Enrichment of Poultry Products with ω3 Fatty Acids by Dietary Supplementation with the Alga *Nannochloropsis* and Mantur Oil

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Experiments were conducted to evaluate the efficiency of the microalga *Nannochloropsis* sp. (*Nanno.*), as a supplement to laying hens' diet, for the production of enriched eggs and meat with ω3 fatty acids (FA). *Nanno.* has a unique FA composition, namely, the occurrence of a high concentration of eicosapentaenoic acid (EPA; 20:5 ω3) and the absence of other ω3 FA. The effect of supplementing diets with *Nanno.* on ω3 FA levels in eggs, plasma, liver, and thigh muscle was compared to that of mantur oil, high in α-linolenic acid (LNA; 18:3 ω3). *Nanno.* is rich also in carotenoids, which may be useful for egg yolk pigmentation. The observed effect of *Nanno.* supplementation on yolk pigmentation was dose responsive, in both the rate of coloration and the color intensity. Addition of enzyme preparations (glucanase plus cellulase or glucanase plus pectinase) slightly elevated the yolk color score. The most prominent changes in the level of ω3 FA in egg yolk were evident when the diets were supplemented with 1% *Nanno.* or mantur lipid extracts. Levels of dietary algal meal (0.1−1.0%) had low and inconsistent effects on the level of yolk ω3 FA. Algal EPA is not accumulated in the liver or in the egg yolk; it is apparently converted and deposited as docosahexaenoic acid (DHA). LNA from mantur oil was partially converted to DHA, and both DHA and LNA were deposited in egg yolks and livers. It is suggested that the absence of DHA and EPA from thigh muscle is due to the small amount of dietary ω3 FA used in this work, compared to other studies, and to the possibility that in laying hens the egg yolk has a priority on dietary FA over that of muscles.

Keywords: *Marine algae; mantur oil; ω3 fatty acids; egg yolk; plasma; liver; thigh muscle; yolk pigmentation*

INTRODUCTION

The importance of long-chain (LC) ω3 fatty acids (FA), eicosapentaenoic acid (EPA; 20:5) and docosahexaenoic acid (DHA; 22:6), in human nutrition is gaining considerable attention due to their role in various physiological processes (Budowsky, 1988; Salem et al., 1996). EPA and DHA are known to reduce the risk of cardiovascular diseases by controlling blood lipid levels (Kinsella et al., 1990) and by reducing platelet reactivity and aggregation (Dyerberg et al., 1978). Salem (1997) suggested that DHA is an essential nutrient for optimal nervous system function, and Simopoulos (1996) indicated the essentiality of ω3 FA for normal growth and development.

EPA and DHA are found in fish and other types of seafood (Simopoulos et al., 1991) and in some microalgae (Herber and Van Elswyk, 1996; Kyle et al., 1992) but are absent from plant food sources. Some oil seeds contain high levels of α-linolenic acid (LNA; 18:3 ω3), and mammals and birds are capable of performing in vivo elongation/desaturation of LNA to produce EPA and DHA (de Gomez et al., 1975). The efficiency of LNA to DHA convertibility increases as the ratio ω6:ω3 decreases. Thus, health authorities of several countries recommend increasing the level of ω3 FA and to reduce the ratio of ω6:ω3 in the diet to approximately 4:1 (Simopoulos, 1996). The ratio ω6:ω3 found in Western cultures is 10−25:1 (Hunter, 1989). The ability of premature infants and hypertensive, diabetic, and elderly people to synthesize LC ω3 FA from LNA is limited. Therefore, a minimal level of preformed EPA and DHA in the diet is required, especially at stages of high demand for these LC FA (Simopoulos, 1996).

The current limited availability and high cost of fish or other EPA and DHA sources in various parts of the world can now be, at least partly, replaced by more popular and less expensive poultry products enriched with these FA. It could convince consumers, who gave up eating eggs because of cholesterol-phobia, to increase egg consumption specifically and poultry meat consumption in general. It was reported by Raper et al. (1992) that the contribution of poultry products, as sources of EPA and DHA to the U.S. food supply, increased from 2 and 5% to 10 and 22%, respectively, during the past 50 years.

The FA profile of eggs and poultry meat lipids is related to the dietary FA composition (Cherian et al., 1996; Dvorin et al., 1998). Fish oil or high LNA oil seeds may be used to enrich poultry products with ω3 FA. The use of DHA containing algae to improve egg quality was recently reported (Herber and van Elswyk, 1996). The microalga *Nannochloropsis* sp. (*Nanno.*) contain ∼20%

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10.1021/jf981361p CCC: $18.00 © 1999 American Chemical Society
Published on Web 11/04/1999

Table 1. Supplements Added to a Basal Diet[a] for the Production of the Experimental Diets

diet	supplements of basal diet
1	1% *Nanno.* meal
2	1% *Nanno.* meal + glucanase and pectinase[b]
3	0.3% *Nanno.* lipid extract
4	1% *Nanno.* lipid extract
5	1% mantur oil
6	basal diet

[a] Basal diet (ingredient %): corn, 20; sorghum, 43; soybean oil meal, 23; fish meal, 1; soapstock of soybean oil, 2; $CaCO_3$, 8.5; DCP, 1.8; mineral and vitamin mixture, 0.29; methionine, 0.15. Calculated composition: energy, 2800 kcal/kg; crude protein, 16.7%; Ca, 3.84%; available P, 0.44%. [b] Finnfeeds International Ltd. Marlborough, U.K. The enzymes were provided in solution and were added at 0.2% of the alga weight, according to the manufacturer's suggestions.

Table 2. Fatty Acid Composition of *Nannochloropsis*, Mantur, and Soybean Soapstock Lipids

fatty acid	*Nanno.* oil	mantur oil	soybean oil soapstock
14:0	4.2		
16:0	14.5	8.6	8.5
16:1	27.6		
18:0	0.8	2.5	3.0
18:1 ω9	5.5	15.1	19.0
18:2 ω6	2.3	14.4	57.3
18:3 ω3	1.7	59.4	9.5
20:4 ω6	4.7		
20:5 ω3	27.7		
total ω6	7.0	14.4	57.3
total ω3	29.4	59.4	9.5
ω6:ω3	0.2	0.2	6.0

lipids, of which 27% are EPA. It was shown that the *Nanno.* EPA can be incorporated into blood and liver lipids in rats consuming diets containing this alga (Sukenik et al., 1994) and into brain lipids of their offsprings (Mokady and Sukenik, 1995). In addition, the alga contains a relatively high level of vitamin E and various carotenoids, mainly violaxantin and β-carotene, which may attain up to 1.5% of the algal dry weight (Lubian et al., 1998; Sukenik et al., 1993, Sukenik, 1999). Both vitamin E and the carotenoids may be useful for enhancing yolk pigmentation and providing antioxidative properties.

The purpose of the present study was to evaluate the effect of supplementing the diets of laying hens with the EPA-rich alga *Nannochloropsis* sp. or with the LNA-rich mantur seed oil (*Matthiola* sp.) on the lipid composition of eggs and various chicken tissues.

MATERIALS AND METHODS

Algal Biomass. *Nannochloropsis* sp. (Eustimatophyceae) was grown in outdoor shallow raceway-type ponds at the facililties of Natural Beta Technologies (NBT) Ltd. (Eilat, Israel) as previously described. The algal lipid extract was prepared from freeze-dried biomass, using a mixture of ethanol/hexane 2:1 (Sukenik et al., 1994). Mantur (*Matthiola* sp., belongs to the Cruciferae family) oil was prepared by cold press extraction of the seeds.

Laying Hens and Diets. Laying chickens (White Leghorn × Rhode Island Red) were kept in individual cages situated in an open shed, in which artificial illumination supplemented daylight to provide 14 h/day of light. Experimental diets were prepared by supplementing a commercial diet by dried *Nanno.* biomass, a lipid extract from this microalga, mantur seed oil, or soybean soapstock as a control (see footnote to Table 1). The FA composition of the supplements is shown in Table 2. Five laying hens were assigned for each treatment, and they received their food in one trough. Each egg was marked daily, weighed, and stored in a cold room (6 °C).

Experiment 1 was designed to preliminarily evaluate the effect of various levels of *Nanno.* biomass on the level of yolk ω3 FA and coloration. Fifteen hens were divided randomly into three groups, and fed a commercial diet to which *Nanno.* meal was added at levels of 0.1, 0.5, and 1.0%.

Experiment 2 was carried out following the evaluation of the potential of commercially available carbohydrate-hydrolyzing enzymes to enhance algal biomass digestibility. The efficacy of several enzymes was examined by in vitro experiments, in which the degree of cell disintegration was studied. On the basis of the in vitro results, glucanase and cellulase preparations were employed in an in vivo experiment. Forty laying hens were divided into eight groups: four groups received the commercial diet to which 0.1% *Nanno.* meal was added, and four groups received 1.0% *Nanno.* meal in their diet. Enzyme preparations were added to the diets as follows: groups 1 of each *Nanno.* concentration did not get any enzyme supplementaion, groups 2 received cellulase, groups 3 received glucanase, and groups 4 received cellulase plus glucanase. (The enzyme preparations were supplied by Finnfeeds International Ltd., Wiltshire, U.K. The enzymes were added at levels of 0.2% of the algal weight, as was suggested by the manufacturer.) The experimental diets were fed during 33 days and then they were replaced by the commercial diet, not supplemented by algae or enzymes, for 10 days.

Experiment 3. Thirty-five laying hens were divided randomly into seven groups of 5 birds each. They were fed the experimental diets (Table 1) during 20 days. Eggs were collected as in all previous experiments, but, in addition, at the end of the experimental period the birds were autopsied and samples of blood plasma, liver, and thigh muscle were collected. The plasma and tissue samples were stored at −20 °C until analyzed for FA composition.

Color Score of Egg Yolks. Individual egg yolks were separated into Petri dishes, and the color was scored visually by five people by comparison with a Hoffman-La Roche color fan.

FA Analysis. Samples of the yolks, liver, and thigh muscle were freeze-dried. FA of these samples and of blood plasma were determined after direct methylation following the procedure described by Miller (1984). The methyl esters were extracted into 1 mL of hexane/diethyl ether (1:1 v/v). The extracts were injected into a Hewlett-Packard 5890A gas−liquid chromatograph equipped with a 25-m fused silica capillary column SP-2330 (Supelco, Bellefonte, PA). The initial oven temperature was 160 °C, and the final temperature was 190 °C. FA identification was performed using external and internal standards.

Statistical Analysis. The effect of dietary treatments was evaluated by analysis of variance. Comparison between individual diets was made using Duncan's multiple-range test.

RESULTS

Egg Production and Size. The average egg production rate (95.1 ± 1.7%) and the average egg weight (66.2 ± 1.1 g) were not affected by the experimental supplements provided with the basic diet.

Coloration of Egg Yolk. The intensity of the yolk color increased almost immediately after diets fortified with algal biomass were provided. The increase was in a dose-dependent manner, reflecting the amount of alga in the diet. The maximal scores of the yolk color, according to the Hoffman-La Roche color fan, were 14, 12, and 9, in the groups receiving 1, 0.5, and 0.1% alga in their diet (experiment 1, data not shown). In experiment 2, two distinct groups were observed for yolk coloration (Figure 1). Maximal color scores were observed in yolks from laying hens fed diets supplemented with 1% *Nanno.* biomass. The color intensity increased rapidly and plateaued around the seventh or eighth day

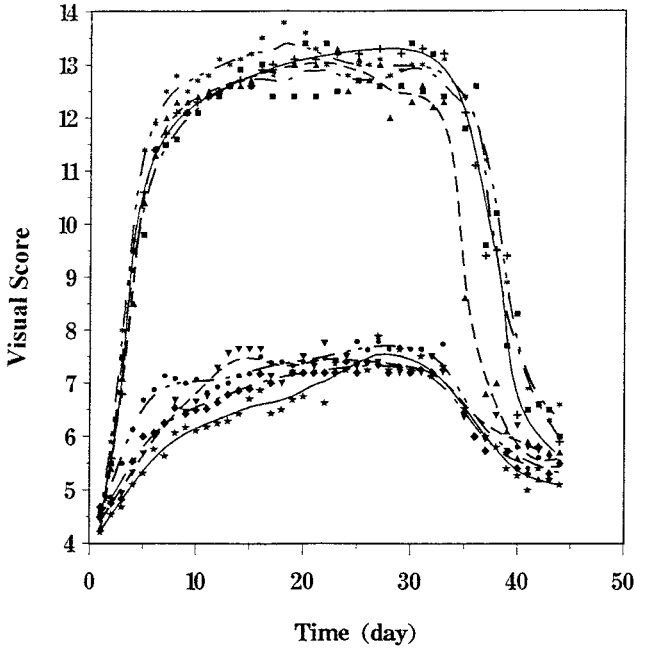

Figure 1. Color intensity of egg yolks from laying hens fed diets containing 0.1 or 1.0% *Nanno.* meal, supplemented with exogenous enzymes: diets 1, no added enzymes; diets 2, glucanase; diets 3, cellulase; diets 4, glucanase plus cellulase. The color was scored according to a Hoffman-La Roche color fan.

on values between 12.4 and 13.5. The effect of the exogenic enzyme supplementation was small, with only a slightly faster coloration rate in the group supplemented with both enzymes—cellulase and glucanase. These scores were ~90% higher than those observed in egg yolks from laying hens fed diets containing only 0.1% alga, in which the maximal scores were between 7 and 7.5. In the group fed diet with no added enzymes, the color score increased slowly to its maximal value on day 26. Supplementing the diet with cellulase and glucanase resulted in a faster coloration rate during the first 10 days, as compared to the other groups. Using either one of the enzymes produced an intermediate rate of egg yolk coloration. Reduction in the color intensity after the hens had been returned to the commercial diet was rapid. However, 12 days after the change of the diets, the yolk color score was still slightly higher than that found at the beginning of the experiment.

In experiment 3, as was shown previously, the intensity of the yolk color increased quickly after feeding of the experimental diets began. The most intense color (visual score 15) was observed when diet 4 (1% *Nanno.* lipid extract) was used (Figure 2). The peak was reached on day 7, earlier than in all the other groups, and was maintained until the end of the experiment on day 20. In groups 1−3 the peaks were reached a few days later. No change in the yolk color was developed when the layers received mantur oil (diet 5) or the basal diet (diet 6). The glucanase and pectinase addition to 1% *Nanno.* diet enhanced yolk coloration (group 2 versus group 1) to a similar value observed for group 3, which received a diet containing 0.3% *Nanno.* lipid extract, the amount of lipids provided by the equivalent amount of 1% *Nanno.* dry meal. The color scores were 15, 11−12.5, and 5 for groups 4, 1−3, and 5, respectively.

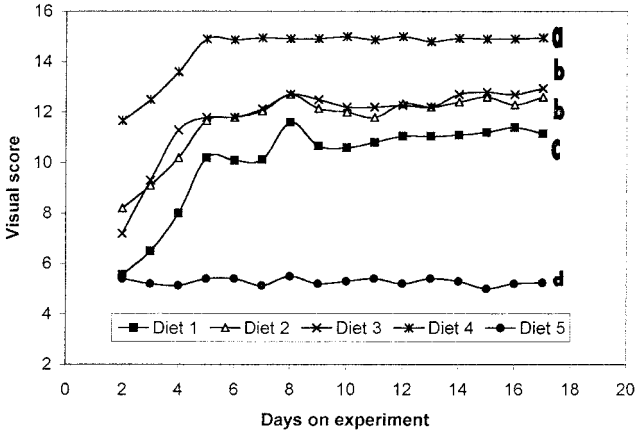

Figure 2. Color intensity of egg yolks from laying hens fed the basal diet with the following supplements: 1, 1% *Nanno.* meal; 2, 1% *Nanno.* plus (pectinase plus glucanase); 3, 0.3% *Nanno.* lipids extract; 4, 1% *Nanno.* lipids extract; 5, 1% mantur oil; 6, basal diet (the curve was essentially the same as for diet 5 and therefore was not included). The color was scored according to a Hoffman-La Roche color fan. Scores on the last day of the experiment marked with different letters differ significantly ($P < 0.05$).

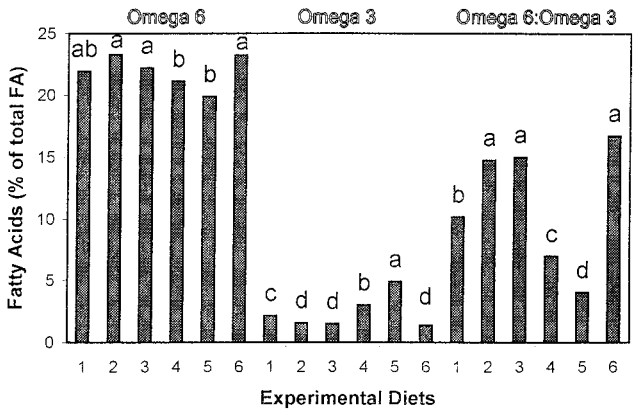

Figure 3. ω6 and ω3 FA and ω6:ω3 ratio in egg yolk lipids from laying hens fed the basal diet with the following supplements: 1, 1% *Nanno.* meal; 2, 1% *Nanno.* plus (glucanase plus pectinase); 3, 0.3% *Nanno.* lipids; 4, 1% *Nanno.* lipids; 5, 1% mantur oil; 6, basal diet. Values marked with different letters differ significantly ($P < 0.05$).

FA Composition of Lipids of Yolk and Various Tissues. In experiments 1 and 2, the addition of 0.1 or 0.5% *Nanno.* meal did not affect the level of DHA in the egg yolk. The addition of 1% *Nanno.* meal elevated the DHA level above its basal value, by ~25% ($P < 0.05$) in experiment 1 and by 35% in experiment 2, when the 1% algal meal diet was supplemented with the enzymes glucanase and cellulase (data not shown). In experiment 3, the greatest changes in FA composition of egg yolk lipids were observed in the groups fed diets supplemented with 1% *Nanno.* or mantur lipids. In these groups, the level of ω6 FA (linoleic plus arachidonic acids) was the lowest, whereas that of ω3 FA (LNA plus EPA plus DHA) was 2−3-fold higher than in control eggs. As a result, the ratio ω6:ω3 dropped from 17 in the control group to 7 or 4 in the 1% *Nanno.* lipids and 1% mantur oil groups, respectively (Figure 3). Feeding diets containing 1% *Nanno.* meal, 1% *Nanno.* meal fortified with enzymes, or 0.3% algal lipid extract had a small and nonsignificant effect on the level of ω6 FA in egg yolk, as compared to that observed in the control group. The level of ω3 FA in the eggs of the 1% *Nanno.*

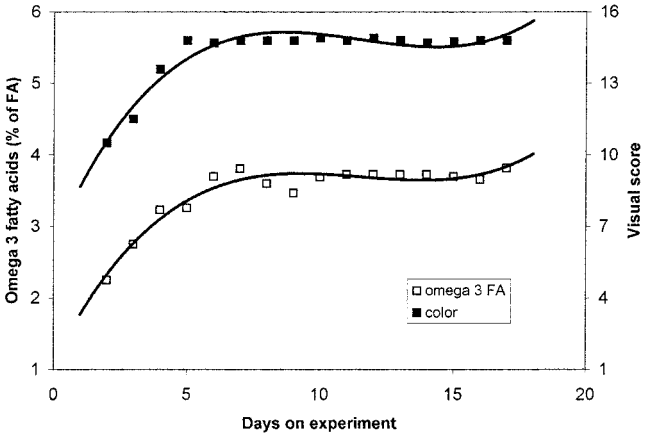

Figure 4. Level of ω3 FA and color score of egg yolk as a function of time of feeding a diet containing 1% *Nanno.* lipids extract.

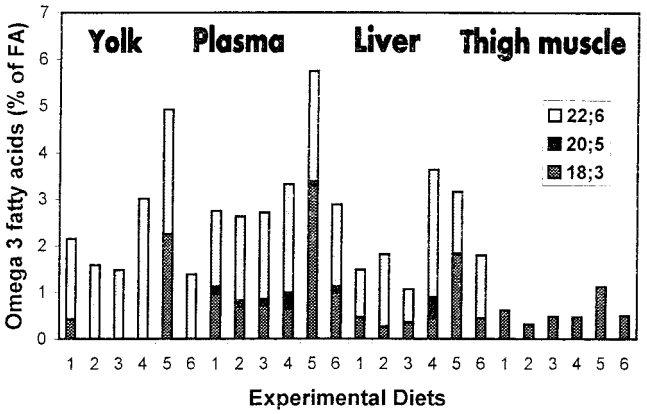

Figure 5. Level of ω3 FA (percent of total fatty acids) in egg yolk, plasma, liver, and thigh muscle of laying hens fed, in addition to a basal diet, the following: 1, 1% *Nanno.* meal; 2, 1% *Nanno.* plus (glucanase plus pectinase); 3, 0.3% *Nanno.* lipids; 4, 1% *Nanno.* lipids; 5, 1% mantur oil; 6, basal diet.

group was ~25% higher than in control eggs, whereas that found in groups 2 and 3, fortified with enzymes or given 0.3% *Nanno.* lipid extract, respectively, did not differ from the controls. The ω6:ω3 ratio in these groups (1−3) varied between 10 and 15.

The level of ω3 FA in egg yolk lipids, in eggs laid by hens fed diet supplemented with 1% *Nanno.* lipids, increased soon after the hens received the experimental diet (Figure 4). The increase was in a pattern similar to changes observed in egg yolk coloration, and both parameters plateaued on day 8.

The distribution of the different ω3 FA in yolk, plasma, liver, and thigh muscle lipids was affected by the dietary source of the ω3 FA (Figure 5). In yolks from laying hens fed a diet containing *Nanno.* biomass or lipids, DHA was the sole ω3 FA [except the yolks of group 1, which contained, in addition to DHA, a small amount of 18:3 FA (0.4% of total FA)]. However, in the group fed mantur oil, the ω3 FA consisted of approximately 50% LNA and 50% DHA. EPA was not detected in yolks from any of the experimental groups.

Blood plasma of the laying hens fed diets supplemented with *Nanno.* meal or lipids contained between 0.66 and 0.97% 18:3, similar to that of the controls (1%) but considerably lower than the level recorded for the mantur group (3.28%). EPA level was low in all groups (0.09−0.14%) except for group 4, which received 1% *Nanno.* lipids and had higher EPA level in the plasma compared to the other groups, (0.32% of total FA, $P < 0.05$). The level of DHA was ~30% higher ($P < 0.05$) in groups 4 and 5, which received diets supplemented with 1% lipids either from *Nanno.* or from mantur, as compared to the control or all other groups, which did not differ from the control DHA level.

The ω3 FA in livers of the *Nanno.*-fed groups consisted of 14−30% LNA and ~86−70% DHA. The distribution of the ω3 FA in the liver of the mantur oil group was 57:43 LNA/DHA. In all groups, the level of EPA was very low or undetectable.

The only ω3 FA found in the thigh muscle was LNA; LCFA, EPA, and DHA could not be detected. The hens fed the mantur oil had the highest level of LNA ($P < 0.05$).

DISCUSSION

Nutritional manipulation of the FA profile of yolk lipids was shown previously (Hargis and Van Elswyk, 1993; Cherian et al., 1996; Halle, 1998). In most of these studies, fish oils, containing EPA and DHA, or flax seed oil, containing LNA, were used to promote efficient deposition of LC ω3 FA in egg yolks. In the study of Herber and Van Elswyk (1996), another source was introduced, namely, a marine alga containing DHA. In the present study the marine alga *Nannochloropsis* sp. was used. This alga, which is rich in EPA and devoid of DHA and LNA, was evaluated as a source of ω3 FA for the production of enriched eggs and meat with essential FA. Furthermore, the unique FA composition of this alga enabled us to study the metabolic fate of dietary EPA in the laying hen and to compare it with LNA-rich oil. The data presented in this study show that the algal EPA is not accumulated in liver, thigh muscle, or egg yolk. Only a small proportion of the dietary EPA was found in the plasma, suggesting that this FA is either catabolized or converted to DHA. The extent of the changes in the liver FA composition (relative to values found in control chickens) reflects both the FA profile of the dietary lipids and the FA biosynthetic capacity of laying hens. The results indicate that dietary EPA and LNA were converted to DHA by the hepatic enzymes and partially deposited in the egg yolk and liver. DHA is the major ω3 LCFA found in yolk lipids, supporting the conclusions of Herber and van Elswyk (1996) that DHA, rather than EPA, was preferentially incorporated into membranes of egg yolk. Application of mantur oil, as a source for LNA, resulted in partial conversion of LNA to DHA, which was found in egg yolk, liver, and plasma, while the rest of the LNA was transferred as is, confirming the findings of Ferrier et al. (1995).

The total amount of ω3 FA provided by the 1% mantur oil diet was approximately twice as much as that provided by the 1% *Nanno.* oil diet. However, the relative amount of ω3 FA in egg yolk lipids was only 60% higher in the mantur group than in the *Nanno.* oil one (4.92 and 3.01%, respectively). Moreover, although in the *Nanno.* oil group 100% of the ω3 FA were in the form of DHA, in the mantur group, DHA and LNA comprised ~50% each of the total ω3 FA. These results clearly suggest that the efficiency of conversion of EPA to DHA is considerably better than that of LNA to DHA.

The effect of dietary FA composition on the FA profile of the yolk lipids is rapid. Changes were evident within a few days following the inclusion of *Nanno.* lipids in the diet, concomitant with variations in egg yolk col-

oration. This phenomenon is due to the fact that the algal carotenoids are fat soluble and deposited in the egg yolk in a manner similar to that of LCFA. Thus, the yolk color may serve as an indicator for the deposition of dietary LCFA in the yolk lipids when algal biomass or lipids are used as a source of these FA. Coloration of egg yolk by deposition of carotenoids is an additional nutritional benefit, because it provides lipophilic antioxidants, which may serve also as a potential source for vitamin A. The occurrence of high levels of these antioxidants may be of special importance considering the enrichment of the egg yolks with LCFA, which are very prone to peroxidation. Furthermore, enhanced coloration may improve the product attractiveness for consumers.

The absence of EPA and DHA from thigh muscle of the laying hens in the present study is contrary to the results of Cherian et al. (1996). These authors showed that following the feeding of diets containing menhaden or flax oil, lipids of white and red meat of laying hens contained DHA and EPA at levels between 0.6 and 6.0% or between 0.2 and 1.4%, respectively. The difference between the two studies is in the level of dietary ω3 FA used: 0.28% EPA in the present study (1% *Nanno.* lipids diet) compared to 0.7% EPAplus DHA (menhaden oil diet) or 0.69% LNA (1% mantur oil diet) compared to 1.54% LNA (flax oil diet) in the cited work. Feeding broilers and laying hens diets containing full-fat flax seeds or flax oil supplying similarly high levels of LNA supported approximately the same levels of ω3 FA in the breast and thigh muscles (Cherian et al., 1996; Krasicka et al., 1998). It seems that in laying hens dietary fat and ω3 FA are preferentially incorporated into egg yolk lipids as compared to muscles. Thus, when the supply of ω3 FA is relatively low (as in the present study), these FA are first deposited in the egg lipids. Only an ample supply of ω3 FA will impose enrichment of muscles with ω3 LCFA.

The absolute levels of total ω3 FA in eggs were 219 and 310 mg/100 g of edible product in the groups fed 1% *Nanno.* lipids or 1% mantur oil, as compared with 143 mg/100 g in the control group. The rest of the groups, which received lower levels of ω3 FA in their diets than those receiving 1% lipids from *Nanno.* or mantur, did not differ from the control in this respect. The levels of ω3 FA in the liver were 153, 228, and 99 mg/100 g and in the muscle 12, 22, and 8 mg/100 g in the groups fed 1% *Nanno.* lipids, 1% mantur oil, or the control group, respectively.

Various sources of ω3 FA including marine algae can be used to enrich poultry products with these essential FA and replace fish or other seafood in human diets. It is important to note that in cases of high need of preformed EPA and DHA, the use of LNA as the only or main dietary source of ω3 FA has its limitations. Therefore, natural sources such as marine oils have a significant advantage in designing the nutritional quality of poultry products according to consumer needs and preferences.

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Received for review December 17, 1998. Revised manuscript received September 30, 1999. Accepted October 7, 1999. Funding for this work was provided by the MAGNET program, Israel Ministry of Commerce and Industry and by the Strauss/Hanauer Memorial Fund Inc.

JF981361P