.76	4.35	0.03	0.11
15	4	7	30.5799	2.48	2.70	4.43	.	.
15	4	8	29.1869	2.56	2.71	4.26	0.03	0.13
16	4	1	29.5756	3.17	3.22	4.79	0.04	0.11
16	4	2	33.1066	3.42	3.17	4.68	0.03	0.11
16	4	3	30.9686	2.46	3.27	4.72	.	.
16	4	4	30.3207	3.33	3.19	4.61	0.03	0.08
16	4	5	27.8588	3.57	3.25	4.42	.	.
16	4	6	26.7574	2.88	3.20	4.57	0.04	0.06
16	4	7	23.1616	3.90	3.32	4.46	.	.
16	4	8	22.0926	3.47	3.41	4.28	0.05	0.08