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Effect of Vegetable Oil Source and L-Carnitine Supplements on Growth Performance, Carcass Characteristics and Blood Biochemical Parameters of Japanese Quails (*Coturnix japonica*)

Research Article

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ABSTRACT

An experiment was conducted to study the effects of soybean, linseed and sunflower oil (various sources of fatty acids) with and without L-carnitine supplements (0 and 50 mg kg^{-1}) on performance, blood biochemical parameters and carcass traits of Japanese quail. One hundred and ninety-two of 7-day old female Japanese quail were randomly assigned to 6 dietary treatments with 4 replicates and fed in the duration of 28 days. A 3 × 2 factorial arrangement (three oil sources and two levels of L-carnitine) was used in a completely randomized design with 8 birds per cage. Feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR) of birds were measured during the experiment. Moreover, 2 birds from each cage (replicate) were randomly selected at the end of the experiment and then the concentration of cholesterol, albumin and total protein of blood sera were measured. Results showed that BWG and FCR were affected by dietary treatments and BWG of quails significantly increased by the addition of L-carnitine to linseed oil treatment ($P<0.05$). Different types of vegetable oil and L-carnitine supplementation had no significant ($P>0.05$) effect on liver and gizzard of quails. Linseed and sunflower oil increased heart weight of quail. Addition of L-carnitine to diet, containing sunflower oil reduced the relative weight of liver and heart. Sunflower oil reduced the concentration of total protein and albumin in blood serum of birds. L-carnitine supplement increased the concentration of total protein and globulin in female blood serum. Data of the present experiment showed that source of dietary oil affected the performance of Japanese quail and also use of different plant oil sources with L-carnitine supplementation affected the blood biochemical parameter of female Japanese quail.

KEY WORDS blood metabolites, carcass characteristics, Japanese quail, L-carnitine, oil source.

INTRODUCTION

In the intensive feeding system of poultry production, oil and fats have commonly been used as energy sources. Some of the advantages of including these oil and fats in diets are decreasing of dust, supplying of essential fatty acids and fat-soluble vitamins and also helps to produce lower heat increment as compared to carbohydrates and proteins (Nobakht *et al.* 2011). Soybean, sunflower and canola oil are some of the important vegetable oils, which are commonly used in poultry diets (Burlikowska *et al.* 2010). The efficient utilization of oil and fat as sources of energy in poultry diets depends on their fatty acids composition (Shahriar *et al.* 2007; Burlikowska *et al.* 2010). It has been reported that by using of linseed, sunflower and olive oil instead of poultry fat, at 1.5, 2 and 3% of diets, im-

proved the body weight and body weight gain (BWG) of quail (El-Yamany *et al.* 2008). Additionally, dietary supplementation of a mixture of canola and soybean oil (2%+2%) in broilers diets improved the FCR (Nobakht *et al.* 2011). Since oil sources have different fatty acid compositions, it seems that using different sources of oils in diets of Japanese quails may have a synergistic effect.

L-carnitine is a non-essential nutrient (Harpaz, 2005) and is extensively utilized as animal feed byproducts (Arslan, 2006). The L-carnitine content of plant products is low, especially in poultry diets which are mainly composed of corn and soybean meal. Methionine and lysine are the precursors of L-carnitine biosynthesis and usually the most important limiting amino acids in poultry nutrition. When diets are not supplemented with these two amino acids, chickens may not be able to synthesize adequate amounts of L-carnitine (Arslan, 2006). L-carnitine is required for the transfer of long-chain fatty acids from the cytosol to the mitochondrial matrix during lipid catabolism (β-oxidation); therefore, it plays a vital role in fat combustion and energy production (Jalali Haji-Abadi *et al.* 2010). Studies with broiler chickens have revealed that supplementing dietary L-carnitine increased the BWG, improved FCR and reduced abdominal fat content in broiler chickens (Rabie *et al.* 1997a; Rabie *et al.* 1997b; Rabie and Szilagyi, 1998). In contrary to this, other studies showed that dietary L-carnitine supplementation did not affect growth performance of Japanese quail (Sarica *et al.* 2005) and broiler chicks (Corduk *et al.* 2007; Kheiri *et al.* 2011).

The contradictory response of birds to dietary supplementation of L-carnitine may be related to different fatty acid composition of oil, which is used in poultry diets. Therefore, this study was conducted to determine the effect of dietary plant oil source, such as soybean, linseed and sunflower oil as a different fatty acid composition and L-carnitine supplementation on growth performance and blood biochemical parameters of Japanese quails.

MATERIALS AND METHODS

Birds and dietary treatment

One hundred and ninety-two 7 day-old female Japanese quails (*Coturnix japonica*) were randomly distributed to 6 dietary treatments with 4 replicates (cages) and 8 birds in each cage base on a 3 × 2 factorial arrangement (three oil sources and two level of L-carnitine) in a completely randomized design. Birds were fed *ad libitum* during 28 days of experiment. The dietary treatments contained two levels of L-carnitine (0 and 50 mg/kg) and three sources of plant oils (soybean, linseed and sunflower). The basal diet was balanced on the basis of corn and soybean meal and formulated to meet nutrient requirements provided by NRC (1994). Each of the dietary treatments group contained 24% crude protein and 2900 kcal ME/kg (Table 1). To supply metabolizable energy for experimental diets, the oil level of all diet was 4%which is supplied by soybean, linseed and sunflower oil.

Table 1 Ingredients and composition of experimental diets

Dietary composition	Without L-carnitine	With L-carnitine
Ingredients (g kg $^{-1}$)		
Basal diet*	947.4	947.4
Oil**	40.0	40.0
Washed sand	12.55	12.475
L-carnitine premix***	0.00	0.125
Premix free L-carnitine****	0.05	0.00
Nutrient composition		
ME(kcal/kg)	2900	2900
Crud protein (%)	24	24
Crud fat (EE) (%)	6.60	6.60
Calcium (%)	0.802	0.802
Available phosphorus (%)	0.305	0.305
Lysine (%)	1.37	1.37
Arginine (%)	1.57	1.57
Methionine (%)	0.405	0.405
Methionine + cysteine (%)	0.778	0.778

* 94.74% basal diet contain: Ground corn: 44.9%; Soybean meal 37% (44% CP); Fish meal: 5%; Wheat bran: 6%; $CaCO_3$: 1.07%; Dicalcium phosphate: 0.07% and Salt: 0.20%; 0.5%-vitamin and mineral premix (provided per kilogram of diet): vitamin A: 7700 IU; vitamin D_3: 3300 IU; vitamin E: 6.6 IU; vitamin K_3: 0.55 mg; Thiamine: 1.5 mg; Riboflavin: 4.4 mg; Pantothenic acid: 22 mg; Niacin: 5.5 mg; Pyridoxine: 3 mg; Choline chloride: 275 mg; Folic acid: 1.1 mg; Biotin: 0.055 mg; vitamin B_{12}: 0.088 mg; Antioxidant: 1 mg; Manganese: 66 mg; Zinc: 66 mg; Iron: 33 mg; Copper 8.8 mg; Iodine 0.9 mg and Selenium,0.3 mg.
** According to experimental diet contain soybean, linseed and sunflower oil.
*** Content: 60% L-carnitine L-tartrate (40% pure L-carnitine).
**** Content: lactose, starch and cellulose microcrystal.

Fatty acid analysis of oils

To determine fatty acids composition of oils, fatty acid methyl esters of oils were analyzed according to the method described by Christie (1990). Fatty acid methyl esters were separated and quantified by gas chromatography (Agilent model 6890, USA) which is equipped with a Flame ionization detector (FID) and a SGE BPX70 column. Nitrogen was used as a carrier gas at a flow of 0.9 mL/min. The injector and detector temperatures were 260 and 300 ˚C, respectively. Individual methyl esters were identified by comparison with known mix fatty acid methyl standards and quantified by comparing their peak area with that of the external standard (Methyl Erucate). Fatty acid composition of soybean, linseed, and sunflower oil are presented in Table 2.

Performance and carcass traits

The body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) were measured during the whole

period of the experiment (1-5 week old). At 35 days of age, two birds were randomly selected from each replicate (cage) then, weighed, slaughtered and weight of some internal organs such as liver, heart and gizzard was determined and expressed as percentage of live body weight.

Table 2 Composition of fatty acids in soybean, linseed and sunflower oils

Fatty acids (%)	Soybean oil	Linseed oil	Sunflower oil
C14:0	0.21	0.11	0.16
C15:0	0.03	0.02	-
C15:1	0.03	0.02	-
C16:0	14.85	4.45	5.24
C16:1	0.17	0.34	0.08
C17:0	0.11	0.08	0.05
C17:1	0.07	0.05	0.04
C18:0	4.36	1.16	4.78
C18:1	25.20	15.76	16.06
C18:2 (ω-6)	47.87	19.18	70.9
C20:0	0.32	0.68	0.38
C18:3 (ω-3)	6.21	55.31	0.39
C20:1	0.19	0.4	0.19
C22:0	0.35	0.13	0.98
C24:0	0.03	0.61	0.36
C24:1	-	1.70	0.39
Saturated	20.26	7.24	11.95
Mono unsaturated	25.66	18.27	16.76
Poly unsaturated	54.08	74.49	71.29
ω-3/ω-6	0.129	2.884	0.0055
Unsaturated/saturated	3.936	12.812	7.368

Blood biochemical parameters

Blood samples (5 cc) were collected from quails during slaughtering from jugular vein for analysis of biochemical parameters. Serum was separated by centrifuge (2000×g for 10 minutes) and was kept in the freezer at -20 ˚C until assay. Total protein, albumin, and cholesterol of blood sera were measured by spectrophotometer enzymatic methods (by Pars Azmoon commercial kits) and globulin was calculated by the differences between concentration of total protein and albumin.

Statistical analysis

The data obtained from the experiment were analyzed using the general linear model (GLM) procedure in the SAS software (SAS, 2004). Significant differences between treatment means were determined using Tukey's HSD test at a probability of ($P<0.05$).

RESULTS AND DISCUSSION

The fatty acid composition is an important criterion to evaluate the use of fat in the intensive feeding of poultry (Burlikowska *et al.* 2010). Fatty acid composition of soybean, linseed, and sunflower oil are presented in Table 2.

Soybean oil has the highest mono-unsaturated fatty acids (especially oleic acid, C18:1) and also the lowest ratio of unsaturated to saturated fatty acids (3.93) in comparison to other oils. The highest linolenic acids (C18:3, ω-3), highest ratio of unsaturated to saturated and also greatest ω-3 to ω-6 fatty acids ratio were seen in linseed oil. It has been reported that using linseed and rapeseed oils, possible to increase the ω-3 fatty acids content in the form of linolenic acid, which is the precursor of the whole ω-3 family (El Yamany, 2008).

Sunflower oil has the highest fatty acid percent of linoleic acid (C18:2, ω-6), as omega 6 fatty acids and therefore this plant oil has the lowest ω-3 to ω-6 fatty acid ratio in comparison to other experimental oils. Base on fatty acid composition of soybean, linseed and sunflower oil are source of omega 9, 3 and 6 fatty acids, respectively.

The effect of dietary oil source, L-carnitine supplement and its interaction on growth performance of Japanese quail is shown in Table 3. The FI of birds was neither affected by oil source, nor by L-carnitine supplementation. Diets which contained soybean oil led to the highest BWG and the lowest FCR quails while L-carnitine supplement had no effect on BWG and FCR.

There was an interaction between oil sources and L-carnitine on BWG of quails as addition of L-carnitine to the diets contained linseed oil improved the BWG of birds. Feeding quails with linseed oil along with L-carnitine supplement led to the lowest FCR of the birds.

The main effects showed that soybean oil improved BWG and FCR of quails. This may be due to the optimum ratio of unsaturated to saturated fatty acids of soybean oil. The important factor affecting the amount of fats metabolizable energy is their digestibility and it is dependent on the length of carbon chain and the degree of saturation of fatty acids (Leeson and Summers, 1997), Moreover, it has been reported that the optimum ratio of unsaturated to saturated fatty acids for maximizing fat digestibility and metabolizable energy value of fat is around 3 to 1 (Lesson and Atteh, 1995).

Also, soybean oil had higher content of oleic acid (C18:1) compared to the other oils (Table 3), in which this fatty acid plays a direct role in the absorption of saturated fatty acids in the lumen and mucosa cells and facilitate their absorption (Leeson and Atteh, 1995). Therefore, soybean oil may improve digestibility, metabolizable energy, BWG and FCR of quails. Scaife *et al.* (1994) fed female broiler chicks by different lipids sources; such as beef tallow, soybean oil, canola oil, marine fish oil or a mixture of these oils and observed improved live body weight using soybean oil while beef tallow fat had the poorest FCR. Dietary L-carnitine supplementation individually had not any effect on FI, BWG and FCR of quails.

Table 3 Effects of dietary oil source and L-carnitine supplementation on feed intake, body weight gain (g.bird^{-1}.day^{-1}) and feed conversion ratio of female Japanese quails

Main factors	Feed intake	Body weight gain	Feed conversion ratio
Oil			
Soybean oil	21.60	5.857^{a}	3.690^{b}
Linseed oil	21.43	5.706^{b}	3.760^{ab}
Sunflower oil	21.85	5.696^{b}	3.840^{a}
L-carnitine level (mg/kg)			
0	21.50	5.712	3.768
50	21.76	5.794	3.759
Treatments			
Soybean oil	21.48	5.832^{a}	3.684^{b}
Linseed oil	21.35	5.571^{b}	3.835^{ab}
Sunflower oil	21.68	5.733^{ab}	3.782^{ab}
Soybean oil + L-carnitine	21.73	5.882^{a}	3.696^{ab}
Linseed oil + L-carnitine	21.51	5.842^{a}	3.683^{b}
Sunflower + L-carnitine	22.04	5.658^{ab}	3.896^{a}
PSEM	0.195	0.061	0.06
Source of variation			
Oil	0.1283	0.0376	0.0442
L-carnitine	0.1283	0.1265	0.8555
Oil × L-carnitine	0.1565	0.0428	0.0452

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
PSEM: pooled standard error of mean.

This finding is in agreement with other researchers (Corduk *et al.* 2007; Lien and Horng, 2001; Kheiri *et al.* 2011) who, reported that L-cranitine supplementation did not have any effect on growth performance of broiler chicks. Sarica *et al.* (2005) demonstrated that dietary L-carnitine supplementation at 0, 30, 40 and 50 ppm did not affect FI, growth and FCR of Japanese quails. Also in another experiment, Sarica *et al.* (2007) showed that using 50 ppm L-carnitine in diet of Japanese quails containing 1% fish oil or sunflower oil had no effect on growth performance. These two reports are in contrast to our results. On the other hand, results of Parsaeimehr *et al.* (2012) showed that using L-carnitine (300 ppm) in diets containing 5% animal fat improved BWG and FCR of broiler chicks compared with diets containing 5% soybean oil. Sayed *et al.* (2001) have demonstrated that addition of L-carnitine (50 ppm) to diet contained 2 and 4% sunflower oil increased FI, BWG and FCR compared to the control group.

In the present study, growth performance of quails improved by using L-carnitine when the diet contained linseed oil as ω-3 fatty acids, and this may be related to improved oxidation of fatty acids in linseed oil by L-carnitine supplement. Therefore, the growth response of birds to L-carnitine supplementation was affected by dietary oil source.

On the other hands, results of Sadeghzadeh *et al.* (2014) showed that optimum production of chicks was occurred by supplementation of 150 ppm L-carnitine and 115% of methionine requirement of female broiler chicks recommended by NRC when the diet contained 3% soybean oil.

According to results of this research, response of quails to L-carnitine supplement may be related to dietary composition such as amino acids level especially methionine and lysine; which are precursors of endogenous biosynthesis of L-carnitine (Arslan, 2006). In addition, type of diet and level of oil also might be effective.

The results indicated that different types of plant oils and L-carnitine supplementation had no significant (P<0.05) effect on body weight, relative weights of liver and gizzard of quails (Table 4). Linseed and sunflower oil significantly increased heart weight and its relative weight of quail (P<0.05). Addition of L-carnitine to the diet contained sunflower oil reduced relative weight of liver and heart. Results of Nobakht *et al.* (2011) showed that sunflower, soybean, and canola oil as well as its combination in chicks' diet had no effect on relative weight of liver in birds. Also, Lien and Horng (2001) showed that supplementation of 160 ppm L-carnitine to broilers diet had no effect on liver weight.

The results of Rabie and Szilagyi (1998) showed that relative weights of liver, gizzard and heart of chicks were not affected by feeding 50 ppm L-carnitine. Other researchers also showed that the relative weight of liver, gizzard, and heart of quails were not affected by supplementation of 0, 30, 40 and 50 ppm L-carnitine (Sarica *et al.* 2005). The absolute and relative heart weights of quails were significantly depressed by carnitine supplemented diets contained sunflower oil compared to other dietary treatments. In the same way, the liver relative weight was also reduced significantly (P<0.05).

Table 4 Effects of dietary oil source and L-carnitine supplementation on live body and some internal organ weight and its relative weight of them to live body of female quails

Main factors	Weight (g)				Relative weight (%)		
	Live body	Liver	Heart	Gizzard	Liver	Heart	Gizzard
Oil							
Soybean oil	227.70	4.717	1.200^{b}	4.317	2.102	0.5415^{b}	1.902
Linseed oil	214.20	4.517	1.600^{ab}	4.900	2.133	0.7482^{a}	2.306
Sunflower oil	235.10	5.167	1.650^{a}	5.000	2.201	0.7068^{a}	2.140
L-carnitine level (mg/kg)							
0	228.20	5.155	1.544	4.989	2.270	0.6784	2.189
50	219.80	4.444	1.422	4.489	2.022	0.6526	2.044
Treatments							
Soybean oil	234.90	5.167	1.200	5.000	2.200^{ab}	0.5097^{c}	2.124
Linseed oil	228.00	4.633	1.600	4.967	2.066^{ab}	0.6974^{abc}	2.198
Sunflower oil	221.70	5.667	1.833	5.000	2.543^{a}	0.8280^{a}	2.244
Soybean oil + L-carnitine	210.50	4.267	1.200	3.633	2.005^{ab}	0.5733^{bc}	1.680
Linseed oil + L-carnitine	200.50	4.400	1.600	4.833	2.201^{ab}	0.7990^{ab}	2.415
Sunflower + L-carnitine	248.50	4.667	1.467	5.000	1.860^{b}	0.5855^{bc}	2.036
PSEM	14.546	0.505	0.161	0.570	0.169	0.05	0.23
Source of variation							
Oil	0.3824	0.4445	0.0312	0.4569	0.8377	0.0033	0.2481
L-carnitine	0.4935	0.1105	0.3709	0.3043	0.0969	0.5396	0.4548
Oil × L-carnitine	0.1535	0.7183	0.4460	0.3044	0.0494	0.0092	0.3767

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
PSEM: pooled standard error of mean.

These results were in accordance with previous studies in which Koksal *et al.* (2011) have observed reduction of heart and liver weights in broiler diets supplemented with 100 ppm carnitine. The reduced weights of the heart and the liver at a lesser extend recorded in L-carnitine supplemented birds which may be related to increasing lipid utilization (Koksal *et al.* 2011).

The results indicated that experimental dietary treatments had significant effect on some blood biochemical parameters of quails (Table 5) (P<0.05). Sunflower oil, in comparison with the other plant oils, reduced the blood level of total protein, albumin and globulin while soybean oil decreased cholesterol level in sera of quails. Dietary L-carnitine supplementation increased the concentration of total protein and globulin, but reduced the cholesterol in the blood sera of female quails.

The lowest concentration of total protein and globulin in quail's serum were found in quails fed on sunflower oil without L-carnitine supplement (P<0.05). On the other hand, the lowest concentration of blood cholesterol was observed in birds that fed on diets containing soybean oil plus L-carnitine.

Supplementations of L-carnitine to the diet contained linseed oil significantly increased globulin and reduced cholesterol level in the serum of quails (Table 5) (P<0.05). Reducing the blood cholesterol of quails using soybean oil may be related to inhibition of hepatic 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA reductase) activity, a key enzyme which is regulating cholesterol synthesis (Lee *et al.* 2003) and or stimulate bile formation and biliary lipid secretion, particularly cholesterol output in bile by lecithin in soybean oil (LeBlanc *et al.* 2003).

L-carnitine supplementation decreased blood cholesterol of quail which is in agreement with those of Rezaei *et al.* (2007) and Parsaeimehr *et al.* (2012). L-carnitine may increase fatty acid oxidation and thus reduced blood cholesterol levels in quails.

On the other hand, the results of Parizadian *et al.* (2011) indicated that quails fed with diet contained L-carnitine supplementation (250 ppm), had lower blood cholesterol compared with the control group.

Sayed *et al.* (2001) also, have demonstrated that addition of L-carnitine (50 ppm) to diet contained 2 and 4% of sunflower oil decreased serum cholesterol compared to the control group.

The plasma cholesterol-lowering effects of L-carnitine would be associated with several possible processes, including increase of cholesterol turnover due to increased conversion of cholesterol to bile acids and biliary excretion or due to modified repartition of whole body cholesterol (Arslan, 2006).

There were some interactions between blood cholesterol level and dietary plant oil as well as L-carnitine supplement. This may be related to fatty acid composition of oils. Sunflower oil reduced protein, albumin and globulin concentration but L-carnitine increased them.

Table 5 Effect of treatments on blood biochemical parameters in female quails

Treatments	Total protein ($g.dL^{-1}$)	Albumin ($g.dL^{-1}$)	Globulin ($g.dL^{-1}$)	Cholesterol ($mg.dL^{-1}$)
Main factors				
Oil				
Soybean oil	3.483^{a}	1.752^{a}	1.732^{ab}	125.0^{b}
Linseed oil	3.433^{a}	1.478^{ab}	1.955^{a}	187.8^{a}
Sunflower oil	2.417^{b}	1.198^{b}	1.218^{b}	188.8^{a}
L-carnitine level (mg/kg)				
0	2.833^{b}	1.401	1.432^{b}	190.6^{a}
50	3.389^{a}	1.551	1.838^{a}	143.8^{b}
Treatments				
Soybean oil	3.067^{ab}	1.677	1.390^{bc}	164.0^{abc}
Linseed oil	3.267^{ab}	1.483	1.783^{ab}	246.7^{a}
Sunflower oil	2.167^{b}	1.043	1.123^{c}	161.3^{abc}
Soybean oil + L-carnitine	3.900^{a}	1.827	2.073^{a}	86.0^{c}
Linseed oil + L-carnitine	3.600^{ab}	1.473	2.127^{a}	129.0^{bc}
Sunflower + L-carnitine	2.667^{ab}	1.353	1.313^{bc}	216.3^{ab}
PSEM	0.314	0.196	0.196	22.173
Source of variation				
Oil	0.0082	0.047	0.0081	0.0208
L-carnitine	0.051	0.367	0.0265	0.0237
Oil × L-carnitine	0.0241	0.136	0.0078	0.0054

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
PSEM: pooled standard error of mean.

The higher concentration level of total protein and globulin by feeding L-carnitine may be related to its protein sparing action (Jalali Haji-Abadi *et al.* 2010) and reduced using of amino acid precursor (lysine and methionine) for L-carnitine biosynthesis.

CONCLUSION

This investigation proved that 50 ppm L-carnitine could improve growth performance of quails which the diet containing 4% linseed oil. The present study also showed that soybean oil improved the FCR of Japanese quail and total protein, albumin and globulin of blood female birds reduced by sunflower oil. Overall, results of this experiment showed that growth performance and blood biochemical response of Japanese quail to dietary supplementation of L-carnitine related to dietary oil source.

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