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POSSIBILITY OF ENRICHING FAYOUMI HEN'S EGG WITH OMEGA-3 FATTY ACIDS (With 6 Tables and 3 Figures)

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إمكانية زيادة الأحماض الدهنية الغير مشبعة (omega-3 FA)
فى بيض الدجاج الفيومى

أمال على راضى ، محمد عبد البارى مندور

تحتل الدهون أهمية خاصة فى غذاء الإنسان وذلك كمصدر للطاقة وتخليق بعض المكونات الحيوية اللازمة لوظائف الجسم والأعضاء. إلا أن زيادتها تسبب كثيرا من أمراض القلب والشرائين. تناول هذا البحث تأثير إضافة زيت السمك (زيت كبد الحوت) بتركيزات مختلفة إلى عليقة الأمهات الأساسية على خواص البيض وأيض الدهون البروتينية وأيض الأحماض الدهنية فى كل من كبد وصفار البيض للأمهات. أجريت هذه التجربة على عدد ١٢٠ دجاجة فيومى تراوحت أوزانها بين ٩٠٠-١٠٠٠ جرام وعند عمر ٢٠ أسبوع قسمت إلى ثلاث مجموعات متساوية ، المجموعة الضابطة تغذت على عليقة أساسية بدون إضافات وتحتوى على ٣٪ زيت بذرة القطن ، المجموعة الثانية والثالثة تغذت على عليقة أساسية بعد إحلال زيت كبد الحوت لزيت بذرة القطن بنسبة ٥٠٪ ، ١٠٠٪ على التوالى . استمرت التجربة شهرين تم خلالها جمع البيض وتقدير خواصه وفى نهاية التجربة تم جمع عينات الدم من المجموعات الثلاث لفصل المصل وتقدير نسبة أيض الدهون البروتينية كذلك عينات الكبد وصفار البيض للتقدير النوعى والكمى للأحماض الدهنية . أسفرت التجربة عن النتائج التالية: زيادة طفيفة فى كتلة البيض ناتجة عن زيادة معنوية فى عدد البيض ، كما أنه ليس هناك إختلاف معنوى فى وزن وسمك البيض كذلك إنخفاض مستوى الدهون الكلية والكوليسترول والجلسريدات الثلاثية والدهون البروتينية خفيفة الكثافة وخفيفة الكثافة جدا فى مصل الدجاج البياض كما زادت الفوسفوليبيدات والدهون البروتينية الثقيلة. كما أن هناك زيادة معنوية تزداد بزيادة تركيز زيت كبد الحوت ($P \leq 0.01$) فى كمية الأحماض الدهنية الغير مشبعة (PUFA) وخاصة (20:5 n-3 , 20:6 n-3) فى كبد وصفار البيض للأمهات التى تقى الإنسان من أمراض القلب.

SUMMARY

To explore the usefulness of fish oil (active EPA-cod liver oil) as a source of eicosapentaenoic acid (EPA, 20: 5n-3), this study was performed on a total of 120 Fayoumi pullets which were randomly allocated into three equal groups (40/ group). The first group was fed on a basal control ration. It contained 3% cotton seed oil, which had high amounts of linoleic acid (18: 2n-6) and little amounts of n-3 polyunsaturated fatty acids. The other two groups were fed on a modified diet in which 50% and 100% of cotton seed oil was replaced by active EPA-cod liver oil (rich in omega-3 polyunsaturated fatty acids), respectively. The results showed that, addition of active EPA-cod liver oil particularly improved egg weight, egg mass production and feed conversion. However, the levels of plasma triacylglycerol, total cholesterol and low density lipoprotein were decreased. A significant increase ($p < 0.01$) in the three different omega-3 polyunsaturated fatty acids α -linolenic, (18:3 n-3), eicosapentaenoic, (EPA, 20:5 n-3) and docosahexaenoic, (DHA, 22:6 n-3) acids were observed in the liver and egg yolk of hens fed on fish oil for two months, the duration of the experiments.

Key words: Hen's egg - Fatty acids - Enrichment.

INTRODUCTION

Beneficial effects of fish oil are mediated by its high contents of long-chain omega (n)-3 fatty acids, mainly eicosapentaenoic acid (EPA, 20: 5 n-3) and docosahexaenoic acid (DHA, 22: 6n-3) (Dyerberg and Jorgensen, 1982). Omega-3 polyunsaturated fatty acids compete with arachidonic acid (AA, 20:4n-6) for the C-2 position of phospholipids in rats (Dyerberg et al., 1986) possibly reducing the formation of vasoconstrictor, prothrombotic and pro-inflammatory metabolites. Fatty acid of n-3 family are, also, known to inhibit the desaturation of n-6 fatty acids (Huang et al., 1992). Subsequent studies have shown a considerable lowering of plasma lipids particularly of triglyceride rich lipoprotein particles in the liver (Nestel et al., 1984 and Wong et al., 1988), increasing beta oxidation (Yamazaki et al., 1987), decreasing activity of esterifying enzymes (Rustan et al., 1988) and diversion to phospholipid formation (Wong et al., 1985) are all mechanisms by which fish oil may

inhibit triacylglycerol and subsequently very low density lipoprotein (VLDL) production, in addition to decreased hepatic phosphatidate phosphohydrolase activity (March *et al.*, 1987). Because the *de novo* synthesis of phosphotidylcholine is required for VLDL secretion (Yao and Vance, 1988) and changes in cytidylytransferase activity could account for decreased VLDL output (Halminski *et al.*, 1991), it has been recognized recently by Farrell, (1993 and 1996) that the intake of n-3 fatty acids significantly ($p < 0.01$) increases high density lipoprotein (HDL) and significantly ($p < 0.05$) decrease LDL. But fish is expensive and often irregularly supplied, it may be contaminated with heavy metals, pesticide residues and contain pathogenic microorganisms, beside, some consumers do not like fish, thus, their dietary supply of the omega-3 polyunsaturated fatty acids is low and having a large imbalance of omega-6 : omega-3 fatty acids. Therefore, the possibility of enriching the hen's eggs with omega-3 fatty acids by modifying the hen's diet with a mixture of edible oils (Fish oil, active EPA-cod liver oil) was proposed.

MATERIALS AND METHODS

Birds:

A total of 120 pullets of Fayoumi breed (20 weeks old) were obtained from Faculty of Agriculture, Alexandria University. The birds were divided into three equal groups (four replicates of 10 birds for each group) and housed in a conventional three tires laying battery (3 birds/ cage of 40 X 40 X 44 cm) throughout the experiment. Feed and water were supplied *ad libitum*. The feed was offered to pullets at, a rate of 50 g/ hen/ day, for 2 months. The ingredient and chemical composition of the basal diet is shown in Table 1.

Table 1: The percentage composition and chemical analysis of the basal diet

Ingredients	Control basal diet	**Chemical analysis	
White corn, ground	63.90	ME (Kcal/kg)	2855
Soy bean meal	20.44	Moisture	9.86
Wheat bran	5.00	Crude protein	18.00
Fish meal	5.00	Ether extract	3.85
Bone meal	1.43	Crude fiber	3.52
Cotton seed oil	3.00	Nitrogen free extract	59.73
Active EPA-cod liver oil	---	Ash	5.04
Lime stone, ground	0.48	Calcium	1.00
Common Salt	0.50	Available phospholrous	0.49
Premix*	0.25	Lysine	1.02
		Methionine + cysteine	0.36

*Premix (Supplied per kilogram final feed): it. A: 10,000 IU, vit. D₃: 2,500 IU, vit. E: 11 mg, vit. K: 1 mg, vit. B₂: 5 mg, vit B₆: 3 mg, choline chloride: 500 mg, pantothenic acid: 11 mg, folic acid: 1 mg, niacin: 30 mg, vit. B₁₂: 10 mg, biotin: 75 mg, Mn: 60 mg, Zn: 45 mg, Fe: 60 mg, Cu: 5 mg, Se: 0.1 mg (Fizer premix).

**Chemical analysis according to NRC (1984).

The sole fat source for the basal diet, was 3% cotton seed oil, which contained high amount of linoleic and little amounts of n-3 polyunsaturated fatty acids. For the treated pullets, the fat source was fish-oil (Active EPA-30 British cod liver oil Ltd.), being supplied by Prof. Farkas Tibor Biological Research Center of the Hungarian Academic of Science, Szeged, Hungary, which contained high levels of linolenic acid and high levels of 18:3 n-3, 20:5 n-3 and 22:6n-3. The first supplemented group was switched to a fish oil replacing (50%) of the cotton seed oil, while the second supplemented group was switched to a fish oil replacing (100%) of the cotton seed oil after 2 weeks feeding on control basal diet. The fatty acid composition of the experimental oils and diets are represented in Table 2.

Table 2: Fatty acid composition of experimental oils and diets
(percent of total fatty acids)

Fatty acid %w/w	Experimental oils		Experimental diets		
			Basal diet	Repletion diets (Active EPA-30)	
	Fish oil (Active EPA-30)	Cotton seed oil		diet 1	diet 2
14:0	5.20	7.70	4.40	4.36	4.33
16:0	15.4	10.7	14.4	14.47	14.54
16:1n-7	7.70	---	---	0.11	0.23
18:0	2.50	4.20	2.10	2.07	2.04
18:1n-9	15.1	23.7	25.6	25.47	25.34
18:2 n-6	1.40	53.4	51.6	50.82	50.34
18:3 n-3	8.50	0.30	1.90	1.98	2.06
20:4 n-6	1.40	---	---	0.02	0.04
20:4 n-3	2.70	---	---	0.04	0.08
20:5 n-3	20.0	---	---	0.30	0.60
22:5 n-6	0.60	---	---	0.01	0.02
22:5 n-3	1.70	---	---	0.02	0.05
22:6 n-3	10.8	---	---	0.16	0.32
Total n-3	42.7	0.30	1.90	2.50	3.11

Basal diet, mixture of 3% cotton seed oil and 0.85% others oils.

Repletion diet (1), mixture of 1.5% active EPA-30 + 1.5% cotton seed oil + 0.85% others oils.

Repletion diet (2), mixture of 3% active EPA-30 + 0.85% others oils.

Fresh diet was prepared every two days and dietary oils were stored in a refrigerator under nitrogen at all times. Food intake was recorded for the 3 groups daily. Water was supplied to pullets through automatic nipples provided in cages. The experimental feeding period using these diets extended for 60 days. The pullets were subjected to an average lighting period of 15 hrs. daily during the whole experimental period.

Body weight of laying pullets in different groups was recorded at the start and end of the experiment.

Eggs produced the various groups were collected daily and average daily egg production, egg weight and egg mass for each group were

calculated. 10 eggs were collected to estimate average egg quality for each group (El-Aggoury et al., 1989).

Blood samples were collected in heparinized tube and kept on ice for plasma separation by centrifugation. Specimens of liver tissues were also collected at the end of the experiment for lipid analysis.

Biochemical analysis of plasma:

Total plasma lipids were determined colorimetrically using sulphophosphovanillin reaction as described by Frings *et al.*, (1972). Phospholipids were determined by the method of Connerty *et al.*, (1961) in which the protein was precipitated by trichloroacetic acid.

Plasma triacylglycerol, total cholesterol and high density lipoproteins were determined by using of enzymatic method of Spin react kits according to the methods of Sidney and Bernard (1973), Zak et al., (1954), and Lopes-Virella et al. (1977), respectively.

Very low density lipoprotein (VLDL) was calculated by division of triacylglycerol/ 5 - mg/dl. Meanwhile, the low density lipoprotein was calculated as total cholesterol- (HDL + VLDL) = mg/dl according to Bauer (1982).

Extraction of total lipids

Liver tissues and egg Yolk were collected from treated and control pullets, and the total lipids were extracted according to Folch *et al.*, (1957). The samples were transferred in the presence of dry methanol containing 5% HCL at 80°C under CO₂ for 2.5 hrs.

Gas chromatography of fatty acid methyl esters:

Methyl esters were separated using a Hewlett- Packard 5890 II gas chromatography, equipped with a capillary column coated with SP2330 of 0.25m thickness (0.25mm I.D. x 30mi CPS-Li Quadrex, New Haven, CT, U.S.A.). High- purity nitrogen was applied as carrier gas with a pressure of 230 KPa. Hydrogen was used at 100 kP a and 280 KP a. The dual column system was programmed from 160°C to 200°C to give partial separation of 18:3 n-3 at a flow rate of 1°C/min. The detector temperature and injector temperature were 250°C and 230°C, respectively. The peaks were identified by means of primary and secondary standards (Fig. 1) and by plotting log relative elution temperature versus the number of carbon atoms (Schmit and Wynne, 1966). The percentage composition was calculated as weight percentage (%w/w) using a Hewlett-Packard 3396A integrator.

Statistical analysis of the recorded data were done using Statistical Analysis System (SAS, 1994 a microcomputer version).

RESULTS

The results are illustrated in Tables 3 - 6 and Figures 1 - 3).

DISCUSSION

Effects of fish oil feeding on the performance and egg quality of Fayoumi pullets:

Average body weight of the layer hens at the start and end of the experiment are shown in Table 3. Statistical analysis of these data revealed no significant differences between all groups at the start of the experiment, while at the end of it, a significant ($P \leq 0.01$) increase was detected in the treated group (100%) compared with the control one. These findings agree with the conclusion of Fortun and Lebas (1994); Xiccato *et al.* (1995) and Esso *et al.* (1997) who found that the inclusion of fats (linseed and sunflower oils) in the diet, even in low proportions, seemed to stimulate the energy intake, feed intake and consequently induced a high litter weigh gain, with a positive effect on the performance of rabbit does.

It can be seen from Table 3. that, the feeding pullets on the different concentrations of fish oil under natural day light, increased the **egg number, egg weight and consequently egg mass** increased with increasing EPA concentrations in the diet. These results are supported by work of March and MacMilan (1990) and El-Katcha (1990) in which other vegetable oils have been tested.

From these results, it could be concluded that the significant effect of feeding fish oil on egg mass may be mainly due to its significant effect on egg number and egg weight (El- Aggoury *et al.*, 1989).

Regardless the effect of fish oil, it was noticed that pullets fed n-3 FA laid eggs without any significant changes in the shell thickness (0.0329 mm) compared to those fed on the basal diet (0.0328) Table 3. It is well known that, shell thickness differed greatly according to the amount of calcium deposited on the formed egg during its maintenance in the uterus. The rate and amount of calcium deposited depends mainly on parathormone and calcitonin hormones (Radwan *et al.*, 1989). Therefore, the present findings are in agreement with the results of Combs and Helbacka (1960) who

concluded that egg shell thickness was not significantly affected by the level of fat in the diet.

Albumin (white) and Yolk weight, there was a non significant decrease in average albumin weight with the increase of EPA concentration in the diets (Table 3). At the same time, the pullets kept under natural photoperiod and fed on the different concentrations 1.5 and 3% of fish oil laid eggs characterized with their higher yolk weight (19.96 and 22.58 mg) compared with those fed on the basal diet (17.97 mg). These results are supported by the observations of March and Amin (1981). Also yolk/albumin ratio increased slightly with increasing fish oil supplementation.

Feed conversion, the addition of fish oil improved feed conversion and efficiency of food utilization for egg production Table 3. The averages were 2.42 and 2.31 in the supplemented groups with 1.5 and 3% fish oil respectively, compared with control group 2.65 ($P < 0.05$). The present results are in agreement with finding of Sell *et al.* (1976), who found that the addition of linseed oil (18:3 n-3 rich oil) improved feed conversion for egg production.

Effect of fish oil on the plasma lipoprotein metabolism in laying hens:

The effects of fish oil (Active EPA-Cod liver oil) on the plasma lipids and lipoprotein levels of laying hens (pullets) fed basal diet (control group) and the two different active EPA concentrations 1.5 and 3.0% (treated groups) are summarized in (Table 4). As shown in this table, the total plasma lipids, cholesterol, low and very low density lipoproteins (LDL and VLDL) and triacylglycerol (TG) were significantly ($P \leq 0.01$) decreased in the pullets fed on fish oil rich in (omega-3 polyunsaturated fatty acids compared to the control group, while the total phospholipids and high density lipoproteins were significantly ($P \leq 0.01$) increased. Such decreases and increases in these parameters agreed with the results of Abdulla (1994), El-Shazly *et al.* (1995) and Farrell (1996) who found that the linolenic acid (18:3n-3) of linseed oil has the same effectiveness in reducing the levels of blood lipids and lipoproteins.

The reduction in hen's plasma TG by dietary fish oil is suggested mainly from the inhibition of hepatic VLDL production (Nestel *et al.*, 1984; Popp-Snijders *et al.*, 1987), and reduction of hepatic synthesis and secretion of TG by decreasing the activity of acyl-coenzyme A and 1,2-diaclyglycerol-acyl transferase (Rustan *et al.*, 1988) as well as increasing proximal beta oxidation activity (Yamazaki *et al.*, 1987).

The highly significant ($P \leq 0.01$) decrease in plasma cholesterol, LDL and VLDL caused by supplementation of active EPA was more clearly apparent with the use of the higher concentration (3%) than with the lower ones (1.5%) Table 4. This appears to be in close agreement with the findings of Shepherd *et al.*, (1980) and Fernandez *et al.* (1992) who concluded that, the diet containing PUFA lowered the LDL-cholesterol concentration in normal subjects. They attributed these findings to the increase in the expression of hepatic receptor for LDL, increased LDL apo-B fraction catabolic rate and decreased LDL-apo-B production. Harris (1989) and Huff and Telford (1989) reported that, the VLDL-Cholesterol significantly reduced after supplementation with salmon oil (rich in omega-3 FA) in normal subjects due to the increased conversion of TG rich apo-B to LDL-apo-B. This is due to enhancement of degradation of TG rich lipoprotein (Ruhling *et al.*, 1984) or to the activation of lipoprotein lipase system which led to decrease of B-lipoprotein (lecithin cholesterol acyltransferase) that mediated the incorporation of cholesterol into HDL and elevation of cholesterol turnover rate which in turn decreased LDL-cholesterol (Erden, 1985).

The hypocholesterolemic effect of fish oil may be attributed to the oxidation of cholesterol to bile acids, interfering with cholesterol absorption through the formation of insoluble mixed crystals (Davis, 1955) and indirect decrease of cholesterol synthesis via decreasing 7- α -hydroxylase activity (Barbara *et al.*, 1977).

Dietary effects of active EPA-30 on fatty acid composition of:

1 - Pullets liver:

The fatty acid compositions of the experimental diets and the liver tissues of laying hens fed experimental diets are listed in Table 2 and 5 respectively. As expected, dietary fatty acid composition greatly affected the composition of hen's liver fatty acids. Significantly higher levels of oleic (18:1 n-9) (29.6 ± 3.8) and linoleic (18:2 n-6) (11.1 ± 3.2) in the liver of the pullets fed the basal diet (3% cotton seed oil) apparently resulted from their abundance in the diet (25.6% and 51.6%) respectively. The considerably higher level of linolenic (18:3 n-3) (8.5) in the fish oil supplemented diets resulted in not only generally high levels of 18:3 n-3 but also in higher levels of long chain n-3 FA (7.3 ± 0.5) especially eicosapentaenoic 20:5 n-3 and docosahexaenoic 22:6 n-3 acids in the liver of fish oil fed hens.

The total n-3 FA for hen's liver fed 1.5% and 3% cod liver oil supplemented diets was 2-fold (6.1 ± 0.6) and 3-fold (7.3 ± 0.5) respectively

higher than that of hens fed basal diet (2.9 ± 0.6). This indicated that dietary linolenic acid had been extensively elongated and desaturated into long chain n-3 polyunsaturated fatty acids, showing the importance of diet on lipogenic processes (Cai and Curtis, 1989).

Regardless the great differences in the 18:2 n-6 levels in the diets which were almost the only exogenous source of n-6 FA for these pullets, the total n-6 FA level in hens fed 1.5% supplemented fish oil was very similar (around 23%), although hens fed 3% supplemented fish oil had significantly lower $\Delta 6$ desaturation (20.2 ± 1.9) compared to those fed cotton seed oil (24.9 ± 3.6). These results suggested that the lower total n-6 FA level in hens fed cod liver oil was due to the shortage of 18:2 n-6 in the diet, rather than the influence of high levels of n-3 FA in that diet. Furthermore, levels of individual long chain n-6 FA namely arachidonic acid (20:4 n-6) for hens fed basal diet was even higher (13.8 ± 1.2) than that for hens fed fish oil (8.9 ± 1.4) which was rich in linolenate. This was an indication that cod liver oil selectively deposit dietary n-6 FA in hen's liver and that the elongation and desaturation of 18:2 n-6 appeared not or at least seriously unaffected by the presence of high levels of 18:3 n-3 in these diets (Huang et al., 1992 and Venkatraman et al., 1992).

2 - Hen's egg yolk:

The values for individual FA of hen's egg yolk are given in Table 6 and Fig. 2. It was clear that, increase in the n-3/n-6 ratio was due to an increase in n-3 with a reciprocal decrease in n-6 FA particularly of 20:4n-6, by reflecting the similar n-3/n-6 reciprocity in the diet (Wainwright et al., 1992). Levels of 20:5n-3 and 22:6n-3 were increased by 2-fold (0.4 ± 0.2) and 3-fold (0.6 ± 0.2) respectively, as dietary n-3FA increased whereas, the levels of monounsaturated fatty acids (16:1n-7 and 18:1 n-9) were significantly decreased with increasing n-3 FA. It was shown recently in rats that with a minimal level of 18:2 n-6 (0.3% of calories) the amount of 20:4n-6 in the brain remained constant and beyond a minimum was independent of the levels dietary 18:3n-3 (Bourre et al., 1988). This suggests that the present effects on 20:4 may be specifically due to the long-chain n-3 FA.

Interestingly, levels of 20:4 n-6 were decreased in both, pullets liver and egg yolk by partial replacement of dietary 18:2 n-6 with n-3 FA. There are two possible mechanisms which may account for this, both related to desaturase activity. It is known that 20:5 n-3 and 22:6 n-3 inhibit $\Delta 6$ and $\Delta 5$ desaturase (Garg et al., 1988) which reduce the formation of 20:4 n-6 from 18:2 n-6, the presence of large amounts of 18:2 n-6 may result in substrate

accumulation due to inhibition of $\Delta 6$ desaturase (Brenner, 1977). In both instances the provision of 18: 3 n-3 would provide the effects of a decline in $\Delta 6$ desaturase activity.

Therefore, it could be concluded that the inclusion of marine fish oil in layer hen diets is of importance, since egg production, egg quality and health of layer are affected by the energy as well as omega-3 FA content of the diets. Also, increasing levels of dietary n-3 FA continues to increase n-3 FA and decrease the n-6 FA levels.

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REFERENCES

- Abdulla, S. E. (1994):* The effect of dietary fish oil and linseed oil on blood lipids in diabetic patients. Ph. D. Thesis (Nutrition) Fac. of Home Economics, Cairo Univ.
- Barbara, C.; Charles, L.; Glen, R. and Raymond, R. (1977):* Interrelated effects of food lipids on steroid metabolism in rats. *J. Nutr.* 107: 1444-1454.
- Bauer, J.D. (1982):* Clinical Laboratory Methods 9th ed. The C.V. Company II 1830 Westline Industrial Missouri 63146. Chapter 33, page 555.
- Bourre, J. M; Bonneil, M.; Dumont, O.; Piciotti, M.; Nalbone, G. and Lafont, H. (1988):* High dietary fish oil alters the brain polyunsaturated fatty acid composition. *Biochem. Physiol.* 37:911-917.
- Brenner, R.R. (1977):* Regulation function of $\Delta 6$ desaturase key enzyme of Polyunsaturated fatty acid synthesis. In Bazan, N. G. Brenner, R. R. and Giusto, N. H. (Eds.). *Function and biosynthesis of lipids.* Plenum Press, New York and London. 85-101.
- Cai, Z. and Curtis, L.R. (1989):* Effect of diet on consumption, growth and fatty acid composition in young grass carp. *Aquaculture.* 81:47-60.

- Connerty, H. V.; Briggs, A. R. and Eaton, E.H.J. (1961):* Clin chem. 7 (37) 580. Cited in H. Varely A.H. Gowenlok and M. Bell (1980) ed. of Practical Clinical Biochemistry 5th ed. Page: 669. William - Hedical Books. LTD, London.
- Combs, G.F. and Helbacka, N.V. (1960):* Studies with laying hen's 1- Effect of dietary fat, Protein levels and other variable in parctical rations. Poul. Sci.39: 271-279.
- Davis, W. W. (1955):* The physical chemistry of cholesterol and β -sitosterol related to the intestinal absorpion of cholesterol. Trans. N. Y. Acad Sci. 18: 123-134.
- Dyerberg, J.and Jorgensen, K. A. (1982):* Marine oils and thrombogenesis. Prog. Lipid. Res. 21: 255-269.
- Dyerberg, J; Bang, H.O. and Stofferson, E. (1986):* Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis. Lancet, ii: 117-119.
- El- Aggoury, S. A.; Gado, M. S. Hanafi. M. S. and Resk Allah, S. A (1989):* Traits of egg quality under the effect of different feeding regimes and lighting programs, 3rd. Egyptian-British conference on: Animal fish and poultry production. Alexandria. October 1989:869-878.
- El- Katcha, M. I. (1990):* Effect of linseed oil and vitamin E in diets on performance and egg quality of hyaline layer hens. Bull. Zool. Egypt. 39:359-372.
- El- Shazly, S.A.M.; Fatouh, I.; Taha, N.; Radi, A. A. and Korshom, M. (1995):* Lipid metabolism in male rats fed diet supplemented with linseed oil and ascorbic acid M. Sc. Thesis. Fac. Vet. Med. Alex. Univ.
- Erden, F. Gulence, S. Torun, M. ; Kocer, Z.; Simsek, B. and Nebioglu, S. (1985):* Ascorbic acid effect on serum lipid fraction in human being. Acta Vitaminol. Enzymol. 7 (112): 131-137.
- Esso, O.H., Badawy, S.A. and Maghraby, N. (1997):* Possible effects of two levels of dietary fat addition on performance and reproductive potentials during puberty and post-parturation in rabbit does. Alex. J. Vet. Sci. 13 (1): 87-97.
- Farrell, D. J. (1993):* Une's designer egg. Poul. International. 66: 61-64.
- Farrell, D. J. (1996):* The problem and practicalities of producing an omega (n-3) fortified egg. World Poultry - Misset 12 (2):39-41.
- Fernandez, M. L.; Lin, E. C.K. and McNamare, D.J. (1992):* Regulation of guinea pig plasma low density lipoprotein kinetics by dietary fat saturation. J. Lipid Res. 33:97-109.

- Folch, J. ; Less, M. and Sloane Stanley, G. H. (1957):* A simple method for the isolation and purification of total lipids from animal. *J. Biol. Chem.* 226:497-509.
- Fortun, L. and Lebas, F. (1994):* Influence of the number of suckling young and feed level on foetal survival and growth in rabbit does. *Ann. Zootech.* 43: 163-171.
- Fringes, C. S.; Frendley, T. W.; Dunn, R. T and Queen, C. A. (1972):* Improved determination of serum lipids by the sulpho-phosphovanillin reaction. *Clin chem.* 18 (7):v673-674.
- Garg, M. L.; Sebokova, E.; Thomson, A. B. R. and Clandini, M. T. (1988):* $\Delta 6$ - Desaturation activity in liver microsomes of rats fed diets enriched with cholesterol and/or n-3 fatty acids. *Biochem. J.* 249:351-356.
- Halminiski, M. A.; Marsh, J. B. and Harrison, E. H. (1991):* Differential effects of fish oil, safflower oil and palm oil on fatty acid oxidation and glycerolipid synthesis in rat liver. *J. Nutr.* 121: 1554-1561.
- Harris, W. (1989):* Fish - oil and plasma lipid and lipoprotein metabolism in humans: a critical review. *J. Lipid. Res.* 30: 786-807.
- Huang, Y.S., Wainwright, P. F., Redden, R.R., Mills, D.E., Bulman-Fleming, B. and Horrobin, D.F. (1992):* Effect of maternal dietary fats with variable n-3/ n-6 rations on tissue fatty acid composition in suckling mice. *Lipids.* 27:104-110.
- Huff, M. W. and Telford, D. E. (1989):* Dietary fish oil increase the conversion of VLDL apo- β to LDL apoB Artherosclerosis. 9: 58-66.
- Lopes-Virella, M. F. Stone, P.; Ellis, S. and Colwell, J. A. (1977):* Cholesterol determination in high density lipoproteins separated by the three different methods. *Clin. Chem.* 23 (5): 882-884.
- March, B. E. and Amin, M. (1981):* Dietary lime stone versus -extra dietary oyster shell as calcium supplement to different layer diets. *Poultry. Sci.* 60: 591-597.
- March, J. B.; Topping, D. L. and Nestl, P. J. (1987):* Comparative effects of dietary fish oil and carbohydrate on plasma lipids and hepatic activities of phosphatidate phosphhydrolase, Diacylglycerol acyltransferase and neutral lipase activities in the rat. *Biochem. Biophys. Acta* 922: 239-243.
- March, B.E. and MacMillan, C. (1990):* Lionleic acid as a mediator of egg size *Poultry. Sci.* 69:634-639.

- Nestel, P. L.; Cammor, W. E.; Reardon, M. F. Cammor, S. Wong S. and Boston, R. (1984):* Suppression by diets rich in fish oil of very low density lipoprotein production in man. *J. Clin Invest.* 74: 82-89.
- National Research Council (1984):* Nutrient Requirements of poultry, 8th Revised Ed. Washington, D.C., National Academy of Sciences.
- Popp-Snijders, G. ; Schouten, J. A.; Heine, R. J.; Van Der Meer, J. and Van Der Venn, E. A. (1987):* Dietary Supplementation of omega-3 polyunsaturated fatty acids improve insulin sensitivity in non- insulin - dependent Diabetes. *diabetes Res.* 4: 141 - 147.
- Radwan, A. A. ; El- Aggoury, S. A. ; Gado, M. S. El- Gendi, G. M. (1989):* Some factors affecting serum calcium and phosphorus levels during the time of egg formation in relation to egg shell quality. 3rd Egyptian British Conference on: Animal, Fish and Poultry Production. Alex. October 1989. 859-868.
- Ruhling, K. ; Heller, R. ; Schauer, I. Schauer, U. J. W. and Thielmann, K. (1984):* Effect of vitamin C on plasma lipid metabolism. *Deutsche-Gesundheits wesen,* 39 (6): 231- 233. (Cited by Nut . Abstract and Review Series A.
- Rustan, A. C.; Nossen, J. O.; Christiansen, E.N. and Drevon, C. A. (1988):* EPA reduces hepatic synthesis and secretion of triacylglycerol by decreasing the activity of acyl-coenzyme A: 1,2 - diacylglycerol acyl transferase *J. Lipid Res.* 29: 1417 - 1426.
- SAS Institute (1994):* SAS / SIAT^R user's guide: Statistics. Ver. 6.04. Fourth Edition. SAS Institute Inc., Cary, NC.
- Schmit, A.J and Wynne, B. (1966):* Relative elution temperature. A simple method for measuring peaks retention in temperature programmed gas chromatography. *J. Chromatogr.* 4:325-332.
- Sell, J. L.; Horani, F. G. and Johnson, R. L. (1976):* The extra-caloric effect of fat in laying hen rations. *Foodstuffs,* 48: 29-39.
- Shepherd, J.; Packard, G.J.; Grundy, S. M. Yeshurm, D.; Gotto, A. M.Jr. and Taunton, O. D. (1980):* Effects of saturated and polyunsaturated fat diets on the chemical composition and metabolism of low density lipoprotein in man *J. Lipids Res.* 21: 91-99.
- Sidney, P.G. and Bernard, R. (1973):* Improved manual spectrophotometric procedure for determination of serum triglycerides. *Clin. Chem.* 19 (9): 1077-1078.

- Venkatraman, J. T.; Toohey, T. and Clandinin, M. T. (1992):* Does a threshold for the effect of dietary omega -3 fatty acids on the fatty acid composition of nuclear envelope phospholipids exist. *Lipid.* 27: 94-97.
- Wainwright, P.E.; Huang, Y. S.; Bulman-Fleming, B. Dalby, D. ; Mills, D. E.; Redden, P. and McCutcheon, D. (1992):* The effect of dietary n-3/n-6 ratio on brain development in the mousies: a dose response study with long- chain n-3 fatty acids. *Lipids.* 27:98-103.
- Wong, S. H. and Marsh, J. B. (1988):* Inhibition of apolipoprotein synthesis and phosphatidate phosphohydrolase by eicosapentaenoic and docosahexaenoic acid in the perfused rat liver. *Metabolism.* 37:1177-1181.
- Wong, S., Reardon, M. and Nestel, P. (1985):* Reduced triacylglycerol formation from long-chain polyenoic fatty acids in rat hepatocytes. *Metabolism.* 34: 900-905.
- Xiccato, G., Paigi-Bini, R., Dalle-Zotte, A., Carazzol, A. and Cossu, M.E. (1995):* Effect of dietary energy level, addition of fat and phosiological state on performance and energy balance of lactating and pregnant rabbit does. *British Society of animal Science*, 61: 387-398.
- Yamazaki, R. K. ; Shen, T. and Schade, G. B. (1987):* A diet rich in n-3 fatty acids increases peroxisomal beta-oxidation activity and lowers plasma triacylglycerols without inhibiting glutathione- dependent detoxification activity in the rat liver. *Biochem, Biophys. Acta* 920:62-67.
- Yoa, Z. and Vance, D.E. (1988):* The active synthesis of phosphotidylcholine is required for very low density lipoprotein secretion from rat hepatocytes. *J. Biol. Chem.* 263:2998-3004.
- Zak, B., Dichenman, R. Whitsee, E., Burnet, H. and Cherney, P. (1954):* Rapid estimation of free and total cholesterol. *Am. J. Path.* 124-130.

Table 3

**Effect of supplemented cod liver oil (Active EPA-30)
on the performance and egg quality of fayoumi pullets**

Parameters	Control (basal diet) Mean \pm S.E	Repletion diets (Active EPA-30)	
		Diet (1) Mean \pm S.E	Diet (2) Mean \pm S.E
Initial body weight (Kg)	0.959 \pm 0.04 ^A	0.957 \pm 0.08 ^A	0.965 \pm 0.06 ^A
Final body weight (Kg)	1.041 \pm 0.05 ^B	1.096 \pm 0.05 ^B	1.170 \pm 0.03 ^A
Average egg weight (gm)	37.88 \pm 3.12	40.1 \pm 1.74 ^A	42.09 \pm 2.50 ^A
Average egg No. (%)	41.44 \pm 0.84 ^B	45.18 \pm 0.52 ^{AB}	48.03 \pm 2.11 ^A
Shell thickness (mm)	0.032 \pm 0.001 ^A	0.0329 \pm 0.001 ^A	0.0327 \pm 0.001 ^A
Albumin weight (mg)	19.92 \pm 2.40 ^A	20.26 \pm 3.63 ^A	19.51 \pm 2.60 ^A
Yolk weight (mg)	17.97 \pm 1.14 ^B	19.96 \pm 2.01 ^{AB}	22.58 \pm 2.17 ^A
Yolk / Albumin	0.912 \pm 0.09 ^A	1.045 \pm 0.33 ^A	1.193 \pm 0.27 ^A
Food conversion	2.65 \pm 0.03 ^A	2.42 \pm 0.05 ^B	2.31 \pm 0.01 ^C

Basal diet, mixture of 3% cotton seed oil and 0.85% other oils

Repletion diet (1) mixture of 1.5% active EPA-30 + 1.5% cotton seed oil + 0.85% other oils

Repletion diet (2) mixture of 1.5% active EPA-30 + 0.85% other oils.

The main values are the average of 10 specimens. Means within the same column with the same superscript are not significantly different at ($P \leq 0.01$)

Table 4

Effect of cod liver oil (active EPA-30) supplementation on plasma lipids and lipoproteins in Fayoumi pullets.

mg/dl	Control Basal diet Mean \pm S.E.	Repletion diets (Active EPA-30)	
		Diet (1) Mean \pm S.E	Diet (2) Mean \pm S.E
Total lipid	558.52 \pm 9.19 ^A	507.22 \pm 4.48 ^B	522.67 \pm 6.20 ^C
Phospholipids	142.82 \pm 2.12 ^C	171.26 \pm 2.44 ^B	183.81 \pm 4.32 ^A
Triacylglycerol	171.43 \pm 2.30 ^A	137.58 \pm 2.06 ^B	100.78 \pm 3.68 ^C
Cholesterol	243.82 \pm 8.27 ^A	198.38 \pm 4.61 ^B	195.88 \pm 2.36 ^B
High density lipoprotein (HDL)	73.24 \pm 2.08 ^C	99.44 \pm 3.27 ^B	138.48 \pm 3.20 ^A
Low density lipoprotein (LDL)	136.39 \pm 6.70 ^A	68.37 \pm 4.47 ^B	38.10 \pm 0.75 ^C
Very low density lipoprotein (VLDL)	34.29 \pm 0.46 ^A	27.52 \pm 0.41 ^B	20.20 \pm 0.75 ^C

Basal diet, mixture of 3% cotton seed oil and 0.85% other oils

Repletion diet (1) mixture of 1.5% active EPA-30 + 1.5% cotton seed oil + 0.85% other oils

Repletion diet (2) mixture of 1.5% active EPA-30 + 0.85% other oils.

The given values are the average of 6 specimens. Means within the same column with the same superscript are not significantly different at (P \leq 0.01)

Table 5

**Effect of cod liver oil (Active EPA-30) supplementation
on the fatty acid composition of the Fayoumi pullets liver**

Fatty acids % w/w	Control Basal diet Mean + S.E	Repletion diets (Active EPA-30)	
		Diet (1) Mean ± S.E	Diet (2) Mean ± S.E
16:0	25.65 ± 1.05 ^B	23.62 ± 1.12 ^B	30.61 ± 0.68 ^A
18:0	13.33 ± 1.74 ^B	17.43 ± 1.12 ^A	13.42 ± 0.87 ^B
T.SFA	38.98 ± 1.31 ^B	41.03 ± 0.52 ^B	44.01 ± 0.32 ^A
16:1 n-7	2.28 ± 0.54 ^A	0.95 ± 0.27 ^B	0.06 ± 0.02 ^B
18:1 n-9	29.59 ± 1.58 ^A	25.70 ± 1.24 ^A	27.36 ± 1.11 ^A
T.MUFA	31.87 ± 1.99 ^A	26.64 ± 1.37 ^B	27.36 ± 0.68 ^{AB}
18:2 n-6	11.09 ± 1.34 ^A	10.35 ± 0.89 ^A	11.23 ± 0.68 ^B
18:3 n-3	0.17 ± 0.05 ^A	0.45 ± 0.04 ^A	0.35 ± 0.05 ^B
20:4 n-6	13.78 ± 0.51 ^A	13.55 ± 1.19 ^A	8.96 ± 0.59 ^B
20:5 n-3	0.58 ± 0.14 ^B	1.31 ± 0.12 ^A	1.17 ± 0.12 ^A
22:6 n-3	2.13 ± 0.24 ^C	4.33 ± 0.22 ^B	5.74 ± 0.19 ^A
T.PUFA	28.27 ± 1.39 ^A	29.97 ± 0.98 ^A	27.12 ± 0.93 ^A
n-3	2.90 ± 0.27 ^C	6.08 ± 0.25 ^B	7.26 ± 0.19 ^A
n-6	24.87 ± 1.49 ^A	23.89 ± 0.74 ^A	20.19 ± 0.79 ^B
n-3/n-6	0.12 ± 0.01 ^C	0.25 ± 0.004 ^B	0.34 ± 0.01 ^A
Sat/Unsat	0.652 ± 0.04 ^C	0.725 ± 0.02 ^B	0.808 ± 0.01 ^A

Basal diet, mixture of 3% cotton seed oil and 0.85% other oils

Repletion diet (1) mixture of 1.5% active EPA-30 + 1.5% cotton seed oil + 0.85% other oils

Repletion diet (2) mixture of 1.5% active EPA-30 + 0.85% other oils.

The main values are the average of 6 specimens. Means within the same column with the same superscript are not significantly different at ($P \leq 0.01$). Only the most common fatty acid are listed.

Table 6

**Effect of cod- liver oil (active EPA-30) supplementation
on the fatty acid composition of the hen's egg yolk**

Fatty acid patterns (w/w%)	Control Basal diet	Repletion diets (Active EPA-30)	
		Diet (1) Mean \pm S.E	Diet (2) Mean \pm S.E
16:0	25.25 \pm 0.83 ^B	26.96 \pm 0.65 ^B	32.06 \pm 0.99 ^A
18:0	9.99 \pm 0.67 ^A	9.11 \pm 0.43 ^A	9.70 \pm 0.57 ^A
T.SFA	37.84 \pm 0.94 ^A	36.07 \pm 0.77 ^A	41.81 \pm 0.87 ^A
16:1 n-7	4.11 \pm 0.50 ^A	4.50 \pm 0.26 ^A	2.48 \pm 0.31 ^B
18:1 n-9	43.16 \pm 1.50 ^A	40.16 \pm 0.51 ^A	32.78 \pm 1.06 ^B
T.MUFA	47.26 \pm 1.89 ^B	44.68 \pm 0.56 ^B	35.27 \pm 1.14 ^A
18:2 n-6	10.78 \pm 0.46 ^B	10.72 \pm 0.42 ^B	12.61 \pm 0.54 ^A
18:3 n-3	0.21 \pm 0.03 ^B	0.36 \pm 0.05 ^A	0.26 \pm 0.03 ^{AB}
20:4 n-6	3.96 \pm 0.31 ^A	2.61 \pm 0.14 ^B	3.03 \pm 0.20 ^B
20:5 n-3	0.22 \pm 0.04 ^B	0.36 \pm 0.03 ^A	0.62 \pm 0.05 ^A
22:6 n-3	1.76 \pm 0.12 ^C	4.67 \pm 0.23 ^B	6.60 \pm 0.19 ^A
T.PUFA	16.89 \pm 0.75 ^C	18.99 \pm 0.49 ^B	22.88 \pm 0.57 ^A
n-3	2.19 \pm 0.12 ^C	5.71 \pm 0.23 ^B	7.53 \pm 0.18 ^A
n-6	14.95 \pm 0.72 ^A	13.24 \pm 0.33 ^B	15.62 \pm 0.50 ^A
n-3/n-6	0.15 \pm 0.01 ^C	0.43 \pm 0.02 ^B	0.48 \pm 0.01 ^A
Sat/Unsat	0.538 \pm 0.03 ^B	0.570 \pm 0.02 ^B	0.722 \pm 0.02 ^A

Basal diet, mixture of 3% cotton seed oil and 0.85% other oils

Repletion diet (1) mixture of 1.5% active EPA-30 + 1.5% cotton seed oil + 0.85% other oils

Repletion diet (2) mixture of 1.5% active EPA-30 + 0.85% other oils.

The given values are the average of 10 specimens. Means within the same column with the same superscript are not significantly different at ($P \leq 0.01$). Only the most common fatty acid are listed.

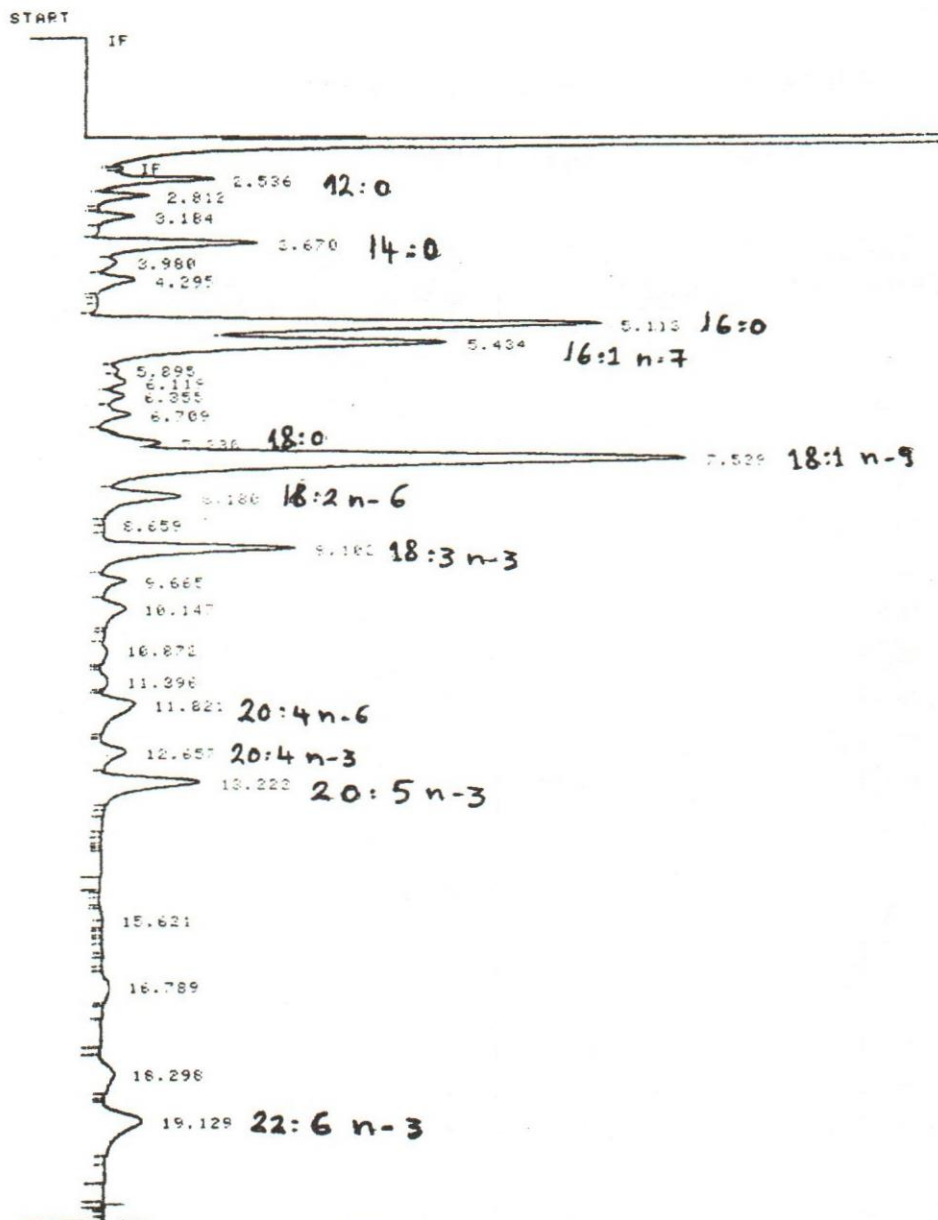


Fig. 1.

Fatty acid patterns (Standard)

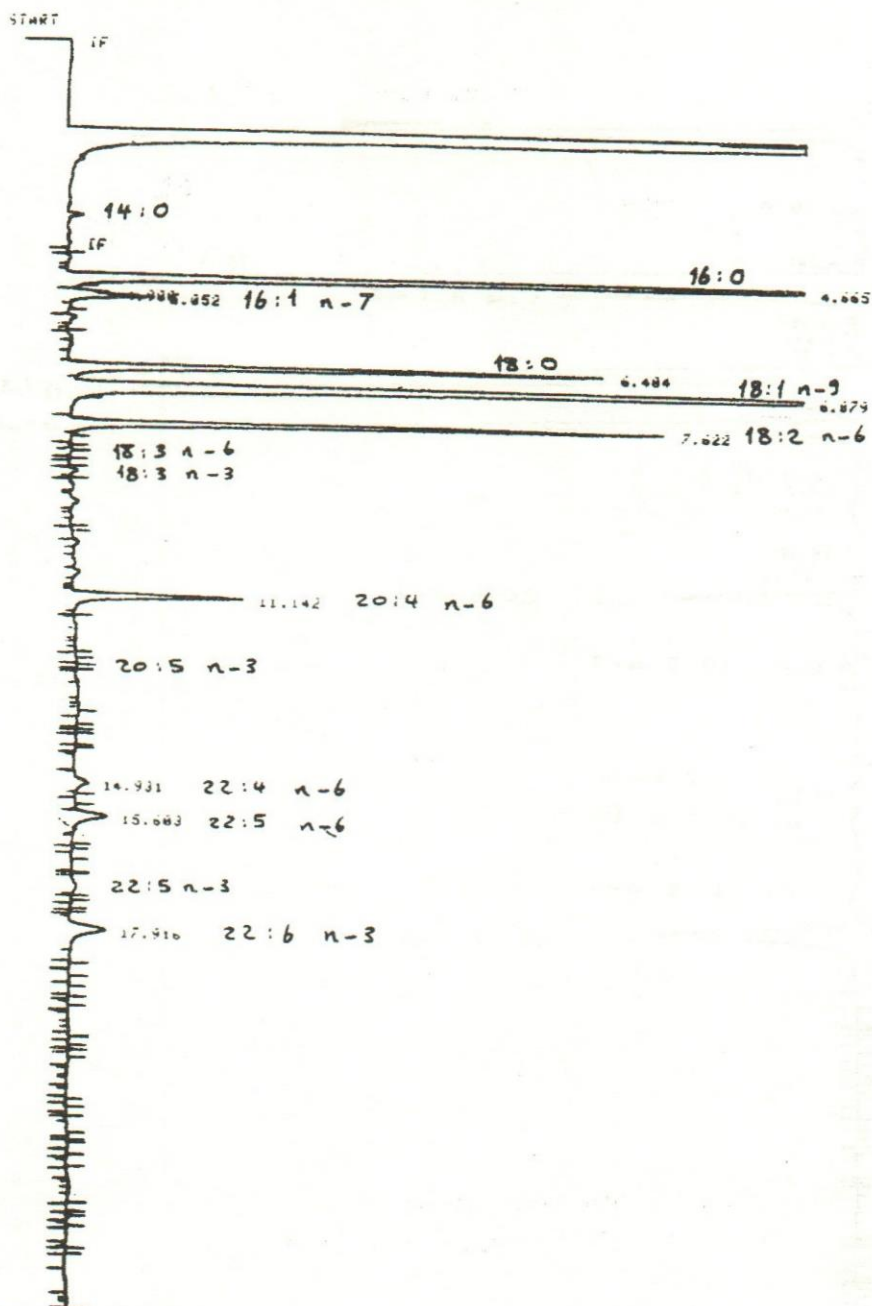


Fig. 2.

Fatty acid composition of egg yolk in hens fed on the basal diet

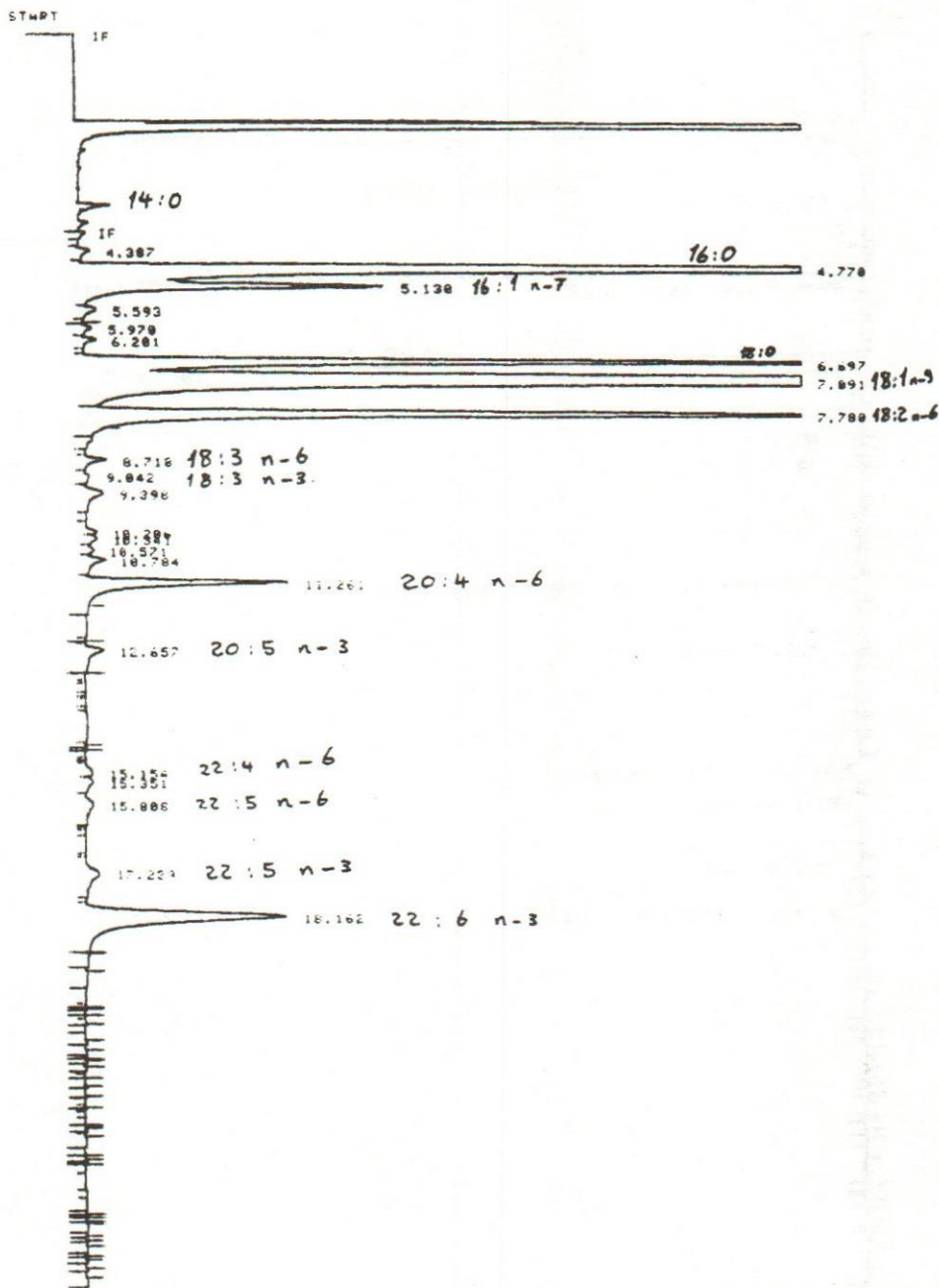


Fig. 3.

Fatty acid composition of egg yolk in hens fed on the fish-oil supplemented diet