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Effect of Dietary Supplementation with Sources of Omega-3 and Omega-6 Fatty Acids on Certain Blood Characteristics of Laying Quail

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Abstract: The objective of this experiment was to determine the effects of dietary supplementation with different fat sources on blood parameters of Japanese quail (*Coturnix coturnix japonica*). Eighty four 7-week old laying quail were randomly assigned to 4 treatment groups (21 birds per group) with 3 replicates for each treatment group and fed for three months on a commercial diet supplemented with 3% of either sunflower oil (T1), flax oil (T2), corn oil (T3) or fish oil (T4). The birds received water and feed *ad libitum* during the experiment. During the last month of experiment blood samples were collected fortnightly from each bird. The first blood samples collection was used to determine fresh blood parameters, while the second blood samples collection was used after pooled blood samples for each replicate of treatment group to determine serum chemistry traits. Dietary supplementation with 3% fish oil (T4) caused a significant ($p < 0.05$) increase in erythrocyte number, Packed Cell Volume (PCV), hemoglobin concentration, Mean Corpuscular Values (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), thrombocyte number and leukocyte number and serum total protein, albumen, globulin, glucose, High Density Lipoprotein (HDL), Alkaline Phosphatase (ALP), calcium and phosphorus concentration, followed by the results of flax oil (T2), whereas sunflower oil (T1) and corn oil (T3) revealed the lowest values respecting these traits. However, adding sunflower (T1) and corn oil (T3) to the diet of laying quail resulted in significant ($p < 0.05$) increase in percentage of reticulocytes and Heterophil to Lymphocyte (H/L) ratio and serum total cholesterol, triglycerides, Low Density Lipoprotein (LDL), uric acid, creatinine, Aspartate Aminotransferase (AST) and alanine aminotransferase (ALT), followed by the results of the flax oil (T2), while fish oil (T4) exhibited the lowest means with relation to these traits. From this experiment it is concluded that dietary fish and flax oils at inclusion level of 3% resulted in significant improvement in blood profile of laying quail. Consequently, fish and flax oils can be added during the laying period to the diet of Japanese quail to enhance general physiological status of these birds.

Key words: Omega-3 and omega-6 fatty acids, blood traits, laying quail

INTRODUCTION

The egg is one of the most complete foods from a nutritional point of view. However, consumers abstain from egg consumption because of relatively high cholesterol content of eggs and the notion that cholesterol rich foods result in coronary heart disease and atherosclerosis. Risk factors for coronary heart disease involve hypertension, obesity and elevated blood cholesterol levels (Basmacoglu *et al.*, 2003). The occurrence of coronary heart diseases was higher in humans who have high blood cholesterol concentration. Genetics, nutrition, sex and age influence blood cholesterol concentrations in humans. During the past 30 years general concerns have concentrated on the relationship between the cholesterol content of diet and the development of coronary heart disease. Furthermore, dietary fat type and fatty acid composition of fat consumed are more important than the quantity of dietary cholesterol consumed (Simopoulos, 2000). Depending on the results of many studies, it was

demonstrated that saturated fatty acids and trans fatty acids cause negative effect on human health, while Polyunsaturated Fatty Acids (PUFA) have a positive effect on human health concerning coronary heart disease (Bhatnager and Durrington, 2003; Meyer *et al.*, 2003). During recent years, consumer demands for more healthy foods supported the interest in modifying the fatty acid profile of eggs. Moreover, many studies and clinical investigations exhibited that omega-3 fatty acids particularly eicosapentaenoic (EPA) acid and Docosahexaenoic (DHA) acid exert beneficial effects on human health. Omega-3 fatty acids are essential for normal growth and development and play important roles in the prevention and treatment of coronary heart disease, hypertension, inflammatory, autoimmune disorders and cancer (Meluzzi *et al.*, 1997; Lewis *et al.*, 2000). On the other hand, the biological effects of the omega-6 fatty acids are largely mediated by their conversion to n-6 eicosanoids that bind to diverse receptors found in every tissue of the body. The

conversion of tissue arachidonic acid (20:4 n-6) to n-6 prostaglandin and n-6 leukotriene hormones provides many targets for pharmaceutical drug development and treatment to diminish excessive n-6 actions in atherosclerosis, asthma, arthritis, vascular disease, thrombosis, immune-inflammatory processes and tumor proliferation. Competitive interactions with the n-3 fatty acids affect the relative storage, mobilization, conversion and action of the n-3 and n-6 eicosanoid precursors (Lands, 2005). Some medical research suggests that excessive levels of n-6 fatty acids, relative to n-3 fatty acids, may increase the probability of a number of diseases and depression (Hibbeln, 2006; Okuyama *et al.*, 2007). Excess n-6 fats interfere with the health benefits of n-3 fats; in part because they compete for the same rate-limiting enzymes. A high proportion of n-6 to n-3 fat in the diet shift the physiological state in the tissues towards the pathogenesis of many diseases: prothrombotic, proinflammatory and proconstrictive (Simopoulos, 2003). However, studies in respect to the effect of omega-3 and omega-6 fatty acids on blood parameters are lacking. Therefore, the present study was conducted to determine the effect of dietary supplementation with different oil sources on blood traits of laying quail.

MATERIALS AND METHODS

Birds and treatments: Eighty four 7-week old Japanese quail (*Coturnix coturnix japonica*) hens were randomly allocated to four experimental treatments. Each treatment consisted of three replicates, with a replicate consisting of seven hens in cage. The cages were kept in closed layer house and the experiment lasted for three months. For the whole period of experiment the quail hens were fed diets containing 3% oil from sunflower (T1), flax (T2), corn (T3), or fish (T4). The birds were allowed free access to food and water. The

experimental diets were formulated to be isoenergetic and isonitrogenous. The ingredient and chemical composition of the experiment diets are presented in Table 1 and their fatty acid composition in Table 2. A regime of 17 h constant lighting and continuous ventilation were provided and all birds were kept under uniform management conditions throughout the experimental period.

Blood samples collection and analysis: At the third month of experiment blood samples were collected fortnightly from each bird included in this experiment between 09:00 and 11:00 h by using heart puncture procedure (Al-Daraji *et al.*, 2008) to ensure a free flow of blood (Pic. 1). The first blood samples collection was used to determine erythrocyte number, Packed Cell Volume (PCV), haemoglobin concentration, Mean Corpuscular Values (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), thrombocyte number, leukocyte number, reticulocytes percentage and Heterophil to Lymphocyte (H/L) ratio. The second blood sample collection was used after pooled blood samples for each replicate in treatment group to evaluate serum chemistry traits. After overnight clotting at 4°C, the samples were centrifuged for 20 min at 4,000 x g. The separated serum was transferred to a commercial laboratory and was analyzed for total protein, albumen, globulin, glucose, total cholesterol, triglycerides, High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), uric acid, creatinine, calcium and phosphorus concentration and the enzyme activities of Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and Alkaline Phosphatase (ALP). Blood traits included in this study were analyzed by using standard methods reported by Al-Daraji *et al.* (2008).

Table 1: Ingredients and chemical composition of the diet fed to laying quails

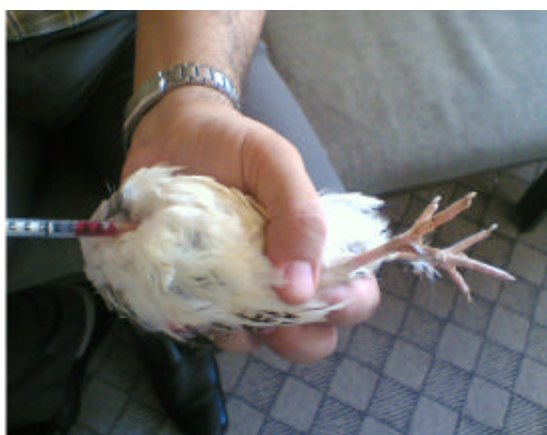
Ingredients (%)	Sunflower oil (T1)	Flax oil (T2)	Corn oil (T3)	Fish oil (T4)
Yellow corn	12.00	12.00	8.50	10.00
Wheat	47.70	47.70	51.50	50.00
Soybean meal	20.00	20.00	19.70	19.70
Protein concentrate*	10.00	10.00	10.00	10.00
Lime stone	7.00	7.00	7.00	7.00
Oil	3.00	3.00	3.00	3.00
Sodium chloride	0.30	0.30	0.30	0.30
Calculated content**				
Crude protein (%)	21.05	21.05	21.10	21.05
Metabolizable energy (kcal/kg)	2888.00	2879.00	2881.00	2885.00
Total calcium (%)	3.60	3.60	3.60	3.60
Available phosphorus (%)	0.30	0.30	0.30	0.30
Methionine (%)	0.35	0.35	0.34	0.34
Lysine (%)	1.00	1.00	0.99	0.99
Cystine (%)	0.27	0.27	0.27	0.27

*Golden protein concentrate provided per kg: : 2500 ME/kg; 40% crude protein; 9% crude fat; 4.5% crude fiber; 9% calcium; 2.3% available phosphorus; 2.3% lysine; 1.25 methionine; 1.8% methionine + cystine; 100000 IU vit A; 10 mg vit B1; 100 mg vit B12; 20 mg vit K3; 50 mg copper; 700 mg manganese; 2 mg selenium; 200 mg vit E; 0.5 mg biotin; 5 mg folic acid; 200 mg niacin; 80 mg pantothenic acid; 10 mg iodine; 25000IU vit D3; 500 mg iron; 10 mg cobalt; 600 mg zinc; 10 mg vit B6.

**Calculated composition was according to NRC (1994)

Table 2: Fatty acid composition (%) of oils included in the diets of laying quails

Numeric name	Common name	T1	T2	T3	T4
C12:0	Lauric acid	-	-	-	0.090
C14:0	Myristic acid	0.060	0.120	0.060	5.410
C15:0	None	0.020	0.080	0.030	0.470
C16:0	Palmitic acid	6.250	6.000	11.010	14.050
C17:0	Margaric acid	0.030	0.110	0.090	1.730
C18:0	Stearic acid	3.580	2.500	1.910	2.870
C20:0	Arachidic acid	0.238	0.500	0.360	0.150
C21:0	None	0.008	0.010	0.010	0.040
C22:0	Behenic acid	0.587	0.230	0.150	0.020
C23:0	None	0.028	0.020	0.020	0.060
C24:0	Lignoceric acid	0.203	0.080	0.160	0.150
C14:1	Myristoleic acid	-	-	-	0.030
C15:1	None	0.010	0.010	-	0.190
C16:1	Palmitoleic acid	0.090	0.400	0.130	8.250
C17:1	None	0.040	0.030	0.040	0.360
C18:1 n9	Oleic acid	23.000	19.000	24.000	21.940
C20:1 n9	Gadoleic acid	0.255	0.280	0.360	11.220
C22:1 n9	Erucic acid	0.007	0.010	0.010	7.650
C24:1 n9	Nervonic acid	0.005	0.020	0.120	2.300
C18:3 n3	Alpha linolenic acid	0.108	57.290	1.260	0.500
C20:3 n3	None	0.025	0.050	0.030	0.050
C20:5 n3	Eicosapentenoic acid (EPA)	0.118	0.630	0.090	10.000
C22:6 n3	Docosahexaenoic acid (DHA)	0.012	0.000	0.020	10.730
C18:2 n6	Linoleic acid	65.000	12.180	60.000	1.020
C18:3 n6	Gamma linolenic acid	0.016	0.020	0.060	0.130
C20:2 n6	11, 14-Eicosadienoic acid	0.155	0.080	0.060	0.190
C22:2 n6	13, 16-Docosadienoic acid	0.155	0.003	0.001	0.380
Total of saturated fatty acids		11.000	9.650	13.800	25.040
Total of mono unsaturated fatty acids		23.400	19.750	24.660	51.940
Total of polyunsaturated fatty acids		65.580	70.550	61.520	23.000
Total of omega-3 fatty acids		0.260	58.270	1.400	21.280
Total of omega-6 fatty acids		65.320	12.280	60.120	1.720
Total of omega-6/total omega-3 fatty acids ratio		251.230	0.210	42.940	0.080



Pic. 1: Blood collection from the quail by using heart puncture method

Statistical analysis: Data were statistically analyzed using the General Linear Model procedure of SAS (2000). Test of significance for the different between means of each classification was done by Duncan's multiple range test (Duncan, 1955).

RESULTS

A comparison between treatment groups showed that T4 group had a highest ($p < 0.05$) means of erythrocyte number, PCV, haemoglobin concentration, MCV, MCH, MCHC, thrombocyte number and leukocyte number, followed by the values of T2 group, whereas T1 and T3 groups recorded the lowest values as regards these traits (Table 3). However, T4 group had significantly ($p < 0.05$) lowest values of reticulocytes percentage and H/L ratio; followed by the results of T2 group, while T1 and T3 groups revealed the highest means in respect of these two traits (Table 3). There was evidence for treatment-related differences concerning serum biochemical parameters. The total protein, albumen, globulin, glucose, HDL, ALP, calcium and phosphorus were higher in T4 group than other treatment groups (T1, T2 and T3). Moreover, T2 group surpasses T1 and T3 groups in relation to these parameters, whereas there were no significant ($p > 0.05$) differences between T1 and T3 groups as concerns all these characteristics (Table 4). On the other hand, the means of serum total cholesterol, triglycerides, LDL, uric acid and creatinine as well as the activities of AST and ALT enzymes were

Table 3: The effect of including different oil sources on blood traits (Mean±SE) of laying quail

Traits	Treatments			
	T1	T2	T3	T4
Erythrocytes ($\times 10^5/\text{mm}^3$)	3.25±0.09c	3.58±0.08b	3.27±1.02c	3.85±1.06a
Packed cell volume (%)	38.10±5.09c	44.20±6.19b	39.0±4.11c	48.5±6.09a
Haemoglobin concentration (g/100 ml)	11.20±2.07c	13.52±3.02b	11.56±5.08c	15.77±2.11a
MCV (fl)	117.2±11.18c	123.4±19.16b	119.26±32.12c	125.97±5.93a
MCH (pg)	34.46±5.80c	37.76±3.19b	35.35±9.17c	40.96±4.09a
MCHC (g/100 ml)	29.39±8.19c	30.58±1.19b	29.64±7.99c	32.51±2.85a
Thrombocytes ($\times 10^3/\text{mm}^3$)	115.0±10.13c	123.1±10.85b	116.3±18.27c	129.3±11.93a
Leucocytes ($\times 10^3/\text{mm}^3$)	17.1±1.8c	20.3±2.3b	17.7±2.1c	23.5±1.9a
Reticulocytes (%)	11.2±1.30a	8.9±0.99b	11.6±2.80a	5.84±0.82c
H/L ratio	0.31±0.03a	0.26±0.09b	0.30±0.06a	0.24±0.08c

T1: Sunflower oil; T2: Flax oil; T3: Corn oil; T4: Fish oil. ^{a,b,c}Values within rows followed by different letters differ significantly ($p < 0.05$)

Table 4: The effect of including different oil sources on serum chemistry characteristics (Mean±SE) of laying quail

Traits	Treatments			
	T1	T2	T3	T4
Total protein (g/100 ml)	3.95±0.31c	5.83±0.67b	4.09±0.28c	6.23±0.82a
Albumen (g/100 ml)	2.23±0.18c	3.75±0.91b	2.39±0.36c	4.09±0.98a
Globulin (g/100 ml)	1.69±0.33c	1.96±2.56b	1.52±0.52c	2.08±0.79a
Glucose (mg/100 ml)	269.3±12.2c	295.2±10.7b	271.5±11.9c	337.1±10.3a
Total cholesterol (mg/100 ml)	199.5±5.8a	182.1±6.9b	197.3±8.0a	162.3±9.2c
Triglycerides (mg/100 ml)	183.2±10.6a	161.7±13.5b	180.9±15.2a	142.3±11.2c
HDL (mg/100 ml)	71.8±3.9c	93.1±4.9b	73.1±4.8c	99.2±2.8a
LDL (mg/100 ml)	91.06±3.2a	56.66±4.9b	88.02±6.8a	34.64±1.9c
Uric acid (mg/100 ml)	12.3±1.1a	9.5±1.3b	11.9±2.6a	8.0±1.8c
Creatinine (mg/100 ml)	0.95±0.06a	0.90±0.08b	0.94±0.03a	0.78±0.09c
AST (U/L)	255.3±15.8a	230.2±11.5b	251.8±22.0a	223.8±10.9c
ALT (U/L)	15.2±1.6a	11.0±2.5b	14.1±4.8a	8.2±3.0c
ALP (U/L)	2285.3±113.8c	2419.5±191.6b	2293.7±177.7c	2597.2±126.9a
Calcium (mg/100 ml)	6.25±0.93c	7.92±1.0b	6.49±0.89c	8.19±1.2a
Phosphorus (mg/100 ml)	3.95±0.61c	4.88±0.80b	3.99±0.72c	5.62±0.91a

T1: Sunflower oil; T2: Flax oil; T3: Corn oil; T4: Fish oil. ^{a,b,c}Values within rows followed by different letters differ significantly ($p < 0.05$)

higher in T1 and T3 groups compared with T2 and T4 groups. However, T4 group denoted the lowest means in regard to these traits in comparison with other treatment groups (Table 4).

DISCUSSION

Several treatments-related differences between treatments groups regarding blood traits included in this study may be explained by the physiological changes in metabolism in laying quail due to fatty acids found in different oils supplemented to the diet of these birds. Inclusion of fish oil in the diet has been shown to increase the proportion of (n-3) PUFA relative to (n-6) PUFA in the tissues of mammals (Schmidt *et al.*, 1991; Whelan *et al.*, 1991) and poultry (Chanmugam *et al.*, 1992; Friedman and Sklan, 1995). Although most research of dietary oils has utilized high levels (>5 g/100 g) of inclusion in the diet, the ratio of (n-3) : (n-6) PUFA appears to be more important in modulating eicosanoid biosynthesis than the absolute concentration of (n-3) PUFA in the diet (Boudreau *et al.*, 1991). Broughton *et al.* (1991) reported that a high concentration of dietary linoleic acid, fish oil supplementation had a minimal

effect on leukotriene production relative to the same concentration of fish oil with lower concentrations of linoleic acid. In chickens fed corn-wheat-soy diets, increasing the (n-3) : (n-6) PUFA ratio from 0.07 (the lowest possible in this type of diet) to 0.33 resulting in much greater liver (n-3) PUFA and much lower (n-6) PUFA (Korever, 1997). Increases in the (n-3) : (n-6) PUFA ratio to 0.66 and to 1.00 resulted in further, although more subtle, increases in hepatic (n-3) : (n-6) PUFA ratio. The enrichment of cell membrane (n-3) PUFA is associated with decreases in the inflammatory response, improvement in growth rate, erythropoiesis and leucopoiesis and increased specific immunity (Korever and Klasing, 1997). The inclusion of fish oil in the diet appears to improve humoral immunity and ameliorate the suppression of the cellular immune response caused by PGE2 (Fritsche *et al.*, 1992). Al-Daraji *et al.* (2010) found that different oil sources supplemented to the diet of laying quail had varying effects on productive and reproductive performance of these birds. This is reflected by the fatty acid composition of these oils added to the diet. Incorporation of these oils, especially fish and flax oils in

the diet may have practical value in improving the productive and reproductive traits of Japanese quail. Arshami *et al.* (2010) reported that long-term effects of feeding diet with 7.5% flaxseed may improve egg production and quality with less feed consumption in laying chicken. Bozkurt *et al.* (2008) indicated that supplementation of fish oil and tallow at the level of 1.5% to the corn-soybean meal diet may affect egg production performance, fertility, egg weight, chick weight, hatch of egg set and specific gravity without any adverse effects on body weight and settable egg characteristics of broiler breeder chickens. The serum chemistry values closely reflected the differences in productive and reproductive performance of birds (Schlotz *et al.*, 2008). Al-Daraji *et al.* (2002 a, b, c) found positive correlation between productive and reproductive performance and plasma total protein, albumen, globulin, glucose, ALP, calcium and phosphorus concentrations and negative correlation between productive and reproductive performance and plasma total cholesterol, uric acid, AST and ALT concentrations.

Conclusion: Under the condition of this experiment it was found that adding fish (T4) and flax (T2) oils to the diet of laying quail resulted in significant improvement in blood profile of these birds compared with sunflower (T1) and corn (T3) oils. Therefore, fish and flax oils can be used as an effective means for enhancing physiological status of laying quail by supplementing their diet with these oils during laying period.

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