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Study the Effects of Different Levels of Energy and L-carnitine on Meat Quality and Serum Lipids of Japanese Quail

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ABSTRACT

Effects of various levels of energy and L-carnitine on meat quality and serum lipids of Japanese quail were examined. This experiment was carried out using 480 quails in a completely randomized design with two levels of energy (2900 and 3100 kcal kg^{-1}) and three levels of L-carnitine (0, 250 and 500 mg kg^{-1}) by factorial arrangement. Four replicates with 20 quails were allocated to each experimental treatment and birds were reared for 42 days. At the end of the experiment, two birds from each experimental unit were selected and after slaughter and separation of carcasses, thigh and breast samples were transferred to the freezer to asses meat quality parameters. The results showed that using of higher levels of energy increased the amount of blood cholesterol and triglyceride ($p<0.05$). The quails were fed with ration containing L-carnitine supplementation, had lower triglyceride in comparison with control group ($p<0.05$). Higher levels of energy increased the amount of crude fat and malonaldehyde in breast samples. The amount of malonaldehyde in breast samples after storage for 90 days and amount of crude fat and malonaldehyde in thigh samples after storage for 30 and 90 days were affected by different levels of L-carnitine, so that using of 250 mg kg^{-1} L-carnitine significantly reduced the amount of malonaldehyde in breast samples and crude fat and malonaldehyde in thigh samples ($p<0.05$). Therefore, it can be concluded that the supplementation of diet with L-carnitine has positive effects on blood triglyceride and meat quality in Japanese quail.

Key words: Japanese quail, malonaldehyde, L-carnitine, peroxidation, antioxidant

INTRODUCTION

Lipid oxidation is the primary cause of rancidity during frozen storage of meat (Ryu *et al*., 2005). Skeletal muscle is particularly susceptible to oxidative reactions, since it contains high concentration of pro-oxidants (transition metals, haem containing proteins such as myoglobin, hemoglobin) and hpid membrane which contain higher percentage of Polyunsaturated Fatty Acids (PUFAs) (Kanner, 1994).

L-carnitine (β-hydroxy γ-trimethyl amino butyrate) is a water-soluble quaternary amine that exists naturally in micro-organisms, plants and animals and is required for the long chain fatty acid transfer from cytoplasm to mitochondrial matrix for subsequent β-oxidation and energy production (Miah *et al*., 2004). L-carnitine is used as feed additive in poultry diets to increase yield and to improve feed efficiency (Rezaei *et al*., 2007). Thus, L-carnitine supplementation to diets reduces

long chain fatty acid availability for esterification to triacylglycerols and storage in the adipose tissue (Xu *et al*., 2003). Feeding diets with supplemental fat to poultry can have distinct economic advantages by providing increased energy levels at a lower cost. This is becoming a general practice in poultry production (Russell *et al*., 2003). Fats added to the diet of fast growing broilers are generally rich in PUFAs (Lauridsen *et al*., 1997). Oils rich in PUFAs have a higher metabolizable energy than animals fats because PUFAs are better digested than saturated fatty acids (Blanch *et al*., 1995). Increasing the PUFAs content of poultry diets increases the proportion of unsaturated fatty acids in meat and other edible parts (Sarica *et al*., 2007). Since lipid oxidation is a major problem in products enriched with n-3 PUFAs, it can be a primary cause of quality deterioration in meat and meat products (Lawlor *et al*., 2003).

L-carnitine has antioxidant properties. It functions by reducing the availability of lipids for peroxidation by transporting fatty acids into the mitochondria for β-oxidation to generate ATP energy (Nouboukpo *et al*., 2010). This reduces the amount of lipids available for peroxidation (Kalaiselvi and Panneerselvam, 1998). Furthermore, L-carnitine, through its antioxidant properties, has been shown to increase the activity and levels of antioxidant enzymes such as superoxide dismutase and glutathione peroxidase in the plasma of poultry (Neuman *et al*., 2002).

Less study has been done to determine whether dietary L-carnitine supplementation can influence the quail meat quality. Sarica *et al*. (2007) reported that dietary L-carnitine supplementation (50 mg kg^{-1} of diet) decreased malonaldehyde amounts in the quail edible meat. The objective of the current study was to determine the effects of different levels of energy and L-carnitine on meat quality and serum lipids of Japanese quail.

MATERIALS AND METHODS

This experiment was conducted using a 2×3 factorial design with two levels of Metabolizable Energy (ME) (2900 and 3100 kcal kg^{-1}) and three levels of dietary L-carnitine (0, 250 and 500 mg kg^{-1}) from Jun 8, 2010 to Nov 10, 2010 at Gorgan University of Agricultural Sciences and Natural Resources, Iran. In this study, 480 Japanese quail were randomly allocated to six dietary treatments. Each treatment had four replicates with 20 birds per cage (100×100 cm). Each cage was equipped with bell-drinker and a feeder. The experimental diets were formulated to meet minimum nutrient requirements of quails, as established by the National Research Council (NRC, 1994). The composition and the calculated nutrient content of the experimental diets are presented in Table 1. Experimental diets (in mash form) and water were provided *ad libitum*. House temperature was maintained at 37°C for the first week and reduced 2°C weekly thereafter. A continuous lighting program was provided during the experiment.

Sample collection: In day 42, 2 mL of blood was collected from jugular vein from 8 birds in each treatment, then two quail from each pen, with body weight similar to pen average body weight, was selected and slaughtered to determine meat quality. The meet was separated manually from bone and then homogenized using a blender with horizontal blades. Samples were then frozen and stored in a freezer at -20°C until further analysis. These samples were analyzed for moisture, pH, Water Holding Capacity (WHC), crude fat and 2-Thiobarbituric Acid Reactive Substances (TBARS). Crude fat was calculated with using standard methods outlined by AOAC (1990). Fat was extracted with ethyl ether using a soxhlet apparatus.

Measurement of lipid oxidation: Lipid oxidation was measured by the 2-thiobarbituric acid distillation method of Tarladgis *et al*. (1960) and results were expressed as 2-Thiobarbituric Acid

Table 1: Composition of experimental diets

Item	Low energy diet	High energy diet
Ingredients (%)		
Corn	52.11	47.68
Soybean meal	41.23	41.97
Soybean oil	1.98	5.67
Fish meal	2.00	2.00
Dicalcium phosphate	0.48	0.49
Limestone	1.20	1.20
Salt	0.30	0.30
Mineral premix	0.25	0.25
Vitamin premix	0.25	0.25
Salinomycin	0.05	0.05
DL-methionine	0.12	0.12
L-carnitine	-	-
Calculated composition		
Metabolizable energy (kcal kg^{-1})	2900.00	3100.00
Crude protein (%)	24.00	24.00
Calcium (%)	0.80	0.80
Available phosphorus (%)	0.30	0.30
Sodium (%)	0.15	0.15
Lysine (%)	1.33	1.33
Methionine (%)	0.50	0.50
Methionine+cystine (%)	0.88	0.88

Each kg of vitamin premix contained: Vitamin A, 3,500,000 IU: Vitamin D_3, 1,000,000 IU: Vitamin E, 9000 IU: Vitamin K_3, 1000 mg; Vitamin B_1, 900 mg; Vitamin B2, 3,300 mg; Vitamin B3, 5,000 mg; Vitamin B5, 15,000 mg; Vitamin B6, 150 mg; Vitamin B9, 500 mg; Vitamin B_{12}, 7.5 mg; Biotin, 500 mg; Choline chloride, 250,000 mg and each kg of mineral premix contained: Mn, 50,000 mg; Fe, 25,000 mg; Zn, 50,000 mg; Cu, 5,000 mg; I, 500 mg; Se, 100 mg

Reactive Substances (TBARS) in mg Malonaldehyde (MDA) kg^{-1} meat. TBARS values were measured on days 30 and 90 for raw quails thigh and breast.

Measurement of the water holding capacity: Water holding capacity was estimated (Castellini *et al.*, 2002) by centrifuging 1 g of the muscles placed on tissue paper inside a tube for 4 min at 1500 g. The water remaining after centrifugation was quantified by drying the samples at 70°C overnight. WHC was calculated as:

$$WHC=\frac{\text{Weight after centrifugation (g)} - \text{Weight after drying (g)}}{\text{Initial weight (g)}}\times 100$$

Measurement of moisture and pH: Moisture was determined using 100°C oven for 16-18 h (AOAC, 1990). Meat pH was determined by blending a 10 g sample in 100 mL distilled water for one minute and pH was measured using a pH meter (Model Inolab pH level-1) (Ensoy *et al.*, 2004).

Serum lipids assay: Blood samples were centrifuged (at, 2,000× g for 10 min) and serum was separated and then stored at -20°C until assayed for measuring serum lipids (cholesterol, High-Density Lipoprotein (HDL) and triglyceride) using appropriate laboratory kits (Gowenlock *et al.*, 1988). Very Low-Density Lipoprotein (VLDL) cholesterol was calculated from triglyceride by dividing the factor 5. The LDL cholesterol was calculated by using the formula:

LDL cholesterol = Total cholesterol-HDL cholesterol-VLDL cholesterol

Statistical analysis: The data obtained from the experiment was analyzed by using SAS (SAS, 1999) statistical programs with the ANOVA. Significant differences among treatment means were separated using Duncan' s multiple range test with a 5% probability (Duncan, 1955).

RESULTS

Serum lipids: Effects of energy and L-carnitine on serum lipids of quails are presented in Table 2. The using of higher levels of energy significantly increased the amount of blood cholesterol, triglyceride and VLDL ($p<0.05$). The quails were fed with ration containing L-carnitine supplementation (250 mg kg^{-1}) had lower triglyceride and VLDL in comparison with control group ($p<0.05$). The effect of L-carnitine on other serum lipids including, cholesterol, High-Density Lipoprotein (HDL) and Low-Density Lipoprotein (LDL) were not significant ($p>0.05$). There was no interaction between energy density and L-carnitine level of diets in terms of serum lipids ($p>0.05$).

Breast meat quality 30 days after slaughter: Table 3 shows the effects of energy density and L-carnitine supplementation on breast meat quality 30 days after slaughter. The effect of energy

Table 2: Effects of energy and L-carnitine on serum lipids of quail

Main effects	Triglyceride	Cholesterol	HDL	VLDL	LDL
ME (kcal kg^{-1})					
2900	159.31^{b}	212.49^{b}	131.24	31.86^{b}	49.38
3100	164.19^{a}	218.008^{a}	132.08	32.83^{a}	53.08
p-valu	0.003	0.008	0.46	0.003	0.07
SEM	1.004	1.31	0.78	0.20	1.39
L-carnitine (mg kg^{-1})					
0	163.36^{a}	217.36	131.87	32.67^{a}	52.81
250	158.88^{b}	212.51	130.85	31.17^{b}	51.01
500	163.01^{a}	215.87	132.26	32.77^{a}	49.88
p-value	0.034	0.12	0.57	0.034	0.48
SEM	1.23	1.61	0.96	0.24	1.71

Mean values in the same column with different superscript letters were significantly different ($p<0.05$) HDL: High density lipoprotein, VLDL: Very low density lipoprotein, LDL: Low density lipoprotein

Table 3: Breast meat quality indexes 30 days after slaughter

Main effects	WHC (%)	pH	Moisture (%)	Crude fat (%)	TBARS
ME (kcal kg^{-1})					
2900	59.14	6.17	73.48	13.31^{b}	0.78^{b}
3100	60.44	6.08	74.29	14.37^{a}	0.98^{a}
p-value	0.14	0.44	0.27	0.015	0.034
SEM	0.60	0.077	0.50	0.28	0.063
L-carnitine (mg kg^{-1})					
0	60.30	6.15	73.72	14.32	0.94
250	59.76	6.10	74.80	13.56	0.82
500	59.31	6.13	73.14	13.66	0.88
p-value	0.64	0.93	0.19	0.26	0.53
SEM	0.73	0.094	0.62	0.34	0.077

Mean values in the same column with different superscript letters were significantly different ($p<0.05$)

on breast meat quality indexes such as WHC, pH and moisture were not significant ($p>0.05$). But the effect of energy on crude fat and TBARS were significant ($p<0.05$), so that quails were fed with ration containing 3100 kcal kg^{-1} ME had higher amount of crude fat and malonaldehyde in breast samples 30 days after slaughter ($p<0.05$). The effect of L-carnitine on breast meat quality indexes such as WHC, pH, moisture, crude fat and TBARS 30 days after slaughter were not significant ($p>0.05$). The interaction between dietary treatments were not significant for breast meat quality indexes 30 days after slaughter ($p>0.05$).

Breast meat quality 90 days after slaughter: Table 4 shows the effects of energy density and L-carnitine supplementation on breast meat quality 90 days after slaughter. The effect of energy on breast meat quality indexes such as WHC, pH and moisture, 90 days after slaughter were not significant ($p>0.05$). But the effect of energy on crude fat and TBARS were significant ($p<0.05$), so that quails were fed with ration containing 3100 kcal kg^{-1} ME had higher amount of crude fat and malonaldehyde in breast samples 90 days after slaughter ($p<0.05$). The effect of L-carnitine on breast meat quality indexes such as WHC, pH, moisture and crude fat 90 days after slaughter were not significant ($p>0.05$). But the effect of L-carnitine on breast TBARS, 90 days after slaughter were significant ($p<0.05$), so that quails were fed with ration containing L-carnitine supplementation (250 mg kg^{-1}) had lower amount of TBARS in comparison with control group ($p<0.05$). The interaction between dietary treatments were not significant for breast meat quality indexes 90 days after slaughter ($p>0.05$).

Thigh meat quality 30 days after slaughter: Effects of energy and L-carnitine on thigh meat quality 30 days after slaughter are presented in Table 5. The effect of energy on thigh meat quality indexes such as WHC, pH and moisture were not significant ($p>0.05$). But the effect of energy on crude fat and TBARS were significant ($p<0.05$), so that quails were fed with ration containing 3100 kcal kg^{-1} ME had higher amount of crude fat and malonaldehyde in thigh samples 30 days after slaughter ($p<0.05$). The effect of L-carnitine on breast meat quality indexes such as WHC, pH and moisture 30 days after slaughter were not significant ($p>0.05$). But the effect of L-carnitine on thigh TBARS and crude fat 30 days after slaughter were significant ($p<0.05$), so that quails were fed with ration containing L-carnitine supplementation (250 mg kg^{-1}) had lower amount of TBARS and crude fat in comparison with control group ($p<0.05$). The interaction between dietary treatments were not significant for thigh meat quality indexes 30 days after slaughter ($p>0.05$).

Table 4: Breast meat quality indexes 90 days after slaughter

Main effects	WHC (%)	pH	Moisture (%)	Crude fat (%)	TBARS
ME (kcal kg^{-1})					
2900	53.79	5.99	76.12	13.47^{b}	1.004^{b}
3100	52.91	6.006	76.45	14.72^{a}	1.23^{a}
p-value	0.39	0.71	0.57	0.02	0.02
SEM	0.70	0.022	0.41	0.25	0.033
L-carnitine (mg kg^{-1})					
0	53.61	5.98	75.97	14.52	1.21^{a}
250	52.98	5.99	76.91	13.79	1.03^{b}
500	53.46	6.03	75.98	13.97	1.10^{ab}
p-value	0.86	0.42	0.34	0.25	0.024
SEM	0.86	0.027	0.50	0.31	0.041

Mean values in the same column with different superscript letters were significantly different ($p<0.05$)

Table 5: Thigh meat quality indexes 30 days after slaughter

Main effects	WHC (%)	pH	Moisture (%)	Crude fat (%)	TBARS
ME (kcal kg^{-1})					
2900	59.10	6.20	73.98	23.002^{b}	1.96^{b}
3100	60.59	6.10	74.41	26.17^{a}	2.18^{a}
p-value	0.11	0.33	0.52	0.004	0.001
SEM	0.63	0.076	0.46	0.52	0.030
L-carnitine (mg kg^{-1})					
0	59.66	6.19	73.98	25.97^{a}	2.20^{a}
250	59.90	6.10	75.19	23.33^{b}	1.95^{b}
500	59.98	6.17	73.41	24.47^{ab}	2.06^{b}
p-value	0.95	0.76	0.10	0.03	0.001
SEM	0.77	0.093	0.56	0.63	0.037

Mean values in the same column with different superscript letters were significantly different ($p<0.05$)

Table 6: Thigh meat quality indexes 90 days after slaughter

Main effects	WHC (%)	pH	Moisture (%)	Crude fat (%)	TBARS
ME (kcal kg^{-1})					
2900	52.26	6.14	75.45	22.78^{b}	2.06^{b}
3100	52.68	6.06	76.26	26.11^{a}	2.61^{a}
p-value	0.70	0.31	0.08	0.01	0.01
SEM	0.76	0.056	0.31	0.47	0.023
L-carnitine (mg kg^{-1})					
0	52.99	6.15	75.50	25.74^{a}	2.56^{a}
250	51.57	6.006	76.61	23.31^{a}	2.34^{b}
500	52.85	6.15	75.46	24.29^{ab}	2.41^{b}
p-value	0.50	0.23	0.08	0.029	0.01
SEM	0.93	0.069	0.38	0.58	0.028

Mean values in the same column with different superscript letters were significantly different ($p<0.05$)

Thigh meat quality 90 days after slaughter: Effects of energy and L-carnitine on thigh meat quality 90 days after slaughter are presented in Table 6. The effect of energy on thigh meat quality indexes such as WHC, pH and moisture were not significant ($p>0.05$). But the effect of energy on crude fat and TBARS were significant ($p<0.05$), so that quails were fed with ration containing 3100 kcal kg^{-1} ME had higher amount of crude fat and malonaldehyde in thigh samples 90 days after slaughter ($p<0.05$). The effect of L-carnitine on breast meat quality indexes such as WHC, pH and moisture 90 days after slaughter were not significant ($p>0.05$). But the effect of L-carnitine on thigh TBARS and crude fat 90 days after slaughter were significant ($p<0.05$), so that quails were fed with ration containing L-carnitine supplementation (250 mg kg^{-1}) had lower amount of TBARS and crude fat in comparison with control group ($p<0.05$). The interaction between dietary treatments were not significant for thigh meat quality indexes 90 days after slaughter ($p>0.05$).

DISCUSSION

Findings in this study are consistent with Rezaei *et al.* (2007) who reported adding L-carnitine to diets significantly decreased the level of serum Triglyceride (TG), cholesterol and VLDL in broiler chicks. L-carnitine may increased fatty acid oxidation and thus reduced blood triglyceride levels in quails. With increasing the transportation capacity of fatty acids to inner mitochondrial membrane,

the serum TG level was reduced. L-carnitine supplementation to diets containing high level of fat, increases oxidation of fatty acids and reduces the secretion of VLDL in liver, thus the level of serum VLDL reduces.

Contrary to present study, Corduk *et al.* (2007) reported that various levels of energy had no significant effect on the blood parameters such as cholesterol and triglyceride. Furthermore, Arslan *et al.* (2004) observed that L-carnitine administration via drinking water did not influence serum total cholesterol, total lipid and triglyceride of Japanese quail. The discrepancies between studies may result from different levels of L-carnitine supplementation, basic carnitine levels in the raw ingredients, the supply or absence of essential amino acids (Rodehutscord *et al.*, 2002), the possible effects of enzymatic breakdown of branched-chain amino acids (Corduk *et al.*, 2007), sparing effects of carnitine with regard to its precursors (lysine and methionine), limited intestinal absorptive capacity of carnitine and its considerable microbial degradation in the intestine (Xu *et al.*, 2003), interspecies differences, age, sex, feeding programand the managerial or environmental conditions of the animals (Celik and Ozturkcan, 2003).

In theory, dietary L-carnitine supplementation could play a role in reducing undesirable carcass fat in poultry. It is suggested that if dietary fat sources rich in long-chain fatty acids have been included in the diets at levels higher than 10 g kg^{-1} and if different levels of L-carnitine were investigated, the role of dietary L-carnitine supplementation in energy metabolism might have been more evident, especially its effects on carcass parameters, abdominal fat content and ether extract contents of total edible meat of quails (Rabie and Szilagyi, 1998).

The abdominal fat and ether extract contents of total edible meat of the quails might have been influenced by differences in the fatty acid composition of dietary fat because L-carnitine has a key role in facilitating the transport of long-chain fatty acids across the inner mitochondrial membrane for β-oxidation to generate ATP, thereby reducing their availability for esterification to triacylglycerols and storage in the adipose tissues (Rabie and Szilagyi, 1998).

These results are in agreement with findings of the previous study Sarica *et al.* (2007). Sarica *et al.* (2007) reported that feeding diets containing L-carnitine significantly decreased malonaldehyde amounts in the edible meat. Also, various levels of energy had no significant effect on the meat quality indexes such as pH and moisture. External factors such as heat, trauma, infection, toxin and exercises can lead to increased free radicals and other Reactive Oxygen Species (ROS) (Halliwell and Gutteridge, 1994). ROS, including hydrogen peroxide, superoxide and hydroxyl, have the potential to induce considerable cell deaths via lipid peroxidation. Decreasing MA amounts of edible meat observed in the present study in response to L-carnitine supplementation might be attributed at least partly to an increased rate of the transport of long-chain fatty acids into the mitochondria. Dietary L-carnitine supplementation promotes the β-oxidation of these fatty acids to generate ATP energy and improves energy utilization (Rabie *et al.*, 1997). Consequently, L-carnitine supplementation to diets reduces the amount of long-chain fatty acids availability for esterification to triacylglycerols and storage in the adipose tissue (De-Beer and Coon, 2009).

CONCLUSION

The supplementation of diet with L-carnitine has positive effects on blood triglyceride and meat quality by reducing MA amount in Japanese quail. But because of there are few research in Japanese quail, further investigation are required to identify the role of L-carnitine in the oxidation of long-chain fatty acids, its antioxidants properties and importance in energy metabolism in Japanese quail.

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