Effects of dietary L-carnitine on the performance and egg quality of laying hens from 65–73 weeks of age

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The effects of L-carnitine supplementation (50–500 mg/kg diet) of a practical layer diet, based on maize, soyabean and wheat, on the performance of laying hens and some indices of egg quality were studied for 8 weeks, using 65-week-old hens kept in cages. Albumen quality (albumen height and Haugh (1937) unit score) was improved, while yolk index and yolk colour score were not affected by dietary L-carnitine. The percentage of egg-white increased and that of egg yolk decreased in response to dietary supplementation of L-carnitine. Dietary L-carnitine did not influence laying performance (egg production rate, mean egg weight, daily feed intake, daily egg mass and feed conversion) or external egg quality measured by egg weight, egg-shape index or by eggshell quality, either measured directly as shell breaking strength or indirectly as shell weight, shell thickness or shell weight per unit surface area. Based on the results of the present study, L-carnitine had a beneficial effect on albumen quality and could modify the components of the edible part of the egg, during the late laying period.

L-Carnitine: Performance: Eggs: Laying hens

There are several factors influencing the quality of eggs before they are laid. Amongst these, the most important factors are nutrition, disease, environment, age and breed or strain of the bird (Moreng & Avens, 1985). Many important egg-quality components deteriorate and become more variable with the age of the bird. Williams (1992) concluded that, excluding disease, the single most important factor affecting albumen quality of freshly-laid egg is the age of the bird that laid it. Nutritional factors can modify egg quality by virtue of their transport into the egg, by inducing metabolic changes that result in the synthesis of compounds which find their way into the egg, or by changing the transport characteristics of the membranes involved in formation of the egg components (Hurwitz, 1987).

L-Carnitine (β-OH-(γ-N-trimethylamino)-butyrate) is a water-soluble quaternary amine which occurs naturally in micro-organisms, plants and animals (Bremer, 1983). In animals, L-carnitine concentrations vary widely in different species (Szilágyi *et al.* 1992) and among tissues (Bremer, 1983; Rinaudo *et al.* 1991). Carnitine is synthesized *in vivo* from the essential amino acids lysine and methionine (Rebouche & Paulson, 1986; Feller & Rudman, 1988). The carnitine biosynthetic pathway also requires Fe^{2+} and a number of vitamins: ascorbate, niacin and pyridoxine (Sándor *et al.* 1983; Leibetseder, 1995).

L-Carnitine plays an important role in energy metabolism. Its major role appears to be the transport of long-chain fatty acids into mitochondria for oxidation (Bremer, 1983; Borum, 1983, 1987).

Several reports on broiler chickens, quails (*Coturnix coturnix*), pigs and fish have demonstrated that growth performance can be improved by feeding supplementary dietary

L-carnitine (Weeden *et al.* 1991; Lettner *et al.* 1992; Schuhmacher *et al.* 1993; Torreele *et al.* 1993).

It has been reported that little L-carnitine is found in cereal grains and their by-products (Tanphaichitr *et al.* 1976; Mitchell, 1978; Borum, 1983). Because cereal grains usually represent the major component of poultry diets, it may be useful to incorporate this compound into the diets. While no or little L-carnitine has been found in eggs (Chiodi *et al.* 1994; Leibetseder, 1995), a high concentration of L-carnitine was found in chick embryo at the first stages of development (Chiodi *et al.* 1994).

It is well documented that rate of lay and egg quality, particularly shell quality, decline as the hen ages (Roland, 1979; Roberts & Brackpool, 1993–4; Larbier & Leclercq, 1994). However, limited literature deals with the effects of L-carnitine supplementation of laying-hen diets on egg quality. Leibetseder (1995) recently produced evidence that the supplementation of a standard layer's ration with either 500 mg L-carnitine or 500 mg nicotinic acid, or a combination of the two compounds, had no effects on egg production, feed intake, body weight or concentrations of serum or yolk cholesterol during the early laying period. However, L-carnitine content of yolks was significantly increased in the supplemented groups.

The objective of the present study was to investigate how far we can sustain normal laying performance and/or egg quality during the late stages of the egg production cycle. The effects of supplemental dietary L-carnitine on laying-hen performance and certain egg-quality indices were investigated during the late laying period.

MATERIALS AND METHODS

Forty 65-week-old Tetra SL laying hens (Hungarian brown hybrid line) were used in the present study. They were randomly divided into four experimental groups of ten birds each and housed in individual laying cages in an open-sided house with a daily photoperiod of 16 h (from 06.00 to 22.00 hours) at the Poultry Research Unit, Institute for Small Animal Research, Gödöllö, Hungary. A basal layer diet was formulated to be adequate in all nutrients for this strain according to the specific breeder guidelines, as recommended by National Research Council (1984). The birds were fed on the basal layer diet supplemented with four levels of L-carnitine (0, 50, 100 or 500 mg/kg diet), for 8 weeks from 65 to 73 weeks of age; then the experiment was terminated. The composition and chemical analyses of the basal layer diet are presented in Table 1. Feed and water were provided *ad libitum* throughout the experimental period.

The basal diet was analysed for DM, crude protein (N × 6·25), crude fibre, diethyl ether-extractable fat and ash using the standard methods described by the Association of Official Analytical Chemists (1980), and for gross energy content by an adiabatic oxygen bomb calorimeter. Concentrations of lysine and methionine were determined by analysis (Aminochrom II type analyzer; Labor Mim, Budapest, Hungary) based on the Moore Stein principle (Moore *et al.* 1958), after HCl hydrolysis (Barocsai, 1990).

Daily records were made of egg production and individual egg weights. Feed consumption and feed conversion determinations were based on measurements over two 28 d periods during the experimental period. The performance of laying hens was evaluated in terms of egg production rate, mean egg weight, daily feed intake, daily egg mass and feed conversion. The latter was calculated both as g feed required to produce 1 g egg and as kg feed required to produce twelve eggs. Mean egg weight was determined by dividing the sum of the individual weights of eggs produced in a given period by the number of those

Table 1. *Composition and proximate analysis of the basal layer diet (g/kg)*

Ingredients	
Yellow maize	455·0
Wheat	200·0
Soyabean meal, 47%	177·3
Lucerne (*Medicago sativa*) meal	65·0
Monocalcium phosphate	11·0
Common salt	3·0
Limestone	82·0
Vitamin and mineral premix*	5·0
DL-Methionine	1·7
Total	1000·0
Calculated analyses	
Crude protein (N × 6·25)	166·2
Crude fibre	40·3
Ca	35·9
P (total)	5·5
Lysine	8·0
Methionine + cystine	6·8
Metabolizable energy (MJ/kg)	11·40
Determined analyses	
DM	945·0
Ash	115·5
Crude protein	166·4
Diethyl ether extract	30·4
Crude fibre	40·0
Lysine (g/kg DM)	8·1
Methionine (g/kg DM)	2·8
Gross energy (MJ/kg DM)	15·55

*Provided (g/kg): Ca 256·6, P 53, NaCl 60, methionine 10, vitamin A 157·5 mg, cholecalciferol 75 mg, vitamin E 472·5 mg.

eggs. Daily egg mass was calculated by dividing the total egg mass produced in a given period by the duration (d) of that period.

Laboratory evaluations of egg quality were performed twice, the first after 3 weeks of treatment and the second at the end of the experiment. Egg-quality measurements were made on all eggs, freshly-collected, laid on two consecutive days. Egg quality was based on determination of external and internal indices, and egg components. The external indices included egg weight, egg-shape index, shell thickness, shell breaking strength and shell weight per unit surface area (SWUSA), and those of interior quality were albumen height, Haugh (1937*a*,*b*) unit score, yolk index and yolk colour score.

Collected eggs were weighed individually and their widths and lengths were measured. Then, they were broken onto a smooth level surface and the height of albumen was determined, away from the chalazae, at the two highest points on opposite sides of the yolk, using a standard tripod micrometer. The average of the two measurements of thick-albumen height together with egg weight were used to compute the Haugh unit score for each individual egg according to Haugh (1937*a*, *b*) and cited later by Larbier & Leclercq (1994), as follows:

$$\text{Haugh units} = 100 \times \log(T - 1{\cdot}7 \times W^{0{\cdot}37} + 7{\cdot}57),$$

where T is thickness of thick-white layer (mm) and W is egg weight (g).

Yolk height was also determined using the same micrometer, while yolk diameter was measured to the nearest 0·1 mm using a steel vernier caliper. Yolk index was calculated as

yolk height × 100 divided by yolk diameter. Yolk colour score was measured using a Roche yolk colour fan (Hartmann, Paris, France). Egg components were determined according to the procedure described by Keshavarz & Nakajima (1995), in which shells of the opened eggs were cleaned carefully of any adhering albumen using paper towels and then the shell and yolk for each egg were weighed separately. The yolk was separated from the albumen and then rolled on a damp paper towel to remove any adhering albumen. The chalazae were also removed before weighing the yolk. Albumen weight was calculated by subtracting the yolk plus shell weight from the total egg weight. Weights of the whole eggs were determined to the nearest 1 g and those of egg components to the nearest 0·1 g.

Egg-shape index was measured as egg width × 100 divided by egg length. The measurement of egg width and length was performed using a wooden apparatus similar to the model described previously by Amer (1972). Shell thickness was measured using a special micrometer (Mitutoy, Tokyo, Japan). Measurements were made at two corresponding positions on the equator of the eggshell and the average was recorded to the nearest 0·001 mm. SWUSA was calculated by dividing shell weight (plus adhering membranes; mg) by the egg surface area (ESA; cm^2). ESA was calculated according to Carter (1975) using the following equation: $ESA = (3{\cdot}9782 \times \text{egg weight}^{0{\cdot}7056}$ (g)).

Data were subjected to statistical analysis using the Statgraphics program (Rockville, 1991). One-way ANOVA was used and differences between means were tested for significance at a confidence level of 95 %.

RESULTS

Data for laying performance, as well as external and internal indices of egg quality, and egg components are presented in Tables 2, 3, 4 and 5 respectively. No mortalities occurred during the study. No significant differences were observed in laying performance (Table 2), external indices of egg quality (except egg-shape index; Table 3), or internal quality of

Table 2. *Effect of dietary* L*-carnitine supplementation on the performance of laying hens from 65–73 weeks of age**

(Mean values for ten hens per dietary treatment)

Criteria of performance	Levels of L-carnitine added (mg/kg)				SED
	0	50	100	500	
Period 1 (65–69 weeks of age)					
Egg production rate† (%)	82·5	79·6	79·6	81·4	1·244
Mean egg wt (g)	60·6	62·4	63·2	61·0	0·541
Daily feed intake (g/hen)	117·2	116·6	117·3	118·0	0·543
Daily egg mass (g/hen)	50·0	49·7	50·3	49·7	0·883
Feed conversion: g feed/g egg	2·37	2·37	2·36	2·42	0·045
kg feed/twelve eggs	1·72	1·77	1·79	1·76	0·029
Period 2 (70–73 weeks of age)					
Egg production rate (%)	77·5	73·6	72·5	78·2	1·203
Mean egg wt (g)	60·8	61·9	62·7	60·5	0·492
Daily feed intake (g/hen)	113·7	113·2	111·7	111·5	0·340
Daily egg mass (g/hen)	47·1	46·0	45·3	47·3	0·726
Feed conversion: g feed/g egg	2·43	2·52	2·49	2·37	0·036
kg feed/twelve eggs	1·72	1·87	1·88	1·72	0·029

* For details of diet and procedures, see Table 1 and pp. 616–618.

† No. of eggs laid per 100 hens per d.

Table 3. *Effect of dietary L-carnitine supplementation on some external indices of egg quality of 65–73-week-old laying hens**

(Mean values with their standard errors for thirteen observations at 3 weeks of treatment and fifteen observations at 8 weeks of treatment)

	Levels of L-carnitine added (mg/kg)							
	0		50		100		500	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
After 3 weeks of treatment								
Egg-shape index†	73·7	0·93	74·1	1·00	73·7	1·27	74·1	0·50
Shell breaking strength (kg)	2·75	0·12	2·75	0·14	2·83	0·10	2·74	0·13
Shell thickness (mm)	0·35	0·008	0·35	0·007	0·36	0·008	0·35	0·007
SWUSA (mg/cm^2)	94·3	1·79	90·3	2·66	91·6	1·71	88·9	2·37
After 8 weeks of treatment								
Egg-shape index†	71·3^a	1·00	74·2^b	0·82	73·7^b	0·56	73·9^b	0·63
Shell breaking strength (kg)	2·63	0·08	2·67	0·10	2·72	0·10	2·52	0·08
Shell thickness (mm)	0·36	0·009	0·35	0·006	0·36	0·009	0·37	0·006
SWUSA (mg/cm^2)	95·7	1·82	95·1	1·91	93·5	1·75	93·7	1·98

^{a,b} Mean values in the same row with different superscript letters were significantly different ($P < 0·05$).
SWUSA, shell weight per unit egg surface area.
* For details of diet and procedures, see Table 1 and pp. 616–618.
† (Egg width × 100) ÷ egg length.

Table 4. *Effect of dietary L-carnitine supplementation on certain indices of internal egg quality of 65–73-week-old laying hens**

(Mean values with their standard errors for thirteen observations at 3 weeks of treatment and fifteen observations at 8 weeks of treatment)

	Levels of L-carnitine added (mg/kg)							
	0		50		100		500	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
After 3 weeks of treatment								
Albumen height, (mm)	6·15	0·22	6·85	0·42	6·92	0·42	6·69	0·33
Haugh unit score†	77·1	1·47	81·1	2·45	81·2	2·60	80·8	1·83
Yolk index‡	43·7	0·74	44·1	0·81	45·5	1·24	44·5	0·72
Yolk colour score§	7·77	0·17	7·46	0·14	7·62	0·14	7·54	0·14
After 8 weeks of treatment								
Albumen height (mm)	5·93^a	0·33	7·20^b	0·33	7·47^{bc}	0·31	8·27^c	0·25
Haugh unit score	74·4^a	2·79	83·3^b	2·19	85·2^{bc}	1·80	89·9^c	1·36
Yolk index	45·1	0·54	44·0	0·73	45·4	0·54	45·1	0·41
Yolk colour score	7·40	0·16	7·27	0·12	7·53	0·19	7·33	0·13

^{a,b,c} Mean values in the same row with different superscript letters were significantly different ($P < 0·001$).
* For details of diet and procedures, see Table 1 and pp. 616–618.
† $100 \times \log(T - 1·7 \times W^{0·37} + 7·57)$, where T is thickness of thick-white layer (mm) and W is egg weight (g).
‡ Yolk height (mm) × 100 ÷ yolk diameter (mm).
§ Measured using a Roche yolk colour fan (Hautmann, France).

Table 5. *Effect of dietary* L-*carnitine supplementation on mean egg weight and egg components of 65–73-week-old laying hens**

(Mean values with their standard errors for thirteen observations at 3 weeks of treatment and fifteen observations at 8 weeks of treatment)

	Levels of L-carnitine added (mg/kg)							
	0		50		100		500	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
After 3 weeks of treatment								
Mean egg wt (g)	61·1	1·36	61·7	1·21	62·5	1·44	61·0	1·60
Shell wt: g	6·82	0·16	6·58	0·21	6·73	0·15	6·42	0·20
% of egg	11·2	0·24	10·7	0·33	10·8	0·23	10·6	0·31
Yolk wt: g	16·6	0·29	17·2	0·38	17·0	0·38	16·4	0·34
% of egg	27·3	0·66	27·8	0·41	27·2	0·43	27·0	0·33
Albumen wt: g	37·6	1·21	38·0	0·90	38·8	1·13	38·1	1·23
% of egg	61·5	0·79	61·5	0·57	62·0	0·54	62·4	0·56
After 8 weeks of treatment								
Mean egg wt (g)	61·7	0·96	61·9	0·55	62·4	0·96	62·8	0·79
Shell wt: g	6·97	0·16	7·01	0·16	6·87	0·14	6·91	0·15
% of egg	11·3	0·21	11·2	0·22	11·0	0·22	11·0	0·24
Yolk wt: g	18·4[a]	0·38	17·1[b]	0·35	17·3[b]	0·25	17·2[b]	0·28
% of egg	29·9[a]	0·59	27·3[b]	0·48	27·7[b]	0·39	27·4[b]	0·49
Albumen wt: g	36·2[a]	0·76	38·4[b]	0·51	38·2[ab]	0·77	38·7[b]	0·79
% of egg	58·8[a]	0·55	61·5[b]	0·56	61·2[b]	0·38	61·6[b]	0·67

[a,b] Mean values in the same row with unlike superscript letters were significantly different ($P < 0·001$).

* For details of diet and procedures, see Table 1 and pp. 616–618.

eggs in terms of yolk index and yolk colour score (Table 4) in response to dietary L-carnitine supplementation. The relative weights of egg yolk and albumen differed significantly ($P < 0·001$), in response to L-carnitine supplementation (Table 5), but only after 8 weeks of supplementation. Internal quality of eggs measured by albumen height and Haugh unit score improved significantly after 8 weeks in all L-carnitine-supplemented groups (Table 4) compared with the controls ($P < 0·001$). L-Carnitine level significantly affected albumen height and Haugh unit score (Table 4). The highest mean values for albumen height and Haugh unit score were achieved by the group of birds fed on the highest level of L-carnitine (500 mg/kg), although the differences between this group and that fed on the diet supplemented with a level of L-carnitine of 100 mg/kg were not statistically significant ($P > 0·05$).

DISCUSSION

It is known that laying performance, and to some extent egg quality, decrease during the late stages of the egg production cycle (Roland, 1979; Moreng & Avens, 1985; Williams, 1992; Roberts & Brackpool, 1993–4; Larbier & Leclercq, 1994).

L-Carnitine plays a well-established role in lipid metabolism, so it may induce some favourable modifications in poultry products, particularly eggs and meat. In this regard, Lettner *et al.* (1992) indicated that feeding diets supplemented with L-carnitine up to 60 mg/kg significantly affected the fatty acid composition of abdominal fat and tended to improve fattening performance of broiler chickens. Leibetseder (1995) found that L-carnitine concentration increased significantly in egg yolks from birds fed on L-carnitine-supplemented diets (500 mg L-carnitine alone or in combination with an equal amount of

nicotinic acid per kg diet), compared with the control. In his trial on broilers, L-carnitine concentrations in tissues (liver, kidney, heart, specific skeletal muscles) were found to increase significantly in response to dietary L-carnitine supplementation.

Our results indicated that dietary L-carnitine supplementation resulted in an improvement in albumen quality (albumen height and Haugh unit score) and caused an alteration in the components of the edible part of the egg. Although egg weight was not affected by dietary L-carnitine, the percentage of egg white increased and that of egg yolk decreased in L-carnitine-supplemented groups compared with the controls.

The mechanism by which this improvement in albumen quality occurred is not yet clear. It has been reported that the ovomucin content of eggs, particularly β-ovomucin, is mainly responsible for the gelatinous properties of the thick-egg-white gel (Robinson, 1987). However, Austic (1977) has shown that eggs removed from the laying hens before their entry into the shell gland contained a greater proportion of thick egg-white and a higher concentration of ovomucin. He also indicated that the variation in ovomucin content in the proportion of thick egg-white of eggs obtained at oviposition seemed to be related to the events of plumping occurring in the shell gland of the laying hen.

The higher values for relative albumen weight of eggs laid by L-carnitine-supplemented groups may be due to the higher metabolic rate in the magnum and/or higher activity of the shell gland of the treated birds compared with the control ones. This suggestion is consistent with the evidence of Larbier & Leclercq (1994) that egg-white consists mainly of water and protein, plus small amounts of minerals, water-soluble vitamins and free glucose. They also stated that albumen proteins are synthesized in the magnum of the laying hen by highly-specialized glandular cells, while it receives the majority of its water subsequently in the uterus (shell gland). Similarly, Roberts & Brackpool (1993–4) stated that the albumen in the magnum is in a concentrated form and represents only half the volume of albumen present in a freshly-laid egg. They also reported that additional fluid (water together with glucose and electrolytes) is added to the albumen, mainly in the shell-gland pouch, to produce the final volume of the albumen.

The decrease in absolute or relative yolk weight for eggs of the supplemented groups may be due to a reduction in the hepatic biosynthesis rate of yolk precursors and/or an alteration in the mode of their transport from the liver into the ovarian follicle and the oocyte, probably caused by L-carnitine. In this connection, it has been reported that 95 % of yolk total lipids is derived from triaglycerol-rich lipoprotein which is synthesized in the liver and transferred into rapidly-developing yolks from the plasma over a period of several days before ovulation. The remaining yolk lipid is derived from the lipovitellin component of plasma vitellogenin (Griffin *et al.* 1984).

The increase in L-carnitine content of eggs has been suggested to be beneficial to the development of the chick embryo (Rinaudo *et al.* 1991). Leibetseder (1995) found that hatching rate was increased from 83 % to 87 % and from 82·4 % to 85·3 % in groups of broiler breeders supplemented with 50 and 100 mg L-carnitine respectively. Generally, under normal nutritional conditions, the proportion of the yolk in the edible part of the egg has been reported to increase with hen age (Larbier & Leclercq, 1994; Rossi & Pompei, 1995).

With respect to effect of dietary carnitine level on the indices studied, a dose-dependent response was observed only in albumen quality after 8 weeks of treatment. While significant differences in albumen quality were found between eggs produced by birds fed on the diets supplemented with L-carnitine levels of 50 *v.* 500 mg/kg, the differences were not significant either between groups supplemented with 50 *v.* 100 mg/kg or those supplemented with 100 *v.* 500 mg/kg. The same trend of response was described

by Weeden *et al.* (1991) who found a linear improvement in feed efficiency of young pigs with increasing dietary carnitine concentration. They fed L-carnitine to starter pigs at levels of 0 or 1000 mg/kg diet from 0 to 2 weeks postweaning, followed by carnitine concentrations of 0, 250 or 500 mg/kg diet for the next 3 weeks. In a previous study on fish, piglets and quail, Schuhmacher *et al.* (1993) concluded that carnitine seemed effective in improving body-weight gain and feed conversion, mainly in groups with diets marginally deficient in lysine and methionine plus cystine respectively. It seems evident that these different expressions of response of animals to dietary carnitine are mainly related to species differences, age, sex, nutritional and physiological status of the animal and the nutrient composition of their diets.

It is noteworthy that there were significant differences in values for egg-shape index after 8 weeks of L-carnitine supplementation of the diet. This trend in response may not be directly related to dietary carnitine, but may reflect indirectly the increase in egg-white percentage in response to dietary carnitine. In this regard, Roberts & Brackpool (1993–4) concluded that egg albumen acts as a 'template' for the deposition of shell membranes, and subsequently eggshell formation.

Based on the results reported here, L-carnitine had a beneficial effect on albumen quality and could modify the components of the edible part of the egg during the late laying period.

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