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High Intake of Linoleic or α-linolenic Acid in Relation to Plasma Lipids, Atherosclerosis and Tissue Fatty Acid Composition in the Japanese Quail

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Abstract: On the basis of a previous study in deceased parrots it was suggested that a high intake of α-linolenic acid might protect against the development of atherosclerosis. To test our suggestion, a feeding experiment was carried out with Japanese quail. Quails are known to develop atherosclerosis when cholesterol is added to the diet. During 80 days, four different diets were fed to groups of 17 or 18 quails. There was a control group that was fed a cholesterol-free diet and the three remaining groups were fed diets fortified with cholesterol. The experimental, cholesterol-rich diets were either rich in saturated fatty acids, linoleic or α-linolenic acid, the exchange of the fatty acids being the only variable. At the end of the experiment, blood was collected for determination of plasma lipids, the degree of atherosclerosis was scored and tissues were collected for fatty acid analyses. Addition of 2% cholesterol to the diet resulted in a two-fold increase of plasma cholesterol and a 10-fold increase in liver cholesterol. Cholesterol feeding induced plaque formation. No significant effect of α-linolenic acid versus either linoleic acid or saturated fatty acids (lauric plus myristic plus palmitic acid) was seen with regard to atherosclerosis and plasma cholesterol. The fatty acid composition of the diets was reflected in the tissue fatty acid composition, but there were significant differences between tissues. It is concluded that, under the conditions of this study, a differential effect on the development of atherosclerosis of α-linolenic acid, linoleic acid and saturated fatty acids could not be demonstrated.

Key words: Atherosclerosis, fatty acids, cholesterol, quail

Introduction

Atherosclerosis is a common disease not only in humans, but also in parrots. The incidence of atherosclerosis in adult parrots may range from 3 to 30 % (Bavelaar and Beynen, 2003a; Dorrestein *et al*., 1977; Fox, 1933; Griner, 1983; Grünberg, 1964; Kempeneers, 1987). Atherosclerosis is mainly located at the beginning of the aorta and brachiocephalic arteries (Fiennes, 1965; Grünberg, 1966; Kempeneers, 1987). It is rarely diagnosed in living parrots, the most common sign being sudden death (Johnson *et al*., 1992), whereas in some cases symptoms such as hind limb paresis, sudden collapses, dyspnea and lethargy can be seen (Johnson *et al*., 1992; Kempeneers, 1987; Phalen *et al*., 1996). So far, there is no treatment known. Thus, it appears important to identify factors that prevent atherosclerosis in parrots.

An elevated plasma cholesterol level is an important risk factor for the development of atherosclerosis in humans and also in animals such as budgerigars, Japanese quails, chickens and rabbits (Consensus Conference, 1985; Finlayson and Hirchinson, 1961; Grundy *et al*., 1982; Grundy, 1986; Hammad *et al*., 1998; Horlick and Katz, 1949; Kakita *et al*., 1972; Kloeze *et al*., 1969; Martin *et al*., 1986; Radcliffe and Liebsch, 1985; Reed *et al*., 1989). The isoenergetic replacement of dietary saturated fatty acids by linoleic acid decreases plasma cholesterol levels and reduces the risk for atherosclerosis (Grundy, 1986; Grundy and Denke, 1990; Grundy *et al*., 1982). In addition to the n-6 polyunsaturated fatty acid, linoleic acid, n-3 polyunsaturated fatty acids may also protect against atherosclerosis (Davis *et al*., 1987; Fann *et al*., 1989; Guallar *et al*., 1999; Hu *et al*., 1999; Sadi *et al*., 1996), but they probably do so because of their anti-thrombotic properties, favorable effects on endothelial function, inhibitory effects on platelet-derived growth factor, hypolipidemic properties and anti-inflammatory actions (Chan *et al*., 1991; Harper and Jacobson, 2001; Ross, 1999; Simopoulos, 2002).

Bavelaar and Beynen (2003a) examined the degree of atherosclerosis in parrots presented for autopsy and found a tendency towards a negative relation between the degree of atherosclerosis and the amount of the n-3 polyunsaturated fatty acid, α-linolenic acid, in muscle and adipose tissue. No relation was found between muscle or adipose tissue linoleic acid and the degree of atherosclerosis. The amounts of α-linolenic acid and linoleic acid in muscle and adipose tissue are directly related to those in the diet (Bavelaar and Beynen, 2003a,b). It was thus suggested that a high α-linolenic acid intake might protect against the development of atherosclerosis in parrots.

To investigate the effect of α-linolenic acid versus either linoleic acid or saturated fatty acids on atherosclerosis

Table 1: The ingredient composition of the basal diet

Ingredients (g/kg)	Basal diet
Soybeans, extracted	500
Fat source*	150
Corn starch	144.7
Wheat	143.6
Glucose	20
Premix[1]	5
Premix[2]	10
Salt	3.6
$Ca(HPO_4)_2.H_20$	7.6
$CaCO_3$	14
Methionine	1.5

*Fat source see Table 2

[1]Trace-element premix contained per kg: 119.5 g $FeSO_4.7H_2O$, 19.0 g MnO_2, 19.2 g $ZnSO_4.H_2O$, 4.7 g $CuSO_4.5H_2O$, 91.6 mg KI, 131.5 mg $Na_2SeO_3.5H_2O$, 2.6 g $NiSO_4.5H_2O$, 0.4 g NaF, 0.3 g $CrCl_3.6H_20$, 0.4 g $SnCl_2.2H_2O$, 40.0 mg NH_4VO_3 and 833.6 g corn starch as carrier

[2]Vitamin premix contained per kg: 0.3 g vitamin A (500 IU/g), 0.04 g vitamin D_3 (500 IU/g), 2.4 g vitamin E (purity 50 %), 0.05 g vitamin K, 10 mg biotin, 25 mg folic acid, 0.3 g vitamin B_{12} (purity 0.1%), 180 g choline chloride (purity 50%), 0.13 g thiamin, 0.18 g riboflavin, 1.1 g niacin, 0.3 g pyridoxin, 2.2 g panthotenic acid (purity 45%) and 812.94 g corn starch as carrier.

a feeding experiment was carried out with Japanese quails, because these birds have been shown earlier to develop atherosclerosis when fed a diet enriched with cholesterol (Morrissey and Donaldson, 1977a; Radcliffe and Liebsch, 1985). Thus, quails were given cholesterol-rich diets containing different blends of oils so that linoleic acid, α-linolenic acid and saturated fatty acids were the only dietary variables. After a feeding period of 80 days, the animals were euthanized, and the degrees of atherosclerosis and plasma lipid concentrations were determined. To assess the fatty acid status, the fatty acid compositions of the various tissues were analyzed. It was anticipated that this study would provide insight into the relative effects of dietary linoleic and α-linolenic acid on atherogenesis and also would provide data to qualify our earlier suggestion (Bavelaar and Beynen, 2003a) that a high intake of α-linolenic acid could protect against the development of atherosclerosis in parrots.

Materials and Methods

The experiment was carried out in the research facility "Achter 't End" and was approved by the animal experiments committee of the Faculty of Veterinary Medicine, Utrecht, The Netherlands.

Animals and experimental procedures: Seventy-five male Japanese quails, aged 6-9 weeks, were purchased from a distributor (Groothandel Kleindieren en Benodigdheden, Harderwijk, The Netherlands). On arrival, the animals were ringed for identification, weighed and randomly divided over 16 wire-floored, suspended cages. They were fed a commercial powdered feed (Volledig Opfokvoer, Van Cooten, Lienden, The Netherlands) for four weeks. Then, at the age of 10-13 weeks, four animals were randomly selected and killed to obtain baseline measurements. Body weights of these animals were 230.5 ± 46.1 g (mean ± SD, n=4). The rest of the animals were given one of the four dietary treatments. Each dietary treatment had four cages with 4-5 animals per cage. During the experiment a 12-hr light, 12 hr-dark light schedule was used and the room temperature was kept at 14-18°C. The quails had free access to feed and tap water. For each cage, an amount covering 25 g of feed per day per animal was put aside and used for feeding for 3-4 days. Fresh feed was given each day, left-overs were collected and weighed to calculate the feed intake per cage for every 3-4 days. Water was refreshed twice a day. The experimental diets were fed for a period of 80 days. After an overnight fast, the animals were killed by cervical dislocation and subsequently blood was collected by decapitation. The blood samples were collected in heparinized tubes. Blood was centrifuged (10.000 x g, 10 min) and the plasma collected and stored at – 20°C until further analyses. From each bird a sample of the breast muscle, thigh muscle and subcutaneous fat was collected and stored at -20°C. The liver was removed, weighed and also stored at -20°C. The heart together with about 1-2 cm of the aorta and the brachiocephalic arteries was isolated from each bird. The arteries were then separated from the heart and from each other, flattened between glass plates and stored in 10 % neutral formalin.

Diet composition and feed analyses: The experimental diets were composed so as to meet the nutrient requirements of quails (Klasing, 1998). The ingredient composition of the diets is given in Table 1. Group A was fed the basal diet without cholesterol. To formulate the diets for groups B, C and D, the glucose component (20 g/kg diet) was replaced by cholesterol on a weight basis. Diets B, C and D differed in fat source so that the amount of the sum of lauric, myristic and palmitic acid, and the amount of either linoleic or α-linolenic acid were the variables. The composition of the fat blends is given in Table 2. The cholesterol-free diet A contained the same fat blend as the cholesterol-rich diet B. Thus, group A versus group B can be considered a negative control group, because cholesterol feeding as the only dietary variable has been shown to raise plasma cholesterol and to promote atherogenesis in quails (Morrissey and Donaldson, 1977a; Ojerio *et al.*, 1972). Comparison of groups B, C and D would give information as to the major objective of this study, i.e. determining the relative effects of linoleic and α-linolenic

Table 2: The composition of the fat sources mixed into the diets*

Ingredients (g/kg)	A	B	C	D
Sunflower oil	125.3	125.3	687.3	--
Olive oil	138.0	138.0	--	100.0
Coconut oil	517.3	517.3	94.7	100.0
Linseed oil	219.3	219.3	218.0	800.0

*Diet A (cholesterol-free, rich in saturated fatty acids) = negative control. Diet B (2.0% cholesterol, rich in saturated fatty acids) Diet C (2.0% cholesterol, rich in linoleic acid). Diet D (2.0% cholesterol, rich in α-linolenic acid)

Table 3: Analyzed macronutrient composition of the diets

	Diet A	Diet B	Diet C	Diet D
Dry matter (g/kg)	906.4	907.4	914.4	917.1
Crude protein (g/kg)	257.6	250.4	258.0	249.5
Crude fibre (g/kg)	28.6	27.4	27.9	27.1
Crude fat (g/kg)	176.3	196.3	200.8	231.1
Crude ash (g/kg)	57.3	56.0	54.4	54.2
Carbohydrates* (g/kg)	386.6	377.3	373.3	355.2
Gross energy†(MJ/kg)	19.6	20.0	20.3	21.0

*Calculated as residual fraction

†The gross energy values (MJ/kg) used were as follows: crude protein 23.8; crude fat 39; carbohydrates 17.

Table 4: Analyzed fatty acid composition of the diets

Fatty acid	Diet A	Diet B	Diet C	Diet D
C12:0	21.4	21.3	3.8	4.0
C14:0	8.5	8.5	1.6	1.7
C16:0	9.1	9.2	7.2	7.3
C18:0	3.2	3.2	3.5	3.6
C18:1 n-9	21.1	21.3	20.9	22.9
C18:2 n-6	17.4	17.3	48.8	17.4
C18:3 n-3	11.4	11.5	11.1	40.1

Expressed as percentage of total fatty acids

C12:0 = lauric acid, C14:0 = myristic acid, C16:0 = palmitic acid, C18:0 = stearic acid, C18:1 n-9 = oleic acid, C18:2 n-6 = linoleic acid and C18:3 n-3 = α-linolenic acid

acid versus saturated fatty acids.

Dry matter, crude protein, crude fibre and crude ash in the diets were analyzed according to the Weende method. Crude fat was extracted from the feed with chloroform:methanol (2:1, v/v) as described by Folch *et al.* (1957) and was weighed and subsequently saponified using methanolic sodiumhydroxide. The constituent fatty acids were converted into their methyl esters using boronitrifluoride in methanol. Fatty acid analyses were performed by gas-liquid chromatography using a flame ionization detector, a Chromopack column (Fused silica, no. 7485, CP.FFAPCB 25 m x 0.32 mm, Chromopack, Middelburg, The Netherlands) and H2 as carrier gas (Metcalfe *et al.*,1966). The individual fatty acids are expressed as weight percentage methyl esters of total methyl esters.

Blood analyses: Plasma total cholesterol, phospholipids and triglycerides were determined with commercial test combinations and the Cobas-Bio centrifugal analyzer (Roche Diagnostics, Basel, Switzerland). For the cholesterol and phospholipid determination, Precinorm U (cat. nr. 171743, Boehringer, Mannheim, Germany) was used as the control serum, and for the triglyceride determination, Precinorm L (cat. nr. 781827) was used. High-density lipoprotein (HDL) cholesterol was determined as soluble cholesterol after precipitation of apo B containing lipoproteins (cat. nr. 543004, Roche, Mannheim, Germany) using the Cobas-Bio autoanalyzer and Precinorm L as the control serum. Low-density lipoprotein (LDL) cholesterol was calculated with the formula of Friedewald *et al.* (1972) as LDL-cholesterol (mmol/l) = total cholesterol (mmol/l)-triglycerides (mmol/l) / 2.2 – HDL-cholesterol (mmol/l). The use of the formula has been validated for the Bobwhite quail (Peebles *et al.*, 1996).

Lipids were extracted from the plasma with the method of Folch *et al.* (1957). Plasma cholesteryl-esters and triglycerides were isolated with prepacked silica sep-pak columns (3 ml / 500 mg, Varian Bond Elut 1210-2041, Allech Associates Inc., Deerfield, IL, USA), using the method of Hamilton and Comai (1988). The fatty acid methylation and analysis occurred as described above.

Determination of atherosclerosis: The arteries were stained in a solution containing equal quantities of dry Sudan III and Sudan IV (Kloeze *et al.*, 1969). After staining, the preparations were differentiated in 70% ethanol, washed in water, and kept in 10% formalin (Kloeze *et al.*, 1969). The arteries were examined for the presence of plaques (elevated areas in the artery wall), spots (points in the artery wall that could reflect the beginning of plaque formation), and the percentage of the artery that colored red after staining. Red coloring indicates fat accumulation in the artery wall. Examination of the arteries was done by F.J.B while she was blinded to treatment modality.

Table 5: Feed consumption and body weight of the groups fed the different groups

	Diet A	Diet B	Diet C	Diet D
Feed consumption / day (g)	$23.7^{a} \pm 1.2$	$22.8^{ab} \pm 1.4$	$21.2^{c} \pm 1.7$	$22.5^{b} \pm 1.5$
Body weight (g) Day 0	245.9 ± 17.7	248.2 ± 29.1	242.6 ± 18.2	248.4 ± 16.1
Day 80	268.1 ± 34.5	259.3 ± 33.5	262.2 ± 23.0	267.0 ± 27.9

Values are mean ± SD. Values that have a different superscript in the same row differ significantly ($P < 0.05$)

Table 6: Plasma lipid values for the baseline and the different dietary treatments

	Baseline	Diet A	Diet B	Diet C	Diet D
Cholesterol (mmol/l)	$6.09^{a} \pm 0.72$	$5.96^{a} \pm 1.07$	$12.24^{b} \pm 4.96$	$10.95^{b} \pm 3.67$	$10.82^{b} \pm 3.55$
Phospholipids (mmol/l)	$3.63^{ab} \pm 0.11$	$3.72^{b} \pm 0.34$	$3.40^{ac} \pm 0.26$	$3.39^{ac} \pm 0.41$	$3.23^{c} \pm 0.23$
Triglycerides (mmol/l)	$1.20^{a} \pm 0.20$	$0.62^{b} \pm 0.15$	$0.37^{c} \pm 0.09$	$0.49^{d} \pm 0.10$	$0.40^{c} \pm 0.13$
HDL cholesterol (mmol/l)	$3.06^{a} \pm 0.09$	$4.57^{b} \pm 0.66$	$4.78^{b} \pm 1.03$	$4.67^{b} \pm 0.77$	$4.53^{b} \pm 0.83$
LDL cholesterol (mmol/l)	$2.76^{ac} \pm 0.92$	$1.15^{a} \pm 0.61$	$7.32^{b} \pm 5.02$	$6.10^{bc} \pm 3.31$	$6.14^{bc} \pm 3.55$
LDL-C/HDL-C	$0.91^{a} \pm 0.92$	$0.27^{b} \pm 0.11$	$1.73^{a} \pm 1.70$	$1.31^{a} \pm 0.64$	$1.43^{a} \pm 0.91$

Values are mean ± SD. Baseline, n=3 or 4; diet A, n=17; diet B, n=18; diet C, n=18; diet D, n=17
Values that have a different superscript in the same row differ significantly ($P < 0.05$)

Table 7: The degree of atherosclerosis for the different dietary treatments

Atherosclerotic changes		Baseline	Diet A	Diet B	Diet C	Diet D
Red coloration	Affected animals	0 (0%)	11 (64.7%)	11 (61.1%)	15 (83.3%)	15 (88.2%)
	Mean percentage of arteries colored	0	27.1	19.2	20.8	35.6
Spots	Affected animals	0 (0%)	5 (29.4%)	8 (44.4%)	14 (77.8%)	5 (29.4%)
	Average number of spots	0	1.2	1.8	2.4	1.8
Plaque formation	Affected animals	0 (0%)	0 (0%)	3 (16.7%)	3 (16.7%)	2 (11.8%)

Tissue analyses: To measure liver cholesterol and triglycerides, thawed liver was homogenized with 4 volumes of 12.5 % ethanol and saponified overnight with ethanolic KOH at 40°C. The cholesterol was then extracted with petroleum ether, dried under nitrogen and dissolved in ethanol prior to determination as described for plasma cholesterol (Carr *et al.*, 1993). Triglycerides were extracted with chloroform:methanol (2:1, v/v) as described by Bligh and Dyer (1959), dried under nitrogen and dissolved in 0.5 ml of 1% Triton X-100 in chloroform. The chloroform was dried under nitrogen at 40°C; 0.25 ml of H20 was added and by placing the tubes in a water bath for 15 min at 37°C the lipid residue was solubilized. Triglycerides were determined as described for plasma (Carr *et al.*, 1993).

The muscle and liver samples were homogenized with the Ultraturrax (T25B, Kika Labortechnik, Staufen, Germany). Total lipids were extracted, saponified, methylated and analyzed for fatty acid composition as described above.

Statistical analyses: To evaluate diet effects, ANOVA was used with diet treatment as factor. If a significant ($P < 0.05$) influence was found, the Least Significant Difference (LSD) test was then used to identify statistically significant differences between the group means. Each animal was considered to be an experimental unit. To test the effect of the diet on atherosclerosis variables, the Chi square test was used. For the relations between specified variables, the Pearson correlations were calculated. The analyses were done with the statistical computer program SPSS (SPSS inc., Chicago, USA).

Results

Diet composition: The analyzed composition of the experimental diets is given in Table 3. With exception of the crude fat content, there was no difference in the analyzed composition between the experimental diets. Because of the added cholesterol, diets B, C and D contained more crude fat than diet A. The analyzed fat level of diet D was somewhat high. The fatty acid composition of the experimental diets is given in Table 4. Diets A and B had a similar fatty acid composition, and were rich in lauric acid, myristic acid and palmitic acid. Diets C and D essentially only differed in the amounts of linoleic and α-linolenic acid.

Feed consumption and body weights: Two animals died during the experiment, the cause being unknown. There were significant differences in feed intake between the different groups (Table 5). Animals in group

Table 8: Liver weight and cholesterol and triglyceride contents for the baseline and the different dietary treatments

	Baseline	Diet A	Diet B	Diet C	Diet D
Weight (g)	$7.0^{a} \pm 0.8$	$3.8^{b} \pm 0.4$	$4.5^{c} \pm 1.4$	$4.6^{c} \pm 1.1$	$4.6^{c} \pm 1.2$
Cholesterol (µmol/g)	$13.1^{a} \pm 1.71$	$9.5^{a} \pm 1.8$	$91.4^{b} \pm 54.3$	$107.5^{b} \pm 70.3$	$84.4^{b} \pm 38.9$
Triglycerides (µmol/g)	$16.3^{a} \pm 5.5$	$6.2^{b} \pm 4.8$	$3.9^{bc} \pm 4.5$	$1.5^{c} \pm 1.3$	$4.0^{bc} \pm 5.1$

Values are mean ± SD

Values that have a different superscript in the same row differ significantly ($P < 0.05$)

Table 9: The fatty acid composition of breast muscle in quails fed the different diets

Fatty acid	Diet A	Diet B	Diet C	Diet D
C12:0	4.7 ± 2.6^{a}	4.1 ± 2.9^{a}	0.7 ± 0.4^{b}	0.6 ± 0.2^{b}
C14:0	3.6 ± 1.1^{a}	3.2 ± 1.1^{a}	0.8 ± 0.2^{b}	0.8 ± 0.1^{b}
C16:0	13.9 ± 1.5^{a}	13.6 ± 1.3^{a}	12.0 ± 1.6^{b}	12.9 ± 1.0^{a}
C18:0	12.0 ± 3.3	14.1 ± 2.8	13.3 ± 3.1	14.1 ± 2.1
C18:1 n-9	20.4 ± 4.5^{a}	19.2 ± 2.1^{ab}	17.0 ± 0.4^{b}	18.8 ± 3.1^{ab}
C18:2 n-6	21.2 ± 1.6^{a}	23.3 ± 0.9^{b}	36.1 ± 4.9^{c}	22.1 ± 1.4^{ab}
C18:3 n-3	4.2 ± 1.1^{a}	3.8 ± 0.8^{ab}	3.2 ± 0.9^{b}	12.0 ± 2.0^{c}
C20:5 n-3	0.8 ± 0.3^{a}	0.9 ± 0.2^{a}	0.0 ± 0.1^{b}	2.3 ± 0.5^{c}
C22:5 n-3	0.7 ± 0.3^{a}	1.0 ± 0.2^{b}	0.7 ± 0.2^{a}	1.2 ± 0.4^{c}
C22:6 n-3	4.2 ± 1.7^{a}	3.2 ± 0.8^{b}	2.6 ± 0.9^{b}	3.1 ± 0.8^{b}

Data are expressed as g methyl esters / 100 g methyl esters. Means ± SD.

Values that have a different superscript in the same row differ significantly ($P < 0•05$)

C12:0 = lauric acid; C14:0 = myristic acid; C16:0 = palmitic acid; C18:0 = stearic acid; C18:1n-9 = oleic acid; C18:2n-6 = linoleic acid; C18:3n-3 = α-linolenic acid; C20:5n-3 = eicosapentanoic acid; C22:5n-3 = docosapentaenoic acid; C22:6n-3 = docosahexaenoic acid

C consumed significantly less feed when compared to the other groups ($P < 0.012$). Birds fed diet A had a significantly higher feed intake when compared to birds in group D ($P = 0.017$). There were no significant differences in body weight between the groups ($P = 0.05$). The animals showed a slight, but significant ($P < 0.001$), gain of body weight (17.8 ± 19.5 g) during the experiment.

Plasma lipids: There was no difference in plasma cholesterol level between animals killed on day 0 (baseline) and those fed the cholesterol-free diet (diet A) for 80 days (Table 6). Inclusion of cholesterol in the diet resulted in a two-fold increase in plasma cholesterol levels. Plasma cholesterol levels of group B were somewhat higher when compared to the other cholesterol-fed groups, but the difference did not reach statistical significance ($P \geq 0.278$). Birds from group A had significantly higher plasma phospholipid levels when compared to the cholesterol-fed animals ($P \leq 0.004$). The cholesterol-fed animals had significantly lower plasma triglycerides levels than the animals fed the cholesterol-free diet ($P \leq 0.003$). Birds fed diet C had significantly higher triglyceride levels than those fed diet B or D ($P \leq 0.03$). The plasma triglyceride levels were highest at baseline and the HDL cholesterol levels were lowest. Feeding cholesterol resulted in a significant increase ($P \leq 0.001$) in plasma LDL-cholesterol levels. Among the cholesterol-fed groups, animals in group B had somewhat higher levels of LDL-cholesterol than animals in the other two groups, but this difference was not significant ($P \geq 0.320$). The ratio LDL/HDL cholesterol was lowest in the animals fed diet A. In animals fed the cholesterol-free diet (diet A), about 77% of total plasma cholesterol was recovered in HDL-cholesterol and 21% in LDL-cholesterol. When cholesterol was added to the diet, about 53% of plasma total cholesterol was found in LDL-cholesterol and only 45% in HDL-cholesterol.

Evaluation of atherosclerosis: Grossly visible plaques were only present in 8 out of the 53 cholesterol-fed birds (Table 7). The quails fed the cholesterol-free diet (diet A) showed no plaques, but showed similar severity of red coloration and spots as did the cholesterol-fed groups. There was no significant difference in the degree of atherosclerosis among the cholesterol-fed groups. Group C had a significantly higher incidence of spots than the other groups ($P = 0.019$). The degree and incidence of red coloration of aorta and brachiocephalic arteries did not differ significantly between the dietary groups. Red coloration was absent at baseline.

Liver: Compared with baseline value liver weight dropped markedly after 80 days of feeding the diets (Table 8). Liver weight was significantly increased after cholesterol feeding ($P \leq 0.04$). There was a significant correlation between plasma total cholesterol and liver weight ($r = 0.41$, $P < 0.001$). The amount of liver cholesterol had increased significantly when cholesterol was added to the diet ($P < 0.001$). There was no

Table 10: The fatty acid composition of thigh muscle in quails fed the different diets

Fatty acid	Diet A	Diet B	Diet C	Diet D
C12:0	5.9 ± 3.7[a]	4.7 ± 2.4[a]	0.9 ± 0.3[b]	0.7 ± 0.3[b]
C14:0	4.1 ± 1.5[a]	3.8 ± 1.2[a]	0.9 ± 0.1[b]	0.8 ± 0.2[b]
C16:0	12.0 ± 1.0[a]	12.1 ± 1.0[a]	9.9 ± 0.9[b]	10.6 ± 0.9[b]
C18:0	12.0 ± 4.0	13.4 ± 3.2	12.6 ± 3.0	14.5 ± 2.8
C18:1 n-9	20.0 ± 4.4[a]	19.0 ± 3.7[ab]	16.9 ± 3.2[b]	17.9 ± 2.7[ab]
C18:2 n-6	22.1 ± 1.8[a]	23.7 ± 1.2[b]	39.9 ± 3.2[c]	22.6 ± 1.4[ab]
C18:3 n-3	4.8 ± 1.0[a]	4.6 ± 0.8[ab]	3.8 ± 0.7[b]	14.7 ± 2.7c
C20:5 n-3	0.6 ± 0.4[a]	0.7 ± 0.4[a]	0.0 ± 0.0[b]	2.2 ± 0.4[c]
C22:5 n-3	1.0 ± 0.3[a]	1.5 ± 0.4[b]	1.0 ± 0.4[a]	1.7 ± 0.3[b]
C22:6 n-3	5.3 ± 2.4[a]	4.6 ± 1.4[a]	3.3 ± 0.9[b]	4.4 ± 0.8[a]

Data are expressed as g methyl esters / 100 g methyl esters.
Means ± SD. Values that have a different superscript in the same row differ significantly (P < 0•05)

Table 11: The fatty acid composition of liver in quails fed the different diets

Fatty acid	Diet A	Diet B	Diet C	Diet D
C12:0	1.5 ± 0.9[a]	1.1 ± 0.8[a]	0.2 ± 0.2[b]	0.1 ± 0.1[b]
C14:0	1.3 ± 0.5[a]	1.3 ± 0.5[a]	0.3 ± 0.1[b]	0.3 ± 0.1[b]
C16:0	13.8 ± 1.5[a]	11.4 ± 1.1[b]	9.2 ± 1.0[c]	8.8 ± 0.7[c]
C18:0	18.9 ± 2.9[a]	15.5 ± 2.2[b]	16.9 ± 2.9[bc]	17.4 ± 2.3[ac]
C18:1 n-9	11.7 ± 2.8[a]	23.4 ± 4.3[b]	17.4 ± 3.0[c]	20.5 ± 2.8[d]
C18:2 n-6	23.1 ± 1.6[a]	23.8 ± 1.9[a]	33.7 ± 3.5[b]	22.8 ± 1.8[a]
C18:3 n-3	3.3 ± 1.2[ab]	3.8 ± 0.7[a]	2.3 ± 0.7[b]	11.5 ± 3.4[c]
C20:5 n-3	1.9 ± 0.6[a]	1.9 ± 0.4[a]	0.8 ± 0.4[b]	4.3 ± 0.5[c]
C22:5 n-3	1.3 ± 0.5[a]	0.9 ± 0.3[b]	0.6 ± 0.2[c]	1.1 ± 0.2[ab]
C22:6 n-3	8.9 ± 1.3[a]	5.0 ± 1.4[b]	3.8 ± 1.0[c]	4.4 ± 0.9[bc]

Data are expressed as g methyl esters / 100 g methyl esters.
Means ± SD. Values that have a different superscript in the same row differ significantly (P < 0•05)

difference among the cholesterol-fed groups. Liver cholesterol content was significantly correlated with either plasma total cholesterol or plasma LDL-cholesterol ($r = 0.80$, $P < 0.001$ for both correlations). Hepatic triglyceride content was significantly higher at baseline ($P < 0.001$). Addition of cholesterol to the diet resulted in a decrease in liver triglyceride level, but only diet C produced significantly lower levels when compared to diet A ($P = 0.001$). Liver triglyceride was significantly correlated with the plasma triglyceride level ($r = 0.58$, $P < 0.001$) and was inversely correlated with either plasma total cholesterol or plasma LDL-cholesterol ($r = -0.40$, $P < 0.001$ and $r = -0.39$, $P < 0.001$, respectively).

Tissue fatty acid composition: The fatty acid composition of the various tissues is given in Tables 9 to 14. Feeding diet A or B resulted in significantly higher levels of lauric acid ($P<0.001$), myristic acid ($P<0.001$) and palmitic acid ($P<0.001$) when compared to diets C and D. Animals fed diet C had significantly higher levels of linoleic acid ($P<0.001$) and significantly lower levels of eicosapentaenoic acid (EPA, C20:5n-3), docosapentaenoic acid (DPA, C22:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) ($P = 0.12$) than the other three groups. Animals in group D had significantly higher levels of α-linolenic acid ($P < 0.001$) and EPA ($P < 0.001$) and significantly lower levels of linoleic acid ($P \leq 0.016$).

Diet B versus diet A only differed with respect to the amount of cholesterol. Tables 9 and 10 show that cholesterol feeding reduced the percentage of DHA, and raised those of linoleic acid and DPA in breast and thigh muscle. Diet B versus diet A increased the levels of α-linolenic and oleic acid in plasma cholesteryl esters, and decreased the levels of the saturated fatty acids, lauric, myristic and palmitic acid, and also lowered the percentages of stearic acid, linoleic acid and DHA (Table 13). In liver tissue (Table 11), the percentages of DPA and DHA were significantly decreased after cholesterol consumption (diet B versus diet A) and that of oleic acid was increased.

There were also differences in the fatty acid composition between the various tissues. Adipose tissue had significantly higher levels of lauric acid ($P < 0.001$) and myristic acid ($P < 0.001$), when compared to the other tissues. Plasma cholesteryl esters (CE) and triglycerides (TG), and liver tissue had the lowest levels of lauric and myristic acid. The amount of palmitic acid differed significantly between the two muscle samples, breast muscle showing the highest value ($P < 0.001$). The level of palmitic acid was highest in plasma TG (P

Table 12: The fatty acid composition of adipose tissue in quails fed the different diets

Fatty acid	Diet A	Diet B	Diet C	Diet D
C12:0	20.5 ± 4.0[a]	22.9 ± 3.7[b]	2.6 ± 0.4[c]	2.9 ± 0.4[c]
C14:0	8.4 ± 0.6[a]	8.6 ± 0.7[a]	1.4 ± 0.09[b]	1.6 ± 0.1[b]
C16:0	8.8 ± 1.0[a]	8.6 ± 1.0[a]	6.9 ± 0.7[b]	7.9 ± 0.6[c]
C18:0	2.9 ± 0.6[a]	3.0 ± 0.4[ab]	3.4 ± 0.6[bc]	3.6 ± 0.6[c]
C18:1 n-9	26.1 ± 2.2[a]	25.0 ± 1.8[b]	23.2 ± 1.0[c]	31.0 ± 0.8[d]
C18:2 n-6	22.0 ± 1.2[a]	21.8 ± 1.7[a]	54.0 ± 1.9[b]	23.8 ± 1.7[c]
C18:3 n-3	6.5 ± 1.0[a]	6.1 ± 0.8[a]	5.6 ± 0.8[a]	25.6 ± 2.4[b]
C20:5 n-3	0.01 ± 0.04[a]	0.01 ± 0.04[a]	0.03 ± 0.01[a]	0.06 ± 0.08[b]
C22:5 n-3	0.0 ± 0.0[a]	0.1 ± 0.03[a]	0.03 ± 0.07[ab]	0.07 ± 0.09[b]
C22:6 n-3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Data are expressed as g methyl esters / 100 g methyl esters.
Means ± SD. Values that have a different superscript in the same row differ significantly (P < 0•05)

Table 13: The fatty acid composition of plasma cholesteryl-esters in quails fed the different diets

Fatty acid	Diet A	Diet B	Diet C	Diet D
C12:0	1.5 ± 0.6[a]	1.0 ± 0.4[b]	0.2 ± 0.2[c]	0.08 ± 0.1[c]
C14:0	2.5 ± 0.3[a]	2.2 ± 0.3[b]	0.7 ± 0.2[c]	0.7 ± 0.1[c]
C16:0	9.3 ± 0.8[a]	5.0 ± 0.5[b]	3.8 ± 0.6[c]	3.6 ± 0.5[c]
C18:0	2.6 ± 0.6[a]	2.0 ± 0.7[b]	1.8 ± 0.3[b]	1.9 ± 0.4[b]
C18:1 n-9	13.2 ± 1.8[a]	33.7 ± 3.9[b]	23.8 ± 1.6[c]	27.4 ± 2.1[d]
C18:2 n-6	49.6 ± 4.1[a]	34.4 ± 3.2[b]	52.3 ± 3.0[c]	27.0 ± 2.7[d]
C18:3 n-3	5.7 ± 0.8[a]	9.2 ± 1.0[b]	5.6 ± 0.9[a]	24.8 ± 2.1[c]
C20:5 n-3	2.4 ± 0.7[a]	2.6 ± 0.6[a]	1.3 ± 0.3[b]	4.4 ± 0.7[c]
C22:5 n-3	0.09 ± 0.2	0.2 ± 0.2	0.1 ± 0.2	0.2 ± 0.2
C22:6 n-3	2.9 ± 0.5[a]	1.2 ± 0.4[b]	1.0 ± 0.4[b]	1.2 ± 0.5[b]

Data are expressed as g methyl esters / 100 g methyl esters.
Means ± SD. Values that have a different superscript in the same row differ significantly (P < 0•05)

< 0.001), and lowest in plasma CE (P < 0.001). Most stearic acid was found in the liver (P < 0.001), while plasma CE had the lowest levels. The amounts of oleic acid were lower in liver and muscle when compared to the other tissues (P < 0.001). Plasma TG had significantly higher levels of oleic acid when compared to plasma CE (P = 0.012). Linoleic acid was abundant in plasma CE when compared to the other tissues (P < 0.001), liver and muscle had the lowest levels (P < 0.001). For α-linolenic acid there was a difference between breast and thigh muscle, the latter having significantly higher levels (P = 0.012). Highest levels of α-linolenic acid were found in adipose tissue (P < 0.001). Plasma CE had the highest levels of EPA, while adipose tissue had the lowest levels (P < 0.001). For both DPA and DHA, breast muscle had significantly lower levels when compared to thigh muscle (P < 0.001). DPA was highest in thigh muscle (P < 0.001) and DHA in liver (P < 0.001); both fatty acids were lowest in adipose tissue (P < 0.001).

Discussion

The major question addressed was whether the feeding of α-linolenic acid instead of linoleic acid or saturated fatty acids would diminish the degree of atherosclerosis in cholesterol-fed quails. The quails fed diet D showed significantly less atherogenic spots in the artery wall than those fed diet C. This observation might indicate that α-linolenic acid versus linoleic acid inhibits cholesterol-induced atherogenesis in quails. However, there was no effect of α-linolenic acid on either red coloration of aorta and brachiocephalic arteries or on plaque formation. Thus, it is difficult to see why α-linolenic acid reduced the number of atherogenic spots, i.e. inhibited the initiation of atherosclerosis, but did not influence the accumulation of lipid in the artery wall and the progression of atherosclerosis measured as plaque formation. Furthermore, no difference in spots was found between diets B and diet D, indicating the α-linolenic acid and saturated fatty acids had no differential effect. Thus, it may be concluded that α-linolenic acid versus either linoleic acid or saturated fatty acids has no effect on atherogenesis. The feeding of α-linolenic acid instead of either linoleic acid or saturated fatty acids did not significantly affect plasma cholesterol. This observation also points at a lack of anti-atherogenic effect of α-linolenic.

The addition of 2.0% cholesterol to the diet indeed resulted in an increase in plasma cholesterol and liver cholesterol, and also induced atherosclerosis. However,the increase in plasma cholesterol was less than expected on the basis of literature data (Toda and Oku, 1995; Yuan *et al.*, 1999). The plasma cholesterol levels of the animals fed the negative control diet A

Table 14: The fatty acid composition of plasma triglycerides in quails fed the different diets

Fatty acid	Diet A	Diet B	Diet C	Diet D
C12:0	0.8 ± 0.6[a]	0.9 ± 0.7[a]	0.2 ± 0.2[b]	0.05 ± 0.2[b]
C14:0	2.3 ± 0.7[a]	2.5 ± 0.5[a]	0.5 ± 0.4[b]	0.5 ± 0.3[b]
C16:0	16.3 ± 1.3[a]	15.0 ± 2.3[b]	12.9 ± 2.0[c]	10.7 ± 0.8[d]
C18:0	5.1 ± 0.7[a]	4.9 ± 0.8[a]	5.4 ± 0.6[ab]	5.7 ± 0.5[b]
C18:1 n-9	32.4 ± 2.1[a]	28.5 ± 3.8[b]	21.2 ± 1.3[c]	25.6 ± 2.6[d]
C18:2 n-6	24.0 ± 1.9[a]	24.2 ± 3.6[a]	42.2 ± 4.0[b]	21.7 ± 2.8[c]
C18:3 n-3	7.3 ± 0.9[a]	7.5 ± 1.4[a]	5.4 ± 1.1[b]	23.2 ± 4.3[c]
C20:5 n-3	1.5 ± 0.5[a]	1.2 ± 0.3[a]	0.5 ± 0.4[b]	2.5 ± 0.6[c]
C22:5 n-3	0.8 ± 0.4	1.1 ± 0.7	0.7 ± 0.5	0.9 ± 0.3
C22:6 n-3	1.9 ± 0.4[a]	1.6 ± 0.7[ab]	1.2 ± 0.7[b]	1.2 ± 0.4[b]

Data are expressed as g methyl esters / 100 g methyl esters.
Means ± SD. Values that have a different superscript in the same row differ significantly ($P < 0.05$)

resembled the values reported in literature, ranging from 3.6 to 7.3 mmol/l (Chamberlain and Belton, 1987; Hammad *et al.*, 1998; Morrissey and Donaldson, 1977b; Ojerio *et al.*, 1972; Siegel *et al.*, 1995; Toda and Oku, 1995). It appears that the quails used in the present study were relatively insensitive to the effect of dietary cholesterol on plasma cholesterol. We used Japanese quail with an average body weight of 264 g and consuming approximately 22.5 g of feed / day. The Japanese quail reported on in the literature had body weights ranging from 100 to 140 g and consumed 14-17 g of feed / day (Howes and Ivey, 1962; Shih, 1983; Toda and Oku, 1995; Wilson, 1961). Thus, the quails used in this study were genetically different from those used by others.

Plaque formation was only seen in cholesterol-fed animals, but only in 8 out of the 53 cholesterol-fed birds. The red coloration and spots found in the artery wall of the quails may be the result of spontaneous atherosclerosis, because cholesterol feeding had no effect on these items. Other authors (Morrissey and Donaldson, 1977a; Shih *et al.*, 1983) found a high incidence of atherosclerosis in Japanese quail challenged with dietary cholesterol. In the current study, no consistent effect of fat type was found on the development of cholesterol-induced atherosclerosis. Other authors did find an effect of n-3 polyunsaturated fatty acids on atherosclerosis in quail. Fann *et al.* (1989) reported that feeding a diet with 10 % fish oil lowered the incidence of cholesterol-induced atherosclerosis. Chamberlain *et al.* (1991), on the other hand, found an increase in fatty streaks when fish oil was added to a low-cholesterol diet, this effect probably being due to the high cholesterol content of the fish oil added to the diet. Sadi *et al.* (1996) investigated the atherogenicity of various oils, and found that perilla oil, which is rich in α-linolenic acid, was less atherogenic than oils rich in linoleic or oleic acid.

Feeding cholesterol resulted in a significant decrease in plasma triglyceride and phospholipid levels. This observation is contrary to literature reports (Chamberlain and Belton, 1987; Hammad *et al.*, 1998; Hoekstra *et al.*, 1998; Radcliffe and Liebsch, 1985; Yuan *et al.*, 1997; Yuan *et al.*, 1998). The observed decrease in plasma triglycerides was paralleled by a decrease in liver triglycerides. This could indicate that hepatic lipoproteins were secreted with a lower level of triglycerides and a higher level of cholesterol. However, Oku *et al.* (1993) investigated the feeding of cholesterol on the composition of lipoproteins and found no effect on triglyceride content of the various lipoproteins.

Without cholesterol added to the diet, HDL was the major lipoprotein transporting cholesterol, since about 77% of total cholesterol was transported by this fraction. This agrees with the literature (Hammad *et al.*, 1998; Nagata *et al.*, 1997; Oku *et al.*, 1993). Addition of cholesterol to the diet did not alter the amount of HDL-cholesterol, but increased LDL-cholesterol, which became the carrier of about 53% of total cholesterol. This shift in lipoprotein profile was also reported by Hammad *et al.* (1998). Other authors have mentioned that VLDL becomes the major transport vehicle in cholesterol-fed birds (Nagata *et al.*, 1997; Oku *et al.*, 1993). As Hammad *et al.* (1998) suggested, the discrepancy is probably due to differences in laboratory determination of lipoproteins. The method used in this study was similar to that used by Hammad *et al.* (1998).

In essence, the different fatty acid compositions of the diets were reflected by the fatty acid composition of the various tissues, including CE and TG in plasma. However, there also were diet effects on fatty acid metabolism in tissues. Feeding diet C rich in linoleic acid decreased the amounts of EPA and DHA in tissues, which is explained by competition between linoleic and α-linolenic acid for the elongation and desaturation enzymes (Raatz *et al.*, 2001; Simopoulos, 1991). Animals from group D fed extra α-linolenic acid showed an increase in EPA content of tissues, indicating that quail can convert α-linolenic acid into EPA. There were differences in fatty acid composition of the tissues for diets A and B, indicating that cholesterol feeding results in changes in fatty acid composition. Addition of

cholesterol led to an increase in oleic acid and a decrease in saturated fatty acids. This was also reported by Muriana *et al.* (1992). It is known that cholesterol feeding tends to decrease membrane fluidity which is compensated for by increased unsaturation of fatty acids (Beynen *et al.*, 1984; Lutz *et al.*,1998).

Differences were also found in fatty acid composition of the different tissues. This can be explained by either selective incorporation or preferential oxidation of fatty acids. For example, the enzyme involved in the incorporation fatty acids into the plasma CE, lecithin-cholesterol acyltransferase (LCAT), has linoleic acid and EPA as preferred substrates (Liu *et al.*, 1995). This results in significantly higher levels of these fatty acids in plasma CE. A high amount of EPA in plasma CE has also been reported by other authors (Harris, 1989; Katan *et al.*, 1997; Plantinga and Beynen, 2003; Zuijdgeest-van Leeuwen *et al.*, 1999). Leaf *et al.* (1995) also reported differences in fatty acid composition between adipose tissue and plasma lipids. They suggested that adipose tissue fatty acid composition could be affected by metabolic conversions occurring between different fatty acids as well as by the preferential release of certain fatty acids from adipose tissue during mobilization.

In conclusion, cholesterol feeding of Japanese quail elevated plasma and liver cholesterol, induced atherosclerosis, and altered fatty acid metabolism. Under the conditions of this study, a differential effect on atherosclerosis of α-linolenic acid, linoleic acid and saturated fatty acids could not be demonstrated.

References

Bavelaar, F. and A.C. Beynen, 2003a. Severity of atherosclerosis in parrots in relation to the intake of a-linolenic acid. Avian Dis., 47: 566-577.

Bavelaar, F. and A.C. Beynen, 2003b. Mathematical relationship between dietary fatty acid composition and either melting point or fatty acid profile of adipose tissue in broilers. Meat Sci., 64: 133-140.

Beynen, A., J.A. Schouten and C. Pop-Snijders, 1984. Compensatory changes in the lipid composition of the erythrocyte membrane. Trends Biochem. Sci., 9: 474.

Bligh, E. and W.J. Dyer, 1959. A rapid method of total lipid extraction and purification. Can. J. Bioch. Physiol., 37: 911-917.

Carr, T., C.J. Andressen and A.L. Rudel, 1993. Enzymatic determination of triglyceride, free cholesterol, and total cholesterol in tissue lipid extracts. Clin. Biochem., 26: 39-42.

Chamberlain, J. and C. Belton, 1987. Effects of long term consumption of fish oil (Maxepa) on serum lipids and arterial ultrastructure in Japanese quail. Atherosclerosis, 68: 95-103.

Chamberlain, J., L.E. Dittmann, S. Hunt, H. Olson and J.R. Cashman, 1991. Effects of long term 2% fish oil supplements on tissue fatty acids, phospholipids, cholesterol, and arterial histology in Japanese quail. Artery, 18: 291-314.

Chan, J., V.M. Bruce and B.E. McDonald, 1991. Dietary alpha-linolenic acid is as effective as oleic acid and linoleic acid in lowering blood cholesterol in normolipidemic men. Am. J. Clin. Nutr., 53: 1230-1234.

Consensus Conference, 1985. Lowering blood cholesterol to prevent heart disease. J. Am. Med. Assoc., 253: 2080-2086.

Davis, H., R.T. Bridenstine, D. Vesselinovitch and R.W. Wissler, 1987. Fish oil inhibits the development of atherosclerosis in rhesus monkeys. Arterioscler, 7: 441-449.

Dorrestein, G., P. Zwart, G.H.A. Borst, F.G. Poelma and M.N. Buitelaar, 1977. Ziekte-en doodsoorzaken van vogels. Tijdschr Diergeneeskd, 102: 437-447.

Fann, J., S.K. Angell, P.D. Cahill, J.C. Kosek and D.C. Miller, 1989. Effects of fish oil on atherosclerosis in the Japanese quail. Cardiovasc Res., 23: 631-638.

Fiennes, R., 1965. Atherosclerosis in Wild Animals. In Comparative Atherosclerosis, pp: 113-126 [J Roberts, and Strauss R, editor].

Finlayson, R. and V. Hirchinson, 1961. Experimental Atheroma in Budgerigars. Nature, 192: 369-370.

Folch, J., M. Lees and G.H. Sloane Stanley, 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226: 497-509.

Fox, H., 1933. Arteriosclerosis in lower mammals and birds: Its relation to the disease in man. In *Arteriosclerosis*, pp: 153-193 [EV Cowdry, editor]. New York: The MacMillan Company.

Friedewald, W., R.I. Levy and D.S. Fredrickson, 1972. Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem. 18: 499-502.

Griner, L., 1983. Pathology of zoo animals; a review of necropsies conducted over a 14-year period at the San Diego zoo an San Diego wild animal park. San Diego: Zoological society of San Diego.

Grünberg, W., 1964. Spontane Arteriosklerose beim Vogel. Bull. Soc. Roy. Zool d'Anv., 34: 21-48.

Grünberg, W., 1966. Arteriosklerose beim Wildtieren. Klin Wochenschr, 43: 479-488.

Grundy, S., 1986. Cholesterol and coronary heart disease: A new era. J. Am. Med. Assoc., 256: 2849-2858.

Grundy, S. and M.A. Denke, 1990. Dietary influences on serum lipids and lipoproteins. J. Lipid Res., 31: 1149-1172.

Grundy, S., D. Bilheimer and H. Blackburn, 1982. Rationale of the Diet-Heart Statement of the American Heart Association. Circulation, 4: 839A-851A.

Guallar, E., A. Aro and F.J.J. Jimenez, 1999. Omega-3 fatty acids in adipose tissue and risk of myocardial infarction: The EURAMIC Study. Arterioscler Thromb Vasc Biol., 19: 1111-1118.

Hamilton, J. and K. Comai, 1988. Rapid separation of neutral lipids, free fatty acids and polar lipids using prepacked silica sep-pak columns. Lipids, 23: 1146-1149.

Hammad, S., H.S. Siegel and H.L. Marks, 1998. Total cholesterol, total triglycerides, and cholesterol distribution and lipoproteins as predictors of atherosclerosis in selected lines of Japanese quail. Comp. Biochem. Physiol. A Mol. Integr. Physiol., 119: 485-492.

Harper, C. and T.A. Jacobson, 2001. The fats of life: the role of omega-3 fatty acids in the prevention of coronary heart disease. Arch Intern. Med., 161: 2185-2192.

Harris, W., 1989. Fish oils and plasma lipid and lipoprotein metabolism in humans: a critical review. J. Lipid. Res., 30: 785-807.

Hoekstra, K., C.R. Nichols, M.E. Garnett, D.V. Godin and K.M. Cheng, 1998. Dietary cholesterol-induced xanthomatosis in atherosclerosis-susceptible Japanese quail. J. Comp. Path., 119: 419-427.

Horlick, I. and L.N. Katz, 1949. The relationship of atheromatosis development in the chicken to the amount of cholesterol added to the diet. Am. Heart J., 38: 336-349.

Howes, J. and W.D. Ivey, 1962. Cortunix quail vor veterinary research. J. Am. Vet. Med. Assoc., 140: 162-163.

Hu, F., M.J. Stampfer and J.E. Manson, 1999. Dietary intake of alpha-linolenic acid and risk of fatal ischemic heart disease among women. Am. J. Clin. Nutr., 69: 890-897.

Johnson, J., D.N. Phalen, V.H. Kondik, T. Tippit and D.L. Graham, 1992. Atherosclerosis in psittacine birds. Proceedings 13th an. Conf. Assoc. Avian Vet., 87-93.

Kakita, C., J. Johnson, R. Pick and L.N. Katz, 1972. Relationship between plasma cholesterol level and coronary atherosclerosis in cholesterol-oil fed cockerels. Atherosclerosis, 15: 17-29.

Katan, M., J.P. Deslypere, A.P.J.M. van Birgelen, M. Penders and M. Zegwaard, 1997. Kinetics of the incorporation of dietary fatty acids into serum cholesteryl esters, erythrocyte membranes, and adipose tissue: an 18 month controlled study. J. Lipid. Res., 38: 2012-2022.

Kempeneers, P., 1987. Atherosclerose bij de papegaai, Utrecht University.

Klasing, K., 1998. Comparative Avian Nutrition. Wallingford: CAB International.

Kloeze, J., U.M. Houtsmuller and R.O. Vles, 1969. Influence of dietary fat mixtures on platelet adhesiveness, atherosclerosis and plasma cholesterol content in rabbits. J. Atheroscl Res., 9: 319-334.

Leaf, D., W.E. Connor, L. Barstad and G. Sexton, 1995. Incorporation of dietary n-3 fatty acids into the fatty acids of human adipose tissue and plasma lipid classes. Am. J. Clin. Nutr., 62: 68-73.

Liu, M., J.D. Bagdade and P.V. Subbaiah, 1995. Specificity of lecithin:cholesterol acyltransferase and atherogenic risk: comparative studies on the plasma composition and in vitro synthesis of cholesteryl esters in 14 vertebrate species. J. Lipid Res., 36: 1813-1824.

Lutz, M., S. Bonilla, J. Concha, J. Alvarado and P. Barraza, 1998. Effect of dietary oils, cholesterol and antioxidant vitamin supplementation on liver microsomal fluidity and xenobiotic-metabolizing enzymes in rats. Ann. Nutr. Metab., 42: 350-359.

Martin, M., S.B. Hulley, W.S. Browner, L.H. Kuller and D. Wentworth, 1986. Serum cholesterol, blood pressure, and mortality, implications from a cohort of 361662 men. Lancet, 2: 933-936.

Metcalfe, L., A.A. Schmitz and J.R. Pelka 1966. Rapid preparation of fatty acid esters from lipids for gaschromatographic analysis. Anal. Chem., 318: 514-515.

Morrissey, R., and W.E. Donaldson, 1977a. Rapid accumulation of cholesterol in serum, liver and aorta of Japanese quail. Poult. Sci., 56: 2003-2008.

Morrissey, R. and W.E. Donaldson, 1977b. Diet composition and cholesterolemia in Japanese quail. Poult. Sci., 56: 2108-2110.

Muriana, F., C.M. Vazquez and V. Ruiz-Gutierrez, 1992. Fatty acid composition and properties of the liver microsomal membrane of rats fed diets enriched with cholesterol. J. Biochem., 112: 562-567.

Nagata, J., G. Maeda, H. Oku, T. Toda and I. Chinen, 1997. Lipoprotein and apoprotein profiles of hyperlipidemic atherosclerosis-prone Japanese quail. J. Nutr. Sci. Vitaminol., 43: 47-57.

Ojerio, A., G.J. Pucak, T.B. Clarkson and B.C. Bullock, 1972. Diet-induced atherosclerosis and myocardial infarction in Japanese quail. Lab. Anim. Sci., 22: 33-39.

Oku, H., M. Ishikawa, J. Nagata, T. Toda and I. Chinen, 1993. Lipoprotein and apoprotein profile of Japanese quail. Biochim. Biophys. Acta., 1167: 22-28.

Peebles, E., J.D. Cheany and K.M. Vaughn, 1996. Changes in gonadal weights, serum lipids, and glucose during maturation of the juvenile Northern Bobwhite quail (Colinus virginianus). Poult. Sci., 75: 1411-1416.

Phalen, D., H.B. Hays, L.J. Filippich, S. Silverman and M. Walker, 1996. Heart failure in a macaw with atherosclerosis in the aorta and brachiocephalic arteries. J. Am. Vet. Med. Assoc., 209: 1435-1440.

Plantinga, E. and A.C. Beynen, 2003. The influence of dietary fish oil vs. sunflower oil on the fatty acid composition of plasma cholesteryl-esters in healthy, adult cats. J. Anim. Physiol. Anim. Nutr., 87: 1-7.

Raatz, S., D. Bibus, W. Thomas and P. Kris-Etherton, 2001. Total fat intake modifies plasma fatty acid composition in humans. J. Nutr., 131: 231-234.

Radcliffe, J. and K.S. Liebsch, 1985. Dietary induction of hypercholesterolemia and atherosclerosis in Japanese quail of strain SEA. J. Nutr., 115: 1154-1161.

Reed, D., J.P. Strong, J. Resch and T. Hayashi, 1989. Serum lipids and lipoproteins as predictors of atherosclerosis. Arteriosclerosis, 9: 560-564.

Ross, R., 1999. Atherosclerosis - An inflammatory disease. New Eng. J. Med., 340: 115-126.

Sadi, A., T. Toda, H. Oku and S. Hokama, 1996. Dietary effects of corn oil, oleic acid, perilla oil, and evening primrose oil on plasma and hepatic lipid level and atherosclerosis in Japanese quail. Exp. Anim. Jap. Ass. Lab. Anim. Sci., 45: 55-62.

Shih, J., 1983. Atherosclerosis in Japanese quail and the effect of lipoic acid. Fed Proc, 42: 2494-2497.

Shih, J., E.P. Pullman and K.J. Kao, 1983. Genetic selection, general characterization and histology of atherosclerosis-susceptible and -resistant Japanese quail.Atherosclerosis, 49: 41-53.

Siegel, H., S.M. Hammad and R.M. Leach, 1995. Dietary cholesterol and fat saturation effects on plasma esterified and unesterified cholesterol in selected lines of Japanese quail females. Poult. Sci., 74: 1370-1380.

Simopoulos, A., 1991. Omega-3 fatty acids in health and disease and in growth and development. Am. J. Clin. Nutr., 54: 438-463.

Simopoulos, A., 2002. Omega-3 fatty acids in inflammation and autoimmune diseases. J. Am. Coll. Nutr., 21: 495-505.

Toda, T. and H. Oku, 1995. Effect of medium-chain fatty acids on cholesterolemia and atherosclerosis in Japanse quails. Nutr. Res., 15: 99-113.

Wilson, W., U.K. Abbott and H. Abplanalp, 1961. Evaluation of Cortunix (Japanese quail) as a pilot animal for poultry. Poult. Sci., 40: 651-657.

Yuan, Y., D.D. Kitts and D.V. Godin, 1997. Influence of dietary cholesterol and fat source on atherosclerosis in the Japanese quail. Br. J. Nutr., 78: 993-1014.

Yuan, Y., D.D. Kitts and D.V. Godin, 1998. Interactive effects of increased intake of saturated fat and cholesterol on atherosclerosis in the Japanese quail. Br. J. Nutr., 80: 89-100.

Yuan, Y., D.D. Kitts and D.V. Godin, 1999. Influence of increased saturated fatty acid intake from beef tallow on antioxidant status and plasma lipids in atherosclerosis-susceptible Japanese quail. Nutr. Res., 19: 461-481.

Zuijdgeest-van Leeuwen, S., P.C. Dagnelie, R. Rietveld, J.W.O. van den Berg and J.H.P. Wilson, 1999. Incorporation and washout of orally administered n-3 fatty acid ethyl esters in different plasma lipid fractions. Br. J. Nutr., 82: 481-488.