L-Carnitine - a feed additive in poultry

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Introduction

Vitamins are defined as a group of complex organic compounds present in small amounts in natural foodstuffs that are essential to normal metabolism and lack of vitamins in the diet causes deficiency disease. Because of the possibility of specific biosynthesis pathways in some species, some “vitamins”, such as carnitine, could be considered only as essential metabolites in these species and dietary sources are not needed.

In 1905, L-carnitine was discovered in great quantities in muscle tissue by Gulewitsch and Krimberg. The amino acid derivative L-carnitine has gained interest in recent years as a potential feed additive for improving domestic animal production because of its metabolic functions. In this context, experiments have been carried out with various domestic birds including broilers, hens, quails, ducks and geese in order to study the effects of carnitine on energy metabolism and on improvement of growth performance.

Chemical structure

Carnitine or β-hydroxy-y-trimethylaminobutyrate is a quaternary amine. It is a very hygroscopic compound easily hydro soluble and has a molecular weight of 161.2 D. Methods of analysis first utilised the bioassay technique using Tenebrio molitor. Other methods developed for carnitine determination include chemical, enzymatic, gas chromatography and radio isotopic procedures.

\[
\begin{align*}
\text{OH} \\
(\text{CH}_3)_3\text{N-CH}_2-\text{CH-CH}_2-\text{COOH}
\end{align*}
\]

Chemical structure of carnitine

Biosynthesis of L-carnitine

Carnitine was synthesized in vivo from lysine and methionine, in the kidney (feline, man), testes (rat), skeletal muscle (sheep), brain (man) and liver in all mammals. During the synthesis, L-lysine provides the
carbon chain and nitrogen atom of carnitine, and L-methionine provides the methyl groups. The conversion of TrimethylLysine to carnitine requires 2 hydroxylations catalysed by 2 specific monooxygenases that use α-ketoglutarate as an electron donor to activate dioxygen. The α-amino acids is cleared into CO2 and succinate. The both enzymes require Fe (II) and are activated by ascorbate.

Endogenous biosynthesis may be sufficient to cover normal requirements in all mammals and bird species, when precursors and cofactors of the L-carnitine is sufficient in the diet. However, this is not the case in neonates (in which, the biosynthesis is not fully developed), under the stress, in animals with the higher performance and when diets are rich in fat.

Carnitine biosynthesis is regulated by the diet, age, and hormonal status of the animal. L-carnitine concentrations vary according to species, tissue types and nutritional status. Dietary carnitine appears to cross rapidly the mucosal intestinal membrane by both passive and active transport mechanisms. Then carnitine is extracted from the portal circulation by the liver and subsequently released into the systemic circulation. Carnitine is not carried in blood in any tightly bound forms, in contrast to other water-soluble vitamins. Free carnitine is excreted in urine, and the principal excretory product is trimethylamine oxide.

**Endogenous synthesis pathway of L-carnitine in mammals**

**Biological functions of L-carnitine:**

Carnitine is required for the transport of long-chain fatty acids from the cytoplasm to the matrix compartment of mitochondria. The Carnitine acyltransferase is the enzyme responsible for this shuttle mechanism and it exists in two forms, the carnitine acyltransferase I (CAT I) and the carnitine acyltransferase II (CAT II). After activation of the long chain fatty acids into acylCoA in cytosol, acyl groups are transferred from coenzyme A to carnitine to form acylcarnitine by the CAT I on the outer surface of the mitochondrial membrane. The carnitine esters move to the inner surface by exchange with free carnitine using an antiport mechanism. The CAT II, located on the inner surface, catalyses the reverse reaction by transferring acyl groups from carnitine to coenzyme A within the mitochondrion, leading to mitochondrial acylCoA and the release of carnitine. Mitochondrial oxidation rate is mainly determined by the capacity of the carnitine transport system. As a consequence, Carnitine is crucial to the shuttle of long-chain fatty acids across the inner mitochondrial membrane and controls the rates of β-oxidation of long-chain fatty acids, playing a pivotal role in energy metabolism. Alterations in carnitine metabolism or concentrations may substantially affect energy production in mitochondria.
Other functions of Carnitine are 1) the buffering and removing of the potentially toxic acyl groups from cells, 2) to regulate the ratios free CoA / acylCoA in 2 separate cellular compartments (cytosol and mitochondria), 3) to regulate gluconeogenesis, fatty acid synthesis and ketone bodies, branched chain amino acid, triglyceride and cholesterol metabolism [50]. In addition, it can exhibit immunomodulatory effects, while growth performance is not improved. The mechanism(s) accounting for the positive effect of L-carnitine on antibody production is not yet fully understood.

Restoration of the cellular L-carnitine content may enhance the lipid metabolism and improve the cellular energy balance. Furthermore, L-carnitine (esters) are known to have free radical scavenging properties. This hypothesis is currently under investigation. L-carnitine is been reported as a hypolipidemic drug, able to reduce the circulating concentrations of cholesterol, triglycerides, free fatty acids, phospholipids, and very low density lipoproteins (VLDL) and to increase the concentrations of high, intermediate, and low density lipoproteins (HDL, IDL, LDL, respectively) in murine. The plasma lipid-lowering effects of L-carnitine would be associated with
several possible processes, including increase of cholesterol turnover due to increased conversion of cholesterol to bile acids and biliary excretion or due to a modified repartition of whole body cholesterol. However, very little is known on the molecular basis of such processes.

**Sources of Carnitine:**

Carnitine occurs naturally in microorganisms, plants and animals. The D-form does not occur in nature but may be obtained by chemical synthesis. The D isomer of carnitine is biologically inactive and it also hinders activities of L-carnitine. At high dosages, D carnitine shows detrimental effects. The major dietary sources are red meat, poultry, fish and dairy products. Animal meals contain 10 to 20 times more L-carnitine than plant-based feedstuffs. However, little L-carnitine is found in cereal grains and their by-products.

**L-Carnitine: Typical contents in feedstuffs (mg/kg)**

<table>
<thead>
<tr>
<th>Feedstuff plant origin</th>
<th></th>
<th>Feedstuff animal origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>5</td>
<td>Animal meal</td>
</tr>
<tr>
<td>Barley</td>
<td>10</td>
<td>Fish meal</td>
</tr>
<tr>
<td>Wheat</td>
<td>5</td>
<td>Feather meal</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>15</td>
<td>Meat bone meal</td>
</tr>
<tr>
<td>Oats</td>
<td>5</td>
<td>Plasmaprotein</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>20</td>
<td>Blood meal</td>
</tr>
<tr>
<td>Rapeseed</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Sunflowers</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Cottonseed</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

In normal physiological and nutritive conditions, L-carnitine requirements are, however, largely covered by dietary sources. Dietary L-carnitine supplementation could improve fatty acid and energy utilisation and therefore gain and feed efficiency, especially in young animals where synthesis is insufficient to cover endogenous requirement. Feeding studies have shown that the performance of animals sharply drops when the L-carnitine content falls below 15-20 mg per kg of feed. Because of the cereal grains usually represent the major component of poultry diets, it may be useful to incorporate this compound into diets. To ensure that poultry receive adequate amounts of L-carnitine, feed recipes should be contained the dosages reported below:
L-carnitine oral supplementation can be realised either in the drinking water or directly in the diet. Since this compound is easily dissolved in water, the drinking water is often preferred to achieve a homogeneous mixture.

**L-carnitine usage in poultry feeding:**

**Dietary supplementation with L-carnitine precursors and cofactors:**

Although plant products are low in carnitine, poultry diets are composed mainly of maize and soybean.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Poultry species</th>
<th>Dose of L-Carnitine, mg/kg diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Laying hens</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>Breeder hens</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>Broiler</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>Turkeys</td>
<td>60</td>
</tr>
<tr>
<td>5</td>
<td>Pigeons</td>
<td>25</td>
</tr>
<tr>
<td>6</td>
<td>Breeding Pigeons</td>
<td>80</td>
</tr>
</tbody>
</table>

Nevertheless, it is generally accepted that endogenous L-carnitine synthesis together with its dietary intake should be sufficient for normal functions. Since carnitine can be biosynthetised endogenously from methionine and lysine, these two amino acids are usually the more important limiting amino acids in poultry nutrition. Methionine and lysine are frequently supplemented in the formulated diets. When diets are not supplemented with these two amino acids, the chicken may synthesize an inadequate amount of carnitine. In a study conducted on fish, piglets and quail, Concluded that carnitine seemed effective for improving body weight gain and feed conversion, mainly in groups with diets marginally deficient in lysine and methionine plus cystine respectively.

The influence of low and high dietary contents of lysine and methionine requirements (20% below or above optimum requirements, respectively) on body weight gain, abdominal fat and carnitine content in some tissues was examined by IBEN et al in broiler chickens. The highest body weight was achieved in groups with optimum dietary contents of the 2 amino acids. In groups with low lysine and methionine supplementation, the abdominal fat content has higher than in the other groups. In another study reported that carnitine concentrations in the liver, kidney, heart and some skeletal muscles significantly increased in response to supplemental dietary L-carnitine.
Effects of dietary L-carnitine supplementation on fat metabolism:

Body fat accumulation results from dietary absorbed fat, endogenous fat synthesis (lipogenesis) and fat catabolism via β-oxidation (lipolysis). Production of broiler chickens with excessive body fat is a problem in the poultry industry. Several factors, such as nutrients and genetics, contribute to the tendency of broilers to accumulate body fat in excess. Therefore, improving carcass composition with additives has become a focus on nutrition research. For example, the dietary fat type affects the metabolism and deposition in broiler chickens. Birds were fed with diets containing either dietary saturated (beef tallow) or polyunsaturated fat (sunflower oil) for 32d. The abdominal fat deposition was significantly lowered in chickens receiving the sunflower oil enriched diet compared the birds fed with the tallow-enriched diet. Furthermore, the specific activities of heart carnitine palmitoyltransferase I and L-3-hydroxyacylCoA dehydrogenase were increased in chicks fed with the polyunsaturated acid enriched diets, indicating a greater rate of β-oxidation, whereas liver fatty acid synthetase activity was depressed, and suggesting hepatic lipogenesis reduction. Postprandial plasma triglyceride concentrations were reduced too, showing that the dietary lipid clearance from the bloodstream to tissues was amplified. In conclusion, Sanz et al. suggested that the lower fat deposition in broilers fed with sunflower oil-enriched diets resulted from the increase of lipid catabolism and the decrease of fatty acid synthesis despite higher dietary fat absorption.

In case of increased metabolic demand the dietary low carnitine supply would became insufficient for metabolic requirements, and animals would became less productive and fatter. Theoretically, supplementing the broiler diet with an adequate content of carnitine would facilitate the fatty acids oxidation, and decrease esterification reactions and triacylglycerol storage in the adipose tissue. Reports on the effects of dietary L-carnitine supplementation on the growth performance and body composition of broiler chickens are conflicting. Some studies have shown that supplemental L-carnitine improved body weight gain and reduced the abdominal fat content of broilers. In the same way, Rabie and Szilagyi reported that L-carnitine supplementation (50 mg / kg) of broiler diets increased breast muscle yield, thigh meat yield and fat content of breast muscle, whereas quantity and percentage of abdominal fat was reduced. Therefore, lower body fat deposition may be attributed to increased fat catabolism or diminished endogenous fatty acid synthesis or both processes. In another study SAYED et al. have demonstrated that addition of L-carnitine (50 mg/kg) to diet containing 2 and 4 % of sunflower oil increased feed intake, weight gain and feed conversion and decreased serum cholesterol and lipid concentrations compared to the control group. Moreover, the association of carnitine and 2% dietary fat has significantly decreased abdominal fat deposits. Carnitine could also increase fatty acid oxidation rates in animals under thermal stress. In
a more recent study on broiler chickens reared under normal temperature or submitted to thermal stress (rapid decrease of temperature from 28°C to 20°C when birds were 14 day old), the L-carnitine supplementation (100 mg/kg) induced marked increases of heart weights, plasma triiodothyronine concentrations and transient elevation of growth hormone, glucose and triglyceride concentrations, particularly in the stressed birds.

The positive effects of L-carnitine supplementation were also evidenced when birds were orally exposed to aflatoxin. Indeed, the percentage of abdominal fat and the plasma lipid concentrations were significantly reduced by L-carnitine (50 mg/kg), particularly in birds exposed to the higher aflatoxin dose (5 mg/kg).

**Effect of dietary supplementation on protein utilisation:**

Rodehutscord *et al.* investigated the effects of L-carnitine supplementation (80 mg/kg diet) in diets containing 4 or 8 % of fat and differently distributed (*ad libitum* in a growth trial, 95 and 85 % of *ad libitum* in a balance trial). The amino acid concentrations of diets were adequate growth and feed conversion tended to increase by about 5 % in the supplemented diets distributed *ad libitum*, and feed conversion was simultaneously improved by carnitine and dietary fat content. However, L-carnitine supplementation has not positively affected the yield of energy utilisation, neither the N accretion nor the dietary protein utilisation efficiency. These authors concluded that endogenous carnitine synthesis is not the limiting factor for energy utilisation in broiler chicken, even at high dietary fat concentration and L-carnitine must not be expected to increase protein utilisation under condition of adequate amino acid contents.

**Effects of dietary L-carnitine supplementation on egg production and egg quality:**

There are several factors influencing the quality and quantity of eggs. Among these, the most important factors are nutrition, disease, age, breed or strain of bird and environment. Many important egg-quality components were deteriorated and become more variable with the age of the bird. Nutritional factors can modify egg quality by inducing metabolic changes. In fact, reports on the effects of dietary L-carnitine on egg production and egg quality of laying hens are limited. Leibetseder has reported that egg hatchability increased from 83 % to 87 % and from 82.4 % to 85.3 % in broiler breeders supplemented by L-carnitine at 50 and 100 mg/kg diet respectively. In another study for the determination of the effects of L-carnitine supplementation (50, 100 or 500 mg/kg diet) on 65-week-old hens kept in cages. Dietary L-carnitine supplementation did not affect the laying performance (egg production rate, mean egg weight, daily feed intake, egg mass and feed conversion) or external egg quality but modified the egg composition. Albumen quality (relative albumen weight) was improved, in supplemented hens, probably because of a higher metabolic rate.
in the magnum and or a higher activity of the shell gland. The increase of albumen could be beneficial for the nutritional point of view and also for improving storage time. Yolk index and yolk colour score did not significantly vary, whereas absolute or relative yolk weights were significantly lowered. L-carnitine has probably induced reduction of hepatic biosynthesis of yolk precursors, or an alteration of their transport from the liver to the ovarian follicle and the oocyte.

The egg production tended to increase, but not significantly, in 44 to 72 weeks old hens dietary supplemented by high L carnitine doses (50 and 100 mg/kg). Moreover, a positive correlation between supplemental L-carnitine amounts in diets and the carnitine concentrations in egg albumen and yolk. In Japanese quails, supplementation with Lcarnitine (500 mg/kg diet) alone or combined with vitamin C (500 mg/kg diet) in diet did not improve growth and carcass yield. However, egg production was significantly enhanced by L carnitine. The optimum performance of birds is obtained in thermoneutral zone, and high and low environmental temperatures depress bird productivity and also affect the quality of products in laying hens exposed to high ambient temperature (35-37 °C 8h and 20-22 °C 16h). The relative albumen weight and height were significantly increased by supplementary L-carnitine (50 mg /kg) in drinking water. However, L carnitine did not affect the growth performance (body weight gain, feed consumption), the laying performance (egg mass and egg weight) the shell quality (weight, thickness, and egg shape index) and the yolk quality (weight and colour score).

**Summary**

Contradictory results were reported from studies on the L-carnitine usage in poultry feeding. Differences in doses of L-carnitine or of its precursors used in the diet, the duration of the supplementation period, supplies of metabolisable energy, fat and glucide cereals of the diet, the sex, the genotype and physiological status of the animals, the breeding and environmental conditions may be responsible for these discrepancies. Exogenous L-carnitine supplementation could be useful in case of metabolic burdens (such as exercise, heat or cold exposure) or when energy demands are elevated (growth in young animals, high zootechnical performance or fat-enriched diet). Moreover, the limited intestinal absorption capacity and its considerable microbial degradation would lead to increase dietary L-carnitine dosages in future investigations.