Copper: A Review

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In reviewing the literature Booth *et al*[1], and Wilson[2] have recorded the different syndromes which have so far been associated with copper deficiency in deer. Familton *et al*[3] have reported copper deficiency to be the most commonly reported trace element deficiency in northern South Island, and in reviewing the laboratory reports since that time it would appear that this is still the case. Fifteen percent of the requests for laboratory diagnosis are related to copper deficiency. Harrison *et al*[4] reported that more than 40% of liver samples collected from Deer Slaughter Premises throughout the South Island had copper levels of less than 100 μmol/kg, with 20% being less than 60 μmol/kg. Mackintosh *et al*[5] concluded that liver copper levels of less than 100 μmol/kg may be considered as indicative of copper deficiency in deer, and that levels of less than 60 μmol/kg are indicative of a high risk for the occurrence of clinical signs of copper deficiency. A recent survey of liver and serum copper levels of deer slaughtered in the Manawatu (Killorn - unpublished) has produced similar results to those of Harrison. On the basis of these results, it would appear that copper deficiency is, or should be a disease of major concern to deer farmers and their veterinarians.

The purpose of this presentation is to attempt to summarise the factors which may be involved in the development of copper deficiency, and hopefully impart a better understanding of the problem to practitioners.

1. COPPER DEFICIENCY

Copper deficiency may be primary or secondary (induced), and the cause of the deficiency may determine the treatment chosen to try to overcome the problem.

Primary Copper Deficiency is caused by inadequate copper intake due to deficient pasture copper levels.

Secondary (induced) Copper Deficiency occurs in the presence of what would normally be an adequate copper intake, but with inhibitory factors decreasing the absorbtion or the availability of copper for normal metabolic functions.

1.1 The influence of soil type on copper levels in plants:

Copper levels in plants do not relate directly to soil parent materials nor the copper levels in soils. They are modified by the changing forms, distribution, and concentration of copper in the soil during soil formation, by the levels of other soil components, and by the age, developmental stage, species, and even the cultivar of the plant[6]. In young soils the development of copper deficiency tends to follow the concentration of copper in the soils and the geochemistry of the parent material. Hence plants generally have low copper levels and copper deficiency occurs on the following soil types:-

(1) Coarse-textured soils formed from acid igneous and sedimentary rocks.

(ii) Soils formed on marine and littoral calcerous deposits.

and (iii) Soils such as peats and mucks with high organic content

or more rarely on
(iv) Fine textured soils developed from acid igneous rocks or sediments.

Generally plants do not develop low copper levels when growing on soils derived from basic igneous rocks.

Plant copper values of 4-6 ppm on mineral soils, and 20-30 ppm on organic soils have been suggested as being deficient for the growth of plants. Harrison *et al*[4] have reported on the soil types in the South Island which are most likely to be associated with copper deficiency in deer.

1.2 Other factors important in determining copper levels in plants:

(i) **Nitrogen** - Copper and nitrogen tend to vary in parallel in plant shoots[7]. Increased nitrates in the soil, or increased N status of plants leads to increased copper absorbtion by the plants.

(ii) **Zinc** - Application of zinc fertilizers has induced low copper in plants [8].

(iii) **Soil pH** - decreased soil pH causes decreased absorbtion of copper by plants. Increased soil pH may increase copper availability, but also tends to increase molybdenum in plants.[9]

(iv) **Age of plants** - Copper is highest in young plants, and in the younger growing parts of plants.

(v) **Plant species** - as a general rule legumes have a higher copper content than grasses.

In copper-sufficient soils, copper concentration is greatest in new leaves. Therefore mowing, grazing, and the use of N fertilizers will ameliorate the decline of copper in pasture plants. On copper deficient soils however, copper concentrations may be highest in older leaves. Copper concentrations are generally greatest in young plants, and decline with the age of the plant.

The range of copper concentrations in leaves and shoots of plants is usually 2-20 ppm, however this may be much greater in copper accumulating plants. Copper levels of less than 3 ppm in whole shoots would indicate that copper is insufficient for plant growth.

1.3 Availability of Copper in plants to Animals

Copper deficiency may occur in animals grazing copper sufficient pastures. This may be because older plants may have a low concentration of copper, or more often because other plant components reduce the absorbtion or utilization of copper.

(a) Organic components.

Phytates, high levels of ascorbic acid, and oxalates, reduce the absorbtion of copper[10,11]. High levels of sulphur-methyl cystine sulphoxide in kale

have been reported to cause copper deficiency in young cattle and sheep fed kale for long periods[12]. Amino acids modify the absorbtion of copper either enhancing or depressing it below the level of copper sulphate depending on the nature of the complex they form with copper. Underwood[10] suggests that such reactions may explain why the availability of copper in diets varies with the amount and type of protein they contain and why copper in hay or dried herbage may be more available to the animal than from fresh green herbage.

(b) Inorganic components.

Calcium, cadmium, iron, molybdenum, sulphur and zinc, all influence the absorbtion of copper from diets. In ruminants Mo and S are particularly important and otherwise safe levels of copper in the diet may be rendered insufficient or toxic through changes in its availability. Soil ingestion giving large amounts of iron in conjunction with high sulphur diets can impair copper absorption in sheep[13] and can cause copper deficiency in cattle[14].

2. NUTRITIONAL REQUIREMENTS FOR COPPER

Copper is a multifunctional element essential for the activity of at least 10 metalloenzymes. The following is a brief resume of the physiological roles of copper:

2.1 **Erythropoiesis:** Anaemia occurs in all species where copper deficiency is severe or prolonged. The primary mechanism in inducing anaemia is thought to be due to a deficiency of cytochrome oxidase leading to an impairment of haem synthesis in hepatocytes and reticulocytes. Decreased erythrocyte survival time has also been reported.[10]

2.2 **Development of connective tissue:** An insufficiency of lysyl oxidase leads to the decreased formation of intermolecular linkages in elastin and collagen. The tensile strength of these proteins depends on the development of these cross linkages.

2.3 **Development of bone:** Malfunctions may again be due to an insufficiency of lysl oxidase resulting in weak cross linkages in collagen (mineralisation of collagen is an important early step in bone growth) and this may lead to deformities through overgrowth and distortion of the epiphyseal plate. The primary lesion may be in osteoblasts where depressed activity occurs leading to disturbed epiphyseal growth or matrix osteoporosis.[15].

2.4 **Central Nervous System:** lesions seen in the CNS are probably due to reduced cytochrome oxidase. This enzyme is necessary to sustain axonal and neuronal integrity. Whether congenital swayback (sheep) is due to neurone lesions with secondary myelin degeneration, or myelin aplasia has not been resolved. Post natal enzootic ataxia is said to be due to myelin aplasia.[16].

2.5 **Immunocompetence:** A deficiency of superoxide dismutase is thought to cause leucocyte malfunctions. Wooliams *et al*[17] have shown marked differences (between copper supplemented and non supplemented lambs, and between lambs from rams selected for high plasma copper concentrations and lambs from rams sclected for low concentrations) in

losses of lambs due to both swayback and to infections.

2.6 **Pigmentation:** Lack of pigmentation in copper deficiency is due to a deficiency of tyrosinase leading to decreased conversion of tyrosine to melanin.

2.7 **The whole animal:** There is no biochemical or physiological explanation for the impairment of complex processes such as growth.

3. COPPER HOMEOSTASIS

There is a need for a continued supply of copper from the diet to maintain physiological processes dependant on cupro-enzyme activity. This need varies as the animal develops. Intakes of copper will vary with the seasonality of plant growth, and changes in food type lead to differences in the availability of copper. Figure 1 shows a simplified model for the changes which take place in animals when the copper intake falls from adequate to grossly inadequate. In this figure four phases are identified.

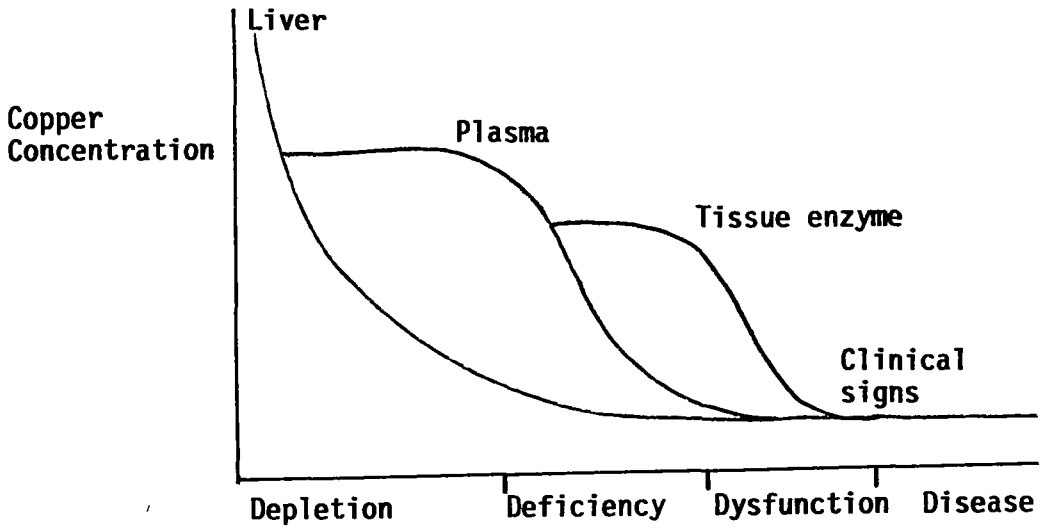
(i) **Depletion** - when intake is inadequate to sustain sufficient stores.

(ii) **Deficiency** - where mobilisation of copper from body stores, coupled with dietary supply fails to maintain circulating levels of copper, but essential functions are maintained.

(iii) **Dysfunction** - where one or more of the physiological processes dependant on copper become impaired.

(iv) **Disease** - where dysfunction becomes manifest in clinical signs.

Figure 1. A model for the changes which occur in mammals when their copper intake falls from an adequate to a grossly inadequate level. (Suttle, 1987[15]).



4. REQUIREMENT FOR COPPER

4.1 Maintenance

The maintenance requirements for copper arise from the need to replace unavoidable losses from the system via urine, faeces, and skin secretions. The replacement of these losses is an obligatory requirement for the maintenance of health, as they may be assumed to occur even if the diet is devoid of copper. Urinary losses probably constitute a significant amount of endogenous loss, even though the amount excreted is very small when compared to faecal excretion. Suttle[18] has estimated that these losses (in sheep) amount to 25% of the need for maintenance. Biliary excretion is often the major source of faecal endogenous copper loss, which consists of unabsorbed secretions from the gastrointestinal tract (bile, saliva, gastric juices). This has been estimated at 75% of the total endogenous loss in adult sheep. There have been no recorded studies on losses through the skin in animals other than man.

4.2 Production

(i) Pregnancy

Copper accumulates in the foetus at an exponential rate, most of this being stored in the liver. Young deer have been reported to be born with very high liver reserves[19] averaging 5589 μmol/kg (wet matter). In addition to the conceptus there is an accumulation of copper in the colostrum which contains 2.5 times the concentration of copper in milk.

(ii) Lactation

The demand for copper in lactation is given by the product of copper concentration in the milk and milk yield. In deer producing 3 litres of milk per day, this may amount to 0.6 mg/day assuming the copper concentration to be intermediate between cattle (.15 mg/l) and sheep (.25 mg/l).

(iii) Growth

Requirements for growth in sheep have been calculated at .8 mg/kg increase in body weight[19], and for calves as 0.6 mg/kg increase in body weight[20]. Again, in the absence of any other data we will assume deer to be intermediate between sheep and cattle (0.7 mg/kg increase in body weight).

The Table below shows estimated gross requirements for deer using the values given above and assuming an availability of copper from the diet of 3.7% as calculated by Freudenberger *et al*[21], but an availability of 80% pre-ruminant.

	Liveweight (kg)	Growth rate+ (kg/day) or Milk Yield++	Net requirement mg/day	Drymatter intake (kg)	Gross dietary requirement (mg/kg DM)
Suckling Fawn	14	0.4+	0.28	0.3	0.9
Weaned Fawn	50	0.3+	0.28	1.8	5.2
Adult Hind	100	0	0.143	2.2	1.7
Pregnant Hind	110	0	0.18	2.5	1.9
Lactating Hind	100	3++	0.743	4	5

5. SUPPLY OF COPPER

5.1 Foetal Reserves

Young deer are born with very high copper reserves (mean 5.598 μmol/kg)[2] and at these levels and in the absence of copper from any other source, these reserves may be adequate for up to 120 days.

5.2 Milk

Though copper concentrations in milk are low, the efficiency of absorbtion in pre-ruminant animals is said to be very high (80%)[23].

5.3 Solid Foods

Ruminants can be faced with 2 major problems: firstly to extract copper from a relatively indigestible food matrix, and secondly, to maintain the released copper in forms available for absorbtion. Anaerobic fermentation in the rumen create conditions unfavourable for the subsequent absorbtion of copper. Sulphide constituents in the diet enter the sulphide pool in the rumen and acting independently or in concert with molybdenum can lower the availability of copper to below 1%. High dietary components of iron can also decrease copper availability.

5.4 Food Intake

The volume of food intake affects both the supply and requirement for copper. During winter the appetite of deer is markedly reduced. However, growth rates are also very low and hence the copper requirements for young deer are also diminished. The impact of decreased food intake either through inappetance or food scarcity will depend on the nature and extent of deficits in supplies of other nutrients.

6. ABSORBTION OF COPPER

Most absorbtion of copper takes place in the acid environment of the abomasum and the small intestine. Copper is not absorbed by simple diffusion as the process shows incidence of saturability. **Figure 2** shows a diagmatic representation of metal ion absorbtion[24].

Absorbtion is dependent on:

(i) Ionisation of the metal in the gut lumen;

(ii) the ion is chelated with protein molecules in the membrane of mucosal cells; and

(iii) these proteins carry bound ions into the cytoplasm.

The carrying of a metal ion into the cell is accomplished by an active process.

Suttle (1975)[25] states that there are six categories of interactions between metals which can influence their absorbtion.

(1) The formation of insoluble complexes - phosphates, sulphides, oxalates, phytates, are all important precipitating substances derived from the diet and are a major factor affecting the intestinal absorbtion of metals. Metals compete for the same ligand. The first step in mineral absorbtion requires the mineral to remain in the ionic state.

(2) Competition for the same transport carriers by chemically similar ions e.g. Copper is preferentially bound to transferrin, the protein transport molecule in mucosa when competing with iron.

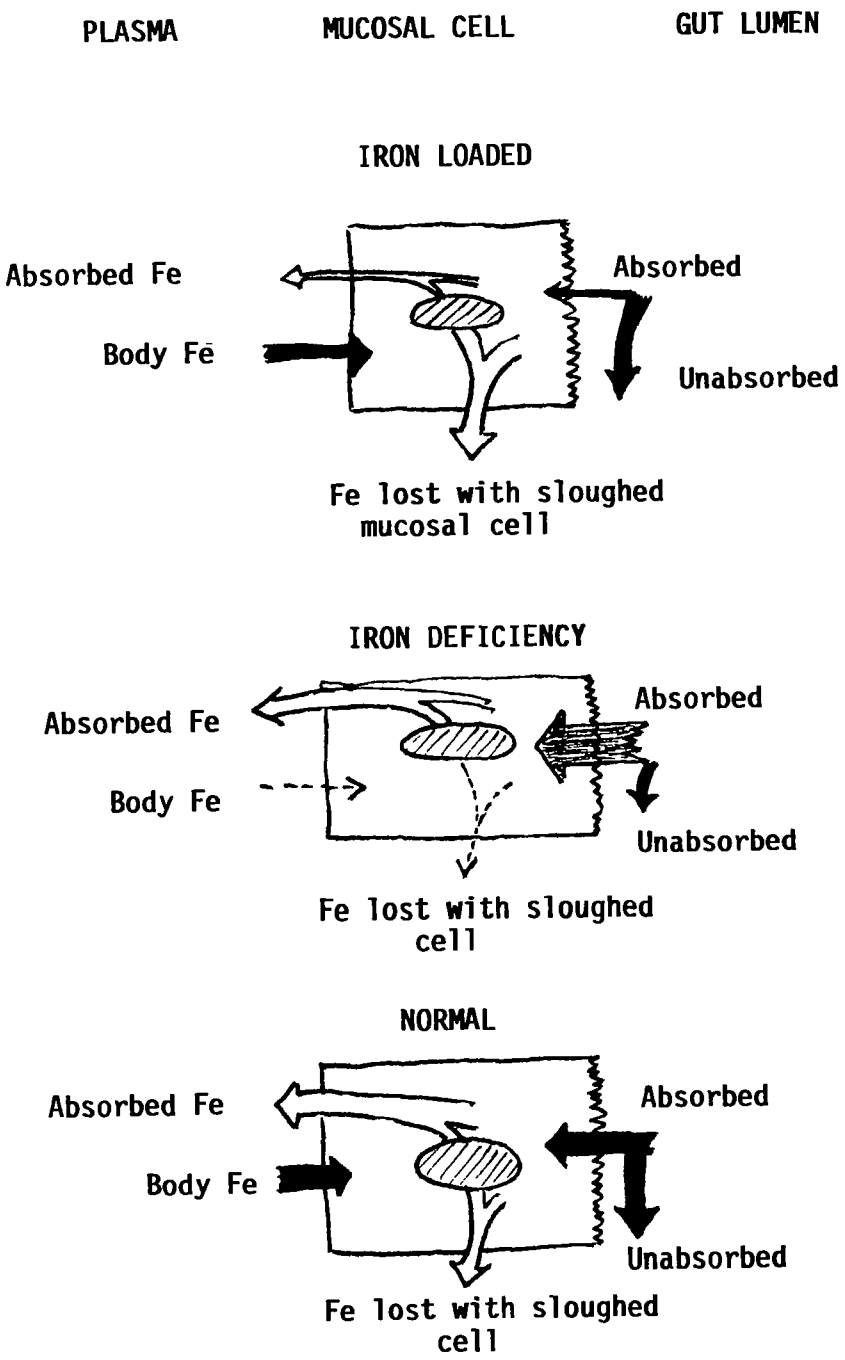
(3) Synthesising of metal binding proteins by the body as a reaction to heavy metal loading e.g. diets rich in zinc and cadmium may induce the synthesis of metallothionine which has a higher affinity for copper than for zinc or cadmium. This means that a large pool of metallothionine in mucosal cells could "trap" copper and slow down its absorbtion.

(4) A change in the metal component of metalloenzymes.

(5) Transport and excretion of minerals.

Metals need to be in the ionic state for absorbtion, and require an acid pH to remain that way. Alternatively they may be chelated and hence remain soluble at a high pH. All metal ions must be capable of binding to protein in the gut because this (Chelation and/or complexing) is an essential step in the intestinal uptake of metal ions. Copper (as for Fe, Zn, Mn, Ca, and Mg) requires a 2 step absorbtion mechanism to move from the gut lumen to the plasma. This is believed to be a carrier protein which carries the ion across the membrane to a larger protein within the cytoplasm (copper-thionine is the transport ligand for copper). Metalloproteins within the cytoplasm serve to store copper as well as transport it to the plasma side. The transport of copper through the cell to plasma appears to involve a special mechanism/mechanisms dependent on metabolic energy.

Figure 2[24] shows a simplified diagram of processes involved with iron absorbtion which may be applicable to other metal ions.



Similarities in mucosal transport of metal ions (Ca, Mg, Fe, Zn, Cu, Mn)

(i) All are absorbed in the acid pH of the upper intestine.
(ii) Solubility (chelation)/ionisation essential for absorbtion
(iii) The ions are attached to the exterior cell membrane, this process requiring an amino acid/peptide molecule.
(iv) Ion binding to peripheral protein requires chelation.
(v) Membrane transport requires energy.
(vi) The minerals are released intracellularly and are re-chelated.

7. TRANSPORT OF COPPER TO THE LIVER

On the serosal side of the gut mucosa, copper is thought to be associated with serum albumin. There is recent evidence that another protein "transcuprein" may be involved with copper transport.

7.1 Uptake of Copper by the Liver

Orally administered copper is quickly cleared from the plasma by the liver. The method of transfer of copper to liver cells is not clearly understood but it is thought that albumin may act by slowing down the rate of transport of copper into hepatocytes.

7.2 Hepatic copper metabolism

In sheep liver there are two major compartments associated with Cu metabolism - a temporary storage compartment for Cu destined for biliary excretion or ceruloplasmin and a longer term storage compartment[25] (Weber, K.M., Boston, R.C., and Leaver, D.D., (1980) Aust.J.Agric.Res., 31, 773, 1980).

Copper is distributed amongst many subcellular organelles.

(i) Microsomal Fraction (approx 18% hepatic Cu) - probably newly synthesized copper proteins.
(ii) Nuclear Fraction (approx 20% hepatic Cu) - may represent temporary copper storage in metalloproteins.
(iii) Large Granule Fraction (approx 20% hepatic Cu) - in lysosomes, mitochondria and peroxisomas and may represent temporary storage or be destined for biliary excretion or

(iv) Cytosol (approx 50% biliary Cu) - in copper carrying proteins metallothionein, copper-zinc superoxide dismutase, and ceruloplasmin.

7.3 Copper Transport in serum and uptake by extrahepatic tissues

Approx 90% of the copper in serum is contained in ceruloplasmin and this copper is not readily exchangeable *in vitro*. The remaining copper is bound to albumin and amino acids which transport copper in the portal circulation.

Ceruloplasmin may act as a transport molecule for copper to the tissues and it has been suggested that ceruloplasmin copper becomes the functional copper of cytochrome oxidase and lysyl oxidase.

Ceruloplasmin, along with other glycoproteins and fibrinogen is an "acute phase reactant" and there are dramatic rises of plasma ceraloplasmin levels as a response to trauma as well as to inflammatory processes. This may reflect a need to boost the delivery of copper to peripheral tissues for complexation into forms that are pharmacologically active.

Ceruloplasmin also has pro and anti-inflammatory properties as well as processing oxidase activities which may regulate the presence in the serum of certain biologically active molecules. It has been shown to be a major antioxidant in the plasma, and inhibits a number of superoxide-mediated reactions preventing auto-oxidation of lipids. Ceruloplasmin is a scavenger of oxygen derived free radicles

and in combination with its antioxidant properties may protect the host from the potential ill effects of local tissue injury.

Other copper proteins with anti inflammatory properties include ascorbate oxidase, laccase, diamine oxidase and superoxide dismutase.

7.4 Metallothionein

Metallothioneins bind copper, zinc, and a number of heavy metals and their synthesis may be stimulated by the presence of these metals. Their role in copper metabolism is not clear. However they may play an important role in copper homeostasis by binding copper and committing it to exit pathways. It is possible they may also act as a store releasing it for cellular processes.

8. COPPER INTERACTIONS

8.1 Chelation

Copper must be in the form of free ions or in the chelated form in order to be absorbed by gut mucosal cells.

8.2 Copper, Molybdenum, Sulphur

Freudenberger *et al* (1987)[21] and Mason *et al* (1984)[26] have suggested that the metabolism of molybdenum may be different in deer than other ruminants. Sulphur may act directly in reducing the availability of copper by the production of insoluble copper sulphide, or in conjunction with molybdenum to form tetrathiomolybdates which bind to protein, and subsequently firmly binds copper and render it unavailable for absorbtion. Thiomolybdates may also be absorbed and bound to plasma albumin. This process modifies the binding sites of copper to plasma albumin and renders it unavailable for uptake by the liver Freudenberger *et al* did not find significant quantities of TEA insoluble copper (albumin/thiomolybdate/Cu complex) in the plasma of deer.

8.3 Copper and Zinc

High concentrations of zinc in the diet lead to reduced copper stores and the development of copper deficiency. This may be due to direct competition of zinc and copper for transport across the cell membrane or because zinc stimulates the production of metallothionein which binds copper in mucosal cells which are sloughed off and excreted.

8.4 Copper and Iron

Reduction in liver copper stores with increased iron in the diet is thought to be dependant on the presence of sulphur. This leads to the formation of iron sulphide in the rumen which is hydrolysed in the abomasum and promotes the formation of insoluble copper sulphide, copper and cadmium.

Cadmium stimulates the production of metallothionein which binds copper.

Levels of minerals which may affect the development of copper deficiency are as follows:

Pasture Copper	values of < 6ppm have been suggested to be deficient
Molybdenum	> 1.5 ppm (depending on pasture Cu levels)
Sulphur	1-5 gm/kg (usually > 4 gm/kg)
Zinc	420 ppm [27]Bremner, I., Young, B.W., Mills, G.F. (1976) Br.J.Nutr 36: 551-561
Iron	> 250 ppm [28]Suttle N.F. (1986) Vet.Rec. 119: 519-522
Cadmium	5-15 ppm [29]Mills, C.F., and Dalgarno A.C. (1972) Nature, 239, 171 [30]Doyle, J.J. and Pfander, W.H. (1975) J. Nutr., 105, 599.

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