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# PROTECTIVE EFFECT OF L-CARNITINE AGAINST OXIDATIVE DAMAGE CAUSED BY EXPERIMENTAL CHRONIC AFLATOXICOSIS IN QUAIL *(COTURNIX COTURNIX)*

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This study was designed to evaluate the effect of L-carnitine supplementation on the plasma malondialdehyde (MDA) and whole blood reduced glutathione (GSH) concentrations in experimentally-induced chronic aflatoxicosis in quails. For this purpose, a total of 80 quails up to 8 weeks old were divided into four equal groups. Group I served as control, Group II was given L-carnitine at the dose of 200 mg/litre in the drinking water for 60 days, Group III was given 60 µg total aflatoxin/kg diet for 60 days, and Group IV was given both 60 µg total aflatoxin/kg diet and 200 mg L-carnitine/litre in the drinking water for 60 days. Aflatoxin treatment caused a significant increase in plasma MDA and a significant decrease in blood GSH concentrations. On the other hand, there was a significant decrease in plasma MDA and a significant increase in whole blood GSH in the L-carnitine-supplemented group. The present study demonstrated that L-carnitine brought about the inhibition of lipid peroxidation by enhancing antioxidant capacity in quails with chronic aflatoxicosis.

**Key words:** L-carnitine, malondialdehyde, reduced glutathione, aflatoxicosis, quail

L-carnitine, synthesised from lysine and methionine, plays a crucial role in fat metabolism by transporting long-chain fatty acids for production of energy via β-oxidation and oxidative phosphorylation. It also facilitates the removal from mitochondria of short- and medium-chain fatty acids accumulated as a result of fat metabolism (Rebouche, 1992). It has been documented that L-carnitine also plays a role in the protection of cell membranes by detoxification of acetyl groups (bonds) and free CoA (Fritz and Arrigoni-Martelli, 1993).

Aflatoxin (AF) is known to cause cell membrane damage through increased lipid peroxidation in laboratory animals (Souza et al., 1999; Rastogi et al., 2001). Protective effects of antioxidant substances such as selenium, some

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vitamins (A, C and E) and their precursors acting as superoxide anion scavenger against mycotoxin-induced damage have well been documented (Atroshi et al., 1999, Galvano et al., 2001).

Lipid peroxidation is an indicator of cell damage caused by toxic effects (i.e. mycotoxins such as aflatoxin), ageing and stress. The induction of lipid peroxidation gave rise to an increase in malondialdehyde (MDA) content. This procedure activates cell-protective antioxidant defence mechanisms. Moreover, it is also well known that there is a negative correlation between lipid peroxidation and reduced glutathione (GSH). Furthermore, GSH also protects the red blood cells against such damage. The measurement of whole blood GSH and plasma MDA concentrations can therefore be used as indicators of oxidative stress (Kalaiselvi and Panneerselvam, 1998).

Although L-carnitine is a well-known antioxidant, there is scarcity of information about its protective effects on lipid peroxidation and the antioxidant system during aflatoxicosis in poultry. Thus, a model experiment was developed to emphasise the protective effect of L-carnitine on lipid-peroxidation-causing effects of chronic aflatoxicosis in quails.

## Materials and methods

In this study, 80 quails up to 8 weeks old were divided into four equal groups. Group I served as control. Animals in Group II received only L-carnitine (Carbitol, Firma DIF, Istanbul, Turkey) at the dose of 200 mg/L in the drinking water; animals in Group III were given only aflatoxin at the dose of 60 µg/kg in feed, and animals in Group IV received aflatoxin (60 µg/kg) via the feed and L-carnitine (200 mg/L) in the drinking water orally for a period of 60 days. Aflatoxin was produced from *Aspergillus parasiticus* NRRL 2999 culture inoculated into sterile polished rice via fermentation by the method of Shotwell et al. (1966). Fermented rice was then steamed to kill the fungi, dried and ground to fine powder. The rice powder was incorporated into the basal diet to provide the required amount of 60 µg aflatoxin (AF) per kg of feed. The diet was then homogenised with a mixer. All groups were fed to meet the recommendations of the National Research Council for quail (Table 1). Water and feed were given to all animals *ad libitum*. The feed and water consumption of animals was calculated daily as 30 g/animal/day for feed and 60 ml/animal/day for water.

Animals were subjected to daily clinical examinations throughout the experiment. Blood samples were taken from the wing vein (vena ulnaris) into EDTA-treated tubes, then separated by centrifugation at 3000 × *g* for 10 min at room temperature and analysed immediately for plasma MDA and whole blood GSH. Analysis of whole blood GSH was carried out by method of Beutler et al. (1963) and plasma MDA was determined by spectrophotometry of the pink-

coloured product of the thiobarbituric acid-reactive substances complex (Yoshoiko et al., 1979).

**Table 1**

Ingredient and nutrient composition of diets (%)*

| Ingredients | Starter period | Grower period |
|---|---|---|
| Corn | 58.20 | 49.90 |
| Soybean meal | 32.10 | 25.00 |
| Fish meal | 7.50 | 4.00 |
| Barley | – | 18.70 |
| Lime stone | 1.10 | 1.20 |
| Dicalcium phosphate | 0.50 | 0.60 |
| Salt | 0.25 | 0.25 |
| Vitamin-mineral premixes** | 0.35 | 0.35 |
| *Chemical analysis* | | |
| Dry matter | 92.40 | 91.45 |
| | % of DM | |
| ME*** kcal/kg | 2895 | 2850 |
| Crude protein | 24.12 | 20.16 |
| Ether extract | 4.14 | 3.79 |
| Crude fibre | 4.27 | 4.58 |
| Ash | 6.02 | 5.11 |

*Carnitine was administered in drinking water as 200 mg/L carnitine group II and IV; **Provided per kg of concentrate: Vitamin A, 21,000 IU; Vitamin $D_3$, 4,200 IU; Vitamin E, 52.5 mg; Vitamin $K_3$, 4.38 mg; Vitamin $B_1$, 5.25 mg; Vitamin $B_2$, 12.25 mg; Vitamin $B_6$, 7 mg; Vitamin $B_{12}$, 0.03 mg; Folic acid, 1.75 mg; D-Biotin, 0.08 mg; Vitamin C, 87.5 mg; Niacin, 70 mg; Cal-D-Pantothenate, 14 mg; Choline chloride, 218.75 mg, Fe, 140 mg; Zn, 105 mg; Cu, 14 mg; Co, 0.35 mg; I, 1.75 mg; Se, 0.26 mg; Mn, 140 mg; ***Metabolisable energy, provided by calculation (NRC, 1984)

Quails in all groups were slaughtered humanely for histopathological examination at the end of the experiment. The liver was kept in formalin. After fixation, the liver was embedded in paraffin wax, cut at 5 μm and stained with haematoxylin and eosin (H × E).

Results were given as mean ± standard error of mean (SEM). Analyses of variance (ANOVA) was used to compare parameters using SPSS software. Differences among groups were analysed by ANOVA with Duncan's test (SPSS, 1993).

## Results

Clinical examination of animals revealed no observable clinical abnormalities in any of the groups. The effects of L-carnitine supplementation on the concentration of plasma MDA and whole blood GSH during experimentally induced chronic aflatoxicosis are presented in Table 2.

**Table 2**

Concentrations of plasma malondialdehyde (MDA) and whole blood reduced glutathione (GSH) in quails exposed to chronic aflatoxicosis (mean ± S.E.)

| Parameter | Groups | | | |
|---|---|---|---|---|
| | Control (Group I) (n = 20) | L-Carnitine (Group II) (n = 20) | Aflatoxin (Group III) (n = 20) | Aflatoxin + L-Carnitine (Group IV) (n = 20) |
| Malondialdehyde (μmol/L) | $2.40 \pm 0.16^{B}$ | $1.69 \pm 0.19^{C}$ | $3.29 \pm 0.22^{A}$ | $2.66 \pm 0.09^{B}$ |
| Reduced glutathione (mmol/L) | $7.03 \pm 0.63^{b}$ | $9.36 \pm 0.78^{a}$ | $5.34 \pm 0.35^{c}$ | $6.57 \pm 0.70^{b}$ |

$^{A,B,C}$Means in the same row with different superscripts differ significantly ($p < 0.001$); $^{a,b,c}$Means in the same row with different superscripts differ significantly ($p < 0.01$)

Mean plasma MDA concentrations were significantly higher in quails of the aflatoxin-treated Group III than those of the other groups ($p < 0.001$). Mean whole blood GSH concentration of the carnitine-treated Group II was significantly higher than that of the other groups ($p < 0.01$) (Table 2).

Histological examination of the liver of AF-fed animals (Group III) revealed swollen hepatocytes, presence of vacuoles unstained with haematoxylin and eosin referring to fatty infiltration in the cytoplasm, and a few necrotising hepatocytes, especially in the diaphragmatic lobe. Quails fed AF + L-carnitine (Group IV) only had swollen hepatocytes, while the liver of L-carnitine-fed animals (Group II) showed no pathological changes.

## Discussion

The toxic effects of aflatoxins in poultry are well documented (Chang and Hamilton, 1982; Rao et al., 1995; Ibrahim et al., 2000; Rosa et al., 2001). However, only few studies have dealt with the effects of aflatoxin on the induction of lipid peroxidation in these species (Souza et al., 1999; Rastogi et al., 2001; Verma and Nair, 2001). This study showed that aflatoxin fed at the dose rate of

60 μg/kg of feed for a 60-day period induced lipid peroxidation in quails, which was supported by increased plasma MDA concentrations. Gutteridge and Halliwell (1990) have reported that high plasma MDA levels may result from an increase in membrane lipid peroxidation or a failure of the antioxidant defence system. In addition, lipid peroxidation can also be a manifestation of AF-induced toxicity. This was also the case in this study as plasma MDA was higher and whole blood GSH lower in quails exposed to chronic aflatoxicosis. These findings are consistent with the previous reports (Mézes et al., 1998; Souza et al., 1999; Rastogi et al., 2001).

Treatment with L-carnitine and AF together significantly decreased MDA concentration ($p < 0.001$) and increased whole blood GSH concentration ($p < 0.01$) in quails. L-carnitine prevents hepatic lipidosis, one of the main symptoms of aflatoxicosis in poultry (Bryden et al., 1979; Merkley et al., 1987). These results were supported by our histopathological findings. It was also reported that antioxidant properties of L-carnitine may be related to the transport of fatty acids into mitochondria for β-oxidation and thus to the decrease of lipid usage and protection of the cell membrane against toxic reactive oxygen species (ROS) and other free radicals (Kalaiselvi and Panneerselvam, 1998).

The cell-protective effect of L-carnitine can be used for inhibiting the genotoxic, mutagenic, and cell proliferative effects of MDA, one of the important toxic aldehydes of lipid peroxidation. The increase of GSH concentration may be due to the decreased lipid peroxidation caused by L-carnitine supplementation, as suggested by Arockia Rani and Panneerselvam (2001). The results presented in this study show similarity with the findings of previous studies on the antioxidant effects of L-carnitine (Atroshi et al., 1999; Arockia Rani and Panneerselvam, 2001).

This study demonstrated that L-carnitine enhanced the blood plasma antioxidant status in quails during chronic aflatoxicosis. The capacity of L-carnitine to elevate GSH concentration was also shown and this antioxidant action may be due to the decrease of lipid peroxidation caused by L-carnitine during chronic aflatoxicosis in quails. Finally, this study has two conclusions. Firstly, it elucidates that L-carnitine supplementation significantly inhibits lipid peroxidation processes induced in the blood by aflatoxin. Secondly, as aflatoxin and its metabolites are likely to be harmful to human health, the consumption of meat, meat products and egg from animals fed aflatoxin-contaminated feed is not recommended. Also, the economic aspects of using L-carnitine for the treatment of aflatoxicosis or for reducing its harmful effects in large poultry production enterprises are open to discussion.

## References

Arockia Rani, P. J. and Panneerselvam, C. (2001): L-carnitine as a free radical scavenger in aging. Exp. Gerontol. **36,** 1713–1726.

Atroshi, F., Rizzo, A., Biese, L., Veijalainen, P., Saloniemi, H., Sankari, S. and Andersson, K. (1999): Fumonisin B1-induced DNA damage in rat liver and spleen: Effect of pretreatment with coenzyme Q10, L-carnitine, α-tocopherol and selenium. Pharmacol. Res. **40,** 459–467.

Beutler, E., Duran, O. and Kelley, B. M. (1963): Improved method for determination of blood glutathione. J. Lab. Clin. Med. **61,** 882–888.

Bryden, W. L., Cumming, R. B. and Balnove, D. (1979): The influence of vitamin A status on the response of chickens to aflatoxin B1 and changes in liver lipid metabolism associated with aflatoxicosis. Br. J. Nutr. **41,** 529–540.

Chang, C. F. and Hamilton, P. B. (1982): Experimental aflatoxicosis in young Japanese quail. Poultry Sci. **61,** 869–874.

Fritz, I. B. and Arrigoni-Martelli, E. (1993): Sites of action of carnitine and its derivatives on the cardiovascular system. Interactions with membranes. Trends Pharmacol. Sci. **14,** 355–360.

Galvano, F., Piva, A., Ritieni, A. and Galvano, G. (2001): Dietary strategies to counteract the effects of mycotoxins: a review. J. Food Protect. **64,** 120–131.

Gutteridge, J. M. C. and Halliwell, B. (1990): The measurement and mechanism of lipid peroxidation in biological systems. Trends Biochem. Sci. **15,** 129–135.

Ibrahim, I. K., Shareef, A. M. and Al-Joubory, K. M. (2000): Ameliorative effects of sodium bentonite on phagocytosis and Newcastle disease antibody formation in broiler chickens during aflatoxicosis. Res. Vet. Sci. **69,** 119–122.

Kalaiselvi, P. and Panneerselvam, C. (1998): Effect of L-carnitine on the status of lipid peroxidation and antioxidants in aging rats. J. Nutr. Biochem. **9,** 575–581.

Merkley, J. W., Maxwell, R. J., Phillips, J. G. and Huff, W. E. (1987): Hepatic fatty acid profiles in aflatoxin-exposed broiler chickens. Poultry Sci. **66,** 59–67.

Mézes, M., Barta, M. and Nagy, G. (1998): Comparative investigation on the effect of T-2 mycotoxin on lipid peroxidation and antioxidant status in different poultry species. Res. Vet. Sci. **66,** 19–23.

NRC (1984): Nutrient Requirements of Poultry. Washington, DC.

Rao, J. R., Sharma, N. N. and Johri, T. S. (1995): Influence of dietary aflatoxin on *Eimeria uzura* infection in Japanese quail. Vet. Parasitol. **56,** 17–22.

Rastogi, R., Srivastava, A. K. and Rastogi, A. K. (2001): Long-term effect of aflatoxin B1 and lipid peroxidation in rat liver and kidney. Effect of picroliv and silymarin. Phytother. Res. **15,** 307–310.

Rebouche, C. J. (1992): Carnitine function and requirements during the life cycle. FASEB J. **6,** 3379–3386.

Rosa, C. A., Miazzo, R., Magnoli, C., Salvano, M., Chiac, S. M., Ferrero, S., Saenz, M., Carvalho, E. C. and Dalcero, A. (2001): Evaluation of the efficacy of bentonite from the south of Argentina to ameliorate the toxic effects of AF in broilers. Poultry Sci. **80,** 139–144.

Shotwell, O. L., Hesseltine, C. V., Stubblefield, R. D. and Sorenson, W. G. (1966): Production of aflatoxin on rice. Applied Microbiol. **14,** 425–429.

Souza, M. F., Tome, A. R. and Rao, V. S. (1999): Inhibition by the bioflavonoid ternatin on aflatoxin B1-induced lipid peroxidation in rat liver. J. Pharmacol. **51,** 125–129.

SPSS for Windows, Release 6.0, Copyright SPSS, Inc., 1989–1993, June 17, 1993.

Verma, R. J. and Nair, A. (2001): Ameliorative effect of vitamin E on aflatoxin-induced lipid peroxidation in the testis of mice. Asian J. Androl. **3,** 217–221.

Yoshoiko, T., Kawada, K. and Shimada, T. (1979): Lipid peroxidation in maternal and cord blood and protective mechanism against activated-oxygen toxicity in the blood. Am. J. Obstet. Gynecol. **135,** 372–376.