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## Effect of Marine and Vegetal Sources on the Hen Diets on the PUFAs and PUFAs n-3 in Laying Hens Egg Yolk and Plasm

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**Abstract:** The present study was aimed to verify the effect of 3% oil addition (soil, corn, canola, linseed, salmon or sardine tuna) chicken diets on the yolk and plasmatic fatty acids composition. 144 Shaver White poultry were housed during four weeks. The inclusion of linseed, soil or corn oil on the diet increased the polyunsaturated fatty acids in yolk and plasm. Linseed oil treatment promoted higher levels of yolk and plasm PUFAs n-3. The EPA and DHA concentration in the plasm were higher in 3% sardine tuna oil and DHA plasm value was increased in both group received fish oils. The plasm fatty acid profile was similar to that found in egg yolk.

**Key words:** Polyunsaturated fatty acids, diet oils, omega-3, plasmatic fatty acids, yolk

### INTRODUCTION

Man has focused in recent decades, greater concern with health and longevity, which creates demand for consumption of healthy foods with lower levels of pesticides, promoting healing or prevention of diseases such as hypercholesterolemia, cardiovascular disease, cancer and diabetes (Simopoulos, 2009).

The coronary heart disease are a major cause of mortality among adults in the U.S. and in Brazil (Ministério da Saúde, 2009), many risk factors relate to diet, especially regarding the quantity and quality of the lipid portion of this. The authors Bang and Dyerberg (1972) were pioneers in showing that Greenland Eskimos rarely suffer from cardiovascular disease and had reduced levels of total cholesterol and LDL (low density lipoprotein) in plasm, due to the consumption of diets rich in polyunsaturated fatty acids omega-3, particularly EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) found in fat and fish oil consumed by them.

In addition to the cardioprotective effect, other benefits have been reported with consumption of these fatty acids, such as anti thrombotic, anti-atherosclerotic, anti-inflammatory protection from dementia, brain development of fetuses and infants up to 3 months of age (Givens and Gibbs, 2008).

The consumption by humans, eggs enriched with polyunsaturated fatty acids, is an alternative to increasing consumption of n-3 in Western countries whose diet is rich in fish, such as Eastern (Shapira *et al.*, 2008; Givens and Gibbs, 2008; Pita *et al.*, 2006; Pita,

2003; Piber Neto, 2006; Carvalho, 2006; Mori, 2001; Lewis, 1996). The modification of the lipid profile of egg can be produced from the inclusion of specific oils in the diet of birds, such as fish oil, and flaxseed or canola oil such as by incorporating the very seeds in the diet of laying hens (Shapira *et al.*, 2008; Souza *et al.*, 2008; Pita *et al.*, 2006; Carvalho, 2006; Mori, 2001 and Lewis, 1996).

On the other hand, Ahn *et al.* (1995) showed that the addition of  $\alpha$ -linolenic acid to the diet of laying hens, promoted deposition of DHA (docosahexaenoic acid) and  $\alpha$ -linolenic acid in the yolk egg and Souza *et al.* (2008); Pita (2003); Galobart *et al.* (2001) and Mori (2001) showed that supplementation of linseed oil or its seeds, the diet significantly increased the concentration of PUFAs n-3 in the egg yolk, consisting mostly of  $\alpha$ -linolenic acid.

Pita (2003) showed that laying hens that received 3% of canola oil in the diet, have yolks with 2.43% of PUFAs n-3, a value much higher than that found in the literature (USDA-0.89%) to birds fed diets based on corn and soybeans.

The lipids that circulate in the plasm of laying hens come from oral consumption, or rather, of the intestinal contribution, hepatic synthesis and mobilization of stored fat in the carcass (Mendonça Junior and Pita, 2005; Grimmering, 1986). Numerous studies show that changing lipid diet promotes changes in the concentrations of fatty acids of the yolk, suggesting change in the behavior of plasm lipids from the dietary modification (Souza *et al.*, 2008; Piber Neto, 2006;

Carvalho, 2006; Pita, 2003; Gomez, 2002; Mori, 2001). The present research was to study the effect of soybean oil, corn, canola, flaxseed, salmon and a mixture of oil sardines and tuna, the birds' diet on the fatty acid profile of yolk and plasm of laying hens.

## MATERIALS AND METHODS

One hundred and forty four hens of the commercial *Shaver White* were used, with initial age of 28 weeks and the experiment lasted for four weeks, being conducted in vivarium birds of the Department of Internal Medicine, Faculty of Veterinary Medicine and Zootecnics, University of São Paulo, located on the campus of the University Campus in São Paulo, approved by the Commission of Bioethics at the University of São Paulo. The diets of laying hens were fed isonitrogenous and isocaloric and offered food and water *ad libitum*, containing 3% of refined oil, canola oil (CAN), linseed (LIN), corn (MIL), soybeans (SOJ), oil crude salmon (SAL) or mixture of industrial crude oil sardines and tuna (SR/AT), constituting a total of six treatments (TRT)-Table 1.

Were utilized weight of one gram of fresh egg yolk and raw or 1 mL of plasm sample for repetition (four egg yolk pool or of four plasm samples pool), according with Folch *et al.* (1957) and Bligh and Dyer (1959), modified by Nielsen (1998), while the saponification of the lipid extract and extraction of fatty acid esters were made according to Hartman and Lago (1973).

The samples were injected into gas chromatograph conditions: 50:1 split injection, 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 and the make-up gas, which is to 30 mL per minute. The temperatures of injector and detector were 250°C and 280°C, respectively.

## RESULTS AND DISCUSSION

While the leading causes of death in the United States of America are heart disease, there is no scientific evidence to show the positive correlation between intake of polyunsaturated fatty acids n-3 and cardioprotective effect from the reduction in the incidence of coronary heart disease (Kinsella *et al.*, 1990; Bang and Dyerberg, 1972). The fatty acids concentration of laying hens in this experiment were significantly changed between treatments and such differences were reflected in the incorporation of different fatty acids in egg yolk.

The treatments CAN, SAL and SR/AT produced eggs yolks and plasms with lower total amount of polyunsaturated fatty acids than the other groups (Table 2 and 3). Thus, the groups SOJ, MIL and LIN had the highest total levels of PUFAs in the egg yolk and plasm, reflecting the high percentage of this category of fatty acids in the diet of laying hens of these treatments, which agrees with the results found by Baucells *et al.*

Table 1: Fatty acid composition of experimental diets (% of total fatty acids)

Rations	Fatty acids (%)					
Fatty acids	SOJ	MIL	CAN	LIN	SAL	SR/AT
Polyunsaturated total (%)	56.29	50.71	36.08	56.96	42.39	45.30
PUFAs n-3 total (%)	5.03	1.32	6.32	30.93	17.60	22.72
α-Linolenic (C <sub>18:3 n-3</sub> )	4.90	1.32	6.17	30.34	1.33	1.93
EPA (%)	0	0	0	0.45	5.73	9.72
DHA (%)	0	0	0	0	7.82	8.85

Table 2: Fatty acid composition of egg yolks (% of total fatty acids), as treatments studied

Treatments	Polyunsaturateds	n-3 <sup>1</sup>	EPA	DHA	α-linolenic
SOJ	24.06 <sup>b</sup> ±0.58	2.09 <sup>ba</sup> ±0.07	0 <sup>a</sup> ±0.00	0.71 <sup>ab</sup> ±0.08	0.90 <sup>bc</sup> ±0.03
MIL	23.01 <sup>b</sup> ±0.57	1.22 <sup>a</sup> ±0.04	0 <sup>a</sup> ±0.00	0.41 <sup>a</sup> ±0.03	0.32 <sup>a</sup> ±0.02
CAN	17.32 <sup>a</sup> ±0.45	2.28 <sup>b</sup> ±0.08	0 <sup>a</sup> ±0.00	1.06 <sup>bc</sup> ±0.08	1.13 <sup>c</sup> ±0.01
LIN	23.66 <sup>b</sup> ±0.44	9.21 <sup>a</sup> ±0.12	0.24 <sup>b</sup> ±0.01	1.45 <sup>b</sup> ±0.09	7.56 <sup>d</sup> ±0.17
SAL	17.33 <sup>a</sup> ±0.44	4.47 <sup>c</sup> ±0.17	0.38 <sup>c</sup> ±0.04	2.91 <sup>d</sup> ±0.16	0.54 <sup>a</sup> ±0.04
SR/AT	17.51 <sup>a</sup> ±0.38	4.90 <sup>d</sup> ±0.22	0.50 <sup>d</sup> ±0.02	3.30 <sup>d</sup> ±0.14	0.56 <sup>ab</sup> ±0.08

<sup>a</sup>Means with different letters in columns denote significant differences (p≤0.05) by Tukey test.

<sup>1</sup>n-3 (fatty acids omega 3 series), EPA (eicosapentaenoic acid), DHA (docosahexaenoic acid), α-linolenic acid (α-linolenic acid).

Table 3: Fatty acid composition of plasma (% of total fatty acids), as treatments studied

Treatments	Polyunsaturateds	n-3 <sup>1</sup>	EPA	DHA	α-linolenic
SOJ	26.43 <sup>b</sup> ±1.20	2.53 <sup>ba</sup> ±0.19	0.17 <sup>a</sup> ±0.08	1.24 <sup>b</sup> ±0.27	0.87 <sup>a</sup> ±0.02
MIL	23.15 <sup>b</sup> ±0.33	0.98 <sup>a</sup> ±0.06	0 <sup>a</sup> ±0.00	0.47 <sup>a</sup> ±0.05	0.33 <sup>a</sup> ±0.03
CAN	17.82 <sup>a</sup> ±0.66	2.53 <sup>b</sup> ±0.09	0 <sup>a</sup> ±0.00	1.34 <sup>b</sup> ±0.09	1.11 <sup>a</sup> ±0.05
LIN	23.19 <sup>b</sup> ±0.81	8.44 <sup>a</sup> ±0.48	0.12 <sup>a</sup> ±0.06	1.91 <sup>b</sup> ±0.11	6.29 <sup>b</sup> ±0.41
SAL	19.28 <sup>a</sup> ±0.96	5.97 <sup>c</sup> ±0.17	0.60 <sup>b</sup> ±0.02	4.24 <sup>c</sup> ±0.15	0.59 <sup>a</sup> ±0.02
SR/AT	19.27 <sup>a</sup> ±0.59	6.14 <sup>c</sup> ±0.26	0.71 <sup>b</sup> ±0.01	4.48 <sup>c</sup> ±0.15	0.52 <sup>a</sup> ±0.04

<sup>a</sup>Means with different letters in columns denote significant differences (p≤0.05) by Tukey test.

<sup>1</sup>n-3 (fatty acids omega 3 series), EPA (eicosapentaenoic acid), DHA (docosahexaenoic acid), α-linolenic acid (α-linolenic acid)

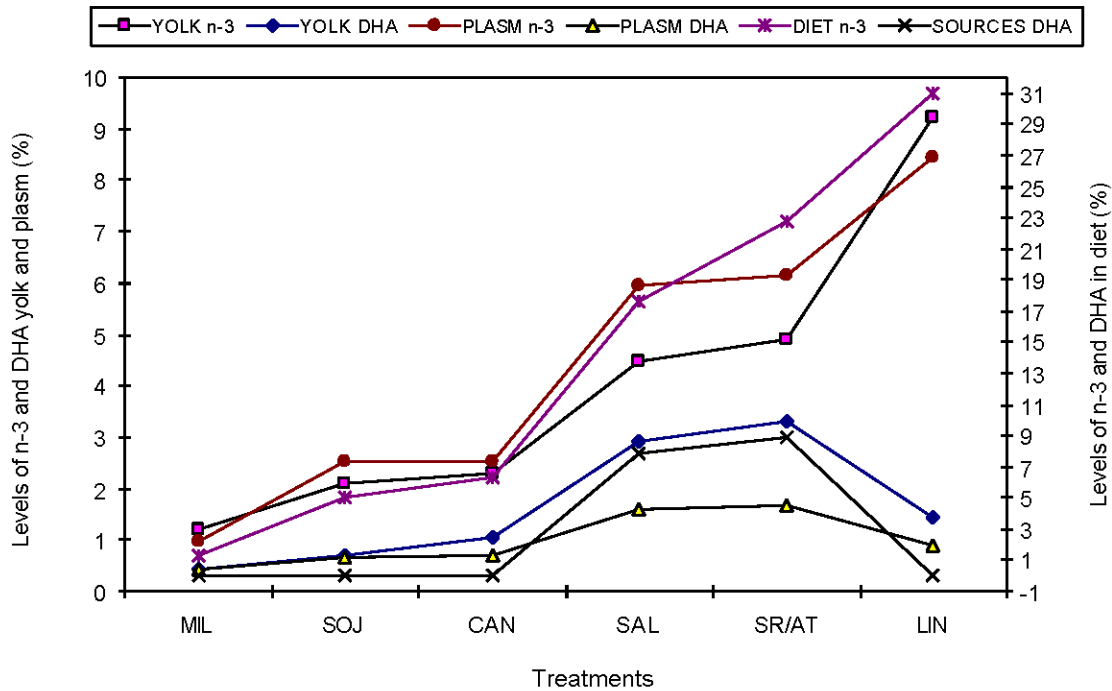


Fig. 1: Effect of concentration of n-3 PUFAs in the diets of treatments on the levels of omega 3 in the yolk and plasma of hens

(2000) and Pita (2003), where the concentration of total polyunsaturated fatty acids were higher in the groups receiving linseed than in the control group and groups receiving canola oil in the diet (Fig. 1).

The highest concentration of PUFAs n-3 in plasm and yolk, was obtained in treatment based on linseed oil, which is in agreement with those reported by Souza *et al.* (2008), Piber Neto (2006), Pita (2003), Mori (2001), Galobart *et al.* (2001) and Baucells *et al.* (2000), who reported higher levels of these fatty acids in the egg yolks from treatments based on flaxseed or its oil, rather than diets based on corn oils, soybeans, canola, sunflower and marines fishes.

Related to  $\alpha$ -linolenic acid in the yolk and plasm, the LIN group received the greatest percentage contents of the acid, which explains the higher concentrations of totals n-3 (Table 2 and 3). These results are in agreement with those found by Souza *et al.* (2008), Pita *et al.* (2006) and Mori (2001), which showed the highest increase of these fatty acids in egg yolk compared to control groups or the addition of other oils rich in polyunsaturated (Fig. 1).

However, when we observe the values obtained for the fatty acids n-3 long-chain, noted that both EPA and DHA were more focused on diets based on oil sardine and tuna, being than the egg yolks from the diets salmon oil showed concentrations of DHA significantly equal to the group SR/AT (Table 2). For the results of plasm EPA and DHA were higher in groups fed with fish oils (SR/AT and SAL) in relation to other treatments (Table 3).

The groups receiving fish oil on plasm and treatment SR/AT in yolk showed the highest concentrations of EPA, results that agree with those of Piber Neto (2006) and Carvalho (2006) who reported higher levels of EPA in eggs yolks from laying hens fed fish oil.

Similarly, the authors Nash *et al.* (1995) found the highest concentrations of this fatty acid in plasm in treatments based on fish meal. There was no deposition of EPA in plasm of birds and egg yolks in the CAN group, which confirms the values found by Pita (2003) and is approaching the levels reported by Baucells *et al.* (2000) equal to 0.08% in egg yolk.

The addition of fish oil promoted higher intakes of DHA in the yolk and plasm of laying hens, which agrees with the studies of Mori (2001), Piber Neto (2006) and Carvalho (2006). Nash *et al.* (1995) observed that in birds submitted to the diet containing 8% fish meal resulted in higher plasm DHA content (9.47 mg/g) than the control group (5.74 mg/g). Pita (2003) pointed out that in birds fed canola oil, the average DHA in the yolk was significantly lower than those fed diets containing ground flaxseed.

Thus, the results of this research led to the conclusion that the modification of dietary fatty acids promoted a similar change in the yolk and plasm of chickens studied. The diets based on fish oil promoted higher levels of EPA and DHA in egg yolk and plasm of laying hens, while the addition of linseed oil increased the concentration of PUFAs n-3 on total yolk and plasm and that this was mainly due to  $\alpha$ -linolenic acid.

## REFERENCES

- Ahn, D.U., H.H. Sunwoo, F.H. Wolfe and J.S. Sim, 1995. Effects of dietary  $\alpha$ -linolenic acid and strains of hen on the fatty acid composition, storage stability and flavor characteristics of chicken eggs. *Poult. Sci.*, 74: 1540-1547.
- Bang, H.O. and J. Dyerberg, 1972. Plasma lipids and lipoproteins in Greenlandic west coast Eskimos. *Acta Medica Scandinavica*, 192: 85-94.
- Baucells, M.D., N. Crespo, A.C. Barroeta, S. López-Ferrer and M.A. Grashorn, 2000. Incorporation of different polyunsaturated fatty acids into eggs. *Poult. Sci.*, 79: 51-59.
- Bligh, E.G. and W.J. Dyer, 1959. A rapid method of total lipid extraction and purification. *Canadian J. Biochem. Physiol.*, 37: 911-917.
- Carvalho, P.R., 2006. Influência da adição de fontes ricas em PUFAS n-3 na dieta de galinhas sobre a composição lipídica do ovo. 2006, Tese de Doutorado. Universidade de São Paulo, Faculdade de Medicina Veterinária e Zootecnia, São Paulo, pp: 283.
- Folch, J., M. Lees and G.H.S. Stanley, 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*, 226: 497-509.
- Galobart, J., A.C. Barroeta, M.D. Baucells and F. Guardiola, 2001. Lipid oxidation in fresh and spray-dried eggs enriched with n-3 and n-6 polyunsaturated fatty acids during storage as affected by dietary vitamin E and  $\alpha$ -tocopherol supplementation. *Poult. Sci.*, 80: 327-337.
- Givens, D.I. and R.A. Gibbs, 2008. Current intakes of EPA and DHA in European populations and the potential of animal-derived foods to increase them. *Proc. Nutr. Soc.*, 67: 273-280.
- Gomez, M.E.B., 2002. Modulação da composição de ácidos graxos poliinsaturados  $\omega$ -3 de ovos e tecidos de galinhas poedeiras, através da dieta. I. Estabilidade oxidativa. Tese de doutorado, Faculdade de Farmácia, Universidade de São Paulo, São Paulo, pp: 129.
- Griminger, P., 1986. Lipid Metabolism. In: *Avian Physiology*. Sturkie, P.D. (Ed.). 4 Edn., New York: Springer, pp: 345-358.
- Hargis, P.S., M.E. Van Elswyk and B.M. Hargis, 1991. Dietary modification of yolk lipid with savelha oil. *Poult. Sci.*, 70: 874-883.
- Hartman, L. and R.C.A. Lago, 1973. Rapid preparation of fatty acid methyl esters from lipids. *Laboratory Practice*, 22: 475-477.
- Kinsella, J.E., B. Lokesh and R.A. Stone, 1990. Dietary n-3 polyunsaturated fatty acid and amelioration of cardiovascular disease: Possible mechanisms. *J. Food Sci. Technol.*, 52: 1-58.
- Lewis, S., 1996. *Avian Biochemistry and molecular biology*. Cambridge: University Press, pp: 272.
- Mendonça Junior, C.X. and M.C.G. Pita, 2005. O ovo como via de eliminação do colesterol. *Farmacologia aplicada à avicultura- boas práticas de manejo de medicamentos*. São Paulo, (Ed.) Roca, pp: 347-358.
- Ministério da Saúde, 2009. Secretaria Executiva. Rede Interagencial de Informações para a Saúde. Indicadores e Dados Básicos para a saúde. Disponível em: <http://tabenet.datasus.gov.br/cgi/ibd2003/matriz.htm>. Acesso em: <jul. 2009.
- Mori, A.V., 2001. Utilização de óleo de peixe e linhaça na ração como fontes de ácidos graxos poliinsaturados  $\omega$ -3 em ovos. Tese de doutorado, Faculdade de Medicina Veterinária e Zootecnia. Universidade de São Paulo, São Paulo, pp: 162.
- Nash, D.M., R.M.G. Hamilton and H.W. Hulan, 1995. The effect of dietary herring meal of the omega-3 fatty acid content of plasma and egg yolk lipids of laying hens. *Canadian J. Anim. Sci.*, 75: 247-253.
- Nielsen, H., 1998. Hen age and fatty acid composition of egg yolk lipid. *Br. Poult. Sci.*, 39: 53-56.
- Piber Neto, E., 2006. Enriquecimento do ovo: Utilização de óleos de peixes e alga marinha como fontes de ácidos graxos poliinsaturados  $\omega$ -3 em rações de galinhas. Dissertação de Mestrado, Faculdade de Medicina Veterinária e Zootecnia. Universidade de São Paulo, São Paulo, pp: 82.
- Pita, M.C.G., 2003. Efeito da suplementação de linhaça, óleo de canola e vitamina E na dieta, sobre as concentrações de ácidos graxos poliinsaturados e alfa tocoferol em ovos de galinha. Dissertação de Mestrado, Faculdade de Medicina Veterinária e Zootecnia. Universidade de São Paulo, São Paulo, pp: 141.
- Pita, M.C.G., E. Piber Neto, P.R. Carvalho and C.X. Mendonça Junior, 2006. Efeito da suplementação de linhaça, óleo de canola e vitamina E na dieta sobre as concentrações de ácidos graxos poliinsaturados em ovos de galinha. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, 58: 925-931.
- Shapira, N., P. Weill and R. Loewenbach, 2008. Egg fortification of n-3 polyunsaturated fatty acids (PUFA): Nutritional benefits versus high n-6 PUFA western diets and consumer acceptance. *IMAJ*, 10: 262-265.
- Simopoulos, A.P., 2009. Evolutionary aspects of the dietary omega-6:omega-3 fatty acid ratio: Medical implications. *World Rev. Nutr. Diet.*, 100: 1-21.
- Souza, J.G., F.G.P. Costa, R.C.R.E. Queiroga, J.H.V. Silva, A.R.P. Schuler and C.C. Goulart, 2008. Fatty acid profile of eggs of semi-heavy layers fed feeds containing linseed oil. *Brazilian J. Poult. Sci.*, 10: 37-44.