

## Effects of Different Oils on the Production Performances and Polyunsaturated Fatty Acids and Cholesterol Level of Yolk in Hens\*

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**ABSTRACT :** In order to understand the effects of different oils on the production performances and polyunsaturated fatty acids and cholesterol level in the yolk, 160 Hexices hens at 42 wks were divided into four groups randomly. Each group fed with control diet (CG), control diet+5% fish oil (FG), control diet+5% palm oil (PG) and control diet+5% soybean oil (SG), respectively. After three weeks' experiment, the results showed that: different oils showed no significant effect on feed/egg weight, egg white weight, body weight, C16, C18:3 n-6 and C20:4 n-6 contents in the yolk ( $p>0.05$ ). But the egg mass of PG was higher than SG ( $p<0.05$ ), the average egg weight of CG was lower than FG ( $p<0.05$ ), and the of PG was lower than FG ( $p<0.05$ ), during the experiment, FG gained more than SG ( $p<0.05$ ), the cholesterol level in yolk of FG was lower than PG and CG ( $p<0.01$ ), meanwhile the C20:5 n-3 content of FG was higher than CG and SG ( $p<0.01$ ), and no C20:5 n-3 was detected in PF, as far as C22:6 n-3 in the yolk was concerned, FG was higher than PG ( $p<0.01$ ), the C18:1 n-9 content of SG was lower than PG ( $p<0.05$ ), the C18:2 n-6 content of SG was the highest than other three groups ( $p<0.01$ ), and CG was the lowest, showed significant to FG ( $p<0.05$ ), the C18:3 n-3 content of FG was higher than SG and PG ( $p<0.05$ ), and the C20:1 n-9 content of FG was higher than other groups ( $p<0.01$ ). The results demonstrated that fish oil could decrease the cholesterol and increase the n-3 fatty acids content in the yolk, and increase the n-3/n-6 level. (*Asian-Aust. J. Anim. Sci.* 2004, Vol 17, No. 6 : 843-847)

**Key Words :** Egg, Yolk, Production Performance, Polyunsaturated Fatty Acids, Cholesterol, Hexics Hen

### INTRODUCTION

Egg is the most nutritious, unadulterated, natural food. Egg will supply about 6.5 g of wholesome protein of high biological value, 5.8 g of emulsified, easily digestible fat, rich in phospholipids needed for brain and other nervous tissue growth and health and supplies only 80 kcal energy. Egg is also a rich source of all essential amino acids, minerals and vitamins (except vitamin C) (Narabari, 2001). To increase egg consumption as a part of healthy eating, scientists are constantly searching for methods to nutritionally enrich the egg. The total protein, fat and sugar content of the egg cannot be alter much but it is possible to manipulate fatty acid composition and levels of minerals, vitamins and certain non-nutrient chemicals (like pigments and antioxidants) in eggs by dietary means (Nash et al., 1995; Ayerza and Coates, 1999).

A number of epidemiological and controlled experiments have reported an inverse relationship between  $\omega$ -3 acid consumption, and risk of cardiovascular, some autoimmune disorders, diabetes, and some types of cancer aside from their important role in neuronal (Bang et al., 1980; Leaf and Kang, 1998; Simopoulos, 2000). Animal

studies showed that lack of  $\omega$ -3 fatty acids can lower learning and visual abilities of animals.

A number of researchers have showed that inclusion high level polyunsaturated fatty acids (PUFAs) of plant oils into hen's feed can increase the PUFAs content in the yolk, especially  $\omega$ -3 PUFAs content, but the results varied. This study was conducted to investigate the effects of fish oil, soybean oil, palm oil on the production performances and PUFAs contents in the egg yolk.

### MATERIALS AND METHODS

#### Experimental procedure, animals and diets

200 healthy Hecics laying hens were selected at 40 weeks of age, following random allocation to the cages, the hens were fed for 30 d, prior to collecting data. Then selected 160 for study, divided them into four groups randomly, each treatment group was represented by ten replicates of ten hens each, and two hens per pen, the trial then lasted three weeks. One group was fed *ad libitum* with control diet, the other three groups were fed *ad libitum* with control diet+5% fish oil (FO), soybean oil (SO) and palm oil (PO) respectively (Table 1), the diets were formulated to contain the same metabolizable energy and protein, the PUFAs composition of different oils were showed in Table 2. Management was according to the Management and Feeding Handbook of Hexics Laying Hens, the room had common ventilation and air conditioning systems and room's temperature was set at  $23.5\pm 3.6^{\circ}\text{C}$  throughout the trial and relative humidity was approximately  $60\pm 5\%$ ,

\* Founded by Jiangxi Provincial Agricultural Office (No. NK2003-X06).

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Received May 21, 2003; Accepted February 16, 2004

**Table 1.** The composition of the experimental diets (%)

Ingredients	Treatments			
	Control	Fish oil	Palm oil	Soybean oil
Corn	68.00	64.60	64.60	64.60
Soybean meal	21.50	20.00	20.00	20.00
Fish meal	3.00	-	-	-
Wheat barn	4.00	7.00	7.00	7.00
Fish oil	-	5.00	-	-
Palm oil	-	-	5.00	-
Soybean oil	-	-	-	5.00
Limestone	1.20	1.20	1.20	1.20
CaHPO <sub>4</sub>	1.70	1.70	1.70	1.70
DL-Met	0.30	0.20	0.20	0.20
Salt	0.30	0.30	0.30	0.30
Pre-mix	1.50	1.50	1.50	1.50
Nutrient level				
Crude protein	18.41	18.02	17.65	18.14
ME, MJ/kg	12.66	12.78	12.79	12.77
DL-Met	0.45	0.46	0.44	0.46
Met+Cys	0.85	0.87	0.86	0.88
Lys	1.02	1.02	1.02	1.02
Cal	3.60	3.60	3.60	3.60
Total P	0.65	0.65	0.65	0.65

illuminating time was 16 h per day and luminance was 10 lx.

### Production performances measurements

Eggs were collected by replicates per day, and egg mass and egg weight were recorded, production performances and feed consumption were calculated each week, each hen was weighted at the end of the experiment. At the last two days of each week, eggs were sampled, and marked group number and laying date on the egg shell, and selected 40 eggs from each group at random, other days sampled 6 eggs per day at each group, and all sampled eggs were stored in refrigerator at 4°C for chemical analysis.

### Chemical analysis

The contents of crude protein (CP), calcium and phosphorus in diets were analyzed according to standard procedures described by the Association of Official Analytical Chemists (1990). Energy values of the diets were calculated as metabolizable energy (ME) according to the methods described by Krogdhal (1985).

Cholesterol in egg yolk was determined spectrophotometrically in Encore Chemistry System (Baker

**Table 2.** Major PUFA levels of different oil

Sorts of PUFA	Fish oil	Palm oil	Soybean oil
C <sub>16:0</sub>	13.24	33.78	8.65
C <sub>16:1</sub>	9.54	0.43	0.54
C <sub>18:1 n-9</sub>	14.56	37.80	26.52
C <sub>18:2 n-6</sub>	14.32	8.02	52.04
C <sub>18:3 n-6</sub>	0.47	0.01	6.02
C <sub>18:3 n-3</sub>	0.52	0.54	8.01
C <sub>20:1 n-11</sub>	0.58	0.30	0.60
C <sub>20:1 n-9</sub>	1.47	0.24	0.42
C <sub>22:1 n-11</sub>	2.60	0.13	0.06
C <sub>22:6 n-3</sub> (DHA)	9.31	0.01	0.12
C <sub>20:5 n-3</sub> (EPA)	11.01	ND	ND

Instruments, UK), using Cholesterol Enzumatique PAP 100, kit, Ref. 61244 from bioMeriedux (France). Fatty acid composition of egg yolk was determined by GLC procedures according to the methods described by Ulbreth and Henninger (1992) for extracted/methylated samples. The fatty acid methyl esters were determined on a Perkin Elmer Autosystem gas chromatograph (Perkin Elmer Corp., Norwalk, CT) with a SGE capillary column no. 5QC/3bpx 70, 0.25, 25+25 m (SGE International Pty. LTD, Ringwood, Victoria, Australia). The results are presented as relative distribution of the individual fatty acids (g 100<sup>-1</sup> of total fatty acids) determined by the percent area.

### Statistical analysis

All data were analyzed by using the General Linear Model Procedures of SAS (1989). Comparison of treatment means was based on Duncan's multiple range test. A significant level of  $p < 0.05$  was applied in all case.

## RESULTS

### Egg production

Hen weight, weight gain, average egg weight, feed to egg ratio, yolk weight, egg mass, egg white weight were presented in Table 3. No significant difference ( $p > 0.05$ ) in feed to egg ratio, egg white weight, end body weight were found among treatments. Egg mass of palm oil group was higher than soybean oil group ( $p < 0.05$ ) and the average egg weight of fish oil group was higher than control group ( $p < 0.05$ ), the yolk weight of fish oil group was higher than palm oil group ( $p < 0.05$ ) and hen weight gain of fish oil group was higher than soybean oil group ( $p < 0.05$ ).

**Table 3.** Effects of different oils on production performances in hens (g)

Treatments	Items							
	Egg mass	Average egg weight	Feed/egg	Egg white weight	Yolk weight	Start body weight	End body weight	Weight gain
Control	623 <sup>ab</sup>	61.53±4.12 <sup>b</sup>	2.56	37.12±2.37	16.97±1.26 <sup>ab</sup>	1,651±12.30	1,660±13.20	9.02±0.95 <sup>ab</sup>
Fish oil	665 <sup>ab</sup>	65.41±3.76 <sup>a</sup>	2.48	39.54±3.01	18.98±1.36 <sup>a</sup>	1,644±15.76	1,665±14.35	11.03±1.01 <sup>a</sup>
Palm oil	780 <sup>a</sup>	63.73±4.01 <sup>ab</sup>	2.55	35.44±2.98	16.36±1.65 <sup>b</sup>	1,647±15.48	1,656±10.52	8.87±0.65 <sup>ab</sup>
Soybean oil	545 <sup>b</sup>	63.34±2.58 <sup>ab</sup>	2.69	36.42±3.25	17.12±2.02 <sup>ab</sup>	1,648±13.25	1,654±11.43	6.05±0.87 <sup>b</sup>

Different lowercase in the same column means significant difference ( $p < 0.05$ ).

**Table 4.** Effects of different oils on the fatty acids (%)

Sorts of PUFA	Control			Fish oil			Palm oil			Soybean oil		
	1st week	2nd week	3rd week	1st week	2nd week	3rd week	1st week	2nd week	3rd week	1st week	2nd week	3rd week
Cholesterol (mg/g)	22.05±1.62 <sup>A</sup>			14.09±0.86 <sup>B</sup>			24.54±2.33 <sup>A</sup>			17.65±2.00 <sup>AB</sup>		
C <sub>16:0</sub>	25.01	24.92	25.03	25.43	24.90	25.07	27.05	26.92	26.03	25.01	24.92	25.03
C <sub>16:1</sub>	2.80	2.83	2.85	2.31	2.25	2.46	3.08	3.43	3.85	2.95	2.94	2.98
C <sub>18:1 n-9</sub>	43.50 <sup>ab</sup>	48.46 <sup>ab</sup>	49.65 <sup>ab</sup>	43.01 <sup>ab</sup>	44.26 <sup>ab</sup>	45.12 <sup>ab</sup>	50.23 <sup>a</sup>	51.11 <sup>a</sup>	52.39 <sup>a</sup>	38.92 <sup>b</sup>	38.65 <sup>b</sup>	39.23 <sup>b</sup>
C <sub>18:2 n-6</sub>	8.50 <sup>bb</sup>	9.42 <sup>bb</sup>	9.47 <sup>bb</sup>	11.45 <sup>ab</sup>	11.98 <sup>ab</sup>	12.46 <sup>ab</sup>	10.95 <sup>abB</sup>	10.31 <sup>abB</sup>	9.57 <sup>abB</sup>	24.05 <sup>A</sup>	24.67 <sup>A</sup>	24.02 <sup>A</sup>
C <sub>18:3 n-6</sub>	0.07	0.07	0.05	0.05	0.06	0.05	0.06	0.05	0.05	0.06	0.07	0.10
C <sub>18:3 n-3</sub>	0.21 <sup>a</sup>	0.19 <sup>a</sup>	0.16 <sup>ab</sup>	0.20 <sup>a</sup>	0.25 <sup>a</sup>	0.24 <sup>a</sup>	0.13 <sup>b</sup>	0.13 <sup>b</sup>	0.12 <sup>b</sup>	0.97 <sup>b</sup>	0.95 <sup>b</sup>	0.94 <sup>b</sup>
C <sub>20:1 n-9</sub>	0.12 <sup>B</sup>	0.12 <sup>B</sup>	0.13 <sup>B</sup>	1.52 <sup>A</sup>	1.61 <sup>A</sup>	1.47 <sup>A</sup>	0.16 <sup>B</sup>	0.16 <sup>B</sup>	0.17 <sup>B</sup>	0.13 <sup>B</sup>	0.14 <sup>B</sup>	0.14 <sup>B</sup>
C <sub>20:2 n-6</sub>	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.23	0.21	0.18
C <sub>20:4 n-6</sub>	1.59	1.45	1.34	1.02	1.15	1.08	1.78	1.66	1.54	1.45	1.52	1.80
C <sub>22:6 n-3</sub>	1.57 <sup>AB</sup>	1.41 <sup>AB</sup>	1.25 <sup>AB</sup>	2.21 <sup>A</sup>	2.14 <sup>A</sup>	2.11 <sup>A</sup>	1.02 <sup>B</sup>	1.03 <sup>B</sup>	0.96 <sup>B</sup>	1.52 <sup>AB</sup>	1.47 <sup>AB</sup>	1.23 <sup>AB</sup>
C <sub>20:5 n-3</sub>	0.01 <sup>B</sup>	0.01 <sup>B</sup>	0.01 <sup>B</sup>	0.62 <sup>A</sup>	0.65 <sup>A</sup>	0.66 <sup>A</sup>	ND	ND	ND	0.01 <sup>B</sup>	0.01 <sup>B</sup>	0.01 <sup>B</sup>
n-3	1.79	1.61	1.42	3.03	3.04	3.01	1.15	1.16	1.08	2.50	2.43	2.18
n-6	10.16	10.94	10.86	12.52	13.19	13.59	12.79	12.02	11.16	25.79	26.47	27.15
n-3/n-6	0.18	0.15	0.13	0.24	0.23	0.22	0.09	0.10	0.10	0.10	0.09	0.08

Different capital letter in the same row at the same week means greatly significantly ( $p < 0.01$ ), different lowercase means significant difference ( $p < 0.05$ ).

#### Fatty acid composition and cholesterol of egg yolk

Table 4 showed the fatty acid composition and the cholesterol content of egg yolk. The cholesterol level of fish oil group was lower than control and palm oil groups ( $p < 0.01$ ). No significant differences among treatments were found for C<sub>16:0</sub>, C<sub>16:1</sub>, C<sub>18:3 n-6</sub> and C<sub>20:4 n-6</sub> ( $p > 0.05$ ). Among control group, fish oil group and palm oil group, no C<sub>20:2 n-6</sub> was detected in the yolk, while in soybean oil group were higher. The C<sub>20:5 n-3</sub> in fish oil group was higher than control and soybean oil group ( $p < 0.01$ ), but no C<sub>20:5 n-3</sub> was detected in palm oil group. the C<sub>22:6 n-3</sub> in fish oil group was higher than palm oil group ( $p < 0.01$ ), the C<sub>18:1 n-9</sub> content of soybean group was lower than palm oil group ( $p < 0.05$ ), as far as C<sub>18:2 n-6</sub> was concerned, soybean oil group was higher than other group ( $p < 0.01$ ), and the control group was the lowest, show significant difference to fish oil group ( $p < 0.05$ ), and the C<sub>18:3 n-3</sub> content of fish oil group was higher than soybean oil and palm oil group ( $p < 0.05$ ), also the C<sub>20:1 n-9</sub> level of fish oil was higher than other groups ( $p < 0.01$ ).

#### DISCUSSION

In this study, the hens were fed with isocaloric and isonitrogenous experimental diets, and the oil supplemental levels were all 5%. The results showed that different oils did not affect feed to egg ratio, egg white weight and body weight ( $p > 0.05$ ), these results were similar to Chen et al. (2003), who found that when supplemented 2% tallow and 2-6% refined cod liver oil in duck diets, no significances were found for feed efficiency, body weight, but when the refined cod liver oil supplemental level was 6%, the yolk weight was lighter than 2-5% oil groups, the plausible reason for this difference was the basal composition and

breed. Zhang et al. (1997) reported when added 8% palm oil, the egg mass was higher than 8% soybean oil and control group, this was similar to this result, they reported that the palm oil could improve the egg production and feed conversion rate was the result of soybean oil contains antinutrients (such as trypsin inhibitors, phytohaemagglutinins). Van Elswyk et al. (1994) reported that the hypolipodmic effect of fish oil might have reduced the hepatic lipogenesis and lipid transport from blood into the developing ova. Oh et al. (1994) reported that hens diets isoenergetically supplemented with 5% fish oil for 8 weeks did adversely influence feed efficiency, body weight or egg production. Kjos et al. (2001) reported that when supplemented 1.8, 8.8, 18.8 and 24.8 g fish silage per kg hen diet, the feed intake decreased significantly to control diet with the fish silage level increased, and when supplemental level was above 8.8 g, the egg mass and egg weight decreased ( $p < 0.01$ ). Baucells et al. (2000) reported even when the fish oil supplemental level was 40 g/kg diet, the egg's production performances did not changed.

The cholesterol level in the yolk of fish oil group was significantly lower than control and palm oil group ( $p < 0.01$ ), this was similar to Yu et al. (1998) and Zhang et al. (1997). Ricardo Ayerza and Wayne Coates (2001) reported that when flaxseed supplemental levels were 2, 2.5 and 5%, no significances were found among control group and experimental groups for cholesterol in egg yolks. Herstad et al. (2000) found that with the fish oil increased, the cholesterol in the yolk decreased. Jiang and Sim (1992) found that when fed rats with n-3 fatty acid-enriched chicken egg, the plasma and serum cholesterol levels were decreased. In this experiment, with the egg weight improved, the cholesterol level was decreased, which was

similar to Choi et al. (2001).

Several factors affect egg composition and lipid profile including bird age, strain and breed. Nevertheless, dietary manipulation still yields the most significant changes to yolk lipid profile (Leskanich and Noble, 1997). Graded levels of dietary saturated and monounsaturated fats have minor effects on the relative egg fatty acid profile (Baucells et al., 2000). In contrast, dietary polyunsaturated fats can cause major changes (Noble et al., 1990) thus allowing for manipulation of yolk lipids to better meet human nutritional requirements. As far as the n-3 polyunsaturated fatty acids levels were concerned, its level in the yolk was proportional to the level in the diet. In this experiment we verified this theory. It is interesting to note that in spite of the higher concentrations of eicosapentaenoic acid (EPA) relative to docosahexaenoic acid (DHA) in fish oil, the concentration of latter found in yolks from hens fed fish oil diet is much greater than the former. This was similar to the results of González-Esquerria et al. (2000) and Nash et al. (1996). The explanation to this finding possibly relates to the birds metabolism of n-3 fatty where conversion of EPA from DHA and vice versa along with tissue specific preferential DHA deposition might occur as reported in mammals (Sprecher et al., 1995). Hargis et al. (1991) reported when the fish oil inclusion was 3% could significantly improve the EPA and DHA concentrations in the egg yolk.

Several studies have shown that an appropriate  $\omega$ -6: $\omega$ -3 ratio must be provided in human diet. Nutritional recommendation suggest a dietary  $\omega$ -6:  $\omega$ -3 ratio of 5:1 (British Nutrition Foundation, 1992; FAO, 1994), or 4:1 (Ministry of Health and Welfare of Japan, cited by Okuyama et al., 1997), or even lower (Simopoulos et al., 1998). The  $\omega$ -6: $\omega$ -3 ratios in the yolks produced by the enriched feeds used in the current trial greatly improve the nutritional quality of the eggs, compared with those of the laying hens fed control diet. The deposition of n-3 fatty acids in the yolk is a gradual process. Yu et al. (1987) reported that n-3 fatty acids concentrations could maintain at a relatively stable level in the yolks after hens fed with salmon fish oil for 8 days, the explanation to this might be the formational time of yolk in the hen need 9 days (Huang et al., 2001). In this study we also found the same phenomenon.

## CONCLUSION

n-3 polyunsaturated fatty acids enriched egg can be produced by supplementation with 5% fish oil without negative effect on laying performances. These eggs may serve as viable dietary alternatives to fish, fish products to provide significant amounts of n-3 PUFAs in human diet.

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