BENEFICIAL IMPACTS OF CHOLINE IN ANIMAL AND HUMAN WITH SPECIAL REFERENCE TO ITS ROLE AGAINST FATTY LIVER SYNDROME

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ABSTRACT

Choline exists in feed ingredients and also synthesized in the body. It is essential for the physiological functions such as performance and lowering liver and body fat. If choline is insufficient in the diet, the liver fat content and abdominal fat content increase causing a metabolic disorder known as a fatty liver syndrome. Thus, dietary supplementation of choline as synthetic choline chloride or through natural herbs to the diets is compulsory. Besides fatty liver syndrome, choline deficiency causes loss of hepatocytes, heart diseases, bone and growth development abnormalities and impairment in kidney functions. The main indication of choline deficiency is raised up the level of liver enzymes like alanine transaminase (ALT). This ALT is usually measured clinically during diagnostic evaluation of hepatocellular injury to fix liver health. Lipotropic nutrients like choline could prevent fatty liver disorders through several mechanisms, such as increased hepatic very low-density lipoproteins (VLDL) secretion. Human and animal studies have reported protective impacts of choline for fatty liver disease (FLD) in addition to coronary heart disease (CVD) prevention from epidemiological data. Nowadays, choline supplementation is below the dietary recommendations because of a lack of understanding the importance of this vital nutrient for human and animal health. In the current review article, literature...
1 Introduction

Choline is a water-soluble micronutrient. Sometimes, it is classified among vitamins like vitamin B group and defined as a natural compound (Alagawany et al., 2016). But, it is not related to vitamins and considered as an essential nutrient due to esteemed much nutritional value. Currently, choline has got great importance in the last years because it can prevent liver fattening, support brain development and play a crucial role in the neural conduction. Choline has an essential role in the synthesis of betalipoproteins and phospholipids. It enables the transportation and burning of fats, which are also associated with its preventive effect against the fatty liver disorder. In some experimental animal models, choline deficiency caused liver fattening and loss of hepatocytes heart-related diseases, bone development abnormalities and impaired kidney functions (Biswa & Giri, 2015). In poultry industry, nutrition represents about 70% of total costs, thus constitutes are a key factor in poultry production. Choline is classified as an essential vitamin for day-old chicks; it is usually added to diets for the purpose of furnishing the body with labile methyl group for formation of creatine and methionine. In addition, it also assists in the prevention of hemorrhagic kidney in different animal models and perosis in turkeys and broilers (Ross et al., 2013). The deficiency of choline is normally noticed in chicks from 1–4 weeks of age. However, the rate of choline synthesis in chickens’ increases in growing chicks over 8 weeks of age (Ross et al., 2013). Corbin & Zeisel, 2012 theorized that laying hen seems to have substantial ability to synthesize choline. The latest authors found that the addition of choline to 100 mg/kg diet did not show any effect on the aspects of egg size, egg production and relative weights of albumin and yolk. Supplementation of choline at 0.4% to broiler diet improved growth traits (Ross et al., 2013). In another study by Emmert & Baker (1997) reported that the addition of choline chloride at the rate of 2000 mg/kg to the broiler diets showed a positive effect on body weight gain of chicks. Methionine is a sulfur amino acid and it is crucial for repair, growth, and metabolism of all tissues and also for reproduction (Ross et al., 2013). The level of methionine in the animal diet is important because of the relationship with the choline needy as a methyl donor and vice versa. Choline can be supplemented in addition to methionine, but will not spare the basic methionine requirements for protein synthesis without the diet contains homo cystine (Ross et al., 2013). Pesti et al. (1981) perceived that the addition of either choline or methionine to basal diet improves growth. Some trials like Huang et al. (2015) were conducted on rats and revealed that choline insufficiency could increase the risk of getting cancer. Many kinds of literature have naked associations between choline metabolism and cancer (Kirienko et al., 2015; Marina et al., 2016). The utilization of herb and lipotropic supplements to the feed decreases the adverse metabolic consequences of the high-calorie diet in poultry farming (Khosravinia et al., 2015; Saeed et al., 2017). Therefore, L-carnitine, vitamin B12, and vitamin E are routinely added to the poultry diet in order to reduce the liver fattening syndrome (Farrokhlyan et al., 2014). The study of Jiang et al. (2014) reported that choline chloride has a lowering effect regarding cholesterol of broiler. Nowadays, the fatty liver syndrome is a metabolic disorder and it is generally encountered in the poultry industry, especially in hens kept in cage farming system. Because birds kept in cages cannot move enough to burn the calories they were taking. During egg lying, the liver, which has already become rather brittle, can easily tear. If the tears occur on large blood vessels, the bird will be susceptible to sudden death as a result of this bleeding. The present review article aimed to give more light on the structure of choline, its sources, functions, and metabolism. In addition, the current article aimed to broaden the knowledge among researchers and poultry breeder about the use of choline on a commercial level to overcome the fatty liver syndrome disorder and its repercussions that are a big threat to the poultry industry.

2 Choline chemical structure

Choline is a quaternary ammonium (also known as trimethyl, β-hydroxy ethyl ammonium) compound found in lipids and its molecular weight is 121.18 (Sheard & Zeisel, 1989) as shown in Figure 1.

\[
\begin{align*}
\text{CH}_3 & \\
\mid & \\
\text{CH}_3 - \text{N}^+ - \text{CH}_2 - \text{CH}_2\text{OH} & \\
\mid & \\
\text{CH}_3 & 
\end{align*}
\]

**Figure 1:** Chemical structure of choline (Sheard & Zeisel, 1989)
3 Different sources and requirements of choline

In 1849, choline was firstly isolated from ox bile (Chole in Greek). Since 1930, the nutritional significance of choline has been recognized and nowadays it’s commonly dietary additive for humans and animals. Choline as chloride or sometimes as other salts like citrate is recognized as not harmful. The chief source of choline in poultry industry can be derived from the green leafy material. Moreover, the study by Song et al. (2012) reported that liver and glandular meal, fish meal and soybean meal are the richest sources of choline in poultry feedstuffs' industry. Vitamins are essential in poultry, animal and human nutrition. The requirement of these nutrients cannot be covered by the native content in feedstuffs, so extra supply is necessary to fulfill the nutrient requirements in the poultry diet (Song et al., 2012). The standard content of choline chloride in various feedstuffs based on chemical composition of crops according to NRC (1994) is given in Table 1. Normal requirement of choline in various poultry species are given in Table 2. Emmert & Baker (1997) assessed choline bioavailability which naturally presents in peanut, soybean meal and rapeseed at rates of 83, 24 and 76 %, respectively. Rape seed meal has high levels of choline (6198 ppm) than those in soybean meal (2218 ppm) and peanut meal (1685 ppm).

4 Functions and synthesis of choline

The functions of choline are categories in four broad terms in the animal body (Zeisel & Niculescu, 2006; Garrow, 2007).

I. Choline is important for metabolism in maintaining and building cells. In addition to, it is required for the maturation of bone cartilage matrix (Figure 2).

II. It plays a key role in the lipid metabolism in the liver. Also, choline suppresses the abnormal deposition of lipids by increasing the consumption of fatty acids or by activating its transport as lecithin in the liver (Xue & Cui, 2001). Choline is considered as a "lipotropic" agent due to its function of acting on lipid metabolism (Corbin & Zeisel, 2012). In the liver of broilers, adding choline (760 mg/ kg diet) reduced fat content (Rama Rao et al., 2001).

III. It is crucial for the formation of acetylcholine that plays an important role in transferring the nerve singles from presynaptic to postsynaptic fibers of sympathetic and parasympathetic nervous systems.

IV. It is considering the vital source of methyl group. It furnishes methyl group for the creation of methionine from homocysteine and of creatine from guanido acetic acid. Zeisel (1990) confirmed that a disturbance in metabolism of methionine results in alterations in metabolism of choline and vice versa. In rats, Kim et al.

### Table 1 Choline chloride content in various feed ingredients

| Feed ingredients                  | Choline in ppm NRC (1994) | Choline in ppm NRC (1997) | Choline in ppm IEEB (1997) |
|-----------------------------------|---------------------------|---------------------------|---------------------------|
| Corn                              | 620                       | 713                       | 200                       |
| Soybean meal (48%)                | 2731                      | 3140                      | 3560                      |
| Fat meat meal (55%)               | 2077                      | 2388                      | 1570                      |
| Corn gluten meal                  | 330                       | 379                       | 660                       |
| Wheat                             | 1002                      | 1152                      | 440                       |

*NRC values are present in choline hydroxide; they have been converted in to choline chloride (equivalent multiply by 1.15). *IEEB: (Institute European de Environment de Bordeaux, F.3300 Bordeaux): results are established on chemical analysis.

### Table 2 Choline requirements in various poultry species according to NRC (1994).

| Avian Species   | Feed                  | Requirement       |
|-----------------|-----------------------|-------------------|
| Broiler Chickens| Cereal grain basal diet| 1300 mg/kg of diet|
| 0-3 weeks       |                       |                   |
| 3-6 weeks       |                       |                   |
| 6-8 weeks       |                       |                   |
| Laying Hen      | Cereal grain basal diet| 105 mg/hen/day   |
| 0-6 weeks       |                       |                   |
| 6-12 weeks      |                       |                   |
| 12-18 weeks     |                       |                   |
| Production stage| Cereal grain basal diet| 105 mg/hen/day   |
| Turkey          | Cereal grain basal diet| 1600 mg/kg of diet|
| 0-4 weeks       |                       |                   |
| 4-8 weeks       |                       |                   |
| 8-16 weeks      |                       |                   |
| 16-20 weeks     |                       |                   |
| 20-24 weeks     |                       |                   |
| Breeding cycle  | Cereal grain basal diet| 800-1000 mg/kg of diet|
Importance of choline in animal and human (1994) observed that the deficiency in folic acid may be caused secondary deficiency of choline in the liver.

V. Increasing the gain of interest for choline and its important role as a methyl donor is possibly the key factor that evaluates how rapidly a diet deficient in choline will bring pathology (Finkelstein et al., 1982). The liver can produce large amounts of betaine-homocysteine methyltransferase under the conditions of methionine-deficient, exclusively in the presence of excess betaine or choline (Emmert & Baker, 1997).

VI. Choline may reduce the risk of hepatic and cardiovascular diseases by being available in local markets as choline-enriched eggs that are favorable needs among consumers (Krishnan, 2010).

5 Choline metabolism pathway

Choline is a key source of methyl group through its metabolite, which takes part in the synthesis of S-adenosyl methionine. Moreover, choline and its metabolites have many biological and physiological functions such as structural integrity, acetylcholine synthesis and signaling roles for cell membranes (Cuccurullo et al., 2017). Choline is a vital nutrient that is essential for neurotransmitter (acetylcholine) synthesis, cell membrane structure, methyl-group metabolism and signaling, and lipid transport. Phosphatidylcholine is synthesized in nucleated cells by the pathway of CDP-choline; this way used choline as the preliminary substrate, and therefore it depends on dietary level of choline. The liver is a unique organ that possesses a second pathway for phosphatidylcholine synthesis; phosphatidylethanolamine N-methyltransferase (PEMT) converts phosphatidylethanolamine (PE) to phosphatidylcholine via 3 sequential methylations using S-adenosylmethionine as the methyl donor (Gibellini & Smith 2010). Figure 3 summarizes the metabolism of choline.

6 Choline digestion

Choline is generally absorbed in the jejunum and ileum parts of the intestine by sodium and an energy-dependent carrier pathway (Veth et al., 2016). After absorption, choline is transferred to the lymphatic circulation basically in lecithin form that linked to chylomicron in the phospholipids tissues (Veth et al., 2016).

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Figure 2 Synthesis and various metabolic functions of choline and related compounds.
7 Incredible impacts of choline

7.1 Choline as Antioxidant

Choline plays an important role in multiple clinical manifestations. The function of choline as methyl is a key importance in maintaining balanced cellular antioxidant defense systems subsequently checking oxidative stress and apoptosis (Table 3). Corbin & Zeisel (2012) illustrated the connection between choline deficiency and development of non-alcoholic fatty liver disease (NAFLD) which may finally progress to hepatocarcinogenesis. The previous study in human as well as in mice confirmed that a deletion of choline-related genes may alter mitochondrial membrane composition owing to choline deficiency. Levels of gut microbiome moderating the availability of choline might enhance the fatty liver disease. These findings established a new understanding that choline is a vital component of diet requirement and gave new insight ways in which many physiological conditions take place (Corbin & Zeisel, 2012).

7.2 Choline as Growth Enhancer

Choline is an essential vitamin for the prevention of perosis as well as for growth performance of poultry species. The choline requirement as demonstrated by growth that was better gained when the diet containing 3467 kcal ME/Kg diet, while the requirement for safeguard against perosis was greater to be about 1900 ppm (Fritz et al., 1967). Nesheim et al. (1971) showed that choline supplementation reduced hepatic fat content compared to control; however no improvements in weight gain for pullets fed corn-soy diet supplemented with choline during 8-20 weeks of age. Agricultural Research Council, 1975 showed that the growing poultry chicks have a requirement for choline of around 1300 mg/kg diet (ARC, 1975).

Lipstein et al. (1977) reported that the chicks that were fed choline up to 520 and 480 mg/kg in basal diets shown good responses as compared to that contained choline at the rate of 400 and 230 mg/kg diet. Pesti et al. (1979; 1980) reported large increases at the rate of 12% in body weight gain with supplementation of choline at 0.04 to 0.39% into the practical type diets for chicks and poults from day-old chicks up to 3 weeks of age.

Derilo & Balnave (1980) showed that growth of broiler was reduced with the low dietary level of choline. However, these effects were highlighted by very low nutritional total sulphur amino acid (TSAA). Increased in the number of mortality and many other pathological changes that are involving in various number of tissues that were observed in birds fed on a low choline diet on the other hand, the same later authors reported that the
Table 3 Antioxidant role of choline

| Test system                        | Observed effects                                                                                                                                                                                                 | References       |
|------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------|
| Male Fisher 344 rats               | Chronic methyl group deficiency due to low levels of dietary choline leads to an imbalance in cellular antioxidant defence systems, increased oxidative stress, and apoptosis.                                   | Ossani et al., 2007; Bagnyukova et al., 2008 |
| Weanling Wistar male rats          | Choline deficiency produces oxidative damage in the liver, heart, kidney, and brain, with an increased lipid peroxidation of subcellular organelles and a decrease in tissue antioxidants.              | Repetto et al., 2010 |
| Weanling male Wistar rats          | Decreased antioxidant content and increased lipid peroxidation are earlier biochemical alterations that precede and lead to histological cell death by necrosis in choline deficiency.          | Schugar et al., 2013 |
| Rodent model (C57BL/6J wild type mice) | Adverse effects of choline deficiency on hepatic mitochondrial structure and function could be linked to the unique signature of hepatic lipid accumulation, inflammation, and cellular and mitochondrial injury induced in mice maintained on a very high fat, protein-restricted, very low carbohydrate and ketogenic diet. | Schugar et al., 2013 |

high dietary concentration of choline (1750 mg/kg feed) boost the requirement for dietary TSAA.

Neither choline nor cystine significantly affected the requirement of methionine or SAA, as estimated by body weight gain (Blachman & Waldroup, 1980). Derilo & Balnave (1980) obtained that when different dietary combinations of choline and TSAA were used with broiler chicks fed purified diet, maximum growth was obtained with a combination of high choline (>1750 mg/kg) and high TSAA (8.49/ kg diet). Anderson & Dobson (1982) showed that increasing the amount of choline supplementation in the avian diet range from 300 to 800 mg/kg feed did not increase the performance or reduce the value of the supplementary methionine. On the other hand, Miles et al. (1983) reported better growths of chicks occurred from the concurrent addition of 0.1% potassium sulfate and 0.066% choline but larger increases were attained from the addition of 0.25% methionine, representing that both choline and sulfate may be involved in sparing methionine in turkeys birds.

The choline requirements of broiler chickens were 1300, 850 and 500 ppm for the starter, grower and finisher periods, respectively (NRC, 1994). Parsons & Leeper, (1984) stated that supplementation of choline or methionine improved the productive performance of laying hens. Yeo et al., (1985) suggested that chicks were given for 4 weeks a diet adequate in all nutrients except sulfur amino acid and with methionine 0.15 and 0.30% and choline 0.05 and 0.10%. They reported that supplementary choline did not increase weight gain except for chicks not given methionine.

Blair et al. (1986) reported that improvement in body weights occurred by fed the chicks with dietary choline at an early stage of age, particularly in the deficiency of supplemental methionine; also, they added that an increase was more difficult to demonstrate at other age. Tillman & Pesti (1986) found that chicks offered a feed that supplemented with L-methionine at 12% gained significantly more than those fed the basal diet, while, poultry chicks that supplemented with choline diets had gained as well as those fed L-methionine.

Andriguetto et al. (1987) studied the effect of diets based on maize meal and soybean oil meal without or with choline supplements at the rate of 200, 400, 600, 800, 1000, 1200, or 1400 mg/kg feed from hatching until 42 days old in male and female chicks. The diets were supplemented with pyridoxine 3.00; folic acid 1.00 and cyanocobalamin 0.030 mg/kg diet. The authors found that weight gain of male and female was not significantly different among groups.

Okolelova et al. (1988) studied the influence of feeding on a basal diet plus choline chloride as liquid, or as 9% mixture with lignin or microcrystalline cellulose, or as a 43% mixture with maize cobs and found that average body weight gain at 49 days old was 1641.4, 1592.0, 1749.6 and 1677.1g, respectively. Krsmanovic et al. (1990) studied the addition of choline without or with 25 or 50 g/kg on broiler diets and found average total body weight gain was 1862, 1867 and 1884g, respectively. Baranova, (1991) pointed out that supplementation of choline chloride in broiler diets increased body weight gain. Sonbol & Habeeb (1991) showed that broiler chicks fed on basal grower diet up to 4 weeks of age (22.14% CP and 2810 kcal ME/Kg) and the basal finisher diet up to 7 weeks of age (18.98%CP and 2912 Kcal ME/Kg) were supplemented with 0.15% methionine +1000 mg/kg choline.
The addition of methionine and choline showed the highest significant live weight (1707g).

Vogt (1992) reported that growth of broiler chicks of 6-weeks old was improved by supplemented choline at levels of 200, 400, 600 and 800 mg/kg diet with corresponding addition DL-methionine at levels of 750, 1500, 2250 and 3000 mg/kg diet, respectively. Mohamed et al., (1994) indicated that addition of choline to corn-soybean meal diets for chicks had a significantly effect on body weight gain. Ryu et al. (1995) showed that broiler chicks were fed on a basal diet supplemented with choline 0, 500 and 1000 mg/kg diet. Body weight was significantly higher when supplemented choline was fed. In two experiments carried out to determine the effect of dietary choline on the performance of broiler chicks. Men-Kin et al. (1996) found that broiler chicks fed on a basal diet containing 23.5-24% CP less methionine and supplemented with choline at levels of 250, 500 or 750 g/ton in experiment 1 or at levels of 1000, 1250 or 1500 g/ton in experiment 2. Jokic et al. (2000) showed that the addition of choline to 950 and 850 mg/kg diet during the starting and finishing period, respectively with 0.20% and 0.15% methionine and 0.10 magnesium sulfate resulted in significantly (P<0.05) increase in body weight gain (from 6.42 to 7.41%) and body mass of chicks from 6.31 to 7.25%. Shrivastav et al. (2004) reported that choline is essential for growth and helpful to prevent leg disorder (perosis) in turkeys.

Simon et al. (1995) demonstrated that extra methionine supplementation above the recommended dose required for broilers chickens to improved their performance including body weight gain and food conversion efficiency, so choline may spare the methionine for broiler's growth (Pesti et al.,1981). Feeding a diet with choline supplementation improved body weight gain and feed efficiency in broilers either alone or in combination with methionine (Combs,1992). The study of Rama Rao et al. (2001) found that choline and methionine should be supplemented to broiler diets at higher levels to gain better results in aspects of health and production. Furthermore, Combs, (1992) postulated that choline is an absolute dietary requirement for broilers, particularly at younger ages as the chick cannot synthesize satisfactory amounts until up to 13 weeks of age. Body weight gain was improved (P<0.01) with increasing choline percent in the diet up to 2000 mg/kg diet, but increasing choline level-up to 2500 mg/kg diet caused a significant decrease in body weight (Slawinska et al., 2014).

7.3 Choline as Immune Booster

Choline is being considered as a member of the B-complex vitamin group and as an essential nutrient for laying hens, broilers and also for other poultry diets for the formation of the phospholipid lecithin found in egg yolk and liver (Maiorano et al., 2012). In animal nutrition, essential nutrients playing an important role on growth performance, meat quality and carcass traits (Kadam et al., 2013) and immune system development (Hhm et al., 2012). Choline showed a positive effect on immune functions by improvements in primary antibody titer of broiler chicks (Maiorano et al., 2012).

7.4 Choline as a Potent Lipotropic Agent

In poultry feed industry, maize is considered the main energy source. Due to non-availability of maize in many developing countries, so nutritionists are always searching for alternative energy sources for animal and poultry feeds. The replacement of maize with pearl millet or broken rice in the diets of broiler breeders and layer caused an accumulation of fat in the liver and abdomen. As shown in Figure 4, choline -as a lipotropic factor- has been established to fix this problem by the donation of methyl in chicken metabolism (Zeisel et al., 2003). Similarly, Rama Rao et al., (2001) reported that birds fed a diet containing choline at the level of 760 mg/kg diet significantly reduced the liver fat. So, choline must be a part of the human and animal diets (Sheard & Zeisel 1989; Gholami et al., 2015). Therefore, it is undertaken that the supplementation of choline in poultry feed can alter the deposition of fat and also the hatching and laying performance of broiler breeders that fed on different sources of energy.

![Figure 4 Effect of choline against fatty liver syndrome](http://www.jebas.org)
Importance of choline in animal and human

removing deposition of fat in the liver or ganad consequently prevents fatty liver disorder. So, these vital properties highly recommend the use of choline as commercially feed additive to cope with the metabolic disorders and enhance bird’s health and productivity.

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Conflict of interest

Authors have no conflict of interest.

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