

AN ABSTRACT OF THE THESIS OF

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Rapid copper depletion without clinical symptoms of copper deficiency was found in cattle under natural Klamath basin conditions. Copper metabolism was influenced by grass species fed to the animals. Tall fescue, Festuca arundinacea Schreb. 'Alta' and 'Fawn', reduced liver copper stores and decreased blood plasma copper and ceruloplasmin activity to a deficiency level in less than four months. Cattle fed during the same time period with quackgrass, Agropyron repens (L.) Beauv., maintained normal blood copper and ceruloplasmin activity levels and increased liver copper stores. Quackgrass was lower than fescue in copper content (4.6 ppm and 6.6 ppm respectively). The average copper/molybdenum ratio was lower in fescue (2.80) than in quackgrass (3.82).

Cattle fed quackgrass grew faster than cattle fed fescue ( $P < 0.001$ ). Copper supplementation of 300, 400 or 1000 mg Cu/head/day (as  $\text{CuSO}_4$ ) did not improve average daily gains. The decrease in daily gains found during July and August in all treatment groups was not alleviated by copper supplementation.

Ceruloplasmin activity values below 130 optical density units are considered subnormal under Klamath basin conditions. Plasma copper

values were less sensitive indicators of beginning copper depletion in this study than were ceruloplasmin activity values. Copper depletion was associated with plasma copper values of 0.08-0.4 ppm, liver copper values of less than 6 ppm dry weight (d.w.) and ceruloplasmin activity values approaching zero. The relationship between plasma copper and ceruloplasmin activity was found to be curvilinear; calculations of plasma copper from ceruloplasmin activity used by other workers were found inaccurate under conditions of this study.

A new test for diagnosis of copper deficiency was developed. The test measures the decrease of uricase (a copper enzyme) activity in the liver and kidneys indirectly by measuring accumulation of uric acid in the blood. (The conversion of uric acid to allantoin is catalyzed by uricase; thus the copper deficiency-caused decrease in uricase activity leads to accumulation of uric acid in the blood.) Three- to four-times higher levels of uric acid were found in copper-depleted cattle when compared to normal cattle.

Increasing copper supplementation from zero to 1000 mg Cu/head/day resulted in an increasing level of copper accumulated in the liver. The relationship between copper supplementation and liver copper was curvilinear, in contrast to results of earlier studies with ruminants. The supplementation of 1000 mg Cu/head/day (approximately 100 ppm, dry feed) resulted in an average liver copper of 400 ppm (d.w.) with no apparent symptoms of copper toxicity. Copper supplementation of 300 mg Cu/head/day was found sufficient to maintain the test animals in positive copper balance. Liver copper accumulation (average of 230 ppm) indicated that long-term copper supplementation of cattle under Klamath

conditions should not exceed 300 mg Cu/head/day; higher levels could result in copper toxicity.

Liver zinc, iron and molybdenum levels were not affected by the different levels of copper supplementation tested in this study.

The influence of water quality on daily gains and on copper metabolism was tested in 1974. Intakes of water from Upper Klamath Lake were lower than intakes of well water ( $P < 0.001$ ). Animals using well water over a 70-day period showed higher gains, but the difference was not significant. Copper metabolism was not affected by differences in water quality.

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in the Klamath Basin

by

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# COPPER DEFICIENCY IN CATTLE IN THE KLAMATH BASIN

## INTRODUCTION

The effect of recent increases in world market demands for grain raised the production cost of meat and other animal products profoundly. One rational way to increase profitability in animal industries is to eliminate wasteful feeding. This can be accomplished through full utilization of feeds by providing the animals with a nutritionally complete and well-balanced diet containing all the nutrients essential for normal animal growth, development and reproduction. In management of range cattle trace elements require as much attention as do major nutrients.

Micronutrient deficiencies or toxicities in plants are caused by a combination of factors, including the amounts of various mineral elements in the parent material from which soils of a particular area developed, the distribution of minerals in the soil profile, and their availability to plants. Climatic and weather conditions, soil management and farming practices are other factors affecting levels and availability of trace elements in soils and plants (Underwood, 1971; Kubota and Alloway, 1972). Range cattle nutrition depends primarily on nutrient levels in the forage. Thus most of the trace element deficiencies in plants result in trace element imbalances of the grazing animals. The Klamath basin of southern Oregon serves as an example of this.

This basin was profoundly affected by volcanic activities. Hundreds of square miles are covered by thick layers of pumice and ashes from volcanic eruptions. The largest, some 7000 years ago, resulted from the eruption of Mt. Mazama. The high temperatures under which ash and pumice particles were formed caused vaporization of large amounts of mineral elements important for both plant and animal nutrition. Erosion caused by torrential rains accompanying and following volcanic eruption filled the lakes and valley floors with the finest pumice particles and ashes. Muck or peat soils formed on the basin's recently drained lakebeds appear to be among those with the most pronounced mineral nutrient imbalances and micronutrient deficiencies (P, S, Mn, Cu, Zn, Se and others), as suggested by results of numerous fertilizer trials (Kubota et al., 1967; Kresge, 1974; Carter, unpublished data).

From both plant production and cattle management standpoints, copper deficiency is a major problem in the Klamath basin. Addition of copper to commercial fertilizers composed of macronutrients has resulted in dramatic vigor and yield improvements in treated oats and wheat (Gross, personal communication; Carter, unpublished data). Range cattle in the Klamath basin frequently exhibit symptoms suggesting copper deficiency, such as loss of hair color, diarrhea and poor gains (Carter, unpublished data). Animals with these symptoms have been found to have low blood and liver copper values (Carter, unpublished data). Copper supplementation corrected the hair color and increased blood and liver copper values in the animals studied; the other

symptoms, such as diarrhea, low gains and generally poor condition, were somewhat alleviated (Carter, unpublished data).

Various levels of copper supplementation have been studied, but the absolute optimal level of supplementation remains unknown. Identification of beginning or marginal copper deficiency without visible symptoms also was a problem. Low copper values in the blood and liver were difficult to interpret. Unavailable forms of copper, such as the copper-molybdenum complex, comprise varying parts of total copper in a sample of animal tissue (Underwood, 1971; Dowdy and Matrone, 1968) and amounts of available copper are unknown.

Poor health and reduced growth rate of cattle in the Klamath basin are thought to be the result of a combination of several factors rather than of a single element deficiency. Copper deficiency is known to be one of these factors. Full understanding of each contributing factor is necessary before effective steps can be taken to improve the health and productivity of cattle in the Klamath basin.

#### Objectives

The general objective of this study was to gain more knowledge of copper deficiency under Klamath basin conditions. The specific objectives were as follows:

- (1) To define 'normal' and 'deficient' copper levels in cattle under Klamath conditions.
- (2) To explore the value of some copper proteins as possible diagnostic tools for detection of early copper deficiency.
- (3) To evaluate different levels of copper supplementation and

their effects on accumulation of copper in the blood and livers of cattle, with observations of possible copper toxicity.

- (4) To derive from results in (1) and (3) recommendations on copper supplementation for Klamath basin conditions.
- (5) To compare tall fescue (Festuca arundinacea Schreb. 'Alta' and 'Fawn') and quackgrass (Agropyron repens (L.) Beauv.) as feeds for beef cattle.
- (6) To evaluate the influence of Upper Klamath Lake water on cattle gains.<sup>1</sup>

Research on these objectives was initiated in the spring of 1973 and continued through 1974. Field studies were conducted at experimental pastures of the Klamath Experiment Station south of Klamath Falls, Oregon. Laboratory work was done at Oregon State University and the University of Idaho.

<sup>1</sup>The need for this study became apparent after analysis of results from 1973.

## LITERATURE REVIEW

Existence of copper in plant and animal tissues has been known for more than one and one-half centuries (Underwood, 1971). Only within the last five decades has it been recognized that copper is essential for normal functioning of plants and animals. Since the early observations that copper is necessary for normal blood formation (Underwood, 1971; Evans, 1973), a wide variety of biological processes have been shown to depend on an adequate supply of dietary copper.

Studies by Neal et al. (1931) indicated that copper deficiency occurs naturally in "salt sick" cattle in Florida. In 1933 Sjollem (1933) identified a disease of sheep and cattle in parts of Holland as copper deficiency. Bennetts and Chapman (1937) found that a disease of lambs called enzootic neonatal ataxia was caused by inadequate intakes of copper from grazing in certain areas of Australia. These pioneering works were followed by demonstrations showing that plants and animals in extensive areas in different parts of the world are affected by simple or conditioned copper deficiency.

The disorders associated with copper deficiency in animals are varied. The symptom most common to all species studied is impaired iron utilization with resulting anemia (Underwood, 1971). Other disorders include depressed growth, depigmentation of hair and wool, abnormal keratinization of wool, bone abnormalities, neonatal ataxia, impaired reproduction, heart failure, cardiovascular defects and gastrointestinal disturbances (Underwood, 1971). The extent to which one or more of these dysfunctions appear depends on many factors, such as

the species, age and sex of the animal and severity and duration of the copper deficiency. Copper utilization in the animal is affected by many other elements, including molybdenum, sulfur (as sulfate) and zinc. The interactions among these elements are again very complex. Thus, so called "area" differences in manifestation of copper deficiency and response to supplementation ultimately may be explained by these difficult-to-assess and often unknown interactions among dietary factors in different environments. Genetic variations in copper metabolism, such as those described by Wiener and Field (1970) in sheep, may be yet another factor contributing to certain "area" differences.

#### LIVER COPPER

After it is absorbed from the intestine, copper becomes distributed throughout the body. In the extrahepatic tissue the metabolism of copper is confined mainly to the normal synthesis and degradation of copper-dependent enzymes. In the liver, copper is incorporated into ceruloplasmin, temporarily stored, or prepared for excretion in the bile (Underwood, 1971; Evans, 1973). According to Underwood (1971), normal cattle and sheep have liver copper levels of 100 to 400 parts per million (ppm). In the western parts of the U.S.A. liver copper levels in cattle are usually lower, often in the range of 30 to 100 ppm (Lesperance and Bohman, 1963; Cook et al., 1966). Most other species have lower normal liver copper levels, usually within the range of 10 to 50 ppm on a dry basis (Underwood, 1971).

Liver copper concentrations in ruminants are sensitive to low dietary copper intake and thus are useful aids in the diagnosis of



copper deficiency. Subnormal liver copper levels occur in animals suffering from copper deficiency (Underwood, 1971; Adams and Haag, 1957).

Copper retention in the liver is influenced by dietary copper intake. Adams and Haag (1957) found that cattle with normal blood copper levels had liver copper values from 30 to 1000 ppm. A similar study in sheep found liver copper levels varying from about 50 to 4000 ppm (MacPherson, Brown and Hemingway, 1964), with no significant change in blood copper values. Dick (1954) studied liver copper storage in groups of sheep ingesting graded increments of copper increasing from 3.6 to 33.6 mg/day over a period of 177 days. The liver copper levels increased steadily from 562 ppm (dry basis) at the lowest to 2340 ppm at the highest intake, and the increase in liver copper storage was linear. Milne and Weswig (1968) found a different effect of copper supplementation on liver copper storage of rats. Rats receiving diets low in copper had significantly reduced liver copper stores. With supplementation, liver copper levels rose to normal and remained normal until an intake of about 1000  $\mu$ g of dietary copper per day was reached. This corresponded to approximately 200 ppm of copper in the ration. When this threshold intake was reached the liver stores of copper increased rapidly, suggesting an overloading of the excretory capacity of the rat. This threshold differs from species to species. Fifty parts per million of dietary copper was sufficient to elevate the liver stores in cotton rats (Milne, 1965). In swine 70 to 130 ppm of dietary copper was needed to reach this threshold (Beck, 1963; Milne, 1968).

These species differences cannot be explained on the basis of normal differences in copper intakes. Beck (1956) has suggested that the threshold differences between species with high and low liver copper levels are caused by different excretory mechanisms. Milne and Weswig (1968) have pointed out that cattle and sheep also probably have an enhanced capacity to bind copper in the liver, because blood copper levels do not rise correspondingly with increased copper intakes in these species, as they do in rats, except at very high intakes.

The concentration of copper in the liver is influenced by several dietary constituents other than copper. The interaction between molybdenum and copper has been studied extensively since the early observation that molybdenum counteracts chronic copper poisoning in sheep and that copper overcomes the toxic effects of high-molybdenum diets in cattle (Underwood, 1971). Dick (1954) has suggested that molybdenum, in the presence of inorganic sulfate, reduces copper retention by reducing copper absorption and increasing copper excretion. Dowdy and Matrone (1968), however, observed that copper absorption in baby pigs is not affected by molybdenum. In addition, Marcilese et al. (1969) demonstrated that sheep fed molybdenum sulfate removed radioactive copper less than half as rapidly from plasma as sheep receiving no molybdenum supplement. These results suggest that molybdenum may interfere with the entry of copper into hepatic and extrahepatic cells.

Whanger and Weswig (1970) demonstrated that cadmium, silver and zinc antagonize copper metabolism within the hepatic cell. These investigators eliminated the competitive effects at the intestinal level by injecting copper into rats maintained on a low-copper diet and

subsequently analyzing the plasma ceruloplasmin activity. The experimental results demonstrated that plasma ceruloplasmin is restored much more readily in rats fed the low-copper diet alone than in animals fed the low-copper diet supplemented with cadmium, silver or zinc. Whanger and Weswig (1970) also have suggested that these elements inhibit ceruloplasmin activity by preventing copper from inducing the apo-ceruloplasmin molecule, by being incorporated into ceruloplasmin in place of copper, or by a combination of both mechanisms.

Whanger and Weswig (1971) later examined the effect of dietary zinc supplements on the subcellular distribution of hepatic copper in rats. Their results indicated that the accumulation of zinc in the liver is accompanied by a decreased concentration of copper within the microsomes and the soluble fraction, while the zinc concentration of these fractions increases significantly. The observation that zinc displaces copper from the microsomes supported the original hypothesis of Whanger and Weswig, since protein biosynthesis is associated mainly with the microsomal fraction. Regarding the interaction of zinc with copper in the soluble fraction, Evans, Majors and Cornatzer (1970) demonstrated that both cadmium and zinc compete with copper for sulfhydryl binding sites on metallothionein from bovine liver. Thus the interaction between copper and other chemically similar transition elements results in part from competition for common binding sites on the hepatic storage protein, metallothionein (Evans, 1973).

## BLOOD COPPER

Bovine erythrocytes contain a blue copper protein, hemocuprein, with a molecular weight of 35,000 and two atoms of copper per molecule (Mann and Keilin, 1938). Erythrocuprein, which is almost colorless, has been isolated from human erythrocytes (Underwood, 1971). The distribution of copper between the plasma and the erythrocytes in normal cattle is almost equal or slightly higher in plasma than in whole blood. Plasma levels are approximately 1.00  $\mu\text{g}$  of copper per milliliter (Underwood, 1971; Adams and Haag, 1957; Bingley and Dufty, 1969). Plasma copper is more labile than corpuscular copper and is a more sensitive and reliable indicator of the copper status of an animal than is whole blood copper (Underwood, 1971).

### Copper in Serum

The copper in serum occurs in two different forms, one firmly and one loosely bound. The former is the blue copper protein, ceruloplasmin, which accounts for most of the copper in serum (about 80% in normal rats, dogs, pigs, sheep and man (Underwood, 1971)). The copper in ceruloplasmin is tightly bound and will not react directly with chelating agents such as sodium diethyldithiocarbamate unless the molecule is first destroyed. The rest of the copper in the serum is loosely bound to protein, probably serum albumin (Underwood, 1971), and will react directly with sodium diethyldithiocarbamate. It is known as the "direct reacting" copper and is believed to constitute true transport copper, while ceruloplasmin is an oxidase enzyme (Underwood, 1971; Cartwright and Wintrobe, 1964). In addition, the serum

contains copper enzymes such as cytochrome oxidase and monoamine oxidase in concentrations that vary with the copper status of the animal (Underwood, 1971; Poole, 1970; Mills and Dalgarno, 1970).

#### COPPER PROTEINS

Within the cells and vascular fluids of the organs copper forms stable complexes and chelates with organic molecules. Several proteins that require copper as part of their molecular structure have been identified. As Frieden (1962) has said ". . . no metal ion surpasses copper salts in their versatility as catalysts for an impressive variety of reactions. . ." This catalytic activity has been found to be enhanced and made more specific when the copper is incorporated in a protein to form a copper enzyme (Underwood, 1971). Cytochrome oxidase, monoamine oxidase, ceruloplasmin, tyrosinase, superoxide dismutase, laccase, ascorbic acid oxidase, uricase,  $\delta$ -aminolevulinic acid dehydrase, dopamine- $\beta$ -hydroxylase and galactose oxidase all have been identified as copper enzymes. Several of the numerous manifestations of copper deficiency in animals are related to decreased tissue concentrations of certain of these enzymes. For example, tyrosinase is essential in the pigmentation process. It catalyzes the first two steps in the synthesis of melanin pigment from tyrosine (Lerner and Fitzpatrick, 1950). Copper deficiency leads to a decreased tyrosinase activity and depigmentation of hair results. Other basic biochemical defects leading to specific disorders found in copper deficiency may be explained similarly.

In animals, many minerals can affect both copper utilization (Underwood, 1971) and direct or indirect induction of certain enzymes (Kovalsky and Vorotnitskaya, 1970). These interrelationships, together with genetic differences in mineral requirements (Wiener and Field, 1970; Wiener, Hall and Hayter, 1973) and different normal levels of individual enzymes, help explain why, under different conditions, such diverse clinical symptoms of copper deficiency are found.

### Ceruloplasmin

Ceruloplasmin was first isolated and characterized by Holmberg and Laurell (1948). It is an  $\alpha_2$  globulin with a molecular weight of 151,000 and contains eight atoms of copper per molecule (Underwood, 1971). In addition, ceruloplasmin is a glycoprotein containing seven percent carbohydrate (Evans, 1973).

Ceruloplasmin behaves as an oxidase with copper as the active group (Milne, 1968). It can oxidize the following types of compounds readily: aromatic polyamines and polyphenols such as p-phenylenediamine; enediols such as ascorbic acid; and several inorganic compounds including  $\text{Fe}^{++}$ ,  $\text{Na}_2\text{S}_2\text{O}_4$ ,  $\text{NH}_2\text{OH}$  and  $\text{K}_4\text{Fe}(\text{CN})_6$ . Because ceruloplasmin is an oxidase, with copper involved in the active site, its activity can be inhibited by KCN,  $\text{NaN}_3$ , NaOCN and KSCN (Milne, 1968).

Since ceruloplasmin is the only oxidase of this kind in mammalian plasma, measurement of the oxidation rate of p-phenylenediamine is a valuable diagnostic technique. The exact nature of the reaction is unknown, but Rice (1962) has suggested that the product is formed

by the condensation of three molecules of p-phenylenediamine. The physiological significance of this reaction is unknown.

Ceruloplasmin is synthesized in the liver (Owen and Hazelrig, 1966). Copper is not incorporated into the globulin until after the metal permeates hepatic cells. Furthermore, copper in ceruloplasmin does not exchange with non-ceruloplasmin copper in vivo (Bush et al., 1956; Scheinberg and Morell, 1957; Sternlieb et al., 1961). Thus ceruloplasmin does not function in transporting ingested copper through the portal blood to the liver (Evans, 1973).

Several investigations have indicated that ceruloplasmin is a multifunctional protein in mammalian physiology. Osaki, McDermott and Frieden (1964) observed that ceruloplasmin oxidizes epinephrine, norepinephrine, serotonin and melatonin. These investigators suggested that ceruloplasmin may function in controlling the plasma levels of certain amines. This hypothesis is supported by the observation that plasma ceruloplasmin concentration is elevated under stress or after exhaustive exercise (Evans, 1973).

In spite of the amount of research done in the last twenty-five years and the amount learned about the ceruloplasmin molecule, very little was known about the biological role of this protein until recent experiments demonstrated that ceruloplasmin is essential in promoting hematopoiesis. For normal hemoglobin production, iron must be mobilized from storage cells in the intestine, liver and reticuloendothelial system and transported to bone marrow cells. Transferrin, which binds ferric iron, is the only known protein that supplies iron to the marrow cells (Jandl and Katz, 1963; Evans, 1973). Since iron entering

the blood from storage cells is in the ferrous state (Moore et al., 1939), the metal must be oxidized prior to incorporation into apotransferrin. Non-enzymic oxidation of ferrous iron is not sufficient to maintain a normal rate of hemoglobin production (Osaki, Johnson and Frieden, 1966).

Curzon and O'Reilly (1960) were the first to observe that ceruloplasmin catalyzes oxidation of ferrous iron. Osaki et al. (1966) later suggested that ceruloplasmin promotes transfer of iron from storage cells to plasma transferrin. This hypothesis has recently been confirmed in vivo. Ragan et al. (1969) found that administration of ceruloplasmin to copper-deficient swine which had adequate iron stores resulted in a rapid increase in plasma iron concentration, while administration of inorganic copper in an amount equivalent to that contained in the ceruloplasmin produced only a minimal increase. Roeser et al. (1970) observed an increase in plasma iron in hypoceruloplasminemic swine immediately after administration of ceruloplasmin. This increase continued at a rate proportional to the logarithm of the ceruloplasmin dose. Copper-deficient swine failed to retain iron in plasma, suggesting that the iron did not become bound to transferrin. Thus swine with copper deficiency, given iron orally, also would have iron deficiency (low storage) as a consequence of impaired mucosal cell-to-plasma iron transfer. Ceruloplasmin was also found highly effective in mobilizing iron from perfused livers (Osaki, Johnson and Frieden, 1971). These observations suggest that ceruloplasmin is the molecular link between copper and iron metabolism.



The transfer of iron from tissues to plasma requires the enzymic oxidation of ferrous iron, and ceruloplasmin is the enzyme that catalyzes the reaction. Osaki et al. (1966) suggested that ceruloplasmin should be designated "ferroxidase" to indicate its enzymatic function. Recently, Topham and Frieden (1970) described the isolation from human serum and the subsequent characterization of a second copper enzyme that oxidizes ferrous iron; this macromolecular enzyme has been designated "ferroxidase II."

### Uricase

Uricase, also called urate  $O_2$  oxidoreductase, urate oxidase or urico-oxidase, is a copper-containing enzyme of key importance in the catabolism of nitrogenous compounds in general and of purines in particular. The enzyme catalyzes conversion of urate to allantoin and  $CO_2$  (Mahler, 1963). Uricase is present in all mammals except the primates. The relationship between uric acid excretion, uricase, and gout in humans has been studied by numerous investigators and reviewed by Keilin (1959). In gouty humans, high levels of uric acid are present in the blood. Since humans have no uricase, conversion of uric acid to urinary allantoin has not been detected (Buzard, Bishop and Talbot, 1954). In other mammals, however, this reaction is very important. Uric acid level in the blood of normal humans is 2.1 to 7.0 mg/dl (American Monitor Corp.). Mammals other than the primates have lower uric acid levels.

There appears to be a correlation between copper content and activity of purified uricase. The protein contains one gram-atom of

copper per mole (Mahler, 1963). The copper content per unit activity remains essentially constant during purification (Mahler, 1963). Uricase activity is inhibited by cyanide and by some heavy metals. Although uricase is a cuproprotein,  $\text{Cu}^{++}$  ions added to the reaction mixture are also inhibitory (Mahler, 1963).

An interesting increase of uricase activity was observed by Kovalsky and Vorotnitskaya (1970) in rats fed a high molybdenum diet and in rats fed a high copper diet. In the former case, the high molybdenum diet induced xanthine oxidase activity and increased uric acid level in tissues; high uric acid was believed to have caused an increase of uricase activity. In the latter case, the high copper diet did not increase uric acid. Uricase activity seemed to be induced directly by excess copper (Kovalsky and Vorotnitskaya, 1970).

#### DIET AND SOME OTHER FACTORS AFFECTING COPPER ABSORPTION AND UTILIZATION

As mentioned in the discussion on liver copper, the availability of copper to the animal is influenced by numerous factors, including levels of molybdenum, sulfate, cadmium, silver, zinc, mercury and calcium. Recent studies also indicate that selenium (Amer et al., 1973) and iron (Campbell et al., 1974) are elements important in copper metabolism. In the studies cited, daily supplementation of iron to young cattle resulted in a significant decrease of liver copper, blood copper, ceruloplasmin and amine oxidase levels during the seven months of treatment (Campbell et al., 1974).

Dietary supplements of ascorbic acid increased the severity of copper deficiency in chicks and rabbits (Evans, 1973). Evans et al.

(1970) demonstrated that ascorbic acid decreases binding of copper by metallothionein from both intestine and liver. Spectral analysis of the protein after addition of ascorbic acid revealed that the vitamin interacts with metallothionein and thereby inhibits the formation of mercaptides (Evans, 1973).

The availability of copper from feed seems to be altered by processing (cooking, drying) (Evans, 1973). Raw herbage appears to be highly effective in increasing the copper stores of copper-deficient rats (Mills, 1954; Mills, 1955; Mills, 1956). In cattle, fresh grass appears to be less favorable. Hartmans and Bosman (1970) found that in the Netherlands, under field conditions, cattle fed hay in winter months had higher liver copper levels than did cattle fed fresh grass or silage of a similar mineral composition. Poor availability of copper from grass, probably as a result of the elevated sulfide concentrations in the rumen, is a possible explanation.

Genetic variation in copper concentrations in body tissues and different requirements of individual genotypes for the amounts of available copper in feed also require consideration. Wiener et al. (Wiener and Field, 1970; Wiener et al., 1973) showed that copper concentrations in whole blood from sheep of hemoglobin type B exceeded those from sheep of hemoglobin type A. The copper concentrations from sheep of hemoglobin type AB were intermediate between those of type A and type B. Different frequencies of allele A in individual breeds would help explain the differences in blood copper concentrations in these breeds.

### Identification of Copper Deficiency in Cattle

Accurate diagnosis of copper deficiency in cattle presents several problems. Different analyses of animal tissues and blood are difficult to interpret because of varying environmental conditions. A simple diagnostic method applicable to all conditions does not exist. Determination of plasma copper levels is one method frequently used as an indicator of copper status in cattle. This method measures the total amount of copper in the blood plasma. Some copper, however, could be present in the plasma as copper-molybdenum complex (Dowdy and Matrone, 1968a; Dowdy and Matrone, 1968b) or in other unavailable forms. Physiological inactivation of copper, resulting in copper deficiency symptoms, also has been suggested recently by Spais et al. (1968). Thus the amount of copper actually available for physiological processes cannot be estimated by this method. Field observations support this theory. Cattle in some areas have relatively low levels of plasma copper, but appear healthy and do not respond to copper supplementation. In other areas, higher plasma copper levels occur, yet the animals have typical clinical symptoms of copper deficiency and benefit from copper supplementation (Underwood, 1971). Therefore, plasma copper normally is used as an indicator of copper deficiency only in combination with other tests.

Copper content of the liver is another useful aid in the diagnosis of copper deficiency. Low liver copper levels have been found in copper-deficient animals of various species, including sheep and cattle grazing copper-deficient pastures (Underwood, 1971). "Normal"

liver copper values in cattle vary. In the western parts of the United States, 30-100 ppm is considered normal (Lesperance and Bohman, 1963; Cook et al., 1966), while in other areas 100-400 ppm is the normal range (Underwood, 1971). Extremely low liver copper levels (<10 ppm) usually indicate copper depletion. However, positive diagnosis of copper deficiency usually cannot be made from this test alone.

In recent years, serum ceruloplasmin activity has been used for the detection of copper deficiency. The method is simple and is not affected by sample contamination (Todd, 1970). Highly significant correlations, with linear increase between ceruloplasmin and various blood copper fractions, have been found by several workers (cited by Todd, 1970). Ceruloplasmin activity determination thus appears to have a value for copper deficiency detection similar to that of plasma copper.

Some copper enzymes other than ceruloplasmin also have been investigated as possible practical indicators of copper deficiency. Mills and Dalgarno (1970, discussion) found a close correlation between whole blood copper concentration and amine oxidase activity. The relationship was so close that tests involving determination of amine oxidase activity appear to have no advantage over assays measuring blood copper content.

A number of published reports (cited by Poole, 1970) have shown that copper-deficient cattle, sheep and rats have lower cytochrome oxidase activities than normal animals. Liver cytochrome oxidase activity was found to be considerably lower in cattle grazing high molybdenum (5-15 ppm) pastures than in a control group treated with

supplemental copper (Poole, 1970). This difference was not reflected by a significant difference in weight gain, although the trend was in favor of the copper-supplemented cattle. Cytochrome oxidase activity in the muscle of normal and copper-deficient rats, sheep and cattle also has been investigated as a possible indicator of copper status (Mills and Dalgarno, 1970). Muscle cytochrome oxidase activity was found to provide a better index of clinical copper status than did plasma monoamine oxidase activity. However, despite the advantage gained by the simple technique of muscle biopsy, muscle cytochrome oxidase was found less accurate for detection of copper deficiency than liver cytochrome oxidase (Mills and Dalgarno, 1970).

It may be concluded that positive identification of copper deficiency under different field conditions will remain difficult until more extensive knowledge of the metabolic roles of different forms of copper in blood, liver and other organs is obtained and the relationships of these roles to the clinical syndrome of deficiency are established. A diagnostic method based on either direct or indirect assays of copper-containing enzymes in tissues appears to be very promising in this regard.

With present techniques, experience in interpretation of results is necessary for each grazing area. Results of laboratory tests, combined with field observations and the responses of the cattle to copper supplementation, are needed to derive "normal" and "deficient" levels in these tests. Once established, these levels will be applicable only to the tested area and as a rule will not be applicable under different field conditions.

## MATERIAL AND METHODS

FIELD EXPERIMENTSStudy Site

Experimental pastures of the Klamath Experiment Station some eight miles south of Klamath Falls, Oregon, were chosen for the study. The site, part of a reclaimed lake bed, is similar to other pastures planted in the muck soils of this area. Grasses appear better adapted to this soil type and to the short, hot summer season than do legumes. Barley, wheat and oats are grown on some of the better muck soils which have been drained and supplemented with macro- and micronutrients such as copper, zinc and manganese. Among the various grasses tested in this area, tall fescue (Festuca arundinacea Schreb. 'Alta' and Festuca arundinacea Schreb. 'Fawn') and quackgrass (Agropyron repens (L.) Beauv.) have been found to produce best yields (Carter, unpublished data).

Experimental Animals

Cattle used in the study were obtained from a local ranch. One-year-old Hereford heifers weighing approximately 250 kilograms were selected. They had been raised on the range and fed hay through the winter. Prior to this study, mineral and trace element supplementation (including copper) was available to the animals at all times. It must be mentioned that, despite mineral supplementation, cattle kept permanently in the Klamath basin do not appear to be healthy. Thus it

is a routine practice to transfer herds periodically to other grazing areas. The cattle in this study appeared to be in better condition than average for animals in the Klamath basin.

The heifers were kept on experimental pastures from the beginning of the grazing season in May. After an adjustment period, they were placed at random in treatment groups.

### Experimental Design

#### Experiment A (1973)

Objectives: a) to evaluate quality of tall fescue (Festuca arundinacea Schreb. 'Alta' and 'Fawn') and quackgrass (Agropyron repens (L.) Beauv.) for beef production;  
b) to compare effects of five different levels of copper supplementation on animals' weight gains and copper status.

Factorial arrangement of ten groups with six animals in each group was used. Animals were assigned to the following treatments: groups 1-5 were fed chopped green fescue (see "Feeding" below); groups 6-10 were fed chopped quackgrass. Five levels of copper supplementation were assigned within each grass treatment as follows:

Groups 1 and 6 - no copper supplementation,

Groups 2 and 7 - 2 cc Cu glycinate injected once in May,

Groups 3 and 8 - 300 mg Cu/head/day (orally) as  $\text{CuSO}_4$ ,

Groups 4 and 9 - 690 mg Cu/head/day (orally) as  $\text{CuSO}_4$ ,

Groups 5 and 10 - 1000 mg Cu/head/day (orally) as  $\text{CuSO}_4$ .



All animals had access to lake water. The experiment was begun on May 29 and continued for 105 days.

#### Experiment B (1974)

Objectives: a) to evaluate quality of tall fescue and quackgrass for beef production;

b) to compare the effects of three levels of copper supplementation on animals' weight gains, copper status and possible copper toxicity.

Six groups with eight animals in each group were assigned to the following treatments: groups 1, 2 and 3 were fed chopped green fescue; groups 4, 5 and 6 were fed chopped quackgrass. Three levels of copper supplementation were fed within each grass treatment as follows:

Groups 1 and 4 - no copper supplementation,

Groups 2 and 5 - 400 mg Cu/head/day (orally) as  $\text{CuSO}_4$ ,

Groups 3 and 6 - 1000 mg Cu/head/day (orally) as  $\text{CuSO}_4$ .

All animals had access to lake water from May until July 1.

After that well water was supplied. The experiment was begun on May 17 and continued for 117 days.

#### Experiment C (1974)

Objectives: a) to evaluate water from Upper Klamath Lake (lake water) as a possible cause of low weight gains of cattle in the Klamath basin;

- b) to assess the effect of lake water on copper metabolism and copper supplementation;
- c) to continue evaluation of tall fescue and quackgrass as in A-a) and B-a).

Forty-eight animals were placed at random in six groups. Animals in groups 1, 2, 3 and 4 were allowed to graze tall fescue (see "Feeding" below); groups 5 and 6 grazed quackgrass. Treatments were divided as follows:

- Group 1 - lake water, 0 Cu, tall fescue,
- Group 2 - well water, 0 Cu, tall fescue,
- Group 3 - lake water, 400 mg Cu/head/day, tall fescue,
- Group 4 - well water, 400 mg Cu/head/day, tall fescue,
- Group 5 - lake water, 400 mg Cu/head/day, quackgrass,
- Group 6 - well water, 400 mg Cu/head/day, quackgrass.

Because of technical difficulties, well water was supplied during the first week of July. Prior to this date all animals had access to lake water. Evaluation of water quality effects thus lasted less than 70 days. Copper supplementation and grasses were evaluated during the entire 117 day season.

### Feeding

Tall fescue (Festuca arundinacea Schreb. 'Alta' and 'Fawn') or quackgrass (Agropyron repens (L.) Beauv.) was fed in all experiments. Selective grazing, with resulting inadequate nutrition in the second half of each grazing rotation, and unequal regrowth of individual pastures leading to irregular rotations of the animals, were eliminated

in experiments A and B. Feeding of chopped grass was considered necessary in these experiments to evaluate influence of copper supplementation on gains. Variation in the quality of grazed grass, with resulting variation in gains, had proved too large in previous years to allow proper assessment of copper influence. Results of this study, however, are fully applicable to grazing conditions of the Klamath basin.

All animals were placed in groups in dry lots and fed mixed chopped grass of uniform quality twice a day. Grass was chopped through the whole season at approximately the same stage of maturity, with frequent irrigation assuring adequate regrowth. Good quality immature grass was fed ad libitum in this experiment.

In experiment C all animals were grazed on young irrigated grass. Pastures were mowed and irrigated after each group rotation. An ample supply of grass was available for grazing at all times.

#### Supplementation

All animals were supplemented by approximately one pound per head per day of rolled barley containing the following mineral compounds:

Dicalcium Phosphate	- 13.93 g	MnSO <sub>4</sub>	- 0.3482 g
Sodium Polyphosphate	- 13.93 g	MgO	- 0.3482 g
NaCl	- 15.324 g	Iodine	- 0.06966 g
ZnSO <sub>4</sub>	- 1.393 g	CoCO <sub>3</sub>	- 0.01393 g

In 1973, copper was added as copper sulfate in a small amount of molasses and fed to designated groups at levels of 300 mg, 690 mg or 1000 mg Cu per head per day. One group receiving no oral copper

supplementation received 2 cc of injected copper glycinate at the beginning of experiment A.

In 1974 copper sulfate was mixed with the other minerals to supplement 400 mg or 1000 mg of copper per day.

### LABORATORY ANALYSES

#### Liver Samples

Liver biopsies were performed on 29 animals at the end of experiment A (1973). In experiment B (1974), four animals were selected at random from each of the six treatment groups and liver biopsies were performed on the first and last days of the experiment. Liver samples were transported to the OSU laboratory and analyzed for copper, zinc, iron and molybdenum. Results were expressed as parts per million, dry weight.

#### Blood Samples

Blood samples were taken from all animals from the vena jugularis at four-week intervals. Two vacutainer tubes were used to collect blood through a stainless steel needle. Heparinized tubes were used for hematocrit reading, then sent to the laboratory for plasma copper and whole blood zinc determinations. A second blood sample in a tube without anticoagulant was allowed to clot, then centrifuged. Separated blood serum was used for determination of ceruloplasmin activity and, in a later part of the experiment, to measure uric acid.

Whole blood zinc was determined from a well-mixed heparinized whole blood sample by atomic absorption. After centrifugation of the whole blood, plasma was removed and used for determination of total plasma copper by atomic absorption.

Ceruloplasmin was assayed as the p-phenylenediamine (PPD) oxidase activity. In this study the method described by Houchin (1958) was adapted for cattle serum by increasing incubation period from 15 to 60 minutes. One-tenth of a milliliter of serum was added to one milliliter of freshly prepared 0.1 percent PPD in acetate buffer (ionic strength 1.2, pH  $5.2 \pm 0.05$ ) at  $37^{\circ}\text{C}$ . After 60 minutes incubation, the reaction was stopped by adding five milliliters of 0.02 percent sodium azide solution. The optical density was measured against a reagent blank at 525  $\mu$  on a spectrophotometer. The optical density value was used to express ceruloplasmin activity as OD units. Optical density can be converted to International Units (Rice, 1962) by the following equation:

$$\text{International Units} = \frac{\text{optical density}}{4} \times (349),$$

where 4 represents change from the original 15 minutes of incubation to the 60 minutes used in this study and 349 is a factor converting the absorbancy of the oxidation product to micromoles of Bandrowski's base formed per minute per liter of plasma under the above conditions. Bandrowski's base is an oxidation product of p-phenylenediamine with the same absorption spectrum as the ceruloplasmin oxidation product (Rice, 1962).

Cattle ceruloplasmin reaction is slower than that of the rat and some other mammals; thus it is doubtful that better precision would be gained by conversion of optical density to International Units by an equation established for faster-reacting ceruloplasmin.

The effect of the increased length of incubation on the cattle ceruloplasmin assay reaction was measured on 40 cattle serum samples in this study. The reaction was linear from 0 to 120 minutes. Several typical samples are shown in Figure 1.

Bingley and Dick (1969) reported that a buffer pH of 5.2 produced a greatly decreased ceruloplasmin oxidase activity in bovine plasma, while a pH of 6.4 was found optimal. To determine whether Houchin's method, using an acetate buffer of pH 5.2 (routinely used for rat plasma ceruloplasmin activity assay at OSU), could also be used for cattle ceruloplasmin assay, acetate buffers were prepared with pH's ranging from 4.4 to 7.2 (as in Bingley's study). One-tenth milliliter of pooled bovine or sheep serum was incubated for 30 minutes with 1.0 ml of each of the buffer-PPD solutions and the reaction stopped by sodium azide as described in Houchin's method. Results are summarized in Figure 2. Highest ceruloplasmin activity was measured at pH 5.4 for both bovine and sheep serum. The pH 5.2 used in Houchin's method therefore was considered to be in a range of good sensitivity for bovine ceruloplasmin activity assay.

Uric acid was determined from serum by a colorimetric uricase-uric acid method (American Monitor Corp.). In this method phosphotungstate reacts with uric acid to form a blue chromophore. A blank was run in

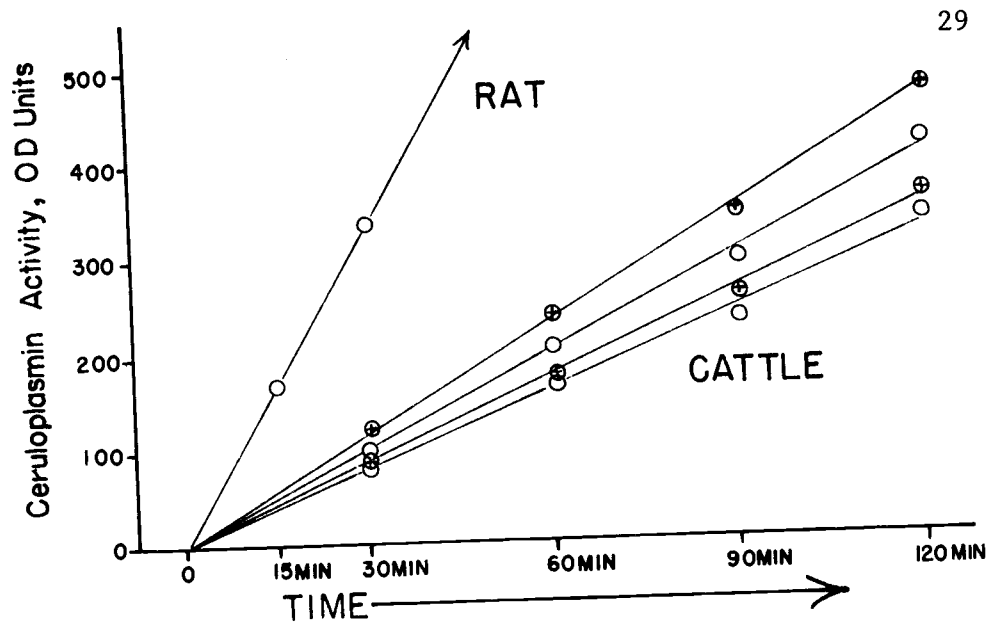


FIGURE 1. The influence of length of incubation on cattle ceruloplasmin activity.

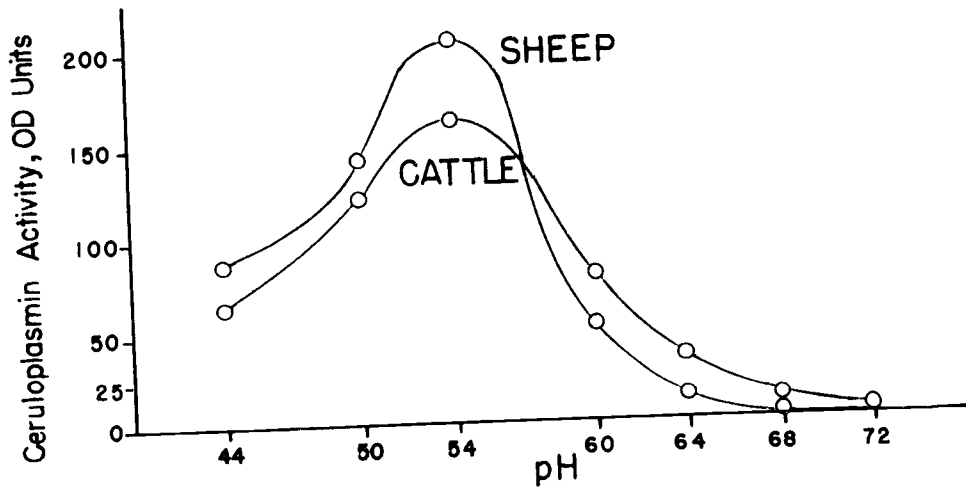


FIGURE 2. The influence of pH on ceruloplasmin activity.

which uric acid was destroyed by the action of uricase. Substances which might interfere, such as glucose, ascorbic acid and glutathione, would contribute equally to test and to blank and thus their effects would be cancelled. In assay, 0.1 ml of serum was mixed with 0.5 ml of buffer and, for the blank, with 0.5 ml of buffer with uricase. After 10 minutes of incubation at 37°C, 1 ml of carbonate reagent and 1 ml of phosphotungstate reagent were added. After an additional 8 minutes of incubation at 37°C, the optical density of the test tube was read on a spectrophotometer set at 750 nm and adjusted to zero optical density with the blank. A standard curve was prepared. Two uric acid standards and human serum with a known uric acid level were assayed with each group of cattle samples to assure accuracy.

Hematocrit was determined shortly after blood samples were obtained. Microhematocrit tubes filled with heparinized blood were centrifuged for five minutes. Volume of red blood cells was expressed as percent of the whole blood sample.



## RESULTS

The cattle in all three experiments remained in good condition through the entire summer period. There were no visible clinical symptoms of copper deficiency in any group. Diarrhea was present in all groups, was not reduced by any treatment, and persisted through the entire season.

FORAGE ANALYSES

Mineral contents of tall fescue and quackgrass, fed during the experimental season of 1974 and determined by atomic absorption are summarized in Table 1. Molybdenum levels were higher in fescue than in quackgrass. This is in agreement with Kresge (1974), who found molybdenum levels for fescue to be 1.5- to 2-times higher than those for quackgrass in the same area in 1972. Molybdenum levels for both grasses were somewhat lower in our experiment than those found by Kresge. At the same time, copper levels in our grass samples were 2-3 ppm higher than in Kresge's. These differences could be explained by a different fertilization program. Similar changes in copper and molybdenum levels were found in this area in the same grass species grown side by side but under different ownership (Kresge, 1974).

Quackgrass (Table 1) had lower molybdenum, copper and manganese levels than fescue. The average Cu/Mo ratio for quackgrass was 3.82, for fescue 2.80. This ratio perhaps can help explain why animals fed quackgrass without Cu supplementation were able to accumulate copper in their livers during the experimental season, while animals fed fescue had their copper reserves depleted.

TABLE 1. Grass analysis, 1974.

Minerals:	Tall Fescue										Quackgrass								
	Average crude protein 15.35 Average acid detergent fiber 33.96										Average crude protein 14.76 Average acid detergent fiber 33.81								
	%				ppm														
	Ca	Mg	K	P	Mn	Zn	Cu	Mo	Cu/Mo	Ca	Mg	K	P	Mn	Zn	Cu	Mo	Cu/Mo	
Date:																			
6-27	.31	.32	1.4	.20	104	20	5.6	2.31	2.42	.16	.20	2.1	.19	44	22	5.4	0.71	7.61	
6-27	.20	.17	1.5	.20	120	24	4.8	2.14	2.24	.12	.18	2.5	.20	47	18	5.2	0.61	8.52	
7-2	.27	.32	2.2	.17	96	24	5.2	3.06	1.70	.26	.31	2.3	.14	41	--	5.4	0.95	5.68	
7-2	.28	.32	1.8	.21	94	22	5.2	2.82	1.84	.15	.15	2.4	.17	37	20	4.2	0.65	6.46	
7-9	.27	.29	--	.20	78	16	4.8	1.70	2.82	.27	.30	1.8	.20	72	20	4.8	1.90	2.53	
7-9	.23	.19	2.1	.16	38	18	4.8	1.02	4.71	.25	.18	2.5	.15	38	20	4.4	1.09	4.04	
7-18	.29	.28	1.1	.18	106	20	5.8	2.52	2.30	.26	.18	1.8	.16	24	14	4.6	1.56	2.95	
7-24	.23	.28	1.3	.16	106	24	8.4	3.40	2.47	.24	.17	3.9	.15	29	28	7.2	2.11	3.41	
8-2	.34	.36	1.6	.24	94	24	6.8	2.18	3.12	.32	.20	2.8	.16	32	20	4.6	2.65	1.74	
8-3	.31	.31	1.6	.21	70	26	6.6	1.50	4.40	.36	.25	5.9	.31	24	28	4.0	0.54	7.41	
8-8	.74	.43	2.0	.24	112	38	13.4	2.89	4.64	.23	.18	3.5	.12	33	22	3.0	0.85	3.53	
8-15	<u>.29</u>	<u>.37</u>	<u>2.3</u>	<u>.24</u>	<u>80</u>	<u>30</u>	<u>7.4</u>	<u>2.58</u>	<u>2.87</u>	<u>.22</u>	<u>.18</u>	<u>1.1</u>	<u>.11</u>	<u>32</u>	<u>24</u>	<u>2.8</u>	<u>0.88</u>	<u>3.18</u>	
Average:	.31	.30	1.7	.20	91	24	6.57	2.34	2.80	.24	.20	2.7	.17	38	21	4.63	1.21	3.82	

LIVER COPPER

Differences in accumulation and storage of copper in the liver as a result of different levels of copper supplementation are presented in Figure 3. At the beginning of experiment B (May 17, 1974) the liver copper level averaged 47 ppm (d.w.) and ranged from 23.5 to 82 ppm. On September 17, when the experiment was terminated, the highest liver copper content was found in the groups supplemented by 1000 mg Cu/head/day (groups B-3 and B-6). The average liver copper content in these groups was 418.8 ppm and ranged from 263 to 604 ppm. The groups supplemented by 400 mg of copper (groups B-2 and B-5) had an average liver copper concentration of 262.7 ppm, with a range of 156 to 326 ppm. Lowest liver copper levels were found in the groups receiving no supplementation of copper (groups B-1 and B-4). The average liver content of these groups was 62.5 ppm of copper, with a range of 5 to 125 ppm. The differences in liver copper accumulation among the treatments supplemented by 1000 mg, 400 mg and 0 mg Cu/head/day are all statistically significant ( $P < .01$ ).

In the groups without copper supplementation, fescue influenced copper metabolism differently than did quackgrass. Fescue-fed animals had reduced liver copper storage, with an average of 23 ppm; the animals fed quackgrass actually accumulated copper in their livers and reached an average of 102 ppm. This difference is statistically significant ( $P < .02$ ).

Figure 4 illustrates liver copper levels at the beginning and at the end of experiment B for each individual animal in groups 1 and

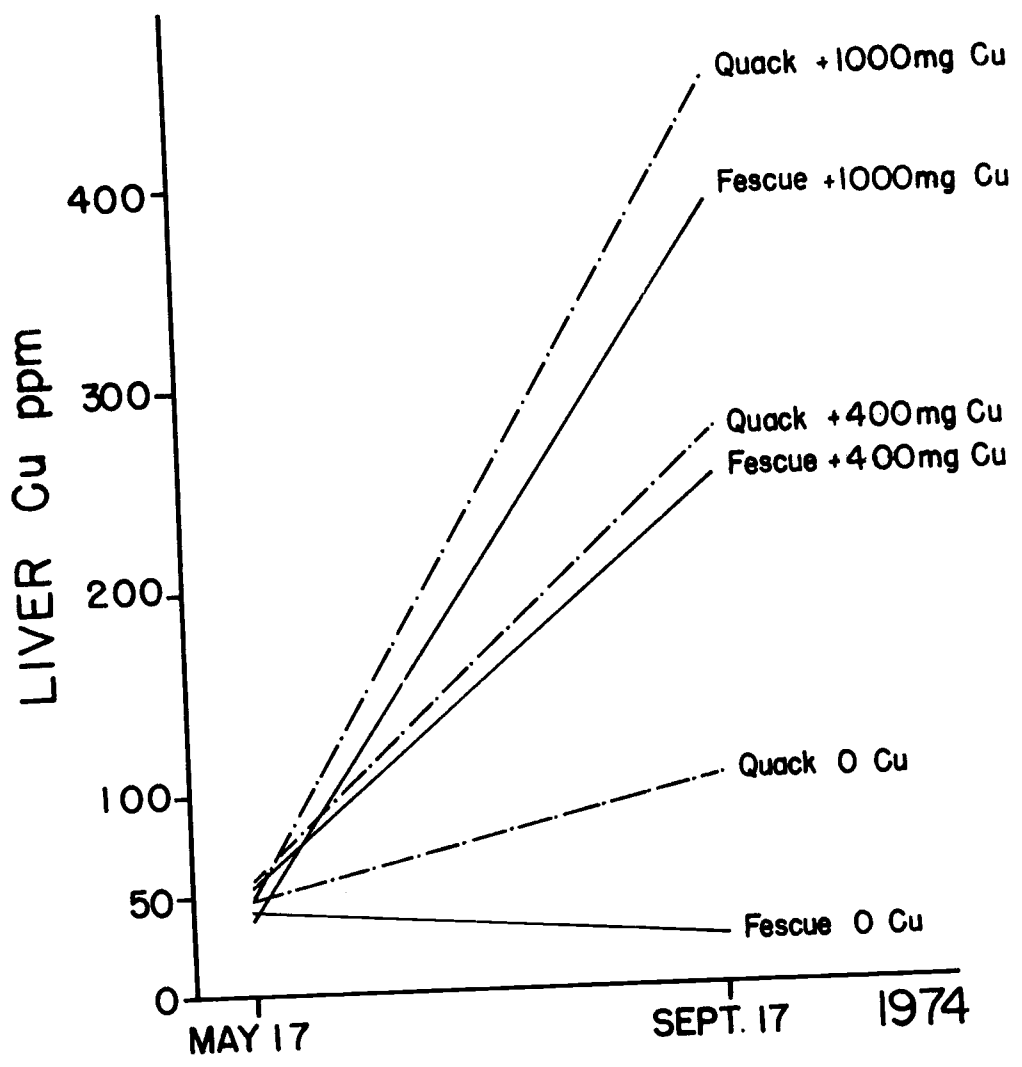


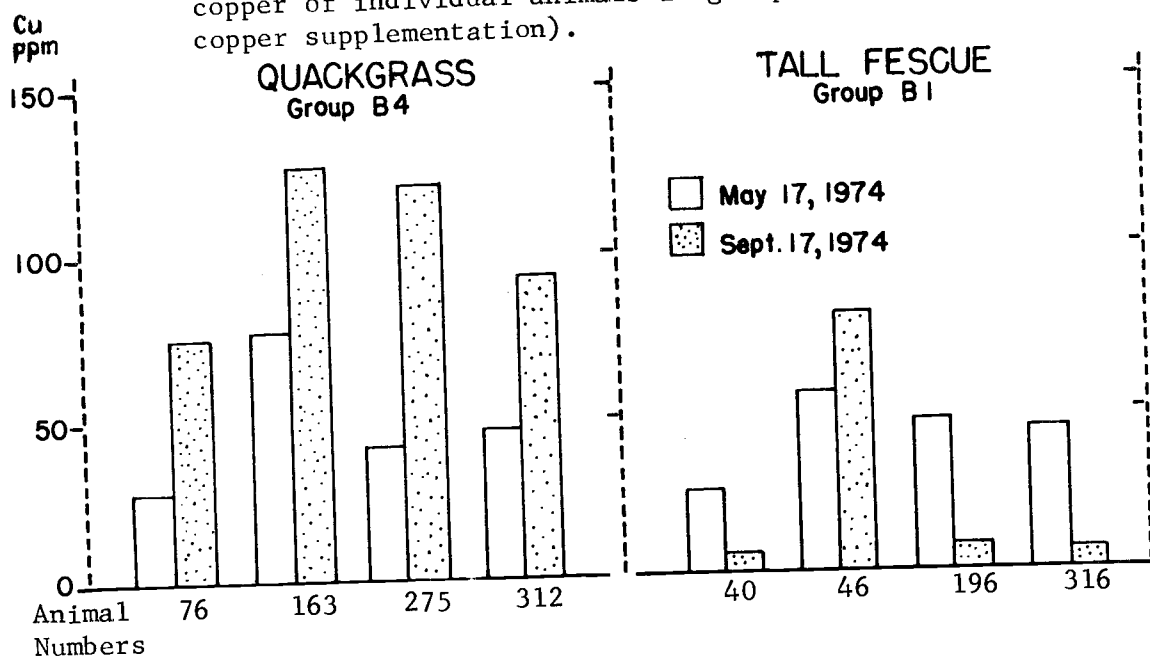
FIGURE 3. The effect of copper supplementation and grass species on liver copper accumulation in cattle from May 17 to September 17, 1974.

4. All animals fed quackgrass increased their liver copper levels, while three of the fescue-fed animals were severely depleted in their liver copper and the fourth one, under the same conditions, accumulated copper. This difference in response of one animal to fescue feeding cannot be explained by a possible liver sample contamination, since plasma copper and ceruloplasmin activity, measured on the same date, were also elevated (Table 2). It is not known whether a genetic difference in copper requirements and utilization or, more probably, a habit of eating soil (as observed on several occasions in this study and also reported by Healy (1965) from New Zealand) was responsible for this discrepancy.

The difference in copper metabolism in animals fed different grasses without copper supplementation perhaps could be explained by differences in the amounts of trace elements in each grass. Quackgrass, which caused accumulation of copper in the liver, contained on the average 4.6 ppm of copper and 1.2 ppm of molybdenum. The copper/molybdenum ratio was 3.82. Tall fescue, which caused copper depletion of the liver and blood, had higher copper and molybdenum contents (6.6 ppm and 2.3 ppm respectively), but the copper/molybdenum ratio was lower (2.8).

Data presented in Figure 3 indicate that fescue caused a decreased rate of liver copper accumulation at all levels of copper supplementation compared to corresponding supplementation levels in quackgrass-fed groups. This decrease was not statistically significant, even though a general trend existed. The somewhat lower molybdenum level in quackgrass, with resulting higher copper/molybdenum ratio,

FIGURE 4. The influence of quackgrass or tall fescue feed on liver copper of individual animals in groups B-1 and B-4 (zero copper supplementation).



Ceruloplasmin, OD Units:	154	152	167	161	0	115	13	3
Plasma Copper, ppm:	0.86	0.79	0.69	0.72	0.18	0.72	0.18	0.08
Liver Copper, ppm:	72.7	125.1	119.0	90.4	4.8	77.4	5.9	4.6

TABLE 2. Ceruloplasmin activity, plasma copper and liver copper levels of individual animals in experimental groups B-1 and B-4 (above).

perhaps could explain the faster liver copper accumulation in quackgrass-fed animals without additional copper supplement. At copper supplementation levels equal to 40 ppm and 100 ppm (dry feed), the 2 or 3 additional ppm of molybdenum in tall fescue would be practically negligible. Thus the decreased rate of liver copper accumulation in fescue-fed groups would have to be caused by other, as yet unidentified, factor(s).

#### LIVER ZINC, IRON AND MOLYBDENUM

Supplementation of zinc in the feed resulted in an increase of zinc in the livers of all animals studied. The average increase was 57 ppm, from the initial 67 ppm to 124 ppm at the end of the experiment. This increase appeared to be quite uniform in all treatments (Table 3) and thus was probably independent of copper intake.

TABLE 3. Average liver zinc and iron levels at the end of experiment B.

Cu Suppl.	<u>Liver zinc, ppm (dry weight)</u>		<u>Liver iron, ppm (dry weight)</u>	
	<u>Quackgrass</u>	<u>Fescue</u>	<u>Quackgrass</u>	<u>Fescue</u>
0 Cu	125.9	113.3	336.8	342.2
400 mg Cu	110.3	138.0	253.2	345.6
1000 mg Cu	131.7	124.9	309.0	338.4

Levels of liver iron are presented in Table 3. As with zinc, liver iron was not affected by any copper treatment. Since liver iron values have been reported to increase in severe copper deficiency (Underwood, 1971), we can assume that copper deficiency was not present

in any treatment group for a sufficient time to cause iron accumulation. Normal hematocrit levels throughout the experiment also indicate that iron metabolism was not impaired.

In September of 1973, the average liver molybdenum level was 3.42 ppm and ranged from less than 1 ppm to 7.8 ppm. Underwood (1971) gives a normal level of molybdenum in the liver as 2-4 ppm, with concentrations of 25-30 ppm for cows ingesting moderately large amounts of molybdenum. Based on these values, it can be assumed that practically all animals tested in the Klamath experiment had normal levels of liver molybdenum.

#### INFLUENCE OF INCREASING COPPER SUPPLEMENTATION ON LIVER COPPER

The influence of different levels of copper supplementation on liver copper levels (experiments A and B) is shown in Figure 5. Animals without copper supplementation had the lowest liver copper stores. With increasing copper supplementation, liver copper levels increased. This increase, however, was not linear, as would be expected for ruminants (Underwood, 1971; Dick, 1954; Milne, 1968). The curve reached the horizontal plateau at approximately 700-800 mg of copper supplementation per animal per day. The data were analyzed by a 2-degree polynomial regression, where

$$\text{liver Cu} = 61.58 + 0.82 (\text{Cu in feed}) - 0.0005 (\text{Cu in feed})^2.$$

$F'_{40} = 12.6$  and the improvement of a curve over a straight line is statistically significant ( $P < .01$ ).

Under the conditions of this experiment, the horizontal part of the curve in Figure 5 was reached when the average value of liver



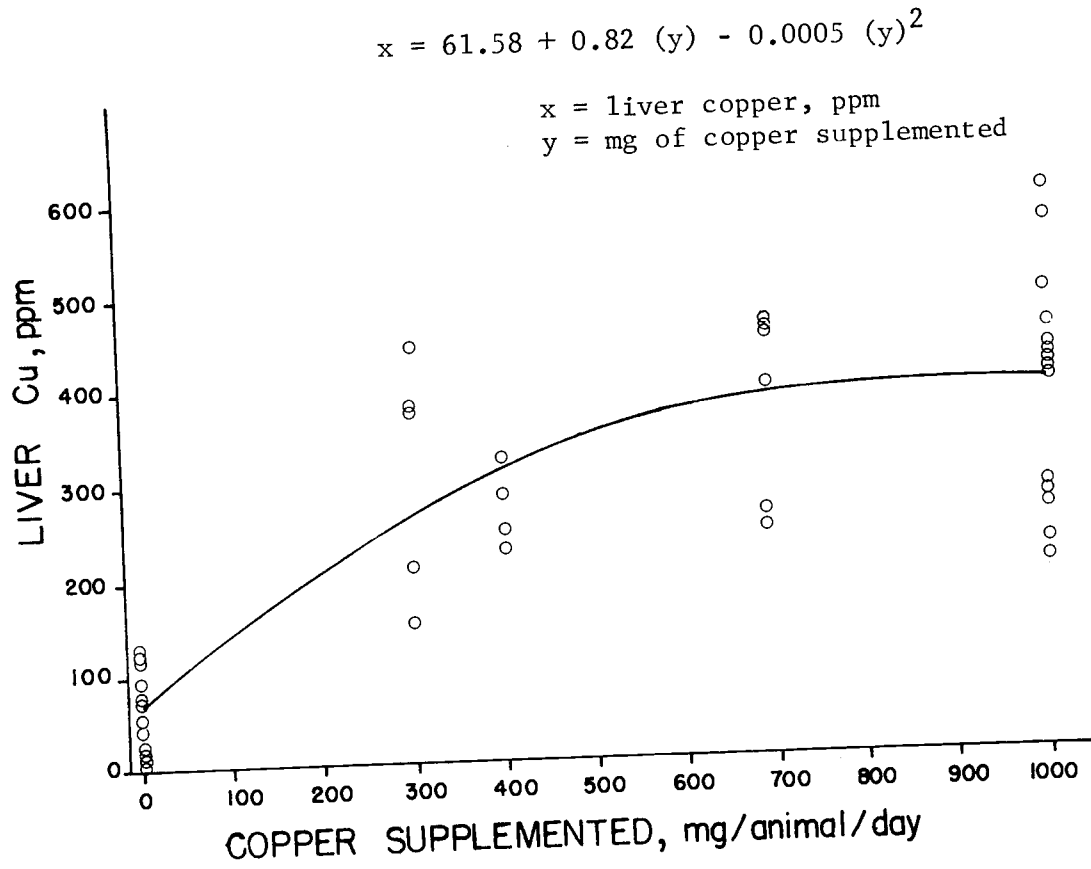


FIGURE 5. Influence of copper intake on liver copper.

copper was approximately 400 ppm, with a range of 200 to 600 ppm. High levels of liver copper in cattle are reported rarely to exceed 1000 ppm (Underwood, 1971; Adams and Haag, 1957). These reports, combined with Figure 5 from this study, indicate that cattle have a different rate of copper accumulation in the liver than do sheep. With increasing copper intake, the copper excretory capacity probably increases, thus slowing the rate of copper accumulation. This hypothesis would help to explain why cattle show not only lower liver copper levels but also considerably more resistance to copper poisoning than do sheep.

#### COPPER IN BLOOD

##### Blood Plasma Copper and Serum Ceruloplasmin Activity

The influence of different levels of copper supplementation on blood plasma copper and ceruloplasmin activity levels is presented in Figures 6, 7, 8 and 9. Animals fed tall fescue without copper supplementation (groups A-1 and B-1) showed progressive decreases in ceruloplasmin activity and plasma copper in both experimental years. These decreases were gradual, so that at the end of the grazing season in September the animals were approaching or had already reached copper deficiency, with plasma copper values of 0.08-0.18 ppm and ceruloplasmin activity near zero in a number of the animals.

An interesting exception in group B-1 was heifer number 46 (see Table 2). Plasma copper and ceruloplasmin activity of this animal decreased at the same rate as in the rest of the group from May to July. Then both values began to rise. The liver biopsy performed in

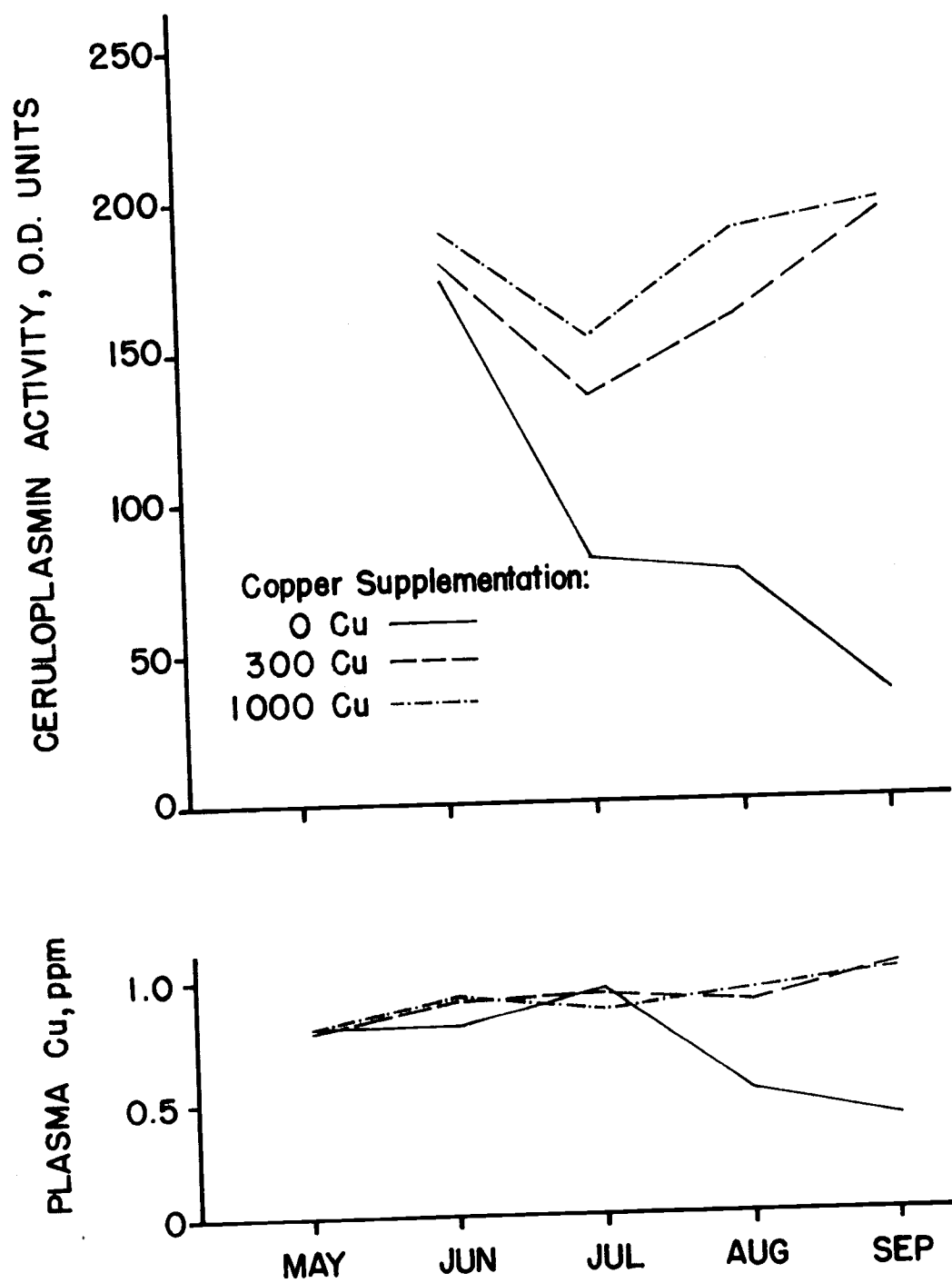


FIGURE 6. Plasma copper and ceruloplasmin activity in experiment A, fescue-fed groups.

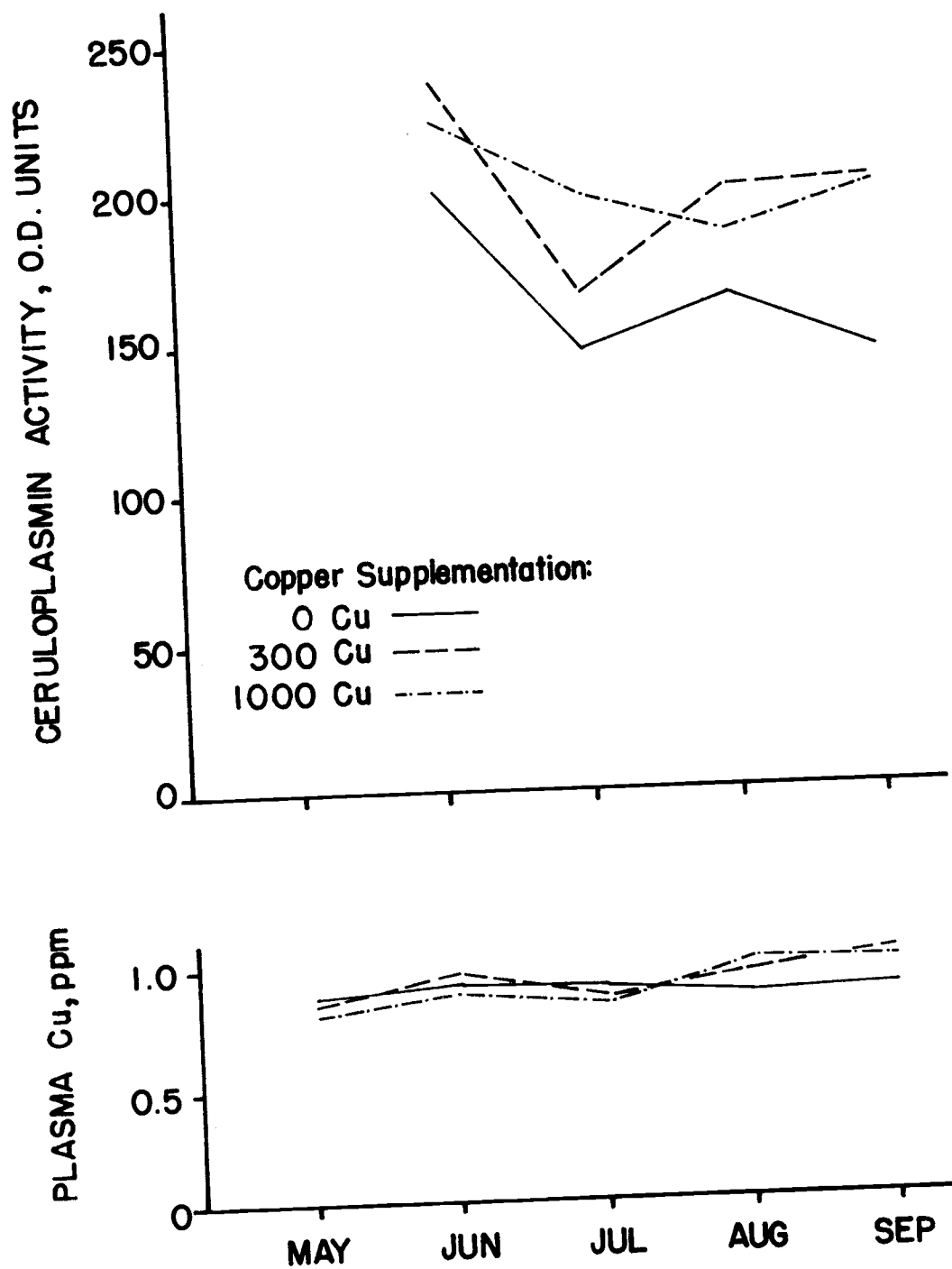


FIGURE 7. Plasma copper and ceruloplasmin activity in experiment A, quackgrass-fed groups.

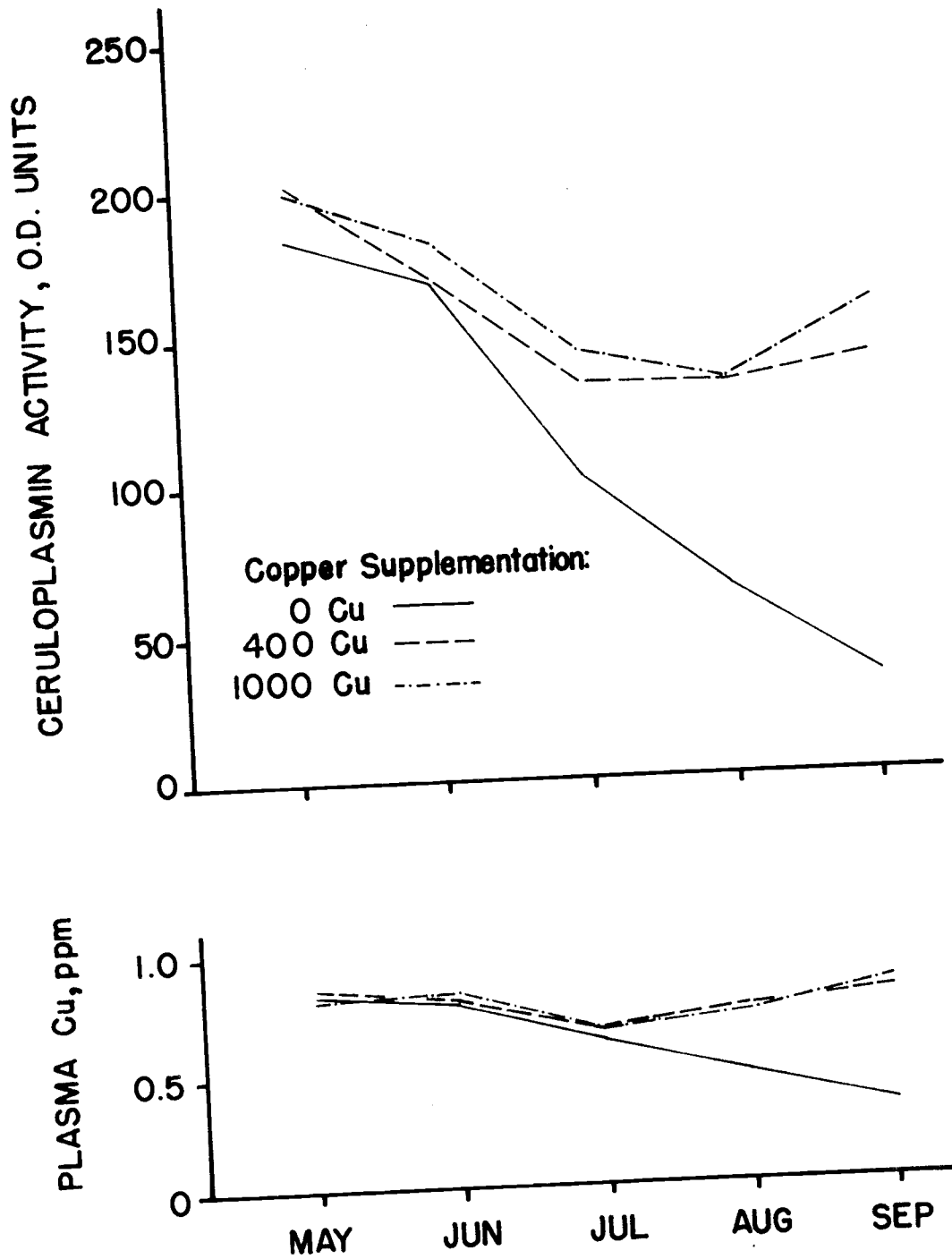


FIGURE 8. Plasma copper and ceruloplasmin activity in experiment B, fescue-fed groups.

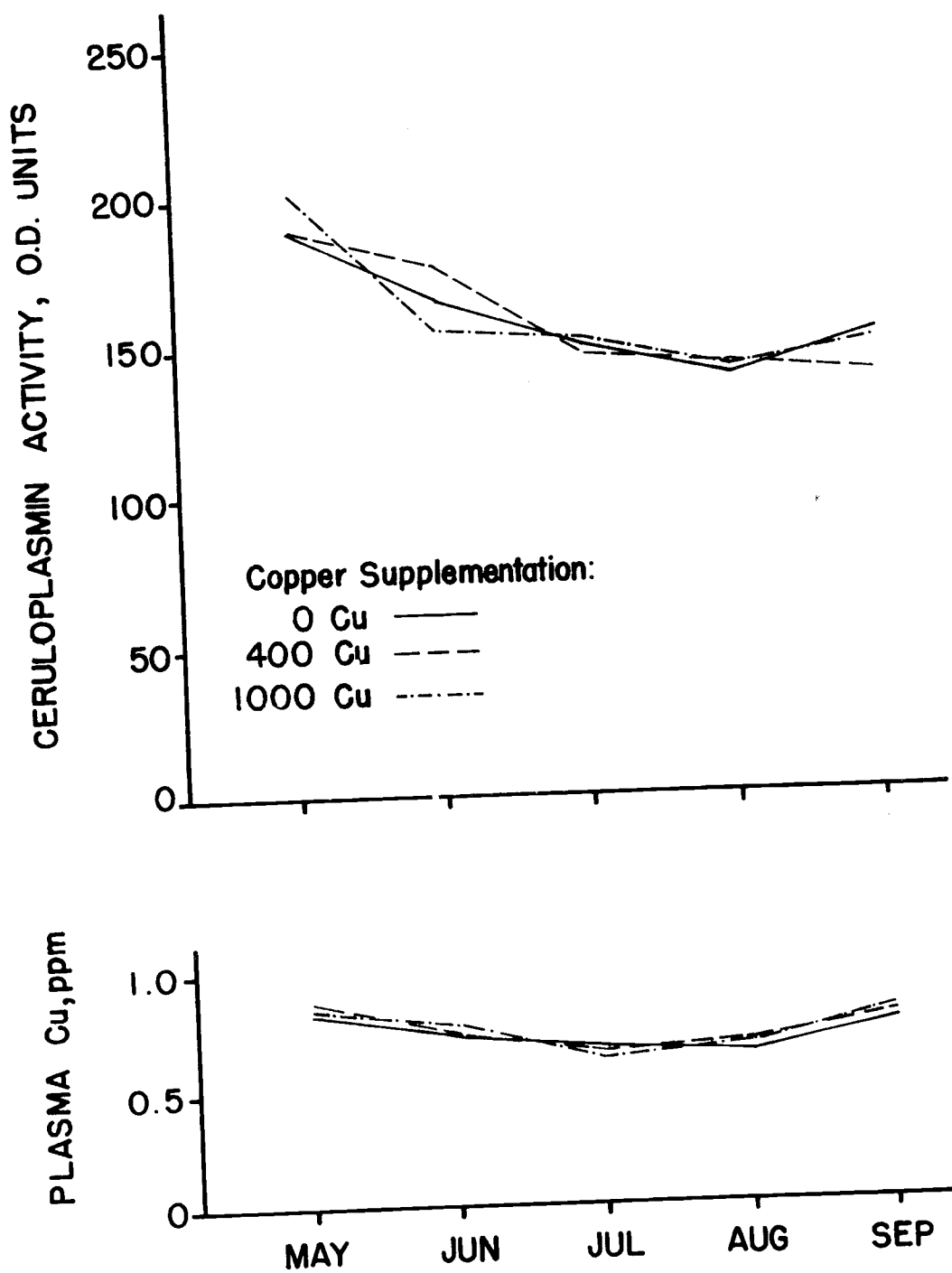


FIGURE 9. Plasma copper and ceruloplasmin activity in experiment B, quackgrass-fed groups.

September revealed some accumulation of copper since May (Figure 4, Table 2). The most probable explanation of this sudden change in copper balance is that the animal began to ingest soil (as discussed in "Liver Copper").

Ceruloplasmin activity appeared to be a better indicator of copper depletion than plasma copper. July blood samples revealed plasma copper levels in unsupplemented animals to be similar to those found in copper-supplemented animals, while ceruloplasmin activity at the same date was already somewhat decreased. This difference, however, was not large enough to justify exclusion of plasma copper analysis from future copper deficiency studies. August and September blood samples revealed progressive decrease in both plasma copper and ceruloplasmin activity.

Feeding of quackgrass without copper supplementation (Figures 7 and 9) did not reduce plasma copper or ceruloplasmin activity compared to similar values of copper-supplemented groups. It can be assumed that animals in this group were in good copper status. Liver biopsies performed in September showed accumulation of copper in the livers of all quackgrass-fed animals.

All groups except unsupplemented fescue-fed groups had similar levels of plasma copper and ceruloplasmin activity throughout the season. It is apparent that animals in these groups were in positive copper balance, and their plasma copper and ceruloplasmin activity levels can be considered normal under Klamath basin conditions. Differences in copper supplementation were reflected only in different copper accumulations in the livers.

A depression in plasma copper and ceruloplasmin activity in July and August appeared in all copper-supplemented groups in 1974 and, to a lesser degree, in 1973. From the initial levels of 0.8-0.9 ppm in May 1974, plasma copper decreased to an average of 0.6-0.7 ppm in July and August and increased again in September to the original spring levels. Ceruloplasmin activity followed a similar pattern: from 200 optical density (OD) units in May of 1974, it decreased to approximately 140 OD units in July and August and then rose again in September.

#### Plasma Copper and Ceruloplasmin Activity Relationship

In this study the relationship between plasma copper and ceruloplasmin activity was found to be curvilinear (Figure 10). This relationship is expressed by the following equation:

$$x = 20.144 + 0.4949 (y) - 0.00082 (y)^2$$

where x represents plasma copper in ppm and y is ceruloplasmin activity in OD units. The relationship is statistically significant ( $P < .0001$ ), with  $R^2 = .739$ . It is apparent from Figure 10 that with increasing ceruloplasmin activity from zero to 100 OD units plasma copper increase is rapid and the relationship almost linear. Ceruloplasmin activity and plasma copper values in this part of the curve represent copper depletion, with approaching copper deficiency at lower levels. In Klamath basin cattle, ceruloplasmin activity levels can be considered normal from 130 to 250 OD units. With increasing ceruloplasmin values in this range, plasma copper increases slowly and does not exceed 1.3 ppm. Ceruloplasmin activity values higher than 250 OD units can be associated with stress, exhaustive exercise (Evans, 1973) or, as



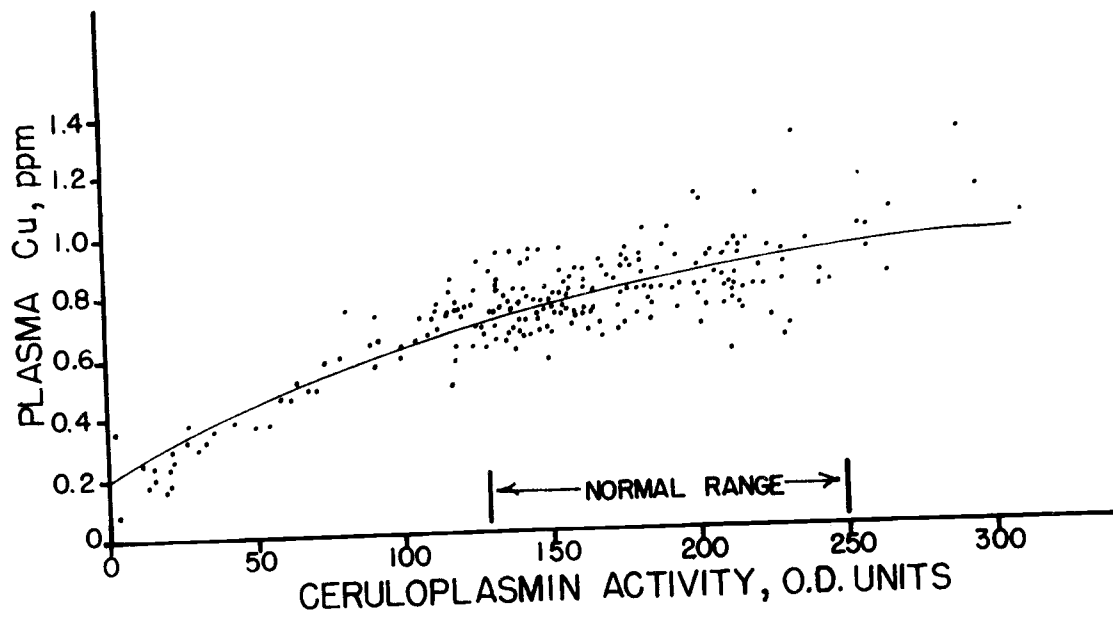


FIGURE 10. Plasma copper and ceruloplasmin activity relationship.

observed in this study, with inflammation. A possible chemical interference with the assay probably also can cause high ceruloplasmin activity readings.

The relationship between plasma copper and ceruloplasmin activity discussed above contrasts with the results observed by Todd (1970), who found this relationship linear and used it to calculate plasma copper from ceruloplasmin. Similar calculations have been reported by others (Bingley and Anderson, 1972).

#### Ceruloplasmin as a Diagnostic Tool in Determining Copper Deficiency

The assay for ceruloplasmin activity in bovine blood serum proved to be easy, accurate and useful under field conditions. The collection and handling of blood samples does not require any special precautions. Contamination usually does not influence the results of this fairly specific enzyme reaction. Storage at room temperature for short periods of time or under refrigeration for several days will not result in a loss of activity. Frozen serum samples can be stored for longer periods of time, but preferably not longer than one or two months. Samples in our study stored frozen for three months did have lower ceruloplasmin activity than fresh serum samples.

Accuracy of the ceruloplasmin activity assay used in this study was very good. Fifty samples were analyzed in duplicates, both simultaneously and on following days. The average difference between two subsamples was 4.4 OD units, which represents 3 percent of the total value.

"Normal" ceruloplasmin activity levels in cattle were tested in two herds from areas without copper deficiency. Oregon State University and University of Idaho supplied beef cattle of apparently normal copper status. Blood samples, taken in July and August 1974 from 30 heifers, showed average ceruloplasmin activity of 205 OD units for OSU cattle and 192 OD units for UI cattle. The range of 150 to 250 OD units was similar in both herds. The summer decrease in ceruloplasmin activity found in the Klamath basin did not occur in these herds.

#### Blood Zinc

In 1973 the average whole blood zinc levels (Table 4) were significantly higher than in 1974 (4.95 ppm and 2.43 ppm respectively). Grass species or copper supplementation did not affect blood zinc levels in any experiment. Zinc supplementation was reflected in a uniform increase of liver zinc, but had no effect on blood zinc.

#### Serum Uric Acid

Uric acid was determined in a total of 80 serum samples taken from different animals between July and September of 1974. Normal values in our study were close to 1.0 mg/dl, and ranged from less than 0.8 to 1.5 mg/dl. In August and September uric acid levels rose to 2-4 mg/dl in groups in which low plasma copper and ceruloplasmin activity indicated progressive copper depletion (groups B-1, C-1 and C-2). Animals from the group with apparently normal copper stores (group B-4) showed normal uric acid levels at the same time.

TABLE 4. Average blood zinc levels for experiments A and B, ppm.

## Experiment A:

Feed: Cu Suppl.	Quackgrass				Tall Fescue			
	July	Aug.	Sep.	Mean	July	Aug.	Sep.	Mean
0 Cu	3.3	6.5	4.6	4.8	3.5	5.3	3.7	4.2
300 mg Cu	4.0	6.6	4.4	5.0	3.6	7.5	4.7	5.3
690 mg Cu	3.4	5.9	4.9	4.7	3.6	7.6	4.9	5.3
1000 mg Cu	<u>3.5</u>	<u>7.8</u>	<u>5.3</u>	<u>5.5</u>	<u>3.6</u>	<u>6.6</u>	<u>5.2</u>	<u>5.1</u>
	Average: 4.97				Average: 4.92			

## Experiment B:

Feed: Cu Suppl.	Quackgrass				Tall Fescue			
	July	Aug.	Sep.	Mean	July	Aug.	Sep.	Mean
0 Cu	2.5	2.5	1.8	2.4	2.5	2.7	1.6	2.4
400 mg Cu	2.5	2.6	1.8	2.4	2.6	2.7	1.7	2.5
1000 mg Cu	<u>3.1</u>	<u>2.7</u>	<u>1.9</u>	<u>2.6</u>	<u>2.5</u>	<u>2.5</u>	<u>1.7</u>	<u>2.4</u>
	Average: 2.46				Average: 2.39			

Changes in uric acid levels of several animals are presented in Table 5.

TABLE 5. Serum uric acid levels in normal and copper depleted cattle.

	Animal Number	June	July	Aug.	Sept.		Animal Number	Sept.
Copper- depleted Group B-1	461	0.7	1.6	1.4	2.05	Copper- depleted Groups C-1 and C-2	18	1.7
	196M	1.85	2.0	2.5	2.15		65	4.05
	316	1.4	1.95	1.95	2.2		161	2.25
							19	2.3
Copper- normal Group B-4	76	1.15	1.65	1.1	1.2		96	3.5
	312	1.3	0.8	1.0	1.2			

Values higher than 2.0 mg/dl may be considered elevated. This finding supports the hypothesis that uricase activity decreased and unconverted uric acid accumulated in blood as a result of a decrease in available copper.

#### DAILY GAINS

Higher daily weight gains were found in groups receiving quackgrass than in fescue-fed groups. The average daily gain for all groups receiving quackgrass was 0.66 kg/day in experiment A and 0.77 kg/day in experiment B. Average daily gains for groups receiving tall fescue were 0.52 kg/day in experiment A and 0.59 kg in experiment B. The differences are statistically significant ( $P < .001$ ).

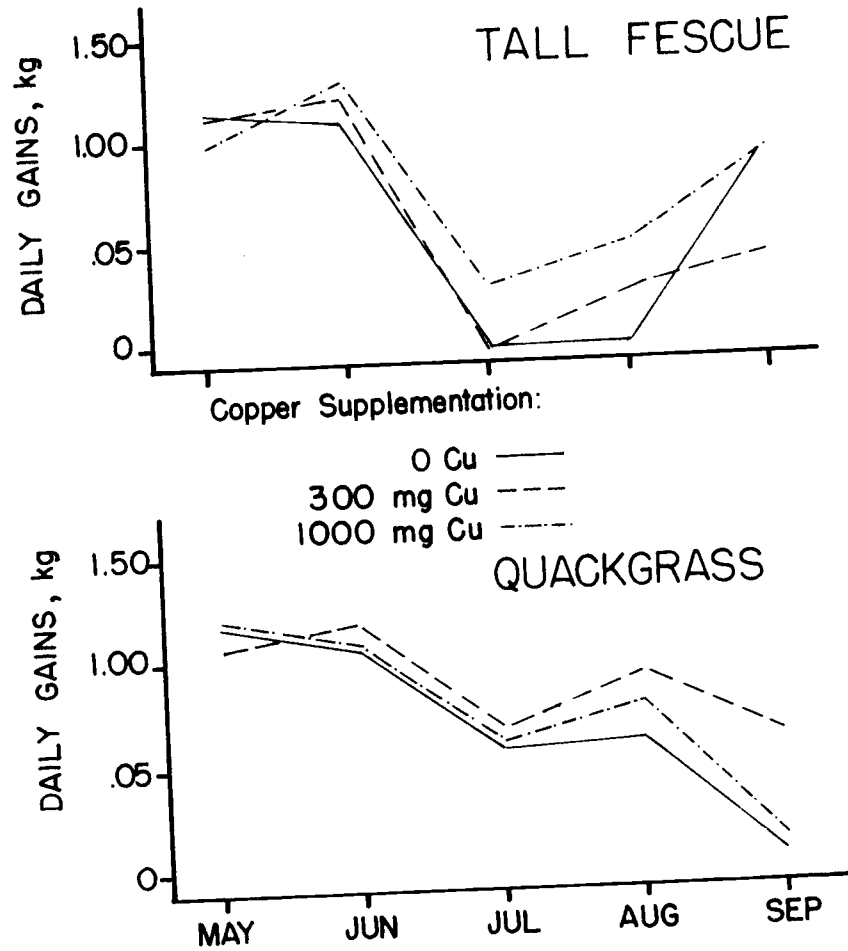
Daily gains were not substantially affected by copper supplementation (Figures 11 and 12). A sharp decrease in gains in July and August appeared in both years in all groups. The lower gains in these months somewhat parallel the decrease in ceruloplasmin activity and plasma copper levels (Figures 6 to 9), but there is no evidence of a link between these two factors.

In 1973 high hematocrit values indicated dehydration of the animals (see "Blood Hematocrit"). In July of 1974 algae-polluted lake water was replaced in experiment B by well water. The decrease in daily gains was slightly less severe in 1974, but still present.

#### Blood Hematocrit

Hematocrits measured in experiment A (1973) revealed progressive hemoconcentration from May to July. The higher hematocrits were then maintained for the rest of the summer. This was true for all groups, including those without copper supplementation where anemia resulting from copper deficiency was expected. The high hematocrits were believed to be the result of dehydration caused by insufficient water intakes.

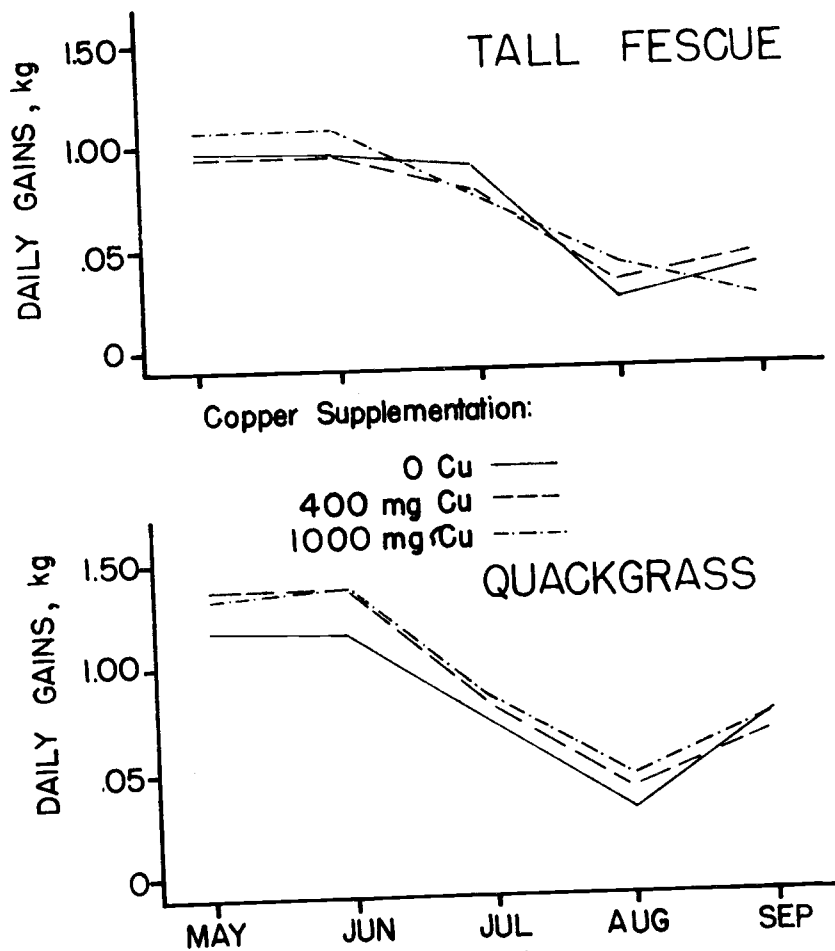
From the beginning of July 1974, well water was supplied to the animals in experiment B and to some groups in experiment C. Figure 13 illustrates the differences in hematocrit values in 1973 and 1974. Each average figure represents the hematocrits of 24 or more animals. In 1974 all hematocrit values were within normal range through the entire season, with none of the apparent hemoconcentration of the previous year. Animals supplied lake water had hematocrit values slightly



Whole season average daily gains:

Tall Fescue		Quackgrass	
0 Cu	0.45 kg	0 Cu	0.54 kg
Cu injected	0.44 kg	Cu injected	0.66 kg
300 mg Cu	0.40 kg	300 mg Cu	0.81 kg
690 mg Cu	0.58 kg	690 mg Cu	0.67 kg
1000 mg Cu	<u>0.69 kg</u>	1000 mg Cu	<u>0.60 kg</u>
Average:	0.52 kg	Average:	0.66 kg

FIGURE 11. Average daily gains in 1973.



Whole season average daily gains:

Tall Fescue		Quackgrass	
0 Cu	0.59 kg	0 Cu	0.72 kg
400 mg Cu	0.60 kg	400 mg Cu	0.78 kg
1000 mg Cu	<u>0.58 kg</u>	1000 mg Cu	<u>0.82 kg</u>
Average:	0.59 kg	Average:	0.77 kg

FIGURE 12. Average daily gains in 1974.



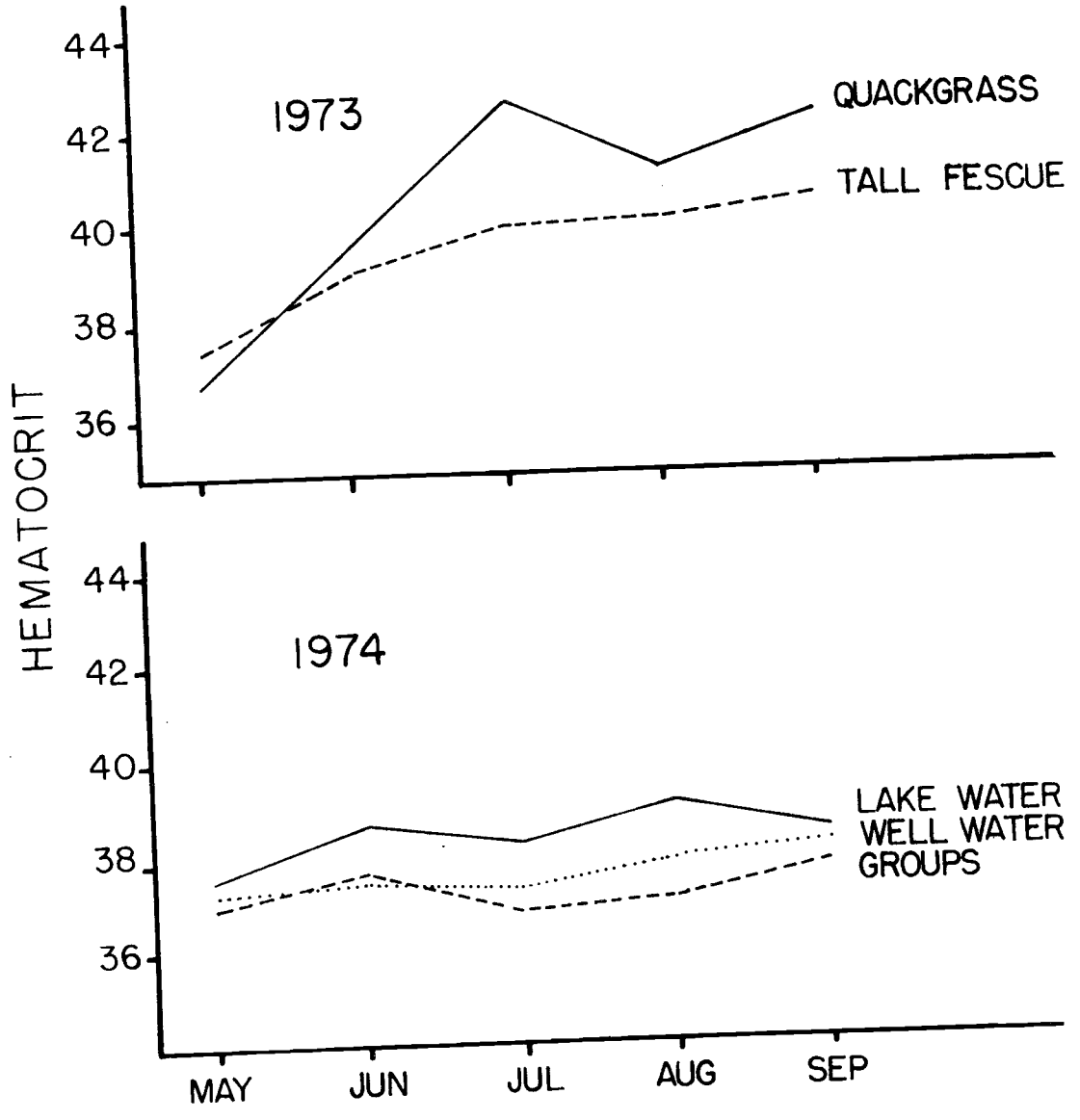


FIGURE 13. Average blood hematocrit values in 1973 and 1974.

higher than animals supplied fresh water (this difference was not statistically significant). The cool summer of 1974, with high precipitation and better quality of lake water, apparently helped the animals to maintain normal levels of body fluids.

#### EFFECT OF WATER QUALITY

The severe reduction in daily gains in the hot summer months was not alleviated by any level of copper supplementation, indicating the presence of other factors responsible for the decrease. Poor quality of available water appeared to be one possible cause.

Water from Upper Klamath Lake is known to be heavily infested by algae. The predominant species in summer months are blue-green algae such as Microcystis, Aphanizomenon and Anabaena (personal communication, Phinney, OSU, 1974). Toxins produced by these algae under favorable conditions have been responsible in many areas for poisoning and death of domestic animals as a result of so-called "algae blooms." A low concentration of the algal toxins or merely poor palatability resulting in low water intakes were considered as possible factors contributing to low cattle weight gains.

Results of a small experiment with rats in the fall of 1973 supported this hypothesis. Water samples collected from Upper Klamath Lake near the end of August 1973 were frozen and later used for four weeks as the only source of water for six growing rats. The average water intake of these rats was 1.58 ml of water per gram of feed. The control group with clean water had an average water intake of 2.01 ml water per gram of feed.

In 1974, experiment C was designed to test the influence of lake water on cattle water intakes and on daily weight gains under natural Klamath basin conditions.

### Cattle Gains

Daily weight gains of cattle in experiment C are presented in Table 6.

TABLE 6. Average daily gains, kg.

Grass Fed	Whole Summer Season of 125 days		July 17-September 10, 55 days	
	Well Water	Lake Water	Well Water	Lake Water
Fescue + 0 Cu	0.46	0.45	0.38	0.26
Fescue + 400 mg Cu	0.44	0.42	0.26	0.14
Quackgrass + 400 mg Cu	0.56	0.54	0.48	0.43

Cattle with access to well water showed slightly higher gains during the 125-day grazing season than did cattle in corresponding groups with access to lake water. This difference, however, was not statistically significant.

Because of technical difficulties, well water was made available to the experimental animals only from the beginning of July. From May to the first week of July all the groups were using lake water. No difference in gains could be observed until after introduction of both types of water. Thus the 55-day grazing period represents the time

when the effects of the difference in water quality were truly tested. The difference in gains was larger during this period than in the total 125-day season, but still not statistically significant ( $P > .05$ ).

### Water Intakes

Water intakes were measured daily for each experimental group in experiments B and C. To eliminate variation caused by measurements taken at irregular intervals and at different times of the day, an average daily water intake for each week was calculated.

Average daily water intakes from July 2 to September 16, 1974 are summarized in Table 7. All three groups receiving lake water had substantially reduced water intakes compared to groups receiving identical feed and well water. The average daily water intake for all animals receiving well water was 29.2 liters per day, while animals receiving lake water had an average intake of 24.4 liters per day. The difference in water intakes was found statistically significant ( $P < .001$ ).

Water intakes of cattle depend on a number of factors, such as age, environment, exercise, lactation, type of diet and feed intake (Church, 1971). In this experiment all of these factors were very similar and water intakes were affected primarily by different water quality.

Since water intakes and the amount of feed consumed are directly related, poor water quality resulting in lowered water intakes would also result in lower feed intake and thus, eventually, in reduced gains. Poor quality of water is thus of considerable importance.

TABLE 7. Animal water intakes in experiments B and C.

Water Intakes in Experiment C (liters/animal/day)

Feed:	Tall Fescue, No Copper		Tall Fescue + 400 mg Cu		Quackgrass + 400 mg Cu	
	Well Water	Lake Water	Well Water	Lake Water	Well Water	Lake Water
Date						
7.2-16	25.3	23.2	23.5	22.4	26.1	22.2
7.17-29	28.5	24.7	26.9	22.7	31.1	26.1
7.31-8.13	24.1	23.2	32.7	23.8	37.9	27.2
8.15-27	27.7	25.3	28.4	22.9	34.1	27.6
8.29-9.10	30.5	23.8	31.0	21.3	33.7	28.9
9.11-16	<u>28.1</u>	<u>27.8</u>	<u>28.4</u>	<u>23.3</u>	<u>31.9</u>	<u>27.0</u>
Average:	27.3	24.3*	28.4	22.7**	32.1	26.3**

Average well water intake (all groups) = 29.2 liters

Average lake water intake (all groups) = 24.4 liters\*\*

Water Intakes in Experiment B (liters/animal/day)

Feed: Date	Quackgrass		
	0 Cu	400 mg Cu	1000 mg Cu
6.29-7.16	30.7	31.6	30.3
7.17-30	37.1	37.3	37.3
7.31-8.13	35.6	36.6	35.3
8.14-27	38.2	37.4	37.7
8.28-9.10	36.2	34.1	36.3
9.11-16	<u>38.6</u>	<u>37.9</u>	<u>40.2</u>
Average:	35.5	35.4	35.5

\*Significant ( $P < .05$ ); \*\*Highly significant ( $P < .01$ )

Water intakes of animals in experiment B for the same period of time also are presented in Table 7. All animals in this experiment were receiving well water and there are only negligible differences in water intakes among groups. Higher total water intakes are correlated with higher consumption of uniform, better-quality feed (chopped grass), reflected in higher daily gains.

### Water Analysis

The odor of lake water pumped for the cattle from the irrigation canal was strong and unpleasant. The lake water in the water trough was dark brown in color, while well water remained clear.

Results of chemical analysis of water samples are presented in Table 8. Well water was of uniform quality throughout the season and was considerably lower in calcium, magnesium and iron content than was lake water. Water collected from Upper Klamath Lake was lower in total hardness and in calcium and magnesium than water from the same lake some 8-10 miles downstream where experimental pastures are located. From these results it may be concluded that mineral content of lake water in irrigation canals in the Klamath basin varies from place to place.

Algae from the canal water were more fragmented than those from Upper Klamath Lake water. Aphanizomenon was predominant through the 1974 season. Microcystis comprised less than one percent, with highest population of 10% at the end of July (Table 8). Toxin was not produced in strong concentrations in 1974. Once a week experimental laboratory mice were fed massive doses of algae collected from Upper Klamath Lake.

TABLE 8. Water analysis.

	Date, 1974	Total Hardness (ppm CaCO <sub>3</sub> )	Ca (ppm)	Mg (ppm)	Fe (ppm)	pH
Well Water	7-18	30	0.80	3.7	0.08	8.2
	8-2	28	0.75	3.3	0.10	7.8
	8-8	22	0.75	3.3	0.10	7.8
	8-23	24	0.80	3.2	0.05	8.0
	8-29	20	0.80	3.2	0.10	7.9
	9-7	24	0.75	3.3	0.08	7.8
Water from Upper Klamath Lake	6-20	28	1.80	4.3	0.25	6.9
	8-2	24	1.50	4.3	0.20	7.4
	8-8	24	1.50	4.2	0.30	7.4
	8-23	30	1.60	4.3	0.25	7.1
Lake Water from Canal (As Used in the Experi- ment)	8-2	58	3.90	9.9	0.15	7.3
	8-8	58	3.75	9.4	0.05	7.2
	8-23	76	5.40	14.6	0.13	7.8
	8-29	98	7.20	16.8	0.10	7.4
	9-7	76	5.40	14.0	0.01	7.5
Algae: Upper Klamath Lake	7-18	100% <u>Aphanizomenon</u>				
	7-24	90% <u>Aphanizomenon</u> , 10% <u>Microcystis</u>				
	8-24	99% <u>Aphanizomenon</u> , 1% <u>Microcystis</u>				
Algae: Water from Canal	7-18	100% <u>Aphanizomenon</u> , fragmented				
	7-24	90% <u>Aphanizomenon</u> , 10% <u>Microcystis</u> , fragmented				
	8-24	99% <u>Aphanizomenon</u> , 1% <u>Microcystis</u> , fragmented				

The mice appeared lethargic and in poor condition compared to control mice, but none died from acute poisoning as had been expected (personal communication, Phinney, OSU).

#### Blood Copper

Plasma copper and serum ceruloplasmin activity (Figure 14) were not affected by differences in water quality. Tall fescue feed without copper supplementation caused gradual copper depletion, with decreased levels of plasma copper and ceruloplasmin activity, while copper-supplemented groups remained in good copper balance. This finding is consistent with the results of experiments A and B.



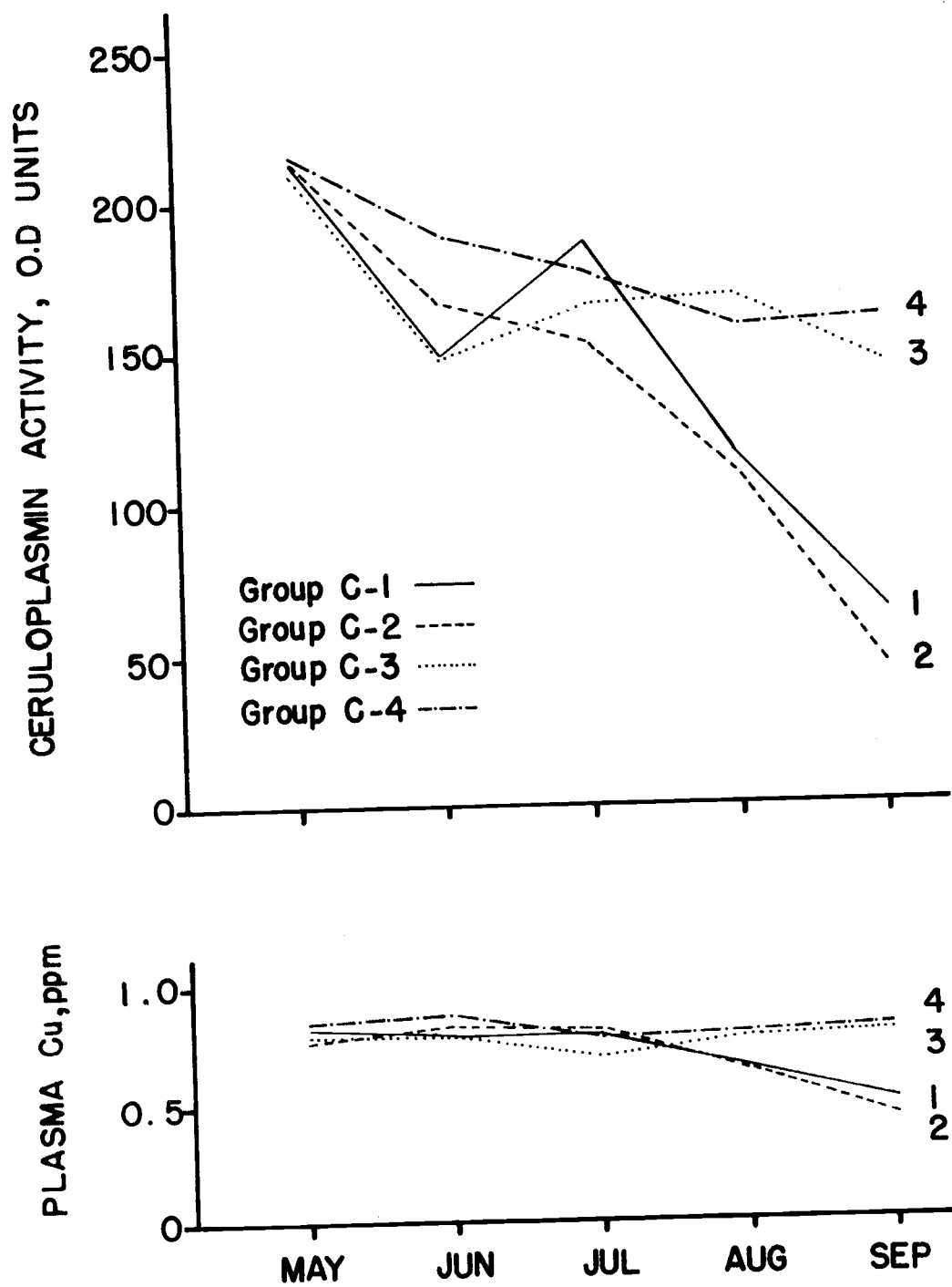


FIGURE 14. Plasma copper and ceruloplasmin activity in experiment C, fescue-fed groups 1, 2, 3 and 4.

## DISCUSSION

Results of forage analyses showed differences between the mineral contents of tall fescue and quackgrass, although both grasses were grown side by side in similar soil and under similar management practices. Fescue, containing higher levels of copper, molybdenum and manganese, caused gradual depletion of copper reserves in experimental animals. Quackgrass, with lower copper, molybdenum and manganese contents, helped the animals to remain in positive copper balance and to increase their liver copper stores.

The basic minimum copper requirement of cattle depends on numerous factors affecting copper metabolism and utilization. Copper/molybdenum ratio is of importance and could be responsible for the difference in the animals' ability to utilize copper from fescue and from quackgrass. It has been suggested by several workers (Bingley and Anderson, 1972; Miltimore and Mason, 1971) that copper/molybdenum ratios below 2.0 cause conditioned copper deficiency in cattle. Quackgrass, with a copper/molybdenum ratio of 3.8, supported a positive copper balance, while fescue, with a copper/molybdenum ratio of 2.8, reduced copper stores to a deficiency level in less than four months. It is possible that a copper/molybdenum ratio higher than the suggested 2.0 is responsible, under Klamath conditions, for copper depletion. This, however, would indicate the presence in the Klamath basin of some other factor influencing copper metabolism.

Results of an extensive survey of mineral levels in forage samples from the Klamath basin (Kresge, 1974; Kubota et al., 1967) show

a wide variation among samples from different farms. Soil pH was found to range from 5.4 to 8.4 (Kresge, 1974). Wide variation also was found in soil minerals. Different soil types, combined with application of different fertilizers, will increase the variation in mineral content of forage samples. In general, however, Kresge (1974) found fescue to have 1.5- to 2-times higher molybdenum levels than quackgrass. This finding is in agreement with our data. In Kresge's study several farms in the Klamath basin produced grass samples with copper levels considerably lower than those found in our study (Kresge, 1974). Copper depletion in cattle on these farms would progress faster than in the cattle in our study and probably even quackgrass would require copper supplementation. Legumes were found to contain considerably higher molybdenum levels than grasses (Kubota et al., 1967) and thus also would require copper supplementation.

Copper accumulation in the liver was apparent at all levels of copper supplementation. The increase of liver copper concentrations with increasing copper supplementation was not linear (Figure 5), as would be expected for ruminants (Underwood, 1971; Dick, 1954). This seemingly conflicts with the data reported by Dick (1954), who found that liver copper levels in sheep increased steadily and that the increase in liver storage was linear with increasing copper intake up to 18.6 mg/head/day. This intake is approximately equivalent to the 150 mg of copper supplemented in our experiment with cattle. At this level, animals in both experiments would be receiving approximately 19 ppm of copper (d.w.) in their diet. When Dick supplemented the sheep with 33.6 mg of Cu/head/day (a level close to the 300 mg Cu supplemented

in our experiment), the increase in liver copper storage was considerably lower, suggesting a curve rather than a straight line.

Basic differences between species in liver copper accumulation are apparent. Thirty parts per million of copper in the diet of sheep (Dick, 1954) resulted in liver copper levels close to 2000 ppm (d.w.) and subsequent death from copper poisoning. The cattle in our study, supplemented by an equal amount of copper, accumulated less than 300 ppm of liver copper. High copper supplementation of some 100 ppm (1000 mg Cu/head/day) resulted in an average of 400 ppm of Cu in cattle livers.

It is interesting to note that in rats Milne and Weswig (1968) found liver copper levels close to 30 ppm on diets with copper supplements ranging from 6 ppm to almost 200 ppm. Figure 15 compares accumulation of copper in livers of sheep (Dick, 1954), cattle (Figure 5 above) and rats (Milne and Weswig, 1968). Sheep accumulate copper rapidly in their livers at relatively low copper intakes and also are very susceptible to copper poisoning. Cattle, with a somewhat slower rate of copper accumulation, are more resistant to copper toxicity, and rats, with low liver copper accumulation, are reported to be extremely tolerant of high copper intakes (Underwood, 1971). This would indicate that copper-excretory capacity varies greatly among species, but the basic response to high dietary copper intakes is not as diametrically different in ruminants and non-ruminants as was thought earlier (Underwood, 1971).

Serum ceruloplasmin activity was found to be a practical and dependable method for routine laboratory use in detection of copper

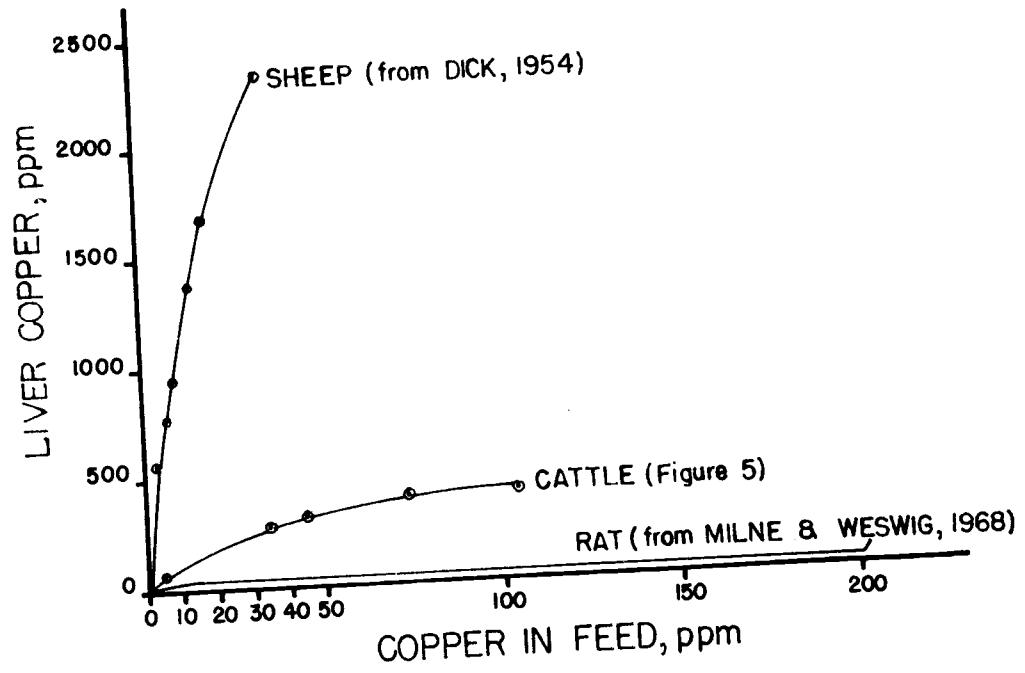


FIGURE 15. Influence of copper intake on liver copper accumulation in different species.

deficiency. The assay may be considered reasonably accurate, danger of sample contamination is small, and sample collection and storage can be handled easily under field conditions. Ceruloplasmin values below 130 OD units may be considered subnormal under Klamath conditions, and progressively lower levels indicate a higher degree of copper depletion. In this study ceruloplasmin activity values below 130 OD units indicated approaching copper deficiency in unsupplemented fescue-fed groups (Figures 7 and 9) four weeks earlier than did plasma copper values. Thus ceruloplasmin activity may be used successfully for routine screening of cattle in copper-deficient areas.

The relationship between plasma copper values and ceruloplasmin activity seen in this study differed from that reported by Todd (1970). The nearly linear increase of plasma copper with increasing ceruloplasmin activity was obtained only for below-normal values less than 100 OD units (Figure 10). At normal ceruloplasmin levels of 130 to 250 OD units, the plasma copper increase slowed substantially. Thus use of ceruloplasmin values to calculate plasma copper in this normal range would be very inaccurate.

Serum uric acid promises to be a useful method for determination of copper deficiency. In normal animals uric acid is converted to allantoin, with uricase catalyzing the reaction. In copper deficiency we may assume that uricase activity, as well as activities of other copper enzymes, will be decreased. Uric acid will be converted to allantoin at a slower rate and unconverted uric acid will accumulate in the blood serum. The elevated uric acid levels of 2 to 4 mg/dl

found in serum of the copper-depleted cattle in this study (Table 5) strongly support this hypothesis.

Norrlind and Kihlberg (1973) used the converse system to measure the amount of dietary purine compounds in rats. The normal level of rat uric acid (1.6 mg%) rose to 3.3 mg% when uricase was chemically inhibited by potassium oxonate. With a higher nucleic acid level in the rat diet, plasma uric acid rose to 11.9 mg%.

The uric acid method appears very promising for practical use. However, there is need to test this technique on animals exhibiting clinical symptoms of copper deficiency and on animals responding to copper supplementation.

Analysis of forage, blood and liver copper data reveals the necessity for copper supplementation in Klamath basin conditions. The high levels of copper supplementation sometimes recommended and used in this area not only appear unnecessary, but could actually become toxic. Three hundred milligrams of copper per head per day is adequate to maintain animals in positive copper balance, while increased liver copper values indicate that even this level is probably too high for long-term supplementation.

Improved weight gains after copper supplementation were not found in this study. All animals had good copper reserves at the beginning of the study and a short period of copper depletion at the end of the experiment was not sufficient to be reflected in decreased gains when compared to supplemented groups. The lower gain in July and August was not alleviated by any level of copper supplementation. It is not known if the temporary reduction of plasma copper and

ceruloplasmin activity also occurring at this time had any connection with lowered gains.

Plasma copper and ceruloplasmin activity reduction occurred in July and August in all groups. Level of copper supplementation had no influence on the decline. Plasma copper values of 0.5 to 0.7 ppm and ceruloplasmin activity of 100 to 130 OD units appeared in animals which showed above-normal liver copper values of some 400 ppm only four weeks later. This reduction was not observed in the "copper-normal" areas of Corvallis, Oregon, and Moscow, Idaho. The cause of the sharp decrease of these blood copper components in Klamath basin cattle and its possible correlation with decreased weight gains is unknown.

The poor quality of Upper Klamath Lake water caused a highly significant decrease in water intakes in cattle when compared to their normal intakes of well water. Slightly higher weight gains were recorded in groups receiving well water, but this difference was not statistically significant.

The wide variation in levels of minerals found in lake water at different locations (Table 8) also appears important. Considerable increase in mineral content occurred in lake water between Upper Klamath Lake and the experimental pastures. The role these minerals possibly may play in trace element metabolism of farm animals deserves attention.



## CONCLUSION

Rapid copper depletion was found in cattle under natural Klamath basin conditions. Copper metabolism appears to be greatly affected by the grass species fed to the animals. Tall fescue, Festuca arundinacea Schreb. 'Alta' and 'Fawn', reduced liver copper stores and decreased blood plasma copper and ceruloplasmin activity to a deficiency level in less than four months. Animals fed over the same time period with quackgrass, Agropyron repens (L.) Beauv., maintained normal blood copper and ceruloplasmin levels and increased liver copper stores. Chemical analysis of both grasses revealed that quackgrass is lower in copper content than fescue (4.6 ppm and 6.6 ppm respectively). The different copper/molybdenum ratios found in fescue and quackgrass may explain the apparent inconsistency between grass copper content and copper metabolism in animals. The average copper/molybdenum ratio is lower in fescue (2.80) than in quackgrass (3.82); however, both ratios are substantially higher than the 2.0 ratio considered by Miltimore and Mason (1971) and Bingley and Anderson (1972) as the upper limit for the molybdenum-caused copper deficiency.

These extreme differences in animal copper metabolism resulting from feeding of two grass species with rather similar copper and molybdenum contents deserve research attention. The results of this study suggest that delimitation of areas copper-deficient for cattle based solely on mineral analysis of forage samples is inadequate. The evaluation of grass species for grazing must include results of studies of animal responses before general recommendations are made. A careful

selection of forages to be grown in copper-deficient areas may reduce deficiency problems.

Beginning copper depletion in cattle is associated with reduced plasma copper and ceruloplasmin activity (Underwood, 1971). Ceruloplasmin activity values below 130 OD units can be considered subnormal under Klamath conditions. Plasma copper values are somewhat less accurate indicators of beginning copper depletion. In this study, ceruloplasmin values decreased below normal levels four weeks earlier than any decline could be detected from plasma copper levels. The assay for ceruloplasmin activity is simple and contamination does not present serious problems. The analysis of ceruloplasmin activity thus appears better suited for diagnosis of copper deficiency than analysis of plasma copper.

The relationship between plasma copper and ceruloplasmin activity in this study was found to be curvilinear; this in contrast to the findings of earlier investigators (Todd, 1970; Bingley and Anderson, 1972). This means that direct calculation of plasma copper from ceruloplasmin activity is inaccurate, especially at high levels.

The uric acid method developed in this study for diagnosis of copper deficiency was found very promising. An elevated serum uric acid indicates a decrease in the activity of the copper protein, uricase. Serum uric acid values two- to three-times higher than normal (0.8 to 1.5 mg/dl) were found in animals with a higher degree of copper depletion. Thus the increased levels of uric acid are probably indicators of physiological copper deficiency and not merely of a decrease in copper reserves as is the case with currently used methods.

Lake water intakes of cattle in the Klamath basin were found to be substantially lower ( $P < 0.001$ ) during summer months than intakes of supplied well water. It is well known that decreased water intake results in lower feed consumption and ultimately in lower weight gain. The quality of water available to the range cattle in the Klamath basin therefore deserves special attention.

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