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Reviews

Copper metabolism and dietary needs

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It has been about 80 years since Cu was first recognized as being important for maintenance of haemoglobin. Since that time requirement values have been established and signs of deficiency and excess well documented. NRC (1994) provide the most comprehensive list of detailed requirement values for Cu for various classes of poultry, yet these lack extensive detail and are predicated on just five publications, the most recent of which was conducted 30 years ago. Requirement values average 6-8 ppm which traditionally is supplied by inorganic salts and especially copper sulphate. Other inorganic sources vary from 40-115% bioavailability of Cu compared to sulphate. Copper is easily complexed with amino acids or proteins, leading to the development of so-called organic sources of copper which are claimed to have better digestibility and/or less formation of insoluble complexes with other minerals in the digesta. Results on effectiveness of organic *vs.* inorganic forms of Cu are variable, although this line of research has led to appreciation of the potential to use much lower levels of supplementation. Since at least 80% of dietary copper appears in the excreta, using lower levels of diet supplementation means reduced Cu in the environment. With less supplementation, knowledge about bioavailability of Cu in major feed ingredients becomes important. Copper in cereals is reported to be 80% available to the bird while that in vegetable proteins is closer to 50% available. Availability from animal proteins is very variable, while corn distiller's grains now provides the most concentrated source of Cu within the major ingredients. Copper levels greatly in excess of requirement, at around 125 ppm, have been shown to improve performance of meat birds and egg layers. The mode of action is unknown although likely relates to antibacterial properties of Cu. Likewise high levels of diet Cu have been shown to reduce cholesterol content of eggs and poultry meat, although this is often at the expense of loss in performance and contribution of more Cu to the environment.

Keywords: copper; requirement; toxicity; lipid metabolism; environment

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Introduction

While copper was shown to be present in animal tissues in the 1880`s, it was not until the late 1920's that both copper and iron were found to be necessary for haemoglobin formation in rats. Although copper is not a constituent of haemoglobin it is present in certain plasma proteins that are involved with iron transport, and a deficiency of copper can result in signs of anaemia. Copper is also a component of other blood proteins, one being erythrocuprein, found in erythrocytes where it is involved in oxygen metabolism. The element also plays a role in many enzyme systems, such as cytochrome oxidase, which is important in oxidative phosphorylation and in certain pigments that can influence feather colour. Copper is present in most body cells, being particularly concentrated in the liver with bile as a major route of excretion.

Interest in copper nutrition was markedly increased in the 1930's when it was demonstrated that certain sheep and cattle diseases were due to a copper deficiency. Studies into these ruminant-specific problems led to extensive mapping of copper deficient areas around the globe and then subsequently the common practice of adding copper supplements to animal diets.

Copper requirement and general metabolism

There is limited information on Cu requirements specified within NRC (1994) and the values that are given, across all species, are based on research in just five publications, the most recent of which is now 30 years old. However, absolute requirements are not much different to modern commercial diet specifications (Leeson and Summers, 2005). NRC (1994) indicated requirements for immature egg strains of 4-5 ppm although no values were given for laying hens. Requirements for meat birds were 6-8 ppm although Cu as a nutrient was missing in tabular data for broiler breeders. Copper requirements may increase when there is immunological stress (Koh *et al.*, 1996). Within regular feed ingredients, NRC (1994) suggest corn distillers with solubles to provide the most concentrated source of Cu with about 50 ppm.

The outer electron structure of metal ions dictates their solubility, reactivity and transport systems. Copper can exist in three states of oxidation and most often, one of two valencies and hence reactivity with other ions and various ligands is inevitable. The main function of Cu is as a cofactor in cytochrome oxidase, lysly oxidase, superoxidase dismutase and cerruloplasmin (Lim and Paik, 2006). As such, there are a number of signs of deficiency ranging from anaemia to inadequate cartilage formation in various tissues. While efficient recycling of Cu by kidney tubules occurs in most animals (Davidson *et al.*, 1974) there is poor recycling from bile (Aoyagi and Baker, 1995b) and so this later route is the main mechanism for clearance when dietary supply exceeds demand and after liver stores have been maximized.

While there can be toxic effects of copper, the mineral is still used periodically as a growth promoter, at 125-250 mg/kg diet (Pesti and Bakalli, 1996; Ewing *et al.*, 1998). The exact mechanism for such enhanced growth has not been elucidated. Certainly the excreta colour of the bird changes markedly, and it is presumed that there will be changes to the gut microflora. In turkeys high levels of diet Cu cause greater frequency of caecal evacuation and in dry weight of caecal contents (Leeson *et al.*, 1997). Viscosity of turkey caecal contents is also much higher when they are fed 250-500 ppm Cu, containing as much as 12,000 ppm Cu.

Aoyagi and Baker (1995b) suggest that the beneficial effect of feeding pharmacological levels of Cu may result from improved digestion of hemicellulose. After feeding 250 mg

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Cu/kg diet, a 15% increase in hemicellulose digestion (22→33%) was observed when feeding corncobs, where diet AMEn increased from -200 kcal/kg to +117 kcal/kg. It was suggested that excess copper stimulates clearing of liver copper into bile through exocytosis of hepatocellular lysozymes. A side effect of such action is increased bile secretion of glucosidases, which could impact carbohydrate digestion. Wideman *et al.* (1996) suggested that the best results from feeding such high levels of Cu occur after 2-3 flocks have been grown on re-used litter, inferring altered microbial status of the litter. Interestingly these researchers also suggest that the occurrence of proventricular hypertrophy due to high levels of Cu are less evident when birds have access to litter as a source of roughage. When feeding ≥50 ppm copper, the majority is lost in the excreta, which is of concern regarding environmental pollution.

Copper sources and availability

Until recently, the main focus was on bioavailability of inorganic sources of copper. Copper sulphate is often used as a standard, although copper chloride, oxide, carbonate and citrate have also been tested and are available commercially. However, over the last few years two other issues have arisen which have refuelled interest in copper source and bioavailability. Firstly, organic sources of copper, which are chelated with amino acids, peptides or proteins, have rekindled interest in bioefficacy of mineral supplements. Secondly, and the most recent area of interest, is consideration of the availability of minerals such as copper in natural feed ingredients. Traditionally, requirements for Cu and other trace minerals are met entirely by supplements with no account of the mineral present in cereals and animal and vegetable proteins. This recent interest stems from attempts at minimizing nutrient supply of Cu and other minerals as a means of reducing excreta levels and so aiding environmental initiatives. While it is easy to measure total levels of Cu in major ingredients, ascertaining bioavailability is a complex procedure.

A typical broiler starter diet will provide around 150% of the requirement for Cu relative to NRC (1994) recommendations. The proportion that is available to the bird is predictable, although values can be quite variable. Such variation may well be impacted by agronomic conditions for cereals and vegetable proteins and by prior nutritional status and processing conditions for animal proteins. As for any nutrient, bioavailability will be affected by parameters used in estimation, which for Cu seem to range from simple production characteristics through to liver and bile accumulation indices.

Aw-Yong *et al.* (1983) suggested bioavailability values for Cu in wheat, corn and barley to be around 80%. Using a bile accumulation index Aoyagi and Baker (1993) showed much lower levels of Cu availability in vegetable proteins. Values were around 40% Cu availability in soybean meal, cotton seed meal and groundnut meal and closer to 50% in corn gluten meal. These lower levels may be due to greater binding of Cu with phytate. In a subsequent study Aoyagi and Baker (1995a) suggested that Cu in soybean meal was only 40% as available as Cu in copper sulphate and that adding phytase to test diets decreased, rather than increased, Cu availability. These authors ascribed the negative effect of phytase on concomitant release of zinc which negatively impacted Cu uptake. Copper in animal proteins seems to be even more variable in availability. It is well known that Cu in pig liver is totally unavailable for poultry and there are reports of Cu deficiency in cats fed porcine liver as a sole source of Cu. Aoyagi *et al.* (1995) found that disrupting the protein structure in porcine liver increased availability, with values of 32%, 46% and 63% seen after autoclaving, acid hydrolysis or protease digestion respectively. In testing other animal proteins Aoyagi *et al.* (1995) showed zero availability in feather meal and only 4% in meat and bone meal manufactured from

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ruminants. Copper in pork meal and poultry by-product meal was respectively 50% and 40% available.

There has been a wealth of information published on bioavailability of inorganic sources and more recently of organic sources of Cu, usually with comparison to copper sulphate, using a range of assessment parameters. Considering chick weight as well as liver Cu levels and haemoglobin status, McNaughton *et al.* (1974) suggested that both cuprous oxide and cuprous iodide were 60% as bio available as sulphate. Pesti and Bakalli (1996) and Ewing *et al.* (1998) both suggested Cu citrate to be more efficacious than Cu sulphate. Using Cu acetate as a standard, Zanetti *et al.* (1991) found carbonate salt to be only 66% as available. Certain aluminosilicates, often used as mycotoxin binders, can sometimes contain moderately high levels of Cu. Xia *et al.* (2004) suggested that the 2.5% Cu within an aluminosilicate was bio available and in addition to growth promotion had a positive impact on intestinal epithelial morphology.

Tribasic copper chloride (TBCC) is now available in commercial quantities and is considered an alternative to copper sulphate, especially in broiler and layer diets. While sulphate is close to 99% soluble in water, TBCC is virtually insoluble at neutral pH increasing to 80% solubility under acidic conditions (Pang and Applegate, 2007). Feeding graded levels up to 390 ppm, Liu *et al.* (2005) suggested TBCC to have a relative value of 134% compared to Cu sulphate when egg weight was the main criteria. Miles *et al.* (1998) proposed a value of 106% for TBCC relative to sulphate. In terms of liver Cu accumulation, TBCC was 109% as efficacious as sulphate (Luo *et al.*, 2005). These authors also suggested that TBCC was less destructive to vitamins in both the feed and within the bird. Liver and plasma vitamin E levels were higher in birds fed TBCC *vs.* sulphate using a diet initially containing 36 IU/kg. Interestingly after storing feed for 21d there was 16% loss of potency of tocopherol when TBCC was used, compared to 40% loss in the feed containing Cu sulphate. Arias and Koutos (2006) suggested 188 ppm Cu from TBCC to have comparable effects to an antibiotic in terms of changes in gut morphology.

Recently considerable interest in testing efficacy of products loosely termed 'organic copper' that are most often chelates with amino acids or proteins. It is often claimed that these chelates are preferentially absorbed and metabolized and provide less chance for antagonism of Cu with other minerals or substrates (Leeson and Summers, 2005). The latter assumption is obviously predicated on these Cu sources remaining chelated until the site of digestion and absorption. Baker *et al.* (1991) ascribed a value of 115% to availability of Cu in a Cu-lysine complex compared to Cu sulphate, although the basal diet used in this study had an uncharacteristically high level of Cu. Miles *et al.* (2003) found a Cu-amino acid chelate to be slightly less efficacious than Cu sulphate. Guo *et al.* (2001) tested a range of Cu complexes in an attempt to predict Cu availability from physical characteristics such as solubility. A Cu-amino acid chelate and 3 different Cu-protein complexes exhibited from 30 to almost 60% free Cu in various solutes, inferring instability of the chelate. Only Cu-lysine seemed to be stable under conditions used in this study. Guo *et al.* (2001) concluded that the best predictor of Cu availability for the chick was solubility at pH 2 inferring that chelation integrity and availability are not correlated. Only Cu-lysine and Cu-amino acid, but none of the Cu-protein products, were more efficacious than Cu sulphate. There have been reports of very low levels of organic minerals being adequate for poultry with a view to reducing mineral excreta output. Nollet *et al.* (2007) showed that broilers fed very low levels of all trace minerals in the form of proteinates, performed as well as birds fed conventional levels as inorganic salts. Likewise Bao *et al.* (2007) suggests that 4 ppm dietary Cu is adequate for birds. In such studies it is important to realize that all minerals are at low levels so as not to induce

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antagonisms. Feeding low levels of Cu in combination with regular levels of other trace minerals, and especially Zn, will likely result in more chance of inducing Cu deficiency.

Copper deficiency

A deficiency of copper usually manifests itself as anaemia, indicating its role in iron metabolism. Iron absorbed as Fe^{++} is usually transported as Fe^{3+}, a conversion which requires ferroxidase enzyme, a component of which is copper. Copper is also important for normal bone formation and in particular cartilage formation.

Copper deficiency results in a wide range of signs in different animal species, and indeed, in the same species different situations are noted. In part, such varied response to Cu deficiency is the result of differential impact on enzyme systems reliant on Cu as a co-factor. Such enzyme systems as cytochrome oxidase involved in electron transport, monoamine oxidases involved in cartilage formation and those involved in deamination of norepinephrine and serotonin activity obviously impart variable responses within the animal. However, anaemia is a common sign noted for all animals, as is growth depression, bone disorders, depigmentation of hair, wool, fur and feathers, demyelination of the spinal cord, fibrosis of the myocardium, and diarrhoea.

Young chicks will become lame in 2 to 4 weeks when fed a copper deficient diet. Bones will be fragile and easily broken, the epiphyseal cartilage becomes thickened, and vascular penetration of the thickened cartilage is markedly reduced. These bone lesions for chickens are quite different from those seen with other farm animals and resemble the bone pathology similar to birds suffering from a vitamin A deficiency. Rucker *et al.* (1975) showed increased bone fragility in chicks fed ≤1 ppm Cu. There was a characteristic reduction in collagen cross-linking caused by either delay or suppression of conversion of soluble to insoluble mature elastin, with rapid degradation of the soluble complex. Rucker and Goethlich-Riemann (1972) also observed low levels of lysyl oxidase in elastin of Cu deficient chicks, with the resultant soluble collagen containing more lysine and less condensed desmosine. O'Dell *et al.* (1966) recorded a 50% reduction in mature insoluble collagen in Cu deficient chicks associated with elevated levels of hydroxyproline and hexosamine. Lilburn and Leach (1980) showed that cartilage from copper deficient chicks oxidized less glucose than normal, and at a rate comparable to that seen in situations of tibial dyschondroplasia. There are similarities in cartilage structure for birds suffering from Tibial Dyschondroplasia (TD) or copper deficiency. Although enzootic neonatal ataxia as occurs in ruminants is not characterized as such with poultry, copper deficient chicks have also been shown to suffer from ataxia and spastic paralysis.

Both copper and iron deficiencies have been reported to result in loss of feather pigmentation (achromotrichia) in New Hampshire chickens and bronze turkeys. A syndrome consisting of achromotrichia, alopecia and dermatitis has been reported as the most readily noted signs of a copper deficiency in rabbits, while a lack of pigment in black-wool sheep, along with loss of wool crimp, is also a common occurrence. While these conditions have been studied for quite some time, the complete clinical picture of a copper deficiency has as yet not been elucidated. The copper containing enzyme polyphenoloxidase, is known to catalyze production of melanin from L-tyrosine, and the lack of wool crimp has been reported to be due to the need for copper in the normal oxidation of cysteine to the S-S- bond required for maintaining the proper protein structure of wool. In their classical study Vohra and Kratzer (1957) attributed depigmentation to reduced tyrosinase activity in the feather pulp of Cu-deficient chicks and poults. Poults were especially susceptible, exhibiting

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depigmentation within 10 d of feeding a Cu-deficient diet and that the condition could not then be corrected by feeding extra Cu.

Studies by Missouri workers in the 1960's showed that a copper deficiency in chickens could produce a dissecting aneurysm of the aorta along with various bone deformities that closely resembled lathyrism. There is some evidence that ß-aminoproprionitrile (BAPN), which has been shown to experimentally produce lathyrism, may act as a chelate in sequestering trace minerals that may prevent lathyrism. Chicks hatched from copper deficient hens have been shown to have no amino oxidase in the aorta or liver. If such chicks are fed a copper deficient diet the enzyme is still not detectable even up to 4 weeks of age, while chicks' fed copper show high amine oxidase levels after the third day of life. Kim and Hill (1966) reported that amino oxidase increases the incorporation of lysine into elastins (desmosines) of the aorta. Hence, the role of copper in elastin formation in the chicken appears to be via amino oxidase, which in turn is involved in the oxidative deamination of the epsilon amino group of lysine. A copper deficiency apparently can result in a reduction in the number of oxidized lysine residues available to condense for the formation of desmosine in cartilage of both the aorta and in bone development. Hill *et al.* (1967) recorded low levels of amine oxidase in newly hatched chicks and that measurable levels were only attained by day 3, although in Cu-deficient birds no measurable enzyme activity was found in the aorta.

Copper deficiency in birds, and especially in turkeys, can lead to aortic rupture. It is likely that the biochemical lesion in the copper-deficient aorta relates to failure to synthesize desmosine as described previously. The lysine content of copper-deficient elastin was observed to be 3 times that seen in control turkeys, suggesting failure to incorporate lysine into the desmosine molecule. In field cases of aortic rupture, many birds have <10 ppm Cu in the liver, compared to 15-30 ppm normally seen in birds of comparable age (Graham, 1977). A high incidence of aortic rupture has been seen in turkeys fed 4-nitrophenylarsionic acid, although the problem could be resolved by feeding higher levels of copper suggesting that products such as 4-nito may simply complex with copper in the digesta. O'Dell *et al.* (1966) reported that while Cu-deficient chicks weighed 30% less than control-fed birds, their aortas were twice as heavy expressed per unit of body weight. Heart (and liver) levels of cytochrome oxidase were also decreased in turkeys fed around just 1 ppm Cu (Simpson *et al.*, 1963) while their erythrocytes are morphologically abnormal.

Low to deficient levels of dietary Cu have also been associated with subtle changes in saturation of body lipid reserves and cholesterol metabolism. Kaya *et al.* (2006) studied haematological parameters and lipid blood profile of broilers fed diets devoid of supplemental Cu. Where 3.5 ppm Cu was provided by cereals and vegetable proteins, total blood cholesterol, trigyceride, and LDL-cholesterol were significantly higher, while ceruloplasmin levels were reduced and prothombin time was increased. It was concluded that Cu deficiency created hypertriglceridaemia, hypercholesteraemia and anaemia.

Copper toxicity

Although levels at 20 times requirement can sometimes provide benefits to health and/or performance of poultry, it is not until levels approaching 100 times requirement are fed that toxicity occurs. Persia *et al.* (2004) using graded levels of supplemental copper chloride suggested toxic levels of Cu to be 640 ppm in terms of weight gain and 780 ppm for feed utilization. Aoyagi and Baker (1993) indicate that with excess Cu intake there is initial accumulation in the liver, but after levels reach 20-25 X normal, Cu is released into circulation leading to haemolysis. Cases of Cu toxicity are quite rare and most usually

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result from accidental overdose of copper sulphate or other copper sources. No major feed ingredients are sufficiently high in Cu to cause toxicity. Ruminants, especially sheep, are much more prone to Cu toxicity that usually occurs due to consumption of forage high in Cu and/or low in molybdenum, or plant material containing alkaloids that promote liver Cu depletion. Cu levels in poultry manure can also be toxic for sheep, especially when broilers or turkeys are fed therapeutic levels of Cu. Cu toxicity in ruminants manifests as haemolysis, suggesting different excretory pathways in poultry, since this effect is rarely seen. Doudoroff and Katz (1953) suggest that as little as 0.01 ppm of Cu in water to be toxic to many freshwater fish species, and hence environmental concerns about Cu in poultry manure.

Jensen and Maurice (1979) indicated the growth rate of chicks to be depressed when feeding 500-700 mg/kg dietary Cu. Wideman *et al.* (1996) describe a case of proventriculitis in young broilers that was caused by feeding just 200 ppm Cu while Poupoulis and Jensen (1976a) described similar signs in 28d broilers fed ≥250 ppm Cu. Henderson and Winterfield (1975) indicated that necrosis and sloughing of the gizzard and proventriculus did not occur in geese until levels of 600 ppm were fed, inferring perhaps a strain effect for this sign of toxicity. Persia *et al.* (2004) likewise suggest a strain effect in response to toxic levels of copper, in that New Hampshire x Columbian chicks are more tolerant of high levels of dietary Cu compared with commercial broiler strains. Wideman *et al.* (1996) indicated proventicular hypertrophy in broilers fed in excess of 200 ppm Cu, and that this was not always associated with growth depression. Hypertrophy was characterized by flattened or irregular papillary orifices and/or thick mucous secretion, which in the gizzard produced a characteristic black discolouration. Birds in this study also exhibited enlarged kidneys, spleen and bursa. Response of broilers to diet Cu may be influenced by general health status of birds. Fox *et al.* (1987) observed a 20 times increase in liver Cu of birds fed diets with 500 ppm Cu, while in *Eimeria* infected birds the increase was 60 times. Interestingly this often toxic level of Cu had no effect on performance of control birds but was very detrimental to those birds with coccidiosis. Wideman *et al.* (1996) also indicated that proventricular hypertrophy was always more evident in experimentally caged birds, attributing the reduced effect in commercial floor reared birds to the presence of litter in the gizzard and proventriculus. Thompson and Weber (1981) were however unable to show any meaningful effect of fibre *per se* on tissue accumulation of Cu under toxicity conditions.

Impaired growth associated with reduced feed intake and reduced water intake seem to be universal signs of copper toxicity. However no one seems to have elucidated cause and effect of these characteristics. Jackson (1977) fed up to 1920 ppm Cu to layers showing that feed and water intake were depressed with levels ≥960 ppm Cu, with an almost linear decline in egg numbers with levels exceeding just 240 ppm. In these layers, blood Cu levels peaked with diet levels of 240 ppm, while all other changes in production and metabolism were rationalized on the basis of reduced feed intake. Pearce *et al.* (1983) suggested changes to hepatic metabolism as well as reduced feed intake to be factors involved in toxic response to Cu by layers. Feeding 1000 ppm Cu altered circulating levels of estradiol and while this seems independent of other factors, the authors recognized that feed intake *per se* impacts hepatic lipogenesis and so hormone balance and activity. Stevenson *et al.* (1983) recorded 20% and 50% reductions in feed intake in response to 500 ppm and 1000 ppm Cu respectively while Griminger (1977) likewise attributed reduced body weight and egg shell quality to reduced nutrient intake in layers fed 2kg copper sulphate per tonne of diet. Ward *et al.* (1994) described a negative linear effect of water Cu level on water intake, recording a 30% reduction in response to consuming water containing 200 ppm Cu. With water

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intake close to double that of feed intake, this toxic effect is comparable to the toxic effect of around 500 ppm diet Cu previously described.

Copper antagonisms and interactions

With copper's ability to exist in numerous valency states, interactions with other minerals and nutrients can occur, which impacts metabolism of copper itself and that of other minerals and chelates. Interaction between copper and other minerals such as zinc, iron and molybdenum are often described as classical examples of mineral interactions that impact the availability of all of these nutrients. A large proportion of copper is absorbed from the duodenum, and in the mucosa is found attached to a protein carrier. Because zinc also binds aggressively to the same carrier, excess of zinc for example can induce a deficiency of copper. Smith and Wright (1975) indicated high levels of circulating molybdenum as a corollary to elevated copper due to the formation of a stable Cu-Mo-protein complex that was not taken up by any tissues. Skrivan *et al.* (2005) found that Zn levels in yolk were reduced as a consequence of feeding 25 ppm Cu. The corollary also occurred in that higher levels of dietary zinc caused less copper in the egg. Adding extra copper resulted in increased Fe deposition in the liver of the laying hens. Liver Cu was only increased when both Zn and Fe were used, and not by supplementing either of these minerals independently (Skrivan *et al.*, 2005). Vohra and Heil (1969) described an antagonistic effect of dietary Zn on hepatic Cu and that Cu itself impacted accumulation of Mn. Additional Zn can also exacerbate the impact of Cu-deficient diets on elastin synthesis within cartilage (Hill *et al.*, 1967).

Because Mo is required to mediate Fe release from the intestinal mucosa, deficiency of Mo or antagonism by other minerals, can cause anaemia (Seelig, 1972). Excess Cu or Mo can cause deficiency of the other and lead to signs of hypochromic microlytic anaemia that are indistinguishable from those of an uncomplicated Fe deficiency. Poupoulis and Jensen (1976a) suggested that high levels of dietary Cu cause varying degrees of gizzard erosion, and that the condition is partly corrected by giving high (0.5 ppm) levels of dietary selenium. In a previous study Jensen (1975) suggested that 800 ppm Cu caused signs of Se deficiency even though the basal diet contained 0.2 ppm of this mineral. There is some indication that elevated levels of diet Cu can alleviate toxicity from heavy metals such as lead (Klauder and Petering, 1975).

The commercial practice of using pharmacological levels of diet Cu for growth promotion in meat birds has been questioned in terms of compatibility with exogenous phytase enzymes. High levels of dietary Cu result in reduction in solubility of the phytic acid complex potentially leading to reduced efficacy of phytase. Banks *et al.* (2004a) suggest that high levels of diet Cu do in fact reduce phosphorus retention, although the mode of action is not via suppression of the efficacy of phytase. Banks *et al.* (2004b) suggested that when using 250 ppm diet Cu phosphorus retention was more impacted by choice of inorganic source. Another commercial feeding practice used some years ago was the addition of arsenicals to broiler diets and again these compounds antagonized Cu metabolism. Czarnecki *et al.* (1984) indicated that 50 ppm of an arsenical reduced liver Cu level by at least 2-4 fold, and that this effect was independent of any changes to feed intake. Since hepatic Cu levels were restored by injectable Cu, but not by additional diet Cu, suggests that the antagonism works within the digesta.

There has been some controversy regarding the impact of high levels of Cu on requirements for sulphur amino acids. Jensen and Maurice (1979) indicated reduced growth rate of chicks when feeding 500-700 mg/kg dietary Cu and that the apparent

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toxicity could be alleviated by feeding 0.4% extra DL-methionine even though the diet was apparently adequate in this amino acid. The authors conclude that methionine and/or a protein are combining with copper, either in the digesta, the intestinal mucosa or the liver, and so rendering it less toxic to the bird. Alternatively one can conclude that high levels of copper increase the birds need for sulphur amino acids. Using purified diets, Robbins and Baker (1980) calculated that 250 ppm and 500 ppm of diet Cu increased the chick's requirement for sulphur amino acids by 13 and 30% respectively. Wang *et al.* (1987) showed 250 ppm Cu to increase the young birds' requirement for methionine by 0.1%. At lower levels of diet Cu, Kashani *et al.* (1986) showed 120 ppm Cu to have no effect on the methionine requirement of poults. Ledoux *et al.* (1987) suggested that the effect of high levels of Cu (≥400 ppm) are simply due to reduced feed intake and hence the need for more of the limiting amino acid in corn-soy diets. Adding 400 ppm or 800 ppm Cu resulted in 9% and 19% reductions in feed intake respectively, and hence the need for correspondingly more nutrients and especially methionine.

Impact of copper on lipid metabolism

Moderately high levels of dietary copper seem to reduce cholesterol levels in eggs and meat products although these effects are sometimes associated with loss of performance. The impact of diet copper on fat saturation of poultry products and eggs is less clear. Bartov (1977) showed no effect of diet Cu on fatty acid saturation of the broiler carcass, although in high-protein, low-energy diets, there was a linear relationship between Cu and skin dry matter content. On the other hand Poupoulis and Jensen (1976b) and Skrivan *et al.* (2000) suggested that as Cu levels increased there was either reduced saturation or increased unsaturation of body lipids. However the changes reported by Skrivan *et al.* (2000), although statistically significant, were of little commercial relevance. In the earlier report of Poupoulis and Jensen (1976b), it was concluded that when changes to growth and feed intake are factored into lipid changes due to 250-500 ppm Cu, then Cu *per se* has no meaningful effect on tissue saturation. Ruiz *et al.* (2000) and Maraschiello *et al.* (2000) provide conflicting reports of the effect of removal of copper from the finisher and withdrawal diets of broilers on oxidative stability of breast and thigh meat.

Moderately high levels of diet Cu seem to have a consistent effect on reducing egg cholesterol content although the quantitative effect is quite variable. In most instances such variability in change in yolk cholesterol is a factor of the assay procedures and resultant variability in baseline levels of cholesterol found in normal eggs. Balevi and Coskun (2004) recorded a 25% reduction in yolk cholesterol in response to feeding 150 ppm Cu although very high levels in control eggs suggest need for review of experimental procedures. Lim *et al.* (2006) recorded only a 7% decline in response to 200 ppm diet Cu, although this was associated with a 23% decline in serum cholesterol of these birds and that interestingly these eggs exhibited improved albumen quality following storage. Pesti and Bakalli (1998) showed slight increase in egg Cu in response to feeding 125 ppm Cu although cholesterol content declined from 11.7 to 8.6mg/g yolk with no further decline from feeding twice this amount of Cu. In this study, virtually all Cu ended up in the excreta. Ankari *et al.* (1998) showed a linear decline in yolk cholesterol from 9.8 to 8.3mg/ml in feeding high levels of Cu. In this study, reduced egg cholesterol was at the expense of 10% reduction in egg production. Lien *et al.* (2004) needed 250 ppm rather than 125 ppm Cu to obtain measurable change in egg composition. Egg and serum cholesterol declined by 20% and 10% respectively, while

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serum Very Low Density Lipoprotein declined 18% and High Density Lipoprotein increased 110%.

Comparable responses have been observed in tissue cholesterol and serum lipid profile of meat birds. Bakalli and Pesti (1995) recorded 12% reduction in plasma cholesterol and 20% reduction of breast muscle of broilers fed 250 ppm Cu. Breast muscle Cu was increased by 15% although levels were still less than 0.5 ppm. As previously recorded with layers, the supplemental Cu resulted in elevated levels of High Density Lipoprotein. In one of two experiments Konjufca *et al.* (1997) showed that feeding 180 ppm Cu resulted in comparable 25% declines in cholesterol in the liver, breast and thigh muscle of broilers, concluding that lower levels of diet Cu cause hypercholesteremia by elevating the levels of hepatic glutathione peroxidase. Likewise Bakalli and Pesti (1995) correlate reduced cholesterol levels with reduced glutathione and also reduced CoA reductase.

Copper and the environment

Regardless of inclusion level, the majority of dietary copper will appear in the excreta, and today this is raising questions concerning environmental sustainability. Pesti and Bakalli (1998) indicated that Cu content of excreta increased from a base level of 35 ppm to 940 ppm when the feed contained 250 ppm Cu. Dozier *et al.* (2003) conclude that at normal diet levels of 4-12 ppm Cu excretion is constant at around 85% of intake, although this increases to at least 95% when pharmacological levels of Cu are used. Topsoil levels of Cu increase by around 12% in response to spreading manure from birds fed 25 ppm rather than 9 ppm Cu and that herbage subsequently grown on this land contained 20% more Cu (Skrivan *et al.*, 2005). Feeding 240 ppm rather than 9 ppm Cu increased the excreta DM from 25 to 400 ppm Cu (Skrivan *et al.*, 2006). These researchers then used this manure as fertilizer dictated by a conventional rate of applying 21 kg N/hectare. Three months later soil level of Cu was 46 ppm Cu *vs.* 25 ppm and herbage subsequently harvested from this land contained twice as much Cu at approximately 19 ppm *vs.* 7 ppm. Dozier *et al.* (2003) conclude that if normal poultry manure is applied to land to accommodate conventional application of nitrogen and phosphorus then the supply of Cu will be at 500% of the needs for most crops. However, this level of application is no where close to the designated saturation' level of 1500 kg Cu/hectare as detailed by the EPA. In fact saturation would take close to 700 years assuming crops removed no Cu from the soil. Any concerns with Cu in the soil therefore relate not to chronic accumulation, but rather accumulation in top soil and associated leaching into watersheds. Kim and Patterson (2003) did observe one positive attribute to high levels of excreta copper, in that it seemed to suppress microbial uricase activity and less ammonia production.

Conclusions

The minimal dietary requirements for Cu for most avian species are around 5-8 ppm when supplied as inorganic salts when all other trace minerals are also provided at levels suggested by NRC (1994). Copper sulphate is usually the standard against which other Cu sources are compared, and such comparisons, depending on measurement criteria, indicate oxides and carbonates to be less available, while Cu chloride has a value of some 10-15% higher potency. So called organic sources of Cu, that are manufactured by chelation with amino acids or proteins are often claimed to have higher potency than inorganic salts, and as such can be provided at lower diet concentrations. Success with

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using such low levels of supplemental Cu (1 ppm) seems predicated on proportional reduction of all trace minerals and not Cu in isolation. When lower levels of Cu are considered as a means of reducing environmental pollution, then the bioavailability of Cu in conventional diet ingredients needs to be considered. Cu in cereals seems to be about 80% available while that in vegetable protein is less and supply from animal proteins is less predictable. The current trend of using more corn distiller's grains is particularly relevant to this feeding situation, because most samples are high in Cu. If there is need to reduce Cu levels in poultry excreta, then there needs to be systematic evaluation of Cu bioavailability in the 6-8 major feed ingredients used world-wide. With requirement at around 6 ppm, it is noteworthy that 125-200 ppm is still sometimes used for broilers, layers and turkeys, ostensibly for microbial control. Performance data is variable, yet very predictably 95% of Cu consumed will appear in the excreta. Cu deficiency is quite rare, and because of various associated enzyme systems, symptoms are variable. Anaemia is often a classical sign of Cu deficiency, and often overtly indistinguishable from that caused by insufficient iron. Cu toxicity is likewise rare and usually the result of error in feed manufacture. While elevated levels of diet Cu can reduce cholesterol level in eggs and poultry meat, this is often at the expense of performance and will always be associated with increased excreta copper levels. In relation to environmental concerns, soil accumulation is less problematic than is potential for leaching and entry of Cu into watercourses.

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