Dietary Levels of Chia: Influence on Yolk Cholesterol, Lipid Content and Fatty Acid Composition for Two Strains of Hens

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ABSTRACT Four hundred fifty H&N laying hens, half white and half brown, were fed for 90 d to compare a control diet to diets containing 7, 14, 21, and 28% chia (*Salvia hispanica L.*) seed. Cholesterol content, total fat content, and fatty acid composition of the yolks were determined 30, 43, 58, 72, and 90 d from the start of the trial. Significantly less cholesterol was found in the egg yolks produced by the hens fed the diets with 14, 21, and 28% chia compared with the control, except at Day 90. Palmitic fatty acid content and total saturated fatty acid content decreased as chia percentage increased and as the trial progressed. Total omega-3 fatty acid content was significantly greater ($P < 0.05$) for both strains for all chia diets compared with the control diet. Total polyunsaturated fatty acid (PUFA) content of the yolks from the chia diets was significantly greater ($P < 0.05$) than from the control diet. Generally, total PUFA content tended to be highest in the yolks of the white hens.

(*Key words*: eggs, chia, omega-3, cholesterol, α-linolenic)

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INTRODUCTION

Coronary heart disease (CHD) is the leading cause of death in the United States and other Western industrialized countries, including Argentina (American Heart Association, 1997; Ministerio de Salud y Accion Social, 1997). Clinical data strongly support the relationship between dietary cholesterol and saturated fatty acids and CHD (American Heart Association, 1990; Bruckner, 1992). Epidemiologic as well as controlled experiments have demonstrated an inverse relationship between CHD and consumption of food rich in omega-3 fatty acids (Bang et al., 1980; Kromhout et al., 1985; Kinsella et al., 1990; Ferretti and Flanigan, 1996; Lorgeril et al., 1996; Pauletto et al., 1996; Leaf and Kang, 1998). The earlier studies were conducted using fish and fish oil as the source of long-chain omega-3 fatty acids. However, more recent studies have used vegetable oil or seeds containing α-linolenic fatty acid, a precursor of omega-3 fatty acids, which is converted to long-chain omega-3 fatty acids and, therefore, can be substituted for fish oils (Simopoulos, 1999).

US egg consumption has declined over the past decade from 256 eggs per capita per year in 1985 to 235 in 1995 (USDA, 1997). The same trend has been reported in Argentina, with annual per capita consumption declining from 160 in 1990 to 128 in 1997 (Lamelas and Asad, 1998). The decline is attributable to consumer concerns about cholesterol, fat sources, types of fatty acids consumed and their relationship to CHD. The American Heart Association (1996) suggests limiting whole egg or egg yolk consumption to 3 or 4 per wk, including those used in cooking.

Several attempts have been made to reverse the decline in egg consumption by changing egg composition through feeding hens modified diets. Hargis (1988) reported that, in general, these efforts have produced only a modest reduction in cholesterol. Incorporation of omega-3 fatty acids in egg yolks by changing hen diets has been more successful and has been reported in a number of studies (Caston and Leeson, 1990; Cherian and Sim, 1991; Caston et al., 1994; Farrell, 1994; Cherian et al., 1995; Nash et al., 1995; Scheideler et al., 1998). These experiments altered the yolk fatty acid profile by adding either flax seed or fish products to the hens' feed.

Clinical investigations that have examined the effect of consumption of omega-3-enriched eggs have shown a significant decline in CHD risk factors (Oh et al., 1991; Ferrier et al, 1992; Sim and Jiang, 1994; Lewis et al., 1998; Van Elswyk et al., 1998). However, the presence of an off-flavor (fishy flavor) in these eggs, which has been noted in a number of sensory evaluations especially when fish oil is used at or greater than 3% of the diet, indicates a strong marketing disadvantage (Marshall et al., 1994a).

There is increasing evidence that an imbalance between omega-6 and omega-3 fatty acids is a major risk factor

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Abbreviation Key: CHD = coronary heart disease; DHA = docosahexanoic acid; EPA = eicosapentaenoic acid; PUFA = polyunsaturated fatty acid; SCWL = Single Comb White Leghorn.

for cancer, cardiovascular, and cerebrovascular diseases and for allergic hyper-reactivity (Okuyama et al., 1997). Omega-6 and omega-3 polyunsaturated fatty acids (PUFA) in human diets compete for the same enzymes but have different biological roles, hence a correct balance between them is of considerable importance (FAO, 1994; Craig-Schmidt and Huang, 1998). Western diets are deficient in omega-3 fatty acids and high in omega-6 fatty acids. The main reason for this imbalance is the growing use of vegetable oils such as sunflower, corn, and safflower, which are rich in omega-6 PUFA (Simopoulos and Robinson, 1998). As a result, dietary omega-3 intakes should be increased.

Ayerza and Coates (1999) used chia (*Salvia hispanica L.*) seed in a hen diet and demonstrated that the eggs produced had a high omega-3 fatty acid content, reduced saturated fatty acid content, low omega-6:omega-3 ratio, and no off-flavor. The trial, however, utilized only one breed (strain) of hens, and chia was fed at only one level. Because of the general success of the study, a more comprehensive trial was undertaken and is reported herein.

MATERIALS AND METHODS

Four hundred fifty H&N laying hens,[2] half white and half brown at 27 wk of age, were selected for the study. These commercial strains were developed from White Leghorn and Red Sex Link breeds, respectively. The hens were housed, three to a cage (30 × 45 × 43 cm high at the front and 38 cm high at the back), in a commercial egg production facility located in San Vicente, Province of Buenos Aires, Argentina. The five diets contained 0, 7, 14, 21, and 28% whole chia seed. Nutrient composition of the diets and chia seed are shown in Table 1. Each diet (treatment) was replicated 15 times, with each replicate comprised of one cage of three hens.

After random allocation to the cages, the hens were fed for 30 d prior to collecting data. The trial lasted 90 d; the white and brown hens received 115 and 120 g of feed/d, respectively. Water was provided ad libitum.

Six eggs from each treatment were randomly selected on Days 30, 43, 58, 72, and 90 for analysis. After selection the eggs were weighed and broken open, and the yolks were separated and weighed. The yolks were then frozen at –18 C and stored until they were analyzed.

Laboratory Analyses

The frozen yolks were analyzed for cholesterol content, total fat content, and fatty acid composition. The procedure was as follows. Each yolk was defrosted and then mixed. Total lipids were extracted according to the method of Folch et al. (1957). Percentage lipid content was determined gravimetrically. Total lipids were converted to fatty acid methyl esters with the IRAM 5-560II method (Instituto Argentino de Racionalizacion de Materiales, 1982). Fatty acid methyl esters were separated and quantified by an automated gas chromathograph (Model 6890, GC[3]) equipped with flame ionization detectors and a 30 m × 530 um i.d. capillary column (Model HP-FFAP[3]). An HP chem station[3] was used to integrate peak areas.

Cholesterol was extracted according to the method of the Association of Official Analytical Chemists procedure 941.09 (1990). The same equipment and procedures that were used for the fatty acids were used to quantify the cholesterol levels, except that the column (Model HP-1 Methyl Siloxane[3]) used was different.

Statistical Analysis

The feeding trial was set up in a randomized block design, and the experimental unit was one cage of three hens. A treatment was comprised of 45 adjacently caged hens, housed three to a cage. Each variable was compared using the generalized linear model analysis of variance technique to assess treatment differences. When the F-value was significant ($P < 0.05$), differences in means were analyzed for significance using Duncan's multiple-range test (SAS Institute, 1988).

RESULTS

Cholesterol and Total Lipids

Results Within Strains. Cholesterol content of the eggs produced by the white hens is presented in Table 2. Significantly ($P < 0.05$) less cholesterol was found in the yolks produced by the hens fed the 14, 21, and 28% chia diets, except at Day 90, compared with the control. In general, cholesterol content decreased as chia content increased; however, the difference was not always significant ($P <$ 0.05). Cholesterol content of the brown hen eggs was not determined because of financial constraints.

Total fat content was not significantly ($P < 0.05$) different among treatments for either strain, except for the white hens at Day 43 (Tables 2 and 3). In this case the diet containing 28% chia produced a higher fat content than did the control diet.

Results Between Strains. Total yolk fat content for the white and brown strains fed the chia diets is presented in Table 4. No significant ($P < 0.05$) differences between the 7 and 21% chia diets were detected over the experimental period. The eggs of hens fed diets containing 14 and 28% chia showed significant ($P < 0.05$) differences between strains on two and one of the measurement dates, respectively.

Fatty Acid Composition

Results Within Strains. Generally, the fatty acid composition of the yolks reflected the dietary fat levels for both strains (Tables 2 and 3). Saturated palmitic acid and total saturated fatty acids decreased as chia percentage

[2]H&H International, Redmond, Washington.
[3]Hewlett Packard Co., Wilmington, DE 20006.

TABLE 1. Nutrient composition of laying hen diets and chia seed

Ingredient	Chia percentage in ration 0%	7%	14%	21%	28%	Chia seed[1]
ME, kcal/kg	2,800	2,800	2,800	2,800	2,800	3,303
Crude protein, %	19.20	19.50	18.50	18.50	18.50	17.1
Lysine, %	0.93	1.26	1.44	1.71	1.95	
Methionine + cystine, %	0.78	0.80	0.83	0.96	1.09	
Threonine, %	0.72	0.94	1.06	1.30	1.46	
Tryptophan, %	0.25	0.43	0.61	0.78	0.95	
Lipids, %	7.00	7.00	10.13	13.00	13.00	32.8
Fiber, %	5.38	5.77	6.00	11.00	11.00	22.1
Calcium, %	4.00	4.00	5.97	4.00	5.59	0.59
Available P, %	0.27	0.28	0.61	0.25	0.52	0.89
Ca:P rate	6.15	6.15	6.00	6.15	6.00	
Xanthaphylls, mg/kg	14.33	14.33	14.33	14.33	14.33	
Miristic	0.02	0.02	0.03	0.01	0.03	0.00
Palmitic	0.61	0.73	0.97	0.85	1.15	6.5
Palmitoleic	0.04	0.04	0.07	0.03	0.06	0.1
Stearic	0.17	0.23	0.37	0.29	0.45	2.9
Oleic	1.02	1.11	1.37	1.07	1.44	7.2
Linoleic	1.14	1.46	1.69	2.00	2.34	20.3
α-Linolenic	0.07	1.50	2.92	4.35	5.78	62.0
Linoleic:α-linolenic ratio	16.3	0.97	0.58	0.46	0.40	0.33
Arachidonic	0.00	0.00	0.00	0.01	0.01	0.3
Gadoleic	0.00	0.00	0.00	0.01	0.01	0.1
Vitamin A, UI/kg	7,350	7,350	7,350	7,350	7,350	
Vitamin D3, UI/kg	2,550	2,550	2,550	2,550	2,550	
Vitamin E, UI/kg	4.2	4.2	4.2	4.2	4.2	
Vitamin B2, ppm	3.6	3.6	3.6	3.6	3.6	
Vitamin B12, ppm	0.0075	0.0075	0.0075	0.0075	0.0075	
Vitamin K3, ppm	1.2	1.2	1.2	1.2	1.2	
Niacin, ppm	18	18	18	18	18	
Pantothenic acid, ppm	4.95	4.95	4.95	4.95	4.95	
Butylated hydroxytoluene	9.9	9.9	9.9	9.9	9.9	
Manganese, ppm	49.5	49.5	49.5	49.5	49.5	
Iron, ppm	30	30	30	30	30	
Zinc, ppm	49.5	49.5	49.5	49.5	49.5	
Copper, ppm	4.95	4.95	4.95	4.95	4.95	
Iodine, ppm	0.3	0.3	0.3	0.3	0.3	
Selenium, ppm	0.15	0.15	0.15	0.15	0.15	

[1]Source: Ayerza and Coates (1999).

increased and as the trial progressed. For the 7% chia diet, however, palmitic acid content at Day 72 and beyond with the white hens, and except at Day 58 for the brown hens, was not significantly different ($P < 0.05$) from the control diet.

Miristic acid levels were erratic throughout the trial. Because this acid composed a very small proportion of the total fatty acid content of the yolks, varying between 0.183 and 0.413% of the total, it does not warrant further discussion. Generally, stearic acid increased in the yolks as chia percentage increased; the trend was stronger with the brown hens.

Total saturated fatty acid content, calculated as the sum of myristic, palmitic, and stearic acids, tended to decrease as chia content increased (Tables 5 and 6). Over the duration of the trial, yolks from both strains fed the 28% chia diet had significantly less ($P < 0.05$) total saturated fatty acids than did yolks from hens that were fed the control diet. Yolks from hens fed the 21% chia diet had significantly less ($P < 0.05$) total saturated fatty acids than did yolks from the control diet at Days 58, 72, and 90 for the brown hens and at all but Day 30 for the white hens. Yolks from hens fed the 14% chia diet had significantly less ($P < 0.05$) saturated fatty acids than the control diet at Days 43, 58, and 72 for the brown hens and at all but Days 30 and 72 for the white hens.

The monounsaturated fatty acids, oleic (which is a greater percentage) and palmitoleic, decreased with increasing percentage of chia in the diet (Tables 2 and 3). For both strains, oleic acid was significantly less ($P < 0.05$) in the yolks produced by hens fed chia, except for Day 90 of the 7% chia diet, than those yolks of hens fed the control. Palmitoleic acid percentage also tended to decrease when hens were fed chia. The decrease, however, was less than that observed for oleic acid, and the difference was not always significantly different between the chia treatments and the control.

Total monounsaturated fatty acid content, calculated as the sum of palmitoleic and oleic acids, was significantly lower ($P < 0.05$) with all chia diets than with the control diet for both strains (Tables 5 and 6). The only exception was the 7% chia treatment at Day 90.

Over the course of the trial, polyunsaturated linoleic acid levels tended to increase with the level of chia in the diet (Tables 2 and 3). The differences between the chia diets and the control diet were not always significant ($P < 0.05$). Linoleic acid content was significantly higher ($P < 0.05$) in the yolks produced by hens fed the 28% chia diet than those from hens fed the control diet, except for Days 72 and 90 in the white hens.

The percentage of arachidonic acid, a polyunsaturated omega-6 fatty acid, decreased for both strains as chia content increased. The difference was significant ($P < 0.05$) at all dates for the 14, 21, and 28% chia diets in the white hens and for 21 and 28% diets in the brown hens compared with the control diet.

Polyunsaturated α-linolenic acid levels increased as chia in the diet increased. The difference between treatments was significant ($P < 0.05$) in the white hens over the duration of the trial, except at Day 90 between the 14 and 21% chia diets. Eggs produced by the brown hens fed chia had significantly higher ($P < 0.05$) levels of α-linolenic acid compared with the control diet; however, differences between the chia diets were not always significant.

Docosahexanoic acid (DHA), a polyunsaturated omega-3 fatty acid, was significantly higher ($P < 0.05$) in the yolks produced by hens fed the chia diets than in those from hens fed the control diet. This result was true in all cases except Day 30 with the 28% chia diet for the white hens and Days 30 and 72 with the 28% chia diet for the brown hens.

Total omega-6 and omega-3 PUFA, calculated as the sum of linoleic and arachidonic fatty acids and the sum of α-linolenic and DHA, respectively, generally were similar to the predominant fatty acid in each group (Tables 5 and 6). Total omega-3 fatty acids were significantly greater ($P < 0.05$) for both strains, for all chia diets during the experiment, compared with the control diet. Increased percentages of chia in the diet tended to increase the omega-3 percentage in the yolks; however, the results were not always significantly different ($P < 0.05$). Eggs from the white hens fed the 28% chia diet contained significantly more ($P < 0.05$) omega-3 fatty acids than did eggs from hens on the other treatments at all five sample dates. For the brown hens, the total omega-3 percentage was not significantly different ($P < 0.05$) between the 21 and 28% chia diets only at Days 58 and 72. Total PUFA content of the yolks, calculated as the sum of the omega-6 and omega-3 fatty acids, was significantly ($P < 0.05$) greater with all of the chia diets than with the control diet, except on Day 90 for the 7% chia diet fed to the white hens.

Addition of chia to the diet resulted in yolk omega-6:omega-3, saturated fatty acid:PUFA, and saturated fatty acid:omega-3 ratios being significantly lower ($P < 0.05$)

TABLE 2. Fatty acid composition, fat content, and cholesterol content of egg yolks from the white laying hens

Day	Treatment	Miristic %[2]	Palmitic %[2]	Palmitoleic %[2]	Stearic %[2]	Oleic %[2]	Linoleic %[2]	Linolenic %[2]	Arachadonic %[2]	DHA[3] %[2]	Total fat %[4]	Cholesterol %[4]
30	0	0.33^{a}	27.13^{a}	3.86^{a}	8.87^{c}	39.28^{a}	12.93^{c}	0.21^{e}	3.39^{a}	1.00^{d}	29.63^{a}	1.40^{a}
	7	0.18^{b}	24.83^{b}	1.67^{c}	14.93^{a}	28.25^{b}	15.17^{b}	2.76^{d}	3.53^{a}	5.48^{a}	29.72^{a}	1.30^{ab}
	14	0.23^{b}	24.13^{b}	1.72^{bc}	15.55^{a}	27.63^{b}	15.23^{b}	5.17^{c}	2.70^{b}	3.97^{b}	29.47^{a}	1.25^{bc}
	21	0.23^{b}	21.62^{c}	1.86^{bc}	13.20^{b}	27.68^{b}	17.33^{a}	9.04^{b}	2.11^{b}	3.08^{c}	29.43^{a}	1.21^{bc}
	28	0.38^{a}	21.22^{c}	2.34^{b}	9.24^{c}	26.97^{b}	18.23^{a}	16.52^{a}	0.84^{c}	1.24^{d}	29.78^{a}	1.18^{c}
	CR[1]	0.07	2.09	0.67	1.81	2.32	1.11	1.58	0.66	0.75	1.65	0.12
43	0	0.39^{a}	25.78^{a}	3.36^{a}	8.43^{a}	40.35^{a}	15.97^{c}	0.39^{e}	2.15^{a}	0.58^{b}	28.53^{b}	1.47^{a}
	7	0.30^{b}	22.58^{b}	3.03^{ab}	8.69^{a}	36.60^{b}	17.63^{b}	5.68^{d}	1.47^{b}	1.58^{a}	29.08^{ab}	1.30^{b}
	14	0.30^{b}	21.08^{b}	2.67^{bc}	8.69^{a}	33.73^{c}	17.46^{b}	10.50^{c}	1.19^{bc}	1.25^{a}	28.87^{b}	1.26^{bc}
	21	0.31^{b}	20.48^{b}	2.38^{cd}	9.44^{a}	29.73^{d}	18.80^{ab}	13.08^{b}	1.16^{bc}	1.61^{a}	28.60^{b}	1.20^{bc}
	28	0.32^{b}	20.55^{b}	2.15^{d}	9.23^{a}	27.37^{e}	19.28^{a}	14.98^{a}	1.09^{c}	1.71^{a}	30.22^{a}	1.17^{c}
	CR	0.07	2.47	0.42	1.14	2.31	1.52	1.52	0.34	0.47	1.41	0.11
58	0	0.38^{a}	26.67^{a}	3.78^{a}	6.99^{b}	41.03^{a}	15.62^{c}	0.36^{e}	2.06^{a}	0.45^{c}	30.14^{a}	1.53^{a}
	7	0.29^{b}	23.60^{b}	2.86^{b}	8.86^{a}	35.73^{b}	16.77^{bc}	4.99^{d}	1.88^{ab}	2.44^{a}	29.10^{a}	1.40^{b}
	14	0.30^{b}	21.42^{bc}	2.30^{bc}	9.34^{a}	32.27^{c}	17.50^{b}	9.38^{c}	1.73^{b}	2.47^{a}	29.50^{a}	1.27^{c}
	21	0.28^{b}	20.38^{cd}	2.49^{c}	8.99^{a}	31.00^{cd}	18.32^{ab}	13.28^{b}	1.25^{c}	1.66^{b}	30.10^{a}	1.23^{c}
	28	0.30^{b}	18.52^{d}	1.20^{c}	8.05^{ab}	28.33^{d}	19.58^{a}	18.42^{a}	0.92^{d}	1.24^{b}	29.88^{a}	1.22^{c}
	CR	0.07	2.54	0.53	1.34	3.65	1.89	2.79	0.29	0.56	1.45	0.10
72	0	0.41^{a}	25.38^{a}	3.46^{a}	8.01^{b}	37.95^{a}	18.12^{a}	0.61^{e}	2.06^{a}	0.69^{c}	NA[5]	1.61^{a}
	7	0.33^{bc}	24.82^{a}	3.26^{a}	9.74^{a}	33.58^{b}	16.68^{b}	4.93^{d}	1.63^{b}	1.95^{a}	NA	1.51^{ab}
	14	0.37^{ab}	22.32^{b}	2.92^{ab}	8.60^{ab}	32.78^{b}	16.58^{b}	10.27^{c}	0.99^{c}	1.58^{ab}	NA	1.47^{b}
	21	0.29^{c}	20.87^{b}	2.92^{ab}	7.99^{b}	30.15^{c}	16.98^{ab}	14.30^{b}	0.76^{c}	1.33^{b}	NA	1.46^{bc}
	28	0.34^{bc}	20.02^{b}	2.41^{b}	9.26^{ab}	27.30^{d}	17.58^{ab}	16.40^{a}	0.76^{c}	1.67^{ab}	NA	1.35^{c}
	CR	0.07	2.52	0.80	1.60	2.80	1.33	1.86	0.41	0.60		0.13
90	0	0.32^{b}	26.00^{a}	2.90^{bc}	7.70^{b}	40.35^{a}	16.25^{a}	0.37^{d}	2.30^{a}	0.73^{b}	30.17^{a}	1.01^{bc}
	7	0.33^{ab}	24.33^{ab}	3.71^{a}	8.36^{ab}	39.35^{a}	14.57^{b}	4.01^{c}	1.39^{b}	1.50^{a}	29.28^{a}	1.27^{a}
	14	0.38^{a}	22.47^{bc}	2.79^{bc}	9.21^{a}	31.70	17.22^{a}	10.89^{b}	1.04^{c}	1.33^{a}	30.15^{a}	1.11^{abc}
	21	0.33^{ab}	22.32^{c}	3.39^{ab}	8.30^{ab}	32.90^{b}	16.28^{a}	11.62^{b}	0.95^{c}	1.30^{a}	30.48^{a}	1.17^{ab}
	28	0.32^{b}	20.63^{c}	2.46^{c}	8.79^{ab}	29.78^{c}	17.22^{a}	15.58^{a}	0.93^{c}	1.27^{a}	30.42^{a}	0.93^{c}
	CR	0.05	2.12	0.71	1.24	3.25	1.46	1.67	0.36	0.33	2.17	0.22

[a–e]Means within a day lacking a common superscript differ ($P < 0.05$) according to Duncan's multiple-range test.

[1]Critical range for mean separation.

[2]Percentage of total fat.

[3]DHA = docosahexanoic.

[4]Percentage of yolk.

[5]NA = Not available.

TABLE 3. Fatty acid composition, fat content, and cholesterol content of egg yolks from the brown laying hens

Day	Treatment	Miristic %[2]	Palmitic %[2]	Palmitoleic %[2]	Stearic %[2]	Oleic %[2]	Linoleic %[2]	Linolenic %[2]	Arachadonic %[2]	DHA[3] %[2]	Total fat %[4]
30	0	0.36^{a}	26.63^{a}	4.22^{a}	7.86^{b}	42.07^{a}	12.65^{d}	0.20^{e}	2.79^{ab}	0.73^{b}	29.70^{a}
	7	0.24^{b}	24.70	2.94^{b}	11.55^{a}	34.23^{b}	13.73^{cd}	2.85^{d}	2.90^{a}	4.38^{a}	30.33^{a}
	14	0.28^{ab}	23.52^{bc}	2.47^{b}	12.63^{a}	31.43^{bc}	14.50^{bc}	5.86^{c}	2.36^{ab}	3.84^{a}	30.85^{a}
	21	0.21^{b}	21.57^{c}	2.26^{b}	13.08^{a}	29.27^{c}	15.73^{b}	8.15^{b}	2.30^{b}	3.59^{a}	31.53^{a}
	28	0.35^{a}	21.97^{c}	2.95^{b}	8.29^{b}	31.15^{bc}	17.27^{a}	13.51^{a}	0.80^{c}	1.01^{b}	31.00^{a}
	CR[1]	0.09	2.75	0.78	2.27	3.33	1.63	2.50	0.60	1.13	2.74
43	0	0.41^{ab}	25.88^{a}	3.97^{a}	7.30^{c}	43.55^{a}	13.82^{c}	0.29^{d}	1.95^{a}	0.42^{b}	28.00^{a}
	7	0.45^{a}	25.48^{a}	4.21^{a}	7.58^{bc}	37.70^{b}	15.40^{bc}	3.94^{c}	1.38^{b}	1.44^{a}	28.03^{a}
	14	0.28^{c}	21.58^{b}	2.97^{b}	8.18^{ab}	36.47^{b}	16.10^{ab}	9.55^{b}	1.00^{cd}	1.35^{a}	27.10^{a}
	21	0.31^{c}	22.58^{b}	3.42^{ab}	8.63^{a}	34.80^{b}	15.13^{bc}	9.95^{b}	1.18^{bc}	1.60^{a}	27.10^{a}
	28	0.35^{bc}	20.40^{b}	2.75^{b}	8.31^{ab}	30.37^{c}	17.77^{a}	14.18^{a}	0.92^{d}	1.23^{a}	27.72^{a}
	CR	0.08	2.99	0.84	0.85	3.46	2.19	2.30	0.25	0.40	1.43
58	0	0.35^{a}	26.62^{a}	4.17^{a}	7.04^{b}	44.15^{a}	12.42^{b}	0.26^{d}	2.11^{a}	0.47^{c}	30.35^{a}
	7	0.33^{ab}	22.42^{b}	3.19^{b}	7.47^{b}	37.90^{b}	17.12^{a}	4.49^{c}	1.88^{a}	2.56^{a}	29.37^{a}
	14	0.32^{ab}	21.37^{b}	2.85^{b}	9.06^{a}	34.00^{c}	16.30^{a}	7.86^{b}	1.93^{a}	2.93^{a}	30.37^{a}
	21	0.30^{ab}	20.87^{b}	3.10^{b}	7.59^{b}	33.37^{c}	16.88^{a}	13.78^{a}	1.15^{b}	1.68^{b}	29.04^{a}
	28	0.27^{b}	20.38^{b}	2.93^{b}	7.01^{b}	32.68^{c}	17.48^{a}	14.29^{a}	1.05^{b}	1.47^{b}	29.32^{a}
	CR	0.07	2.26	0.65	1.32	3.35	1.38	3.24	0.43	0.57	2.03
72	0	0.37^{a}	25.73^{a}	3.93^{a}	8.38^{b}	40.70^{a}	14.63^{b}	0.50^{e}	2.29^{a}	0.76^{d}	NA[5]
	7	0.33^{ab}	24.62^{a}	3.53^{a}	10.61^{a}	34.35^{bc}	14.33^{b}	3.88^{d}	2.18^{a}	2.68^{a}	NA
	14	0.32^{ab}	21.10^{b}	3.50^{a}	8.16^{b}	36.02^{b}	14.57^{b}	8.86^{c}	1.11^{b}	1.84^{b}	NA
	21	0.29^{b}	20.10^{b}	2.99^{b}	8.45^{b}	32.48^{c}	16.93^{a}	13.65^{b}	0.86^{b}	1.32^{c}	NA
	28	0.37^{a}	21.70^{b}	2.68^{b}	8.82^{b}	28.87^{d}	17.20^{a}	15.28^{a}	0.76^{b}	1.10^{cd}	NA
	CR	0.06	2.26	0.56	0.88	2.70	1.28	1.64	0.52	0.50	
90	0	0.34^{a}	25.45^{a}	3.27^{ab}	7.90^{ab}	42.22^{a}	14.18^{c}	0.29^{d}	2.74^{a}	0.86^{b}	NA
	7	0.33^{a}	24.37^{a}	3.49^{a}	7.37^{b}	40.85^{a}	14.00^{c}	4.15^{c}	1.43^{b}	1.64^{a}	NA
	14	0.32^{a}	21.75^{b}	3.57^{a}	8.13^{ab}	35.85^{b}	15.47^{bc}	8.80^{b}	1.17^{bc}	1.54^{a}	NA
	21	0.31^{a}	20.88^{b}	3.52^{a}	8.35^{ab}	34.60^{b}	16.78^{ab}	10.43^{b}	1.03^{c}	1.46^{a}	NA
	28	0.33^{a}	21.13^{b}	2.87^{b}	8.76^{a}	26.67^{c}	17.47^{a}	16.60^{a}	0.95^{c}	1.58^{a}	NA
	CR	0.05	1.84	0.66	1.19	2.52	1.89	1.83	0.41	0.33	

[a–e]Means within a day lacking a common superscript differ ($P < 0.05$) according to Duncan's multiple-range test.

[1]Critical range for mean separation.

[2]Percentage of total fat.

[3]DHA = docosahexanoic.

[4]Percentage of yolk.

[5]NA = not available.

than those of the control diets for both strains on all dates. As the chia percentage increased, the ratios improved numerically, but they were not always significantly different ($P < 0.05$).

Results Between Strains. A comparison of yolk fatty acid contents produced by the white and brown strains fed the chia diets is presented in Table 4; the combined values are presented in Table 7. Irrespective of strain and diet, no significant difference ($P < 0.05$) was detected between strains for total saturated fatty acid content of the yolks, except on Day 30 for 7% chia diet and Day 90 for 14 and 21% chia diets (Table 7). In each case, the brown hens produced yolks with a lower total saturated fatty acid content than the white hens (Table 7). The lower saturated fatty acid content found with the 7 and 14% chia diets could be related to the lower stearic acid content recorded on the same days; however, this was not true for the 21% chia diet.

Total monounsaturated fatty acid content was numerically highest for the brown hens on all dates and for all treatments, except Day 90 with the 28% chia diet (Table 7). Significant differences ($P < 0.05$) were observed in only 13 of the 24 cases. Differences in total monounsaturated fatty acid content paralleled the oleic fatty acid difference found between strains. This difference arose because oleic acid is the major constituent.

Chia in the diet yielded a significant difference ($P < 0.05$) in the incorporation of omega-3 PUFA between strains on only 1 d with each treatment (Table 7), with exception of the 28% chia treatment, which had 2 d with significant differences ($P < 0.05$) between strains. In all five instances, the brown hens exhibited lower incorporation of omega-3 PUFA than the white hens. α-Linolenic acid, a major component of total omega-3 PUFA, was significantly higher ($P < 0.05$) on Days 43, 72, and 90 for the white hens than for the brown hens fed the control diet. Over the experimental period, α-linolenic acid was significantly higher ($P < 0.05$) only on 1 d for the white hens within each chia treatment (Table 4), and it was a different day in each case.

The difference between strains in terms of omega-6 PUFA incorporation decreased slightly as chia percentage increased (Table 7). The number of dates significantly different ($P < 0.05$) between strains were 3, 3, 1, 1, and 2 for the control, 7, 14, 21, and 28% chia diets, respectively. In all cases, the brown hens had lower omega-6 PUFA incorporation. A similar behavior was observed for lino-

TABLE 4. Comparison of fatty acid composition, fat content, and cholesterol content of egg yolks between two strains of laying hens

Treatment	Day	Strain	Miristic %[2]	Palmitic %[2]	Palmitoleic %[2]	Stearic %[2]	Oleic %[2]	Linoleic %[2]	Linolenic %[2]	Arachiadonic %[2]	DHA[3] %[2]	Total fat %[4]
7	30	Brown	0.24^{a}	24.70^{a}	2.94^{a}	11.55^{b}	34.23^{a}	13.73^{b}	2.85^{a}	2.90^{b}	4.38^{a}	30.33^{a}
		White	0.18^{b}	24.83^{a}	1.67^{b}	14.93^{a}	28.25^{b}	15.17^{a}	2.76^{a}	3.53^{a}	5.48^{a}	29.72^{a}
		CR[1]	0.05	1.62	0.74	1.58	2.17	0.85	0.56	0.34	1.30	1.43
	43	Brown	0.45^{a}	25.48^{a}	4.21^{a}	7.58^{b}	37.70^{a}	15.40^{b}	3.94^{b}	1.38^{a}	1.44^{a}	28.03^{a}
		White	0.30^{b}	22.58^{a}	3.03^{b}	8.69^{a}	36.60^{a}	17.63^{a}	5.68^{a}	1.47^{a}	1.58^{a}	29.08^{a}
		CR	0.10	3.75	0.75	0.84	3.29	1.96	0.83	0.25	0.37	1.24
	58	Brown	0.33^{a}	22.42^{a}	3.19^{a}	7.47^{b}	37.90^{a}	17.12^{a}	4.49^{a}	1.88^{a}	2.56^{a}	29.37^{a}
		White	0.30^{a}	23.60^{a}	2.86^{a}	8.86^{a}	35.73^{b}	16.77^{a}	4.99^{a}	1.88^{a}	2.44^{a}	29.10^{a}
		CR	0.06	2.06	0.48	1.17	2.00	0.72	0.58	0.26	0.32	1.55
	72	Brown	0.33^{a}	24.62^{a}	3.53^{a}	10.61^{a}	34.35^{a}	14.33^{a}	3.88^{a}	2.18^{a}	2.68^{a}	NA[5]
		White	0.33^{a}	24.82^{a}	3.26^{a}	9.74^{a}	33.58^{a}	16.68^{a}	4.93^{a}	1.64^{a}	1.95^{a}	NA
		CR	0.08	3.42	0.93	2.35	3.74	1.38	1.66	0.86	0.75	
	90	Brown	0.33^{a}	24.37^{a}	3.49^{a}	7.37^{a}	40.85^{a}	14.00^{a}	4.15^{a}	1.43^{a}	1.64^{a}	NA
		White	0.33^{a}	24.33^{a}	3.71^{a}	8.36^{a}	39.35^{a}	14.57^{a}	4.01^{a}	1.39^{a}	1.50^{a}	NA
		CR	0.07	2.44	0.88	1.69	3.11	1.51	1.06	0.29	0.47	
14	30	Brown	0.28^{a}	23.52^{a}	2.47^{a}	12.63^{b}	31.43^{a}	14.50^{a}	5.86^{a}	2.36^{a}	3.84^{a}	30.85^{a}
		White	0.23^{a}	24.13^{a}	1.72^{b}	15.55^{a}	27.63^{b}	15.23^{a}	5.17^{a}	2.70^{a}	3.97^{a}	29.47^{b}
		CR	0.05	2.92	0.43	2.78	3.06	1.72	1.66	0.56	1.20	1.06
	43	Brown	0.28^{a}	21.58^{a}	2.97^{a}	8.18^{a}	36.47^{a}	16.10^{a}	9.55^{a}	1.00^{a}	1.35^{a}	27.10^{b}
		White	0.30^{a}	21.08^{a}	2.67^{a}	8.69^{a}	33.73^{b}	17.47^{a}	10.50^{a}	1.19^{a}	1.25^{a}	28.87^{a}
		CR	0.04	1.37	0.64	0.98	2.45	1.77	1.63	0.31	0.41	1.34
	58	Brown	0.32^{a}	21.37^{a}	2.85^{a}	9.06^{a}	34.00^{a}	16.30^{b}	7.86^{a}	1.93^{a}	2.93^{a}	30.37^{a}
		White	0.31^{a}	21.42^{a}	2.30^{b}	9.34^{a}	32.27^{a}	17.50^{a}	9.38^{a}	1.73^{a}	2.47^{a}	29.50^{a}
		CR	0.04	1.30	0.52	2.04	2.34	1.18	2.65	0.61	1.04	1.14
	72	Brown	0.32^{a}	21.10^{a}	3.50^{a}	8.16^{a}	36.02^{a}	14.57^{b}	8.86^{a}	1.11^{a}	1.84^{a}	NA
		White	0.37^{a}	22.32^{a}	2.92^{a}	8.60^{a}	32.78^{b}	16.58^{a}	10.27^{a}	1.00^{a}	1.58^{a}	NA
		CR	0.08	2.45	0.59	1.09	2.92	1.27	2.13	0.35	0.58	
	90	Brown	0.32^{b}	21.75^{a}	3.57^{a}	8.13^{b}	35.85^{a}	15.47^{b}	8.80^{b}	1.17^{a}	1.54^{a}	NA
		White	0.38^{a}	22.47^{a}	2.79^{b}	9.21^{a}	31.70^{b}	17.22^{a}	10.89^{a}	1.04^{a}	1.33^{a}	NA
		CR	0.04	1.13	0.49	0.87	2.26	1.65	1.88	0.21	0.27	
21	30	Brown	0.21^{a}	21.57^{a}	2.26^{a}	13.08^{a}	29.27^{a}	15.73^{b}	8.15^{a}	2.30^{a}	3.59^{a}	31.53^{a}
		White	0.23^{a}	21.62^{a}	1.86^{a}	13.20^{a}	27.68^{a}	17.33^{a}	9.04^{a}	2.11^{a}	3.08^{a}	29.43^{a}
		CR	0.05	1.73	0.50	2.40	2.06	1.43	3.16	0.85	1.00	0.94
	43	Brown	0.31^{a}	22.58^{a}	3.42^{a}	8.63^{a}	34.80^{a}	15.13^{b}	9.95^{b}	1.18^{a}	1.59^{a}	27.10^{a}
		White	0.31^{a}	20.48^{a}	2.38^{b}	9.44^{a}	29.73^{b}	18.80^{a}	13.08^{a}	1.16^{a}	1.61^{a}	28.60^{a}
		CR	0.07	2.76	0.76	1.13	3.49	2.16	2.78	0.23	0.40	1.60
	58	Brown	0.30^{a}	20.87^{a}	3.10^{a}	7.59^{b}	33.37^{a}	16.88^{a}	13.78^{a}	1.15^{a}	1.68^{a}	29.04^{a}
		White	0.28^{a}	20.38^{a}	2.49^{a}	8.99^{a}	31.00^{a}	18.32^{a}	13.28^{a}	1.25^{a}	1.66^{a}	30.10^{a}
		CR	0.08	2.61	0.66	0.84	5.14	2.03	4.15	0.27	0.49	2.19
	72	Brown	0.29^{a}	20.10^{a}	2.99^{a}	8.45^{a}	32.48^{a}	16.93^{a}	13.65^{a}	0.86^{a}	1.32^{a}	NA
		White	0.29^{a}	20.87^{a}	2.92^{b}	7.99^{a}	30.15^{a}	16.98^{a}	14.30^{a}	0.76^{a}	1.33^{a}	NA
		CR	0.07	2.03	0.61	0.52	2.47	1.17	2.44	0.21	0.49	
	90	Brown	0.31^{a}	20.88^{a}	3.52^{a}	8.35^{a}	34.60^{a}	16.78^{a}	10.43^{a}	1.03^{a}	1.46^{a}	NA
		White	0.33^{a}	22.32^{a}	3.39^{a}	8.30^{a}	32.90^{b}	16.28^{a}	11.62^{a}	0.95^{a}	1.30^{a}	NA
		CR	0.04	1.23	0.67	0.94	2.44	1.95	1.62	0.12	0.26	
28	30	Brown	0.35^{a}	21.97^{a}	2.95^{a}	8.29^{a}	31.15^{a}	17.27^{a}	13.51^{b}	0.80^{a}	1.01^{a}	31.00^{a}
		White	0.37^{a}	21.22^{a}	2.34^{a}	9.24^{a}	26.97^{b}	18.23^{a}	16.52^{a}	0.84^{a}	1.24^{a}	29.78^{a}
		CR	0.14	3.57	0.87	1.46	1.71	1.37	2.79	0.21	0.36	2.08
	43	Brown	0.35^{a}	20.40^{a}	2.75^{a}	8.31^{a}	30.37^{a}	17.77^{b}	14.18^{a}	0.92^{a}	1.23^{a}	27.72^{b}
		White	0.32^{a}	20.55^{a}	2.15^{a}	9.23^{a}	27.37^{b}	19.28^{a}	14.98^{a}	1.09^{a}	1.71^{a}	30.22^{a}
		CR	0.10	3.02	0.55	1.16	2.77	1.19	2.67	0.31	0.65	1.32
	58	Brown	0.27^{a}	20.38^{a}	2.93^{a}	7.01^{a}	32.68^{a}	17.48^{b}	14.29^{a}	1.05^{a}	1.47^{a}	29.32^{a}
		White	0.30^{a}	18.52^{a}	2.00^{b}	8.05^{a}	28.33^{b}	19.58^{a}	18.42^{a}	0.92^{a}	1.24^{a}	29.88^{a}
		CR	0.06	2.07	0.49	1.18	3.90	1.35	4.38	0.25	0.34	2.01
	72	Brown	0.37^{a}	21.70^{a}	2.68^{a}	8.82^{a}	28.87^{a}	17.20^{a}	15.28^{a}	0.76^{a}	1.10^{b}	NA
		White	0.34^{a}	20.02^{a}	2.41^{a}	9.26^{a}	27.30^{a}	17.58^{a}	16.40^{a}	0.76^{a}	1.67^{a}	NA
		CR	0.05	1.70	0.48	0.64	2.04	1.38	1.21	0.14	0.54	
	90	Brown	0.33^{a}	21.13^{a}	2.87^{a}	8.70^{a}	29.78^{a}	17.47^{a}	16.60^{a}	0.95^{a}	1.58^{a}	NA
		White	0.32^{a}	20.63^{a}	2.46^{a}	8.79^{a}	26.67^{b}	17.22^{a}	15.58^{a}	0.93^{b}	1.27^{b}	NA
		CR	0.04	2.33	0.66	0.81	2.36	1.28	2.72	0.15	0.29	

[a–b]Means within a treatment-day grouping lacking a common superscript differ ($P < 0.05$) according to Ducnan's multiple-range test.
[1]Critical range for mean separation.
[2]Percentage of total fat.
[3]DHA = docosahexanoic.
[4]Percentage of yolk.
[5]NA = not available.

leic acid, a major component of total omega-6 PUFA (Table 4).

Generally, total PUFA content tended to be highest in the yolks of the white hens (Table 7). This strain effect was significantly different ($P < 0.05$) only in 13 of the 25 cases, however. Three cases were found with each of the control, 7, 14, and 28% treatments, and one case was found with the 21% chia treatment.

Yolk saturated:omega-3 fatty acid ratio showed few statistically significant differences ($P < 0.05$) between strains (Table 7). Two exceptions were Days 43 and 72 for the 7 and 28% chia diets, respectively. In both cases, the white hens produced lower saturated:omega-3 ratios than the brown hens.

Omega-6:omega-3 ratios were similar over the duration of the feeding period for all treatments, except on Days 43 and 58 for the 7% chia diet and Day 30 for the 14% chia diet (Table 7). The saturated:PUFA ratio did not show many significant differences ($P < 0.05$) between strains over the experimental period; only five exceptions were found for the 25 cases. In each instance the white hens gave a lower ratio than the brown hens.

DISCUSSION

Epidemiological studies, as well as controlled studies conducted with animals and humans, suggest that increasing polyunsaturated omega-3 fatty acid intake reduces CHD, as well as autoimmune and inflammatory diseases (Dyerberg et al., 1978; Hennekens et al., 1990; Nettleton, 1995). Coronary heart disease is reduced because omega-3 fatty acids lower triglyceride and cholesterol serum contents, two factors that increase the risk of CHD (Lorgeril et al., 1994; Eristland et al., 1995; Noakes et al., 1996).

Ferrier et al. (1992) showed that consumption of α-linolenic-enriched eggs resulted in a significant decrease (35%) in human serum triglyceride levels, after 1 wk, with no change in total or high-density lipoprotein cholesterol levels detected. The eggs were produced by including ground flaxseed in the hens' diet.

Cholesterol

Compared with the control diet, significantly lower ($P < 0.05$) yolk cholesterol was found with the 14, 21, and 28% chia diets fed to the white hens. This result was different from trials in which hens were fed flaxseed, and differences in yolk cholesterol content were not found (Caston and Leeson, 1990; Scheideler and Froning, 1996). Lall and Slinger (1973) and Bartov et al. (1969) reported increased yolk cholesterol when high levels of polyunsaturated fats were added to hen diets.

TABLE 5. Total saturated, monounsaturated (mono), polyunsaturated, omega-6, and omega-3 fatty acids and their ratios with the white hens

Day	Treatment	Saturated %[2]	Mono %[2]	Omega-6 %[2]	Omega-3 %[2]	PUFA[3] %[2]	Omega-3/Omega-6	SFA[4]/PUFA	SFA/Omega-3
30	0	36.33^{b}	43.15^{a}	16.32^{c}	1.20^{d}	17.53^{d}	14.13^{a}	2.09^{a}	31.99^{a}
	7	39.95^{a}	29.92^{b}	18.69^{ab}	8.24^{c}	26.94^{c}	2.28^{b}	1.48^{b}	4.86^{b}
	14	39.91^{a}	29.35^{b}	17.93^{b}	9.14^{c}	27.07^{c}	1.97^{b}	1.48^{b}	4.39^{b}
	21	35.05^{b}	29.54^{b}	19.44^{a}	12.12^{b}	31.56^{b}	1.63^{b}	1.11^{c}	2.94^{b}
	28	30.83^{c}	29.31^{b}	19.08^{ab}	17.76^{a}	36.84^{a}	1.09^{b}	0.84^{d}	1.77^{b}
	CR[1]	2.04	2.76	1.51	1.52	2.25	1.61	0.15	5.27
43	0	34.61^{a}	43.71^{a}	18.11^{c}	0.97^{e}	19.09^{e}	18.66^{a}	1.82^{a}	35.80^{a}
	7	31.57^{b}	39.63^{b}	19.10^{abc}	7.26^{d}	26.37^{d}	2.65^{b}	1.20^{b}	4.39^{b}
	14	30.07^{b}	36.41^{c}	18.66^{bc}	11.74^{c}	30.40^{c}	1.60^{c}	1.00^{c}	2.59^{bc}
	21	30.23^{b}	32.11^{d}	19.96^{ab}	14.70^{b}	34.66^{b}	1.36^{c}	0.88^{cd}	2.08^{c}
	28	30.10^{b}	29.52^{e}	20.37^{a}	16.69^{a}	37.06^{a}	1.23^{c}	0.82^{d}	1.82^{c}
	CR	2.60	2.41	1.61	1.48	2.60	0.65	0.16	2.26
58	0	34.04^{a}	44.81^{a}	17.67^{c}	0.81^{e}	18.48^{e}	22.13^{a}	1.87^{a}	43.60^{a}
	7	32.75^{ab}	38.60^{b}	18.64^{bc}	7.43^{d}	26.08^{d}	2.52^{b}	1.26^{b}	4.43^{b}
	14	31.06^{bc}	34.57^{c}	19.23^{abc}	11.85^{c}	31.08^{c}	1.64^{bc}	1.00^{c}	2.66^{b}
	21	29.66^{c}	33.49^{cd}	19.57^{ab}	14.94^{b}	34.51^{b}	1.34^{bc}	0.87^{cd}	2.05^{b}
	28	26.86^{d}	30.33^{d}	20.50^{a}	19.65^{a}	40.16^{a}	1.06^{c}	0.68^{d}	1.39^{b}
	CR	2.78	3.89	1.86	2.58	3.58	1.24	0.23	6.63
72	0	33.80^{ab}	41.41^{a}	20.18^{a}	1.29^{e}	21.47^{e}	15.64^{a}	1.58^{a}	26.24^{a}
	7	34.89^{a}	36.84^{b}	18.31^{b}	6.88^{d}	25.19^{d}	2.72^{b}	1.39^{b}	5.25^{b}
	14	31.29^{bc}	35.71^{bc}	17.57^{b}	11.85^{c}	29.42^{c}	1.50^{c}	1.07^{c}	2.67^{c}
	21	29.15^{c}	33.07^{c}	17.75^{b}	15.63^{b}	33.38^{b}	1.15^{c}	0.88^{d}	1.91^{c}
	28	29.62^{c}	29.71^{d}	18.35^{b}	18.07^{a}	36.42^{a}	1.02^{c}	0.81^{d}	1.64^{c}
	CR	3.13	3.26	1.53	1.69	2.31	0.63	0.16	1.50
90	0	34.02^{a}	43.25^{a}	18.55^{a}	1.09^{d}	19.64^{c}	17.26^{a}	1.74^{a}	32.04^{a}
	7	33.02^{ab}	43.06^{a}	15.96^{b}	5.50^{c}	21.45^{c}	2.95^{b}	1.55^{b}	6.16^{b}
	14	32.05^{bc}	34.49^{bc}	18.25^{a}	12.22^{b}	30.47^{b}	1.51^{c}	1.06^{c}	2.68^{bc}
	21	30.95^{cd}	36.29^{b}	17.23^{ab}	12.92^{b}	30.15^{b}	1.34^{c}	1.03^{c}	2.40^{bc}
	28	29.75^{d}	32.24^{c}	18.15^{a}	16.85^{a}	35.00^{a}	1.09^{c}	0.85^{d}	1.79^{c}
	CR	1.99	3.40	1.58	1.70	2.75	1.29	0.17	3.98

[a–e]Means within a day lacking a common superscript differ ($P < 0.05$) according to Duncan's multiple-range test.

[1]Critical range for mean separation.

[2]Percentage of total fat.

[3]PUFA = polyunsaturated fatty acid.

[4]SFA = saturated fatty acid.

TABLE 6. Total saturated, monounsaturated (mono), polyunsaturated, omega-6, and omega-3 fatty acids and their ratios with the brown hens

Day	Treatment	Saturated %[2]	Mono %[2]	Omega-6 %[2]	Omega-3 %[2]	PUFA[3] %[2]	Omega-6/Omega-3	SFA[4]/PUFA	SFA/Omega-3
30	0	34.86[a]	46.28[a]	15.44[b]	0.93[e]	16.37[c]	17.16[a]	2.14[a]	39.17[a]
	7	36.49[a]	37.17[b]	16.63[ab]	7.23[d]	23.86[b]	2.36[b]	1.53[b]	5.16[b]
	14	36.42[a]	33.90[bc]	16.86[ab]	9.70[c]	26.55[b]	1.75[b]	1.39[bc]	3.83[b]
	21	34.86[a]	31.52[c]	18.03[a]	11.74[b]	29.77[a]	1.57[b]	1.19[c]	3.09[b]
	28	30.61[b]	34.10[bc]	18.07[a]	14.52[a]	32.58[a]	1.27[b]	0.96[d]	2.20[b]
	CR[1]	3.62	3.58	1.68	2.23	3.35	1.80	0.24	5.33
43	0	33.59[a]	47.52[a]	15.76[b]	0.71[d]	16.47[d]	23.24[a]	2.07[a]	50.71[a]
	7	33.52[a]	41.91[b]	16.78[ab]	5.38[c]	22.16[c]	3.15[b]	1.54[b]	6.38[b]
	14	30.04[b]	39.44	17.10[ab]	10.91[b]	28.01[b]	1.58[b]	1.08[cd]	2.78[b]
	21	31.53[ab]	38.22[c]	16.31[b]	11.54[b]	27.85[b]	1.44[b]	1.18[c]	2.91[b]
	28	29.05[b]	33.12[d]	18.69[a]	15.41[a]	34.10[a]	1.24[b]	0.86[d]	1.93[b]
	CR	2.86	3.53	2.30	2.35	4.07	3.61	0.32	10.59
58	0	34.01[a]	48.32[a]	14.52[b]	0.72[d]	15.25[c]	20.34[a]	2.27[a]	48.52[a]
	7	30.21[b]	41.09[b]	19.00[a]	7.05[c]	26.04[b]	2.70[b]	1.16[b]	4.29[b]
	14	30.74[b]	36.85[c]	18.23[a]	10.79[b]	29.02[b]	1.73[bc]	1.06[bc]	2.92[b]
	21	28.75[bc]	36.47[c]	18.03[a]	15.46[a]	33.49[a]	1.20[c]	0.87[c]	1.93[b]
	28	27.66[c]	35.61[c]	18.54[a]	15.76[a]	34.29[a]	1.24[c]	0.82[c]	1.88[b]
	CR	2.50	3.49	1.35	3.05	3.74	1.52	0.26	6.51
72	0	34.48[a]	44.63[a]	16.92[ab]	1.26[d]	18.18[d]	13.55[a]	1.91[a]	27.88[a]
	7	35.56[a]	37.88[bc]	16.51[b]	6.56[c]	23.07[c]	2.56[b]	1.55[b]	5.58[b]
	14	29.58[b]	39.51[b]	15.67[b]	10.70[b]	26.37[b]	1.50[c]	1.13[c]	2.84[c]
	21	28.84[b]	35.48[c]	17.80[a]	14.97[a]	32.76[a]	1.20[c]	0.88[d]	1.95[c]
	28	30.89[b]	31.55[d]	17.96[a]	16.38[a]	34.34[a]	1.10[c]	0.90[d]	1.90[c]
	CR	2.28	2.66	1.34	1.75	2.26	0.80	0.18	2.77
90	0	33.69[a]	45.49[a]	16.92[ab]	1.15[d]	18.07[d]	15.12[a]	1.88[a]	30.21[a]
	7	32.06[b]	44.34[a]	15.43[b]	5.79[c]	21.22[c]	2.72[b]	1.53[b]	5.72[b]
	14	30.20[c]	39.42[b]	16.64[ab]	10.33[b]	26.97[b]	1.63[bc]	1.12[c]	2.95[bc]
	21	29.55[c]	38.12[b]	17.81[a]	11.89[b]	29.70[b]	1.51[bc]	1.01[cd]	2.53[bc]
	28	30.23[c]	29.53[c]	18.42[a]	18.18[a]	36.60[a]	1.03[c]	0.83[d]	1.69[c]
	CR	1.75	2.77	1.91	1.92	3.09	1.60	0.19	3.57

[a–d]Means within a day lacking a common superscript differ ($P < 0.05$) according to Duncan's multiple-range test.
[1]Critical range for mean separation.
[2]Percentage of total fat.
[3]PUFA = polyunsaturated fatty acid.
[4]SFA = saturated fatty acid.

The decrease in cholesterol found in the chia study could arise because of the fiber content of the chia. Chia seed contains 60.9% total dietary fiber, on a dry basis, with 7% being soluble and 55.9% insoluble (Weber et al., 1991). Hargis (1988) reported that fiber influences cholesterol metabolism of laying hens by decreasing absorption of cholesterol, binding with the bile salts in the intestinal tract, shortening intestinal transit time, and increasing fecal sterol excretion. The hypocholesterolemic effect of dietary fiber has been reported for other animals as well (Shen et al., 1998).

The reduction in cholesterol content found herein, up to 20%, is probably not significant in practical terms to the poultry industry, because the Food and Drug Administration in the United States (1997) has stated that for a product to legally claim "less" or "reduced" amounts of a nutrient, it has to have 25% less than the normal amount of that nutrient. Given the 20% reduction that was found, however, additional tests should be conducted to examine the potential of chia for cholesterol reduction, as it may be possible to reach the 25% threshold.

Lipid Content and Composition

With the exception of one date for the white hens, no difference was detected in total fat content of the yolks among treatments throughout the trial for either strain. The same finding was reported for yolks from hens fed 8 and 16% flaxseed diets (Cherian and Sim, 1991; Cherian et al., 1995) and 30% chia (Ayerza and Coates, 1999). The reason for the difference in yolk lipid content, found at Day 43 for the white hens fed the 28% chia diet, is not evident. It may, however, be related to sample size.

Yolk lipid composition depends on a combination of de novo liver lipid synthesis, hepatic uptake, and the incorporation of lipid components from the diet (Sim and Qi, 1995). The effect of dietary fats on yolk fatty acid profiles has been demonstrated with the magnitude of the change varying with fat source (Caston and Lesson, 1990; Cherian and Sim, 1991; Jiang et al, 1992; Caston et al., 1994; Cherian et al., 1995; Nash et al., 1995; Herber and Van Elswyk, 1996; Scheideler and Froning, 1996). Those trials, however, did not include chia. A significant finding of the research reported herein was the beneficial effect chia had on the saturated palmitic and polyunsaturated omega-3 fatty acid contents of the yolks.

Saturated Fatty Acids

The decrease in palmitic acid in the chia trial was greater for both strains than was the decrease reported

TABLE 7. Comparison of total saturated, monounsaturated (mono), polyunsaturated, omega-6, and omega-3 fatty acids and their ratios between two strains of laying hens

Treatment	Day	Strain	Saturated %[2]	Mono %[2]	Omega-6 %[2]	Omega-3 %[2]	Omega-6/Omega-3	PUFA[3]	SFA[4]/PUFA	SFA/Omega-3
7	30	Brown	36.49[b]	37.17[a]	16.63[b]	7.23[a]	2.36[a]	23.86[b]	1.53[a]	5.16[a]
		White	39.95[a]	29.92[b]	18.70[a]	8.24[a]	2.28[a]	26.94[a]	1.48[a]	4.86[a]
		CR[1]	1.82	2.41	0.85	1.15	0.39	1.41	0.12	0.81
	43	Brown	33.52[a]	41.91[a]	16.78[b]	5.38[b]	3.15[a]	22.16[b]	1.54[a]	6.38[a]
		White	31.57[a]	39.63[a]	19.10[a]	7.26[a]	2.65[b]	26.37[a]	1.20[b]	4.39[b]
		CR	3.71	3.07	2.09	0.97	0.39	2.84	0.31	1.31
	58	Brown	30.21[a]	41.09[a]	19.00[a]	7.05[a]	2.70[a]	26.04[a]	1.16[a]	4.29[a]
		White	32.75[a]	38.60[b]	18.64[a]	7.43[a]	2.52[b]	26.08[a]	1.26[a]	4.43[a]
		CR	2.68	1.79	0.77	0.47	0.17	0.99	0.14	0.54
	72	Brown	35.56[a]	37.88[a]	16.51[b]	6.56[a]	2.56[a]	23.07[b]	1.55[a]	5.57[a]
		White	34.89[a]	36.84[a]	18.31[a]	6.88[a]	2.72[a]	25.19[a]	1.39[a]	5.25[a]
		CR	4.64	4.11	1.44	1.34	0.57	2.02	0.26	1.72
	90	Brown	32.06[a]	44.34[a]	15.43[a]	5.79[a]	2.72[a]	21.22[a]	1.53[a]	5.72[a]
		White	33.02[a]	43.06[a]	15.96[a]	5.50[a]	2.95[a]	21.46[a]	1.55[a]	6.16[a]
		CR	1.85	3.33	1.57	1.39	0.45	2.85	0.23	1.47
14	30	Brown	36.42[a]	33.90[a]	16.86[a]	9.70[a]	1.75[b]	26.55[a]	1.39[a]	3.83[a]
		White	39.91[a]	29.35[b]	17.93[a]	9.14[a]	1.97[a]	27.07[a]	1.48[a]	4.39[a]
		CR	3.83	3.31	1.66	1.26	0.2	2.59	0.23	0.77
	43	Brown	30.04[a]	39.44[a]	17.10[a]	10.91[a]	1.58[a]	28.01[a]	1.08[a]	2.78[a]
		White	30.07[a]	36.41[b]	18.66[a]	11.74[a]	1.60[a]	30.40[a]	1.00[a]	2.59[a]
		CR	1.59	2.3	1.78	1.67	0.2	2.94	0.14	0.45
	58	Brown	30.74[a]	36.85[a]	18.23[a]	10.79[a]	1.73[a]	29.02[b]	1.06[a]	2.92[a]
		White	31.06[a]	34.57[a]	19.23[a]	11.85[a]	1.64[a]	31.08[a]	1.00[a]	2.66[a]
		CR	2.62	2.7	1.07	1.88	0.37	1.94	0.13	0.71
	72	Brown	29.58[a]	39.51[a]	15.67[b]	10.70[a]	1.50[a]	26.37[b]	1.13[a]	2.84[a]
		White	31.29[a]	35.71[b]	17.57[a]	11.85[a]	1.50[a]	29.42[a]	1.07[a]	2.67[a]
		CR	2.21	2.85	1.39	2.07	0.31	2.49	0.15	0.58
	90	Brown	30.20[b]	39.42[a]	16.64[a]	10.33[b]	1.63[a]	26.97[b]	1.12[a]	2.95[a]
		White	32.05[a]	34.49[b]	18.25[a]	12.22[a]	1.51[a]	30.47[a]	1.06[a]	2.68[a]
		CR	0.88	2.49	1.65	1.76	0.26	2.68	0.11	0.5
21	30	Brown	34.86[a]	31.52[a]	18.03[a]	11.74[a]	1.57[a]	29.77[a]	1.19[a]	3.09[a]
		White	35.05[a]	29.54	19.40[a]	12.12[a]	1.63[a]	31.56[a]	1.11[a]	2.94[a]
		CR	3.6	2.45	1.61	2.4	0.34	2.87	0.2	0.82
	43	Brown	31.53[a]	38.22[a]	16.31[b]	11.54[b]	1.44[a]	27.85[b]	1.18[a]	2.91[a]
		White	30.23[a]	32.11[b]	19.96[a]	14.70[a]	1.36[a]	34.66[a]	0.88[b]	2.08[a]
		CR	3.01	3.83	2.25	2.76	0.16	4.91	0.28	0.84
	58	Brown	28.75[a]	36.47[a]	18.03[a]	15.46[a]	1.20[a]	33.49[a]	0.87[a]	1.93[a]
		White	29.66[a]	33.49	19.57[a]	14.94[a]	1.34[a]	34.51[a]	0.87[a]	2.05[a]
		CR	2.88	5.57	1.91	3.93	0.27	5.25	0.17	0.56
	72	Brown	28.84[a]	35.48	17.80[a]	14.97[a]	1.20[a]	32.76[a]	0.88[a]	1.95[a]
		White	29.15[a]	33.07	17.75[a]	15.63[a]	1.15[a]	33.38[a]	0.88[a]	1.91[a]
		CR	1.88	2.88	1.3	2.45	0.19	2.97	0.13	0.41
	90	Brown	29.55[b]	38.12	17.81[a]	11.89[a]	1.51[a]	29.70[a]	1.01[a]	2.53[a]
		White	30.95[a]	36.29	17.23[a]	12.92[a]	1.34[a]	30.15[a]	1.03[a]	2.41[a]
		CR	1.15	2.98	1.94	1.65	0.21	2.93	0.12	0.4
28	30	Brown	30.61[a]	34.10	18.07[a]	14.52[b]	1.27[a]	32.58[b]	0.96[a]	2.20[a]
		White	30.83[a]	29.31[b]	19.08[a]	17.76[a]	1.09[a]	36.84[a]	0.84[a]	1.77[a]
		CR	2.59	2.26	1.5	2.94	0.2	4.11	0.2	0.64
	43	Brown	29.05[a]	33.12[a]	18.69[b]	15.41[a]	1.24[a]	34.10[a]	0.86[a]	1.93[a]
		White	30.10[a]	29.52[b]	20.37[a]	16.69[a]	1.23[a]	37.06[a]	0.82[b]	1.82[a]
		CR	2.58	2.92	1.35	2.68	0.23	3.2	0.13	0.42
	58	Brown	27.66[a]	35.61	18.54[b]	15.76[a]	1.24[a]	34.29[b]	0.82[a]	1.88[a]
		White	26.86[a]	30.33	20.50[a]	19.65[a]	1.06[a]	40.16[a]	0.68[a]	1.39[a]
		CR	1.48	4.27	1.3	4.35	0.31	5.07	0.15	0.61
	72	Brown	30.89[a]	31.55[a]	17.96[a]	16.38[b]	1.10[a]	34.34[b]	0.90[a]	1.90[a]
		White	29.62[a]	29.71[a]	18.35[a]	18.07[a]	1.02[a]	36.42[a]	0.81[b]	1.64[b]
		CR	1.99	2.31	1.47	1.43	0.13	1.88	0.08	0.21
	90	Brown	30.23[a]	29.53[b]	18.42[a]	18.18[a]	1.03[a]	36.60[a]	0.83[a]	1.69[a]
		White	29.74[a]	32.24[a]	18.15[a]	16.85[a]	1.09[a]	35.00[a]	0.85[a]	1.79[a]
		CR	1.98	2.63	1.21	2.81	0.15	3.51	0.12	0.33

[a–b]Means within a treatment-day grouping lacking a common superscript differ ($P < 0.05$) according to Duncan's multiple-range test.
[1]Critical range for mean separation.
[2]Percentage of total fat.
[3]PUFA = polyunsaturated fatty acid.
[4]SFA = saturated fatty acid.

by Marshall et al. (1994b) when feeding diets containing 1.5% menhaden oil to Single Comb White Leghorn (SCWL) hens. Also the decrease was greater than that reported by Abril and Barclay (1998) when feeding 165 mg/d of DHA obtained from an algae-like microorganism (*Schizochytrium sp.*) combined with 2.5% flaxseed, when fed to SCWL laying hens.

Other research, however, in which hens were fed diets rich in omega-3, obtained by adding flaxseed (Caston and Leeson; 1990; Cherian et al., 1995) or menhaden fish oil (Van Elswyk et al., 1992), have found no change in palmitic content of the yolks. The inhibition of fatty acid synthesis in the liver is greater during digestion of unsaturated fats, rather than saturated fats (Sim and Qi, 1995). Discrepancies between tests in terms of palmitic acid content could be attributed to different degrees of lipogenesis reduction brought about by the different PUFA contents of the diets. Also, when palmitic acid is released from the synthetase complex, it can be esterified into complex lipids, elongated to stearic acid, or desaturated to palmitoleic acid. Different enzymes are involved in each reaction, and the regulation and control of these secondary pathways is poorly understood. It is known, however, that high concentrations of stearic acid (from the palmitic acid elongation process) in tissue will be found in vivo only when they are derived from dietary sources (Nelson, 1992).

The findings of the current chia study suggest that the reduced palmitic acid content in the egg yolks is feed dependent. The absence of a significant difference (P < 0.05) in palmitic acid content between eggs from the 7% chia diet and control diet on some dates for both strains could indicate that the quantity of PUFA incorporated into the ration by the chia at these levels is at the lower limit that can produce a consistent decrease in lipogenesis.

The reduction in palmitic acid found (up to 30.6%) could indicate an additional health advantage for these omega-3-enriched eggs. Dietary saturated fatty acids are independent risk factors associated with CHD; their negative effects on blood low-density lipoprotein cholesterol are stronger than the effects of dietary cholesterol (American Heart Association, 1988; Mayo Clinic, 1997).

In 80% of the cases, a significant difference (P < 0.05) in yolk stearic acid content was detected between the control and the chia diets. As reported by Nash et al. (1995), stearic acid generally increases as α-linolenic acid content increases. This increase occurred in the current study. In contrast, other authors indicated no change (Van Eswyk et al., 1992; Cherian et al., 1995) or a small decrease (Caston and Leeson, 1990; Marshall et al., 1994b) in stearic acid content as the diets became richer in omega-3 fatty acids. Some significant differences (P < 0.05) in stearic acid content between strains were observed herein, with the brown hens showing the lowest quantity in those cases, which could indicate genetic dependence. Stearic acid, however, is considered much less hypercholesterolemic than palmitic acid (Nelson, 1992; Katan et al., 1995) or not hypercholesterolemic at all (Bonanome and Grundy, 1988; Grundy, 1997) and, hence, is of lessor importance in terms of its influence on health.

The chia diets affected not just the quantity, but also the quality of the total saturated fatty acid content of the yolks. As chia percentage increased, the fatty acid profile improved as evidenced by the change in the relationship of the palmitic and stearic acid contents, and the significant reduction (P < 0.05) in total saturated fatty acids. Neither of these changes has been reported in other trials in which hens were fed omega-3-enriched diets by adding feedstuffs other than chia. The total saturated fatty acid changes reported herein reflect an advantage with the chia diets over diets with 3% menhaden fish oil (Van Elswyk et al. 1992), 1.5% menhaden fish oil, and 2.4 or 4.8% golden marine algae (Herber and Van Elswyk, 1996). Those diets did not decrease yolk total saturated fatty acid content. The chia diets also had an advantage over diets with 4, 8, and 12% herring fish meal (Nash et al., 1995), which increased yolk saturated fatty acid content.

The chia diets lowered yolk total saturated fatty acid content by up to 21.1% at Day 58 for the white hens and by 19.2% at Day 30 for the brown hens with the 28% chia diet. This reduction is greater than was found with diets with 1.5% menhaden fish oil (Marshall et al., 1994b) or 10, 20, or 30% flaxseed (Caston and Lesson, 1990), which reduced total saturated fatty acid content by 11.6, 1.2, 8.4, or 13%, respectively. However, compared with hens receiving 165 mg/d of DHA obtained from an algae-like microorganism combined with 2.5% flaxseed (Abril and Barclay, 1998), chia gave a similar decrease in total saturated fatty acid content but a higher palmitic:stearic acid ratio. The explanation for this result could be related to the 12 IU of vitamin E/hen per d that supplemented the combined algae and flax diets. This amount of vitamin E is 12 times greater than that recommended by the National Research Council (1994).

Cherian et al. (1996) reported a significant decrease (P < 0.05) in yolk total saturated fatty acid content with hens fed flax oil plus 423.4 μg/g of tocopherols, when compared with eggs from hens fed flax alone. Thus, the decreased saturated fatty acid content found in the eggs produced by the hens fed chia could be related to the potent antioxidant activity caused by the chlorogenic and caffeic acids (Taga et al., 1984) in the chia.

In the chia study, total saturated fatty acid content was significantly lower for the brown hens on 20% of the days with the 7, 14, and 21% chia diets, compared with the white hens. As no significant difference was detected in the control and 28% chia diets, the results suggest that at low to medium chia levels the brown hens tended to produce yolks with a lower saturated fatty acid content than the white hens. This genetic dependence could have important implications for commercialization.

Monounsaturated Fatty Acids

The concomitant decrease in total monounsaturated fatty acids, which occurred with both strains as total omega-3 PUFA increased, was similar to the change found in the plasma of laying hens fed 8 and 16% flaxseed diets (Cherian and Sim, 1991). Decreased yolk oleic acid content was found as flaxseed content increased from 10 to 20% and then to 30% (Caston and Leeson, 1990). This decrease in oleic acid could be related to the inhibition effect of PUFA against Δ9-desaturase activity, resulting in the formation of monounsaturated oleic acid. This interaction has been reported in other animals (Brenner, 1974; Garg et al., 1988c). Oleic acid can be formed from stearic acid through an enzymatic desaturation process (Nettleton, 1995); however, stearic acid from dietary sources is not easily converted to oleic acid. This desaturation is depressed with polyunsaturated rich diets, as has been reported for in vitro experiments (Brenner and Peluffo, 1966).

The stearic acid content was higher in the chia diets than in the control diets. The entrance of dietary (preformed) fatty acids into the pathways of lipid metabolism is different from that of fatty acids synthesized de novo (Nelson, 1992). Thus, the increase in the stearic and the decrease in the oleic acid found as chia in the diet increased are probably related processes and agree with reports that lipogenesis is depressed in diets rich in PUFA (Brenner, 1974).

The lack of a significant difference ($P < 0.05$) in total monounsaturated fatty acid content between hens fed the 7% chia diet at Day 90 and hens fed the control diet, with both strains, suggests that the omega-3 fatty acid provided by this amount of chia is below that which can produce a change in eggs. In a previous experiment (Ayerza and Coates, 1999), a 30% chia diet produced no significant difference ($P > 0.05$) in total monounsaturated fatty acid content compared with the control diet; however, a different breed was used. Genetic effects on fatty acid relationships have been documented (Cherian et al., 1995). Such an interaction is evident when comparing the white and brown hens in this experiment and considering the results of the previous chia trial.

Omega-6 Fatty Acids

The most notable fatty acid differences found between the brown and white strains was in total omega-6 PUFA and total monounsaturated fatty acids, whose major components are linoleic and oleic acids, respectively. These results could indicate a genetic influence on chia utilization and the storage of dietary fats, with the brown hens being more efficient than the white hens in utilizing dietary oleic and linoleic acids. Similar results, in terms of genetic differences, were found by Nash et al. (1995) between two commercial SCWL genotypes, when fed herring meal at 40, 80, and 120 g/kg of feed. Scheideler et al. (1998) reported similar results among Babcok B300, DeKalb Delta, and Hy-Line W-36, when fed flaxseed at 10% of the diet.

The decline in arachidonic acid found in the yolks of the white hens has been reported for other breeds fed diets enriched with α-linolenic acid (Cherian and Sim, 1991; Cherian et al., 1995). The decline in arachidonic acid content has been suggested to be a result of inhibition of the synthesis of this fatty acid when hens receive diets high in α-linolenic acid (Craig-Schmidt et al., 1987).

Linoleic content of the diets increased as chia content increased (Table 1), whereas the arachidonic acid content of the yolks decreased for both strains when fed these diets. The change can be attributed to a decrease in the linoleic:α-linolenic ratio in the diet, which arises because of competition between these two families of fatty acids for Δ6 and Δ5 enzymes involved in the processes of desaturation and elongation (Hayek and Reinhart, 1998). A low ratio of omega-6 to omega-3 fatty acids consumed is more effective in inhibiting omega-6 fatty acid elongation in other animals than is the absolute amount of omega-3 fatty acid consumed (Boudreau et al., 1991). The decrease in metabolic rates found as chia increased was probably related to the α-linolenic acid increase; thus, overloading the hen diets with omega-3 fatty acids inhibited arachidonic deposition in the yolks.

The difference in arachidonic acid incorporation between strains could be due to a diet × strain interaction. Enzyme-limiting capacity in the biosynthetic pathway in laying hens when modifying PUFA has been suggested by Scheideler et al. (1998). They showed that different strains have different capacities to utilize the Δ6 and Δ5 enzymes to desaturate and elongate linoleic and linolenic acids under different omega-6:omega-3 ratios.

Omega-3 Fatty Acids

The increase in the long-chain omega-3 fatty acid DHA in the yolks could be due to desaturation and elongation of α-linolenic acid in the hen livers, because chia seed lacks DHA (Ayerza, 1995). As was previously reported (Caston and Lesson, 1990; Sim and Jiang, 1994; Cherian et al., 1996; Ayerza and Coates, 1999), laying hens can convert α-linolenic acid to its elongated metabolite DHA and then deposit it in the yolk.

For both strains, the maximum DHA levels were reached with the 7% chia diet. It has been established that longer-chain omega-3 fatty acids such as eicosapentaenoic acid (EPA) and DHA exercise inhibitory effects on Δ6- and Δ5-desaturase enzymes that are involved in desaturation and elongation of α-linolenic acid (Garg et al., 1988a,b). The implications of preferential tissue concentration of particular omega-3 fatty acids are only beginning to be understood. It is interesting to note that physiological studies fail to demonstrate a specific function for α-linolenic acid in humans and poultry, but that they act as substrates for the synthesis of EPA and DHA (National Research Council, 1994; Nettleton, 1995). It appears that increasing chia beyond 7% in the diet provides no substantial advantage in terms of conversion of α-linolenic to DHA. This effect indicates, perhaps, a biological regulation for tissue incorporation of this long-chain omega-3 fatty acid.

Eicosapentaenoic acid (EPA), another metabolite formed during the desaturation and elongation of α-linolenic acid, was not detected in any treatment, for either strain. This type of relationship between α-linolenic acid from a plant source and its long-chain metabolites has been reported (Cherian et al., 1995; Scheideler et al., 1998a). Cherian and Sim (1991) found EPA acid incorporation into egg yolks in quantities of only 0.12, 0.15, and 0.11% of the total fatty acids from hens fed 8 and 16% flax and 16% canola seed, respectively. The formation of EPA is a step in the conversion of α-linolenic acid to DHA (Voss et al., 1991; Craig-Schmidt and Huang, 1998). An absence of EPA in the chia study could be attributed to an error in chromatographic interpretation, rather than to an actual absence, because the levels of this fatty acid are very small.

Various increases in α-linolenic and DHA of yolks from hens fed flaxseed and sea products have been reported. Adding omega-3 rich materials to hen feeds has shown increases in total omega-3 fatty acids of 610% in eggs from hens fed 1.5% dietary menhaden oil (Marshall et al., 1994b). Increases of 142, 180, and 224% have been shown for eggs from hens fed 4, 8, and 12% herring fish meal, respectively (Nash et al., 1995). Increases of 269, 339, and 483% have been shown in eggs from hens fed 1.5% menhaden fish oil or 2.4 or 4.8% golden marine algae, respectively (Herber and Van Elswyk, 1996). Increases in total omega-3 fatty acids of 339, 538, and 594% have been observed in eggs from hens fed 0.5, 1.5, and 3.0% menhaden fish oil, respectively (Van Elswyk et al., 1995); 436% in eggs from hens fed 3% menhaden fish oil (Van Elswyk et al., 1992); 477, 655, and 253% in eggs from hens fed 8% flax, 16% flax, and 16% canola seeds, respectively (Cherian and Sim, 1991). Increases in total omega-3 fatty acids of 174, 241, 366, 273, and 496% have been observed in eggs from hens fed 1.5% menhaden fish oil, whole flax seed at 5% and 15% and ground flax seed at 5% and 15%, respectively (Ayamond and Van Elswyk, 1995). At the high end, increases of 657 and 513% in total omega-3 fatty acids were found for six strains of hens fed a 17% flax seed diet (Cherian et al., 1995) and 538, 983, and 1,306% in eggs from hens fed 10, 20, and 30% flax seed, respectively (Caston and Leeson, 1990).

Comparatively, eggs produced by the hens fed the 7, 14, 21, and 28% chia diets gave an average increase in omega-3 fatty acid content of 658 and 670%, 1,059 and 1,099%, 1,310 and 1,375%, and 1,659 and 1,682% for the white and brown hens, respectively. The brown hens gave greater percentage increases than were cited in any other reports. The change found with the 28% chia diet in the current trial was also greater than that found in the eggs produced by the Issa Brown hens fed the 30% chia diet in a previous experiment (Ayerza and Coates, 1999). As the breeds and fatty acid contents used in the two experiments were different, the variation in the results may be due to a genetic × diet interaction.

Total omega-3 fatty acids incorporated into the yolks (Table 8), with both strains of hens fed chia, was greater than that reported with hens fed 0.5, 1.5, and 3% menhaden fish oil, which had contents of 7.19, 11.4, and 12.6 mg/g of yolk, respectively (Van Elswyk et al., 1995). With the exception of the 7% chia diet, the values were also higher than those reported with diets containing 1.5% menhaden oil, 5% and 15% whole flaxseed, and 5% and 15% ground flaxseed, which gave omega-3 contents of 8.57, 11.87, 17.99, 13.45, and 24.42 mg/g of yolk, respectively (Ayamond and Van Elswyk, 1995). Also the values were higher than those reported in egg yolks from hens fed 1.5% menhaden fish oil or 2.4 or 4.8% natural marine microalgae, which gave total omega-3 fatty acid levels of 10.5, 10.6, or 13.7 mg/g of yolk, respectively (Herber and Van Elswyk, 1996).

Total omega-3 fatty acid content found in the yolks of the hens fed chia plateaued after Day 30 in both strains. In other studies, the omega-3 acid level stabilized within 9 to 12 d of the start of the trial with diets containing 3% α-linolenic acid derived from flaxseed and on Day 8 with 1.2% EPA plus 5.4% of DHA derived from pacific salmon oil (Yu and Sim, 1987; Sim and Qi, 1995). Analyses must be undertaken earlier in chia feeding trials to determine stabilization time for omega-3 incorporation into yolks.

In general, increasing chia levels in the diet produced similar changes in yolk fatty acid content of both strains. However, the magnitude of change in most of the fatty acid contents was small. The same finding was reported for yolks obtained from six breeds of laying hens fed diets containing two levels of α-linolenic acid provided by feeding menhaden oil and flax oil (Cherian et al., 1995). Scheideler et al. (1998) suggested that not only were differences in hen breed responsible for changes in the egg fatty acid profiles found with different diets but also that a fiber × fat utilization interaction exists. When different hen breeds were given more insoluble fiber, one breed responded with an increased α-linolenic acid content, another breed showed a decrease, and a third breed showed no change. In the chia experiment as chia amounts increased, the α-linolenic acid and the fiber contents increased simultaneously (Table 1).

No significant difference ($P < 0.05$) was detected between strains in terms of capacity to incorporate α-linolenic acid into the egg yolks or in desaturation and elongation capacity in terms of producing DHA. Scheideler et al. (1998) reported that Hisex White hens (a European breed) had increased α-linolenic and DHA levels in the eggs compared with Hy-Line, DeKalb, and Babcock (American breeds or strains), when fed the same diet. It has been suggested that because European breeds have larger livers compared with American breeds, they may have more capacity to elongate α-linoleic acid and change it to DHA (D. Fernandez, 1998, Llafe S.A. 25 de Mayo 158 of 34, Bs. As. Argentina, personal communication). The strains tested in the chia trial were of American origin, and hence the response is closer to other American breeds and strains than to European breeds.

Although the ability of laying hens to incorporate omega-3 fatty acids into the yolk has been shown to exhibit a genetic × diet interaction (Scheideler et al., 1998; Cherian et al., 1995), this relationship does not appear to be consistent with the results of the chia trial. In the experiment by Cherian et al. (1995), which compared six breeds of laying hens, α-linolenic acid incorporation into

TABLE 8. Total polyunsaturated omega-3 and omega-6 fatty acid contents in milligrams per egg and milligrams per gram of yolk

		Brown hens				White hens			
Day	Treatment	Omega-3 mg/egg	Omega-6 mg/egg	Omega-3 mg/g yolk	Omega-6 mg/g yolk	Omega-3 mg/egg	Omega-6 mg/egg	Omega-3 mg/g yolk	Omega-6 mg/g yolk
30	0	NA	NA	2.76^{e}	45.60^{c}	NA[2]	NA	3.57^{d}	48.29^{b}
	7	NA	NA	21.95^{d}	50.49^{bc}	NA	NA	24.50^{c}	55.60^{a}
	14	NA	NA	29.89^{c}	52.01^{ab}	NA	NA	26.94^{c}	52.80^{ab}
	21	NA	NA	37.02^{b}	56.90^{a}	NA	NA	35.61^{b}	57.24^{a}
	28	NA	NA	44.91^{a}	55.97^{ab}	NA	NA	53.04^{a}	56.80^{a}
	CR[1]			6.78	6.25			5.51	5.21
43	0	38.07^{d}	853.34^{a}	1.98^{d}	44.16^{a}	47.98^{e}	892.27^{b}	2.78^{e}	51.61^{c}
	7	273.98^{c}	849.42^{a}	15.09^{c}	47.12^{a}	347.09^{d}	912.18^{ab}	21.18^{d}	55.63^{bc}
	14	565.43^{b}	883.73^{a}	29.56^{b}	46.34^{a}	596.31^{c}	946.44^{ab}	33.82^{c}	53.88^{bc}
	21	626.06^{b}	875.14^{a}	31.52^{b}	44.40^{a}	742.58^{b}	1007.78^{ab}	42.01^{b}	57.04^{ab}
	28	852.14^{a}	1030.42^{a}	42.88^{a}	51.85^{a}	859.04^{a}	1047.40^{a}	50.45^{a}	61.59^{a}
	CR	187.4	198.8	7.61	7.75	105.0	152.2	4.41	5.49
58	0	40.54^{d}	816.19^{b}	2.19^{d}	44.16^{b}	41.84^{d}	934.12^{ab}	2.41^{e}	53.67^{b}
	7	379.03^{c}	1021.05^{a}	20.70^{c}	55.80^{a}	340.23^{c}	838.06^{b}	21.69^{d}	53.47^{b}
	14	567.54^{bc}	946.07^{ab}	32.81^{b}	55.33^{a}	596.50^{b}	993.79^{ab}	33.85^{c}	56.38^{ab}
	21	923.14^{a}	1061.79^{a}	45.45^{a}	52.12^{a}	724.84^{b}	967.52^{ab}	44.08^{b}	58.86^{ab}
	28	884.38^{a}	1045.57^{a}	46.25^{a}	54.37^{a}	986.11^{a}	1030.67^{a}	58.72^{a}	61.28^{a}
	CR	203.8	159.1	9.87	6.05	188.6	165.9	8.93	29.04
90	0	NA	NA	NA	NA	56.78^{d}	964.17^{a}	3.31^{d}	56.09^{a}
	7	NA	NA	NA	NA	245.13^{c}	711.71^{b}	16.06^{c}	46.69^{b}
	14	NA	NA	NA	NA	596.22^{b}	891.71^{a}	36.85^{b}	55.12^{a}
	21	NA	NA	NA	NA	649.23^{b}	866.08^{a}	39.28^{b}	52.48^{ab}
	28	NA	NA	NA	NA	821.27^{a}	887.19^{a}	51.12^{a}	55.21^{a}
	CR					93.88	127.3	4.76	6.69

$^{a–e}$Means within day lacking a common superscript differ (P < 0.05) according to Duncan's multiple range test.

[1]Critical range for mean separation.

[2]NA = not available.

yolks was significantly different (P < 0.05) only between two groups of hens. One group was composed of White Leghorn, Light Sussex Barred Rock, and Rhode Island Red, and the second group included Brown Leghorns and New Hampshire hens. In this experiment, no significant difference (P < 0.05) between breeds and incorporation of total omega-3 PUFA into the egg yolks was found.

Nash et al. (1995) reported that a genotype × dietary interaction existed, because rates of incorporation of EPA and DHA were different between two hen genotypes of SCWL hens fed four levels of fish meal. The results, however, were highly variable, and no systematic trend could be discerned. Similarly, the minimal differences detected in the chia study could be related to physiological mechanisms of omega-3 PUFA incorporation into yolks between the white and brown hens, but the mechanisms were not sufficiently dissimilar to produce a consistent variation.

Omega-3 incorporation into the yolks was greater for the white hens than for the brown. With the 28% chia diet, the white hens incorporated up to 25% more total omega-3 fatty acids than the brown hens. This difference suggests the white hens could continue to incorporate omega-3 PUFA into the egg yolk beyond the point at which chia starts to decrease egg production. This finding agrees with previous the chia trial (Ayerza and Coates, 1999) in which production decreased, yet omega-3 incorporation increased.

Omega-6:Omega-3 Ratio

Results of several studies have shown that omega-3 rich diets lower human risk of CHD (Kinsella et al., 1990; Turini et al., 1994). In addition, an adequate dietary omega-6:omega-3 ratio must be provided. Nutritional recommendations suggest a dietary omega-6:omega-3 fatty acid ratio of 5:1 (Simopoulos, 1989; Canada Health and Welfare, 1990; British Nutrition Foundation, 1992; Food and Agricultural Organization, 1994), or 4:1 (Ministry of Health and Welfare of Japan, cited by Okuyama et al., 1997), or even less (Simopoulos and Robinson, 1998). Western diets do not provide these ratios, mainly due to their high omega-6 fatty acid content (British Nutrition Foundation, 1992).

Yolk omega-3 levels for the hens fed the 14, 21, and 28% chia diets were higher than those from eggs produced in Greece by range-fed hens (265 mg/g of yolk) and were considerably higher than those levels from eggs obtained from US supermarkets and analyzed by Simopoulos and Salem (1992). The high value found with the range-fed hens was presumed to arise because the hens were consuming green leafy vegetables, fresh and dried fruits, insects, and worms (Simopoulos and Salem, 1992).

The omega-6:omega-3 ratio (extremes of 13.55:1 to 23.23:a) found in the yolks from the hens fed the control diet in the chia trial is similar to the ratio (20:1) found in common eggs in the US (American Egg Board, 1981). Addition of chia to the diet significantly reduced the omega-6:omega-3 ratio, which greatly improved the nutritional quality of these eggs compared with those produced by hens fed the control diet.

Saturated Fatty Acid:PUFA Ratio

Inclusion of chia in the diet resulted in a lower saturated fatty acid:PUFA ratio in the yolks, compared with the control. Several nutritional studies report the relationship between saturated fatty acids and the risk of CHD and the need to reduce consumption of these fatty acids. Chia improved the saturated fatty acid:PUFA ratio and put it more in line with the 1:1 recommendation made by two health organizations: the American Heart Association (1990) and Canada Health and Welfare (1990).

In conclusion, adding chia to laying hen diets is an alternative way of increasing the concentration of omega-3 PUFA in egg yolks. Although this and other experiments (Cherian et al., 1995) indicate that the ability of laying hens to incorporate different fatty acids is genetically dependent, Hrdinka et al. (1996) suggest that the composition of the dietary fat affects its absorption and utilization and that there can be synergistic effects between different fatty acids in the adipose tissues of broiler chicks. This synergism could be another reason for the differences in omega-3 and saturated fatty acid incorporation found between hens fed chia and those reported for hens fed other sources of omega-3.

The results of this study show that chia seed could be an alternative to flaxseed and sea sources to produce eggs more acceptable to the health conscious public. It could also help to reverse the declining per capita consumption of eggs that has taken place in recent years.

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REFERENCES

Abril, R., and W. Barclay, 1998. Production of docosahexaenoic acid-enriched poultry eggs and meat using an algae-based feed ingredient. Pages 77–88 *in*: The Return of ω3 Fatty Acids into the Food Supply. A. P. Simopoulos, ed. Karger AG, Basel, Switzerland.

American Egg Board, 1981. Eggcyclopedia. American Egg Board, Park Ridge, IL.

American Heart Association, 1988. Dietary guidelines for healthy American adults: A statement for physicians and health professionals. Arteriosclerosis 8:221A.

American Heart Association, 1990. The Cholesterol Facts. American Heart Association, and The National Heart, Lung, and Blood Institute, Special Report. Circulation 81:1721–1733.

American Heart Association, 1996. Eggs: AHA scientific/medical position. Heart and Stroke A-Z Guide. American Heart Association, Dallas, TX.

American Heart Association, 1997. 1997 Annual Report. American Heart Association, Dallas, TX.

Association of Official Analytical Chemists, 1990. Cholesterol in Eggs. Official Methods of Analysis, II(941.09). AOAC International, Gaithersburg, MD.

Ayamond, W. M., and M. E. Van Elswyk, 1995. Yolk thiobarbituric acid reactive substances and n-3 fatty acids in response to whole and ground flaxseed. Poultry Sci. 74:1388–1394.

Ayerza, R., 1995. Oil content and fatty acid composition of chia (*Salvia hispanica* L.) from five northwestern locations in Argentina. J. Am. Oil Chem. Soc. 72:1079–1081.

Ayerza, R., and W. Coates, 1999. An omega-3 fatty acid enriched chia diet: its influence on egg fatty acid composition, cholesterol and oil content. Can. J. Anim. Sci. 79:53–58.

Bang, H. O., J. Dyerberg, and H. M. Sinclair, 1980. The composition of the Eskimo food in northwestern Greenland. Am. J. Clin. Nutr. 33:2657–2661.

Bartov, I., S. Bornstein, and P. Budowski, 1969. Variability of cholesterol concentration in plasma and egg yolk of hens and evaluation of the effects of some dietary oils. Poultry Sci. 48:1276–1281.

Bonanome, A., and S. M. Grundy, 1988. Effect of dietary stearic acid on plasma cholesterol and lipoprotein levels. N. Engl. J. Med. 318:1244–1248.

Boudreau, M. D., P. S. Chanmugam, S. B. Hart, S. H. Lee, and D. H. Hwang, 1991. Lack of dose response by dietary n-3 fatty acids at a constant ratio of n-3 to n-6 fatty acids in suppressing eicosanoid biosynthesis from arachidonic acid. Am. J. Clin. Nutr. 54:111–117.

Brenner, R. R., 1974. The oxidative desaturation of unsaturated fatty acids in animals. Mol. Cell. Biochem. 3:41–52.

Brenner, R. R., and R. O. Peluffo, 1966. Effect of saturated and unsaturated fatty acids on the desaturation *in vitro* of palmitic, stearic, oleic, linoleic, and linolenic acids. J. Biol. Chem. 241:5213–5219.

British Nutrition Foundation, 1992. Unsaturated fatty acids: Nutritional and physiological significance. British Nutrition Foundation's Task Force, London, England.

Bruckner, G., 1992. Fatty acids and Cardiovascular Diseases. Pages 735–752 *in*: Fatty Acids in Foods and Their Health Implications. C. K. Chow, ed. Marcel Dekker, Inc., New York, NY.

Canada [dept of] Health and Welfare. 1990. Nutrition Recommendations. Canadian Government Publishing Center, Ottawa, Canada.

Caston, L., and S. Leeson, 1990. Research Note: Dietary flax and egg composition. Poultry Sci. 69:1617–1620.

Caston, L. J., E. J. Squires, and S. Leeson, 1994. Hen perfomance, egg quality, and the sensory evaluation of eggs from SCWL hens fed dietary flax. Can. J. Anim. Sci. 74:347–353.

Cherian, G., and J. S. Sim, 1991. Effect of feeding full fat flax and canola seeds to lying hens on the fatty acid composition of eggs, embryos and newly hatched chicks. Poultry Sci. 70:917–922.

Cherian, G., S. X. Li, and J. S. Sim, 1995. Dietary α-linolenic acid and lying hen strain: Fatty acids of liver, adipose tissue, white meat, dark meat, and egg yolk. J. Agric. Food Chem. 43:2553–2559.

Cherian, G., F. W. Wolfe, and J. S. Sim, 1996. Dietary oils with added tocopherols: Effects on egg or tissue tocopherols, fatty acids, and oxidative stability. Poultry Sci. 75:423–431.

Craig-Schmidt, M. C., S. A. Faircloth, and J. D. Weete, 1987. Modulation of avian lung eicosanoids by dietary omega-3 fatty acids. J. Nutr. 117:1197–1206.

Craig-Schmidt, M. C., and M. C. Huang, 1998. Interaction of n-6 and n-3 fatty acids: Implications for supplementation of infant formula with long-chain polyunsaturated fatty acids. Pages 63–84 *in*: Lipids in Infant Nutrition. Y. S. Huang and A. J. Sinclair, ed. AOCS Press, Champaign, IL.

de Lorgeril, M., S. Renaud, N. Mamelle, P. Salen, J. L. Martin, L. Monjaud, J. Guidollet, P. Touboul, and J. Delaye, 1994. Mediterranean alpha-linolenic acid-rich diet in secondary prevention of coronary heart disease. Lancet 343:1454–1459.

de Lorgeril, M., P. Salen, J. L. Martin, N. Mamelle, I. Monjaud, P. Touboul, and J. Delaye, 1996. Effect of a Mediterranean type of diet on the rat of cardiovascular complications in patients with coronary artery disease. J. Am. College Card. 28:1103–1108.

Dyerberg, J. H., O. Bang, E. Stoffersen, S. Moncada, and J. R. Vane, 1978. Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis? Lancet ii:117–121.

Eristland, J., H. Arnesen, I. Seljeflot, and A. T. Hostmark, 1995. Long-term metabolic effects of n-3 polyunsaturated fatty acids in patients with coronary artery disease. Am. J. Clin. Nutr. 61:831–836.

Farrell, D. J., 1994. The fortification of hens' eggs with ω-3 long chain fatty acids and their effect in humans. Pages 386–401 *in*: Egg Uses and Processing Technologies: New Developments. J. S. Sim and S. Nakai, ed. CAB International, Wallingford, England.

Ferretti, A., and V. P. Flanigan, 1996. Antithromboxane activity of dietary Alpha-linolenic acid: A pilot study. Prostaglandins Leukot. Essent. Fatty Acids 54:451–455.

Ferrier, L. K., L. Caston, S. Leeson, E. J. Squires, B. Celi, L. Thomas, and B. J. Holub, 1992. Changes in serum lipids and platelet fatty acid composition following consumption of eggs enriched in alpha-linolenic acid (LnA). Food Res. Int. 25:263–268.

Folch, J., M. Lees, and G.H.A. Sloane-Stanley, 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497–507.

Food and Agricultural Organization, 1994. Fats and oils in human nutrition: Report of a joint expert consultation. Food and Nutrition Paper N:57. FAO, Rome, Italy.

Food and Drug Administration of the United States, 1997. A Food Labeling Guide—Appendix A: Definitions of Nutrient Content Claims. Center for Food and Safety and Applied Nutrition, Washington, DC.

Garg, M. L., E. Sebokova, A. B. Thomson, and M. T. Clandinin, 1988a. Δ6-desaturase activity in liver microsomes of rats fed diets enriched with cholesterol and/or ω-3 fatty acids. Biochem. J. 249:351–356.

Garg, M. L., A. B. Thomson, and M. T. Clandinin, 1988b. Effects of dietary cholesterol and/or ω-3 fatty acids on lipid composition and Δ5-desaturase activity of rat liver microsomes. J. Nutr. 118:661–668.

Garg, M. L., E. Sebokova, A. Wierzbicki, A. Thomson, and M. T. Clandinin, 1988c. Different effects of dietary linoleic and α-linolenic acid on lipid metabolism in rat tissues. Lipids 23:847–851.

Grundy, S. M., 1997. What is the desirable ratio of saturated, polyunsaturated, and monounsaturated fatty acids in the diet? Fats and oil consumption in health and disease. Am. J. Clin. Nutr. 66(4s):988–990.

Hargis, P. S., 1988. Modifying egg yolk cholesterol in the domestic fowl—a review. World's Poult. Sci. J. 44:15–29.

Hayek, M. G., and G. A. Reinhart, 1998. Utilization of ω3 fatty acids in companion animal nutrition. Pages 176–185 *in*: The Return of ω3 Fatty Acids into the Food Supply. A. P. Simopoulos ed. Karger AG, Basel, Switzerland.

Hennekens, C. H., J. E. Buring, and S. L. Mayrent, 1990. Clinical and Epidemiological Data on the Effects of Fish Oil in Cardiovascular Disease. Pages 71–85 *in*: ω-3 Fatty Acids in Health and Disease. R. S. Lees and M. Karel, ed. Marcel Dekker, Inc., New York, NY.

Herber, S. M., and M. E. Van Elswyk, 1996. Dietary marine algae promotes efficient deposition of n-3 fatty acids for the production of enriched shell eggs. Poultry Sci. 75:1501–1507.

Hrdinka, C., W. Zollitsch, W. Knaus, and F. Lettner, 1996. Effects of dietary fatty acid pattern on melting point and composition of adipose tissues and intramuscular fat of broiler carcasses. Poultry Sci. 75:208–215.

Instituto Argentino de Racionalizacion de Materiales, 1982. Aceites y grasas vegetales y animales: Metodo rapido de preparacion de esteres metilicos de acidos grasos para su analisis por cromatografia en fase gaseosa. Instituto Argentino de Racionalizacion de Materiales, Buenos Aires, Argentina.

Jiang, Z., D. U. Ahn, L. Lander, and J. S. Sim, 1992. Influence of feeding full-flax and sunflower seeds on internal and sensory qualities of eggs. Poultry Sci. 71:378–382.

Katan, M., P. Zock, and R. Mensink, 1995. Dietary oils, serum lipoproteins, and coronary heart disease. Am. J. Clin. Nutr. 61(Suppl):1368–1373.

Kinsella, J. E., B. Lokesh, and R. A. Stone, 1990. Dietary n-3 polyunsaturated fatty acids and amelioration of cardiovascular disease: Possible mechanisms. Am. J. Clin. Nutr. 52:1–28.

Kromhout, D., E. B. Bosschieter, and C. De Lezenne Coulander, 1985. The inverse relation between fish consumption and 20-year mortality from coronary heart disease. N. Engl. J. Med. 312:1205–1209.

Lall, S. P., and S. J. Slinger, 1973. Nutritional evaluation of rapeseed oils and rapeseed soapstocks for laying hens. Poultry Sci. 42:394–396.

Lamelas, K. F., and A. Asad, 1998. Avicultura en cifras. Secretaria de Agricultura, Ganaderia, Pesca y Alimentacion, Buenos Aires, Argentina.

Leaf, A., and J. X. Kang, 1998. ω3 fatty acids and cardiovascular disease. Pages 24–37 *in*: The Return of ω3 Fatty Acids into the Food Supply. A. P. Simopoulos, ed. Karger AG, Basel, Switzerland.

Lewis, N. M., K. Schalch, and S. E. Scheideler, 1998. Incorporation of ω3 fatty acid-enriched eggs in low fat diets of hypercholesterolemic humans. Page 229 *in*: The Return of ω3 Fatty Acids into the Food Supply. A. P. Simopoulos, ed. Karger AG, Basel, Switzerland.

Marshall, A. C., K. S. Kubena, K. R. Hinton, P. S. Hargis, and M. E. Van Elswyk, 1994a. N-3 fatty acid enriched table eggs: A survey of consumer acceptability. Poultry Sci. 73:1334–1340.

Marshall, A. C., A. R. Sams, and M. E. Van Elswyk, 1994b. Oxidative stability and sensory quality of stored eggs from hens fed 1.5% menhaden oil. J. Food Sci. 59:561–563.

Mayo Clinic. 1997. Eggs: Lower cholesterol eggs on the way. Mayo Foundation for Medical Education and Research. http://www.mayo.edu. Accessed 1998.

Ministerio de Salud y Accion Social, 1997. Estadisticas Vitales: Informacion basica. Gobierno de la Republica Argentina, Buenos Aires, Argentina.

Nash, D. M., R.M.G. Hamilton, and H. W. Hulan, 1995. The effect of dietary herring meal on the omega-3 fatty acid content of plasma and egg yolk lipids of laying hens. Can. J. Anim. Sci. 75:245–253.

National Research Council, 1994. Nutrient Requirements of Poultry. National Academy Press, Washington, DC.

Nelson, G. J., 1992. Dietary Fatty Acids and Lipid Metabolism. Pages 437–471 *in*: Fatty Acids in Foods and their Health Implications. C. K. Chow, ed. Marcel Dekker, Inc., New York, NY.

Nettleton, J. A., 1995. ω-3 Fatty Acids and Health. Chapman & Hall, New York, NY.

Noakes, M., P. J. Nestel, and P. M. Clifton, 1996. Modifying the fatty acid profile of dairy products through feedlot technology lowers plasma cholesterol of humans consuming the products. Am. J. Clin. Nutr. 63:42–46.

Oh, S. Y., J. Ryue, C. H. Hsieh, and D. E. Bell, 1991. Eggs enriched in omega-3 fatty acids and alterations in lipid concentrations in plasma and lipoproteins, and in blood pressure. Am. J. Clin. Nutr. 54:689–695.

Okuyama, H., T. Kobayashi, and S. Watanabe, 1997. Dietary fatty acids—The n-6/n-3 balance and chronic elderly diseases excess linoleic acid and relative n-3 deficiency syndrome seen in Japan. Prog. Lipid Res. 35:409–457.

Pauletto, P., M. Puato, M. G. Caroli, E. Casiglia, A. E. Munhambo, G. Cazzolato, G. Bittolo, M. Bon, T. Angelli, and A. C. Pessina, 1996. Blood pressure and atherogenic lipoprotein profiles of fish-diet and vegetarian villagers in Tanzania: The Lugalawa study. The Lancet 348:784–788.

SAS Institute. 1988. SAS/STAT User's Guide. Release 6.03 Edition. SAS Institute Inc., Cary, NC.

Scheideler, S. E., and G. W. Froning, 1996. The combined influence of dietary flaxseed variety, level, form, and storage conditions on egg production and composition among vitamin E-supplemented hens. Poultry Sci. 75:1221–1226.

Scheideler, S. E., G. W. Froning, and D. Jaroni, 1998. Factors affecting $\omega 3$ fatty acid deposition from dietary flaxseed and elongation of C18:3 to C22:6 in the egg. Page 230 *in*: The Return of $\omega 3$ Fatty Acids into the Food Supply. A. P. Simopoulos, ed. Karger AG, Basel, Switzerland.

Shen, H., L. He, R. L. Price, and M. L. Fernandez, 1998. Dietary soluble fiber lowers plasma LDL cholesterol concentrations by altering lipoprotein metabolism in female guinea pigs. J. Nutr. 128:1434–1441.

Sim, J. S., and Z. Jiang, 1994. Consumption of ω-3 PUFA enriched eggs and changes of plasma lipids in human subjects. Pages 414–420 *in*: Egg Uses and Processing Technologies: New Developments. J. S. Sim and S. Nakai, ed. CAB International, Wallingford, England.

Sim, J. S., and G. H. Qi, 1995. Designing poultry products using flaxseed. pages 315–333 *in*: Flax. S. C. Cunnane and L. U. Thompson, ed. Flaxseed in Human Nutrition. AOCS Press, Champaign, IL.

Simopoulos, A. P., 1989. General recommendations on dietary fats for human consumption. Pages 403–404 *in*: Dietary $\omega 3$ and $\omega 6$ Fatty Acids: Biological Effects and Nutritional Essentiality. C. Galli and A. P. Simopoulos, ed. NATO Scientific Affairs Division and Plenum Press, New York, NY.

Simopoulos, A. P., 1999. Essential fatty acids in health and chronic disease. Am. J. Clin. Nutr. 70:560s–569s.

Simopoulos, A. P., and J. Robinson, 1998. The omega plan. Harper Collins Publishers, New York, NY.

Simopoulos, A. P., and N. Salem, 1992. Egg yolk as a source of long-chain polyunsaturated fatty acids in infant feeding. Am. J. Clin. Nutr. 55:411–414.

Taga, M. S., E. E. Miller, and D. E. Pratt, 1984. Chia seeds as a source of natural lipid antioxidants. J. Am. Oil Chem. Soc. 61:928–931.

Turini, M. E., W. S. Powell, S. R. Beher, and B. J. Holub, 1994. Effects of a fish-oil and vegetable-oil formula on aggregation and ethalonamine-containing lysophospholipid generation in activated human platelets and on leukotriene production in stimulated neutrophils. Am. J. Clin. Nutr. 60:717–724.

United States Department of Agriculture, 1997. Agricultural Statistics 1997. United States Government Printing Office, Washington DC.

Van Elswyk, M. E., P. L. Dawson, and A. R. Sams 1995. Dietary menhaden oil influences sensory characteristics and headspace volatiles of shell eggs. J. Food Sci. 60:85–89.

Van Elswyk, M. E., S. D. Hatch, G. G. Stella, P. K. Mayo, and K. S. Kubena, 1998. Poultry-based alternatives for enhancing the $\omega 3$ fatty acid content of American diets. Pages 103–115 *in*: The Return of $\omega 3$ Fatty Acids into the Food Supply. A. P. Simopoulos, ed. Karger AG, Basel, Switzerland.

Van Elswysk, M. E., A. R. Sams, and P. S. Hargis, 1992. Composition, functionality, and sensory evaluation of eggs from hens fed dietary menhaden oil. J. Food Sci. 57:342–349.

Voss, A., M. Reinhart, S. Sankarappa, and H. Sprecher, 1991. The metabolism of 7,10,13,16,19-Docosapentaenoic acid to 4,7,10,13,16,19-Docosahexaenoic acid in rat liver is independent of a 4-desaturase. J. Biol. Chem. 266:19995–20000.

Weber, C. W., H. S. Gentry, E. A. Kohlepp, and P. R. McCrohan, 1991. The nutritional and chemical evaluation of chia seeds. Ecol. Food Nutr. 26:119–125.

Yu, M. M., and J. S. Sim, 1987. Biological incorporation of n-3 polyunsaturated fatty acids into chicken eggs. Poultry Sci. 66(Suppl. 1):195. (Abstr.).