BUTTERWORTH HEINEMANN

Flavonoids increase tissue essential fatty acids in vitamin E–deficient chicks

Kenneth J. Jenkins and A.S. Atwal

Centre for Food and Animal Research, Research Branch, Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada

This study assessed the effect of dietary flavonoids (quercetin, morin, rutin, silymarin), a simple phenolic (ferulic acid), and vitamin E on lipid composition of selected tissues taken from chicks fed a low vitamin E, low essential fatty acid (EFA) diet. Dietary supplementation with vitamin E had no influence on lipid classes in blood plasma, liver, or breast muscle or on their fatty acid composition. Similarly, the phenolics had no effect on lipid classes in these tissues but quercetin, morin, and ferulic acid had marked effects on the fatty acid composition of tissue lipids, notably by reducing 18:1 and 20:3 n-9(triene indicator of EFA deficiency), and increasing 18:2 n-6, 20:4 n-6, and total n-6 fatty acids. These phenolic antioxidants appear to have promoted the production and/or the conservation of EFA in the EFA- and vitamin E–deficient chicks. (J. Nutr. Biochem. 6:97–103, 1995.)

Keywords: flavonoids; essential fatty acids; tissue lipids; chicks

Introduction

Flavonoids are naturally occurring benzo-γ-pyrone derivatives widely distributed in fruits, vegetables, nuts, seeds, flowers, and bark.[1] The human dietary consumption of flavonoids in the United States was estimated in 1976 as about 1 g/day.[2] However, recent data obtained on edible parts of foods and from food disappearance studies, using modern HPLC analytical methods, indicate that this was a gross overestimate.[3] Dutch flavonoid intakes have been estimated as only 24 to 26 mg daily.[3] Although flavonoids have not been shown to have a primary metabolic function in plants, they do appear to have a role in plant defense against disease and predation.[4] In animals there have been numerous reports indicating that flavonoids can have beneficial effects. For example in humans they have been widely used for providing protection against hepatoxic agents[5] and for reducing capillary fragility and permeability problems.[6]

Some beneficial effects of flavonoids have been found in lipid metabolism apparently related to their antioxidant properties.[6] These have included a reduction of serum lipids and cholesterol,[7,8] protection against peroxidation in human platelets,[5] and inhibition of lipoxygenase and prostaglandin synthetase activities.[9] There are also reports of flavonoids altering arachidonic acid metabolism.[10] In view of these later reports on lipid effects, we utilized an ongoing investigation on the protective efficacy of dietary flavonoid antioxidants against nutritional muscular dystrophy in vitamin E (antioxidant)–deficient chicks[11] to study whether these flavonoids altered lipid metabolism, as indicated by changes in lipid composition of selected tissues. Seven of the 10 treatment groups in the feeding experiment were chosen to provide blood plasma, liver, and breast muscle samples at the end of the 33-day feeding period. Analyses included lipid classes and composition of fatty acids in the major lipid classes of the blood plasma and tissues collected.

Methods and materials

Animals, diets, tissue samples

Seventy 1-day-old unsexed chicks (Centre for Food and Animal Research meat strain 31; Chambers et al.[12]) were utilized for this

Contribution No. 2238, Centre for Food and Animal Research, Agriculture and Agri-Food, Canada.
Address reprint requests to Dr. Kenneth Jenkins at the Center for Food and Animal Research, Research Branch, Agriculture and Agri-Food Canada, Ottawa, Ontario, K1A 0C6 Canada.
Received July 1, 1994; accepted September 22, 1994.

Nutritional Biochemistry 6:97–103, 1995

655 Avenue of the Americas, New York, NY 10010

0955-2863/95/$10.00
SSDI 0955-2863(94)00018-H

study. Housing consisted of electrically heated wire-floored battery brooders, with 23 hr of light provided daily. Feed and water were provided for ad libitum consumption. A commercial starter ration was fed for the first 3 days, the birds randomized into lots of 10 chicks each, and then fed the various treatment diets for 33 days. The composition of the basal diet is shown in *Table 1*. The linoleic acid content was low to prevent the development of encephalomalacia in addition to muscular dystrophy. The dietary treatments were: basal diet, ferulic acid, morin, quercetin dihydrate, rutin trihydrate, silymarin, and a natural source of vitamin E as d-α-tocopheryl acetate. Ferulic acid is not a flavonoid but a simple phenolic; it was included as it is also a phenolic antioxidant that occurs commonly in plant foods and feeds. The flavonoids and simple phenolic were fed at the 1,000 ppm level in view of the report by Machlin et al.[13] that antioxidants, other than vitamin E, were required at this concentration to prevent muscular dystrophy in the chick. This was not an abnormally high intake for chicks as the concentration of polyphenolics in natural poultry grower rations frequently reaches 900 ppm[11] for humans in the United States, which would be about 2,000 ppm dietary flavonoids if the old intake estimate of 1 g of flavonoids/day,[2] is used. However, our 1,000 ppm level would be high for humans if the average daily intake of flavonoids for all humans is in reality close to the recent Dutch domestic estimate of only 24 to 26 mg daily.[3]

At the end of the experiment, blood samples were collected in heparinized tubes and centrifuged for plasma. The birds were killed by CO_2 asphyxiation and samples were taken of liver and breast muscle. Plasma and tissues were stored at −25°C. Scoring of birds for incidence and severity of nutritional muscular dystrophy were as described.[11]

Lipid analyses

Liver and muscle samples were frozen and pulverized at dry ice temperature and then extracted with chloroform:methanol (2:1) according to Kramer and Hulan.[14] All the lipid classes were separated by thin-layer chromatography using silica gel H coated plates,[15] and identified after spraying the plates with 2′,7′-dichlorofluorescein. The corresponding spots were scraped off, methyl heptadecanoate was added as an internal standard, and they were transmethylated directly using anhydrous HCl/methanol (5% by weight). The resulting methyl esters were analyzed by gas liquid chromatography.[16] The liver classes analyzed in the plasma, liver, and muscle samples were phosphatidylcholine (PC), cholesterol, cholesterol esters (CE), triglycerides, sphingomyelin, free fatty acids, lysophosphatidylcholine, phosphatidylethanolamine (PE), phosphatidylinositol, phosphatidylserine, and cardiolipin. The fatty acid composition of lipid classes was determined for plasma PC, CE, liver and muscle PC, and PE.

Table 1 Composition of low vitamin E basal diet and fatty acid composition of dietary lard

Ingredients	Weight %	Fatty acids*	
		Type	Weight %
Glucose	66.2	14:0	1.2
Casein, vitamin free	20.0	16:0	24.8
Cellulose	3.0	16:1 n-9	2.5
Lard, vitamin E-stripped	4.0	18:0	15.5
Glycine	0.6	18:1 n-9	40.8
L-arginine · HCl	1.0	—	—
Salts, premix, macro†	4.0	18:2 n-6	8.7
Salts, premix, micro‡	0.2	18:3 n-3	0.26
Vitamin premix§	1.0	20:4 n-6	0.13

*Relative abundance of fatty acids with minor fatty acids not included. Number of carbon atoms: number of double bonds, *n-x* where *n* is the chain length of the fatty acid, and *x* is the number of carbon atoms from the last double bond to the terminal methyl end.
†Macro salts mixture (g/100 g of diet): $CaHPO_4 \cdot 2H_2O$ 1.87; $CaCO_3$ 0.65; KH_2PO_4 0.69; MgO 0.08; NaCl 0.60.
‡Micro salts mixture (mg/100 g of diet): $FeSO_4 \cdot 7H_2O$ 41.4; $MnSO_4 \cdot H_2O$ 33.3; KI 0.26; $CuSO_4 \cdot 5H_2O$ 1.67; ZnO 6.0; $Na_2SeO_4 \cdot 10H_2O$ 0.047.
§Vitamin premix (mg/100 g of diet): niacin 5; calcium pantothenate 2; thiamine · HCl 1; riboflavin 1; pyridoxine · HCl 0.45; folic acid 0.4; menadione 0.05; D-biotin 0.02; vitamin B_{12} 0.002; vitamin A palmitate 500 IU; vitamin D_3 38 ICU; choline chloride 150.

Statistics

A randomized, one-way design was used with treatments composed of seven dietary treatments of 10 chicks each. Data were subjected to ANOVA and treatment means separated by Duncan's multiple range test[17] using 5% probability.

Results

The lipid classes analyzed for plasma, liver, and muscle samples are not presented as there were no changes in their concentrations ($P > 0.05$) as a result of the flavonoid, simple phenolic, or vitamin E treatments.

The concentrations of fatty acids in various tissue lipids are presented in *Tables 2, 3,* and *4*. As the main effects of the treatments were on the concentrations of the unsaturated fatty acids (UFA), the fatty acids are presented in the sequence in which they occur in the metabolic pathways for UFA (*Figure 1*).

There were virtually no differences in the tissue and plasma lipid fatty acid composition data between the low vitamin E basal and the vitamin E–supplemented group (*Tables 2, 3,* and *4*) indicating that vitamin E deficiency and the development of muscular dystrophy did not affect the formation of UFA and the uptake of fatty acids in general. There were, however, very consistent and striking effects of quercetin, morin, and ferulic acid on the fatty acid composition of the tissue lipids analyzed (*Tables 2, 3,* and *4*). Their effects were also similar within each fatty acid series. For example in the n-9 series there was a definite reduction in 18:1 and in most instances lowered 20:2, and 20:3. This suggests a reduction in Δ9-desaturase activity (which forms 18:1) and reduced activity of the desaturases and elongases that form the subsequent n-9 fatty acid metabolites. In the n-6 series, the quercetin, morin, and ferulic acid treatments increased the 18:2 and 20:3 fatty acids, and total n-6 fatty acids for all six lipids analyzed. The most marked increases were for 18:2 and 20:4 in muscle PE and liver PE, and total n-6 fatty acids for all six lipids. The 22:4 and 22:5 fatty acids, which were in low concentrations in all lipids, were generally increased. The 18:3 fatty acid, also in low concentrations, was increased in plasma CE. The results indicate increased incorporation of the 18:2 n-6 precursor and enhanced production of the n-6 UFA metabolites. The n-3 series fatty acid concentrations were very small because of the low dietary intake of 18:3 n-3 (0.01% of diet, *Table 1*). However, as was found for the n-6 series, there was a tendency for the quercetin, morin, and ferulic acid treatments to increase the n-3 UFA in many of the lipids. The

Table 2 Fatty acid composition of blood plasma phosphatidylcholine and cholesteryl esters*

Dietary Treatments	Fatty Acids									
	16:0	16:1 n-7	18:0	18:1 n-9	20:2 n-9	20:3 n-9	22:3 n-9	18:2 n-6	18:3 n-6	20:3 n-6
Plasma PC										
Basal	23.0	0.04	21.3^{c}	39.2^{a}	0.35^{a}	6.2^{a}	0.14	0.53^{c}	0.02	0.27^{b}
Ferulic acid	23.9	0.03	27.5^{a}	29.6^{b}	0.19^{b}	4.2^{c}	0.12	4.4^{b}	0.04	1.0^{a}
Morin	20.8	0.04	23.7^{b}	32.3^{b}	0.23^{b}	5.3^{b}	0.15	5.2^{ab}	0.05	1.3^{a}
Quercetin	21.4	0.03	24.1^{b}	31.9^{b}	0.20^{b}	5.0^{bc}	0.14	5.6^{a}	0.06	1.5^{a}
Rutin	21.2	0.06	20.6^{c}	42.3^{a}	0.35^{a}	4.9^{bc}	0.12	0.59^{c}	0.02	0.24^{b}
Silymarin	23.8	0.05	19.6^{c}	39.5^{a}	0.30^{a}	6.4^{a}	0.14	0.83^{c}	0.03	0.30^{b}
Vitamin E	21.8	0.05	20.5^{c}	41.2^{a}	0.34^{a}	6.4^{a}	0.13	0.50^{c}	0.02	0.19^{b}
SE	1.2	0.01	0.8	2.4	0.02	0.3	0.01	0.3	0.01	0.05
Plasma CE										
Basal	12.8	0.09	4.0	65.9^{a}	0.07	0.66^{bc}	0.0	1.3^{b}	0.05^{b}	0.19^{b}
Ferulic acid	13.1	0.08	3.8	53.9^{b}	0.03	0.56^{d}	0.0	12.1^{a}	0.26^{a}	0.99^{a}
Morin	14.4	0.12	4.0	51.7^{b}	0.06	0.54^{d}	0.0	12.2^{a}	0.24^{a}	0.97^{a}
Quercetin	13.6	0.10	4.4	53.5^{b}	0.06	0.58^{cd}	0.0	13.1^{a}	0.29^{a}	1.2^{a}
Rutin	11.7	0.11	3.2	66.5^{a}	0.05	0.67^{b}	0.0	1.2^{b}	0.05^{b}	0.17^{b}
Silymarin	12.6	0.08	3.6	65.8^{a}	0.09	0.77^{a}	0.0	2.0^{b}	0.09^{b}	0.23^{b}
Vitamin E	12.0	0.09	3.3	67.8^{a}	0.06	0.64^{bc}	0.0	1.3^{b}	0.05^{b}	0.21^{b}
SE	0.9	0.02	0.5	2.2	0.02	0.03	—	0.4	0.02	0.04

Dietary Treatments	Fatty Acids									
	20:4 n-6	22:4 n-6	22:5 n-6	Sum n-6	18:3 n-3	20:5 n-3	22:5 n-3	22:6 n-3	Sum n-3	Ratio triene:AA†
Plasma PC										
Basal	0.38^{c}	0.05^{c}	0.07^{b}	1.5^{c}	0.01	0.10	0.03^{b}	0.16^{c}	0.40^{c}	16.3^{b}
Ferulic acid	1.2^{b}	0.13^{b}	0.13^{a}	7.2^{b}	0.02	0.11	0.04^{b}	0.19^{bc}	0.60^{b}	3.5^{c}
Morin	1.9^{a}	0.20^{a}	0.17^{a}	9.1^{a}	0.04	0.14	0.07^{a}	0.26^{ab}	0.82^{a}	2.8^{c}
Quercetin	1.9^{a}	0.21^{a}	0.15^{a}	9.8^{a}	0.02	0.16	0.09^{a}	0.29^{a}	0.86^{a}	2.6^{c}
Rutin	0.25^{c}	0.04^{c}	0.03^{b}	1.4^{c}	0.01	0.11	0.03^{b}	0.12^{c}	0.37^{c}	19.6^{b}
Silymarin	0.35^{c}	0.06^{c}	0.05^{b}	1.8^{c}	0.01	0.14	0.03^{b}	0.12^{c}	0.39^{c}	18.3^{b}
Vitamin E	0.23^{c}	0.03^{c}	0.03^{b}	1.2^{c}	0.01	0.09	0.02^{b}	0.10^{c}	0.32^{c}	27.8^{a}
SE	0.1	0.02	0.02	0.4	0.008	0.03	0.01	0.03	0.04	1.6
Plasma CE										
Basal	0.19^{b}	0.0	0.0	1.8^{b}	0.03^{b}	0.10^{b}	0.0	0.0	0.28^{c}	3.5^{b}
Ferulic acid	0.75^{a}	0.0	0.0	14.2^{a}	0.17^{a}	0.19^{a}	0.0	0.0	0.46^{b}	0.75^{c}
Morin	0.80^{a}	0.0	0.0	14.2^{a}	0.13^{a}	0.17^{ab}	0.0	0.0	0.61^{a}	0.68^{c}
Quercetin	0.87^{a}	0.0	0.0	15.6^{a}	0.15^{a}	0.22^{a}	0.0	0.0	0.47^{b}	0.67^{c}
Rutin	0.13^{b}	0.0	0.0	1.5^{b}	0.04^{b}	0.11^{b}	0.0	0.0	0.49^{b}	5.2^{a}
Silymarin	0.20^{b}	0.0	0.0	2.5^{b}	0.03^{b}	0.14^{ab}	0.0	0.0	0.27^{c}	3.9^{b}
Vitamin E	0.18^{b}	0.0	0.0	1.7^{b}	0.03^{b}	0.21^{a}	0.0	0.0	0.38^{bc}	3.6^{b}
SE	0.04	—	—	1.1	0.02	0.03	—	—	0.04	0.2

*Values are means for 10 chicks/dietary treatment with samples run in duplicate. Relative abundance of the major fatty acids in weight percent. Fatty acid notation is number of carbon atoms: number of double bonds (omega notation). Values in the same column without a common superscript are significantly different ($P < 0.05$) based on ANOVA and the Duncan test. SE = standard error of mean.
†Ratio of 20:3 n-9 (triene) to 20:4 n-6 (arachidonic acid).

sum n-3 values were increased significantly ($P < 0.05$) for all lipids analyzed.

The incidence and relative severity of muscular dystrophy were: controls 10/10, 3.8; ferulic acid 7/10, 1.9; morin 7/10, 1.7; quercetin 6/10, 2.1; rutin 10/10, 4.0; silymarin 10/10, 3.1; and vitamin E 0/10, 0.0 (scoring methods given in original paper[11]). Thus, ferulic acid, morin, and quercetin, the treatments that caused differences in fatty acid composition of tissue lipids, also provided partial protection against muscular dystrophy. At the same time, however, vitamin E provided complete protection against the disorder but caused no fatty acid changes. This would suggest that complete protection by vitamin E and partial protection by the flavonoids might have resulted from different mechanisms, or that flavonoid protection against dystrophy was not related to the lipid compositional changes.

The basal diet fed was chosen because of its reliability in promoting the development of muscular dystrophy in chicks.[11] The linoleic acid (18:2 n-6) content of the diet, supplied mainly by the vitamin E–stripped lard, was found by analysis as 0.35%, considerably lower than the 0.8% requirement level suggested by Watkins.[18] The low linoleic

Table 3 Fatty acid composition of liver phosphatidylcholine and phosphatidylethanolamine*

Dietary Treatments	Fatty Acids									
	16:0	16:1 n-7	18:0	18:1 n-9	20:2 n-9	20:3 n-9	22:3 n-9	18:2 n-6	18:3 n-6	20:3 n-6
Liver PC										
Basal	22.7	0.08	17.7^{d}	43.5^{a}	0.31^{a}	4.0^{a}	0.08	0.48^{b}	0.04^{c}	0.18^{b}
Ferulic acid	21.5	0.06	22.1^{ab}	35.3^{b}	0.20^{b}	3.1^{b}	0.08	4.7^{a}	0.08^{b}	1.2^{a}
Morin	22.5	0.07	21.0^{abc}	34.4^{b}	0.24^{b}	3.2^{b}	0.09	4.4^{a}	0.04^{c}	1.3^{a}
Quercetin	20.8	0.04	23.7^{a}	33.1^{b}	0.20^{b}	3.5^{b}	0.11	5.7^{a}	0.12^{a}	1.6^{a}
Rutin	21.7	0.07	19.2^{bcd}	44.0^{a}	0.30^{a}	2.9^{b}	0.06	0.58^{b}	0.03^{c}	0.19^{b}
Silymarin	22.2	0.07	17.8^{cd}	43.0^{a}	0.34^{a}	4.8^{a}	0.10	0.82^{b}	0.05^{c}	0.28^{b}
Vitamin E	22.9	0.08	17.2^{d}	44.2^{a}	0.31^{a}	4.1^{a}	0.09	0.70^{b}	0.05^{c}	0.22^{b}
SE	1.4	0.02	1.1	2.0	0.02	0.2	0.02	0.4	0.01	0.1
Liver PE										
Basal	11.5^{ab}	0.05	21.5^{b}	33.8^{a}	0.52^{a}	14.4^{a}	0.28	0.90^{b}	0.04	0.33^{b}
Ferulic acid	9.8^{b}	0.03	29.7^{a}	20.9^{b}	0.26^{b}	6.2^{c}	0.32	6.9^{a}	0.07	1.5^{a}
Morin	10.2^{b}	0.05	30.9^{a}	20.0^{b}	0.25^{b}	5.7^{c}	0.34	6.2^{a}	0.05	1.3^{a}
Quercetin	9.6^{b}	0.02	32.8^{a}	17.8^{b}	0.24^{b}	5.3^{c}	0.32	7.2^{a}	0.09	1.5^{a}
Rutin	12.2^{a}	0.04	22.2^{b}	35.3^{a}	0.49^{a}	11.3^{b}	0.23	1.2^{b}	0.03	0.37^{b}
Silymarin	11.3^{ab}	0.03	23.2^{b}	31.4^{a}	0.52^{a}	14.6^{a}	0.30	1.5^{b}	0.08	0.43^{b}
Vitamin E	11.4^{ab}	0.04	23.1^{b}	33.7^{a}	0.47^{a}	13.6^{a}	0.30	1.1^{b}	0.03	0.33^{b}
SE	0.7	0.01	1.4	1.8	0.03	0.7	0.02	0.5	0.02	0.07

Dietary Treatments	Fatty Acids									
	20:4 n-6	22:4 n-6	22:5 n-6	Sum n-6	18:3 n-3	20:5 n-3	22:5 n-3	22:6 n-3	Sum n-3	Ratio triene:AA†
Liver PC										
Basal	0.33^{b}	0.02^{c}	0.04^{c}	1.1^{b}	0.01	0.10^{b}	0.02^{b}	0.14^{c}	0.34^{c}	12.1^{a}
Ferulic acid	1.6^{a}	0.13^{b}	0.16^{b}	8.2^{a}	0.02	0.14^{a}	0.06^{a}	0.33^{b}	0.73^{b}	1.9^{b}
Morin	1.9^{a}	0.11^{b}	0.27^{a}	8.2^{a}	0.03	0.14^{a}	0.04^{a}	0.34^{b}	0.72^{b}	1.7^{b}
Quercetin	2.3^{a}	0.20^{a}	0.21^{b}	10.4^{a}	0.01	0.16^{a}	0.08^{a}	0.49^{a}	0.97^{a}	1.5^{b}
Rutin	0.33^{b}	0.02^{c}	0.03^{c}	1.2^{b}	0.01	0.10^{b}	0.02^{b}	0.08^{c}	0.27^{c}	8.8^{a}
Silymarin	0.49^{b}	0.03^{c}	0.06^{c}	1.7^{b}	0.01	0.11^{b}	0.02^{b}	0.14^{c}	0.39^{c}	9.8^{a}
Vitamin E	0.38^{b}	0.02^{c}	0.05^{c}	1.4^{b}	0.01	0.11^{b}	0.02^{b}	0.14^{c}	0.32^{c}	10.8^{a}
SE	0.2	0.01	0.02	0.7	0.006	0.01	0.007	0.03	0.04	1.3
Liver PE										
Basal	5.2^{b}	0.07^{b}	0.11^{b}	6.6^{b}	0.03	0.39^{b}	0.05^{b}	0.45^{c}	1.2^{b}	2.8^{a}
Ferulic acid	13.1^{a}	0.34^{a}	0.38^{a}	22.3^{a}	0.02	0.57^{a}	0.16^{a}	0.93^{ab}	2.1^{a}	0.47^{b}
Morin	12.5^{a}	0.40^{a}	0.44^{a}	21.0^{a}	0.04	0.46^{b}	0.16^{a}	0.68^{b}	1.8^{a}	0.46^{b}
Quercetin	15.2^{a}	0.41^{a}	0.39^{a}	24.9^{a}	0.02	0.56^{a}	0.21^{a}	1.05^{a}	2.3^{a}	0.35^{b}
Rutin	5.1^{b}	0.07^{b}	0.09^{b}	6.9^{b}	0.02	0.40^{b}	0.06^{b}	0.36^{c}	1.1^{b}	2.2^{a}
Silymarin	5.7^{b}	0.09^{b}	0.12^{b}	7.9^{b}	0.01	0.41^{b}	0.07^{b}	0.45^{c}	1.2^{b}	2.6^{a}
Vitamin E	4.7^{b}	0.06^{b}	0.12^{b}	6.4^{b}	0.04	0.41^{b}	0.05^{b}	0.43^{c}	1.2^{b}	2.9^{a}
SE	1.3	0.03	0.02	1.6	0.008	0.03	0.02	0.1	0.2	0.3

*Values are means for 10 chicks/dietary treatment with samples run in duplicate. Relative abundance of the major fatty acids in weight percent. Fatty acid notation is number of carbon atoms: number of double bonds (omega notation). Values in the same column without a common superscript are significantly different ($P < 0.05$) based on ANOVA and the Duncan test. SE = standard error of mean.
†Ratio of 20:3 n-9 (triene) to 20:4 n-6 (arachidonic acid).

acid content was necessary to avoid the development of encephalomalacia and dystrophy together. The symptoms of essential fatty acid (EFA) deficiency have been described as: retarded growth, increased water consumption, reduced resistance to disease, enlarged liver, elevated concentrations of eicosatrienoic acid (triene; 20:3 n-9) and reduced arachidonic acid (20:4 n-6) in tissues.[18] Of these we only studied the effect of the diets on 20:3 n-9 and 20:4 n-6 concentrations in tissue lipids. However, these results (*Tables 2, 3,* and *4*) did provide strong evidence that the chicks were EFA-deficient and the effects of the quercetin, morin, and ferulic acid on fatty acid composition should be considered in that light. The 20:3 n-9 concentrations in the lipids were very high, relative to low 20:4 n-6, resulting in high 20:3 n-9 to 20:4 n-6 ratios, indicative of EFA deficiency. Holman[19] has suggested that EFA deficiency is present when the ratio in tissue lipids exceeds 0.4. The quercetin, morin, and ferulic acid treatments markedly reduced this ratio for all lipids analyzed (*Tables 2, 3,* and *4*), mainly by increasing 20:4 n-6 synthesis but also to some extent by reducing 20:3 n-9. This suggests that these compounds may have the potential to reduce EFA deficiency in

Table 4 Fatty acid composition of breast muscle phosphatidylcholine and phosphatidylethanolamine*

Dietary Treatments	Fatty Acids									
	16:0	16:1 n-7	18:0	18:1 n-9	20:2 n-9	20:3 n-9	22:3 n-9	18:2 n-6	18:3 n-6	20:3 n-6
Muscle PC										
Basal	26.6	0.13	7.2	49.6^{a}	0.29^{a}	1.3	0.17	0.84^{c}	0.02	0.24^{b}
Ferulic acid	25.4	0.11	7.4	43.5^{b}	0.20^{b}	1.1	0.19	5.8^{b}	0.05	1.0^{a}
Morin	31.3	0.11	8.1	38.7^{c}	0.19^{b}	1.2	0.20	5.6^{b}	0.04	1.0^{a}
Quercetin	28.7	0.08	7.5	38.1^{c}	0.20^{b}	1.4	0.22	7.3^{a}	0.06	1.4^{a}
Rutin	29.5	0.13	6.5	48.6^{a}	0.24^{ab}	0.7	0.13	1.1^{c}	0.02	0.19^{b}
Silymarin	28.6	0.15	6.3	47.8^{ab}	0.31^{a}	1.1	0.14	1.1^{c}	0.02	0.25^{b}
Vitamin E	30.6	0.13	6.7	49.3^{a}	0.25^{ab}	0.8	0.13	0.90^{c}	0.03	0.19^{b}
SE	2.0	0.03	0.8	1.5	0.03	0.2	0.04	0.4	0.01	0.2
Muscle PE										
Basal	6.8	0.17	13.0^{c}	35.4^{a}	0.74^{a}	10.3^{a}	1.1	1.4^{b}	0.08	0.69^{b}
Ferulic acid	8.4	0.12	19.3^{a}	25.8^{b}	0.39^{c}	4.5^{c}	0.90	6.1^{a}	0.05	1.4^{a}
Morin	7.2	0.15	18.7^{ab}	26.2^{b}	0.41^{c}	4.9^{c}	1.0	5.7^{a}	0.05	1.4^{a}
Quercetin	6.4	0.17	17.9^{ab}	25.6^{b}	0.40^{c}	5.3^{c}	1.1	5.9^{a}	0.05	1.6^{a}
Rutin	6.6	0.14	15.3^{bc}	38.1^{a}	0.70^{a}	7.6^{b}	0.82	2.0^{b}	0.08	0.69^{b}
Silymarin	6.4	0.14	13.6^{c}	35.7^{a}	0.75^{a}	8.6^{ab}	1.0	1.6^{b}	0.09	0.60^{b}
Vitamin E	8.6	0.12	13.2^{c}	39.0^{a}	0.66^{a}	8.3^{ab}	0.80	1.5^{b}	0.08	0.56^{b}
SE	0.8	0.03	1.3	1.9	0.02	0.7	0.1	0.5	0.01	0.1

Dietary Treatments	Fatty Acids									
	20:4 n-6	22:4 n-6	22:5 n-6	Sum n-6	18:3 n-3	20:5 n-3	22:5 n-3	22:6 n-3	Sum n-3	Ratio triene:AA†
Muscle PC										
Basal	0.20^{b}	0.05^{c}	0.03^{b}	1.4^{c}	0.0	0.10^{c}	0.06^{c}	0.13^{ab}	0.31^{cd}	6.6^{a}
Ferulic acid	0.67^{a}	0.17^{b}	0.06^{b}	7.9^{b}	0.03	0.15^{b}	0.09^{b}	0.16^{a}	0.56^{a}	1.7^{b}
Morin	0.71^{a}	0.19^{b}	0.09^{a}	7.8^{b}	0.03	0.12^{bc}	0.08^{b}	0.11^{ab}	0.45^{b}	1.6^{b}
Quercetin	1.1^{a}	0.23^{a}	0.05^{b}	10.4^{a}	0.04	0.19^{a}	0.12^{a}	0.15^{a}	0.65^{a}	1.3^{b}
Rutin	0.14^{b}	0.03^{c}	0.02^{b}	1.5^{c}	0.01	0.10^{c}	0.04^{c}	0.05^{c}	0.28^{d}	4.7^{a}
Silymarin	0.18^{b}	0.05^{c}	0.02^{b}	1.7^{c}	0.01	0.09^{c}	0.04^{c}	0.08^{bc}	0.22^{d}	6.0^{a}
Vitamin E	0.12^{b}	0.03^{c}	0.02^{b}	1.3^{c}	0.02	0.09^{c}	0.05^{c}	0.08^{bc}	0.38^{bc}	6.6^{a}
SE	0.2	0.01	0.01	0.8	0.007	0.01	0.007	0.02	0.03	0.8
Muscle PE										
Basal	3.9^{c}	0.44^{b}	0.23^{b}	6.8^{b}	0.02	0.43^{c}	0.34^{c}	0.85	1.9^{b}	2.6^{ab}
Ferulic acid	7.1^{b}	0.89^{a}	0.31^{ab}	15.8^{a}	0.05	0.67^{b}	0.54^{b}	0.95	2.7^{a}	0.64^{c}
Morin	8.6^{a}	1.2^{a}	0.36^{a}	17.4^{a}	0.05	0.67^{b}	0.64^{a}	0.92	2.8^{a}	0.57^{c}
Quercetin	8.6^{a}	1.0^{a}	0.37^{a}	17.6^{a}	0.03	0.83^{a}	0.67^{a}	1.1	3.2^{a}	0.62^{c}
Rutin	3.2^{c}	0.32^{b}	0.16^{b}	6.4^{b}	0.02	0.47^{c}	0.26^{c}	0.67	1.6^{b}	2.2^{b}
Silymarin	3.3^{c}	0.35^{b}	0.17^{b}	6.1^{b}	0.05	0.46^{c}	0.27^{c}	0.70	1.7^{b}	2.9^{ab}
Vitamin E	2.7^{c}	0.27^{b}	0.12^{b}	5.2^{b}	0.02	0.44^{c}	0.24^{c}	0.50	1.3^{b}	3.2^{a}
SE	0.4	0.09	0.03	1.3	0.009	0.05	0.03	0.1	0.2	0.2

*Values are means for 10 chicks/dietary treatment with samples run in duplicate. Relative abundance of the major fatty acids in weight percent. Fatty acid notation is number of carbon atoms: number of double bonds (omega notation). Values in the same column without a common superscript are significantly different ($P < 0.05$) based on ANOVA and the Duncan test. SE = standard error of mean.
†Ratio of 20:3 n-9 (triene) to 20:4 n-6 (arachidonic acid).

chicks. On the other hand, these treatments did not improve weight gains over the controls; in fact none of the dietary treatments affected weight gains, feed intake, or feed efficiency.[11]

Discussion

There were two main objectives of this feeding study with flavonoids. The first was to determine whether commonly occurring food flavonoid antioxidants would alter the development of nutritional muscular dystrophy (NMD), a condition arising in chicks from vitamin E (antioxidant) deficiency. It was found that quercetin, morin, and ferulic acid provided partial protection against the disorder.[11] The second objective, pursued in the present study, was to investigate reports that flavonoids can affect lipid metabolism in animals.[6–9] The diet fed to produce NMD required not only a low vitamin E content but a low concentration of EFA (about 45% of requirement) to prevent the onset of encephalomalacia as well as dystrophy.

In contrast to reports[7,8] that dietary flavonoids can reduce serum triglycerides and cholesterol in animals, we

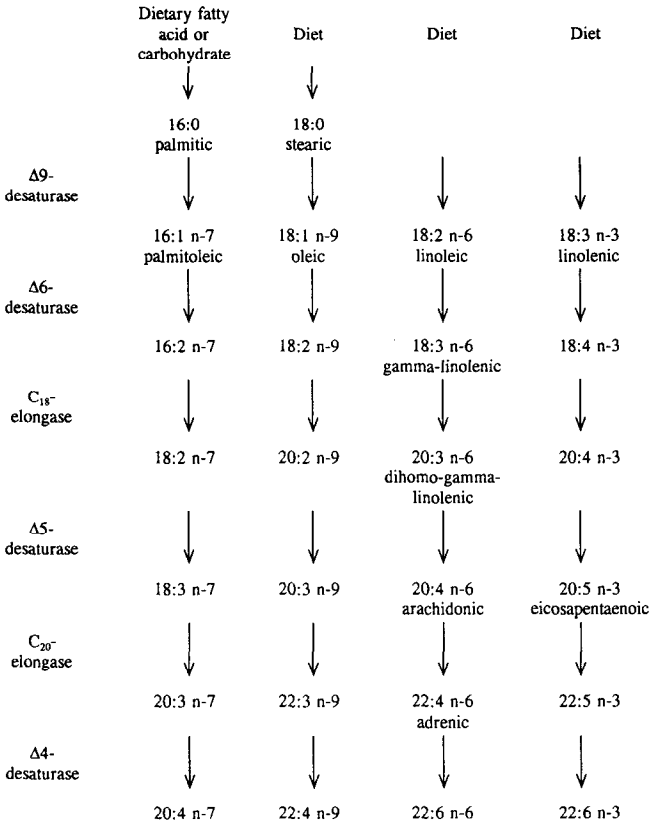

Figure 1 Major metabolic pathways for the unsaturated fatty acids showing location of desaturase and elongase activities.

found that none of the flavonoids or simple phenolic (ferulic acid) altered chick serum lipids or lipid classes in liver and muscle. It was also found that vitamin E deficiency did not alter blood and tissue lipid classes or their fatty acid composition. However, quercetin, morin, and ferulic acid (but not rutin or silymarin) produced striking changes in the fatty acid composition of the tissue lipids, with the main effects on the UFA which were formed metabolically by the desaturase and elongase reactions. These enzymes act preferentially on the n-3 family of fatty acids, then the n-6, and finally the n-9 family.[19] In EFA deficiency, where there are low concentrations of n-3 and n-6 fatty acids in tissue lipids, the emphasis is on n-9 fatty acid metabolism and an elevated production of the triene 20:3 n-9.[19] This occurred in this study indicating that indeed the chicks were EFA deficient. The unexpected finding was that two flavonoids (quercetin, morin) and the simple phenolic (ferulic acid) acted toward reversing this process. The production of most of the n-9 fatty acid metabolites in plasma and tissue lipids was decreased and the n-6 fatty acid metabolites increased. Particularly notable was an increased uptake of the 18:2 n-6 (linoleic acid) UFA precursor in all lipids and 20:4 n-6 (arachidonic acid) in liver PE and muscle PE. The n-3 fatty acid metabolites only occurred in trace amounts in the lipids because of the very low dietary level of 18:3 n-3 (linolenic acid) but they also showed an obvious tendency to be increased.

Thus it appears from this study that quercetin, morin, and ferulic acid tend to promote the production of and/or conserve EFA in the vitamin E–EFA–deficient chick. Whether this phenolic effect on fatty acid composition also occurs in vitamin E adequacy is presently under investigation. It is notable that rutin did not affect fatty acid composition but quercetin did although rutin is simply a glycoside of quercetin. This suggests that the chicks either did not absorb rutin to any extent or were ineffective in hydrolyzing the glycosidal bond. We tested only a few flavonoids in this study; it would be of interest to investigate whether some of the numerous other common food and feed phenolic antioxidants have a similar effect as quercetin, morin, and ferulic acid on EFA metabolism.

As our study showed that flavonoids can increase tissue lipid arachidonic acid, it is interesting that there are reports that flavonoids can influence the availability and metabolism of arachidonic acid.[10] In vitro studies have shown that several flavonoids including quercetin can inhibit phospholipase A_2 which is primarily responsible for the hydrolysis and release of arachidonic acid from membrane phospholipids and also inhibits 5-lipoxygenase which produces the leukotrienes from arachidonic acid. It was suggested[10] that the well-known antiinflammatory activity produced in animals by quercetin and some other flavonoids results from the inhibition of the proinflammatory 5-lipoxygenase metabolites of arachidonic acid. Thus, the higher lipid arachidonic acid concentrations produced by dietary flavonoids and the simple phenolic in our study might have been related to reduced release of arachidonic acid from lipids and/or a lowered utilization of the fatty acid either for eicosanoid production or for use as a source of energy.

Acknowledgments

The authors gratefully acknowledge the excellent technical assistance of G. Griffith with the feeding experiment and the lipid analyses.

References

1 Wollenweber, E and Dietz, V.H. (1981). A review on: occurrence and distribution of free flavonoid aglycones in plants. *Phytochemistry* **20,** 869–932

2 Kühnau, J. (1976). The Flavonoids. A class of semi-essential food components: their role in human nutrition. *World Rev. Nutr. Diet* **24,** 117–191

3 Hertog, M.G.L., Hollman, P.C.H., Katan, M.B., and Kromhart, D. (1993). Estimation of daily intake of potentially anticarcinogenic flavonoids and their determinants in adults in The Netherlands. *Nutr. Cancer* **20,** 21–29

4 Blum, U. (1986). Plants defend themselves chemically. In *Research for Tomorrow, 1986 Yearbook of Agriculture* (J.J. Crowley ed.), p. 139–142, US Department of Agriculture, Washington, DC, USA

5 Koch, H.P. and Löffler, E. (1985). Efficacy of individual flavonoids on lipid peroxidation in human platelets. *Meth. Find. Exp. Clin. Pharmacol.* **7,** 13–18

6 Fahey, G.C. and Jung, H.J.G. (1980). Phenolic compounds in forages and fibrous feedstuffs. In *Toxicants of Plant Origin,* Vol. 4, p. 123–190, CRC Press, Boca Raton, FL, USA

7 Beretz, A. and Cazenave, J.P. (1988). The effect of flavonoids on blood-vessel wall interactions. In *Plant Flavonoids in Biology and Medicine II; Biochemical, Cellular, and Medicinal Properties,* p. 187–200, Alan R. Liss, Inc., NY, USA

8 Kato, N., Tosa, N., Doudou, T., and Imamura, T. (1983). Effects

of dietary quercetin on serum lipids. *Agric. Biol. Chem.* **47,** 2119–2120

9 Larson, R.A. (1988). The antioxidants of higher plants. *Phytochemistry* **27,** 969–978

10 Welton, A.F., Hurley, J., and Will, P. (1988). Flavonoids and arachidonic acid metabolism. In *Plant Flavonoids in Biology and Medicine II: Biochemical, Cellular, and Medicinal Properties,* p. 301–312, Alan R. Liss, Inc., NY, USA

11 Jenkins, K.J., Collins, F.W., and Hidiroglou, M. (1992). Efficacy of various flavonoids and simple phenolics in prevention of nutritional myopathy in the chick. *Poultry Sci.* **71,** 1577–1580

12 Chambers, J.R., Bernon, D.E., and Gavora, J.S. (1984). Synthesis and parameters of new populations of meat-type chickens. *Theor. Appl. Genet.* **69,** 23–30

13 Machlin, L.J., Gordon, R.S., and Meisky, K.H. (1959). The effect of antioxidants on vitamin E deficiency symptoms and production of liver "peroxide" in the chicken. *J. Nutr.* **67,** 333–343

14 Kramer, J.K.G. and Hulan, H.W. (1978). A comparison of procedures to determine free fatty acids in rat heart. *J. Lipid Res.* **19,** 103–106

15 Kramer, J.K.G., Fouchard, R.C., and Farnworth, E.R. (1983). A complete separation of lipids by three-directional thin layer chromatography. *Lipids* **18,** 896–899

16 Kramer, J.K.G., Fouchard, R.C., and Jenkins, K.J. (1985). Differences in chromatographic properties of fused silica capillary columns, coated, crosslinked bonded, or crosslinked and bonded with polyethylene glycols using complex fatty acid methyl ester mixtures. *J. Chromatogr. Sci.* **23,** 54–56

17 Duncan, D.B. (1955). Multiple range and multiple F test. *Biometrics* **11,** 1–42

18 Watkins, B.A. (1991). Importance of essential fatty acids and their derivatives in poultry. *J. Nutr.* **121,** 1475–1485

19 Holman, R.T. (1978). Essential fatty acid deficiency in animals. In *CRC Handbook Series in Nutrition and Food.* M. Recheigl, Jr., ed. Section E, vol. 11, p. 491–515, CRC Press, Inc., West Palm Beach, FL, USA