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Modulation of Plasma Levels and Percentages of Incorporation of ω-3 PUFAs in Egg Yolk under the Influence of Supplementation Sources Rich in Omega 3 to Diet of Laying Hens

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Abstract: Hundred forty-four Shaver White laying hens were used over a 4 week experimental period to investigate the effect of 3% of soybean oil, corn oil (MIL), canola oil, flaxseed oil (LIN), salmon oil (SAL) or tuna and sardine oil (SR/AT) added to the diets, upon the fatty acid egg yolk composition, blood plasma levels and incorporation time of each fatty acid into the egg yolk. Hens were allocated into 72 cages and the experimental design was a 6 x 6 randomized factorial model. Hens fed 3% of different oils, responded with increased polyunsaturated fatty acids omega 3 (ω-3 PUFAs), except for corn oil. The addition of flaxseed, soybean or corn oil into the diet increased the PUFAs levels into the egg yolk and in the blood plasma. Adding tuna and sardine oil into the diet increased the concentration of yolk saturated fatty acids. The levels of ω-3 PUFAs were increased in the tuna and sardine oil treatment, while the flaxseed oil increased the plasma fatty acids. The deposition of 349.28 mg/yolk of α-linolenic fatty acids (ALA) was higher in the group fed LIN, while the higher equal to 157.13 mg DHA/yolk was observed in group SR/AT. In the plasma, deposition increased from 0.33% (MIL) for 6.29% ALA (LIN), while that of DHA increase of 0.47% (MIL) for 4.24% (SAL) and 4.48% (SR/AT) and of 0.98% (MIL) for 6.14% (SR/AT) and 8.44% (LIN) of ω-3 PUFAs. The percentage of EPA into the yolk and plasma was higher for the hens fed 3% tuna and sardine oil diet, as well as the levels of yolk DHA. The concentration of DHA into the plasma was higher for the salmon and tuna/sardine oil treatments. The PUFAs yolk decreased during the first eight days of experiment, while the ω-3 PUFAs increased during the same period. The concentration of ALA increased until ten days of experiment, while the percentage of EPA and DHA increased up to the eighth experimental day.

Key words: DHA, fish oil, omega-3, plasma fatty acids, eggs, yolks

INTRODUCTION

Polyunsaturated Fatty Acids (PUFAs): In last decades, has been proven that diets with adequate amounts of omega 3 polyunsaturated fatty acids (ω-3 PUFAs) play an important role in the prevention and treatment of various diseases. Supplementation, dietary ω-3 PUFAs has also been associated with reduced cardiovascular diseases, cancer and ulcerative colitis, may also protect patients with pre-neoplastic lesions of the colon (Teitelbaum and Walker, 2001). Both, ω-3 PUFAs-EPA ($C_{20:5\ \omega-3}$, eicosapentaenoic) and DHA ($C_{22:6\ \omega-3}$, docosahexanoic) - are potent anti-inflammatory agents and show no side effect, being used successfully in the treatment of autoimmune inflammatory diseases such as psoriasis and inflammatory arthritis in addition to possessing antithrombotic effect (Shapiro, 2003). Was also shown that the DHA is a fatty acid essential for brain, visual and cognitive function development of human newborns (Dangour *et al*., 2010).

The importance of the ω-3 family - DHA, DPA ($C_{22:5\ \omega-3}$, docosapentaenoic) and EPA - of PUFAs for the development of the central nervous system - phospholipids of the brain, where it functions in the maintenance of membrane fluidity, permeability, ion conduction and release of acetyl choline - in both mammalian and avian species is unequivocal (Neuringer *et al*., 1988; Neuringer, 2000). Large amounts of DHA are required by the brain during the late gestational and early postnatal periods when cellular differentiation, active synaptogenesis and membane biogenesis take place. Deficiency of ω-3 fatty acids has been reported to cause impaired learning defects, visual abnormalities and polydipsia in nonhuman primates (Connor *et al*., 1991).

The diet should supply precursors, α-Linolenic (ALA), EPA or DHA. Considering the importance of ω-3 PUFA in the development of the central nervous system, the study of Noble and Cocchi (1990) comparing the magnitude of

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changes in the fat acid composition of Phosphotidylethanolamine (PE) and Phosphatidylcholine (PC) fraction of brain and liver tissue of newly hatched chicks from eggs enriched with ω-3, ω-6, or ω-9 fatty acids.

Physiologically the importance long-chain ω-3 PUFAs (LC ω-3 PUFAs) - EPA and DHA - are obtained from the elongation metabolites of the essential ALA. In the human, the conversion of ALA to LC ω-3 PUFAs is inefficient (0.5% at 10% to EPA). Although present in marine mammals and fishes, os PUFAs are under represent in the traditional western diet. It is assumed that the optimal ratio of ω-6 to ω-3 fatty acids in the human nutrition is around 3:1, studies estimate that the actual ratio ω-6:ω-3 ranges between 10:1 and 15:1 (Moran, 1996; Simopoulos, 2000; 2009). The most ω-3 PUFAs of egg yolk are located in the position sn-2 of phospholipids, ie, the DHA and EPA may occupy the position sn-2 of PC and PE (Schreiner *et al*., 2004).

Animals and humans are unable to synthesize ALA. Derivatives by synthesis from alpha-linolenic acid, especially EPA and DHA, are components of cell membranes of organs and tissues including those who perform functions that are vital to the retina, neurons, regulators and hormonal modulators, hormones, among others, therefore essential vital functions. Its deficiency can lead to metabolic disorders subclinical or clinical depending upon of the function they implement. Unlike mammals, chickens have a rudimentary lymphatic system. Thus, chylomicrons are absorbed directly into the portal blood or transport to liver for further synthesis and subsequent tissue deposition (Cherian *et al*., 1996a).

Yolk chichen eggs formation and plasma lipoproteins: In formation and accumulation of lipid yolk, Noble (1987) inferred that on average, an egg 60 grams contains about 6 grams of fat. The sequential maturation from egg yolk occurs within the ovary, in the range of 24 h and there is a hierarchy of follicular maturation, with 4 to 6 developing follicles. The effort required to sustain the metabolic formation of lipid yolk is centered in the organized synthesis and transport system. The weight and lipid content of the liver are dramatically increased in laying hen. The total concentration of lipid increases 2 to 3 times higher than that found in immature hen, in particular Triglycerides (TG). The liver changes are in response to egg production and are under hormonal influence. The lipids are accumulated in the liver by stimulating the synthesis of fatty acids, in contrast to mammals in which it is associated with adipose tissue (Fisher and Leveille, 1957; Leveille *et al*., 1975).

The efficient conversion of ALA to EPA and DHA is probably limited by the competition for delta-6-desaturase (removal of hydrogen atomfrom the carbon chain by delta-6-desaturase), the enzyme essential for the first step in the desaturation if ω-3 and ω-6 fatty acid. Two carbons are then added by an elongase.This desaturation-elongation cycle continues until the production of PUFAs, $C_{22:4\ \omega-6}$ or $C_{22:6\ \omega-3}$, reaches completion (Nettleton, 1995).

The transport of fatty acids in the bird is different from mammals. Long-chain fatty acids and monoglycerides are esterified to triglycerides in the endoplasmic reticulum of cells of the intestinal mucosa. The new synthesized TG coalesce into drops which receive a layer of phospholipids (PC or PE), a thin layer of protein, more free cholesterol to form a chylomicron. In birds, the rich lipoproteins in resynthesized lipoproteins are absorbed directly into the system from the portal circulation and transported to the liver, while in mammals, after carried the extracellular space, the transport of chylomicrons is via the lymphatic system, from which are carried the bloodstream. The laying hens liver, in non-posture, has an mean of 3% to 5% of wet weight in fat. The laying hen's liver, in posture, has an average of 10-15% dry weight. Feeding large amounts of dietary fat depresses liver lipogenesis in the laying hen. High dietary fat levels results in patterns of fatty acid incorporation into liver and egg yolk TG that resemble the fatty acid composition of the dietary fat. TG: in liver: 72% and in yolk: 70%. Phospholipids: in liver: 9% and in yolk: 21% (Ivy and Nesheim, 1973).

Most of the yolk components are derived from the blood plasma. The major plasma precursors are vitellogenic and a specialized type of TG-rich lipoprotein found in abundance in the laying hen. There are synthesized in the liver, in response to oestrogen stimulation, transported to the ovary and transferred to the growing oocyte, most probably by a selective mechanism. The accumulation of triglyceride in the plasma of the laying hens is a result of an oestrogen-induced increase in the rate of synthesis of Very Low Density Lipoprotein (VLDL) lipoproteins in liver. VLDL lipopoteins from the plasma of laying hens are different to those from immature hens or roosters. The concentration mean was of 138 mg VLDL-TG/100 mL non estrogenized versus 5.025 mg VLDL-TG/100 mL of plasma in one estrogenized birds (Kudzma *et al*., 1975).

The plasma of the hen not lay and young rooster, contains the levels around of 360 mg HDL, 120 mg Low Density Lipoprotein (LDL) and 60 mg VLDL/dL. The laying hen's plasma contains the levels around of 140 to 180 mg High Density Lipoprotein (HDL), 200 mg LDL and 1400 to 5500 mg VLDL/dL, representing an increase of VLDL in plasma from 23 to 91 times (Griffin *et al*., 1984).

The effect of dietary Herring Meal (HM) on plasma lipids and egg yolk of laying hens was studied by Nash *et al*. (1995) at four periods in the laying cycle. Plasma total lipids were inversely related to dietary levels while HM ω-6 ($p<0.01$) and ω-3 ($p<0.001$) fatty acid levels were positively and inversely related. The levels of total lipids

differed ($p<0.01$) among treatments ranged from 44.4 mg (control, 0% HM), 38.5 mg (4% HM), 39.1 mg (8% HM) and 38.9 (12%) mg lipid/mL in plasma.

The yolk of the egg contains large amounts of a lipoprotein which has similar density characteristics to plasma low density lipoproteins. In chickens liver, the fatty acid specificity of triglyceride synthetase closely paralleled the mammalian systems; the insaturated fatty acid mainly occupied the 2-position (EPA and DHA in position sn-2 of phospholipid) while the saturated fatty acids and unsaturated fatty acids of trans configuration occurred at the 1, 3 positions of the triglycerides (Bickerstaffe and Annison, 1970). Egg yolk triglyceride showed a similar distribution of fatty acids. Lipid deposition occurs during the later stages of ovum formation determines that it contains the majority of the yolk lipid. PC and PE are by far the major phospholipid components. In the PE, phosphatidyl serine and PC fractions palmitic and stearic acids together account for about 50% of the total fatty acids. In the cholesteryl ester, triglyceride and total phospholipids fractions, oleic acid is the major fatty acid present. Palmitic and stearic acids together account for more than one-third of the fatty acids; substancial levels of linoleic acid are present in each.

Lipid composition of the yolk: About 33% of the total weight of the yolk and 60-65% of its dry matter content. However, as can be seen later, under conditions where particular specialized diets have been fed extensive changes can be observed in both the lipid and fatty acid compositions of the yolk. The proportions of major lipids (% weight of total) in the plasma of the laying hen is 3.9% cholesterol esters, 62.9% of triglycerides, 25.4% of phospholids, 1% of free fatty acids, 6.8% of free cholesterol. The composition of phospholids is: 17.7% of PE, 2.2% of phosphatidyl inositol, 70.9% of PC, 3.6% of sphingomyelin and 5.6% of lyso-phosphatidyl choline (Leskanich and Noble, 1997). DHA and EPA occupy the position sn-2 of PE or PC.

The hepatic synthesis is responsible for the supply of lipids needed for the formation of egg yolk, being that the lipids deposited in adipose tissue are mobilized only in extreme circumstances of low-energy diets. The fatty acid composition of yolk can be modified via the nutrition of poultry, especially if change the source, or amount of oils and fats in the diet (Cherian *et al*., 1996b; Hargis *et al*., 1991; Grobas *et al*., 2001; Ahn *et al*., 1995; Scheideler and Froning, 1996; Cherian and Sim, 1991; 1992; 2001; Mori, 2001). Inversely, age and strain of the bird, seems little to influence the fatty acid composition of yolk (Nielsen, 1998), although, Grobas *et al*. (2001) and Scheideler *et al*. (1998) have demonstrated significant differences in the concentrations of some fatty acids such as palmitic acid, stearic acid, linoleic acid and DPA, between commercial strains.

The fatty acid composition of eggs can be modified through the hen's diet (Hargis and Van Elswyk, 1993). Linoleic acid should be reduced in the egg due to their abundance in the western human diet. Thus, the enrichment of eggs with ω-3 PUFA has practical relevance. Eggs enriched with EPA and DHA could be a source of these ω-3 polyunsaturated fatty acids for people not consuming fish products. The recommended daily intake of total ω-3 polyunsaturated fatty acids has been set at 1.5% of the dietary energy, which is equivalent to 0.65 g EPA +DHA, 2.22 g ALA and Sat<8% per day adult, being 2000 Kcal per diet (Simopoulos *et al*., 2000).

The fatty acids of the series ω-3 long chain are typically found in fish oils and their derivatives, as well as in some marine algae. Since linoleic acids and ALA acid are present in large amounts in some vegetable oils such as flaxseed, canola and soybean (Mori, 2001; Pita, 2003). The incorporation of ω-3 PUFAs in the yolk, both relative to the DHA as at the EPA, increases as a result of adding fish oil to the diet of laying hens (Mori, 2001; Piber Neto, 2006; Carvalho, 2006; Carvalho *et al*., 2009), being that the refining of the oil can promote significant losses of ω-3 fatty acids (Briz, 1997).

The modification of the lipid profile of egg yolk can be produced from the inclusion of specific oils in the diet of hens such as fish oils flaxseed oils and even through the incorporation of the own seeds in the diet of laying hens. Numerous studies show that the change of lipid diet promotes change the concentrations of fatty acids of the yolk, which suggests a change in behavior at the level of plasmatic lipids from the dietary change (Gomez, 2003; Pita, 2003; Piber Neto, 2006; Carvalho, 2006; Mori, 2001; Lewis, 1996; Lewis *et al*., 2000).

This research has the objective to study the effect of the oils of soybean, corn, canola, flaxseed, salmon and a mixture of oils from sardines and tuna, the birds' diet on the fatty acid profile of yolk and plasma of laying hens.

It also has the purpose to analyze the incorporation of the main fatty acids in the yolk during the experimental period, aiming to define the minimum period required for full incorporation of dietary fatty acids, is carried out in different treatments.

MATERIALS AND METHODS

One hundred forty-four 28-week-old *Shaver White* commercial line of laying hens in this experiment were used.

Installations and equipments: The trial was conducted in the vivarium of birds from the Department of Clinical Medicine, Faculty of Veterinary Medicine and Zootechnic, University of São Paulo, localized in the Campus of the University City, São Paulo.

Table 1: Composition of experimental

Ingredients	SOJ	MIL	CAN	LIN	SAL	SR/AT
Corn	56.96	56.96	56.96	56.96	56.96	56.96
Soybean meal (48%)	27.27	27.27	27.27	27.27	27.27	27.27
Soybean oil	3.00	-	-	-	-	-
Corn oil	-	3.00	-	-	-	-
Canola oil	-	-	3.00	-	-	-
Linseed oil	-	-	-	3.00	-	-
Salmon oil	-	-	-	-	3.00	-
Sardine/tune oil	-	-	-	-	-	3.00
DL-methionine	0.11	0.11	0.11	0.11	0.11	0.11
Sodium chloride	0.39	0.39	0.39	0.39	0.39	0.39
Limestone	10.43	10.43	10.43	10.43	10.43	10.43
Dicalcium phosphate	1.59	1.59	1.59	1.59	1.59	1.59
Vitamin premix (*)	0.10	0.10	0.10	0.10	0.10	0.10
Mineral premix (*)	0.15	0.15	0.15	0.15	0.15	0.15
Determined analysis						
Ether extract (%)	4.38	4.65	4.62	4.68	4.42	4.46
α-Linolenic acid (%)	0.47	0.49	0.49	3.52	3.43	3.68
Analysis calculated						
Metabolizable energy (kcal/kg)	2,800.00	2,800.00	2,800.00	2,800.00	2,800.00	2,800.00
Crude protein (%)	18.00	18.00	18.00	18.00	18.00	18.00
Metionina (%)	0.40	0.40	0.40	0.40	0.40	0.40
Methionine + cystine (%)	0.70	0.70	0.70	0.70	0.70	0.70
Calcium (%)	4.50	4.50	4.50	4.50	4.50	4.50
Phosphorus total (%)	0.63	0.63	0.63	0.63	0.63	0.63
Phosphorus available (%)	0.40	0.40	0.40	0.40	0.40	0.40
Crude fiber (%)	2.48	2.48	2.48	2.48	2.48	2.48

(*) Premix vitamin-mineral supplies (per ton of diet): vitamin A 8,000000 UI, vitamin D_3 2,500000 UI, vitamin E 10,000 UI, vitamin K_3 2,500 mg, vitamin B_1 1,000 mg, vitamin B_2 5,000 mg, vitamin B_6 1,500 mg, vitamin B_{12} 12,000 mcg, pantothenic acid 8,000 mg, folic acid 500 mg, nicotinic 25,000 mg, selenium 150 mg

The birds in the experiment were housed in a uniform manner, as to body weight, egg weight and laying rate, immediately before the start of the experiment, 72 cages of dimensions 0.45m x 0.25m x 0.45m being placed two per cage, forming six treatments with three replicates of eight birds, where each replicate was represented by a set of four cages, in the food trough in trough-shaped and type *nipple* drinkers.

The experiment was carried in the course of the second semester of 2004 and the birds received water and food daily ad libitum and were subjected to a total of 16 hours of light.

For the analysis of ether extract, the experimental rations, we used the apparatus of Sohxlet and incubator Fanem® model SE 315.

To determine the fatty acid profile of oils used in diets of experimental diets, of the yolks and of plasma, was used a gas chromatograph Varian® brand, model CP 3800, equipped with flame ionization detector and coupled to the system Workstation Star Chromatography, where the column used was fused silica capillary CP-Wax 52CB (Chrompack) with 30m length, 0.25 mm in diameter and 0.25 mm of polyethylene glycol.

Experimental rations: The birds used in the experiment, were fed diets isocaloric and isonitrogenous, containing 3% of one of the oils: Refined Canola Oil (CAN), Linseed (LIN), corn (MIL), soybeans (SOJ) and crude Salmon (SAL) and industrial mix of crude oil Sardines plus Tuna (SR/AT), Totaling Six Treatments (TRT). To the crude was added Santoquim® for protection of oxidative fatty acids. The rations, based corn and soybeans, were formulated according to the nutritional requirements of NRC (1994), free from any ingredients of animal origin (Table 1). All oils used in the experiment were analyzed for fatty acid profile and the experimental diets produced, according to Table 2. It was added antioxidant Santoquin Roche® at a concentration of 500 mg/kg in oils in salmon and in sardines-tuna mixture.

Fatty acids in egg yolk: During the experiment were collected four eggs per replicate for lipid phase extraction and subsequent measurement of fatty acid content of yolk. This collection was performed on alternate days in the second, fourth, sixth, eighth and tenth day of experiment, in addition, a collection made on the thirtieth day trial. The yolks were separated, weighed and homogenized in order to obtain a sample for repetition ("pool" of four yolks), the every two days.

A gram of *in nature* egg yolk to lipid extraction was used, according to Folch *et al.* (1957) and Bligh and Dyer (1959), modified by Nielsen (1998), whereas the saponification of the lipid extract and the extraction of esters acids were made according to Hartman and Lago (1973). The samples were stored in a freezer at -80°C for around of 30 days.

Table 2: Fatty acid composition of the oils, rations and concentration of fatty acids ω-3 long-chain (ω-3 LC) oils used in the experimental diets (% of total fatty acids)

	Fatty acids (%)					
Fatty acids (Analyzed)	SOJ	MIL	CAN	LIN	SAL[a]	SR/AT[a]
Oil (composition)						
Saturated total (%)	14.83	15.70	7.47	12.63	27.22	37.04
Monounsaturated total (%)	24.54	35.90	62.48	24.39	31.50	24.02
Polyunsaturated total (%)	59.94	48.45	30.11	62.95	37.94	37.02
PUFAs ω-3 total (%)	5.71	0.79	8.31	49.51	32.66	31.19
Relation P/S	4.04	3.09	4.03	4.98	1.39	1.00
Miristic ($C_{14:0}$)	0.06	0.03	0.04	0.04	4.74	9.29
Palmitic ($C_{16:0}$)	0.00	0.00	0.00	0.00	0.45	0.82
Palmitoleic ($C_{16:1\ n-7}$)	0.07	0.12	0.17	0.07	7.29	10.19
Estearic ($C_{18:0}$)	3.22	2.10	2.00	6.01	4.76	4.24
Oleic ($C_{18:1}$)	24.20	35.46	60.90	24.18	23.37	12.01
α-Linolenic ($C_{18:3}$)	5.53	0.74	8.14	49.26	1.30	0.46
Rations (total diet)						
Saturated total (%)	16.93	16.45	11.73	14.89	21.98	29.83
Monounsaturated total (%)	26.62	32.71	52.59	27.83	36.32	25.18
Polyunsaturated total (%)	56.29	50.71	36.08	56.96	42.39	45.30
PUFAs ω-3 total (%)	5.03	1.32	6.32	30.93	17.60	22.72
Relation P/S	3.33	3.08	3.08	3.83	1.93	1.52
ALA (%, $C_{18:3\ ω-3}$)	4.90	1.32	6.17	30.34	1.33	1.93
Oil (ω-3 LC)[a]						
EPA (%)	0.05	0.00	0.00	0.00	10.92	15.91
DPA (%)	0.00	0.00	0.00	0.00	4.65	0.67
DHA (%)	0.00	0.02	0.17	0.14	15.61	13.95

[a]Added antioxidant Santoquin Roche® at a concentration of 500mg/kg
[b]ω-3 LC: Composition of the original sample ω-3 PUFAs of long chain

Completed the experiment with the birds, the solvent of the stored samples was evaporated under a stream of nitrogen and dry sample, rediluted in hexane and injected into the gas chromatograph. The conditions of operation of the device were: injection split 50:1, column temperature 150°C for 15 min, scheduled to 210°C at a rate of 3°C per minute. As carrier gas was used with nitrogen flow rate of 1.5 ml per minute, as the make-up gas, which is to 30ml per minute. The temperatures of injector and detector were 250°C and 280°C, respectively.

The determination of qualitative composition was made by comparison of retention times of peaks with the standards of fatty acid esters, using area normalization, expressed as a percentage by mass for the quantitative determination. The external standard of fatty acid esters used in this experiment was of the Supelco® 189-19.

Together with the analysis of fatty acids, was performed total lipid by gravimetry from a sample by repetition (Folch *et al*., 1957; Bligh and Dyer, 1959).

Fatty acids in the plasma of laying hens: After the end of the experiment, were proceeded to collect plasma from two birds per repetition, obtained a pool of two plasmas. The blood was collected through the jugular vein, which is in a total of 10 ml per pool.

To prevent the formation of a clot, heparin was used during the collection. Subsequently, we proceeded to the centrifuge for five minutes in a refrigerated centrifuge, where the serum was separated.

Was used in a milliliter of serum lipid phase separation of this, according to Folch *et al.* (1957) and Bligh and Dyer (1959), modified by Nielsen (1998), while saponification of the lipid extract and the extraction of esters of fatty acids were made according to Hartman and Lago (1973). The samples were stored in a freezer at -80°C for 30 days.

The sample injection in gas chromatography, occurred after solvent evaporation under a stream of nitrogen and redilution in hexane. The unit operated under the following conditions: injection split 50:1, column temperature of 150°C for 15 min, scheduled to 210°C at a rate of 3°C per minute. Nitrogen was used both as carrier gas with a flow rate of 1.5 ml per minute as a make-up gas, which is to 30 ml per minute, being employed temperatures 250°C and 280°C of detector.

Statistical analysis of results: The randomized block design with three replicates per treatment was employed and followed the procedures of analysis of variance described by Snedecor and Cochran (1980). Two criteria were used: sources of PUFAs (different oils) and retention time (DAY) of fatty acids in the yolks (6 x 6). The Tukey test was applied to the contrast between means. Statistical analysis was processed through the use of Statistical Analysis System software (SAS, 1985).

RESULTS

Lipid composition of egg yolk: Table 3 shows the percentage of saturated fatty acids, monounsaturated,

Table 3: Means and respective Errors of the mean (Em), reported for groups of fatty acids (% total lipid) and of the main fatty acids (% total lipid) present in the yolks of laying hens and their relationships, according to the treatments on the last day trial

TRT	SAT[a]	MONO	POLY	P/S	ω-3	ALA[b]	EPA	DPA	DHA
SOJ	34.38[b*]	41.48[a]	24.06[b]	0.70[c]	2.09[b**]	0.90[bc]	0.00[a]	0.40[b]	0.71[ab]
Em	±0.30	±0.60	±0.58	±0.02	±0.07	±0.03	±0.00	±0.08	±0.08
MIL	34.05[b]	42.96[ab]	23.01[b]	0.68[c]	1.22[a]	0.32[a]	0.00[a]	0.40[b]	0.41[a]
Em	±0.55	±1.07	±0.57	±0.01	±0.04	±0.02	±0.00	±0.08	±0.03
CAN	31.31[a]	51.44[d]	17.32[a]	0.55[b]	2.28[b]	1.13[c]	0.00[a]	0.09[a]	1.06[bc]
Em	±0.33	±0.77	±0.45	±0.01	±0.08	±0.01	±0.00	±0.03	±0.08
LIN	31.01[a]	45.50[bc]	23.66[b]	0.76[d]	9.21[e]	7.56[d]	0.24[b]	0.00[a]	1.45[c]
Em	±0.13	±0.32	±0.44	±0.02	±0.12	±0.17	±0.01	±0.00	±0.09
SAL	35.04[bc]	47.91[c]	17.33[a]	0.49[ab]	4.47[c]	0.54[a]	0.38[c]	0.00[a]	2.91[d]
Em	±0.70	±0.63	±0.44	±0.01	±0.17	±0.04	±0.04	±0.00	±0.16
SR/AT	37.04[c]	45.56[bc]	17.51[a]	0.47[a]	4.90[d]	0.56[ab]	0.50[d]	0.42[b]	3.30[d]
Em	±0.53	±0.81	±0.38	±0.01	±0.22	±0.08	±0.02	±0.03	±0.14

*Means with different letters in columns denote significant differences ($p \leq 0.05$) by Tukey test; Em: Error of the mean.
[a]ω-3 (Series ω-3 fatty acid), POLY (Polyunsaturated), MONO (Monounsaturated), SAT (Saturated), P/S (Polyunsaturated to Saturated). [b]ALA (α-Linolenic acid), EPA (Eicosapentaenoic acid), DPA (Docosapentaenoic), DHA (Docosahexaenoic Acid); Em: Error of the mean

polyunsaturated, total PUFAs ω-3, Polyunsaturated/ Saturated ratio (P/S), ALA, EPA, DPA and DHA of the egg yolk of laying hens collected by the end of the experiment of the different experimental groups.

PUFAs: The different treatments studied demonstrated significant changes ($p \leq 0.05$) in levels PUFAs in egg yolk. The groups that received CAN (17.32%), SAL (17.33%) and SR/AT (17.51%) produced yolks with less total amount of acids, compared with others: SOJ (24.06%), MIL (23.01%) and LIN (23.66%) (Table 3).

Series of ω-3 PUFAs, showed significant differences ($p \leq 0.05$) between treatments and the less content mean of 1.22% noted in MIL, which differed from the other groups. Intermediate values were reflected in means 2.09% (SOJ) and 2.28% (CAN), which revealed significant differences in relation to other groups. Content somewhat higher levels equal 4.47% were found in eggs from SAL, a value significantly different that the mean of 4.90% from SR/AT and other groups. The higher deposition of ω-3 fatty acids in egg yolk equal the 9.21% was recorded in the LIN group, considering all treatments (Table 3).

Note if that quantities were deposited significantly ($p \leq 0.05$) higher ALA in the yolks of the LIN group (7.48%), followed by CAN (1.13%) and SOJ (0.91%). The lowest levels of this acid were found in buds from MIL (0.33%) and SAL (0.61%). The deposition of EPA in the yolks only occurred in the LIN group (0.24%), SAL (0.38%) and SR/AT (0.50%), all values being significantly ($p \leq 0.05$) different and in the other groups. The levels of DPA in the yolks studied were significantly ($p < 0.05$) higher in treatment SR/AT (0.42%), MIL (0.40%) and SOJ (0.40%) in compared to other groups. DHA suffered significant increase ($p \leq 0.05$) of its content in egg yolk to the SAL (2.91%) and SR/AT (3.30%) compared to other treatments. However, the groups receiving linseed oil (1.45%) and canola oil (1.06%) had intermediate deposition of DHA in the yolk. The MIL group had the lowest average (0.41%) among the treatments (Table 3).

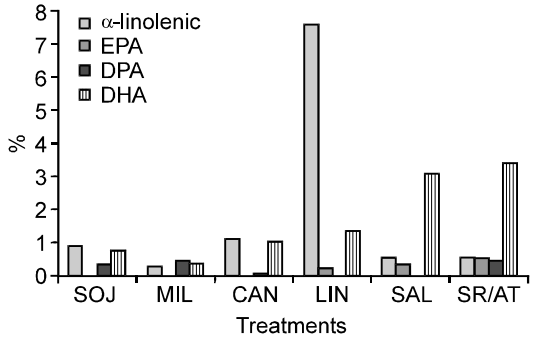

Fig. 1: Levels of ω-3 fatty acids in the yolk in the last experimental day, according to the treatments studied

The concentrations of ω-3 PUFAs in the egg yolk conformed the treatments are showed in Fig. 1. The addition of Linseed Oil (LIN) and fish oils (SAL; SR/AT) to the diet increased the levels of these fatty acids in the yolks. The Fig. 9 shows also the relationship between the concentration of ALA and DHA deposited in the yolk and in the plasma. The figure shows the close relationship between plasma concentrations and those present in the yolks from each treatment.

Time of incorporation of fatty acids in egg yolk: The percentages of saturated, monounsaturated, polyunsaturated, ratio Polyunsaturated/Saturated (P/S), the egg yolk of laying hens, according to the sources of fat and collection days during the experiment are demonstrated in Table 4. The analysis of variance of saturated fatty acids, monounsaturated, poly-unsaturated, total ω-3 PUFAs and relationship Polyunsaturated/Saturatted (P/S), the egg yolk linking if the lipid sources with the day of collection are presented in Table 5.

The behavior of total ω-3 PUFAs during the experiment are shown in Table 6, while Fig. 2 promotes the view of the existing values in table previously mentioned.

Table 4: Profile of the main fatty acids of egg yolk (% of total lipid), according to the sources and experimental days

TRT	SAT	MONO	POLY	ω-3	P/S
Sources					
SOJ	33.06^{b*}	43.78^{a}	23.22^{c}	1.60^{b}	0.70^{c}
MIL	32.55^{ab}	44.47^{a}	22.86^{a}	1.14^{a}	0.71^{c}
CAN	31.67^{a}	49.63^{c}	18.74^{c}	1.78^{b}	0.59^{b}
LIN	31.59^{a}	45.48^{b}	22.94^{c}	6.02^{d}	0.73^{c}
SAL	34.80^{c}	45.69^{b}	19.57^{b}	3.26^{c}	0.57^{ab}
SR/AT	35.16^{c}	45.63^{b}	19.35^{ab}	3.44^{c}	0.56^{a}
Days of treatment					
Day 2	32.01^{a}	46.31^{b}	21.71^{c}	1.12^{a}	0.68^{b}
Day 4	32.83^{b}	45.62^{ab}	21.56^{bc}	1.64^{b}	0.66^{b}
Day 6	32.90^{b}	45.37^{a}	21.68^{c}	2.90^{c}	0.66^{b}
Day 8	33.87^{c}	45.29^{a}	20.90^{ab}	3.77^{d}	0.62^{a}
Day 10	33.51^{bc}	46.16^{ab}	20.29^{a}	3.85^{d}	0.62^{a}
Day 30	33.71^{c}	45.94^{ab}	20.53^{a}	3.96^{d}	0.61^{a}

*Means with different letters in columns denote significant differences ($p \leq 0.05$) by Tukey test

Table 7 shows the percentages of major fatty acids found in plasma from hens fed the experimental diets, namely, palmitic, palmitoleic, stearic, oleic, linolenic acid, EPA, DPA and DHA, according with the fat sources and collection days. Figures 3 and 4 refer to the table afore mentioned.

Table 8 shows the analysis of variance of major fatty acids from egg yolks according to the days of egg collection and the lipid sources used in chicken feed.

Tables 9 and 10 shows the percentages of α-linolenic fatty acids, EPA and DHA from egg yolks of chickens, according to the sources of dietary fatty acids during the experimental period, whereas in Fig. 5, 6 and 7 are plotted graphically the values contained in Tables 9 and 10, respectively.

ω-3 PUFAs in the egg yolk: The total ω-3 fatty acid was deposited in lower quantities in the treatment MIL (1.14%) compared to the other, whereas the SOJ (1.60%), CAN (1.78%), SAL (3,26%) and SR/AT (3.44%) groups indicated intermediate values significantly and SAL and SR/AT different from the others, while the addition of LIN (6.02%) promoted the highest concentration total ω-3 egg yolk during the experiment (Table 4, Fig. 3).

Regarding the day of egg collection, was shown that the yolks analyzed on days 2 (1.12%), 4 (1.64%) and 6 (2.90%) had lower levels of n- 3 total compared to other days, being different between themselves. Since the eggs collected on days 8, 10 and 30 denote concentrations equal to each other significantly, indicating that after the eighth day of the experiment, the concentration of total ω-3 in egg yolk did not change until the end of the study (Table 4, Fig. 3). The analysis of variance of ω-3 fatty acids total (Table 5) denotes statistical significance ($p \leq 0.01$) for both sources and for days of collection and demonstrates the significant interaction between them. With respect to each of the sources of fatty acids used, it became apparent stabilization of the levels of ω-3 total DAY 4 (SOJ) DAY 6

Table 5: Analysis of variance of main fatty acids of the yolk, according to the sources and studied experimental days

		F values				
Sources of variation	G.L	SAT	MONO	POLY	ω-3	P/S
Sources (F)	5	32.17**	46.12**	67.22***	454.32**	49.53**
Days (D)	5	6.57**	1.98^{ns}	5.90**	211.48**	6.27**
F x D	25	4.83**	5.99**	6.78**	39.38**	5.19**
Residue	72	-	-	-	-	-
Total	107	-	-	-	-	-

**Significant at 1% ($p \leq 0.01$)

Table 6: Average contents of ω-3 fatty acids in egg yolk total (% of total lipids) and their Error of the mean (Em) according to the sources and time of experiment

	ω-3 Total					
Trt	Day 2	Day 4	Day 6	Day 8	Day 10	Day 30
SOJ	1.09^{a*}	1.41^{ab}	1.79^{ab}	1.40^{ab}	1.81^{ab}	2.09^{b}
Em[1]	±0.19	±0.16	±0.21	±0.08	±0.16	±0.08
MIL	1.11^{a}	1.19^{a}	1.16^{a}	1.13^{a}	1.04^{a}	1.22^{a}
Em	±0.29	±0.15	±0.26	±0.25	±0.22	±0.02
CAN	1.06^{a}	1.38^{ab}	1.80^{bc}	2.06^{c}	2.08^{c}	2.28^{c}
Em	±0.15	±0.10	±0.17	±0.08	±0.10	±0.10
LIN	1.21^{a}	2.43^{b}	6.10^{c}	8.33^{d}	8.81^{d}	9.21^{d}
Em	±0.20	±0.11	±0.30	±0.27	±0.16	±0.21
SAL	1.12^{a}	1.82^{a}	3.30^{b}	4.06^{bc}	4.80^{c}	4.47^{c}
Em	±0.10	±0.25	±0.20	±0.17	±0.32	±0.22
SR/AT	1.13^{a}	1.60^{a}	3.26^{b}	4.90^{c}	4.86^{c}	4.90^{c}
Em	±0.09	±0.19	±0.25	±0.44	±0.21	±0.30

*Means with different letters in rows denote significant differences ($p \leq 0.05$) by Tukey test; [1]Em: Error of the mean

Table 7: Profile of the main fatty acids of egg yolk (% of total lipid) according to the sources and experimental days used

	Fatty acid levels in yolk							
TRT	PALM	PALTOL	ESTE	OLEI	ALA	EPA	DPA	DHA
Sources								
SOJ	24.06^{c*}	2.48^{a}	8.43^{ab}	41.00^{a}	0.70^{c}	0.000^{a}	0.22^{a}	0.63^{b}
MIL	23.71^{c}	2.34^{a}	8.31^{ab}	41.70^{ab}	0.37^{a}	0.006^{a}	0.24^{a}	0.46^{a}
CAN	23.13^{b}	2.24^{a}	8.02^{a}	47.06^{d}	0.85^{d}	0.000^{a}	0.15^{a}	0.75^{b}
LIN	22.49^{a}	2.35^{a}	8.57^{bc}	42.88^{c}	4.73^{e}	0.079^{b}	0.12^{a}	1.05^{c}
SAL	24.88^{d}	2.92^{b}	9.08^{d}	42.47^{bc}	0.51^{b}	0.240^{c}	0.18^{a}	2.26^{d}
SR/AT	25.16^{d}	3.29^{c}	8.90^{cd}	42.00^{b}	0.53^{b}	0.320^{d}	0.24^{a}	2.26^{d}
Days of treatment								
Day 2	23.42^{a}	2.34^{a}	7.93^{a}	43.68^{d}	0.44^{a}	0.000^{a}	0.13^{a}	0.51^{a}
Day 4	23.62^{ab}	2.31^{a}	8.49^{b}	43.00^{bcd}	0.64^{b}	0.021^{a}	0.25^{a}	0.67^{b}
Day 6	23.69^{ab}	2.74^{b}	8.52^{b}	42.35^{ab}	1.32^{c}	0.089^{b}	0.21^{a}	1.25^{c}
Day 8	24.31^{c}	2.85^{b}	8.92^{b}	42.14^{a}	1.64^{b}	0.178^{c}	0.21^{a}	1.70^{d}
Day 10	24.02^{bc}	2.61^{ab}	8.84^{b}	43.25^{cd}	1.81^{e}	0.186^{c}	0.14^{a}	1.65^{d}
Day 30	24.37^{c}	2.76^{b}	8.61^{b}	42.70^{abc}	1.83^{e}	0.187^{c}	0.22^{a}	1.64^{d}

*Means with different letters in columns denote significant differences ($p \leq 0.05$) by Tukey test

Table 8: Analysis of variance of main fatty acids of the yolk, according to the sources and studied experimental days

	F values							
Sources of variation	G.L	PALM	PALTOL	ESTE	OLEI	ALA	EPA	DPA	DHA
Sources (F)	5	31.29**	11.44**	6.67**	69.86**	1522.29**	141.02**	1.45^{ns}	251.97**
Days (D)	5	4.49*	3.36^{ns}	5.41**	4.93**	193.00^{88}	50.83**	1.16^{ns}	104.96**
F x D	25	4.65**	2.94**	1.79^{ns}	7.63**	123.86**	14.27***	0.96^{ns}	22.11**
Residue	72	-	-	-	-	-	-	-	-
Total	107	-	-	-	-	-	-	-	-

*Significant at 5% ($p \leq 0.05$); **Significant at 1% ($p \leq 0.01$)

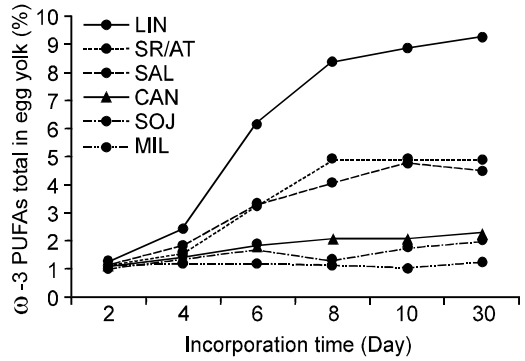

Fig. 2: Average concentrations of ω-3 fatty acids in egg yolk total (% of total lipid) according to the sources and time of incorporation

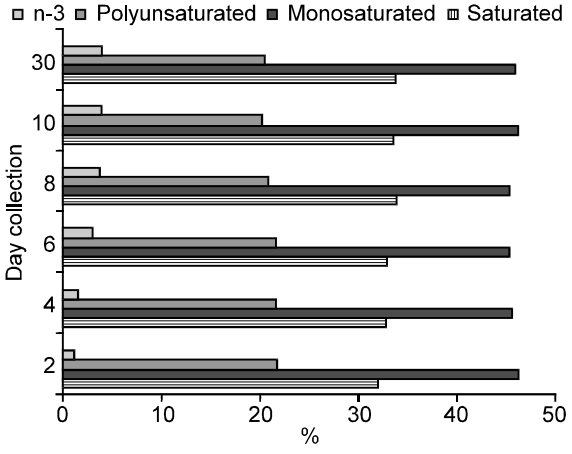

Fig. 3: Effect of day of experiment on the incorporation and fatty acid profile of the yolk of eggs produced during the experiment

(CAN) and DAY 8 (LIN, SAL and SR/AT) and these values were increased progressively until the days mentioned. The ALA was incorporated into the yolks, so significantly different between treatments, being much higher in the group fed with LIN (4.73%) (Table 7). Regarding the trial period, there was significant difference between growing days: DAY2 (0.44%), DAY4 (0.64%), DAY6 (1.32%) and DAY8 (1.64%) until the tenth day: DAY10 (1.81%), where there was a stabilization of their levels by the end of the study in DAY30 (1.83%) (Table 7, Fig. 4). These values promoted significance in the analysis of variance ($p \leq 0.01$) for both sources and collection days and showed significant interaction between them (Table 8). When associated each of the sources of fatty acids for the study period, increase in the levels of polyunsaturated progressive in the yolks of treatments was noted in groups SOJ (DAY10), CAN (DAY10) and LIN (DAY8) (Table 9, Fig. 5).

The fatty acids LC ω-3 long-chain EPA and DHA significantly change between different treatments and between days of collection of eggs, while the DPA not

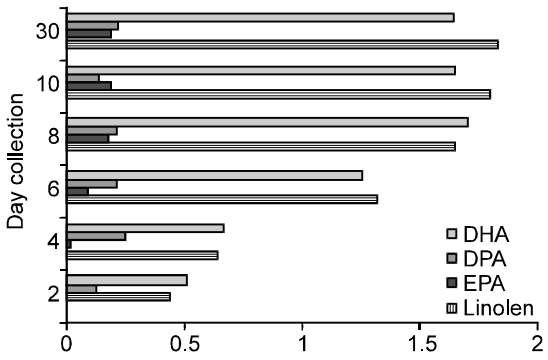

Fig. 4: Effect of day of experiment on the incorporation and profile of the main fatty acids in the yolk of eggs produced during the experiment

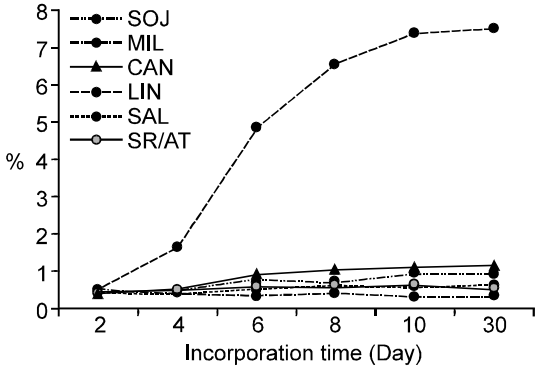

Fig. 5: Concentration of ALA in egg yolk (% of total lipid) according to the sources and time of incorporation

denoted a significant difference between treatments or between collection days (Table 7, Fig. 4). Thus, the acid EPA suffered significant increase in the yolks from the DAY4 for DAY6 (0.089%) and for DAY8 (0.178%), where it stabilized until the end of the experiment in DAY30 (0.187%). However, when assessing specifically the behavior of each source during the experiment, it is noted that the EPA concentrations increased up to DAY10 (LIN) and DAY8 (SAL and SR/AT), followed by the stable end of the study (Table 10; Fig. 7).

Very similarly, the treatments promoted different levels of DHA in egg yolk and the concentrations of this acid in the yolk were significantly different during the day and growing collection: DAY2 (0.51%), DAY4 (0.67%) and DAY (1.25%), suffering from significant stabilization of the eighth experimental day: DAY8 (1.70%), DAY10 (1.65%) and DAY30 (1.64%). In function of these values, analysis of variance acids EPA and DHA (Table 7) denote statistical differences ($p \leq 0.01$) for sources and collection days and showed significant interaction between them (Table 8). The levels of DHA stabilized in DAY8 in the treatments CAN, LIN, SAL and SR/AT (Table 10, Fig. 5).

Table 9: Levels of α-linolenic acid in egg yolk (% of total lipids) and their respective Errors of the mean (Em), according to the sources and time-trial (day)

	α-linolenic acid					
TRT	Day 2	Day 4	Day 6	Day 8	Day 10	Day 30
SOJ	0.39^{a}	0.49^{a}	0.73^{b}	0.72^{b}	0.89^{c}	0.91^{c}
Em	±0.23	±0.02	±0.10	±0.15	±0.55	±0.39
MIL	0.51^{a}	0.38^{a}	0.31^{a}	0.38^{a}	0.32^{a}	0.33^{a}
Em	±0.15	±0.03	±0.05	±0.06	±0.06	±0.03
CAN	0.40^{a}	0.53^{a}	0.90^{b}	1.03^{ab}	1.10^{c}	1.13^{c}
Em	±0.25	±0.44	±0.12	±0.36	±0.87	±0.14
LIN	0.52^{a}	1.60^{a}	4.84^{b}	6.55^{c}	7.36^{c}	7.48^{c}
Em	±0.11	±0.19	±0.32	±0.23	±0.18	±0.29
SAL	0.42^{ab}	0.38^{a}	0.52^{ab}	0.58^{ab}	0.55^{ab}	0.61^{b}
Em	±0.18	±0.04	±0.08	±0.06	±0.02	±0.01
SR/AT	0.39^{a}	0.46^{a}	0.58^{a}	0.58^{a}	0.65^{a}	0.51^{a}
Em	±0.02	±0.04	±0.06	±0.02	±0.10	±0.12

*Means with different letters in rows denote significant differences ($p \leq 0.05$) by Tukey test; Em: Error of the mean

Table 10: Levels of DHA and EPA in egg yolk (% of total lipids) and their respective errors of the mean (Em), according to the sources and time of experiment

TRT	Day 2	Day 4	Day 6	Day 8	Day 10	Day 30
DHA						
SOJ	0.53^{a}	0.54^{a}	0.72^{a}	0.59^{a}	0.66^{a}	0.71^{a}
Em	±0.06	±0.05	±0.04	±0.00	0.10	±0.07
MIL	0.45^{a}	0.47^{a}	0.47^{a}	0.53^{a}	0.40^{a}	0.41^{a}
Em	±0.03	±0.03	±0.02	±0.03	±0.07	±0.05
CAN	0.51^{a}	0.58^{ab}	0.70^{abc}	0.86^{cd}	0.81^{bc}	1.06^{d}
Em	±0.01	±0.04	±0.05	±0.02	±0.06	±0.08
LIN	0.49^{a}	0.57^{a}	1.06^{b}	1.43^{c}	1.33^{c}	1.45^{c}
Em	±0.03	±0.07	±0.06	±0.06	±0.05	±0.12
SAL	0.54^{a}	1.02^{a}	2.40^{b}	3.48^{c}	3.25^{c}	2.91^{bc}
Em	±0.02	±0.05	±0.06	±0.23	±0.28	±0.19
SR/AT	0.51^{a}	0.87^{a}	2.12^{b}	3.33^{c}	3.45^{c}	3.30^{c}
Em	±0.04	±0.09	±0.22	±0.39	±0.27	±0.18
EPA						
SOJ	0.00	0.00	0.00	0.00	0.00	0.00
Em	0.00	0.00	0.00	0.00	0.00	0.00
MIL	0.00^{a}	0.00^{a}	0.00^{a}	0.02^{a}	0.02^{a}	0.00^{a}
Em	0.00	0.00	0.00	0.02	±0.02	0.00
CAN	0.00	0.00	0.00	0.00	0.00	0.00
Em	0.00	0.00	0.00	0.00	0.00	0.00
LIN	0.00^{a}	0.00^{a}	0.05^{a}	0.08^{a}	0.10^{ab}	0.24^{b}
Em	0.00	0.00	±0.03	±0.04	±0.05	±0.02
SAL	0.00^{a}	0.06^{a}	0.19^{ab}	0.44^{c}	0.46^{c}	0.38^{bc}
Em	0.00	±0.05	±0.06	±0.23	± 0.28	±0.19
SR/AT	0.00^{a}	0.07^{a}	0.29^{b}	0.53^{c}	0.54^{c}	0.50^{bc}
Em	0.00	±0.04	±0.03	±0.07	±0.07	±0.04

*Means with letters distinct in lines denote significant differences ($p \leq 0.05$) by Tukey test; Em: Error of the mean

The birds that received fish oil in diet consumed 290 mg of DHA/day (SAL) and 380 mg of DHA/day (SR/AT), promoting the incorporation of 142.90 mg DHA/yolk (SAL) and 157.13 mg of DHA/yolk (SR/AT) (Table 11).

In the group, LIN, daily intake of 1380 mg of ALA has provided incorporation of 349.28 mg of the same acid in egg yolk and biotransformation of 63.11 mg of fatty acid DHA in the egg.

Table 11: Comparison of the levels of DHA and ALA found in the diet and incorporated in the yolk of egg

	Ingestion		Yolk[a]	
TRT	DHA (mg/day)	ALA[b] (mg/day)	DHA (mg/yolk)	ALA (mg/yolk)
SOJ	Traces	210	24.42	41.58
MIL	Traces	60	35.39	14.78
CAN	Traces	270	64.17	52.21
LIN	Traces	1380	63.11	349.28
SAL	290	50	142.90	24.95
SR/AT	380	80	157.13	25.87

[a]Values calculated from the fixed weight of 14 g/yolk and fat percentage in yolk.
[b]ALA: α-linolenic fatty acid

Table 12: Means and errors of the mean (on) of the main fatty acids and ω-3 PUFAs totals (% total lipid) present in blood plasma of hens according to the treatments

TRT[a]	PALM	PALTOL	ESTE	OLEI	ALA	EPA	DPA	DHA	ω-3
SOJ	24.71^{ab*}	2.41^{a}	8.92^{a}	36.99^{a}	0.87^{a}	0.17^{a}	0.16^{a}	1.24^{b}	2.53^{b}
Em	±0.52	±0.12	±0.39	±0.89	±0.02	0.08	±0.04	±0.27	±0.19
MIL	25.31^{abc}	2.08^{a}	8.95^{a}	39.93^{ab}	0.33^{a}	0.00^{a}	0.04^{a}	0.47^{a}	0.98^{a}
Em	±0.36	±0.11	±0.15	±0.80	±0.03	±0.00	±0.02	±0.05	±0.06
CAN	24.18^{ab}	2.04^{a}	8.72^{a}	46.48^{c}	1.11^{a}	0.00^{a}	0.08^{a}	1.34^{b}	2.53^{b}
Em	±0.24	±0.04	±0.28	±0.41	±0.05	±0.00	±0.04	±0.09	±0.09
LIN	23.02^{a}	2.54^{a}	9.10^{a}	41.75^{b}	6.29^{b}	0.12^{a}	0.12^{a}	1.91^{b}	8.44^{d}
Em	±0.38	±0.27	±0.37	±1.25	±0.41	±0.06	±0.06	±0.11	±0.48
SAL	27.98^{c}	3.79^{b}	9.08^{a}	38.34^{ab}	0.59^{a}	0.60^{b}	0.44^{b}	4.24^{c}	5.97^{c}
Em	±1.45	±0.14	±0.50	±0.60	±0.02	±0.02	±0.01	±0.15	±0.17
SR/AT	27.26^{bc}	3.98^{b}	8.91^{a}	39.03^{ab}	0.52^{a}	0.71^{b}	0.37^{b}	4.48^{c}	6.14^{c}
Em	±0.87	±0.11	±0.32	±0.72	±0.04	±0.01	±0.01	±0.15	±0.26

*Means with different letters in columns denote significant differences ($p \leq 0.05$) by Tukey test;
[a]TRT (treatment), PALM (Palmitic), PALTOL (Palmitoleic), ESTE (Stearic), OIL (Oleic acid), ALA (α-linolenic acid), EPA (Eicosapentaenoic Acid), DPA (Docosapentaenoic), DHA (Docosahexaenoic); ω-3: omega-3 PUFAs; Em: Error of the mean

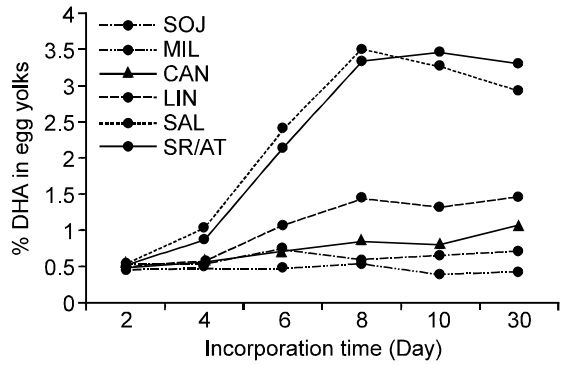

Fig. 6: Concentration of DHA in egg yolk (% of total lipid) according to the sources and time of incorporation

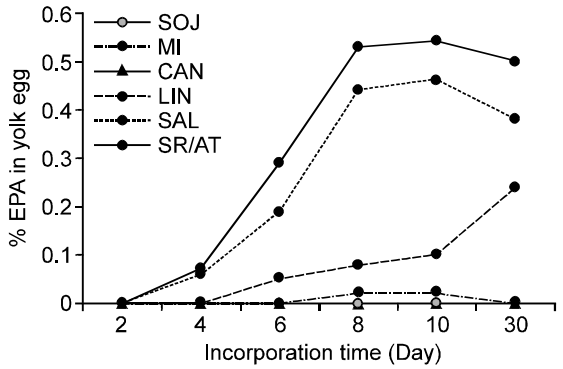

Fig. 7: Levels of EPA in egg yolk (% of total lipid) according to the sources and time of incorporation

Plasmatic fatty acids: Percentages of major fatty acids found in plasma from hens fed the experimental diets, which were: palmitic, palmitoleic, stearic, oleic, ALA, EPA, DPA and DHA and ω-3 PUFAs are listed in Table 12.

PUFAs: The total amount of polyunsaturated fatty acids ω-3 series, showed significant difference ($p \leq 0.05$) between treatments, being the lower (0.98%) in the group fed with MIL and the higher (8.44%) in the LIN treatment. Been demonstrated also significant differences between the midpoints SOJ (2.53%), CAN (2.53%), SAL (5.97%) and SR/AT (6.14%) when contrasted with the other groups (Table 12).

The observed changes in average values of ALA in plasma of the birds were very sharp, showing average significantly higher for hens fed LIN (6.29%) compared with those obtained in other treatments, ranging between 0.33% and 1.11% (Table 12).

The PUFAs, series ω-3 long chain, EPA had higher values in groups fed with fish oils, being SAL (0.60%) and SR/AT (0.71%) compared to other treatments (Table 12).

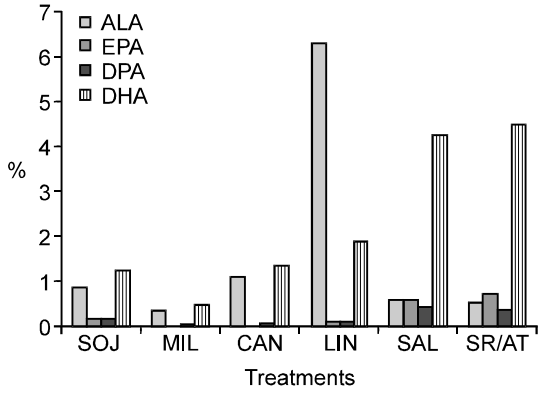

Fig. 8: Levels of ω-3 PUFAs fatty acids in plasma of hens in the last experimental days, according to the treatments studied

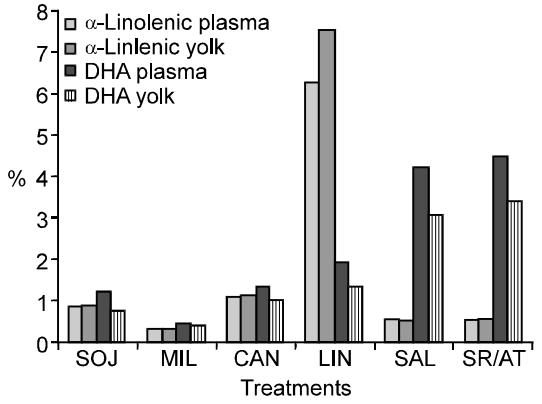

Fig. 9: Comparison between levels of the main ω-3 PUFAs in plasma of hens and in the egg yolk, according to the treatments studied

In relation to the Docosapentaenoic Acid (DPA) in plasma of birds studied, significantly higher values were denoted in the groups receiving SAL (0.44%) and SR/AT (0.37%) when in contrast to the other. Treatments SOJ, MIL, CAN and LIN were not significantly different between themselves (Table 12).

Similarly, DHA increased significantly in plasma of birds who received salmon oil (4.24%) and sardine/tuna (4.48%), when compared to treatments containing vegetable oils. However, the groups receiving LIN (1.91%), CAN (1.34%) and SOJ (1.24%) had intermediate levels of DHA in plasma, with the group MIL (0.47%), presented the lowest average among the treatments studied (Table 12).

The Fig. 8 shows values of total ω-3 PUFAs in plasma, according to the treatment. Note that the addition of linseed oil and fish oils to the diet increased the levels of these fatty acids in plasma of hens.

The comparison between the levels of DHA and ALA percentage in the yolk and plasma of laying hens demonstrates a direct relationship in all treatments in the present experiment (Fig. 9).

DISCUSSION

In the present research, the confrontation of the results of the determinations of fatty acid profiles in the diets, plasma and egg yolks showed highly significant changes in profiles of fatty acids ω-3 PUFAs in both plasma and egg yolks of laying hens (Tables 3-12; Fig. 1-9). These findings are consistent with studies conducted by several authors and at different times. The changes noted above in this research are enabled by a set of complex mechanisms, since the hormone until the cell in laying hen, which adjust for the reproductive phase. Thus, McIndoe (1971) inferred that the LDL, predominantly in the plasma of the laying hen, (estrogenic phase) have in mean 11% protein, 66% TG, 23% phospholipid (72% lecithin, 19% cephalin and small amounts of lysophospholipids and sphingomyelin) and 4% cholesterol. This author declared that, if the increased triglyceride concentration in laying hen liver represents lipoprotein synthesized for yolk formation, a large increase in the concentration of phospholipid would be expected since 30% of yolk lipid is phospholipid. The increased triglyceride may therefore be excess of requeriment or be synthetized and stored prior to incorporation into a lipoprotein precursor of rapid turnover. Still, on the pattern of lipogenesis in the laying hen, the onset of lay in the hen is accompanied by marked increases in the concentrations of plasma lipids and phosphoproteins. The total plasma lipids ranged from 0.2-0.5 g/100 mL to 10-14 g/100 mL. According to Heald and Badman (1963), the changes are gratest in the 14 day preceding egg laying, when the values for plasma Free Fatty Acid (FFA) may increased from between 0.2 and 0.5 meq./L to 0.5 meq./L to 4.0 meq./L. When laying starts, the plasm FFA has been found to fall sharply to between 0.75 and 1.5 meq./L and the total lipids to between 1.5 and 3.0 g/100 mL.

The eggs yolk lipids are derived from circulanting plasma lipoproteins, which are synthesized in the liver in response to the hormonal changes which accompany the onset of lay. The yolk of the egg contains large amounts of a lipoprotein which has similar density characteristics to plasma low density lipoproteins. The changes in total contents of n-3 PUFAs in the present study are similar to those described by Annison (1983), to mention that the composition of plasma (VLDL, LDL, HDL) in mg/100 mL of serum in male 99 weeks) was 33.1 mg, 69.9 mg and 590 mg/100 mL of serum, respectively. The composition of plasma (VLDL, LDL, HDL) em mg/100 mL of serum 5.480 mg, 198 mg and 144 mg/100 mL of serum, respectively, in the laying hens. The content (relative to protein content = 100): Phospholipid in the VLDL, LDL and HDL in percentage:

76.8, 78.1 and 86.2, respectively, in male and 175, 115 and 100 in the laying hens. TG in the VLDL, LDL and HDL in percentage: 20.3, 30.9 and 5.1 in male and 481, 194 and 25.6 in laying hens.

Moreover, the limitations of ω-3 PUFAs to the maximum of 6.14% (DHA) and 8.44% (ALA) in plasma and 9.21% (LIN) and 4.90% (SR/AT)ω-3 PUFAs in the yolk egg laying hens in present research, found endorse in the statements of Bickerstaffe and Annison (1970) to inferred that in chickens liver, the fatty acid specificity of triglyceride synthetase closely paralleled the mammalian systems; the unsaturated fatty acid mainly occupied the sn 2-position (EPA and DHA in sn-2 position of phospholipid) while the saturated fatty acids and unsaturated fatty acids of trans configuration occurred at the sn-1and sn-3 positions of the triglycerides. Lipid deposition occurs during the later stages of ovum formation determines that it contains the majority of the yolk lipid. PC and PE are by far the major phospholipid components. In the PE, phosphatidyl serine and PC fractions palmitic and stearic acids together account for about 50% of the total fatty acids. In the cholesteryl ester, triglyceride and total phospholipids fractions, oleic acid is the major fatty acid present. Palmitic and stearic acids together account for more than one-third of the fatty acids; substancial levels of linoleic acid are present in each (Annison, 1983).

At present research, in parallel to the increase of ω-3 PUFAs in plasma was observed the rise of these ω-3 PUFAs in the yolk, thus agreeing with that reported by Noble (1987) and Leskanich and Noble (1997) in which the lipid composition of the yolk are about 33% of the total weight of the yolk and 60-65% of its dry matter content. However, as can be seen later, under conditions where particular specialized diets have been fed extensive changes can be observed in both the lipid and fatty acid compositions of the yolk. According to Noble *et al.* (1986; 1990a,b; 1996) the proportions of major lipids (% weight of total) in the yolk is distributed in: Lipid: 63,1% of TG, 29.7% of phospholipids, 4.9% free cholesterol, 0.9% free fatty acids and 1.3% of cholesteryl esters. Between the phospholipids, the main are: 69.1% of PC, 23.9% of PE, 2.7% of phosphatidyl serine, 1.0% of sphingomyelin and 3.2% of others.

According to Jy-L *et al.* (1976), the concentrations and lipid compositions of the fractions in the plasma in VLDL, LDL and HDL of non-laying and laying hens showed remarkable changes in its composition. In the non-laying is 61 mg (VLDL), 150 mg (LDL) and 205 mg (HDL) versus 1225 mg (VLDL), 125 mg (LDL) and 100 mg (HDL)/100 mL plasma on the total of lipids in laying hens. The concentrations and lipid compositions of the fractions in the plasma in VLDL, LDL and HDL of non-laying is 65% (VLDL), 24.5% (LDL) and 4.6% (HDL) versus 64.6% (VLDL), 50.4% (LDL) and 23.4% (HDL) to triglycerides on the total percentage de lipids in laying hens. Nevertheless, the authors above also mentioned that the concentrations and lipid compositions of the fractions in the plasma in VLDL, LDL and HDL is 21.7% (VLDL), 49.7% (LDL) and 54.7% (HDL) in non-laying versus 29.5% (VLDL), 38.2% (LDL) and 50.1% (HDL) in laying hens to phospholipids on the total percentage de lipids.

For enrichment of plasma (Table 12; Fig. 8 and 9) and egg yolk formation and accumulation of yolk lipid observed in the present research (Tables 3-11; Fig. 1-7) had similar considerations in studies of Kudzma *et al.* (1975) in which the liver changes are in turn accompanied by marked increases in the concentrations of the plasma lipids, in particular triglycerides. Total plasma lipid concentration increases from 200-500 mg/100 mL in the immature hen to a level which may exceed 2000 mg/mL in the laying hen. The changes in the plasma lipid concentration which precede egg laying are associated almost wholly with a dramatic rise in the concentration of the TG-rich VLDL fraction as a result of increased synthesis within the liver (Kudzma *et al.*, 1975). Fundamental physical and chemical structural differences have been shown to exist in the very low density lipoproteins synthesis for yolk formation (Kudzma *et al.*, 1979; Griffin, 1992), some of which are clearly orientated towards a specific function in the lipid transfer from plasma to yolk and possibly embryonic nutritional requeriments.

Lipid composition of egg yolk: In laying birds, the ingested triglycerides are hydrolyzed in the intestine to fatty acids and monoglycerides in the intestinal lumen and absorbed by the hepatic portal system and transported through portomícrons to the liver. The hepatic system is responsible for the synthesis of VLDLs to transport fatty acids through the plasma until the egg yolk. The VLDLs are incorporated into the yolk in intact form and this characteristic, unique to lipid metabolism of poultries, enable that the fatty acid profile of eggs can be manipulated in proportion to the content and quality of dietary fat (Mendonça *et al.*, 2000; Mendonça and Pita, 2005; Leeson and Summers, 2001; Cherian *et al.*, 1996b).

In the present research, the lipid composition of fatty acids in egg yolk, suffered significant alteration by substitution of source of dietary fatty acids (Table 3), agreeing with the results observed by Pita (2003), Piber Neto (2006), Carvalho (2006), Mori (2001), Aymond and Van Elswyk (1995), Cherian *et al.* (1996b), Grobas *et al.* (2001).

Noble *et al.* (1990a) stated that fat from egg yolk, is predominantly unsaturated, due to high levels of monounsaturated (20.1%) and PUFAs (45.7%). According to these authors, the P/S fat of egg yolk - 0.59 -, meets fully the recommendations of the Committees of the British Nutrition for healthier eating and may vary from 0.32 to 0.45.

In present study, as for ω-3 PUFAs in total, the high concentration was obtained for treatment based on LIN (9.21%) significantly different from the other groups, being MIL (1.22%), SOJ (2,09%), CAN (2.28%), SAL (4.47%) and SR/AT (4.90%) (Table 3). These values are in agreement with the results presented by Baucells *et al*. (2000), Pita (2003), Piber Neto (2006), Mori (2001), Galobart *et al*. (2001a), who reported higher levels of totals ω-3 PUFAs in the egg yolks from treatments based on flaxseed oil, rather than diets based on various oils such as corn, soybean, canola, sunflower, fish and marine sources. This is due to high contents of ALA in flaxseed oil.

Consequently, the higher concentration of ALA in egg yolk was reported in the group receiving LIN (7.56%), which differed significantly from the mean for eggs from hens fed diets with added MIL (0.32%) or fish oil, beind SAL (0.54%) and SR/AT (0.56%) (Table 3). These results agree with those presented by Baucells *et al*. (2000) where contents were 4.87%, 0.73%, 0.44% and 0.18% ALA in the yolks of hens fed diets added flaxseed oil, canola oil, fish or soybeans, respectively. Cherian and Sim (1992) found that hens fed diets containing 10% rapeseed oil or 10% flaxseed had values of ω-3 PUFAs in the egg yolk total of 2.83% and 8.29%, respectively, the ALA in the form of ω-3 most abundantly found in both cases. Carvalho (2006) and Piber Neto (2006) does not denote a significant difference in the concentration of fatty acid by adding salmon oil or mixture of sardines and tuna in the diet of hens compared to control groups and enriched with microalgae in the diet of hens.

In the present studied, was observed deposition of EPA in the yolks in treatments that received linseed or fish oil and that the group of mixture of oils (SR/AT), has provided the largest concentration of EPA, significantly different from the average earned for the Salmon Oil (SAL) and Linseed Oil (LIN) (Table 3). These results differ partly from those reported by Piber Neto (2006), thay noted no significant difference regarding the deposition of EPA in the yolk among the treatments with sardine and tuna oil or salmon oil, however, the incorporation of this fatty acid ω-3 was significantly higher than in control. Likewise, these results agree with the findings of Carvalho (2006), what showed higher levels of EPA in egg yolks from birds fed diets containing 0.80-2.4% of salmon oil, compared to control. In studies of Baucells *et al*. (2000), on the other hand, diets enriched with 5% of oils: fish, flaxseed, canola or soy promoted incorporation of 0.92%, 0.25%, 0.08% and 0.02% EPA in the yolk eggs, respectively, results superior to those denoted in the present experiment where values were 0.50%, 0.38%, 0.24%, 0% and 0% EPA in the yolks to the groups that received oil Sardines/Tuna (SR/AT), Salmon (SAL), Linseed (LIN), Canola (CAN) and Soybean (SOJ) oils, respectively. However, it should be noted that the experiment Baucells *et al*. (2000) used a higher percentage amount of oil that in the present study. Pita (2003) also found no incorporation of EPA in the yolks of hens fed diets with added 6% canola oil.

The supplementation of the mixture of fish oils (SR/AT) and diets containing corn or soybean oils, promoted higher incorporation of DPA in the experimental yolks (Table 10), results that, which corroborate the found by Baucells *et al*. (2000), who observed levels of 0.47%, 0.29%, 0.09% and 0.06% of DPA in the yolks of hens fed diets rich in 5% fish oil, flaxseed, canola or soybean, respectively.

The addition of salmon oil and mixture of sardines/tuna promoted higher incorporation of DHA in egg yolk, followed by eggs of groups fed flaxseed and canola oils, compared to treatments SOJ and MIL. Numerous studies indicate higher amounts of DHA in the yolk from diets containing fish oil (Table 3), to the detriment of others. Mori (2001), Piber Neto (2006) and Carvalho (2006) found higher values of DHA in the yolk of eggs from hens fed diets containing oil sardine/tuna or salmon at the expense of those with corn oil or no added oils or enriched with microalgae. Pita (2003) in laying hens fed canola oil pointed out value average 0.83% DHA in egg yolk, significantly lower to that observed for laying hens subjected to diets containing ground flaxseed and more lower than the income for the present study, being 1.06% (CAN) and 1.45% (LIN) of DHA.

If consider that the average values of DHA per egg and from birds fed diets containing salmon or tuna and sardines oils, are located at 142.90 mg for the first group and 157.13 mg for the last. The ingestion of an egg, meets at 65% and 71.4% respectively, the minimum requirements of an adult male (220 mg) according to the tables of the RDA (Recommended Dietary Allowance) cited by the NRC (1989), Simopoulos *et al*. (2000) and Simopoulos (2000, 2009). Such results if approximate of the values reported by Carvalho (2006).

Time of incorporation of fatty acids in egg yolk: In the present research, the fatty acid profile of egg yolk during the period of feeding of hens allowed to observe that the incorporation of total fatty acids does not occur in the two first days of experiment. The incorporation requires a period of about 10 days for the levels of different dietary fatty acids to stabilize in the yolks.

There are in mean, five to seven egg yolks preformed in the ovary of laying hens in the follicular hierarchy, which allows the understanding that the incorporation of new nutrients to the egg and consequently the yolk after administration of new diet, can only occur in its entirety from the fifth to seventh day after the delivery of the experimental diet (Griminger, 1986).

Moreover, Hargis *et al*. (1991) stated that the measured in the hen consumes new diet with additional sources of

lipids, the fatty acid profile of the egg is gradually changed, with more changes marked are observed between 12 and 15 days after introduction of the new diet.

ω-3 polyunsaturated fatty acids (ω-3 PUFAs): The total ω-3 PUFAs in the egg yolk, during the experimental period, behaved much like those found on the last day of the experiment. Thus, smaller amounts were found embedded in the MIL group (1.14%), intermediate values in treatments SOJ (1.60%), CAN (1.78%), SAL (3.26%) and SR/AT (3.44%) and highest in those egg yolks from hens fed with LIN (6.02%). Regarding the collection days for eggs, there was a gradual increase in the levels to stabilize the eighth day (Table 11). This behavior was common treatments for CAN, LIN, SAL and SR/AT, while for the group SOJ was recorded from the stable value day2 (Table 13, Fig. 11). These results differ from those reported by Hargis *et al.* (1991) that when administering fish oil to laying hens observed stabilization of the levels of total ω-3 in the yolks from the third experimental week.

Likewise, Nash *et al.* (1995), with use of increasing levels of fish meal (HM), the percentage of ω-3 PUFAs increased ($p<0.001$) of 1.9% (control) for 3.6% (12% HM) of ω-3. The EPA has increased from 0.27 mg (0.07% EPA, Control) for 1.29 mg/g (0.29% EPA, 12% HM) of lipid in the plasma. The DHA increased from 5.74 mg (1.27% EPA, Control) for 11.77 mg/g (2.73% EPA, 12% HM) of lipid in the plasma. The lipids increased from 0.02% (Control, 0.13 mg/g) to 0.21% (12% HM, 1.46 mg) of EPA. In the yolk of 1.01% (Control, 7.1 mg/g) of DHA to 2.69% (12% HM, 18.8 mg/g) of DHA in egg yolk. The plasma lipids were analyzed at each of the four periods in the laying cycle, being to 169, 211, 253 and 287 days. Yolk lipids also were analysis at each of the four periods in the laying cycle.

The levels of ALA in the yolks suffered a gradual increase until the tenth day of the experiment, remaining from this time constant until the end of the trial (DAY30) (Table 7). Treatments SOJ and CAN had the concentrations of fatty acid, stabilized at this day, while LIN showed a significant increase until the eighth day of the experiment (Table 9; Fig. 5). However, Hargis *et al.* (1991) observed continuous incorporation of this acid in the yolk only after four weeks of experimental diet administration to laying hens.

The incorporation of ω-3 LC - EPA and DHA - in egg yolk, showed differences judged significant, between the sources of fatty acids studied and days of collection of eggs. Moreover, the DPA did not differ significantly, in both cases (Table 7). The increase of EPA in the yolks, began on the DAY4 (0.021%) rising gradually until the eighth, stabilizing if by the end of the experiment, this behavior remain well explained in groups receiving fish oil in diet (Table 10; Fig. 7).

DHA, in turn, had their levels increased from DAY2, being that the concentrations remained increased until the DAY8 when it stabilized (Table 7), values which are characterized in treatments CAN, LIN, SAL and SR/AT (Table 10; Fig. 6). The results here presented corroborate those found by Yu and Sim (1987) that by adding salmon oil to the diet of hens, observed maximum incorporation of EPA and DHA in the eighth experimental day. On the other hand, Hargis *et al.* (1991) noted maximum levels in the yolk of EPA and DHA after 1 week and 3 weeks, respectively, administration of feed containing 3% fish oil to laying hens. However, these findings disagree of mentioned by Van Elswyk *et al.* (1994), that to feeding hens with 3% menhaden oil in the diet obtained increasing concentrations of DHA during the first three weeks and stabilized after the fourth experimental week.

Similarly to the results of present research, Mori (2001) had verified levels of 99 mg DHA/yolk to adding 2% fish oil and evaluated the eight weeks experimental, Piber Neto (2006) was found that the use of 1% of salmon oil or 1.2% of sardine oil in the diet of laying hens, promoted incorporation of 1.9% and 1.97% DHA in egg yolk, respectively, the four-week trial and Herber and Van Elswyk (1996) to add 1.5% menhaden oil in the diet of laying hens, promoted daily intake of 155 mg of DHA by bird, being that around of 89% of this concentration has been incorporated into the yolk egg of laying hens, around eigth weeks experimental.

The increase in the total content of ω-3 PUFAs in the egg yolk was proportional to intake, being 24.42 mg (SOJ), 142.90 mg (SAL) and is 157.13 mg DHA/yolk (SR/AT). However, these levels were exceeded by 349.28 mg ALA/yolk (LIN) versus 14.78 mg/yolk (MIL) egg of layers (Table 11). Similar results were presented Piber Neto (2006), when utilized a mixture of salmon plus sardines/tuna (92.40 mg/yolk) starting of diet containing 1.20 g DHA/kg ration evaluated around twenty eight days experimental.

Fatty acid in blood plasma: In the present study, all birds showed an increase of ω-3 PUFAs in plasma by effect of different treatments and sources studied. The levels increased of 0.98% (MIL) for 5.97% (SAL), 6.14% (SR/AT) and 8.44% (LIN) of ω-3 PUFAs on the lipid total in plasma by effect of treatments and sources. Between the fatty acids, the DHA increased of 0.47% (MIL) for 4.24% (SAL) and 4.48% (SR/AT), followed by 6.29% ALA (LIN) on the lipid total (Table 12; Fig. 8 and 9).

The most of these components of yolk are derived from blood plasma. The largest, are the precursor plasma vitellogenins and a specialized type of triglyceride-rich lipoproteins in abundance on laying hen. They are synthesized in the liver in response to estrogen stimulation, transported to the ovary and transferred to the oocyte growth, probably by a selective mechanism.

The size of the load required of the liver, cardiovascular system and the ovary can be seen from a consideration of the total quantity of material produced during the yolk of the egg laying. (Romanoff and Romanoff, 1949). The hen which lays 328 eggs during the first year of the cycle produces about 5.9 kg of egg yolk, most of them being the yolk lipoprotein and protein, this represents around 2.0 kg of total lipid (Scheuermann and Bellaver, 1995).

Plasmatic fatty acids have changed significantly between treatments, being that such differences reflected if in the incorporation of different fatty acids in the yolks. The composition of body fat of animals is usually a reflection of the profile of fatty acids ingested (Chen *et al*., 1965).

ω-3 PUFAs: The addition of different oils in the diet of birds changed markedly, the concentration of total ω-3 PUFAs in plasma of studied birds (Table 12). With regard to the levels of PUFAs in the plasma of birds, groups that were fed diets supplemented with canola oil, salmon and sardines/tuna (CAN, SAL, SR/AT) had averages of these fatty acids significantly higher than the other treatments. Thus, the percentage in plasma in present study of 2.53% (CAN), 2.53% (SOJ), 5.97% (SAL), 6.14% (SR/AT) and 8.44% (LIN) versus only 0.98% (MIL), ($p \leq 0.05$) - Table 12, showed parallely results similar those obtained for the egg yolk 2.28% (CAN), 2.09% (SOJ), 4.47% (SAL), 4.90% (SR/AT) and 9.21% (LIN) versus so only the lower value 1.22% (MIL), ($p \leq 0.05$) (Table 3). Mean values as high as 8.44% ω-3 (LIN) in the plasma and 9.21% ω-3 (LIN) in the yolk presented in this research, were also observed by Nelson and Ackman (1988), when mentioned that the absorption and the transport of ω-3 PUFAs are similar to other ω-3 PUFA-LC. Still, in present research, the higher percentage of DHA were found in treatments SAL and SR/AT, both equal 4.24% (SAL) and 4.48% (SR/AT) in plasma (Table 12), as in the egg yolk 2.91% (SAL) and 3.30% (SR/AT) (Table 3), resulting these in significantly different from the other groups.

While the main causes of death in the United States are due to coronary heart disease, there are plenty of scientific evidence indicating the existence of positive correlation between intake of ω-3 PUFAs and cardio protective effect from the reducing the incidence of coronary heart disease (Kinsella *et al*., 1990; Bang and Dyerberg, 1980).

Observations similar to the present study were detected by Herold and Kinsella (1986) when added source of ω-3 PUFA diet rats. Effect of feeding corn oil and fish oil containing diets for two week on the fatty acid composition of plasma phospholipids with 5% de sardine oil in the diet of rats were of: 8.53% EPA and 10.10% DHA fatty acid content of plasma phospholipids.

If the increased triglyceride concentration in laying hen liver represents lipoprotein synthesized for yolk formation, a large increase in the concentration of phospholipids would be expected since 30% of yolk lipid is phospholipids. The increased triglyceride may therefore be excess of requirement or be synthetized and stored prior to incorporation into a lipoprotein precursor of rapid turnover (McIndoe, 1971).

Plasmatic concentrations of ALA were more elevated in groups fed with linseed oil, followed by hens receiving canola oil (Table 12), levels, these, in matche with those found in the respective yolks eggs (Table 3). According to Brenner *et al*. (1969) the excess of ALA in the diet provides reduced synthesis of arachidonic acid from linoleic acid, due to competition between the ALA and linolenic acids by Δ-6 desaturase, an enzyme involved in the desaturation of these acids, that would have high affinity for this last fatty acid. Excess ALA in diet containing linseed oil (LIN), limited the synthesis of arachidonic acid, resulting in low values of this acid in egg yolk and plasma of experimental hens.

The fish oils promoted a higher concentration of EPA (SAL = 0.60% and SR/AT = 0.71%) in plasma of laying hens, followed by treatments that received soy oil (0.17%) or flaxseed (0.12%), whereas those fed canola oil or corn is not presented detectable concentration of this acid in plasma (Table 12). These results are partly consistent with the EPA concentrations found in egg yolk, where the highest values were assigned to groups receiving fish oil equal 0.38% (SAL) and 0.50% (SR/AT) followed by the group fed diet supplemented with 0.12% linseed oil - Table 3 -, not being possible to detect the EPA in the diets with soybean oil, corn and canola. The results of this experiment show coherence with those found by Nash *et al*. (1995), where the EPA levels in plasma were higher in treatments based on fishmeal, than in the control group based in soybeans.

In this research, the levels of DPA in plasma of the hens studied were higher in the groups receiving fish oil (SAL = 0.44%, SR/AT = 0.37%) than in the other, being related with the concentrations obtained for the egg yolks of hens in this same case study of treatment with oil sardine/tuna (0.46%) (Table 12). However, the levels of DPA in plasma from hens fed diets containing oils from flaxseed, canola, soy, corn or salmon are not agreement with those found in the eggs yolk of the same hens (Table 3) by authors mentioned above.

With regard to DHA, there was concordance of values identified in present research with those found by Nash *et al*. (1995), in hens submitted the diet containing 8% fish meal that presented higher levels of plasma DHA (9.47 mg/g) than the control group (5.74 mg/g). In the present study also showed a significant increase of DHA in plasma of hens fed fish oil (SAL = 4.24%, SR/AT = 4.48%), being that the treatments added to vegetable oil had values intermediaries of this fatty acid, except in regarding the corn oil, which promoted the smallest concentration of DHA in plasma of hens (Table 12). These results are consistent with those found in egg

yolk, whose levels had variation from 0.77% to 3.40% of DHA, being that the fish oils promoted major incorporation of this acid fatty in yolk. The authors demonstrated still, a linear relationship between the amounts of ω-3 PUFA diet and their levels in plasma of birds; so, the measure which increased the concentration of this in diet, promote if a higher transport of ω-3 in the plasma.

The similarity between the fatty acid composition of plasma and egg yolk, with the levels dietary of these, suggests direct deposition of dietary fatty acids in plasma and consequently in the yolks (Sim and Bragg, 1977; Marshall *et al*., 1994).

In birds and in humans, the metabolic conversion of ALA in ω-3-LC is more slow and inefficient (Nettleton, 1991; Hargis and Van Elswyk, 1993). For this reason, the employment of canola and flaxseed in the diet of laying hens provided enrichment of egg yolk mainly ALA, being that only small part of EPA and DHA was incorporated into the egg yolk, consequent to desaturation and elongation of chain ALA (Pita, 2003; Farrel, 1994; Marshall *et al*., 1994).

Conclusion: The results of the present study allowed the following conclusion:

Laying hens showed high predisposition to absorb and transfer the ω-3 PUFAs of the diet for the tissues and for egg yolk. The levels increased in the diet directly reflected in the increase of ω-3 PUFAs in plasma and fat of egg yolk in a minimum time interval between the intake of PUFAs and the tissual enrichment.

Flaxseed oil, rich in ALA, was responsible for high concentrations of ω-3 PUFAs in egg yolk, while feeding with corn oil, resulted in values much reduced of this group of fatty acids in the yolk.

The addition of linseed oil to the diet of hens promoted the higher percentage of incorporation of ALA to the egg yolk.

The higher concentrations of EPA and DHA in egg yolk were obtained with the supplementation of sardines and tuna oil (SR/AT) and Salmon (SAL) oils.

The addition of fish oils (SAL) and (SR/AT) promoted the incorporation of 142.9 mg DHA/egg yolk and 157.13 mg DHA/egg yolk, values these that attend the 65% and 71.4%, respectively, of the requirements minimum of an adult male human.

The levels of ω-3 PUFAs in egg yolk were significantly increased until the eighth experimental day, remaining constant thereafter until the end of the study.

The utilization of sources of fish oils promoted a gradual decrease of total PUFAs in the egg yolk until the eighth day of study, being that after of this period, no significant alteration occurred in the values of these fatty acids.

The addition of Linseed Oil (LIN) to the diet of hens provided a gradual increase in concentrations of ALA in egg yolk during the experimental period, with the obtaining of plateau from the tenth day. Since the inclusion of Soybean Oil (SOJ) and Canola (CAN) to the ration determined increased more discreet of the levels of this fatty acid in the yolk, with the plateau reached to ten days of assay.

The birds fed with PUFA-rich sources of ω-3 fatty acid (LIN, SAL and SR/AT) determined increased levels of EPA and DHA in egg yolk with the plateau being reached on the eighth day of trial.

So overall, the behavior of plasmatic ω-3 fatty acids was coherent with that found for the yolk of eggs, in the same experimental period.

The addition of linseed oil to the diet of birds promoted larger amounts of ω-3 PUFAs and ALA in the blood plasma of birds.

The oils in salmon and sardine/tuna added to the diets of hens, promoted the higher levels of EPA and DHA in blood plasma of laying hens.

For all sources of PUFAs studied, was possible to observe consistency in the fatty acids profiles of yolk and blood plasma of hens. Levels of EPA and DHA in blood plasma and egg yolk of laying hens was directly proportional to the levels of ω-3 PUFAs utilized in the diet.

Significant effects were observed for the increase of all ω-3 PUFAs in plasma and egg yolk contrasting significantly with the control.

Significant interactions between sources and days were observed for the increase of all ω-3 PUFAs in plasma and egg yolk of laying hens.

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