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DSM in Animal Nutrition & Health

Choline

Properties and Metabolism

Choline is a beta-hydroxyethyltrimethylammonium hydroxide. Pure choline is a colorless, viscous, strongly alkaline liquid that is notably hygroscopic. Choline is soluble in water, formaldehyde and alcohol, and has no definite melting or boiling point. The chloride salt of this compound, choline chloride, is produced by chemical synthesis for use in the feed industry, although there are other forms. Choline chloride consists of deliquescent white crystals, which are very soluble in water and alcohols. Aqueous solutions are almost pH neutral. Choline is ubiquitously distributed in all plant and animal cells, mostly in the form of the phospholipids phosphatidylcholine (lecithin), lysophosphatidylcholine, choline plasmalogens and sphingomyelin–essential components of all membranes (Zeisel, 1990). Lecithin is the predominant phospholipid (>50%) in most mammalian membranes (Zeisel, 2006). In the lung, desaturated lecithin is the major active component of surfactant (Brown, 1964), lack of which results in a respiratory distress syndrome in premature infants. Choline is a precursor for the biosynthesis of the neurotransmitter acetylcholine. Glycerophosphocholine and phosphocholine are storage forms for choline within the cytosol and the principal forms found in milk (Rohlfs et al., 1993).

Choline is present in the unsupplemented diet mainly in the form of lecithin, with less than 10% present either as the free base or as sphingomyelin. Choline is released from lecithin and sphingomyelin by digestive enzymes of the gastrointestinal tract, although 50% of ingested lecithin enters the thoracic duct intact (Chan, 1991). Choline is released from lecithin by hydrolysis in the intestinal lumen. Both pancreatic secretions and intestinal mucosal cells contain enzymes capable of hydrolyzing lecithin in the diet. Within the gut mucosal cell, phospholipase A1 cleaves the alpha-fatty acid, and phospholipase B cleaves both fatty acids. Quantitatively, digestion by pancreatic lipase is the most important process (Zeisel, 2006). The net result is that most ingested lecithin is absorbed as lysophosphatidylcholine.

Choline is absorbed in the jejunum and ileum mainly by an energy and sodium dependent carrier mechanism. Only one-third of ingested choline in monogastric diets appears to be absorbed intact. Absorbed choline is transported into the lymphatic circulation primarily in the form of lecithin bound to chylomicra. It is transported to the tissues predominantly as phospholipids associated with the plasma lipoproteins. The remaining two-thirds of choline is metabolized by intestinal microorganisms to trimethylamine, which is excreted in the urine between six and 12 hours after consumption (De La Huerga and Popper, 1952). In ruminants, dietary choline is rapidly and extensively degraded in the rumen from studies with both sheep (Neill et al., 1979) and cattle (Atkins et al., 1988; Sharma and Erdman, 1988). Estimates of rumen degradation have ranged from 85% to 99%. In in vivo studies with dairy cows, in which choline intake was increased up to 303 grams per day over controls, there was only a 1.3 gram per day increase in choline flow to the duodenum (Sharma and Erdman, 1988).

Work with sheep (Neill et al., 1979) and goats (Emmanual and Kennelly, 1984) suggests that ruminants must metabolize and utilize choline in a different manner than monogastric animals. Choline absorption must be very limited in all ruminants because of: (1) almost complete degradation of dietary choline in the rumen; (2) only limited supplies from any rumen protozoa that might escape rumen degradation; and (3) the complete absence of choline in rumen bacteria.

Functions

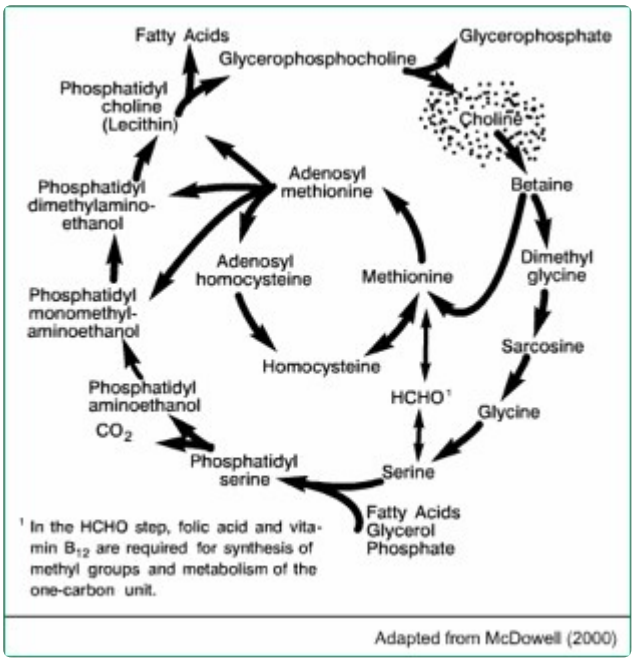
Choline functions in four broad categories in the animal body (Zeisel, 2006; Garrow, 2007):

(a) It is a metabolic essential for building and maintaining cell structure. As a phospholipid component, choline is a structural part of lecithin (phosphatidylcholine), of certain plasmologens and the sphingomyelins. Lecithin is a part of animal cell membranes and lipid transport moieties in cell plasma membranes. Choline is required as a constituent of the phospholipids needed for normal maturation of the cartilage matrix of the bone. Various metabolic functions and synthesis of choline are depicted in Figure 16-1.

Epidermal barrier function is a critical attribute of mammalian skin. The barrier is responsible for preventing skin-associated pathologies through controlling egress of water and preventing ingress of environmental agents. Barrier function was measured using transepidermal water loss (TEWL). It was found that a combination of pantothenic acid, choline, nicotinamide, histidine and inositol, when fed at supplemented concentrations, was able to significantly reduce TEWL in dogs after nine weeks (Watson et al. 2006).

Choline has been shown to influence brain structure and function. For rodents, choline was critical during fetal development, where it influences stem cell proliferation and apoptosis, thereby altering brain structure and function. Memory is permanently enhanced in rodents exposed to choline during the latter part of gestation (Zeisel and Niculescu, 2006).

Figure 16-1: Metabolic Pathway for the Synthesis of Choline and Related Compounds



(b) Choline plays an essential role in fat metabolism in the liver. It prevents abnormal accumulation of fat (fatty livers) by promoting its transport as lecithin or by increasing the utilization of fatty acids in the liver itself. Choline is thus referred to as a "lipotropic" factor due to its function of acting on fat metabolism by hastening removal or decreasing deposition of fat in liver. In broilers, liver fat content was reduced by adding choline at 760 mg per kg (345 mg per lb) of diet for birds fed different energy sources (Rao et al., 2001).

(c) Choline is essential for the formation of acetylcholine, a substance that makes possible the transmission of nerve impulses. Acetylcholine is the agent released at the termination of the parasympathetic nerves. As acetylcholine, there is transmission of nerve impulses from presynaptic to postsynaptic fibers of the sympathetic and parasympathetic nervous systems.

(d) Choline is a source of labile methyl groups. Choline furnishes labile methyl groups for formation of methionine from homocystine and of creatine from guanidoacetic acid. Methyl groups function in the synthesis of purine and pyrimidine, which are used in the production of DNA. Methionine is converted to S-adenosylmethionine in a reaction catalyzed by methionine adenosyl transferase. S-adenosylmethionine is the active methylating agent for many enzymatic methylations. A disturbance in folic acid or methionine metabolism results in changes in choline metabolism and vice versa (Zeisel, 2006). The involvement of folic acid, vitamin B12, and methionine in methyl group metabolism, and of methionine in de novo choline synthesis, may allow these substances to substitute in part for choline. A severe folic acid deficiency has been shown to cause secondary liver choline deficiency in rats (Kim et al., 1994).

The demand for choline as a methyl donor is probably the major factor that determines how rapidly a diet deficient in choline will induce pathology. The pathways of choline and 1-carbon metabolism intersect at the formation of methionine from homocysteine. Methionine is regenerated from homocysteine in a reaction catalyzed by betaine-homocysteine methyltransferase, in which betaine, a metabolite of choline, serves as the methyl donor (Finkelstein et al., 1982). Large increases in chick hepatic betaine-homocysteine methyltransferase can be produced under methionine-deficient conditions, especially in the presence of excess choline or betaine (Emmert et al., 1996). To be a source of methyl groups, choline must be converted to betaine. Betaine has been shown to perform methylation functions as well as choline in some cases. However, betaine fails to prevent fatty livers and hemorrhagic kidneys.

Since choline contains biologically active methyl groups, methionine can partly be spared by choline and homocysteine. Research with lactating dairy cattle suggests that a high proportion of dietary methionine is used for choline synthesis (Erdman and Sharma, 1991).

Requirements

Choline, unlike most vitamins, can be synthesized by most species, although in many cases, not in sufficient amounts or rapidly enough to satisfy all the animal's needs. Requirement recommendations assume that the diets of animals contain adequate methionine, folate, and vitamin B12. For laboratory animals, the primary basis for these recommendations is the level of choline required to prevent fatty liver, a phenomena that happens quickly (one to several days) after the consumption of a diet devoid of choline (Garrow, 2007).

When intake of precursors or accessory factors, such as methionine, vitamin B12, or folic acid is insufficient, dogs and cats have been shown to require dietary choline. Young animals also show a higher need for choline than adults. Dog and cat requirements for choline have been determined through the use of purified diets, and recommendations often do not take into account bioavailability from feedstuffs, individual animal variation or effects of other dietary factors.

Dietary factors such as methionine, betaine, myo-inositol, folic acid and vitamin B12 or the combination of different levels and composition of fat, carbohydrate and protein in the diet, as well as the age, sex, caloric intake and growth rate of animals, all have influence on the lipotropic action of choline and thus the requirement of this nutrient (Mookerjea, 1971; DuCoa L.P., 1994). Dietary betaine can spare choline, since choline functions as a methyl donor by forming betaine. In relation to protein level, a larger choline effect on litter size and piglet and litter weight was observed for gilts fed a 12% protein diet than for those fed a 16% protein diet (Maxwell et al., 1987).

Studies have shown that vitamin B12 and folic acid reduce the requirement for choline in chicks and rats (Welch and Couch, 1955). Folic acid and vitamin B12 are required for the synthesis of methyl groups and metabolism of the one-carbon unit. Biosynthesis of a labile methyl group from a formate carbon requires folic acid, while vitamin B12 plays a role in regulated transfer of the methyl group to tetrahydrofolic acid (THF). Therefore, marked increases in choline requirements have been observed under conditions of folic acid and/or vitamin B12 deficiencies.

The two principal methyl donors functioning in animal metabolism are choline and methionine, which contain "biologically labile methyl groups" that can be transferred within the body. This phenomenon is called

transmethylation. Therefore, dietary adequacy of both methionine and choline directly affects requirements of each other. Other than exogenous sources of methyl groups from choline and methionine, methyl group formation from de novo synthesis of formate carbons is reduced with folic acid and/or vitamin B12 deficiencies.

Most animals, including dogs and cats, can synthesize sufficient choline for their needs, provided enough methyl groups are supplied. As an example, methionine in the pig can completely replace that portion of the choline needed for transmethylation. Young poultry, on the other hand, are unable to benefit from methionine or betaine as a dietary replacement for choline unless methylaminoethanol or dimethylaminoethanol is in the diet, as young poultry appear unable to methylate aminoethanol when fed a purified diet (Jukes, 1947). Later studies showed that the chick can synthesize microsomal methylaminoethanol and choline from S-adenosylmethionine but, unlike the pig, at an insufficient rate in relation to needs (Norvell and Neshein, 1969). In growing chicks, 50% of the dietary choline requirement must be suppled as choline per se, but the remaining 50% could be replaced by betaine (Dilger et al., 2007).

The metabolic need for choline can be supplied in two ways: either by dietary choline or by choline synthesis in the body, which makes use of labile methyl groups. For selected species, body synthesis sometimes cannot take place fast enough to meet choline needs for rapid growth, and thus clinical signs of deficiency result. Since choline functions in prevention of fatty livers and hemorrhagic kidneys, it does not act as a true vitamin, since choline is incorporated into phospholipids (via cytidine diphosphocholine). Therefore, unlike a typical B-vitamin, the choline molecule becomes an integral part of the structural component of liver, kidney or cartilage cells (Scott et al., 1982).

In general for some species, males are more sensitive to choline deficiency than females (Wilson, 1978). Growth hormone seemed to increase the choline requirement in rats independent of its ability to promote growth and increase food intake (Hall and Bieri, 1953). Cortisone and hydrocortisone have been reported to decrease severity of renal necrosis, and hydrocortisone reduced the amount of liver lipid in choline-deficient rats (Olson, 1959).

Excess dietary protein may increase the young animal's choline requirement. Diets high in fat aggravate choline deficiency and thus increase the growing animal's requirement. Fatty liver is generally enhanced by fats containing a high proportion of long-chain saturated fatty acids. Choline deficiency develops to a greater degree in rapidly growing animals, with deficiency lesions more severe in these animals.

For dogs and cats, choline is only required in the diet when its synthesis from methionine is limiting. This is especially true when high-fat diets are fed, and extensive lipid transport is required (Ralston Purina, 1987).

A. Requirements in Dogs

Since choline was first described as a lipotropic agent in dogs, much of the data regarding choline requirements have been derived from dogs fed purified diets in experiments conducted in the 1940s. In puppies, high-protein diets (40% protein) did not require supplemental choline, whereas a diet with 19% casein induced a deficiency (Schaefer et al.,1941). Supplementation of choline at 50 mg per kg (22.7 mg per lb) of body weight prevented deficiency. With a 15% protein diet, 20 mg per kg (9.1 mg per lb) of body weight failed to prevent deficiency, whereas 100 mg per kg (45.5 mg per lb) did (Fouts, 1943). According to the current NRC recommendation (2006) the choline requirement for all classes of dogs is 1,700 mg per kg (773 mg per lb) of diet. This requirement is supported by the data of McKibbin et al. (1944), where weanling puppies were fed from 0 to 1,500 ppm of supplemental choline in purified diets. The Association of American Feed Control Officials (AAFCO, 1992) suggests a requirement of 1200 mg per kg (545.5 mg per lb) of diet for all classes of dogs.

B. Requirements in Cats

Growing kittens fed a purified diet with 42% casein and 24% hydrogenated coconut oil responded to a 0.1% choline supplement with reduced fatty infiltration of the liver and improved growth. However, a 0.5% supplement showed maximum reduction in liver lipid (Carvalho da Silva et al., 1959b). Anderson et al. (1979), also using purified diets, showed maximum growth at 0.1% supplemental choline and no additional growth response at

0.3% supplemental choline. Meanwhile, Schaeffer et al. (1982), using diets containing soy protein, showed maximum growth in kittens at 0.24% choline, where six graded levels of choline from 0.04% to 0.34% were fed. According to the current NRC (2006) the choline requirement for all classes of cats is 2,550 mg per kg (1,159 mg per lb) of diet. AAFCO (2007) recommends 2,400 mg per kg (1,091 mg per lb) of diet for all classes of cats.

Sources

All naturally occurring fats contain some choline, and thus, it is supplied by all feeds that contain fat. Egg yolk, glandular meats, and brain are the richest animal sources; germ of cereals, legumes and oilseed meals are the best plant sources (DuCoa L.P., 1994). Corn is low in choline, with wheat, barley and oats containing approximately twice as much choline as corn. Since betaine can spare the requirement for choline, it would be useful to know the concentrations of betaine in feeds. Unfortunately, most feedstuffs contain only small amounts of betaine. However, wheat and wheat by-products apparently contain over twice as much betaine as choline. Thus, the choline needs of swine or poultry fed wheat-based diets would be much lower than those fed diets based on other grains. Sugarbeets are also high in betaine.

Little is known of the biological availability of choline in natural feedstuffs. Using a chick assay method, soybean, canola, and peanut meals were found to contain a substantial proportion of unavailable choline (Emmert and Baker, 1997). Dehulled regular soybean meal and whole soybeans were tested and appeared to range in availability from 60% to 75% (Molitoris and Baker, 1976). However, soybean lecithin products are equivalent to choline chloride in bioavailability (Emmert et al., 1997). Canola meal, although three times as rich in total choline as soybean meal, has less bioavailable choline (Emmert and Baker, 1997). In their work with chicks, production of trimethylamine (resulting from bacterial degradation of choline) in the intestine was greater in chicks fed canola meal than in those fed soybean meal.

Commercially, choline is produced by chemical synthesis, and choline salts are used in dietary supplementation. Choline is available as chloride (86.8%) and bitartrate (48%) salts. Choline chloride is available for feed use as the 70% liquid or 50% to 60% dry dilutions. The 70% liquid is very corrosive and requires special storage and handling equipment. It is not suitable for inclusion in concentrated vitamin premixes but is most economical when added directly to concentrate feed mixtures.

Choline from dietary sources of supplemental sources, such as choline chloride would not be of value to ruminants since rumen microorganisms almost completely destroy dietary choline. Choline supplements are only of value if they are resistant to rumen degradation. Recently, a rumen-protected choline product has become available (Erdman and Sharma, 1991).

Deficiency

The most common signs of choline deficiency in a number of species include poor growth, fatty livers, perosis, hemorrhagic tissue (particularly in kidneys and certain joints), and hypertension. In general, severity of clinical signs in animal species is influenced by other dietary factors, including methionine, vitamin B12, folic acid and dietary fat. When feed intake and consequently growth are depressed by choline deficiency, severity of choline deficiency is then reduced. Research information is very limited on detection methods to determine choline status of animals. Often the best indicators of status or need for choline is observation of pathology attributable to choline deficiency (e.g., fatty livers) for particular species, as well as beneficial performance responses when diets are supplemented with the vitamin. Tissue levels of choline or its functional metabolites can be determined to evaluate choline status. There is evidence of a reduction of acetylcholine in brains, kidneys and intestines of rats deprived of choline six days after weaning. Choline administered to rats either by injection or by diet causes a dose-related increase in brain acetylcholine (Kuksis and Mookerjea, 1984). Studies on the mechanism of liver fat accumulation have suggested that this is related to a lack of lecithin synthesis. With a choline deficiency, the hepatic phosphatidylcholine:phosphatidylethanolamine ratio is reduced, and is thus a means of evaluating choline status.

For dogs, a liver function test as measured by delayed bromsulfalein elimination could be the basis of determining choline status (McKibbin et al., 1944; 1945). Plasma phosphatase activity and blood prothrombin times (impaired vitamin K function) were also elevated in the choline-deficient puppies (NRC, 1985). For cats, hypoalbuminemia (abnormally low level of albumin in the blood plasma) was reported by Mansur Guerios and Hoxter (1962), but was not found by Schaeffer et al. (1982).

A. Deficiency in Dogs

Dogs were important in the early history of determining the nutritional significance of choline. Following the discovery of insulin in 1922 by Banting and Best, it was observed that fatty degeneration of the liver associated with insulin deprivation in dogs could be corrected by feeding either raw pancreas or lecithin. In 1932 choline was discovered to be the active component of pure lecithin previously shown to prevent the fatty liver "lipotropic effect" in rats (Best et al., 1934). Choline deficiency in dogs is associated with a loss of body weight, vomiting, an increase in fat content of the liver, and death (NRC, 2006). Betaine, considered to be only a methyl donor, was found to have a similar lipotropic effect in dogs and rats. Dutra and McKibbin (1945) described the pathology of "uncomplicated" choline deficiency in young puppies. They reported decreased growth and degeneration and fatty infiltration of the liver, causing impaired liver function. There were atrophic changes of the thymus. Choline-deficient dogs with fatty livers show an increased rate of hepatic phospholipid synthesis following choline supplementation (NRC, 2006).

B. Deficiency in Cats

Kittens deficient in choline have decreased food intake and growth rate and increased lipid content of the liver (Carvalho da Silva et al., 1959b; Anderson et al., 1979; Schaeffer et al., 1982).

Fortification Considerations

Response to dietary supplementation of choline is dependent on age of animals, protein and sulfur amino acid intake, and levels of dietary choline and other choline-sparing nutrients. Unlike most vitamins, choline can be synthesized by dogs and cats when labile methyl compounds such as betaine and methionine are present in adequate amounts in the diet. Thus, protein-rich diets can actually reduce choline requirements. Generous use of animal by-products rich in methionine and choline for pet foods should preclude a choline deficiency. In view of the sparing effect of methionine and the widespread distribution of choline in plant and animal materials, it is most unlikely that a dog or cat will become choline deficient under normal circumstances. Methionine furnishes methyl groups for choline synthesis for most species, including dogs and cats. Choline, however, is effective only in sparing methionine, which otherwise would be used to make up for a choline shortage. Methionine is not used for choline synthesis if there is an adequate level of dietary choline. In formulating pet diets that are high in grains and low in animal products, methionine is frequently one of the most limiting amino acids. Therefore, it would be impractical for marginal quantities of methionine to be wasted for synthesis of the vitamin when supplemental choline can be provided more economically.

Choline has been shown to prevent organ injury and improves survival during endotoxemia. Choline or CDP-choline attenuates coagulation abnormalities and prevents the development of acute disseminated intravascular coagulation in dogs during endotoxemia (Yilmaz et al., 2010). Oral administration of a choline compound, referred to as silibinin-phosphatidycholine complex, has been shown to increase granulocyte glutathione content and phagocytic function, both of which would be potentially beneficial in cats with diseases associated with oxidative stress (Webb et al., 2009).

Choline chloride is stable in multivitamin premixes but is highly destructive to various other vitamins in the premix (Frye, 1978; Gadient, 1986). Choline is stable during processing and storage in pressure-pelleted and extruded feeds. Since the material is hygroscopic, containers supplying choline should be kept closed when not in use.

Vitamin Safety

Experimental animal toxicity data on clinical signs of choline overdosage include salivation, trembling, jerking, cyanosis, convulsions and respiratory paralysis. Estimates of the oral LD50 of choline chloride in rats varied from 3.4 to 6.7 g per kg (1.5 to 3.0 g per lb) of body weight (Chan, 1991). Insufficient data are available to support precise estimates of maximum tolerable dietary levels of choline for dogs, and there are no available data for cats. Studies with dogs suggest a low tolerance for choline chloride and lecithin in that species. Adverse effects have been reported for levels of choline chloride equivalent to three times the apparent choline requirement (NRC, 1987). Davis (1944a) showed that administering daily dietary equivalent of 150 mg of choline to dogs resulted in a maximum number of erythrocyte reductions that took place after 12 to 25 days. Davis (1944b) found that choline chloride induced a hyperchromic anemia in about 15 of 17 dogs. The anemia was produced by giving the dogs single doses of 10 mg per kg (4.5 mg per lb) per day of choline chloride by stomach tube.