Effects of supplementary dietary L-carnitine on performance and egg quality of laying hens fed diets different in fat level

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Abstract

The present study aimed to examine the effects of dietary L-carnitine supplementation on performance parameters and egg quality measurements of white Leghorn hens at two dietary fat levels. Two hundred 22-weeks old white Leghorn hens were randomly distributed into 40 cages of five birds each. Two basal diets different in added fat level (0 or 3%) were formulated and supplemented with incremental levels of L-carnitine (0, 50, 100, 150 mg/kg diet). The experiment lasted 98 d (two weeks for adaptation and 12 weeks as the main experimental period). At the final day of trial, ten randomly selected hens per treatment were euthanized to measure abdominal fat content. Dietary inclusion of 3% soybean oil caused a significant (P<0.05) increase in egg weight and egg mass, and decrease in feed consumption by the birds. Daily energy intake, however, was not affected by dietary fat supplementation. Except of feed conversion ratio, none of performance parameters were found to be influenced by dietary fat by carnitine interaction. Feed conversion ratio improved (P<0.05) when L-carnitine was supplemented to diets contained in 3% added fat. The albumen height and subsequently Haugh unit were improved (P<0.05) by dietary supplementation of L-carnitine, particularly the level of 150 mg/kg; however, eggshell quality indexes (thickness and breaking strength) were not affected by dietary L-carnitine inclusion, but influenced (P<0.05) by fat supplementation of diets. Moreover, dietary addition of fat increased abdominal fat percentage and supplementary dietary L-carnitine significantly (P<0.05) decreased abdominal fat and yolk cholesterol contents. From the present results, it can be seen that although the supplemental L-carnitine had no considerable effect on most performance parameters, it had a beneficial impacts on lipid metabolism and internal egg quality indexes of 24 to 36 wk-aged laying hens.

Introduction

L-carnitine (β-hydroxy-γ,N-trimethylamino-butrate), which its chemical structure was determined in 1927 by Tomita and Sendju, is a highly polar quaternary amine naturally found in microorganisms, plants and animals. This betaine derivative plays an important role in fatty acids oxidation (Bremer, 1983). Although L-carnitine participates in several metabolic reactions, its most widely known function is probably its ergogenic action resulting from the involvement of L-carnitine in fat metabolism (Zeyner and Harmeyer, 1999). L-carnitine is indispensable for the transport of long chain fatty acids from the cytosol into the mitochondrial matrix where the β-oxidation of fatty acids occurs (Bremer, 1983). For catabolism, long-chain free fatty acids are activated in the cytosol to form acyl-CoA esters of different chain length. Because the inner mitochondrial membrane is impermeable to these thioesters of coenzyme A, the acyl moieties are shifted from CoA bond to L-carnitine to form acylcarnitine esters, and are able to transport into the mitochondrial matrix, thereby being made available for the β-oxidation and releasing of acetyl moieties into the citric acid cycle. Thus, energy production from high-energy long-chain fatty acids strongly depends on the carrier function of L-carnitine (Zeyner and Harmeyer, 1999).

Poultry diets frequently contain a high amount of cereals (particularly maize), which have considerable quantity of lipids but very low L-carnitine contents (Baumgartner and Blum, 1997). Some researches have been suggested that the supplementary dietary L-carnitine caused significant increases in body weight gain and improved feed conversion ratio (FCR) in broiler (Rabie et al., 1997a, 1997b, 1997d). In contrast, other studies in poultry reported that dietary L-carnitine supplementation did not affect broiler and laying hen performance (Cartwright, 1986; Barker and Sell, 1994). Dietary energy level is usually increased by the addition of fat (Rabie and Szilagyi, 1998). Several studies have shown that increasing dietary energy or supplementing fat decreased feed intake and improved FCR of laying hens (Zou and Wu, 2005). Feeding dietary fat decreases liver lipogenesis in the laying hen (Naber and Biggert, 1989). Because of the L-carnitine role in energy metabolism and lipid oxidation, and the role of dietary fat in providing energy demands and reducing liver lipogenesis in laying hens, we hypothesized that the interaction between dietary fat and L-carnitine is able to improve FCR and to decrease egg yolk cholesterol and triglycerides contents. Therefore, the objectives of this study were to examine the effects of supplemental L-carnitine and dietary fat level on the performance and egg quality of layers at the early stage of lay.

Materials and methods

General protocol

Two hundred 22-weeks old Hy-Line W36 strain white Leghorn hens were randomly distributed into 40 cages of 45×45×40 cm in dimensions, with five hens per each cage replicates. Eight dietary treatments were designed with 25 birds each (5 replicates of 5 birds). The trial was carried out in a completely randomized design with a 2×4 factorial arrangement of treatments. The two basal diets contained 0 or 3% added fat, each supplemented with 0, 50, 100 or 150 mg/kg L-carnitine. Ingredients and nutrient composition of experimental diets are shown in Table 1. The basal diets were formulated to meet all nutrients recommendations by National Research Council (1994) for laying hens. The experiment lasted 98 d (two weeks for pre-experiment adaptation period and 12 weeks as the main recording period), when the hens were 36 weeks-old. The birds were kept under standard practices, had free access to feed and water, and photoperiod was 16 h/d of light. Egg production was recorded daily. Feed consumption was recorded at bi-weekly intervals. Similarly, egg weight and albumen height were measured at the end of each two week interval. Yolk lipids were extracted from 20
eggs per treatment by the method described by Folch et al. (1957) as modified by Washburn and Nix (1974). Yolk cholesterol and triglycerides were measured by the colorimetric method (ERBA CHEM-5, Beijing Biochemical Instrument Company, Beijing, China). At the end of the trial (36 wk of age), ten randomly selected hens per treatment were euthanized to measure abdominal fat content.

Statistical analysis

Data were analyzed using the General Linear Models (GLM) procedure of SAS Statistical System (SAS Institute, 1999). The following model was assumed in the analysis of all studied parameters: \( Y_{ijk} = \mu + A_i + B_j + (AB)_{ij} + e_{ijk} \), where \( Y_{ijk} \) is observed value for a particular character; \( \mu \) is overall mean; \( A_i \) is the effect of the \( i \)th level of supplemental L-carnitine; \( B_j \) is the effect of the \( j \)th level of dietary fat; \( (AB)_{ij} \) is the interactive effect between L-carnitine and dietary fat level; and \( e_{ijk} \) is random error associated with the \( ijk \)th recording. Significant differences among treatment means were separated by Duncan’s multiple range test (Duncan, 1955) at the P<0.05 level.

Results and discussion

The different egg production characteristics are presented in Table 2. As shown, dietary supplementation with L-carnitine and dietary added fat had no significant effects on egg production. This finding agrees those of Rabie et al. (1997a, 1997c), who reported that dietary L-carnitine supplementation did not influence laying performance (egg production, egg weight and egg mass). Similarly, Harms et al. (2000), Wu et al. (2005) and Zou and Wu (2005) demonstrated that egg production was not affected by supplemental fat or dietary energy. Contrary to these observations, Grobas et al. (1999b) reported that the supplementary dietary fat increased egg production from 38 to 61 weeks of age. In the present trial, no changes in body weight were observed because the experiment was terminated at 36 weeks of age, the age at which birds do not gain weight. This trend was evidenced in the study by Grobas et al. (1999b). It appears that the birds don’t utilize higher energy from fat-supplemented diets to gain weight and/or lay greater egg number compared with the groups fed on diets without additional fat supplement. The lack of higher energy utilization from fat-supplemented diets led both groups (with or without fat) to have similar egg production rates.

Interactive effect between L-carnitine and dietary added fat was not significant for egg production during the overall experimental period. The average egg weight and egg mass among the levels of supplemental L-carnitine showed no statistical differences (Table 2), but the inclusion of fat into the diets significantly (P<0.05) increased egg weight and mass. It appears that the improvements in egg weight and mass by dietary inclusion of 3% soybean oil may be, in part, due to the increase in dietary energy content (2,600 vs 2,720 in diets contained 0 or 3% added fat, respectively). Of course, the egg weight response to dietary 3% soy oil may be attributed to the presence of higher linoleic acid concentrations in soy oil-supplemented diets. It has been well demonstrated that the high levels of dietary linoleic acid increase egg size (March and MacMillan, 1990). Similarly, Keshavarz and Nakajima (1995), Harms et al. (2000), Bohnsack et al. (2002) and Wu et al. (2005) reported that increase in dietary fat level caused improvements in hen’s egg weights. However, several researchers (Summers and Leeson, 1983; Zou and Wu, 2005) observed that neither egg weight nor egg mass was affected by supplemental fat or dietary energy. The differences in research results reported by various authors might be, in part, due to the differences in dietary fat source used. In this experiment, vegetable oil (soy oil) was used that is contained in high amounts of linoleic acid (NRC, 1994; Leeson and Summers, 1997).

Neither feed nor energy intakes were affected by dietary L-carnitine supplementation (Table 2). However, feed intake was significantly (P<0.05) declined by supplementing dietary fat (Table 2). This event may be attributed to the increase in dietary energy concentration. This response agrees with the fact that under ad libitum feeding condition, birds consumed feed primarily to satisfy their energy requirements (Leeson et al., 1993). The present observation was in agreement with those of Rabie and Szilagyi (1998), Grobas et al. (1999a, 1999b) and Wu et al. (2005), who reported that the supplemental fat had a marked effect on feed intake, so that feed consumption was significantly (P<0.05) reduced with increasing dietary energy density.

Feed conversion ratio was not affected by incremental levels of L-carnitine (Table 2). This result was in agreement with those of Rabie et al. (1997a, 1997c), who reported that the addition of L-carnitine to the diet of laying hens did not influence FCR values. However, several studies with broilers (Rabie et al., 1997b, 1997d; Rabie and Szilagyi, 1998) indicated that the additional exogenous L-carnitine could improve FCR. Addition of 3% fat to

### Table 1. Composition and nutritive value of two basal experimental diets (with or without additional fat).

| Ingredients (%) | 0 | Fat level (% of diet) |
|-----------------|---|----------------------|
| Corn            | 62.10 | 56.40 |
| Barley          | 5.00   | 5.00   |
| Soybean meal    | 21.53 | 24.23 |
| Soybean oil     | -      | 3.00   |
| Oyster shell    | 9.60   | 9.60   |
| Dicalcium phosphate | 0.90 | 0.90 |
| Vitamin premix\(\alpha\)-tocopheryl acetate), 11 U; vitamin E (as \(\alpha\)-tocopheryl acetate), 11 U; vitamin K, 2 mg; vitamin B; 2 mg; vitamin B; 4 mg; vitamin B; 2.4 mg; vitamin B; 0.015 mg; pantothenic acid, 10 mg; niacin, 34 mg; folic acid, 0.5 mg; biotin, 0.15 mg; choline chloride, 140 mg; °Provided per kg of diet: manganese, 80 mg; copper, 8 mg; indine, 0.86 mg; selenium, 0.3 mg; zinc, 80 mg; iron, 75 mg. |
| DL-methionine   | 0.12   | 0.12   |

## Calculated analysis

| ME, kcal/kg | 2600 | 2720 |
| CP, %       | 14.50 | 15.14 |
| Met, %      | 0.36  | 0.38  |
| Met + Cys, %| 0.58  | 0.60  |
| Lys, %      | 0.74  | 0.78  |
| Ca, %       | 3.33  | 3.34  |
| P available,%| 0.30 | 0.31 |
| Na, %       | 0.15  | 0.15  |

*Provided per kg of diet: vitamin A (as retinyl acetate), 8800 U; vitamin D, 2500 U; vitamin E (as \(\alpha\)-tocopheryl acetate), 11 U; vitamin K, 2 mg; vitamin B; 2 mg; vitamin B; 4 mg; vitamin B; 2.4 mg; vitamin B; 0.015 mg; pantothenic acid, 10 mg; niacin, 34 mg; folic acid, 0.5 mg; biotin, 0.15 mg; choline chloride, 140 mg; °Provided per kg of diet: manganese, 80 mg; copper, 8 mg; indine, 0.86 mg; selenium, 0.3 mg; zinc, 80 mg; iron, 75 mg. |

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the diets significantly (P<0.01) decreased FCR. Consistent with this, Zou and Wu (2005) and Wu et al. (2005) reported that increase in dietary fat content improved FCR. Furthermore, FCR values were affected (P<0.05) by supplementary L-carnitine by dietary fat interaction, so that supplementation of experimental diets with 50 mg/kg L-carnitine improved FCR when the diets already were added by 3% soybean oil. This observation was similar to that of Rabie and Szilagyi (1998) with broiler chicks, who reported that supplemental L-carnitine was more effective at the higher level of energy produced by dietary fat. L-carnitine plays an important role in lipid and energy metabolism. Its major role appears to be the transport of long-chain fatty acids into the mitochondria for oxidation (Borum, 1983, 1987; Bremer, 1983). As the higher energy diets in the present trial had 3% added fat, probably the addition L-carnitine might improved the availability of diet energy by body cells. Increase in energy usage improves other nutrient utilization such as protein, amino acids, calcium and phosphorus (Zou and Wu, 2005), consequently lowers FCR values. The later evidence shows a synergistic effect of L-carnitine at the higher energy (fat) levels of laying hens diets.

L-carnitine supplementation reduced relative weight of abdominal fat as a proportion of live body weight (Table 2). This result is similar to those of Rabie et al. (1997d), Rabie and Szilagyi (1998), and Xu et al. (2003), who reported that abdominal fat content significantly decreased in broilers when fed on L-carnitine-supplemented diets. This response concurs together with the L-carnitine role in biological systems. L-carnitine induces oxidation of fatty acids, whereby decrease fatty acids availability for esterification to triacylglycerols and storage in the adipose tissues (Lien and Hornig, 2001; Xu et al., 2003). Addition of 3% fat to the diets significantly (P<0.05) increased abdominal fat percentage, probably because with increasing dietary fat, fatty acids amounts for storage in the adipose tissues were increased. Supplementary dietary L-carnitine was interacted with dietary fat level for abdominal fat content so that, dietary inclusion of 3% soy oil increased abdominal fat when the L-carnitine supplement was absent from the diets. On the other hand, addition of L-carnitine to the diets contained 3% added fat decreased (P<0.05) abdominal fat percentage, however, opposite trend was seen for diets without additional fat. The exact reason or explanation for this finding remains to be elucidated; however, it seems that in diets without additional fat, liver lipogenesis is increased (Naber and Biggert, 1989). Probably, L-carnitine induced fat transportation from liver whereby reduced the triglycerides supply of liver for incorporating in egg yolk (as seen in Table 3). Naturally, excess fats migrated from liver, incorporate within other tissues with adipose tissues (abdominal fat) are preferred.

The influences of dietary fat level and incremental L-carnitine levels on egg quality measurements are shown in Table 3. L-carnitine had no significant effect on shell thickness and eggshell breaking strength; however, the addition of 3% fat to the diets caused a significant (P<0.05) increase in shell thickness and shell breaking strength. Presumably, the improvements of egg shell quality measurements by dietary added fat may be related to efficiently digestion and absorption of vitamin D_3. Vitamin D_3 is a fat soluble vitamin and is one of the main factors in increasing shell thickness. Increasing shell thickness and shell strength may be, in part, due to the development of mycelia structures by dietary fat inclusion (Scott et al., 1982; McDowell, 2000). Improvement of mycelia structures increases the efficient absorption of vitamin D_3, causing therefore an increased shell thickness and strength. This result agrees reports by Grobas et al. (2001), who observed that dietary fat improved eggshell quality indexes.

Our observations indicated that albumen height and subsequent Haugh unit score were significantly (P<0.05) increased by increasing dietary L-carnitine levels. Birds fed on the highest L-carnitine level (150 mg/kg) had the

### Table 2. Influences of L-carnitine supplementation of diets differing in fat content on performance parameters and abdominal fat of laying hens from 24 to 36 wk of age.

| Fat level (% of diet) | L-carnitine (mg/kg) | Egg production (%) | Egg weight (g) | Egg mass (g/d per hen) | Feed intake (g/d per bird) | FCR (g feed: g egg) | Energy intake (kcal/d) | Abdominal fat (% of LBW) |
|----------------------|---------------------|--------------------|----------------|-----------------------|----------------------------|---------------------|-----------------------|------------------------|
| 0                    | 0                   | 87.00              | 57.71          | 50.20                 | 101.2                      | 2.01^a              | 263.1                 | 1.95^c                 |
| 50                   | 88.30               | 57.72              | 50.95          | 102.0                 | 2.00^b                      | 265.2               | 2.47^b                |
| 100                  | 88.72               | 57.29              | 50.83          | 101.7                 | 2.00^b                      | 264.4               | 2.44^b                |
| 150                  | 88.51               | 57.27              | 50.72          | 102.1                 | 2.01^b                      | 265.5               | 2.34^c                |
| abc Means with unlike superscripts letters within the column of each classification (interaction, fat level or L-carnitine) are significantly (P<0.05) different. ns: not significant; □*P<0.05; □**P<0.01. |
| 0                    | 89.00               | 58.41              | 51.98          | 97.6                  | 1.88^b                      | 265.5               | 3.69^c                |
| 50                   | 87.24               | 59.09              | 51.56          | 95.4                  | 1.85^c                      | 259.5               | 2.00^c                |
| 100                  | 87.42               | 58.22              | 50.88          | 94.9                  | 1.87^b                      | 258.1               | 2.49^b                |
| 150                  | 89.00               | 58.21              | 51.80          | 96.1                  | 1.86^b                      | 261.4               | 2.43^c                |
| abc Means with unlike superscripts letters within the column of each classification (interaction, fat level or L-carnitine) are significantly (P<0.05) different. ns: not significant; □*P<0.05; □**P<0.01. |
| 0                    | 88.13               | 57.51              | 50.67^b        | 101.8^b               | 2.01^a                      | 264.7               | 2.38^b                |
| 3                    | 88.17               | 58.49^a            | 51.59^b        | 96.0^b                | 1.86^b                      | 261.1               | 2.57^b                |
| 0                    | 88.00               | 58.11              | 51.13          | 99.4                  | 1.94                        | 264.4               | 2.52^b                |
| 50                   | 87.77               | 58.42              | 51.26          | 98.7                  | 1.93                        | 262.5               | 2.28^b                |
| 100                  | 88.07               | 57.79              | 50.90          | 98.3                  | 1.93                        | 261.5               | 2.46^b                |
| 150                  | 88.76               | 57.81              | 51.30          | 99.1                  | 1.93                        | 263.6               | 2.33^b                |
| Probability:         |                     | ns                 | ns             | ns                    | ns                          | ns                  | ns                    |
| Fat × L-carnitine    |                     | ns                 | ns             | ns                    | ns                          | ns                  | ns                    |
| SEM                  | 1.510               | 0.381              | 1.110          | 2.586                 | 0.018                       | 7.736               | 0.140                 |
Table 3. Influences of L-carnitine supplementation of diets differing in fat content on egg quality indexes of laying hens during a period of 24–36 wk of age.

| Fat level (% of diet) | L-carnitine (mg/kg) | Albumen height (mm) | Haugh unit | Shell thickness (mm) | Shell strength (kg/cm²) | Yolk cholesterol (mg/g yolk) | Yolk TG (mg/g yolk) |
|-----------------------|---------------------|---------------------|------------|----------------------|-------------------------|-----------------------------|---------------------|
| 0                     | 0                   | 7.63                | 87.73      | 0.410                | 3.47                    | 12.4<sup>a</sup>            | 298.0<sup>a</sup>   |
| 50                    | 7.70                | 87.65               | 0.415      | 3.42                 | 11.4<sup>b</sup>        | 297.8<sup>a</sup>           | 11.5<sup>b</sup>    |
| 100                   | 7.77                | 88.35               | 0.412      | 3.52                 | 11.5<sup>b</sup>        | 290.6<sup>ab</sup>          | 11.4<sup>b</sup>    |
| 150                   | 7.81                | 90.54               | 0.410      | 3.39                 | 11.0<sup>b</sup>        | 287.0<sup>b</sup>           | 11.4<sup>b</sup>    |
| 3                     | 0                   | 7.55                | 86.74      | 0.414                | 3.46                    | 12.4<sup>a</sup>            | 294.4<sup>a</sup>   |
| 150                   | 7.84                | 88.74               | 0.416      | 3.55                 | 10.8<sup>b</sup>        | 288.5<sup>abc</sup>         | 11.4<sup>b</sup>    |
| 0                     | 7.82                | 88.58               | 0.412<sup>b</sup> | 3.45<sup>b</sup>    | 11.7                    | 293.4<sup>b</sup>           | 11.4<sup>b</sup>    |
| 3                     | 7.73                | 87.81               | 0.414<sup>a</sup> | 3.52<sup>a</sup>    | 11.4                    | 291.8<sup>b</sup>           | 11.4<sup>b</sup>    |
| 0                     | 7.59<sup>b</sup>    | 87.23<sup>b</sup>   | 0.412      | 3.47                 | 12.4<sup>a</sup>        | 296.2<sup>b</sup>           | 11.4<sup>b</sup>    |
| 50                    | 7.72<sup>b</sup>    | 87.50<sup>b</sup>   | 0.414      | 3.50                 | 11.4<sup>b</sup>        | 295.0<sup>b</sup>           | 11.4<sup>b</sup>    |
| 100                   | 7.78<sup>ab</sup>   | 88.40<sup>b</sup>   | 0.412      | 3.50                 | 11.2<sup>b</sup>        | 291.3<sup>b</sup>           | 11.4<sup>b</sup>    |
| 150                   | 8.00<sup>b</sup>    | 89.64<sup>b</sup>   | 0.413      | 3.47                 | 11.2<sup>b</sup>        | 287.8<sup>b</sup>           | 11.4<sup>b</sup>    |

Probability:
- Fat level: ns, ns, **, * P<0.05.
- L-Carnitine: **, ns, ns, ** P<0.05.
- Fat x L-Carnitine: ns, ns, ns, * P<0.05.
- SEM: 0.200, 0.957, 0.001, 0.040, 0.500, 4.00

*Means with unlike superscripts letters within the column of each classification (interaction, fat level or L-carnitine) are significantly (P<0.05) different. ns: not significant; *P<0.05.

Conclusions

In conclusion, it appears that dietary L-carnitine supplementation has beneficial impact on abdominal fat deposition and internal egg quality measurements (albumen height and Haugh unit). Egg yolk cholesterol and triglycerides contents might also reduced by L-carnitine supplementation of laying hens’ diets. It is evident that the advantageous effects of supplementary L-carnitine are more pronounced when the diets are contained in the higher fat levels.

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