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Efficiency of PUFAs Incorporation from Marine Sources in Yolk Egg's Laying Hens

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Abstract: Two hundred eighty-eight 32-wk-old Hisex White laying hens were used in this research during a 10 week period, arranged in a 2 x 5 completely randomized factorial design, with three replicates of eight birds per treatment. Two groups: fish oil (OP) and Marine Algae (AM) with five DHA levels (120, 180, 240, 300 and 360 mg/100 g diet) were assigned including two control groups, birds fed corn and soybean basal diet (CON) and a diet supplemented with AM (AM420) to study the efficiency of egg yolk fatty acid enrichment. The Arachidonic Acid (AA), Linoleic (LA) and PUFAs n-6 mean values ranged, respectively, 98.71 mg, 987.70 mg and 1108.92 mg/yolk for the hens fed the CON diet and 38.87 mg, 734.22 mg and 802.79 mg/yolk for the OP360 group. The percentage of AA incorporation (% INC) in egg yolk decreased linearly with the increase of DHA levels in the diets supplemented with OP and AM, from 4.81% (CON) to 2.57% (OP360) and 3.51% (AM420). The efficiency (%) of DHA incorporation into the yolk decreased linearly with increasing of DHA levels in the diet supplemented with OP and AM, from 85.11% (OP120) and 65.28% (AM120) to 49.45% (OP360) and 34.06% (AM420). The levels of DHA in the egg yolk of birds supplemented with OP had significant increase from 22.64 mg/yolk (CON) to 187.91 mg/yolk in OP360 group. PUFAs n-3 means into the eggs were significantly ($p<0.05$) increased when the groups CON (62.16 mg/yolk) and OP360 (218.62 mg/yolk) were compared.

Key words: PUFAs n-3, hens' eggs, DHA, fish oil, marine algae, PUFAs n-6

INTRODUCTION

The main components of the hen's egg are: water (74%), protein (13%), fat (11%), carbohydrates (1%) and other nutrients with minor percentage including minerals, vitamins and carotenoids (Romanoff and Romanoff, 1949). Egg's macrostructure is approximately 9-12% of shell, 60% of albumen and 30-33% of yolk (Stadelman, 1995). Yolk lipid content is around 33% (Cotteril and Geiger, 1977) including 63.3% of triacylglycerols, 29.7% of phospholipids (phosphatidylcholine: 73%; phosphatidiletanolamine: 15%) and 5.2% of total cholesterol (Leskanich and Noble, 1997).

Currently, selected lines of commercial laying hens of *Gallus gallus domesticus* L. genus produce more than 328 eggs per laying cycle (Scheuermann and Bellaver, 1995) and egg weight mean around 60 grams (Li-Chan *et al*., 1995). It is necessary to balance diet to get equilibrium of protein, carbohydrates, lipids, vitamins and minerals to increase the intake of macro and micro essential nutrients for maintenance and formation of laying hen (Stadelman and Pratt, 1989). If we consider the egg as a vital cell, which will generate the embryo, it must contains every necessary nutrient to develop a new life (Griminger, 1986; Etches, 1996).

Nutritionally, egg is considered strategic for modification of human and animal diet leading to development food with functional or nutraceutical properties. Surai and Sparks (2001) and Zeidler (1998) considered that benefits of improving egg quality with increasing polyunsaturated fatty acids omega-3 (PUFAs n-3), vitamin E, carotenoids and selenium concentrations were reflected on research results aiming to enrich eggs. Yolk has high fat quantity, this fat may have changed the chemical composition of its fatty acids (Yu and Sim, 1987; Griffin, 1992; Sim, 2000). Knowledge of these properties is important, because can allow adoption of strategies to modify fatty acids characteristics.

Some of these fatty acids, called long chain PUFAs of n-3 series, eicosapentaenoic (EPA, $C_{20:5n-3}$) and Docosahexaenoic (DHA), ($C_{22:6n-3}$), participate as constituents of biological membranes and organelle cells, acting important biochemical functions at molecular level how cell-to-cell signaling and precursors of substances with hormonal regulators properties autocrine and paracrine (Phetteplace and Watkins, 1989). Prostaglandins, thromboxanes and leukotrienes of series 3 and 5 come from EPA. Brain normal development and prevention of many diseases as Cardiovascular Diseases (CVD), diabetes, lupus, psoriasis, cancer (Nettleton, 1995) and visual disturbances (Neuringer *et al*., 1984) are related to the increase of EPA and DHA in the diet.

Surai and Sparks (2001) reported eggs fortification with 200 mg/yolk of PUFAs n-3. Hargis *et al.* (1991) used 3% of fish oil and obtained over 180 mg/egg of EPA + DHA. Abril and Barclay (1998) used 300 mg and 600 mg DHA from marine microalgae/hen/day and enriched eggs showed 172.8 and 243 mg of total PUFA n-3, respectively. Herber and Van Elswyk (1996) supplemented laying hens diet with 2.4% and 4.8% of marine microalgae *Schizochytrium* sp. and fortified eggs showed 9.5 mg/g and 11.5 mg/g of PUFAs n-3 yolk, respectively.

This research aimed to study the influence of diet supplementation with rising quantity of fish oil and marine algae levels on yolk's PUFAs n-3 profile and the efficiency of DHA incorporation egg laying hens subjected to corn and soybean meal basal diet.

MATERIALS AND METHODS

The research was conducted in the experimental poultry vivarium of the School of Veterinary Medicine and Animal Science of University of São Paulo. In this study, a randomized design with 288 Hisex White laying hens with 32 weeks of age, distributed in 12 treatments with three replicates of eight birds, housed in cages of 0.45 m x 0.25 m x 0.45 m, with two birds per cage. Feed and water were provided ad libitum with chicken feeder and nipple drinker. Hens received 16 h of light daily.

Experimental diets: Experimental diets of 12 treatments administered to laying hens, isocaloric and isonitrogenous, were formulated in accordance with requirements set by the NRC (1994). The control group was composed of corn and soybean meal basal diet, while diets to others treatments were supplemented with salmon oil (OP) or Marine Algae (AM) (Table 1). Treatments from two to six DHA increasing levels were added from salmon oil (*Salmo salar*) at concentrations of 120 mg (0.80% OP), 180 mg (1.20% OP), 240 mg (1.60% OP), 300 mg (2.00% OP) and 360 mg DHA/100 g diet (2.40% OP), while those of seven to twelve contained marine algae (*Schizochytrium* sp) percentage rising of 120 mg (0.50% AM), 180 mg (0.75%), 240 mg (1.00% AM), 300 mg (1.25% AM), 360 mg (1.50% AM) and 420 mg DHA/100 g of feed (1.75% AM). OP (salmon) and AM were added of 200 ppm of antioxidant butyl hydroxy toluene.

Composition of total PUFAs n-3 series from sources of fish oil (OP) and Marine Algae (MA) were, respectively: EPA ($C_{20:5\ n-3}$) -11.44% and 2.74% , DPA ($C_{22:5\ n-3}$) - 4.43% and 17.08%, DHA ($C_{22:6\ n-3}$) -15.03% and 42.38%. Relations of n-6/n-3 total from sources OP and AM were of 0.24 and 0.05, respectively. Fish oil (salmon) was added of antioxidant, Butyl-hydroxy-toluene (BHT), in amount of 200 mg/kg. Marine algae's ether extract was 56.20%. Marine algae and corn oil contained antioxidant added by the supplier.

Analysis of fatty acids of egg yolk: In the eighth week trial, four eggs were collected by replicate. Yolks were separated and we obtained weight per unit. After, buds were homogenized in order to obtain a sample for repetition (pool made up of four buds). This way was obtain three samples per treatment. Tests were conducted from a gram of fresh and raw yolk in each sample using the methodology described by Bligh and Dyer (1959), Folch *et al.* (1957), modified by Nielsen (1998) and AOAC (1970). Technique described by Hartman and Lago (1973) was utilized to saponification of lipid extract and to obtain fatty acid esters of samples. Then, the sample was solubilized with hexane and proceeded with injection of 1 (one) mL of solution for determining fatty acid methyl-esters profile using gas chromatography technique.

For evaluation of fatty acid profiles of AM, fish oil and corn oil of diets and yolk (Table 1-6) used the gas chromatography technique with utilize of Varian CP 3800 chromatograph equipped with flame ionization detector and connected to the system "Workstation Star Chromatography". We applied capillary column of fused silica, CP-WAX 52CB (Chrompack), long 30 m, diameter 0.25 mm and 0.25 µm of polyethylene. Operating conditions were: injection "split", 50:1 ratio, column temperature: 150°C for 15 min, set up to 210°C in a ratio of 3°C per minute; carrier gas: nitrogen with a flow rate of 1,5 mL per minute; gas "make-up": Nitrogen 30 mL per minute; injector temperature: 250°C; detector temperature: 280°C. External standards were used containing the profile of fatty acid methyl-esters 189-19 of Supelco®_. Were used internal standard ethyl ester of docosahexaenoic acid Sigma®_cis - 4.7.10.13.16.19 to 99% (D-2661, 10mg).

Statistical analysis: For statistical evaluation of results was used a factorial arrangement design with three replicates per treatment according to the procedures described by Snedecor and Cochran (1967), using two criteria for classification [sources of polyunsaturated fatty acids: fish oil (OP) and Marine Algae (AM)] and levels of Docosahexaenoic Acid (DHA) in diets containing fish oil and marine algae: 120, 180, 240, 300 and 360 mg/100 g. In order to compare treatments previously mentioned with the control group formed by birds fed with corn and soybeans basal diet and with an other group consisting of hens subjected to diet containing AM content of 420 mg of DHA/100 g in diet has been established an analyze of variance involving total of twelve treatments. The Tukey test was applied to analyze the difference between means. Statistical analysis was performed using the software "Statistical Analysis System (SAS, 1994) adopting the level of 5% significance.

RESULTS

Results showed changes in the fatty acid composition of yolk. expressed in percentage of total fatty acids, for total

Table 1: Composition of experimental diets

Treatment	CON	OP120	OP180	OP240	OP300	OP360	AM120	AM180	AM240	AM300	AM360	AM420
Ingredients (%)												
Corn meal	53.79	52.53	52.53	52.53	52.53	52.53	51.61	51.68	51.76	52.06	52.42	52.78
Soybean meal	28.3	27.85	27.85	27.85	27.85	27.85	27.79	27.68	27.57	27.62	27.71	27.8
Wheat meal	3.17	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	4.45	3.75	3.05
Corn oil	3.00	2.20	1.80	1.40	1.00	0.60	3.00	3.00	3.00	3.00	3.00	3.00
Fish oil	-	0.80	1.20	1.60	2.00	2.40	-	-	-	-	-	-
Marine algae	-	-	-	-	-	-	0.5	0.75	1	1.25	1.5	1.75
DL- methionine	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27
Choline chloride	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12
Sodium chloride	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Limestone	9.60	9.48	9.48	9.48	9.48	9.48	9.96	9.75	9.53	9.48	9.48	9.48
Dicalcium phosphate	1.30	1.30	1.30	1.30	1.30	1.30	1.30	1.30	1.30	1.30	1.30	1.30
Vitamin premix	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Mineral premix	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Analysis determinated												
Ether extract (%)	4.01	4.06	4.12	4.43	4.15	4.2	4.46	4.6	4.63	4.74	4.8	5.28
Eicosapentaenoic acid (EPA,%)	-	1.84	2.73	4.00	5.46	6.26	0.21	0.20	0.05	0.29	0.28	0.39
Docosapentaenoic acid (DPA, %)	-	0.09	0.1	0.12	0.17	0.15	0.5	1.05	1.05	2.17	1.82	2.79
Docosahexaenoic acid (DHA, %)	-	2.69	4.11	5.18	6.95	7.44	1.62	2.9	2.87	3.68	4.73	7.18
Analysis calculated												
Metabolizable energy (kcal/kg)	2750	2750	2750	2750	2750	2750	2750	2750	2750	2750	2750	2750
Crude protein (%)	17	17	17	17	17	17	17	17	17	17	17	17
Methionine (%)	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55	0.55
Methionine + Cystine (%)	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84	0.84
Calcium (%)	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Phosphorus total (%)	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.61
Phosphorus available (%)	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37
Crude fiber (%)	3.40	3.48	3.48	3.48	3.48	3.48	3.46	3.45	3.44	3.41	3.37	3.36

*Vitamin premix supplies (per kg of diet): vitamin A, 8000 IU, vitamin D3, 2500 IU, vitamin E, 10 mg, vitamin K3, 2.5 mg, thiamine, 1 mg, riboflavin, 5 mg, pyridoxine, 1.5 mg, 0.5 mg folic acid, vitamin B12, 12 mcg, nicotinic acid, 25 mg, pantothenic acid, 8 mg; methionine, 0.9 g choline, 0.1 g. **Mineral premix supplies (per kg of diet): iron, 50 mg; copper, 10 mg, zinc, 50 mg, manganese, 80 mg, iodine, 1 mg, cobalt, 1 mg, selenium, 0.15 mg

saturated fatty acids (SAT.%), monounsaturated (MUFAs, %). polyunsaturated (PUFAs, %). PUFAs n-3 and n-6 (%) and the n-6/n-3 ratio Polyunsaturated:Saturated (P/S), among treatments. OP's and AM's sources and levels studied according to yolk weight. total lipids (%) and yolk fat influenced by addition of increasing levels of DHA are presented in Table 3-6.

The highest concentrations in increasing order of SAT into egg yolk were myristic<stearic<palmitic and only the stearic acid ($C_{18:0}$) did not show significant differences among treatments judged by variance analysis.

Total levels of SAT (%) in the yolk were not influenced significantly by addition of DHA increasing levels. In turn, the highest significantly mean was obtained with the use of OP source (34.79%) compared to that reported with the use of MA (33.49%) (Table 4).

Among the three MUFAs in the yolk, investigated in this study (Table 5), only the variable quantity of 0.24-0.29% acid gadoleic ($C_{20:1n-9}$) did not show significant differences between treatments judged by variance analysis, MUFAs concentrations in ascending order were gadoleic<palmitoleic<oleic.

For the oleic acid. the fatty acid in more quantity into yolk, variance analysis presented significant differences between treatments being responsible for this contrasts those among OP180 (μ = 42.80%), AM360 (μ = 38.54%) and AM420 (μ = 38.02%). Total levels of MUFAs (%) into yolk were not influenced by OP's and AM's levels in the diet (Table 3). While, mean of 45.29% (OP) to total acids was higher ($p<0.05$) than mean of 42.28% (AM) for total MUFAs into yolk with OP source in diet.

There were means of major fatty acids into yolk with percentage of 13.21% of palmitic, 2.09% of stearic, 29.71% of oleic, 49.22% of linoleic, 1.67% of γ-linolenic and total percentage summation of 15.99% of SAT, 30.32% of MUFAs, 51.02% of PUFAs and 50.88% of PUFAs n-6 in the experimental diet on control diet (CON) (Table 2). Unlike on total lipids of yolk, means of 25.03% of palmitic, 2.61% of palmitoleic, 7.89% of stearic, 39.90% of oleic, 17.60% of linoleic, 1.76% of arachidonic, 33.21% of SAT, 42.78% of MUFA, 20.88% of PUFAs and 19.77% of PUFAs n-6 (Table 3) into egg yolk. Therefore, in accordance with the above measures we were seen increasing the percentage of palmitic, palmitoleic, stearic, oleic and PUFAs n-3 and sharp decrease of linoleic into yolk. In general, the increase of fatty acids in yolk in relation to percentages analyzed in the diet (Table 2) is due to: SAT: palmitic and stearic; MUFAs: palmitoleic and oleic and PUFAs n-3: EPA, DHA and DPA. Moreover, the reduction is due to total PUFAs mainly the linoleic (Table 3).

Table 2: Fatty acids Composition (% of total fatty acids) of experimental diets

Treatments	CON	OP120	OP180	OP240	OP300	OP360	AM120	AM180	AM240	AM300	AM360	AM420
OP (%)	0	0.80	1.20	1.60	2.00	2.40	0	0	0	0	0	0
AM (%)	0	0	0	0	0	0	0.50	0.75	1.00	1.25	1.50	1.75
	Fatty acid content (%)											
Myristic ($C_{14:0}$)	0.29	1.03	1.59	2.07	3.18	2.36	0.37	0.68	0.67	0.88	1.08	1.62
Palmitic ($C_{16:0}$)	13.21	13.51	14.53	14.61	16.85	15.69	13.24	13.69	14.17	14.60	14.89	15.96
Palmitoleic ($C_{16:1\ n-7}$)	0.27	1.77	2.35	3.07	4.47	3.34	0.26	0.13	0.12	0.14	0.12	0.13
Stearic ($C_{18:0}$)	2.09	2.45	2.76	2.90	3.00	2.97	2.14	2.05	2.02	2.01	2.05	1.80
Oleic ($C_{18:1\ n-9}$)	29.71	28.03	29.66	28.42	24.16	22.99	28.73	27.02	29.58	25.79	26.82	24.61
Linoleic ($C_{18:2\ n-6}$)	49.22	42.43	38.20	34.14	31.29	34.10	45.54	45.10	44.57	45.51	43.62	41.17
Alpha-linolenic ($C_{18:3\ n-3}$)	0.14	0.11	0.14	0.18	0.20	0.20	0.10	0.07	0.07	0.12	0.15	0.16
Gema- linolenic ($C_{18:3\ n-6}$)	1.67	1.90	1.78	2.00	2.24	2.30	2.18	1.56	1.65	1.66	1.43	1.35
Arachidic ($C_{20:0}$)	0.41	0.38	0.36	0.32	0.24	0.29	0.40	0.42	0.42	0.41	0.42	0.40
Gadoleic ($C_{20:1\ n-9}$)	0.30	0.67	0.88	0.86	0.95	0.89	0.36	0.30	0.33	0.35	0.32	0.30
Arachidonic ($C_{20:4\ n-6}$)	-	0.17	0.24	0.31	0.42	0.36	0.15	0.16	0.15	0.20	0.26	0.38
EPA ($C_{20:5\ n-3}$)	-	1.85	2.74	4.00	5.46	6.26	0.21	0.20	0.31	0.29	0.28	0.39
Erucic ($C_{22:1\ n-9}$)	0.05	0.33	0.23	0.34	-	0.38	0.41	0.36	0.18	0.23	0.16	0.12
Unidentified	2.64	2.59	0.34	1.50	0.41	0.29	3.78	4.31	1.82	1.96	1.84	1.62
DPA ($C_{22:5\ n-3}$)	-	0.10	0.10	0.12	0.18	0.15	0.50	1.05	1.04	2.17	1.82	2.79
DHA ($C_{22:6\ n-3}$)	-	2.69	4.11	5.18	6.95	7.44	1.63	2.89	2.87	3.68	4.73	7.18
Saturated total	15.99	17.37	19.23	19.89	23.27	21.32	16.15	16.83	17.29	17.90	18.45	19.79
Monounsaturated total	30.32	30.80	33.12	32.68	29.57	27.60	29.77	27.81	30.22	26.50	27.43	25.16
Polyunsaturated total	51.02	49.24	47.31	45.93	46.75	50.80	50.30	51.05	50.67	53.63	52.29	53.43
n-6 total	50.88	44.49	40.22	36.45	33.96	36.75	47.86	46.83	46.38	47.37	45.30	42.90
n-3 total	0.14	4.75	7.09	9.48	12.79	14.05	2.44	4.22	4.29	6.27	6.99	10.53
n-6/n-3 ratio	355.70	9.38	5.67	3.85	2.66	2.62	19.61	11.09	10.81	7.55	6.49	4.08
P/S ratio	3.19	2.84	2.46	2.31	2.01	2.38	3.12	3.03	2.93	3.00	2.83	2.70
Fat total	4.01	4.06	4.12	4.43	4.15	4.20	4.46	4.60	4.63	4.75	4.80	5.28

Experimental phase continued with pronounced change of fatty acid content into yolk in function of increased DHA levels of OP and AM sources in the diet of birds. The mean increased from 1.11% (CON) to 3.85% (OP360) and 3.18% (AM420) of PUFAs n-3 into yolk.

Marine sources influenced all fatty acids percentage except the acid linolenic (Table 4), being different ($p<0.05$) the total mean of 2.95% (OP) and 2.39% (AM) of PUFAs n-3 into yolk to analyzing sources.

At first sight, it was observed that the yolk contains around 20% of PUFAs acids despite the requirement of the diet, it showed levels around 50% to corn and soybean basal diet. After the addition of seafood sources rich in PUFAs, the incorporation of n-3 was done of increasing way a second phospholipids levels and that according to literature is found mainly in ethanolamine. As the phospholipid fraction is around 30% of total fatty acids this explains, in part, the predominance of PUFAs n-6 mainly to triacylglycerols fraction into yolk that occupies approximately 70% of fatty acids compounds in egg yolk. The PUFAs n-3 made up between 1.87% (AM120) and 3.85% (OP360) of total PUFAs fraction. The increase of PUFAs n-3 from OP and AM sources is due to PUFAs n-6, which major reductions were to linoleic acid and arachidonic acid in differences ($p<0.05$) observed among levels, sources and control treatment (Table 3 and 4).

The influence of sources and levels shows further reduction percentages into yolk to linoleic acid - 14.76% AL or 814.13 mg/yolk. arachidonic acid -0.89% AA or 49.04 mg/yolk. PUFAs -19.07% or 54.46 mg/g yolk. PUFAs n-6-6.11% or 888.50 mg/yolk and relations n-6/n-3 (5.79%) and P/S (0.54%) when add the OP source compared with AM source in the diet of birds (Table 4 and 6). Furthermore, the OP source was responsible for greater enrichment of 142.33 mg DHA/egg yolk and 163.29 mg PUFAs n-3/gema.

Major incorporation of 62.44% of DHA in OP source against 49.45% of DHA in AM source. The greater efficiency of DHA incorporation in yolk occurred at levels near the intake of 120 mg (75.19%) to 180 mg (68.39%) and the least to 240 mg (46.78%) DHA/100 g diet when we observed drastic reduction of consumption (Table 5, Fig. 1).

Consumption and incorporation means of OP source compared to AM source, were lower of 1572.11 mg PUFAs n-6/bird/day and lower of 3.12% AA, respectively. Paradoxically, the biggest incorporation mean of 52.40% (OP) and lower mean of 49.28% (AM) of AL into yolk (Table 6).

DHA mean (%) in egg yolk showed significant increases to mean values when increasing levels of DHA from OP source were add in diet of laying hens (Table 5). Moreover, increasing levels of DHA from AM source in the diet. DHA into yolk means showed differences ($p<0.05$) in relation to mean of 0.40% (CON) (Table 5). It was observed to DHA mean (%) a linear response with increasing levels of DHA from OP of AM sources to treatments studied and the opposite occurred with AA (Fig. 1).

Definitely, the sources influenced total PUFAs n-3 in egg yolk. Mean value of 2.95% (OP) of total PUFAs n-3

Table 3: Fatty acids Composition (% of total fatty acids) of the yolk, according to the treatments studied

Treatments	CON	OP120	OP180	OP240	OP300	OP360
OP (%)	0	0.80	1.20	1.60	2.00	2.40
AM (%)	0	0	0	0	0	0
Fatty acid	**---------- Fatty acid content (%) ----------**					
Myristic ($C_{14:0}$)	0.28^{e*}	0.33^{cde}	0.36^{bcde}	0.40^{bc}	0.42^{ab}	0.49^{a}
Palmitic ($C_{16:0}$)	25.03^{abc}	25.38^{abc}	25.27^{abc}	25.59^{abc}	26.27^{ab}	26.70^{a}
Palmitoleic ($C_{16:1\ n-7}$)	2.61^{bcd}	2.80^{abcd}	3.46^{a}	3.23^{abc}	3.73^{ab}	3.62^{a}
Stearic ($C_{18:0}$)	7.89^{a}	9.82^{a}	7.83^{a}	8.65^{a}	8.02^{a}	8.40^{a}
Oleic ($C_{18:1\ n-9}$)	39.90^{ab}	41.19^{ab}	42.80^{a}	41.59^{b}	41.34^{ab}	41.66^{ab}
Linoleic ($C_{18:2\ n-6}$)	17.60^{abcd}	15.83^{bcde}	14.96^{de}	15.39^{cde}	14.64^{de}	12.99^{e}
Alpha-linolenic ($C_{18:3\ n-3}$)	0.15^{a}	0.11^{a}	0.14^{a}	0.13^{a}	0.15^{a}	0.13^{a}
Gema-linolenic ($C_{18:3\ n-6}$)	0.41^{abc}	0.39^{abc}	0.43^{abc}	0.48^{ab}	0.47^{ab}	0.52^{a}
Gadoleic ($C_{20:1\ n-9}$)	0.28^{a}	0.27^{a}	0.28^{a}	0.29^{a}	0.28^{a}	0.29^{a}
Arachidonic ($C_{20:4\ n-6}$)	1.76^{a}	1.20^{abcd}	0.88^{bcd}	0.81^{cd}	0.87^{bcd}	0.69^{d}
EPA ($C_{20:5\ n-3}$)	0^{e}	0.08^{ed}	0.14^{cd}	0.17^{bc}	0.25^{b}	0.35^{a}
DPA ($C_{22:5\ n-3}$)	0.56^{a}	0.07^{d}	0.05^{d}	0.04^{d}	0.02^{d}	0.06^{d}
DHA ($C_{22:6\ n-3}$)	0.40^{f}	1.87^{de}	2.29^{cd}	2.43^{bcd}	2.99^{ab}	3.32^{a}
Saturated total (S)	33.21^{a}	35.53^{a}	33.46^{a}	34.64^{a}	34.72^{a}	35.60^{a}
Monounsaturated total (M)	42.78^{abcd}	42.26^{abcd}	46.54^{a}	45.10^{abc}	45.00^{abc}	40.68^{d}
Polyunsaturated total (P)	20.88^{abc}	19.56^{bc}	18.88^{c}	19.47^{bc}	19.39^{bc}	18.06^{c}
n-6 total	19.77^{abc}	17.42^{bcd}	16.26^{d}	16.69^{cd}	15.97^{d}	20.19^{ab}
n-3 total	1.11^{f}	2.13^{de}	2.62^{cd}	2.77^{bcd}	3.41^{ab}	3.85 ^{a}
n-6/n-3 ratio	17.50^{a}	8.17^{cd}	6.39^{de}	6.02^{def}	4.70^{f}	3.72^{f}
P/S ratio	0.63^{abc}	0.55^{bc}	0.56^{bc}	0.56^{bc}	0.56^{bc}	0.51^{c}
	---------- Means of PUFAs in the yolk (mg/yolk) ----------					
AA	98.71^{a}	66.67^{abcd}	49.70^{bcd}	43.66^{cd}	46.29^{bcd}	38.87^{d}
DHA	22.64^{f}	104.80^{cde}	129.58^{bcd}	130.78^{bcd}	158.61^{ab}	187.91^{a}n-3
Total	62.16^{e}	119.54^{cde}	148.16^{bcd}	149.39^{bcd}	181.15^{ab}	218.22^{a}
Treatments	AM120	AM180	AM240	AM300	AM360	AM420
OP (%)	0	0	0	0	0	0
AM (%)	0.50	0.75	1.00	1.25	1.50	1.75
Fatty acid	**---------- Fatty acid content (%) ----------**					
Myristic ($C_{14:0}$)	0.30^{ed}	0.37^{bcd}	0.33^{cde}	0.36^{bcde}	0.42^{ab}	0.43^{ab}
Palmitic ($C_{16:0}$)	24.18^{c}	25.94^{abc}	24.47^{bc}	25.62^{abc}	25.57^{abc}	25.81^{abc}
Palmitoleic ($C_{16:1\ n-7}$)	2.37^{d}	2.48^{cd}	2.43^{cd}	2.25^{d}	2.53^{cd}	2.42^{cd}
Stearic ($C_{18:0}$)	8.11^{a}	7.82^{a}	7.90^{a}	8.00^{a}	8.03^{a}	8.89^{a}
Oleic ($C_{18:1\ n-9}$)	40.62^{ab}	38.98^{ab}	40.83^{ab}	39.14^{ab}	38.54^{b}	38.02^{b}
Linoleic ($C_{18:2\ n-6}$)	19.03^{a}	18.21^{abc}	18.46^{abc}	18.41^{abc}	18.71^{ab}	18.42^{abc}
Alpha-linolenic ($C_{18:3\ n-3}$)	0.12^{a}	0.13^{a}	0.11^{a}	0.14^{a}	0.12^{a}	0.13^{a}
Gema-linolenic ($C_{18:3\ n-6}$)	0.32^{c}	0.39^{abc}	0.32^{c}	0.36^{bc}	0.40^{abc}	0.36^{bc}
Gadoleic ($C_{20:1\ n-9}$)	0.27^{a}	0.26^{a}	0.25^{a}	0.26^{a}	0.24^{a}	0.24^{a}
Arachidonic ($C_{20:4\ n-6}$)	1.73^{a}	1.53^{a}	1.51^{ab}	1.53^{a}	1.79^{a}	1.41^{abc}
EPA ($C_{20:5\ n-3}$)	0.01^{e}	0.02^{e}	0.02^{e}	0.02^{e}	0.05^{e}	0.06^{ed}
DPA ($C_{22:5\ n-3}$)	0.36^{d}	0.34^{bc}	0.27^{bc}	0.31^{bc}	0.24^{c}	0.28^{bc}
DHA ($C_{22:6\ n-3}$)	1.38^{e}	2.00^{de}	1.79^{de}	2.26^{cd}	2.28^{cd}	2.72^{abc}
Saturated total (S)	32.59^{a}	34.14^{a}	32.71^{a}	33.99^{a}	34.04^{a}	35.12^{a}
Monounsaturated total (M)	43.25^{abcd}	41.71^{bcd}	43.52^{abcd}	41.64^{bcd}	41.31^{cd}	40.68^{d}
Polyunsaturated total (P)	22.96^{a}	22.63^{ab}	22.46^{ab}	23.04a	23.60^{a}	23.37^{a}
n-6 total	21.08^{a}	20.14^{ab}	20.29^{ab}	20.29^{ab}	20.90^{a}	20.19^{ab}
n-3 total	1.87^{e}	2.49^{cde}	2.18^{de}	2.74^{bcd}	2.70^{bcd}	3.18^{abc}
n-6/n-3 ratio	11.25^{b}	8.16^{cd}	9.40^{cd}	7.41^{cd}	7.81^{cd}	6.36^{de}
P/S ratio	0.70^{a}	0.67^{ab}	0.69^{a}	0.67^{ab}	0.69^{a}	0.67^{ab}
	---------- Means of PUFAs in the yolk (mg/yolk) ----------					
AA	96.40^{a}	89.68^{a}	82.39^{ab}	80.83^{abc}	100.14^{a}	77.79 ^{abc}
DHA	77.02^{e}	116.95^{bcde}	98.01^{de}	119.69^{bcde}	127.54^{bcde}	149.75^{abc}
Total	104.18^{bcd}	145.68^{bcd}	119.24^{cde}	145.11^{bcd}	150.40^{bcd}	175.32^{abc}

*Means with different letters in rows indicate significant differences ($p<0.05$) by Tukey test

differed significantly from mean of 2.39% (AM) into yolk. Between levels, the influence was recorded the lowest mean of 2.00% (120 mg) and the biggest of 3.27% of PUFAs n-3 (360 mg DHA) into yolk (Table 4).

The means of total in mg/yolk and mg/g yolk, DHA consumption (mg/bird/day) and DHA incorporation (%) according to weight and fat of yolk showed effects of treatments and sources studied. Among sources, the

Table 4: Fatty acids Composition in egg yolk (% of total fatty acids), according to the soures and levels studied

	Sources		Nivels				
Fatty acid	OP	AM	120	180	240	300	360
Myristic ($C_{14:0}$)	0.40^{a*}	0.35^{b}	0.31^{c}	0.36^{BC}	0.36^{BC}	0.39^{AB}	0.46^{A}
Palmitic ($C_{16:0}$)	25.84^{a}	21.15^{b}	24.78^{B}	25.61^{AB}	25.03^{AB}	25.94^{AB}	26.13^{A}
Palmitoleic ($C_{16:1\ n-7}$)	3.29^{a}	2.41^{b}	2.58^{A}	2.97^{A}	2.82^{A}	2.81^{A}	3.07^{A}
Stearic ($C_{18:0}$)	8.54^{a}	7.97^{a}	8.96^{A}	7.82^{A}	8.28^{A}	8.01^{A}	8.22^{A}
Oleic ($C_{18:1\ n-9}$)	41.71^{a}	39.62^{b}	40.90^{A}	40.89^{A}	41.20^{A}	40.24^{A}	40.09^{A}
Linoleic ($C_{18:2\ n-6}$)	14.76^{b}	18.56^{a}	17.43^{A}	16.58^{A}	16.92^{A}	16.52^{A}	15.85^{A}
Alpha-linolenic ($C_{18:3\ n-3}$)	0.12^{a}	0.12^{a}	0.12^{B}	0.13^{AB}	0.12^{B}	0.14^{A}	0.12^{AB}
Gema-linolenic ($C_{18:3\ n-6}$)	0.45^{a}	0.35^{b}	0.36^{A}	0.40^{A}	0.40^{A}	0.41^{A}	0.46^{A}
Gadoleic ($C_{20:1\ n-9}$)	0.28^{a}	0.25^{b}	0.26^{A}	0.27^{A}	0.27^{A}	0.27^{A}	0.26^{A}
Arachidonic ($C_{20:4\ n-6}$)	0.89^{b}	1.61^{a}	1.46^{A}	1.21^{A}	1.16^{A}	1.20^{A}	1.24^{A}
EPA ($C_{20:5\ n-3}$)	0.20^{a}	0.02^{b}	0.04^{A}	0.08^{A}	0.09^{A}	0.14^{A}	0.20^{A}
DPA ($C_{22:5\ n-3}$)	0.04^{b}	0.30^{a}	0.21^{A}	0.19^{A}	0.15^{A}	0.17^{A}	0.15^{A}
DHA ($C_{22:6\ n-3}$)	2.57^{a}	1.94^{b}	1.63^{B}	2.14^{AB}	2.10^{AB}	2.63^{A}	2.80^{A}
Saturated total	34.79^{a}	33.49^{b}	34.06^{A}	33.80^{A}	33.67^{A}	34.35^{A}	34.81^{A}
Monounsaturated total	45.29^{a}	42.28^{b}	43.75^{A}	44.13^{A}	44.31^{A}	43.32^{A}	43.44^{A}
Polyunsaturated total	19.07^{b}	22.93^{a}	21.25^{A}	20.75^{A}	20.96^{A}	21.21^{A}	20.83^{A}
n-6 total	16.11^{b}	20.53^{a}	19.25^{A}	18.19^{A}	18.49^{A}	18.13^{A}	17.55^{A}
n-3 total	2.95^{a}	2.39^{b}	2.00^{C}	2.55^{ABC}	2.47^{BC}	3.07^{AB}	3.27^{A}
n-6/n-3 ratio	5.79^{b}	8.80^{a}	9.71^{A}	7.27^{AB}	7.71^{AB}	6.05^{B}	5.76^{B}
P/S ratio	0.54^{b}	0.68^{a}	0.62^{A}	0.61^{A}	0.62^{A}	0.61^{A}	0.59^{A}

Means with different letters in rows indicate differences ($p<0.05$) by Tukey test

highest mean of 142.33 mg at OP source differed significantly from mean of 107.84 mg DHA/egg yolk at AM source. For medium among levels, the highest mean of 139.15 mg (300 mg) and 157.72 mg DHA/egg yolk (360 mg DHA) differed ($p<0.05$) from the lowest mean of 90.90 mg DHA/egg yolk (120 mg DHA) (Table 5). The highest mean of 9.28 mg (OP) and the mean of 7.97 mg to PUFAs n-3/g yolk from AM source did not show statistic differences ($p>0.05$) among sources (Table 5). For total PUFAs n-3 (ALA + EPA + DHA + DPA) the highest mean of 218.22 mg/ yolk (OP360) showed difference ($p<0.05$) compared with means of other treatments. except for OP300 and AM420.

Polyunsaturated fatty acids omega-6 (PUFAs n-6) into yolk: Decreasing mean content of 17.60% (CON) to 12.99% (OP360) of AL and 1.76% (CON) to 0.69% (OP360) of AA were observed with increasing levels of PUFAs n-3 in the diet of laying hens (Table 3).

In relation to increasing levels of OP in the diet, linear responses were obtained for AA mean and DHA mean expressed by the regression equations shown in Fig. 1. For sources the highest mean of 20.53% (AM) to total PUFAs n-6 differed ($p<0.05$) from the lowest of 16.11% (OP) in egg yolk. Among sources, DHA increasing levels determined the best ($p<0.05$) relation of 0.54 (OP) against mean of 0.68 P/S (AM) to AM source (Table 4). Means of yolk weight (g), total lipids (%) and yolk fat (g) did not show significant differences to treatments, sources or levels studied (Table 5). Mean contents of LA. γ-linolenic acid and arachidonic acid in egg yolk were influenced ($p<0.05$) by sources in the diet (Table 6).

DISCUSSION

According to Noble *et al.* (1990), the standard of post egg involves sequential maturation of "ova" or yolk in a range of approximately 24 h. Metabolic efforts to sustain supply of lipids for yolk formation are achieved by a single system of transport and synthesis highly organized (Griffin *et al.*, 1984; Sim, 1998). With diets poor in fat, the most part of fatty acids into yolk is result of synthesis "*de novo*" from carbohydrates, while to diets rich in fat, the most part comes from dietary fatty acids (Naber and Biggert, 1989).

Evaluation of yolk lipid composition in this study allowed to observe the striking influence of diet on changes of final composition of fatty acids in yolk fat by effect of treatments, sources and DHA levels in the layer diet. These results are in agreement with Van Elswyk (1997), Abril and Barclay (1998). Abril *et al.* (2000) and Galobart *et al.* (2002) that reported the relationship between diet composition and profile of fatty acids in egg yolk of laying hens.

In this experiment, the largest contributors to sum of saturated fatty acids (%) into yolk were mean of 25.03% of palmitic acid (CON) and 7.89% of stearic acid (CON), this was also observed by Jiang and Sim (1993 and 1994) (Table 3).

According to Guardiola *et al.* (1994) there is a natural tendency to balance the fat composition of egg yolks from birds fed conventional diet. Thus, approximately 33% would be composed mainly of saturated fatty acids, as palmitic and stearic. Also, to diets supplemented with PUFAs n-3 sources, total saturated fatty acid means (%) is around one third (33%) cited by the authors above (Table 4).

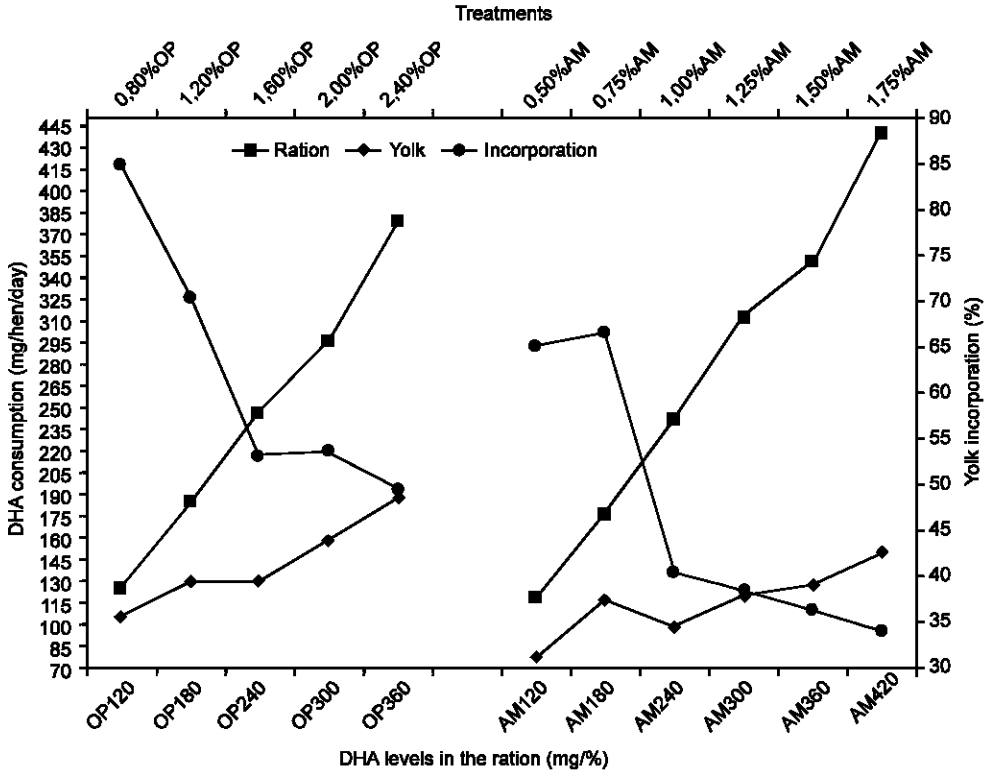

Fig. 1: DHA incorporation into yolk according to treatments, sources and levels of OP or AM in the diet

Unlike the saturated fatty acids, MUFAs incorporation in diets can increase their level in yolk at the expenses, mainly of myristic, palmitic, stearic and palmitoleic fatty acids (Pankey and Stadelman, 1969). Typically, egg coming from hens fed conventional diet (corn/soy), the oleic acid ($C_{18:1\ n-9}$) is the fatty acid in a higher percentage (Guardiola *et al*., 1994), this is in agreement with the observed by Hargis *et al.* (1991). Van Elswyk *et al.* (1998, 2000) and Jiang and Sim (1993 and 1994). In this study, MUFAs with the highest percentages into egg yolk (Table 5) were means of 42.80% for oleic acid (OP180) and 3.73% for palmitoleic acid (OP300). For treatments with increasing levels of DHA from OP and AM sources in the diet, did not observe significant differences in relation to mean of 42.78% total MUFAs of control group. However, the sources have influenced the means difference ($p<0.05$) of 45.29% (OP) and of 42.28% (AM) (Table 6). These are consistent with results of this research, observations indicated by Watkins and Elkin (1992) and Pankey and Stadelman (1969), that MUFAs source added in diet, such as olive oil (high content in C18: 1 n -9), influence significant changes in MUFAs yolk composition at the expense of fatty acids: mainly myristic, palmitic, stearic and palmitoleic. In this research, we found that means of fatty acids mentioned above were ($p<0.05$) modified by OP source (Table 2- 4). Results of PUFAs n-3 inclusion with 20 carbons (EPA. $C_{20:\ 5\ n-3}$) and 22 carbons (DHA. $C_{22:6\ n-3}$) in diet of laying hens from OP source showed direct reflection on fatty acids composition, with a significant fortification to PUFAs n-3 in egg yolk, this is in accordance with the data presented in several studies conducted with various sources of PUFAs n-3 (oil: fish. flaxseed. canola. omega; seeds: flaxseed. canola. meal: fish. algae) in different countries with different location and at diverse continents, for example we can cite studies carried out by Jiang *et al.* (1991), Leskanich and Noble (1997), Marshall *et al.* (1994a,b) and Sim (2000). In this experiment was observed a decrease of 26.19% of linoleic acid concentration in yolk with relation to mean decreasing variations of 17.60% (CON) to 12.99% (OP360), which indicated that the third largest fatty acid of the yolk (LA, $C_{18:2\ n-6}$) was partially replaced by PUFAs n-3 into the yolk (Table 1), by treatments influence with increasing of DHA levels from OP source in the diet.

However, in opposite to high levels of PUFAs n-6 incorporation in egg yolk represented mainly by LA (CON -17.60%). Wheeler *et al.* (1959) found evidence that, for example, the omega-3 alpha-linolenic and their derivates, EPA and DHA, with rich diet containing linseed oil, was lower than 15%. CHEN *et al.* (1965) observed that when linseed oil was added in the diet, linoleic acid was incorporated with more quantity in triacylglycerols fraction than in phospholipids fraction. Instead, EPA and

Table 5: Medium PUFAs of n-3 (eg mg/yolk) and total PUFAs n-3 (mg/yolk), consumption of DHA (mg/bird/day), incorporation (%), weight of yolk (g), total lipids (%) of fat and yolk (g) of the average total yolk lipids, according to the sources and levels studied

	Sources		Levels				
Fatty Acids	OP	AM	120	180	240	300	360
	Means levels of PUFAs n-3 in yolk						
EPA (mg/ yolk)	11.08^{a*}	1.22^{b}	2.41^{A}	4.84^{A}	5.10^{A}	7.27^{A}	11.12^{A}
DPA (mg/ yolk)	2.73^{b}	16.94^{a}	12.06^{A}	11.20^{A}	8.44^{A}	9.01^{A}	8.46^{A}
DHA (mg/ yolk)	142.33^{a}	107.84^{b}	90.90^{B}	123.27^{AB}	114.39^{AB}	139.15^{A}	157.72^{A}
ALA (mg/ yolk)	7.13^{a}	6.91^{a}	6.47^{A}	7.60^{A}	6.37^{A}	7.69^{A}	7.00^{A}
n-3 total (mg/ g yolk)	9.28^{a}	7.97^{a}	6.69^{A}	8.64^{A}	8.33^{A}	10.26^{A}	9.21^{A}
n-3 total (mg/ yolk)	163.29^{a}	132.92^{b}	111.86^{C}	146.92^{ABC}	134.32^{BC}	163.13^{AB}	184.31^{A}
Consumption of DHA (mg/hen/day)	245.98^{a}	239.68^{a}	120.91^{E}	179.80^{D}	244.11^{C}	303.58^{B}	365.74^{A}
Incorporation of DHA (%)	62.44^{a}	49.45^{b}	75.19^{A}	68.39^{A}	46.78^{B}	46.17^{B}	43.19^{B}
Yolk weight (g)	16.31^{a}	16.71^{a}	16.75^{A}	17.05^{A}	16.17^{A}	15.87^{A}	16.70^{A}
Lipids totals (%)	33.77^{a}	33.21^{a}	33.28^{A}	33.64^{A}	33.64^{A}	33.28^{A}	33.62^{A}
Fat of yolk (g)	5.51^{a}	5.54^{a}	5.57^{A}	5.73^{A}	5.43^{A}	5.30^{A}	5.61^{A}

*Means with different letters in rows show significant differences ($p<0.05$) by Tukey test

Table 6: Mean content (mg / yolk) of PUFAs n-6 LA (C18: 3 n-6), GLA (C18: 3 n-6), AA (C20: 4 n-6) and total n-6 PUFAs 6 (mg/g yolk and mg/yolk), consumption of PUFAs n-6 and percentage of incorporation of LA and AA, the yolk weight, lipids total fat and the egg yolk according to the sources and levels studied

	Sources		Levels				
Fatty acids	OP	AM	120	180	240	300	360
	Means levels of n-3 fatty acids in the yolk						
AL (mg/ yolk)	814.13^{b*}	1030.23^{a}	975.33^{A}	952.01^{A}	919.59^{A}	874.11^{A}	889.84^{A}
GLA (mg/ yolk)	25.33^{a}	19.94^{b}	20.02^{A}	23.35^{A}	21.78^{A}	21.86^{A}	26.16^{A}
AA (mg/ yolk)	49.03^{b}	89.88^{a}	81.53^{A}	69.69^{A}	63.02^{A}	63.56^{A}	69.50^{A}
n-6 total (mg/ g yolk)	54.46^{b}	68.21^{a}	64.11^{A}	61.22^{A}	62.18^{A}	60.33^{A}	58.83^{A}
n-6 total (mg/ yolk)	888.50^{b}	1140.06^{a}	1076.90^{A}	1045.06^{A}	1004.40^{A}	959.54^{A}	985.51^{A}
Consumption PUFA n-6	1572.11^{b}	2118.16^{a}	1923.70^{A}	1829.90^{A}	1814.50^{A}	1819.50^{A}	1838.10^{A}
Incorporation AL (%)	52.40^{a}	49.28^{a}	50.83^{A}	52.76^{A}	51.50^{A}	50.27^{A}	48.84^{A}
Incorporation AA (%)	3.12^{b}	4.30^{a}	4.22^{A}	3.67^{A}	3.54^{A}	3.50^{A}	3.64^{A}
Weight of yolk (g)	16.31^{a}	16.71^{a}	16.75^{A}	17.05^{A}	16.17^{A}	15.87^{A}	16.70^{A}
Lipids totals (%)	33.77^{a}	33.21^{a}	33.28^{A}	33.64^{A}	33.64^{A}	33.28^{A}	33.62^{A}
Fat of yolk (g)	5.51^{a}	5.54^{a}	5.57^{A}	5.73^{A}	5.43^{A}	5.30^{A}	5.61^{A}

*Means with different letters in rows show significant differences ($p<0.05$) by Tukey test

DHA were held exclusively in phospholipids, preferably in phosphatidiletanolamine fraction, this was also verified by Cherian and Sim (1991) and Jiang *et al.* (1991). Fat of yolk is composed of 29.7% phospholipids, 63.3% triacylglycerols and 4.9% cholesterol free. Phospholipids (mean of 23.9% phospha-tidiletanolamine and 69.1% phosphatidylcholine) with the lowest level in egg show consequently the lowest percentage of EPA and DHA incorporation in tissues and in egg yolk (Leskanich and Noble, 1997). Analyzing use of supplements rich of DHA (Table 1) in this experiment, the greatest efficiency of DHA incorporation in egg yolk was 85.11% (OP120) to the birds that had mean intake of 123.57 mg of DHA/bird/day and showed eggs enrichment with 104.80 mg DHA/egg yolk. Incorporation efficiency showed a linear decrease to OP source ($Y = -8.7443 X + 88.4970$, $R2 = 0.85$) and AM ($Y = -7.1074 X + 71.7190$, $R2 = 0.79$) with the increased of DHA levels in the diet, presenting reduced efficiency of DHA incorporation to 49.45% (OP360) and 34.03% (AM420) in egg yolk. With these DHA levels of 360 mg (OP360) and 420 mg (AM420) from OP and AM sources in the diet, respectively, mean intakes of 380 mg DHA (OP360) and 440.21 mg DHA/bird/day (AM420) and eggs fortification with 187.92 mg of DHA + 19.70 mg of EPA/yolk, make up the total 218.22 mg of total PUFAs n-3 (OP) and 175.32 mg of total PUFAs/yolk (AM) (Table 3), we observed highlight of the greatest efficiency from OP source in the diet of birds (Table 4). Values of eggs enrichment with PUFAs n-3 are in accordance to Cherian *et al.* (1996) and Cherian and Sim (2003) that reported incorporation, respectively, 3.8% and 4.5% of DHA in egg yolks with 3.5% of savelha oil in the diet of laying hens. In this research, means ranged of 1.87% (OP120) to 3.85% (OP360) and therefore discordant results presented by SIM (1998) who reported that using flax seed in the diet the production of eggs enriched with total PUFAs n-3 were 7-12% of yolk lipids. The author also reported that observed levels of fatty acids in eggs in the following order: α-linolenic> DHA> DPA> EPA. The total PUFAs n-3 reached 600 mg/egg and by the same author, as a consequence, the ratio of fatty acids

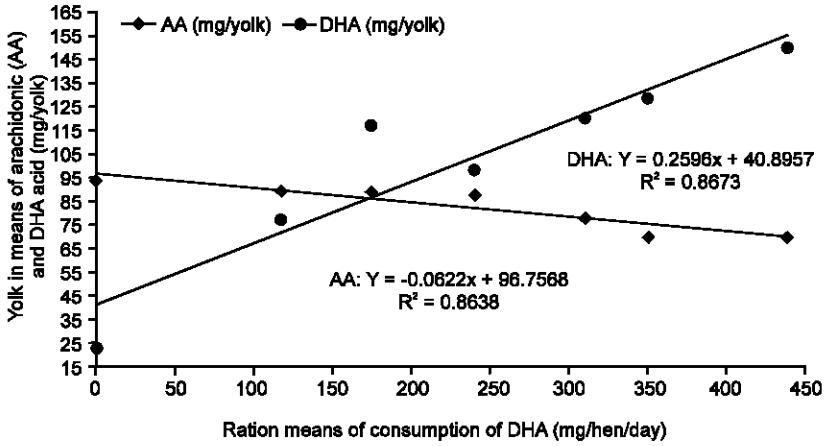

Fig. 2: Means of Arachidonic Acid (AA) and DHA into yolk how function of DHA consumption in the diet (mg/hen/day)

n-6: n-3 was reduced ($p<0.05$) in eggs enriched with n-3. Human adult requirements for PUFAs according to Simopoulos *et al.* (1994) based on the "Recommended Dietary Allowance" (RDA) in the decisions of Nutrition Subcommittee added to NRC (1989), would be a minimum of 220 mg of DHA and 220 mg of EPA/day. Results of this experiment include the supply of minimum requirements to an adult human in 85% of DHA (OP360) and 18% of PUFAs n-6 (OP360). Similarly, additional utilization of AM source in the diet of laying hens proved the possibility of obtaining draft eggs enriched with PUFAs containing mean yolk values of 149.75 mg of DHA, 175.32 mg of PUFAs n-3 and 1113.20 mg of PUFAs n-6 (AM420). The intake of one egg per day (AM source) supplied the daily requirements of approximately 68% of DHA and 25% of PUFAs n-6 of an adult human. according to requirements table of RDA (Table 5 and 6).

The decrease of LA in the diet (Table 2), showed with the increased of OP and AM levels in the diet riched in PUFAs n-3, influenced reduction of AA concentration into yolk, were observed a decrease of 60.8% to mean of 0.69% (OP360) in relation the mean of 1.76% of AA (CON) in yolk (Table 1), this is agreeing with observations of Mori (2001) when he used OP content of 2% in the diet of laying hens.

Results of this experiment are consistent with Huang *et al.* (1990) that fed hens with 3% of fish oil (15.87% EPA. 6.81% DHA) for 4 weeks. They noted a significant difference reducing the mean of 1.96% AA (CON) to 0.87% AA in yolk, when treatment contained 3% of fish oil. The authors also found an increase of PUFAs n-3 into egg yolk, adding that laying hens through specialized metabolic and physiological process, have great ability to absorb, to convert and to deposit fatty acids in the diet, in tissues and in the egg yolk. When chicken gets diet rich in LA or γ-linolenic, it reflects in egg yolk preponderance of AA (Hermier, 1997). By elongation and desaturation mechanism LA can be converted to γ-linolenic at dihomo-γ-linolenic acid and AA.

The highest LA concentrations in the diet would result to the highest AA levels. Moreover, according to the authors above in cellular level. EPA and DHA effectively are incorporated into phospholipids of membranes, because they move AA and also inhibit enzymes related to desaturation and elongation stages which are common both n-6 and n-3 series. These authors also considered that possibly a decrease of LA in diet would reduce, too, AA content resulting from substrate reduction for formation of its precursors.

Inhibition of delta-5-desaturase also shows influence on limit of AA incorporation because it interferes in the conversion of dihomo-γ-linolenic acid to AA. According to Von Schacky and Dyerberg (2001), the great affinity of acyl transferase for n-3 series compared to n-6 series, shows to be favorable to EPA and DHA incorporation. However, results of this experiment are consistent with the statements made by Watkins *et al.* (2001), that the decrease of n-6:n-3 in the diet, consequently lead to the decrease of this relationship in egg yolk or in animal tissue.

PUFAs n-6 into yolk: With levels increasing of OP source in the diet of laying hens, we noted a decrease of total PUFAs n-6 means in yolk of 17.42% (OP120) to 14.21% (OP360), which represents, between this value and the control group, a reduction of 28.12% of PUFAs n-6 total into yolk (Table 2). Results of this experiment are consistent with a significant number of experiments realized which intended to modify content of PUFAs n-6 and PUFAs n-3 in egg yolk, and among those cited in the literature, one can mention the work of Wheeler *et al.* (1959), Adams *et al.* (1989), Van Elswyk (1993), Surai and Sparks (2001), Galobart *et al.* (2002) and Tsuzuki *et al.* (2003) in which were evidenced that the sum of

PUFAs n-6 is the third largest group of fatty acids into yolk and it can be represented in ascending order by linoleic<palmitic<oleic acids greater quantity in egg yolk (Table 2). According to Cherian and Sim (1991) and Jiang *et al.* (1991), LA incorporates in triacylglycerols and phosphatidylcholine fraction of phospholipids tissue.

Whereas AA levels in egg yolk dependent of LA content and of relationship between n-6: n-3 in the die, hence significant decrease of AA (mg/yolk) and incorporation percentage of AA into yolk on treatments with increasing OP levels in the diet may be considered effect of decreasing OP consumption in the diet (Table 3 and 4). Different behavior for AA was found on treatments with increasing DHA levels of AM source and maintained constant content of 3% corn oil in the diet, we did not observe significant decrease in relation to the control group. Results of this experiment are consistent with Washburn (1979), Barclay *et al.* (1994) and Zeller *et al.* (2001) when they reported mean values of 53.30 mg AA/yolk with supplementation of marine algae (165 mg DHA/hen/day) in the diet of laying hens. The authors above also found that equally to other sources of PUFAs n-3 and PUFAs n-6, the PUFAs n-3 from marine algae and the PUFAs n-6 from algae and other additional sources in the diet showed 9.20% and 10.87% of total PUFAs n-3 and n-6 incorporated into phospholipids and 2.12% and 12.13%, of total PUFAs n-3 and n-6 incorporated into triacylglycerols, respectively. These features explain the lowest incorporation of PUFAs n-3 and the highest incorporation of PUFAs n-6 on fatty acids into yolk in this experiment, and in other studies which the composition of PUFAs n-6 and n-3 are similar to these results, findings reported by Jiang *et al.* (1991), Leskanich and Noble (1997) and Sim (2000).

Moreover, the results of this research are consistent with the observations of Watkins *et al.* (2001). LA concentration in food influences the enzyme delta-6-desaturase, shifting the reaction towards the conversion to higher concentrations of series PUFAs n-6 by the competition of different amounts of substrate (Hwang, 1989).

According to Nettleton (1995) and Simopoulos *et al.* (1999), the proportion of PUFAs n-3 formed are variable and dependent of relationship between PUFAs n-3 and PUFAs n-6 by initial reflection on the competition of the delta-6-desaturase enzyme in interconversion of their respective precursors of 18°C.

Conclusion: The increase of PUFAs n-3 of OP source in the diet determined linear response to the increase of Docosahexaenoic Acid (DHA) in egg yolk. Total percentage of PUFAs n-3 into the yolk increases with supplementation of OP and AM sources in the diet of laying hens. OP source was more effective, highlighting the best mean of PUFAs n-3 (218.22 mg/yolk), however, both marine enrichment sources of PUFAs n-3 showed similar fortification of egg yolk in mg/g yolk.

The possibility to manipulate fatty acids composition in egg yolk fat is favorable to increase PUFAs n-3 through additional fatty acid sources in the diet of laying hens.

The efficiency of DHA incorporation by the poultry showed an inverse relationship of DHA level into the diet (85.11% for 120 mg DHA/100 g ration and 49.45% of incorporation with 360 mg DHA/100 g of diet). These results allow the implementation of strategies to enrich eggs of poultry production. Regarding the OP addition in the diet of laying hens, it was noted that is possible obtain PUFAs enriched eggs with mean values of 187.91 mg of DHA, 218.22 mg of PUFAs n-3 and 802.79 mg of PUFAs n-6 per egg using OP to provide 360 mg DHA/100 g of diet.

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