



The Effects of Dietary Supplementation of L-carnitine and Humic Substances on Performance, Egg Traits and Blood Parameters in Laying Hens

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ABSTRACT : This experiment was carried out to determine the effects of supplementation of L-carnitine and humic substances alone or in combination in laying hen diets on performance, egg traits and blood parameters. A total of 180 IGH type brown laying hens aged 22 weeks were employed in a completely randomized block design with one control group and three treatment groups. Each group was divided into five replicates as subgroups, each comprising 9 hens. The diets of the first, second and third treatment groups were supplemented with 0.1 g/kg L-carnitine, 1.5 g/kg humic substances (Farmagülator[®] Dry Plus) and 0.1 g/kg L-carnitine+1.5 g/kg humic substances, respectively. The experimental period lasted 18 weeks. Feeding supplemental carnitine, humic substances or carnitine+humic substances resulted in increases in body weight gain ($p<0.05$). Dietary treatments did not significantly affect daily feed intake, daily metabolizable energy intake, egg production, egg weight, feed efficiency, mortality, egg shape index, egg breaking strength, egg shell thickness, egg albumen index, egg yolk index, egg Haugh unit and the percentages of egg shell, albumen and yolk. Supplementation of humic substances reduced egg yolk cholesterol as mg per g yolk and mg per yolk ($p<0.05$). Blood serum parameters were not affected by the supplementation of carnitine, humic substances or carnitine+humic substances. The results in this study demonstrated that humic substances supplementation reduced egg cholesterol without adverse effects on performance, egg traits and blood parameters of laying hens. It was concluded that the usage of L-carnitine alone or in combination with humic substances in diets had no beneficial effects in laying hens. (**Key Words :** L-carnitine, Humic Substances, Laying Hen, Performance, Egg Traits, Blood Parameters)

INTRODUCTION

L-carnitine and humic substances are used as feed additives in poultry diets to increase yield and to improve feed efficiency.

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). Several reports on broilers and pigs have demonstrated that growth performance can be improved by feeding supplementary dietary L-carnitine (Weeden et al.,

1991; Lettner et al., 1992). There are limited articles about the effects of carnitine supplementation of laying hen diets on laying performance and egg quality (Leibetseder, 1995; Rabie et al., 1997; Çelik et al., 2004).

Humic substances include humus, humic acid, fulvic acid, ulmic acid and trace minerals. Humic substances have been shown to contain a wide variety of molecular components such as polysaccharides, fatty acids, polypeptides, lignins, esters, phenols, ethers, carbonyls, quinones and lipids. Humic acids are not soluble in water under acid conditions but are soluble under alkaline conditions (Pettit, 2004). Humic substances have many beneficial effects like antibacterial, antiviral and antiinflammatory effects. They also improve immune system, reduce odour in faeces, cause a reduction in stress and play a role in liver function (Islam et al., 2005). There are limited articles about dietary supplementation of humic substances in laying hens. The use of humic substances in animal feeds brings a number of advantages for animal health and productive performance (Eren et al., 2000; Yörük et al., 2004; Küçükersan et al., 2005).

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Received February 2, 2006; Accepted May 30, 2006

Table 1. Ingredients and chemical composition of the diets (g/kg)

Ingredient	Control group	Treatment groups		
		Carnitine	Humic substances	Carnitine +humic substances
Corn	569.5	569.5	569.5	569.5
Barley	70.0	69.9	68.5	68.4
Soyabean meal	267.0	267.0	267.0	267.0
Limestone	76.5	76.5	76.5	76.5
Dicalcium phosphate	10.0	10.0	10.0	10.0
Salt	2.5	2.5	2.5	2.5
Vitamin-mineral premix ^a	2.5	2.5	2.5	2.5
DL-methionine	1.5	1.5	1.5	1.5
Lysine	0.5	0.5	0.5	0.5
L-carnitine	-	0.1	-	0.1
Humic substances (Farmagülatör® Dry Plus)	-	-	1.5	1.5
Chemical composition (Analyzed per kg)				
Dry matter (g)	903.0	907.8	912.5	904.0
Metabolizable energy ^b (MJ)	11.40	11.34	11.38	11.46
Crude protein (g)	170.8	172.5	172.7	171.9
Crude fibre (g)	27.0	26.5	26.5	26.0
Calcium (g)	34.0	36.5	33.0	33.5
Phosphorus (g)	5.8	5.8	5.8	5.9
L-carnitine ^c (mg)	8.0	108.0	8.0	108.0

^a Composition per 2.5 kg: 12,000,000 IU vitamin A, 2,400,000 IU vitamin D₃, 30 g vitamin E, 2.5 g vitamin K₃, 2.5 g vitamin B₁, 6 g vitamin B₂, 4 g vitamin B₆, 20 mg vitamin B₁₂, 25 g niacin, 8 g calcium-D-panthotenate, 1 g folic acid, 50 g vitamin C, 50 mg D-biotin, 150 g choline chloride, 1.5 g canthaxanthin, 0.5 g apo carotenoic acid ester, 80 g Mn, 60 g Zn, 60 g Fe, 5 g Cu, 1 g I, 0.5 g Co, 0.15 g Se.

^b Metabolizable energy content of diets was estimated according to Leeson and Summers (2001).

^c Carnitine content of diets was calculated based on L-carnitine content in the ingredients reported by Baumgartner and Blum (1997).

As far as we know there is no published study about the interaction of dietary humic substances and carnitine in laying hens. It was hypothesized that these two feed additives either alone or in combination would enhance performance and egg traits by acting on nutrient metabolism in laying hens. Measuring blood parameters could be more useful to detect some metabolic effects of L-carnitine and humic substances. Therefore the aim of this study was to determine the effects of the dietary L-carnitine and humic substances alone or in combination on laying performance, egg traits and blood parameters in laying hens.

MATERIALS AND METHODS

Animals and diets

A total of 180 IGH type brown laying hens aged 22 weeks with uniform body weight were used in this study. Laying hens were employed in a completely randomized block design with one control group and three treatment groups, each containing 45 hens. Each group was divided into five replicates as subgroups, each comprising 9 hens. They were placed into cages randomly. Feed and water were provided for *ad libitum* consumption and the diets were presented in mash form. The experiment was run for 18 weeks.

The ingredients and chemical composition of the diets are presented in Table 1. The diets of the first, second and

third treatment groups were supplemented with 0.1 g/kg L-carnitine, 1.5 g/kg humic substances (Farmagülatör® Dry Plus, Farmavet International Inc., Kocaeli 41400, Turkey) and 0.1 g/kg L-carnitine+1.5 g/kg humic substances, respectively. L-carnitine and humic substances were supplemented by replacing the same amount of barley. Farmagülatör® Dry Plus contained 8% moisture, 35% sodium humate, 6% fulvic acid, 6% trace minerals, 20% SiO₂ and 25% kaolin.

Traits measured

Nutrient composition of diets were determined according to the AOAC (1990). Metabolizable energy levels of diets were estimated using a prediction equation (Leeson and Summers, 2001):

$$\text{ME (kcal/kg)} = 53 + 38 [(\text{crude protein, \%}) + (2.25 \times \text{ether extract, \%}) + (1.1 \times \text{starch, \%}) + (\text{sugar, \%})]$$

The levels of L-carnitine in the diets were calculated based on the L-carnitine content in the ingredients reported by Baumgartner and Blum (1997).

Hens were weighed individually at the beginning and at the end of the experiment. Mortality was recorded as it occurred. Eggs were collected daily and egg production was

Table 2. The effects of dietary supplementation of L-carnitine and humic substances on laying performance (mean±standard error)

	Control group	Treatment groups		
		Carnitine	Humic substances	Carnitine +humic substances
Initial body weight (g)	1,824±18	1,791±17	1,768±15	1,793±28
Body weight gain (g)	197±27	294±21*	285±21*	294±23*
Feed intake (g/hen-d)	125±2	122±2	122±1	121±2
ME intake (MJ/hen-d)	1.43±0.02	1.39±0.02	1.39±0.02	1.38±0.02
Hen-day egg production (%)	88.9±1.5	91.1±1.0	93.1±0.7	90.5±1.3
Egg weight (g)	59.6±0.8	59.3±0.5	59.5±0.4	59.3±0.6
Feed efficiency (kg feed/kg egg)	2.37±0.04	2.27±0.04	2.20±0.02	2.26±0.05

n = 5 per group. * p<0.05, treatment groups compared with control group by Dunnett test.

calculated on a hen-day basis. Eggs were weighed two times a week individually. Feed intake was recorded biweekly and calculated as g per hen per day. The value of feed efficiency was calculated as kg feed per kg egg. Metabolizable energy intake was determined as MJ per hen per day.

To determine the egg traits 15 eggs were collected randomly from each group (3 eggs from each replicate) at the first day of the 3rd, 8th, 13th and 18th week of the experiment (as a total 60 eggs per group during the experiment). Individual eggs were weighed and their shape index, shell breaking strength and shell thickness were measured. Then the values of yolk height, albumen height, yolk width, albumen width and albumen length were determined. By using these values yolk index, albumen index and Haugh unit were calculated (Card and Nesheim, 1972). Egg internal quality and shell quality analyses were completed within 24 h of the eggs being collected. At the end of the experiment 30 eggs per group (6 eggs from each replicate) were randomly chosen to determine yolk cholesterol. Eggs were boiled for 5 minutes. The weight of whole egg, egg shell, yolk and albumen were recorded. The percentage values of shell weight, yolk weight and albumen weight were calculated. Cholesterol was extracted according to the method of the AOAC (1990) procedure 941.09. Yolk cholesterol was determined as mg per g yolk and mg per yolk.

At the end of the experiment blood samples were collected from the *Vena brachialis* under the wing from 15 hens randomly chosen from each group (3 from each replicate) and centrifuged at 3,000 g for 10 min. Serum was collected and stored at -20°C for determination of serum parameters. Serum concentrations of total protein, triglyceride, cholesterol, very low density lipoprotein (VLDL) and levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and creatine kinase (CK) were determined by Hitachi autoanalyser (Hitachi Ltd, Tokyo Serial Number 1238-23) using their accompanying commercial kits.

Statistical analyses

Statistical analyses were done using the SPSS

programme (SPSS Inc., Chicago, IL, USA). Each group in the trial consisted of five replicates in a complete randomized block design experiment. The normality of data distribution was checked using the Kolmogorov-Smirnov test. The means of initial body weight and egg weight in each replicate in each group were used as covariates for statistical analysis of all response variables. One-way ANOVA was performed to examine differences among groups. When significant interaction was found, a follow-up test using Dunnett was performed. Level of significance was taken as p<0.05. Values were given as mean±standard error (Dawson and Trapp, 2001).

RESULTS AND DISCUSSION

During the experimental period one laying hen died in the group fed diets containing L-carnitine. No mortality was seen in other groups. Similarly, some researchers found that mortality rate in broilers and laying hens was not affected by the supplementation of humate (Eren et al., 2000; Yalçın et al., 2003; Özçelik and Yalçın, 2004; Yörük et al., 2004) and carnitine (Rabie et al., 1997; Özçelik and Yalçın, 2004; Yalçın et al., 2005) or carnitine+humate (Özçelik and Yalçın, 2004; Yalçın et al., 2005). Dietary carnitine supplementation has been shown to improve livability due to more efficient utilization of fatty acids in heart muscle (Daşkıran and Teeter, 2001).

The effects of carnitine and humic substances on laying performance are shown in Table 2. The supplementation of carnitine, humic substances or carnitine+humic substances to the diets of laying hens resulted in increases in body weight gain (p<0.05). The results are in agreement with the results of studies involving broilers fed diets supplemented with carnitine (Rabie and Szilagyi, 1998; Kita et al., 2002; Çelik and Öztürkcan, 2003). The improvement in body weight gain caused by dietary L-carnitine supplementation may be partially explained by an increase in the concentration of plasma insulin-like growth factor-I, which consists of 70 amino acids and has the potency to stimulate body weight gain (Kita et al., 2002). The mechanism by which humic substances affect poultry performance is

Table 3. The effects of dietary supplementation of L-carnitine and humic substances on egg traits of laying hens (mean \pm standard error)*

	Control group	Treatment groups		
		Carnitine	Humic substances	Carnitine +humic substances
Egg shape index	78.5 \pm 0.3	79.1 \pm 0.3	78.5 \pm 0.3	79.3 \pm 0.3
Egg shell breaking strength (kg/cm ²)	2.98 \pm 0.06	3.13 \pm 0.06	2.97 \pm 0.07	2.98 \pm 0.07
Egg shell thickness (μ m)	412 \pm 4	413 \pm 4	408 \pm 5	410 \pm 4
Egg albumen height (mm)	6.44 \pm 0.06	6.41 \pm 0.06	6.61 \pm 0.07	6.51 \pm 0.07
Egg albumen index	8.67 \pm 0.13	8.64 \pm 0.12	8.93 \pm 0.15	8.95 \pm 0.16
Egg yolk index	46.7 \pm 0.3	46.3 \pm 0.3	46.4 \pm 0.4	47.0 \pm 0.4
Egg Haugh unit	80.3 \pm 0.4	80.2 \pm 0.4	81.3 \pm 0.4	80.8 \pm 0.4

n = 60 per group. * No significant differences among groups, One-way ANOVA.

Table 4. The effects of dietary supplementation of L-carnitine and humic substances on percentages of egg components and egg yolk cholesterol (mean \pm standard error)

	Control group	Treatment groups		
		Carnitine	Humic substances	Carnitine +humic substances
Shell percentage (%)	11.6 \pm 0.3	11.8 \pm 0.3	11.7 \pm 0.3	11.4 \pm 0.2
Albumen percentage (%)	60.6 \pm 0.4	61.4 \pm 0.4	61.1 \pm 0.5	61.1 \pm 0.5
Yolk percentage (%)	27.8 \pm 0.4	26.9 \pm 0.5	27.3 \pm 0.5	27.5 \pm 0.4
Yolk cholesterol (mg/g yolk)	14.5 \pm 0.6	14.3 \pm 0.5	12.8 \pm 0.3*	13.4 \pm 0.5
Total yolk cholesterol (mg/yolk)	243 \pm 9	230 \pm 9	211 \pm 6*	222 \pm 8

n = 30 per group. * p<0.05, treatment group fed diet containing humic substances compared with control group by Dunnett test.

largely unknown. There are limited number of articles show that humic substances promote growth by altering partitioning of nutrient metabolism (Parks et al., 1986; Stepchenko et al., 1991). In some studies, L-carnitine supplementation of the diets of laying hens (Leibetseder, 1995; Çelik et al., 2004), laying quails (Yalçın et al., 2005) and broilers (Buyse et al., 2001; Daşkiran and Teeter, 2001; Lien and Horng, 2001; Özçelik and Yalçın, 2004), and humate supplementation of the diets of laying quails (Yalçın et al., 2005) and of broilers (Yalçın et al., 2003; Özçelik and Yalçın, 2004) did not alter the body weight at the end of the experiment.

Daily feed intake and daily metabolizable energy intake values were not affected by dietary treatments. Similar to the results of the present study, carnitine (Leibetseder, 1995; Rabie et al., 1997; Çelik et al., 2004; Yalçın et al., 2005), humate (Yörük et al., 2004; Yalçın et al., 2005) and carnitine+humate (Yalçın et al., 2005) supplementation had no effect on the feed intake of laying hens and laying quails. However, some researchers (Tancho, 1999; Hayırlı et al., 2005) reported that dietary humate supplementation increased feed intake in laying hens.

There were no differences in egg production among the groups in the present study. The results of the present study are also in agreement with the results of some researchers who found that carnitine (Leibetseder, 1995; Rabie et al., 1997; Çelik et al., 2004; Yalçın et al., 2005), humate (Küçükersan et al., 2005; Yalçın et al., 2005) and carnitine+humate (Yalçın et al., 2005) had no effect on egg

production of laying hens and laying quails. However, egg production values of laying quails fed 0.5 g/kg L-carnitine (Bayram et al., 1999) and of laying hens fed 1 and 2 g/kg humate (Tancho, 1999; Yörük et al., 2004; Hayırlı et al., 2005) were higher than those of the control group. The mean values of egg weight were not affected by dietary treatments. Similar to the results of the present study, carnitine (Rabie et al., 1997; Çelik et al., 2004; Kita et al., 2005), humate (Tancho, 1999; Yörük et al., 2004; Hayırlı et al., 2005; Küçükersan et al., 2005; Yalçın et al., 2005) and carnitine+humate (Yalçın et al., 2005) supplementation had no effect on the egg weight of laying hens and laying quails. However carnitine supplementation of laying quail diets increased egg weight (p<0.01) compared to the control group in the study of Yalçın et al. (2005).

There were no differences in the values of feed efficiency among the groups in the present study. These results are in agreement with the results of some studies of the supplementation of carnitine (Rabie et al., 1997; Çelik et al., 2004; Özçelik and Yalçın, 2004; Yalçın et al., 2005), humate (Yalçın et al., 2005) and carnitine+humate (Özçelik and Yalçın, 2004; Yalçın et al., 2005) in poultry. However some researchers found improvements in feed efficiency for hens fed humate at levels of 1 and 2 g/kg (Tancho, 1999; Yörük et al., 2004; Hayırlı et al., 2005) in the diets compared with hens fed a control diet.

The effects of dietary supplementation of carnitine and humic substances on egg traits are shown in Table 3 and the effects on percentages of egg components and egg yolk

Table 5. The effects of dietary supplementation of carnitine and humic substances on blood serum parameters of laying hens (mean \pm standard error)*

	Control group	Treatment groups		
		Carnitine	Humic substances	Carnitine +humic substances
Total protein (g/L)	54.8 \pm 1.7	53.7 \pm 1.0	56.7 \pm 0.9	54.9 \pm 1.1
Triglyceride (g/L)	13.9 \pm 0.6	14.6 \pm 0.7	14.6 \pm 0.6	15.0 \pm 0.6
Cholesterol (mmol/L)	4.57 \pm 0.38	4.74 \pm 0.34	4.27 \pm 0.21	4.19 \pm 0.27
VLDL (g/L)	2.84 \pm 0.11	3.00 \pm 0.10	2.96 \pm 0.11	3.03 \pm 0.10
ALT (U/L)	6.53 \pm 0.72	6.73 \pm 0.84	4.87 \pm 0.46	4.80 \pm 0.47
AST (U/L)	190 \pm 9	186 \pm 12	188 \pm 6	178 \pm 5
Creatine kinase (U/L)	1,123 \pm 135	1,181 \pm 168	1,324 \pm 114	1,117 \pm 126

VLDL: very low density lipoprotein, ALT: alanine aminotransferase, AST: aspartate aminotransferase.

n = 15 per group. * No significant differences among groups, One-way ANOVA.

cholesterol are shown in Table 4. In this study, feeding supplemental carnitine, humic substances or carnitine+humic substances had no effect on egg shape index, egg shell breaking strength, egg shell thickness, shell percentage, albumen height, albumen index, albumen percentage, yolk index, yolk percentage and Haugh unit. Yalçın et al. (2005) also reported that the feeding of supplemental carnitine, humate or carnitine+humate to laying quails had no effect on egg-shell thickness, shell percentage, yolk index, yolk percentage and albumen percentage. Similar to the results of the present study, egg shell thickness was not affected by carnitine (Rabie et al., 1997) and humate (Yörük et al., 2004) supplementation in laying hens, but Tancho (1999) concluded that egg shell thickness was increased with humate supplementation. The values of albumen height and Haugh unit were increased by carnitine (Rabie et al., 1997; Kita et al., 2005) and by humate (Yalçın et al., 2005) supplementation in laying hens and quails, respectively. In contrast to these results, Hayırlı et al. (2005) indicated that albumen index and Haugh unit decreased ($p < 0.01$) linearly as humate supplementation level increased. In disagreement with the present study, Rabie et al. (1997) reported that L-carnitine supplemented diets increased ($p < 0.01$) albumen percentage and decreased ($p < 0.01$) yolk weight and yolk percentage after 8 weeks of treatment.

Supplementation of carnitine to the laying hen diets had no effect on egg cholesterol content. Similar to the result of the present study, carnitine supplementation had no effects on egg yolk cholesterol content of laying hens (Leibetseder, 1995) and laying quails (Yalçın et al., 2005).

Humic substances supplementation reduced egg yolk cholesterol as mg per g yolk and mg per yolk ($p < 0.05$) in the present study. Combination of carnitine and humic substances in diets caused a nonsignificant reduction in egg cholesterol level. The mechanism of this effect is not known. Further studies should be done to detect the interaction between carnitine and humic substances.

The effects of carnitine and humic substances supplementation on blood serum parameters of laying hens are shown in Table 5. It was observed in the present study

that there were no differences among groups in serum levels of total protein, triglyceride, cholesterol, VLDL, ALT, AST and CK. These results are in agreement with the results of Yalçın et al. (2005). Serum cholesterol levels of laying hens (Leibetseder, 1995) and broilers (Lien and Horng, 2001) were not affected by the administration of L-carnitine in the feed. In contrast to the present study, carnitine supplementation increased plasma cholesterol (Eder, 2000), reduced serum triglyceride (Xu et al., 2003) and humate supplementation increased serum total protein and decreased serum triglyceride and VLDL concentrations (Hayırlı et al., 2005).

Different responses to supplementary carnitine and humic substances from other studies might be due to the species, age, sex, plane of nutrition, nutrient composition of the diet, levels of L-carnitine or its precursors, levels of humic substances in the diet, the duration of supplementation or environmental conditions.

As a result, humic substances supplementation reduced egg cholesterol content without adverse effects on performance, egg weight, other egg traits and blood parameters of laying hens. The usage of L-carnitine alone or in combination with humic substances in diets had no beneficial effects. Further studies are needed to understand these situations and to clarify the cholesterol-depressing effect in the egg yolk due to dietary humic substances.

ACKNOWLEDGEMENTS

This study was supported by Ankara University Research Fund (Project No. 2002 08 10 042). We thank Professor Dr. Osman Saraçbaşı for their assistance with data analysis.

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