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Lipid Profile of the Yolk under the Influence of Supplementaries Sources Rich in Ω-3 PUFAs in the Diet of Laying Hens in the Time

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Abstract: Two hundred eighty-eight 32-wk-old Hisex White laying hens were used in this research during a 10 weeks 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 effect of time 0, 2, 4, 6 and 8 weeks (wk) on the efficiency of egg yolk fatty acid enrichment. The means varied ($p<0.01$) of 17.63% (OP360) to 22.08% (AM420) is the total Polyunsaturated Fatty Acids (PUFAs) and 45.8 mg/g (OP360), 40.37 mg/g (OP360, 4 wk) to 65.82 mg/g (AM420) and 68.79 mg/g/yolk (AM120, 8 wk) for n-6 PUFAs. On the influence of sources and levels in the times, the means of n-3 PUFAs increased by 5.58 mg/g (AM120, 2 wk) to 14.16 mg/g (OP360, 6 wk) when compared to average of 3.34 mg PUFAs Ω-3/g/yolk (CON). Usually, the means DHA also increased from 22.34 (CON) to 176.53 mg (μ, OP360), 187.91 mg (OP360, 8 wk) and 192.96 mg (OP360, 6 wk) and 134.18 mg (μ, OP360), 135.79 mg (AM420, 6 wk), 149.75 mg DHA (AM420, 8 wk) per yolk. The opposite was observed for the means AA, so the effect of the sources, levels and times, decreased ($P<0.01$) of 99.83 mg (CON) to 31.99 mg (OP360, 4 wk), 40.43 mg (μ, OP360) to 61.21 mg (AM420) and 71.51 mg AA / yolk (μ, AM420). Variations of the average weight of 15.75g (OP360) to 17.08g (AM420) yolks of eggs de 32.55% (AM420) to 34.08% (OP360) of total lipids and 5.28 g (AM240) to 5.84 g (AM120) of fat in the yolk were not affected ($p>0.05$) by treatments, sources, levels and times studied. Starting of 2 week, the hens increased the level of n-3 PUFAs in the egg yolks, being expressively increased ($p<0.01$) until 4 weeks, which after the increased levels of n-3 PUFAs tended to if stabilize around of time of 8 experimental weeks, when it was more effective saturation of the tissues and yolk.

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

INTRODUCTION

Essential fatty acids are those that the animal or human organism does not synthesize and are required to be administered at feeding. Thus, the fatty acid a-linolenic (ALA, $C_{18:3\ \omega-3}$) and linoleic (AL, $C_{18:2\ \omega-6}$) are considered essential (Balnave, 1970; Watkins, 1991; Whitehead, 1981). From the ALA, there is synthesis of EPA and DHA. The EPA is the precursor of the E3 series prostanoids and prostaglandins, which modulate the reaction to not inflammatory.

In opposite, of fatty acid arachidonic (AA, $C_{20:4\ n-6}$) derived from elongate AL, derive the E2 series of inflammatory leukotrienes, prostaglandins and thromboxane. The balance between these two types of response to aggression, produces a balanced organism, among the states, normality and reaction to aggression by pathogens (Watkins, 1991; Von Schacky and Dyerberg, 2001; Simopoulos, 2009). Besides produce tasty meat that surpassing all expectations in culinary, hens also distinguished itself by producing "super omega 3 eggs" (Noble, 1987; Leskanich and Noble, 1997; Grobas *et al*., 2001; Galobart *et al*., 2002).

Evaluated the eggs, exceeded all expectations about the ability to incorporate PUFAs in the yolk lipids. Besides the high biological value of lecithin and phospholipid lecithin, now enriched with omega 3 long chain, they become part of a select class of nutrients with ability nutraceutical (Jiang and Sim, 1992). The effective power of such a modulator different phospholipids on the cell membranes and as a cellular signal as is well known (Bang and Dyerberg, 1972; Watkins *et al*., 2001; Cherian and Sim, 1991; 1993; Simopoulos *et al*., 1999).

Unlike mammals, birds, the digestive system is much simplified and rudimentary. The small intestine of mammals, the following lipids absorbed by the lymph, which after they pass into the thoracic duct from which

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follow the bloodstream. In birds, the lipids are transported from the intestinal cell directly into the bloodstream (Beitz and Allen, 1984; Freeman, 1984).

Ever a specie of the only once, contributing to the improvement of nutritional status in human and veterinary medicine (Zeidler, 2003), as the laying hen (*Gallus gallus domesticus*, Linneaus, 1758). Small in size, but a giant in production capacity (Scheuermann and Bellaver, 1995; Briz, 1997), beat all records, When all at once, incorporated 528 mg of omega PUFAs in egg yolk, to be supplemented with flax seed (*Linum usitatissimum* L.) to diet (Cherian and Sim, 1991), or around of 200 mg DHA and 20 mg EPA (Hargis *et al*., 1991; Huang *et al*., 1990; Carvalho, 2006).

Scientifically proven the possibility of changing of the chemical composition of fatty acids of plasmatic lipoprotein (Pita, 2007) and egg yolk of laying hens (Carvalho, 2006; Piber Neto, 2006), innovation is very well received by scientific community worldwide. The high impact and the major gains from this innovation were for new ways through new concepts, paradigm of nutrition recovery for the use, for the benefit of the human population (Bang and Dyerberg, 1972).

Naturally, such scientific advances are very desirable, since the immense damage public health with effects on congestion hospital emergencies especially for care cardiovascular disease. Unbalanced diets in three main categories of saturated, monounsaturated and polyunsaturated fatty acid has resulted in serious consequences to lipid metabolism (Simopoulos *et al*., 1999; Simopoulos, 2009; Von Schacky and Dyerberg, 2001).

The advancement of research in animal nutrition, especially in layer, aiming at enriching their products with omega-3 PUFAs (Scheuermann and Bellaver, 1995; Briz, 1997), enabled, new insights into the physiological behavior of omega-3 PUFAs in long chain, EPA ($C_{20:5\ n-3}$, eicosapentaenoic acid) and DHA ($C_{22:6\ n-3}$, docosa-hexaenoic acid), to enrich phospholipids of Phosphatidylcholine (PC) and Phosphatidyl-ethanolamine (PE), with numerous actions on the modulating capacity (Simopoulos *et al*., 1999), the various organ systems, among others, the immunocompetent (balanced inflammatory response or less), vascular (reduced platelet aggregation), nervous (increase the threshold of synaptic intercellular signal) skeletal and visceral (Watkins, 1971; Von Schacky and Dyerberg, 2001).

Disease prevention, including the cardiovascular system, showing statistically significant both from the standpoint of public health, such as financial aid to countries in the west has been cited by many authors from different continents as possible to be reduced when applying preventive programs targeted nutrition, which will be provided in the diet balanced in all nutrients including especially the omega-3 PUFAs, aiming to achieve a reduction in rates of n-6 PUFAs: n-3 PUFAs from the current >15:1 for 3:1 (Simopoulos *et al*., 1999; Simopoulos, 2009). This also seems to be the best way to choose, surely, by presenting a lower cost and greater benefit.

Among the animal species, the laying hens emerged in front in the research with magnificent results. Satisfactorily, this species enriched with high levels of n-3 PUFAs the egg products and tissue, with wide application in industry through of oil-rich yolk long chain Omega 3 PUFAs, EPA and DHA, from phospholipids FC and FE, in addition to other derivatives available for consumption and industrialization (Hwang, 1989; Jiang and Sim, 1994; Leskanich and Noble, 1997).

At the turn into the new millennium was marked by new and important victories of humanity especially in science. The mastery of techniques, dependent on multi disciplinary areas (Briz, 1997) of knowledge, was to consecrate the "epic of Omega 3 PUFAs".

The knowledge of nutritional and metabolic diseases caused by imbalance or deficiency of these fatty acids in the diet or contributes to a significant improvement in the overall framework of preventive measures to be adopted by Western communities with problems of balance of total PUFAs (n-6: n-3) diet (Simopoulos, 2009).

The applicability, extracted from experience with animals provided significant advances. The scenario of changes in public health is still very expressive close to what can be done if governments implement the strategies of nutrition for advances in human nutrition (Zeidler, 2003), under the form of policy measures comprehensive and preventive actions collectively (mass).

In the area of metabolic and deficiency disease caused by unbalanced diets on lipids, the new millennium was covered with large contributions and advances in science, lacking only the governmental counterpart in the western countries, where severe problem and the promotion of mass public policies aimed at preventing nutritional deficiencies and metabolic disorders in humans. The elucidation of endemics deficiency in omega-3 PUFAs was significantly contributed to the understanding of many nutritional disorders in the area of vascular diseases in humans, especially heart disease.

Jiang and Sim (1992) administered diets containing 10% linseed as a rich source of n-3 PUFAs and obtained the plateau around 2 weeks of the new food. Rats that received the yolks enriched with n-3 incorporated significant amounts of plasma and tissues (liver) after 4 weeks of starting the diet. Lopes-Ferrer *et al*. (1999) showed the effectiveness of the incorporation of n-3 PUFAs of origin of 8.2% linseed oil (LO) and 8.2 fish oil (FO) in diet of the broilers at 5 weeks experimental, 21.61% (OP) and 23:56% (LO) total n-3 PUFAs in the tissues of broiler were incorporated.

Chang and Huang (1990) studied the effects of rate PUFA and MUFA on the Saturated (SAT) fatty acids on human plasma and liver lipid concentration in different PUFAs diet, MUFA and SAT. After the trial period observed significant changes in the plasma profile of the individuals.

Pita (2003) studied the effect of time on the fatty acid content of plasma and yolk of laying hens fed diets enriched with additional sources of n-3 PUFAs, showed increasing content from the sixth day and plateau around 30 days.

Piber (2006) noted that by adding a mixture of sardine oil and tuna diet of laying hens showed high levels of PUFAs to the 30 experimental days.

Carvalho (2006) noted that with increasing levels of sources of salmon oil to the diet of laying hens proportionally the levels of n-3 PUFAs in the yolk increased significantly on the seventh day reaching a plateau after 4 weeks and stabilizing after 5 weeks experimental.

The present research had as objective to measure time in weeks, the effect of supplementation rich in increasing levels of n-3 PUFAs in the diet of laying hens on the content of n-3 PUFAs in egg yolk and analyze the behavior of the levels of PUFAs in the yolk over time.

MATERIALS AND METHODS

The research was conducted in the experimental poultry vivarium of the School of Veterinary Medicine and Animal Science of University of Sao 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%

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	28.30	27.85	27.85	27.85	27.85	27.85	27.79	27.68	27.57	27.62	27.71	27.80
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	-	-	-	-	-	-	0.50	0.75	1.00	1.25	1.50	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	4.20	4.46	4.60	4.63	4.74	4.80	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	0.10	0.12	0.17	0.15	0.50	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	2.90	2.87	3.68	4.73	7.18
Analysis calculated												
Metabolizable energy (kcal/kg)	2750.00	2750.00	2750.00	2750.00	2750.00	2750.00	2750.00	2750.00	2750.00	2750.00	2750.00	2750.00
Crude protein (%)	17.00	17.00	17.00	17.00	17.00	17.00	17.00	17.00	17.00	17.00	17.00	17.00
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

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 (%)	0.00	0.80	1.20	1.60	2.00	2.40	0.00	0.00	0.00	0.00	0.00	0.00
AM (%)	0.00	0.00	0.00	0.00	0.00	0.00	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
α-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
γ-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

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 initial time (0), 2, 4, 6 experimental weeks were collected four eggs for each of the three replicates of eight birds, totaling 12 eggs. Then the march was continued analytically, to extract and determine the profile of fatty acids in the fat from the pool of egg yolks.

Likewise, in the time trial of 8 weeks, four eggs per replicate were collected for obtain a pool of each experimental unit and thereafter, the determination of fatty acids in three replicates.

Yolks were separated and we obtained weight per unit. After, yolks were homogenized in order to obtain a sample for repetition (pool made up of four yolks). 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, 10 mg).

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

Table 3: Fatty acid composition (%) and results shown below in mg/g/yolk for PUFAs Ω-6 and PUFAs Ω-3 acids, in the times 0, 2, 4, 6 and 8 experimental weeks, according to the treatments studied

Treatments	Times (weeks)	CON	OP120	OP180	OP240	OP300	OP360
OP (%)		0	0.80	1.20	1.60	2.00	2.40
Fatty acids		Fatty acids content					
Total SAT (%)	0	32.52[1]	32.75	31.36	32.92	33.51	30.67
	2	35.20	34.55	36.18	33.13	34.96	36.04
	4	34.82	34.46	34.31	35.30	35.59	36.63
	6	35.58	34.39	34.58	33.83	35.48	36.82
	8*	33.21^{a}	35.53^{a}	33.46^{a}	34.64^{a}	34.72^{a}	35.60^{a}
	μ	34.70	34.73	34.63	34.23	35.19	36.27
	dp	1.33	1.00	1.76	1.01	0.83	2.55
Total MUFAs (%)	0	45.89	44.58	45.24	44.95	44.38	43.23
	2	43.81	46.81	45.89	46.33	45.63	45.30
	4	45.79	46.66	47.76	46.62	45.01	46.27
	6	44.16	47.54	47.18	47.06	46.14	45.03
	8	42.78^{abcd}	42.26^{abcd}	46.54^{a}	45.10^{abc}	45.00^{abc}	45.57^{ab}
	μ	44.14	45.82	46.84	46.28	45.45	45.54
	dp	1.34	2.15	1.00	0.94	0.67	1.13
Total PUFAs (%)	0	21.29	22.30	20.44	21.72	21.59	21.54
	2	20.43	18.29	17.45	19.96	19.04	18.17
	4	18.97	18.50	17.51	17.59	18.24	16.68
	6	19.74	17.69	17.87	18.63	17.76	17.61
	8	20.88^{abc}	19.56^{bc}	18.88^{c}	19.47^{bc}	19.39^{bc}	18.06^{c}
	μ	20.01	18.51	17.93	18.91	18.61	17.63
	dp	0.92	1.82	1.26	1.54	1.48	1.84
Total PUFAs Ω–6	0	65.93	68.70	60.82	66.62	67.95	68.34
(mg/g/yolk)	2	65.11	53.28	45.54	53.87	53.31	48.19
	4	56.02	50.86	47.13	45.59	43.69	40.37
	6	61.28	52.05	49.36	49.93	47.48	45.82
	8	66.89^{a}	59.44^{ab}	54.13^{ab}	56.09^{ab}	53.75^{ab}	48.92^{b}
	μ	62.33	53.91	49.04	51.37	49.56	45.83
	dp	65.93	68.70	60.82	66.62	67.95	68.34
Total PUFAs Ω–3	0	3.39	3.36	2.86	3.01	3.67	3.00
(mg/g/yolk)	2	4.25	6.18	8.41	9.40	9.91	12.70
	4	3.50	6.36	7.36	8.97	9.55	11.86
	6	4.26	6.89	8.68	9.43	11.28	14.16
	8	3.75^{e}	7.29^{dc}	8.78^{bcd}	9.31^{bcd}	11.48^{ab}	13.27^{a}
	μ	3.94	6.68	8.31	9.28	10.56	13.00
	dp	0.38	0.51	0.65	0.21	0.97	0.97
Yolk weight (g)		16.90	16.43	16.90	16.06	15.75	16.41
Total lipids (%)		33.85	34.03	33.32	33.60	33.56	34.80
Yolk fat (g)		5.61	5.59	5.64	5.39	5.32	5.64

*Means (three replicates) with different letters in the same row denote significant difference ($p<0.05$) by Tukey test.
[1]Absolute value, resulting from a determination by the treatment time in weeks

Table 4: Results shown below in mg/yolk arachidonic (AA), EPA, DPA and DHA acid at 0, 2, 4, 6 and 8 experimental weeks, according to the treatments

Treatments	Times (Weeks)	CON	OP120	OP180	OP240	OP300	OP360
OP (%)		0	0.80	1.20	1.60	2.00	2.40
Fatty acids		Fatty acid content					
AA (mg/yolk)	0	73.53	85.64	57.51	71.08	83.59	69.58
	2	110.09	60.55	59.80	45.80	52.97	48.91
	4	82.83	62.05	50.57	44.31	39.67	31.99
	6	107.67	69.78	59.17	52.29	48.24	41.96
	8*	98.71^{a}	66.67^{abcd}	49.70^{bcd}	43.66^{cd}	46.29^{bcd}	38.87^{d}
	μ	99.83	64.76	54.81	46.52	46.79	40.43
	dp	15.89	10.03	4.84	11.51	17.14	14.38
EPA (mg/yolk)	0	0.00	0.00	0.00	0.00	0.00	0.00
	2	0.00	3.69	7.41	8.91	10.74	17.03
	4	0.00	3.72	8.07	8.74	10.57	16.33
	6	0.00	3.88	6.17	8.76	11.69	18.81
	8*	0.00^{e}	4.59^{cde}	8.29^{bcd}	9.38^{bc}	13.44^{b}	19.70^{a}
	μ	0.00	3.97	7.49	8.95	11.61	17.97
	dp	0.00	1.81	3.45	4.01	5.32	8.15

Table 4 cont.

Treatments	Times (Weeks)	CON	OP120	OP180	OP240	OP300	OP360
OP (%)		0	0.80	1.20	1.60	2.00	2.40
DPA (mg/yolk)	0	19.08	19.40	14.70	17.56	20.73	17.33
	2	33.05	4.74	2.98	1.79	0.00	0.00
	4	27.58	3.67	2.83	1.89	1.92	1.51
	6	34.87	5.30	3.79	2.25	2.08	1.91
	8*	31.50[a]	4.14[d]	2.58d	2.22[d]	1.33[d]	3.38[d]
	µ	31.75	4.46	3.05	2.04	1.33	1.70
	dp	6.27	6.71	5.23	6.94	8.71	7.09
DHA (mg/yolk)	0	28.33	28.51	25.00	23.50	27.70	23.95
	2	31.53	87.03	121.82	133.78	138.58	170.15
	4	22.95	90.44	104.91	123.65	128.86	155.10
	6	28.25	96.50	125.51	137.38	156.50	192.96
	8*	22.64[f]	104.80[cde]	129.58[bcd]	130.78[bcd]	158.61[ab]	187.91[a]
	µ	26.34	94.69	120.46	131.40	145.64	176.53
	dp		30.36	43.71	48.52	54.19	69.86

*Means (three replicates) with different letters in the same row denote significant difference ($p<0.05$) by Tukey test.
[1]Absolute value resulting from a determination by the treatment time in weeks

Table 5: Fatty acid composition (%) and results shown below in mg/g/yolk for PUFAs Ω-6 and PUFAs Ω-3 at 0, 2, 4, 6 and 8 experimental weeks, according to the treatments studied

Treatments	Times (weeks)	CON	AM120	AM180	AM240	AM300	AM360	AM420
AM (%)		0	0.50	0.75	1.00	1.25	1.50	1.75
Fatty acids		---------- Fatty acids content ----------						
Total SAT (%)	0	32.52*	31.72	32.78	32.35	32.62	32.64	31.87
	2	35.20	33.11	35.16	34.37	34.59	34.66	34.57
	4	34.82	34.05	34.54	34.18	35.05	34.99	35.24
	6	35.58	32.32	34.81	32.49	35.15	35.01	33.77
	8*	33.21[a]	32.59[a]	34.14[a]	32.71[a]	33.99[a]	34.04[a]	35.12[a]
	µ	34.70	33.02	34.66	33.44	34.70	34.68	34.68
	dp	1.33	0.88	0.92	0.97	1.04	0.99	1.38
Total MUFAs (%)	0	45.89	46.53	45.23	44.74	45.21	44.19	44.80
	2	43.81	45.22	42.97	43.99	43.14	44.58	43.38
	4	45.79	45.11	43.78	43.50	43.36	43.17	44.17
	6	44.16	45.33	44.15	43.07	42.56	43.96	43.11
	8*	42.78[abcd]	43.25[abcd]	41.71[bcd]	43.52[abcd]	41.64[bcd]	41.31[cd]	40.68[d]
	µ	44.14	44.73	43.15	43.52	42.68	43.26	42.84
	dp	1.34	1.18	1.32	0.64	1.31	1.30	1.57
Total PUFAs (%)	0	21.29	21.14	21.51	22.40	21.68	22.22	22.37
	2	20.43	21.20	21.21	21.19	21.74	20.27	21.22
	4	18.97	20.16	21.11	21.69	20.92	21.31	19.95
	6	19.74	21.82	20.34	22.98	20.72	20.56	22.19
	8*	20.88[abc]	22.96[a]	22.63[ab]	22.46[ab]	23.04[a]	23.60[a]	23.37[a]
	µ	20.01	21.54	21.32	22.08	21.61	21.44	21.68
	dp	0.92	1.03	0.83	0.70	0.91	1.35	1.29
Total Ω–6 PUFAs	0	65.93	62.76	64.38	62.70	62.88	67.13	74.23
(mg/g/yolk)	2	65.11	62.67	57.78	64.58	66.39	61.85	61.12
	4	56.02	55.49	55.26	60.57	55.91	56.46	54.36
	6	61.28	67.68	64.57	68.62	57.81	58.41	65.09
	8	66.89[a]	68.79[a]	68.76[a]	68.28[a]	66.91[a]	68.76[a]	67.98[a]
	µ	62.33	63.99	62.86	65.82	60.21	61.21	62.48
	dp	4.81	6.06	6.19	3.77	5.71	5.41	5.90
Total Ω–3 PUFAs	0	3.39	3.84	2.94	2.87	2.58	3.64	3.06
(mg/g/yolk)	2	4.25	5.58	6.61	7.45	9.04	8.64	9.50
	4	3.50	4.95	6.49	7.18	8.43	8.33	9.13
	6	4.26	6.02	6.87	7.48	7.77	8.73	9.98
	8	3.75[e]	6.10[ed]	8.50[bcd]	7.36[cd]	9.06[bcd]	8.84[bcd]	10.70[abc]
	µ	3.94	5.66	7.12	7.37	8.58	8.64	9.83
	dp	0.38	0.53	0.94	0.14	0.61	0.22	0.68
Yolk weight (g)		16.90	17.90	16.28	16.00	17.00	16.38	17.08
Total lipids (%)		33.85	33.97	33.70	33.00	32.87	33.67	32.55
Yolk fat (g)		5.61	5.84	5.47	5.28	5.59	5.51	5.56

*Means (three replicates) with different letters in the same row denote significant difference ($p<0.05$) by Tukey test.
[1]Absolute value, resulting from a determination by the treatment time in weeks

Table 6: Results shown below in mg/yolk arachidonic acid (AA), EPA, DPA and DHA at 0, 2, 4, 6 and 8 experimental weeks, according to the treatments studied

Treatments	Times (Weeks)	CON	AM120	AM180	AM240	AM300	AM360	AM420
AM (%)		0	0.50	0.75	1.00	1.25	1.50	1.75
Fatty acids		Fatty acids content						
AA (mg/yolk)	0	73.53	79.90	73.70	69.96	53.73	88.76	124.31
	2	110.09	89.03	91.13	84.80	82.83	68.78	74.77
	4	82.83	81.91	85.21	85.51	75.55	67.84	61.21
	6	107.67	97.25	89.84	91.85	74.29	71.43	72.25
	8*	98.71^{a}	96.40^{a}	89.68^{ab}	82.39^{ab}	80.83^{abc}	100.14^{a}	77.79^{abc}
	µ	99.83	91.15	88.97	86.14	78.38	77.05	71.51
	dp	15.89	8.00	7.18	8.03	11.58	14.38	24.43
EPA (mg/yolk)	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	2	0.00	0.00	0.00	0.00	1.61	2.41	2.83
	4	0.00	0.00	0.00	0.00	1.59	2.88	0.00
	6	0.00	0.00	0.00	1.19	0.00	2.56	2.30
	8*	0.00^{e}	0.24^{e}	1.40^{e}	0.82^{e}	1.11^{e}	2.54^{de}	2.90^{de}
	µ	0.00	0.06	0.35	0.50	1.08	2.60	2.01
	dp	0.00	0.11	0.63	0.57	0.81	1.17	1.48
DPA (mg/yolk)	0	19.08	14.50	15.00	15.83	14.53	22.34	17.62
	2	33.05	17.35	18.15	15.82	18.97	13.79	15.11
	4	27.58	15.26	15.44	15.96	14.28	11.92	13.55
	6	34.87	22.04	19.33	18.46	17.18	14.15	15.62
	8*	31.50^{a}	19.98^{b}	19.83^{b}	14.67^{bc}	16.69^{bc}	13.54^{c}	15.42^{bc}
	µ	31.75	18.66	18.19	16.23	16.78	13.35	14.93
	dp	6.27	3.17	2.22	1.39	1.95	4.11	1.45
DHA (mg/yolk)	0	28.33	20.27	25.38	21.25	18.71	31.36	23.48
	2	31.53	65.43	85.88	99.16	113.00	113.07	127.04
	4	22.95	61.06	88.71	93.53	113.06	114.31	124.14
	6	28.25	70.47	90.02	98.86	102.48	127.94	135.79
	8*	22.64^{f}	77.02^{e}	116.95^{bcde}	98.01^{de}	119.69^{bcde}	127.54^{bcde}	149.75^{abc}
	µ	26.34	68.50	95.39	97.39	112.06	120.72	134.18
	dp	3.84	22.37	33.73	34.13	42.20	40.58	50.50

*Means (three replicates) with different letters in the same row denote significant difference ($p<0.05$) by Tukey test.
[1]Absolute value, resulting from a determination by the treatment time in weeks

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 Saturated (% SAT), Monounsaturated (% MUFAs), Polyunsaturated (% PUFAs), n-6 PUFAs (mg/g), n-3 PUFAs (mg/g), arachidonic (AA, mg/yolk), docosahexaenoic (DHA, mg/g), docosapentaenoic (DPA, mg/yolk) and eicosapentaenoic (EPA, mg/yolk) by effect of treatments, sources e levels studied. 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 3-7 and Fig. 1-5.

Totals fatty acid (%): Total saturated (%) in the times showed averages ranging from 33.02%±0.88 (AM120) to 36.27%±2.55 (OP360) of SAT in the yolk indicating no influence treatments and levels on the times for 2, 4, 6 and 8 weeks studied (Table 3 and 5), however, between sources, OP showed significantly higher means ($p<0.01$) than the AM (Table 7).

Monounsaturated (MUFAs): The total MUFA (%) showed significant variation in means 42.68%±1.31 (AM300) to 46.84%±1.00 (OP180) (Table 3 and 5), being significantly higher for the source OP ($p<0.01$), independent of the times studied (Table 7).

Polyunsaturated (PUFAs): The total PUFAs (%) showed significant variation from medium to 17.63±1.84% (OP360) to 22.08%±0.70 (AM240), which was significantly higher ($p<0.01$) to the source AM (Table 3 and 5).

Ω–6 PUFAs: The total n-6 PUFAs ranged from 45.8 mg/g (OP360), 40.37 mg/g (OP360, 4 weeks) to 65.82 mg/g (AM420) and 68.79 mg/g/yolk (AM120, 8 weeks), being the higher mean ($p<0.01$) obtained at the source AM (Table 3, 5 and 7).

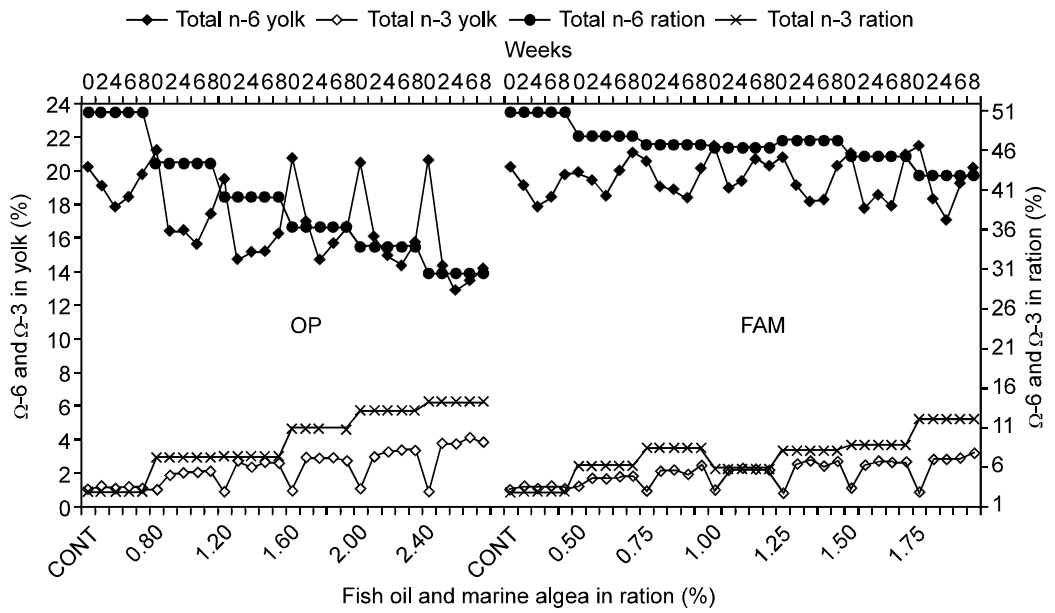

Fig. 1: Evolution levels of W-6 and W-3 in the yolk in function of levels of PUFAs in the ration

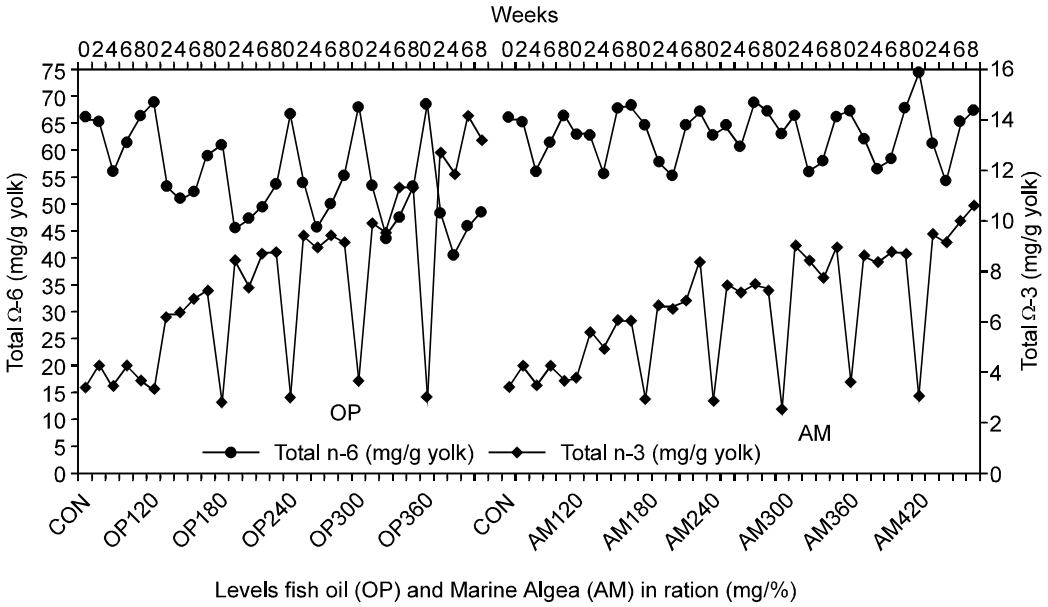

Fig. 2: Total Ω-6 and Ω-3 fatty acids in egg yolk (mg/g/yolk) in 0, 2, 4, 6 and 8 experimental weeks, according to the sources and treatments studied

Ω-3 PUFAs: The total n-3 PUFAs varied ($p<0.01$) significantly between sources and levels of 5.58 mg/g (AM120, 2 wk) to 14:16 mg/g (OP360, 6 wk) versus average 3.34±0.84 mg PUFAs n-3/g/yolk (CON). Between times, significant interaction ($p<0.01$) was observed for an average of n-3 source in OP (Table 7).

Totals fatty acid in yolk (mg/yolk): The mean of 99.83 mg (CON) decreased to 31.99 mg (OP360, 4 wk), 40.43±14:38 mg (µ, OP360) to 61.21 mg (AM420) and 71.51±24.43 mg AA/yolk (µ, AM420). Was significant ($p<0.01$) the effect of OP on AM, however, there was no interaction (Table 7) between sources and levels ($p>0.05$).

Mean ±69.86 mg 22.34 (CON) increased to 176.53 mg (µ, OP360), 187.91 mg (OP360, 8 wk) and 192.96 mg (OP360, 6 wk) and 134.18±50.50 mg (µ, OP360) 135.79 mg (AM420, 6 wk), 149.75 mg DHA (AM420, 8 wk) per

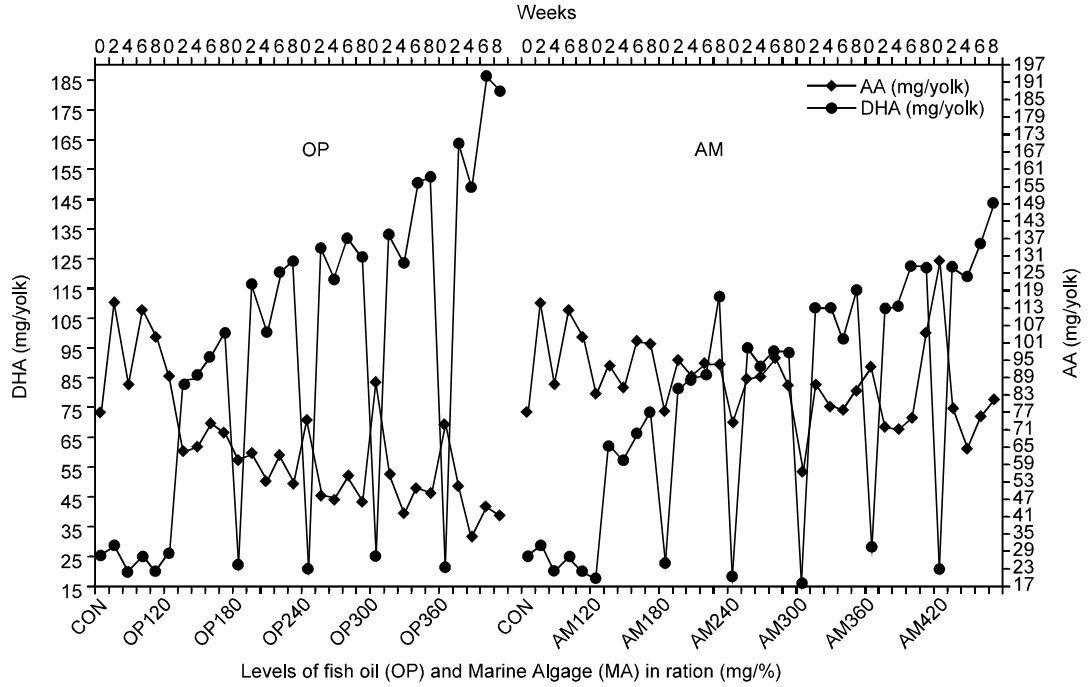

Fig. 3: Total fatty acids arachidonic ($C_{20:4\ \omega-6}$) and docosahexaenoic ($C_{22:6\ \omega-3}$) in egg yolk (mg/yolk) at 0, 2, 4, 6 and 8 experimental weeks, according to sources and treatments studied

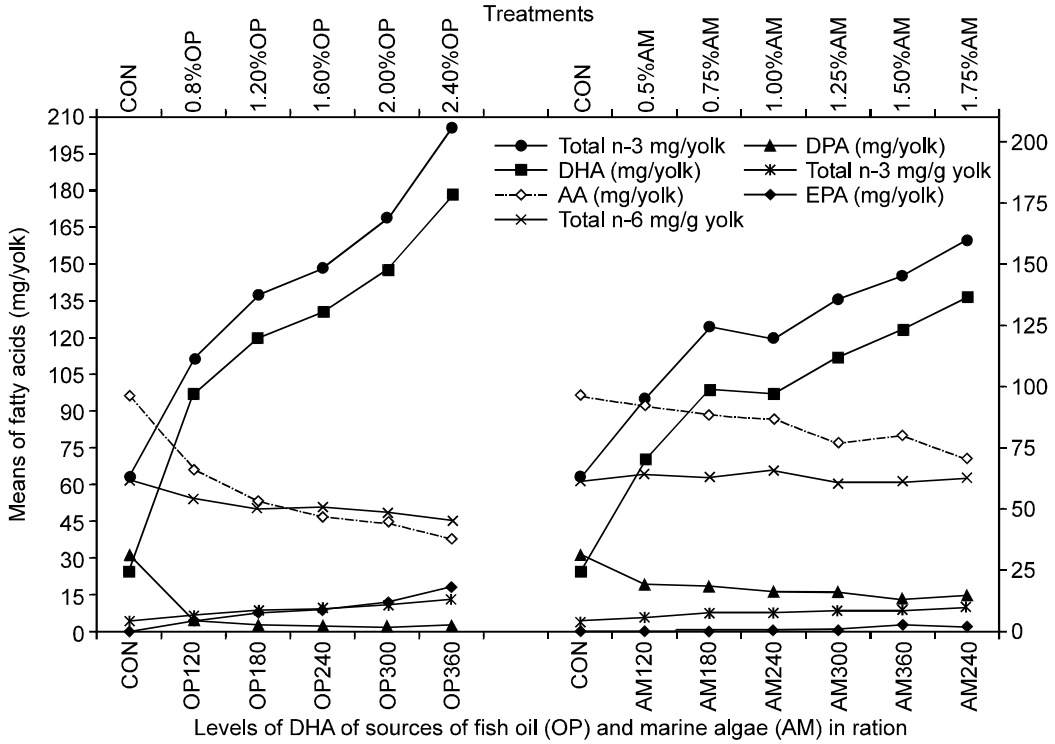

Fig. 4: Means fatty acids in times of 2, 4, 6 and 8 experimental weeks, according to the levels of DHA source of fish oil and marine algae in the diet (mg/100 g)

yolk. Significant effects ($p<0.01$) were observed for the levels and sources, being more effective for the OP. However, there was no significant interaction between these.

Table 7: Analysis of variance of the fatty acid contents of the yolk, according to the sources and levels studied

Sources variation	G.L	SAT	MUFAs	PUFAs	Ω-6 PUFAs	Ω-3 PUFAs	AA	EPA	DPA	DHA
		%			mg/g/yolk					
		F-values								
Sources (F)	1	9.78**	29.12**	79.07**	45.00**	33.79**	71.01**	226.57**	443.44**	50.04**
Levels (N)	4	0.97^{ns}	0.47^{ns}	0.21^{ns}	1.25^{ns}	21.93**	1.89^{ns}	19.71**	4.17*	21.42**
F x N	4	2.16^{ns}	1.78^{ns}	1.01^{ns}	0.64^{ns}	3.44*	1.25^{ns}	11.42**	3.37*	1.91^{ns}
Error	20	-	-	-	-	-	-	-	-	-
Total	29	-	-	-	-	-	-	-	-	-

*Significant at 5% level; **Significant at 1% level; ^{ns}not significant

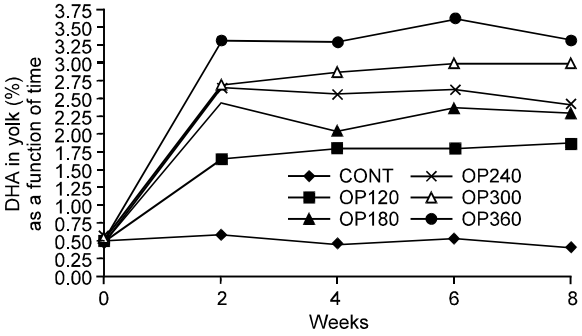

Fig. 5: Levels of DHA in the yolk in 8 weeks on the diet of hens supplemented with fish oil

The mean 3.69 mg (OP120, 2 wk) increased for 19.70 mg (OP360, 8 wk) and 0.24 mg (AM120, 8 wk) to 2.90 mg (AM420, 8 wk) and 2.30 mg EPA (AM420, 6 wk) per yolk.

As medias de 1.33 (OP300, 8 weeks) increased for 3.38 mg (OP360, 8 weeks) and for 15.42 mg (AM420, 8 weeks) and 19.98 mg DPA (AM120, 8 weeks) per yolk.

The mean of 1.33 (OP300, 8 wk) increased for 3.38 mg (OP360, 8 weeks) and 15.42 mg (AM420, 8 wk) and 19.98 mg DPA (AM120, 8 wk) per yolk.

The variations of the means of weight of 15.75 g (OP360) to 17.08 g (AM420) for the yolks, 32.55% (AM420) to 34.08% (OP360) for totals lipids and 5.28 g (AM240) to 5.84 g (AM120) of fat in the yolk were not influenced ($p>0.05$) between treatments, sources and levels studied (Table 3 and 5).

DISCUSSION

The composition of experimental diets in n-3 and n-6 PUFAs, influenced, once for all the chemical composition of yolk lipids. As a general rule, the extent to which the levels of n-3 PUFAs of marine origin were increased in all treatments the hens responded with increased levels of n-3 PUFAs and a decrease of n-6 PUFAs in the egg yolk (Table 1-7, Fig. 1-5).

The chemical composition of fatty acids of the treatments utilized, showed increment the contents of Ω–3 PUFAs and DHA, decreased of the n-6/n-3 ratio in the final ration of laying hens (Table 2) and increase significant for the levels of W-3 PUFAs, EPA and DHA in egg yolk (Table 3-7; Fig. 1-5).

The sources and levels of Ω-3 PUFAs of marine origin, influencied significantly the enrichment of egg yolk Ω-3 PUFAs. The times of 0 (initial), 2, 4, 6 and 8 weeks showed influence significantly the enrichment of tissues and yolks, being that after 2 weeks of administration of the diets was possible to show significant increases of Ω-3 PUFAs in the yolks.

Likewise, the results of this research, Mori (2001) about the eighth week, Pita *et al.* (2006) around 4 weeks of experimental and Piber (2006), at 4 weeks experimental showed significant additions of Ω-3 PUFAs in the yolk eggs, after supplementation with marine cocktails, rich in Ω-3 PUFAs in the diet of laying hens. Similarly, Hargis *et al.* (1991), Leskanich and Noble (1997) and Cherian and Sim (1991 and 1993) found the possibility of modifying the fat composition of egg yolk, favorable to increased of Ω-3 PUFAs in the human diet, besides the obtain olein phospholipid (phosphatidyl-olein) of yolks rich in Ω-3 PUFAs, EPA and DHA, widely used in industrial and children's formulations (Noble, 1987; Nash *et al.*, 1995; Leskanich and Noble, 1997; Pita *et al.*, 2006).

Totals fatty acid (%): At present research, treatment and time in weeks did not influenced the percentage of SAT totals, which stood around 33% to 36% of SAT, agrees with that described by Briz (1997) and Carvalho (2006) that the SAT suffer only minimal variation in the composition of total fatty acids, by influence of the addition of different fats the diets of laying hens (Table 3 and 5).

Monounsaturated (MUFAs): The total MUFA (%) showed variation in average around 42 to 46 (Table 3 and 5), being lower for the source AM ($p<0.01$) independent of the times studied (Table 7). Just as observed in present research, Watkins *et al.* (1997), Grobas *et al.* (2001) and Baucells *et al.* (2000) reported that the expense of MUFA especially, in higher levels in the yolk - oleic acid - occurs the increased incorporation of n-3 PUFAs in the fat of egg yolk.

Polyunsaturated (PUFAs): The total PUFAs (%) showed variation in average around 17% (OP360) to 22% (AM240), being the source the OP responsible for larger reductions ($p<0.01$) in the content of total PUFAs in the

fat composition of yolk (Table 3, 5 and 7). Agreeing with the results of the present research, Griminger (1986), Chang and Huang (1990), Jiang and Sim (1992), Cherian and Sim (1993) and Mori (2001) observed that hens respond favorably to reduction of PUFAs total by influence of the change of lipid profile of the diet.

Ω-6 PUFAs: The total Ω-6 PUFAs, ranged from 45.8 mg/g (OP360), 40.37 mg/g (OP360, 4 wk) to 65.82 mg/g (AM420) and 68.79 mg/g/yolk (AM120, 8 wk), being the higher means ($p<0.01$), obtained from the source AM (Table 3, 5 and 7).

During the time of achievement of present research, the experimental time in weeks among all the acids examined, the total content of Ω-6 PUFAs were the ones that showed the greatest reductions in average 62.33 mg/g (CON) to 45.83 mg/g/yolk (OP360) by influence of the source OP in diet, being that the inverse was observed with total n-3 PUFAs, in which the average increased from 3.94 mg/g (CON) to 13 mg/g (OP360) and 9.83 mg/g/yolk (AM420) by influence from both sources OP and AM. Like finding, in the present study are in agreement with that mentioned by Watkins *et al.* (1997) and Briz (1997) and Pita (2007), that in the desaturation and elongation of the chain of 18 C acids, Linoleic (AL) and Linolenic Acid (ALA), in the competition for enzyme delta-6-desaturase, by difference of substrate, the reaction is displaced in favor of the conversion of the n-3 PUFAs - EPA (C 20:5 n-3) and DHA (C 22:6 n-3) - in detriment of Arachidonic (AA, $C_{20:4\ n-6}$). Mean time (2, 4, 6 and 8 wk) decreased from 99.83 mg (CON) for 40.43 mg (OP360) and 71.51 mg AA/yolk (AM 420), being the source OP more effective ($p<0.05$) in the reduction of AA in egg yolk in the times in weeks.

Moreover, at present, research, besides the small contribution of EPA and DPA, the largest contribution to the total n-3 PUFAs, in effect, treatment, sources and levels in different times (0, 2, 4, 6 and 8 weeks) studied, was observed for total DHA, which increased from 26.34 mg (CON) to 176.53 mg (OP360) and 134.18 mg/yolk (AM420), being the source of OP and levels of DHA in the diet , those responsible for such increases ($p<0.01$) in Ω-3 PUFAs (Table 3, 5 and 7; Fig. 1-5). Likewise evidenced by this study, similar results were presented in the experiments with laying hens mentioned by Mori (2001), Piber (2006), Pita (2007), which when utilized marine sources of omega-3 PUFAs, reported significant increases in total Ω-3 PUFAs, taking as a contribution especially in this significant addition, mainly increased to the DHA in the chemical composition of the fat in egg yolk.

The parameters of the weight of yolks, percentage of total lipids in the yolk and amounts of fat in egg yolk, have not influence ($p>0.05$) treatments, sources, levels and times (0, 2, 4, 6 and 8 weeks) studied in the present research (Table 3 and 5). According to these results, several authors (Cherian and Sim, 1991; 1993; Mori, 2001; Piber, 2006; Pita, 2007) mentioned that only the fat composition chemical of the yolk were significantly altered by the influence of marine sources to supplement the diet of laying hens.

Conclusion: Among marine sources, supplementary to the diet of laying hens, fish oils (OP) determined higher enrichment of Ω-3 PUFAs in egg yolk. Analyzing at the times, from 2 weeks of adding OP the diet of laying hens, eggs were obtained with significant levels of DHA and Ω-3 PUFAs. The source of marine algae (AM) supplement the diet, despite showing minimally lower levels of DHA and Ω-3 PUFAs in the fat of the yolk compared to OP source, both had similar response times in relation to the enrichment of eggs with Ω-3 PUFAs.

The levels of Ω-3 PUFAs increased significantly in time, culminating with plateau after the fourth week of the administration of supplemental rich in Ω-3. Based on whether these results, can be infer that the eggs were enriched after two weeks and from 4 weeks the increase of PUFAs was minimal as of this time which in practice shows availability of commercializing eggs containing Ω-3 PUFAs about 14 days and obtaining satisfactory levels of Ω-3 PUFAs after 28 days in order to provide for the consumer.

The operationalization of such agribusiness with a view to the consumer aware of healthy products certainly if will with basing in scientific research results, interpreted according to different sources of enrichment used in cocktails of supplements to laying hens. Knowing if as in poultry production, the study of the time factor and levels of enrichment with Ω-3 PUFAs in weeks it is vital for decision at the time of the commercialization of enriched eggs, the results of this present research, if lining of characteristics practices to inform the behavior of hens subjected to different cocktails of enriching in the diet and the best time in weeks estimated to obtain eggs enriched with Ω-3 PUFAs.

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