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 altered 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 ω-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 ω-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 ω-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 Hecics Laying Hens, the room had common ventilation and air conditioning systems and room’s temperature was set at 23.5±3.6°C throughout the trial and relative humidity was approximately 60±5%,
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 Instruments, UK), using Cholesterol Enzymatique 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 100⁻¹ 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, egg white 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 1. The composition of the experimental diets (%)

| Ingredients     | 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        |
| CaHPO4          | 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        |

Table 2. Major PUFA levels of different oil

| Sorts of PUFA   | Fish oil | Palm oil | Soybean oil |
|-----------------|----------|----------|-------------|
| C16:0           | 13.24    | 33.78    | 8.65        |
| C16:1           | 9.54     | 0.43     | 0.54        |
| C18:1:9         | 14.56    | 37.80    | 26.52       |
| C18:1:6         | 14.32    | 8.02     | 52.04       |
| C18:1:9         | 0.47     | 0.01     | 6.02        |
| C18:3:9         | 0.52     | 0.54     | 8.01        |
| C20:1:0         | 0.58     | 0.30     | 0.60        |
| C20:1:9         | 1.47     | 0.24     | 0.42        |
| C22:1:11        | 2.60     | 0.13     | 0.06        |
| C22:6:3 (DHA)   | 9.31     | 0.01     | 0.12        |
| C20:5:3 (EPA)   | 11.01    | ND       | ND          |

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

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

Different lowercase in the same column means significant difference (p<0.05).
Table 4. Effects of different oils on the fatty acids (%)

| Types of PUFA | Control | Fish oil | Palm oil | Soybean oil |
|--------------|---------|----------|----------|------------|
| Cholesterol  | mg/g    |          |          |            |
| C16:0        | 22.05±1.62 A | 14.09±0.86 B | 24.54±2.33 A | 17.65±2.00 AB |
| C18:0        | 25.01   | 24.92    | 25.03    | 25.43      |
| C18:1 n-9    | 2.80    | 2.83     | 2.85     | 2.31       |
| C18:2 n-6    | 8.50±0  | 9.42±0   | 9.47±0   | 11.45±0   |
| C18:3 n-3    | 0.07    | 0.07     | 0.05     | 0.05       |
| C16:1        | 43.50 ab | 48.46 ab | 49.65 ab | 43.01 ab   |
| C18:2 n-6    | 0.21 a  | 0.19 a   | 0.16 b   | 0.20 a     |
| C18:3 n-3    | 0.12±0  | 0.12±0   | 0.13±0   | 1.52±0     |
| C20:1 n-9    | ND      | ND       | ND       | ND         |
| C20:2 n-6    | 1.59    | 1.45     | 1.34     | 1.02       |
| C20:3 n-3    | 0.01 B  | 0.01 B   | 0.01 B   | 0.62 A     |
| n-3          | 10.16   | 10.94    | 10.86    | 12.52      |
| n-6          | 0.18    | 0.15     | 0.13     | 0.24       |
| n-3:n-6      | 2.33 A  | 17.65    | 1.62 A   | 1.41 A     |

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 C16:0, C18:0, C18:1 n-9 and C20:4 n-6 (p>0.05). Among control group, fish oil group and palm oil group, no C20:2 n-6 was detected in the yolk, while in soybean oil group were higher. The C20:5 n-3 in fish oil group was higher than control and palm oil groups (p<0.01). Among soybean group was lower than control and palm oil group (p<0.05), as far as C18:3 n-3 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 C18:3 n-3 content of fish oil group was higher than soybean oil and palm oil group (p<0.05), also the C20:1 n-9 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% oils group, the plausible reason for this 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 Eiwijk et al. (1994) reported that the hypolipidemic 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). Bauells 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 if dietary saturated and monounsaturated fats have minor effects on the relative 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-Esquerra et al. (2000) and Nash et al. (1996). The explanation to this finding possibly relates to the birds tissue specific preferential metabolism of n-3 fatty acids 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 DHA deposition might occur as reported in mammals. González-Esquerra, R. and S. Lesson. 2001. Alternatives for enrichment of eggs and chicken meat with omega-3 fatty acids. Can. J. Anim. Sci. 81:295-305.

Several studies have shown that an appropriate ω-6:ω-3 ratio must be provided in human diet. Nutritional recommendation suggest a dietary ω-6: ω-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 ω-6:ω-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 server as viable dietary alternatives to fish, fish products to provide significant amounts of n-3 PUFAs in human diet.

**REFERENCES**

Association of Official Analytical Chemists. 1990. Official methods of analysis. 15th ed. AOAC, Arlington, VA.

Bang, H. O., J. Dyerberg and H. M. Sinclair. 1980. The composition of the Eskimo food in northwestern Greenland. Can. J. Anim. Sci. 33:2657-2661.

Baucells, M. D., N. Crepo and A. C. Barroeta. 2000. Incorporation of different polyunsaturated fatty acids into eggs. Poult. Sci. 79:51-59.

British Nutrition Foundation. 1992. Unsaturated fatty acids: nutritional and physiological significance. British Nutrition Foundation’s Task Force, London, England.

Chen, Tian-Fwu and Hsu Jenn-Chung. 2003. Incorporation of n-3 long-chain polyunsaturated fatty acids into duck eggs yolks. Asian-Aust. J. Anim. Sci. 16(4):565-569.

Choi, S. H., K. T. Song and H. R. Oh. 2001. Cholesterol contents and fatty acids composition of chukar, pheasant, guinea and quail egg yolk. Asian-Aust. J. Anim. Sci. 14(6):831-836.

Food and Agricultural Organization. 1994. Fat and oils in human nutrition: Report of a joint expert consultation. Food and Nutrition Paper N: 57 FAO, Rome, Italy.

González-Esquerra, R. and S. Lesson. 2001. Alternatives for enrichment of eggs and chicken meat with omega-3 fatty acids. Can. J. Anim. Sci. 81:295-305.

Hargis, P. S., M. E. Van Elswyk and B. M. Hargis. 1991. Dietary modification of yolk lipid with menhaden oil. Poult. Sci. J. 70:874-883.

Herstad, O., M. Øverland and A. Haug. 2000. Reproductive performance of broiler breeder hens fed n-3 fatty acid-rich fish oil. Acta. Agric. Scand Sect A, Anim. Sci. 50:121-128.

Huang Beiying and Yang Ning. 2001. Effects of dwarf gene on incorporation of dietary fatty acids into yolks of laying hens. Acta Veterinaria et Zootecnica Sinica. 32(6):499-504 (in Chinese).

Jiang, Z. and J. S. Sim. 1992. Effects of dietary n-3 fatty acid-enriched chicken eggs on plasma and tissue cholesterol and fatty acids composition of rats. Lipids, 27:279-284.

Kjos, N. P., O. Herstad, A. Skrede and M. Øverland. 2001. Effects of dietary fish silage and fish fat on performance and egg quality of laying hens. Can. J. Anim. Sci. 81:245-251.

Krogdhal, A. 1985. Fish viscera silage as a protein source for poultry. 1. Experiments with layer-type chicks and hens. Acta. Agri. Scand. 35:3-23.

Leaf, A. and J. X. Kang. 1998. ω 3 fatty acids and cardiovascular disease, Pages 24-37 in A P Simopoulos, ed. The return of long-chain polyunsaturated fatty acids into eggs. Poult. Sci. 79:51-59.

Leskanich, C. O. and R. C. Noble. 1997. Manipulation of the n-3 polyunsaturated fatty acid composition of avian eggs and meat. World’s Poult. Sci. J. 53:155-183.

Narabari, D. 2001. Nutrionally enriched eggs. Poult. Inter. 40(10):22-30.

Nash, D. M., R. M. G Hamilton, K. A. Sanford and H. W. Hulan. 1995. The effect of dietary herring meal on the omega-3 fatty acid content of plasma and egg lipids of laying hens. Can. J. Anim. Sci. 75:247-253.

Nash, D. M., R. M. G Hamilton, K. A. Sanford and H. W. Hulan. 1996. The effect of dietary menhaden meal and storage on the
omega-3 fatty acids and sensory attributes of egg yolk in laying hens. Can. J. Anim. Sci. 76:377-383.

Noble, R. C., M. Cocchi, E. Turchetto. 1990. Egg fat - a case of concern? World's Poult. Sci. J. 46:109-118.

Oh, S. Y., H. H. Lin, J. Ryue and D. E. Bell. 1994. Effects of dietary eggs enriched in omega-3 fatty acids as a wholesome food. J. Applied Nutr. 46:14-25.

Ricardo Ayerza and Wayne Coates. 2001. Omega-3 enriched eggs: The influence of dietary α-linolenic fatty acid source on egg production and composition. Can. J. Anim. Sci. 81:355-362.

Okuyama, H., T. Kobayashi and S. Watanabe. 1997. Dietary fatty acids: The n-6/n-3 balance and chronic elderly diseases. Excess linoic acid and relative n-3 deficiency syndrome seen in Japan. Prog Lipid Res. 35:409-457.

SAS Institute Inc. 1989. The SAS® System for Windows™ Release 6.12. Copyright © 1989-1996 SAS Institute Inc. Cary NC 27513, USA.

Simopoulos, A. P. 2000. Symposium: role of poultry products in enriching the human diet with n-3 PUFA. Human requirement for n-3 polyunsaturated fatty acids. Poult. Sci. 79:961-970.

Sprecher, H., D. L. Luthria and B. S. Mohammed. 1995. Reevaluation of the pathways for the biosynthesis of polyunsaturated fatty acids. J. Lipid Res. 36:2471-2477.

Van Elswyk, M. E. Hargis, J. D. Williams and P. S. Hargis. 1994. Dietary menhaden oil contributes to hepatic lipidosis in laying hens. Poult. Sci. 73:653-662.

Yu Huiming, Li Defa and Guang Wutai. 1998. Effect of different fat sources on the nutrients retain, body composition and serum metabolites for broilers. Acta Veterinaria et Zootechnica Sinica. 29(4):304-314 (in Chinese).

Yu, M. M. and J. S. Sim. 1987. Biological incorporation of n-3 polyunsaturated fatty acids into chicken eggs. Poult. Sci. 66(Suppl.1):195 (Abstr.).

Zhang Liying, Wang Bowei and Shang Huet al. 1997. Effects of different plant oils on hens production performances and PUFA and cholesterol contents in the yolk. Feed Research. 8:7-8 (in Chinese).