Nutritionally Important Fatty Acids in Hen Egg Yolks from Different Sources

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ABSTRACT

Egg samples were collected from six different sources across Canada, and the yolks from those samples were analyzed for fatty acid composition using gas chromatography. Three yolk samples were from regularly fed chickens from three different Canadian egg processing plants, and the other three samples were from chickens fed with special diets. The specially fed chicken yolk samples were collected from three different Canadian egg producers. The three egg yolk samples from specially fed chickens had a significantly higher linolenic acid and docosahexaenoic acid content than the three regularly fed chicken yolk samples ($P < 0.05$). However, the arachidonic acid levels in the regularly fed chicken yolk samples were significantly higher ($P < 0.05$). In general, there was no significant difference among the three egg sources in each group. There was some variation in the fatty acid levels during different seasons for each source, but the difference was not statistically significant in most cases.

(Key words: polyunsaturated fatty acids, egg yolk, gas chromatography, fatty acid composition, nutrition)

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INTRODUCTION

Consumption of polyunsaturated fatty acids has been reported to reduce the risk of atherosclerosis and stroke and to promote infant growth (Clandinin et al., 1989; Ferrier, 1992). Ferrier (1992) observed a 50% increase in blood platelet docosahexaenoic acid (DHA) level for five volunteers in response to consumption of omega-3-modified eggs compared with control eggs. DHA is effective in slowing platelet adhesion and, therefore, may reduce the risk of heart disease. Von Schacky et al. (1999) investigated the effect of dietary omega-3 fatty acids on coronary atherosclerosis in a randomized, double-blind, placebo-controlled trial. The participants consumed omega-3 fatty acids of fish oil concentrate (55% eicosapentaenoic acid and DHA) at an intake of 6 g/d for 3 mo and then 3 g/d for 21 mo. The fish oil concentrate intake at this rate modestly mitigated the course of coronary atherosclerosis compared with a placebo with a fatty acid composition resembling that of the average European diet. Johansen et al. (1999) studied the effect of supplementation with omega-3 fatty acid on soluble markers of endothelial function in patients with coronary heart disease. They reported that supplementation with omega-3 fatty acids significantly decreased hemostatic markers of atherosclerosis.

Different feeds, such as flaxseed, safflower oil, perilla oils, marine algae, fish, fish oil, and vegetable oil have been added to chicken feeds to increase the n-3 fatty acid content in the egg yolk (Jiang et al., 1991; Herber and Van Elswyk, 1996; Kim et al., 1997; Van Elswyk, 1997; Chae et al., 1998). The nutritional manipulation of the diets of laying hens to include sources of n-3 fatty acids promotes the deposition of these nutrients into egg yolk (Van Elswyk, 1997). n-3 fatty acid-rich eggs may provide an exciting alternative food source for enhancing consumer intake of these proposed healthful fatty acids. Van Elswyk (1997) reported that eggs from hens fed with 15 g menhaden oil/kg were considered acceptable by trained flavor panelists. Evaluation of the eggs during storage verified that the shelf life of the enriched eggs was comparable to that of typical eggs.

Many specially fed chicken egg brands are available in Canadian supermarkets including Born 3 Eggs, Vita Plus Eggs, and Formula 3 Eggs. Omega-3 fatty acid-enhanced eggs are also available in the US market under various brand names such as Gold Circle Farms Eggs, EggPlus, and The Country Hen Better Eggs.

The objective of the current study was to compare the nutritionally important fatty acid compositions between egg yolks from chickens fed with a special diet and egg yolks from chickens fed with a regular diet. The levels of linolenic acid ($\text{C}_{18:3}$), arachidonic acid ($\text{C}_{20:4}$), and DHA ($\text{C}_{22:6}$) were compared among the egg yolks from the different sources.

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Abbreviation Key: DHA = docosahexaenoic acid.
MATERIALS AND METHODS

Materials

Egg samples were collected from six different sources. Dried egg yolk samples from chickens on a regular diet were from processing plants located in Abbotsford, British Columbia (Regular 1); St. Mary’s, Ontario (Regular 2); and Winnipeg, Manitoba (Regular 3). The three specially fed chicken egg yolk sources included Special 1, Special 2, and Special 3 from various regions in Canada. The premium vegetarian diet for Special 1 contained a unique combination of ingredients such as flaxseed, wheat, corn, and soybean meal. The diets for Special 2 and Special 3 could not be released because of proprietary reasons. The egg samples from chickens on a special diet were purchased from supermarkets and were hand-broken. The yolk was separated from the white (albumen) and spray-dried using a pilot-scale dryer. Three different batches each of the egg yolk samples from the six sources were prepared in the months of July 1997, September 1997, November 1997, and January 1998.

Fatty acid methyl esters and boron trifluoride methanol (BF₃, 14%) were purchased from Sigma Aldrich Canada Ltd. All other solvents and reagents used were either analytical grade or HPLC grade and were used without further purification.

Methods

The fatty acids from the egg yolk were derivatized to fatty acid methyl esters following the AOCS official method Ce 2-66 (1997). A sample egg yolk powder (0.25 g) was introduced to a 100-mL Kimax flask with T-shaped joint 20/40 outer neck followed by 5 mL of 0.5 M sodium hydroxide in methanol. A condenser was attached, and the mixture was heated in steam for 10 min. Then, 5 mL of boron trifluoride-methanol was added slowly through the top of the condenser and boiled for 2 min more. Next, 2 mL of hexane was added through the condenser, and the mixture was boiled for 1 additional min. The flask and condenser were removed from the heat and cooled. The condenser was then separated from the flask, and 15 mL of saturated sodium chloride was added to the flask. The flask was stoppered and shaken vigorously for 30 s while the solution was still tepid. The mixture was centrifuged at 10,000 × g for 10 min. A volume of 0.1 mL of the supernatant was then diluted with 0.9 mL of hexane. The hexane solution was dried with a small amount of anhydrous sodium sulfate and passed through a 0.22-µm syringe filter. A 1-µL sample was injected into the gas chromatograph within 30 min of preparation.

A gas chromatograph4, equipped with a split/splitless injector (250 C) and a flame ionization detector (250 C), was used. The separation was carried out on an HP INNOWAX column4 (30 m length, 0.25 mm i.d., 0.25-µm film thickness). Flow rates were: air 450 mL/min, hydrogen 40 mL/min, and make-up helium 45 mL/min. The oven temperature was held at 180 C for 8 min, increased to 225 C at a rate of 10 C/min, and held at this temperature for 28 min. The total run time was 41 min. During the run, the carrier gas helium was maintained at a constant flow. The component peaks were recorded on a Hewlett-Packard ChemStation4 and were identified by comparison to retention times of the standards. Statistical analysis was carried out using SigmaStat.5 Two-way ANOVA was used to compare the effect of yolk sources and time of sampling on the contents of linoleic acid (C₁₈:3), arachidonic acid (C₂₀:₄), and DHA (C₂₂:₆). When a significant difference was found among the mean values, Tukey’s test was used for multiple comparison of the means to determine which two groups were significantly different. SigmaPlot6 was used for graphing.

RESULTS AND DISCUSSION

The study shows that the fatty acid composition was greatly affected by the source of the egg yolk. The results follow for the three polyunsaturated fatty acids (C₁₈:3, C₂₀:₄, and C₂₂:₆) of great interest.

Linolenic Acid

The average C₁₈:3 content of the samples from all sources is presented in Figure 1. Egg yolks from chickens on special diets had a significantly higher C₁₈:3 content than the regular egg yolks (P < 0.01). There was no statistically significant difference among the egg yolks from the hens on special diets (P > 0.05). Within the egg yolks from hens on regular diets, the difference in C₁₈:3 contents was

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4Niro Atomizer, DK-2860 Soeborg, Denmark.
5Oakville, Ontario, Canada, L6H 9Z9.
6Hewlett-Packard, Model 6890, Atlanta, GA 30319.
significant for the three sources \((P < 0.05)\). Regular 1 had a significantly higher \(C_{18:3}\) content than Regulars 2 and 3. No significant difference was found among the sampling times \((P = 0.403)\). This result indicates that \(C_{18:3}\) content did not vary significantly over season.

**Arachidonic Acid**

The \(C_{20:4}\) contents for the samples from the six sources are presented in Figure 2. Unlike \(C_{18:3}\), specially fed chicken egg yolk had a lower \(C_{20:4}\) content than the regularly fed chicken eggs. Two-way ANOVA showed that the difference was significant \((P < 0.05)\). Among the three specially fed chicken egg yolk sources, Special 2 had a significantly lower \(C_{20:4}\) content than Specials 1 and 3 \((P < 0.05)\). There was no significant difference between the latter two \((P > 0.05)\). Among the three regular egg yolk samples, Regulars 2 and 3 had a significantly higher \(C_{20:4}\) content than Regular 1 \((P < 0.05)\). There was no significant difference between Regulars 2 and 3 \((P > 0.05)\).

The difference in the mean value among the samples from different sampling times was greater than would be expected by chance after allowing for the effects of difference in sources \((P = 0.017)\). However, multiple comparison did not show any of the groups to be significantly different \((P > 0.05)\).

Matthews and Van Holde (1990) suggested that linoleic acid and its metabolites decreased the production of \(C_{20:4}\) and its metabolites, displaced \(C_{20:4}\) in the phospholipids, or both. Jiang et al. (1991) also observed a negative relationship between \(C_{18:3}\) and \(C_{20:4}\) and between \(C_{20:4}\) and longer-chain n-3 fatty acids in total yolk lipids, triglycerides, phosphatidylcholine, and phosphatidylethanolamine. They reported that the enzymatic pathway for the synthesis of \(C_{20:4}\) from \(C_{18:3}\) was shared by the n-3 fatty acids, and \(C_{18:3}\) inhibited the n-6 desaturase, thereby reducing the conversion of \(C_{18:3}\) to \(C_{20:4}\).

**DHA**

Figure 3 presents the comparison of \(C_{22:6}\) contents of the yolk samples from the six sources. It can be seen that specially fed chicken egg yolk had a much higher \(C_{22:6}\) content than regularly fed egg yolk. The difference was statistically significant \((P < 0.05)\). Among the three specially fed yolk sources, Special 2 had a significantly higher \(C_{22:6}\) content than Specials 1 and 3 \((P < 0.05)\). There was no significant difference between the average of the latter two \((P > 0.05)\). Within the three regular egg yolk sources, Regular 1 has a significantly higher average \(C_{22:6}\) content than Regulars 2 and 3 \((P < 0.05)\). No significant difference was found between the latter two \((P > 0.05)\). For \(C_{22:6}\), the difference in the mean values among the samples from the different sampling times was greater than would be expected by chance after allowing for the effects of differences in sources \((P < 0.05)\). The results indicated that \(C_{22:6}\) contents vary during different seasons.

As with \(C_{18:3}\), there was a negative relationship between the levels of \(C_{22:6}\) and \(C_{20:4}\). Garg et al. (1988) and Kinsella et al. (1990) reported that higher levels of longer-chain n-3 fatty acids, such as \(C_{22:6}\), might hinder the incorporation of \(C_{20:4}\) into phosphatidylcholine and phosphatidylethanolamine.

**CONCLUSIONS**

The results from fatty acid composition analysis showed that egg yolk from the three special sources had much higher \(C_{18:3}\) and \(C_{22:6}\) contents. However, the three regular yolk sources had a much higher \(C_{20:4}\) content. There was some variation in the fatty acid levels for all of the sources over time, but the difference was not statistically significant in most cases. For the specially fed chicken yolk samples, small sample size may have contributed to the variation.
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