

Comparison of *n*-3 fatty acid sources in laying hen rations for improvement of whole egg nutritional quality: a review

BY MARY E. VAN ELSWYK

Department of Poultry Science, Graduate Faculty of Nutrition, Texas A&M University, Texas Agricultural Experiment Station, College Station, TX, 77843-2472, USA

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. *n*-3 Fatty acid-rich eggs may provide an exciting alternative food source for enhancing consumer intake of these proposed healthful fatty acids. Care must be taken when designing *n*-3 fatty acid-rich poultry rations, however, to assure that the resulting egg fatty acid profile is useful for promoting consumer health yet maintaining egg sensory quality. In study 1 laying hens were fed on diets supplemented with graded levels of menhaden oil (MO), rich in both eicosapentaenoic acid (EPA; 20:5*n*-3) and docosahexaenoic acid (DHA; 22:6*n*-3), for 4 weeks to determine maximal yolk fatty acid deposition attainable without sensory compromise. Yolk fatty acids were analysed for an additional 4 weeks, post-MO removal, to investigate yolk *n*-3 fatty acid tenacity. Dietary MO levels between 15 and 30 g/kg yielded the greatest yolk *n*-3 fatty acid content; however, only eggs from birds fed with 15 g MO/kg were considered acceptable by trained flavour panelists. Evaluation of eggs from hens fed with 15 g MO/kg during storage verified that the shelf-life of enriched eggs was comparable with that of typical eggs. In study 2, graded levels of whole or ground flaxseed were used for the deposition of linolenic acid (LNA; 18:3*n*-3) and to determine *in vivo* production of DHA from dietary LNA for yolk deposition. Flaxseed form influenced yolk *n*-3 fatty acids only when given at 150 g/kg diet. *In vivo* production of DHA, while significant, was not enhanced by increasing the level of dietary flaxseed nor by grinding the seed. In the third study, a DHA-rich natural marine alga (MA) was investigated as an *n*-3 fatty acid supplement. Despite similar DHA profiles, dietary MA was found to be more efficient for yolk DHA deposition than dietary MO. These studies suggest that there are numerous viable *n*-3 fatty acid supplements for poultry rations. It must be realized, however, that the fatty acid profile of the final product varies substantially depending on which supplement is fed.

Docosahexaenoic acid: α -Linolenic acid: Eggs

The growing role of diet in both the progression and prevention of disease has led to the convergence of consumer and governmental attention on the health quality of food. Governmental guidelines include recommendations to reduce total fat and cholesterol in the diet to maintain cardiovascular health (United States Department of Agriculture, 1993). Due, at least in part, to consumer concern with dietary cholesterol a significant decline in per capita whole-egg consumption has been observed during the past several decades in the United States. Attempts to modify whole-egg cholesterol content to meet demands of health conscious consumers have been largely unsuccessful. More than 30 years of genetic, pharmacological, and nutritional research have failed to yield a consistent, economical means of lowering shell egg cholesterol content (Hargis, 1988). It has been hypothesized that when whole-egg cholesterol content is reduced below that sufficient to support an embryo, the hen will cease production (Hargis, 1988). Fortunately, the tide of public and medical opinion concerning the role of dietary cholesterol in the initiation and progression of heart disease has begun to turn. A recent study conducted by Ginsberg *et al.* (1994) indicated that normolipidaemic individuals may consume up to four eggs daily, as part of a

moderate-fat diet, without experiencing significant adverse changes in their blood lipids. The findings of this study are not novel and support an emerging hypothesis that dietary cholesterol and blood cholesterol are not well correlated in the normolipidaemic individual and perhaps even the hyperlipidaemic individual. Therefore, modified health guidelines allow the consumption of up to four eggs per week in a typical consumer diet. Where health guidelines have become more stringent, however, is in the area of total dietary fat.

Total dietary fat has been targeted for decrease in consumer diets by most international agencies, although within the target of dietary fat, some fats such as the polyunsaturated fats are targeted for an increase. The *n*-3 fatty acids (*n*-3 FA) characteristic of fish and certain oilseeds have been recognized for their role in reducing the risk of CHD and stroke while promoting the neurological development of preterm infants (Connor *et al.* 1991; Nettleton, 1993). Findings by Kromhout *et al.* (1985) suggest that ingesting a minimum of 30 g fish/d is associated with 50 % reduction in risk for occlusive heart disease and stroke (Keli *et al.* 1994). Governmental attentions to these epidemiological findings have prompted the issuance of daily guidelines for the consumption of these important *n*-3 FA. Health and Welfare Canada has instituted a recommended nutrient intake of 3 % of total dietary energy from essential fatty acids to include linolenic acid (LNA; 18:3*n*-3). Recommendations from the European Community favour a minimum contribution of 0.5 % *n*-3 FA to total dietary energy with a maximum contribution of 5 %, providing no clear delineation of the distribution between LNA and the longer chain *n*-3 FA characteristic of fish, eicosapentaenoic acid (EPA; 20:5*n*-3) and docosahexaenoic acid (DHA; 22:6*n*-3). While governmental recommendations for clearly specified amounts of these important nutrients in our diet continue to be in progress, the identification of food sources in the consumer diet is critical to the consumer's ability to meet these health guidelines. Currently in the US, despite proposed health benefits to be attained from consuming fish, individuals consume 14 g fish/d or less. Enhancing the dietary availability of these potentially healthful fatty acids through fatty acid modification of commonly consumed products has therefore been encouraged by health professionals (Nettleton, 1991, 1993; Simopolous & Salem, 1991).

Recently, eggs have gained attention as an alternative to fish and oilseeds as a source of *n*-3 FA (Hargis & Van Elswyk, 1993). The enrichment of laying-hen rations with marine oils, algae or oilseeds, such as flax, readily promotes the deposition of *n*-3 FA into egg yolk. The present paper will discuss the various dietary sources of *n*-3 FA useful for enrichment of laying-hen rations and provide guidelines on how these sources can best be used in the development of a healthful, *n*-3 FA-rich whole egg. Study 1 will refer to a series of experiments designed to determine the usefulness of the fish oil, menhaden oil (MO), for the production of acceptable *n*-3 FA-rich whole eggs. Study 2 will review a series of experiments investigating the use of flaxseed as a source of *n*-3 FA in laying hen rations. Study 3 will provide information on the use of a novel marine microalga, DHA GoldTM (OmegaTech Inc., Boulder, CO, USA), for the production of DHA-rich whole eggs.

EXPERIMENTAL STUDIES

Study 1. Influence of graded levels of dietary menhaden oil on egg yolk n-3 fatty acids

Objectives. The objectives of the experiments outlined in this study were (1) to determine the minimum level of dietary MO needed to obtain maximal yolk *n*-3 FA incorporation without compromising sensory quality; (2) to determine the tenacity of yolk *n*-3 lipids following removal of dietary *n*-3 lipids; and (3) to evaluate the storage stability of eggs from hens fed with MO compared with eggs from hens fed on a typical diet.

Materials and methods. Single Comb White Leghorn (SCWL) hens (n 27 per treatment) were fed on diets containing either 0, 5, 10, 15, 20, 25 or 30 g MO/kg (Zapata Protein Inc., Reedville, VA, USA). Diets were stored frozen in airtight containers until use. Diets were fed for a minimum of 4 weeks. The 4-week feeding period was used to ensure that the incorporation of n -3 FA into yolk would be complete (Hargis *et al.* 1991). Following weekly egg collection and fatty acid determination for 4 weeks, n -3 FA enrichment was removed from all diets and eggs collected weekly to determine depletion of yolk n -3 FA. Briefly, to determine fatty acid content, yolks (n 10 per treatment) were pooled (n 3/treatment per week) and three 1 g portions were obtained from each pool. Yolk samples were extracted by the method of Folch *et al.* (1957) and extracts were saponified and transesterified using methanolic BF_3 . Methyl esters were extracted using hexane. Fatty acid methyl ester (FAME) mixtures (Alltech, Deerfield, IL, USA) were used to identify fatty acid retention times. A methyl ester of the fatty acid 17:0 (Alltech) was used as a standard for quantification.

For the determination of egg yolk flavour quality, a series of flavour panels using both consumers and trained panelists were conducted. In consumer panels (n 2), panelists (n 150/panel) were provided with both hard boiled and scrambled egg samples from hens fed on either 0 or 30 g MO/kg. Duo-trio flavour difference tests were conducted by the method of Larmond (1977). Before receiving samples, participants were briefly instructed on evaluation of flavour using the duo-trio test. In each sitting, panelists were presented with three room-temperature, hard boiled samples for the first comparison, followed by three warm, scrambled samples for a second comparison. A reference sample was randomly alternated between samples from both treatment groups throughout each trial. Participants were given distilled water and instructed to rinse their mouths between samples.

Trained sensory panels involved the utilization of twelve individuals previously trained to detect flavour intensity according to the Spectrum method (Meilgaard *et al.* 1989). Before the egg panels, participants were trained specifically in the evaluation of scrambled egg and fish oil flavours. Panelists evaluated scrambled egg samples for ten aroma, nine taste, and six aftertaste characteristics using an intensity scale of 0 (not present), 0.5 (threshold), 1 (slight), 2 (moderate) to 3 (strong).

To evaluate the lipid stability of eggs from hens fed with MO during storage, eggs were collected and stored for yolk thiobarbituric acid (TBA) analysis and consumer flavour analysis. Eggs were collected during a 4-week period and collections staggered 1 week to facilitate analysis of all storage lengths within 24 h of the baseline collection to avoid variation due to assay. Lengths of storage times were chosen in an effort to simulate potential duration of storage of whole eggs by consumers. Determination of yolk lipid oxidation products was conducted according to the method of Tarladgis *et al.* (1960), as modified by Rhee (1978), for quantification of malonaldehyde and other thiobarbituric acid reactive substances (TBARS). To avoid the generation of oxidation products during the procedure, 0.5% propyl gallate-EDTA and ethoxyquin were used as aqueous and lipid-soluble antioxidants respectively.

Study 2. Influence of dietary flaxseed form and level on yolk n-3 fatty acids

Objectives. The specific objectives in this study were (1) to determine if flaxseed form influences the deposition of yolk n -3 fatty acids; and (2) to elucidate the level of dietary flaxseed most useful for incorporation of marine-type (EPA + DHA) fatty acids into egg yolk.

Materials and methods. SCWL pullets, aged 22 weeks, were randomly assigned to six diets (n 21 per treatment, n 7 per replicate). Diets included a typical maize-soyabean

layer ration or a typical ration designed to include 50 g flaxseed/kg (whole or ground), or 150 g flaxseed/kg (whole or ground). A 15 g MO/kg diet was used as a marine-type *n*-3 FA control. Diets were formulated to be both isoenergetic and isonitrogenous. Diets were stored frozen in airtight containers before use. Egg-yolk fatty acids were determined at the end of a 4-week feeding period, as described earlier.

Study 3. Dietary marine algae for the efficient deposition of yolk docosahexaenoic acid

Objectives. The specific objectives of this study were (1) to evaluate the influence of feeding a novel marine microalga (MA) on laying-hen egg production variables and (2) to compare the efficiencies of MO and MA for yolk *n*-3 fatty acid deposition.

Materials and methods. Two feeding experiments were conducted. One experiment used 24-week-old SCWL hens (*n* 20 per treatment) fed on isoenergetic, isonitrogenous rations designed to include 24 or 48 g DHA GoldTM/kg, 15 g MO/kg, or no added *n*-3 fatty acids. The second experiment used the same dietary regimens fed to 56-week-old laying hens. On a weight basis, 15 g MO/kg provided 13.5 g/100 g total fatty acids as EPA and 9.1 g/100 g total fatty acids as DHA. The MA used was devoid of EPA and contained 74 g DHA/kg on a dry-weight basis. The *n*-3 FA-rich ingredients and enriched diets were stored frozen in airtight containers until use. All diets were mixed bi-weekly, diets were fed *ad libitum* daily, and residual feed was removed from the feed troughs weekly.

In each experiment, yolk fatty acids were determined weekly for 4 weeks using pooled yolks from each treatment. To prepare pools (*n* 5/treatment per week), yolks (*n* 3/pool) were pooled and blended. Two 1 g yolk samples from each pool were extracted by the Folch *et al.* (1957) method. Tridecylic acid (13:0; Alltech) (10 mg) was added to each 1 g sample via the Folch solvents to serve as an internal standard. Extracted samples were directly saponified with 0.5 M-NaOH in methanol and transesterified using 100 g/l methanolic-HCl. Samples were injected onto a 50 m 007 series bonded phase fused silica 0.53 mm inside diameter capillary column (Alltech) with a 1 µm film thickness and separated using a Varian 3600 model gas chromatograph. FAME mixtures were used for identification of fatty acid retention times.

Study 1. Results and discussion

Egg yolk *n*-3 FA were significantly enhanced by all *n*-3 FA rich diets, except the 5 g MO/kg ration, as early as week 1 (Table 1).

Eggs from hens fed on the 25 and 30 g/kg diets reached a plateau in *n*-3 FA content by week 3 of the feeding trial. Lower levels of MO supplementation, however, continued to accumulate yolk *n*-3 FA weekly. Although not shown, egg yolk *n*-3 FA accumulation was at the expense of yolk *n*-6 fatty acids, predominately arachidonic acid (20:4*n*-6). The two lower levels of MO supplementation, 5 and 10 g/kg, resulted in statistically similar yolk *n*-3 FA contents that were significantly greater than control but lower than those contents achieved by the higher levels of MO supplementation. The greatest yolk *n*-3 FA deposition was obtained by feeding diets that contained 15–30 g MO/kg. The *n*-3 FA contents of eggs from hens fed on these diets were not statistically different from each other. The incorporation of yolk *n*-3 FA up to 200 mg/yolk is significant as this amount of *n*-3 FA is comparable with that found in a 100 g serving of lean cold-water fish. Considering that most egg consumers eat two eggs as a serving, the equivalent of 400 mg marine-type *n*-3 FA could be obtained from a typical serving of these eggs.

On withdrawing dietary n-3 FA from the laying-hen diets, a significant 20 % decline in yolk n-3 FA was observed from week 4 to week 5 regardless of enrichment level (Table 1). A further 20 % reduction in yolk n-3 FA was observed between week 5 and week 6, however, this decline began to plateau at week 7. At week 8, all treatments had significantly lower total yolk n-3 FA as compared with week 4, however, only the 15, 10 and 5 g/kg treatments yielded yolk n-3 FA contents similar to control. Eggs from hens fed on the higher levels of MO still contained an average of 75 mg total n-3 FA per yolk.

Consumer flavour analysis was conducted using eggs from the highest level of MO enrichment (30 g/kg) only. We used a duo-trio test to determine if a group of consumer panelists could detect a flavour difference between eggs from hens fed with MO and those from hens fed on a typical ration. Panelists differentiated between n-3 FA-enriched and control scrambled eggs ($P < 0.001$) but not between hard boiled eggs (Van Elswyk *et al.* 1992). Additionally, several consumer comments referred to a fish-like flavour when eggs from hens fed on 30 g MO/kg were served scrambled. The volatility of flavour compounds characteristic of fish oils was believed to be enhanced during the mixing of egg samples before scrambling as well as during exposure to high heat during scrambling. Each scrambled sample was presented warm while hard-boiled samples were equilibrated to room temperature before serving. The presentation of warm scrambled samples was believed to increase the sensory availability of volatile flavour compounds thus contributing to the ability of consumers to distinguish egg samples from hens fed on 30 g MO/kg. In an effort to evaluate which level of dietary MO would provide an acceptable egg product, regardless of preparation method, eggs from several of the lower MO enrichment levels were investigated using trained sensory panelists.

Trained panelists evaluated scrambled egg samples from hens fed on diets containing 5, 15, or 30 g MO/kg, as compared with those from hens fed on a typical ration. Of the twenty-five sensory attributes evaluated, only five were significantly influenced by level of dietary MO (Van Elswyk *et al.* 1995). Therefore results reported of the effects of dietary MO on egg sensory quality are limited to fishy aroma, fishy taste, fishy aftertaste, sweet taste, and medicinal taste (Table 2). Sweet taste was significantly lower in 5 g/kg eggs as compared with the other treatments. Fishy taste, fishy aroma, and fishy aftertaste increased, generally in a dose-response fashion, as level of dietary fish oil increased. Generally, eggs from hens fed with 30 g MO/kg were found to be significantly greater than controls in all the fishy attributes, while those from hens fed on the intermediate levels of MO were not statistically discernible from control eggs. In addition to exhibiting higher fishy attribute scores, eggs from hens fed with 30 g MO/kg diet also exhibited a significantly greater medicinal taste as compared with control eggs. In an effort to condense their sensory evaluation of n-3 FA-rich eggs into one overall opinion, panelists were asked to rank egg samples on an overall flavour scale. Only eggs from hens fed on the 30 g MO/kg diet received significantly lower overall flavour scores as compared with control eggs. These data indicated that eggs from hens fed on a diet containing 15 g MO/kg may produce the most desirable egg on both a sensory and nutritional basis.

The final experiment in this study investigated the storage capabilities of eggs from hens fed with 15 g MO/kg diet. TBA numbers for yolk from stored whole eggs indicated no effects of storage and no interactions between dietary and storage treatments (Marshall *et al.* 1995). However, a consistent and significant dietary treatment effect was observed. The TBA numbers of yolk from eggs of hens fed with 15 g MO/kg diet were greater each week, even at the 24 h baseline collection, compared with values for yolk from control-fed hens. The origin of such lipid oxidation products in the egg yolk remains to be determined. Potentially, hens may have consumed the lipid oxidation products in the feed and deposited

Table 2. Effect of graded levels of dietary menhaden oil on sensory characteristics of scrambled eggs (Modified from Van Elswyk *et al.* 1995)

Menhaden oil in diet (g/kg)	Sensory characteristics					
	Overall quality*	Fishy aroma†	Sweet taste†	Fishy taste†	Medicinal taste†	Fishy aftertaste†
0	7.6 ^a	0.4 ^b	2.4 ^a	0.3 ^c	0.06 ^b	0.3 ^b
5	7.4 ^a	0.4 ^b	1.6 ^b	0.6 ^{bc}	0.01 ^b	0.8 ^{ab}
15	7.0 ^a	0.8 ^{ab}	2.1 ^a	0.9 ^{ab}	0.09 ^{ab}	0.7 ^{ab}
30	6.3 ^b	1.0 ^a	2.1 ^a	1.2 ^a	0.23 ^a	1.1 ^a

^{a,b,c} Mean values within a column not sharing a common superscript letter were significantly different, $P < 0.05$.

* Rating scale of 0–10 where 0 = inedible, 5 = edible, 10 = very good.

† Intensity scale of 1–3 where 0 = not present, 0.5 = threshold, 1 = slight, 2 = moderate, 3 = strong.

them in the egg yolks. Alternatively, the oxidative deterioration of *n*-3 FA may have been initiated in the hen's liver and oxidation products may have been directly transported from the liver along with other lipophilic materials for yolk deposition. The persistence of TBA values during storage of eggs from both dietary treatments seems to indicate that lipid oxidation products, regardless of origin, once deposited in the yolk were stable during storage. Although TBA values are frequently a predictor of flavour acceptability, consumers in our study did not report any significant differences between flavour scores for stored 15 g MO/kg eggs and control eggs. Therefore, the persistent level of TBARS present in eggs from hens fed on diets containing 15 g MO/kg was not associated with deleterious change in sensory quality during storage. Storage stability of eggs enriched with *n*-3 FA was found to be comparable with that of typical eggs.

Study 2. Results and discussion

At the end of the 4-week feeding period, total yolk *n*-3 FA increased in response to all *n*-3 FA-enriched diets regardless of enrichment source (Aymond & Van Elswyk, 1995). As dietary flaxseed increased, total yolk *n*-3 FA increased in a linear fashion. At the 50 g/kg level of flaxseed, total yolk *n*-3 FA deposition was not significantly different whether the seed was fed whole (12 mg/g yolk) or ground (13 mg/g yolk). However, ground flaxseed at 150 g/kg diet resulted in a significantly greater deposition of total *n*-3 FA in yolk (24 mg/g yolk) than did 150 g whole flaxseed/kg (18 mg/g yolk). Whether whole or ground, flaxseed resulted in significantly greater total yolk *n*-3 FA deposition than 15 g MO/kg (9 mg/g yolk). Importantly, however, the advantage of dietary flaxseed for yolk *n*-3 FA deposition was the result of increasing yolk LNA. The 15 g MO/kg diet and the ground 150 g flaxseed/kg diet resulted in a 2–3 mg/yolk greater deposition of marine-type *n*-3 FA (EPA + DHA) than the other diets. This distinction of egg yolk *n*-3 FA profiles is important as most clinical and epidemiological studies demonstrating health benefits from dietary *n*-3 FA in human subjects suggest that EPA and DHA are the most bioactive *n*-3 FA. Eggs containing predominately LNA have been proposed to be less biopotent for mediating health benefits characteristic of consuming fish.

Study 3. Results and discussion

Feed consumption was not significantly altered by feeding diets rich in MA. On average, hens consumed 103 g feed daily. In terms of dietary *n*-3 FA, this equated to 345 mg/d of EPA + DHA for hens fed with 15 g MO/kg, 186 mg DHA/d for hens fed with 24 g MA/kg, and 365 mg DHA/d for hens fed with 48 g MA/kg. No changes in egg production were noted in the first experiment; however, in the second experiment feeding 48 g MA/kg diet to older hens reduced egg production by the end of the feeding trial (Herber & Van Elswyk, 1996). In the first experiment, a reduction in yolk weights was observed in response to the 15 g MO/kg diet and the 48 g MA/kg diet. Depression of yolk weights in response to dietary *n*-3 FA has been previously reported in the literature, typically reductions of about 1 g are noted. Interestingly, the 24 g MA/kg diet did not reduce yolk weights (Table 3). In the second experiment, with reproductively mature hens, no changes in egg or yolk weights were observed in response to any *n*-3 FA-rich diet.

At the conclusion of the 4-week feeding trial, eggs from hens fed with 15 g MO/kg and 24 g MA/kg diets contained similar total yolk *n*-3 FA, 9.4 and 9.5 mg/g yolk respectively. These findings are interesting, however, as hens fed on 15 g MO/kg diet were supplied with 189 mg more total *n*-3 FA/d than hens fed on 24 g MA/kg diet. The highest level of incorporation of *n*-3 FA was attained by the 48 g MA/kg treatment (12.2 mg/g yolk). It is important to note, however, that doubling the amount of alga in the diet (i.e. from 24 g to 48 g/kg) did not result in doubled *n*-3 FA deposited in the yolk, indicating that optimal utilization of DHA is below that supplied by a ration containing 48 g MA/kg.

The MO used in the 15 g MO/kg diet supplied a greater proportion of its *n*-3 FA as EPA, however, the primary *n*-3 FA deposited was DHA. MO-fed hens were supplied 155 mg DHA daily and essentially all was deposited into the yolk. For example, in week 4 hens fed with 15 g MO/kg deposited 8.1 mg DHA/g yolk. Assuming an average yolk weighs 17 g, total yolk DHA deposited would be 138 mg. This would account for 89% of the dietary DHA supplied to hens fed on 15 g MO/kg. However, EPA deposition never exceeded 12 mg per total yolk and accounted for less than 5% of the total EPA supplied.

Based on these findings, utilization of MO as an *n*-3 FA source in poultry rations may need re-evaluation. As a result of the efficient deposition of yolk DHA, its direct dietary supplementation in a source such as MA may represent a more efficient means of utilizing dietary *n*-3 FA for the production of *n*-3 FA-enriched whole eggs. Furthermore, supplying DHA directly in the form of MA also provides naturally-occurring carotenoids which may

Table 3. Influence of dietary marine algae on yolk weight (g), first experiment (Data from Herber & Van Elswyk, 1996. Reproduced with the permission of *Poultry Science*.)

(Mean values with their standard errors for twenty eggs per treatment)

Time on experimental diet (weeks)	Dietary treatment							
	Control		15 g Menhaden oil/kg		24 g Marine algae/kg		48 g Marine algae/kg	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1	13.3 ^{a,x}	0.2	12.8 ^{ab,y}	0.3	12.9 ^{a,z}	0.2	12.3 ^{b,z}	0.2
2	13.7 ^{a,x}	0.3	13.5 ^{a,x,y}	0.3	14.1 ^{a,x,y}	0.2	12.6 ^{b,y,z}	0.3
3	13.4 ^{a,x}	0.6	13.8 ^{a,x,y}	0.7	13.8 ^{a,y}	0.2	13.1 ^{a,y}	0.3
4	13.6 ^{b,x}	0.2	14.2 ^{ab,x}	0.2	14.7 ^{a,x}	0.3	13.9 ^{b,x}	0.2

^{a,b} Mean values within a row not sharing a common superscript letter were significantly different, $P < 0.05$.

^{x,y,z} Mean values within a column not sharing a common superscript letter were significantly different, $P < 0.05$.

enhance oxidative stability to both *n*-3 FA-enriched poultry rations and resulting poultry products. Further investigations of MA will include a detailed examination of the sensory quality and oxidative stability of *n*-3 FA-rich eggs and feeds that have been enriched using MA.

CONCLUSIONS

Numerous dietary supplements are available and effective for enhancing yolk *n*-3 FA. Importantly, however, *n*-3 FA profiles vary dramatically depending on the dietary source utilized. Further study of the influence of yolk fatty acid profile on human health variables must be undertaken to determine the optimal laying-hen feeding regimen for the production of nutritionally enhanced eggs.

REFERENCES

- Aymond, W. M. & Van Elswyk, M. E. (1995). Yolk thiobarbituric acid reactive substances and *n*-3 fatty acids in response to whole and ground flaxseed. *Poultry Science* **74**, 1388–1394.
- Connor, W. E., Neuringer, M. & Reischick, S. (1991). Essential fatty acids: the importance of *n*-3 fatty acids in the retina and brain. *Nutrition Reviews* **50**, 21–29.
- Ginsberg, H. N., Karmally, W., Siddiqui, M., Holleran, S., Tall, A. R., Rumsey, S. C., Deckelbaum, R. J., Blaner, W. S. & Ramakrishnan, R. (1994). A dose-response study of the effects of dietary cholesterol on fasting and postprandial lipid and lipoprotein metabolism in healthy young men. *Arteriosclerosis and Thrombosis* **14**, 576–586.
- Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* **226**, 497–509.
- Hargis, P. S. (1988). Modifying egg yolk cholesterol in the domestic fowl – a review. *World's Poultry Science* **44**, 17–29.
- Hargis, P. S. & Van Elswyk, M. E. (1993). Manipulating the fatty acid composition of poultry meat and eggs for the health conscious consumer. *World's Poultry Science* **49**, 251–264.
- Hargis, P. S., Van Elswyk, M. E. & Hargis, B. M. (1991). Dietary modification of yolk lipid with menhaden oil. *Poultry Science* **70**, 874–883.
- Herber, S. M. & Van Elswyk, M. E. (1996). Dietary marine algae promote efficient deposition of *n*-3 fatty acids for the production of enriched shell eggs. *Poultry Science* **75**, 1501–1507.
- Keli, S. O., Feskens, E. J. M. & Kromhout, D. (1994). Fish consumption and risk: the Zutphen study. *Stroke* **25**, 328–332.
- Kromhout, D., Bosschieter, E. B. & Coulander, C. (1985). The inverse relationship between fish consumption and 20-year mortality from coronary heart disease. *New England Journal of Medicine* **312**, 1205–1209.
- Larmond, E. (1977). *Laboratory Methods for Sensory Evaluation of Food*. Publication no. 1637. Ottawa: Canadian Department of Agriculture.
- Marshall, A. C., Sams, A. R. & Van Elswyk, M. E. (1995). Oxidative stability and sensory quality of stored eggs from hens fed 1.5% menhaden oil. *Journal of Food Science* **59**, 561–563.
- Meilgaard, M., Civille, G. C. & Carr, B. T. (1989). *Sensory Evaluation Techniques*, 1st ed., p. 135. Champaign, IL: AOCS Press.
- Nettleton, J. A. (1991). *N*-3 fatty acids: comparison of plant and seafood sources in human nutrition. *Journal of the American Dietetic Association* **91**, 331–337.
- Nettleton, J. A. (1993). Are *n*-3 fatty acids essential nutrients for fetal and infant development? *Journal of the American Dietetic Association* **93**, 58–64.
- Rhee, K. S. (1978). Minimization of further lipid peroxidation in the distillation 2-thiobarbituric acid test of fish and meat. *Journal of Food Science* **43**, 1776–1778.
- Simopolous, A. P. & Salem, N. (1991). Egg yolk as a source of long-chain polyunsaturated fatty acids in infant feeding. *American Journal of Clinical Nutrition* **55**, 411–414.
- Tarladgis, B. G., Watts, B. M. & Younathan, M. T. (1960). A distillation method for the quantitative determination of malonaldehyde in rancid foods. *Journal of the American Oil Chemists' Society* **37**, 44–48.
- United States Department of Agriculture (1993). *Nutrition: Eating for Good Health*. Agriculture Information Bulletin no. 685. Washington, DC: USDA.
- Van Elswyk, M. E., Dawson, P. L. & Sams, A. R. (1995). Dietary menhaden oil influences sensory characteristics and headspace volatiles of shell eggs. *Journal of Food Science* **60**, 85–89.
- Van Elswyk, M. E., Sams, A. R. & Hargis, P. S. (1992). Composition, functionality, and sensory evaluation of eggs from hens fed dietary menhaden oil. *Journal of Food Science* **57**, 342–344, 349.