Journal Home page : www.jeb.co.in ★ E-mail : editor@jeb.co.in

JEB
Journal of Environmental Biology

ISSN: 0254-8704
CODEN: JEBIDP

Gamma-linolenic acid egg production enriched with hemp seed oil and evening primrose oil in diet of laying hens

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Publication Info

Paper received:
5 March 2013

Revised received:
22 July 2013

Accepted:
11 October 2013

Abstract

This study was carried out to find out the effect of supplying gamma linolenic acid (GLA) on laying performance and egg quality. A hundred twenty of 30 weeks old hyline brown laying hens with 98% of egg production were completely randomized to 4 different treatment groups by 30 hens (the control group fed with the diet containing beef tallow, 3 treatment groups fed with the diet containing corn oil, the diet containing hemp seed oil and the diet containing evening primrose oil, respectively), and their laying performance and egg production were investigated for 5 weeks. Intake of hemp seed oil or evening primrose helped to increase the retention rate of GLA, which was transmigrated into eggs from blood. GLA was not detected in the blood samples of control group and treatment group fed diet containing corn oil, while it was significantly increased in the blood samples of the treatment groups fed with diet containing hemp seed oil and diet containing evening primrose oil, respectively. GLA retention was not observed in the eggs produced respectively by control group and treatment group fed with diet containing corn oil, whereas it was significantly increased in the eggs produced by the treatment group fed with diet containing hemp seed oil by 1.09% and the treatment group fed with diet containing evening primrose oil by 4.87%. This result suggests that GLA-reinforced functional eggs can be produced by adding hemp seed oil and evening primrose oil to the feed for laying hens and feeding them with it. It is thought that further researches and clinical trials on biochemical mechanism related to atopic dermatitis should be conducted in future.

Key words

Egg performance, Egg quality, Evening primrose oil, Gamma linolenic acid, Hemp seed oil

Introduction

Gamma fatty acids are newly emerging lipid-derived bio-materials that can help to improve lipid metabolism and prevent metabolic disease and inflammation. Atopic skin disease, which is prevalent in children and is believed to result from a combination of genetic and environmental factors, is recognized as a serious illness. Therefore, functional foods, which help to prevent this condition, are required to be developed. Gamma fatty acids refer to gamma linolenic acid (GLA, 18:3n-6) and dihomo-gamma linolenic acid (DGLA, 20:3n-6) which are the intermediary metabolites of linoleic acid(18:2n-6), the parent fatty acid of omega-6 (Kim *et al.*, 2012; Tso *et al.*, 2012). It is known that human blood contains 5~6 g of lipid per liter, including 1,500 mg l^{-1} of linolenic acid and its metabolites, 25 mg/L of GLA, 100 mg l^{-1} of DGLA and 400 mg l^{-1} of arachidonic acid, and human breast milk is known to be high in GLA (Miles *et al.*, 2004). It has been reported that GLA content of human breast milk after a week of lactation is about 0.35~1.0% per liter of breast milk lipid, and colostrum, the breast milk produced by the mammary glands of mammals in late pregnancy, also contains similar level of GLA (Demmelmair *et al.*, 2001). Since human breast milk contains about 33g of lipid per liter, a 5kg breastfed infant, who consumes about 800ml of breast milk per day, takes in about 25~65 mg of GLA per kg (Park, 2008). Human breast milk is also high in DGLA, occupying 0.3~0.4% of total lipid. Therefore, a breast fed baby takes in about 20~26 mg of

DGLA kg^{-1} day^{-1} (Thijs *et al.,* 2000).

It can be thought that atopic-dermatitis is more prevalent in formula-fed infants because the incidence of atopic dermatitis increases in people who have lack of GLA (Andreassi *et al.,* 1997; Yasumoto *et al.,* 1997; Thijs *et al.,* 2000). It has been newly demonstrated that GLA supply is effective in drastic reduction of dysmenorrhea, prevention of skin disease such as eczema, anti-aging protection of skin in women using cosmetics, prevention of cardiovascular disease, cerebrovascular disease and hypertension by improving blood lipid metabolism, hypertension, obesity, rheumatoid arthritis, atopic dermatitis, enhancement of immunity, prevention of obesity and prevention of osteoporosis in menopause women (Barre, 2001; Demmelmair *et al.*, 2001; Park, 2002; Park and Zammit, 2003; Menendez *et al.*, 2005; Kim *et al.*, 2012). The maximum daily dose of gamma fatty acids, considering the safety of gamma fatty acids, is reported to be 300~360 mg, or 6~7 mg kg^{-1} in an adult woman who is 50 kg in weight (Leicer *et al.,* 2000). Gamma fatty acids were first found in breast milk, they have been also found in hemp seed oil (1-6%), evening primrose oil (7~10%), black currant oil (15~20%) and borage oil (18~25%) in a form of GLA (Fan and Chapkin, 1998; Miles *et al.*, 2004; Foster *et al.*, 2010; Nykiforuk *et al.*, 2012). There are some reports about the effect of GLA on egg, specially on the quality. For instance it has been suggested that GLA is rapidly metabolized to arachidonic acid in the body and incorporated into the yolk (Furuse *et al.*, 1992).

It was reported that gamma fatty acids of chickens could be reinforced with hemp seed oil and evening primrose oil (Park, 2007). Gamma fatty acids are hardly found in animal food such as eggs, and it was also difficult to find results of such study on gamma fatty acids as conducted with animal food. If GLA is insufficient in a human body due to a lack of Δ6-desaturase activity and various factors suppressing Δ6-desaturase activity even though GLA is normally produced, he will be susceptible to a variety of diseases (Duche and Rkste, 2001; Kim *et al.*, 2012). To increase GLA intake through everyday food, GLA of eggs can be reinforced. This study was conducted to investigate the feeding effects of dietary GLA on haying performance and egg quality and to contribute the production of highly functional eggs with reinforced GLA.

Materials and Methods

Experimental design : One hundred twenty Hyline brown hens which were 16 weeks old were purchased from Eunkwan farm (Gyunggido Ahnsung, Republic of Korea). Hens were completely randomized into four treatment groups with three replicates of 10 animals per group in three-layer wire cages. These groups were : Control group (beef tallow as saturated fatty acids), CO (corn oil as n-6 fatty acids), HSO (hemp seed oil as low GLA 1.68%) and EPO (evening primrose oil as high GLA 8.03%). When they were 30 week old reaching 98% of egg production, laying performance and egg quality were evaluated for 5 weeks. Lighting during the laying period was adjusted to 17 hr, and the experimental diet was provided *ad libitum* and they had free access to water.

Preparation of diet : Experimental diet was prepared according to NRC feeding standard (1994), and composed of corn grain 51.30%, soybean meal 11.70%, wheat bran 18.00%, corn gluten meal 5.20%, fat 5.00%, limestone 7.30%, tricalcium phosphate 0.80%, salt 0.30%, DL-methionine 0.10% and growth promotors 0.30%. Beef tallow and CO were obtained from conventional markets, and HSO and EPO were purchased from a Chinese company (Rolf M. Wunder & Co.) via Sinwoo Trading Co. Ltd. (Seoul Republic of Korea). To prevent oxidation of unsaturated fatty acids in vegetable oils, vitamin E (α-tocopheryl acetate) was used as a natural antioxidant. 50 mg of vitamin E was added to 1 kg of oil. Each content of crude protein (16.25%) and metabolizable energy (2,700 kcal kg^{-1}) was adjusted to same level.

Laying performance and egg quality : Feed intake was assessed on weekly basis to calculate the average daily feed intake per hen, and daily egg production was checked at 15:00 hour every day. The eggs produced daily were weighed together each day. Three eggs, whose weight were close to the average weight, were selected from each replicate pen of each treatment group to evaluate the egg quality. The automatic egg quality tester (Robotmation Egg Multi Tester-EMT-5200, Japan) was used to evaluate egg shell breaking strength, egg shell thickness, Haugh unit and York color.

Determination of blood lipid profile : Blood samples were collected from wing veins of laying hens (5 hens per pen in each treatment group) using heparinized syringes. The blood samples were centrifuged for 15 min at 3,000 rpm to separate plasma. Triglyceride and total cholesterol content in the plasma were determined by commercial enzyme kits (Asan pharmaceutical, Seoul, Republic of Korea) (Park, 2011).

Determination of fatty acid composition : 10 eggs per replicate pen in each treatment group were boiled, and egg yolks were separated to analyze fatty acids. Fatty acid composition was assessed according to the method devised by Hwangbo *et al.* (2009), and it could be briefly described as follows; 200 ml of organic solvent mixture (chloroform: methanol=2:1) and 6 ml of 0.88% KCl were added to 5 g of egg yolk and the mixture was homogenized by agitating it with an ultra turrex at 2,500 rpm for 3 min and after the first centrifugation of lipids, this process was repeated 3 times more to extract lipid. The extracted lipid was finally concentrated by using a stream of nitrogen gas. The concentrated lipid fraction (4-5 mg) was placed in a saponification reactor and 1 ml of 0.5 N methanolic NaOH (2 g NaOH per 100 ml methanol) was added thereto. Then it was heated for 15 min and cooled. After cooling, 2 ml of BF_3-methanol, a methylation reagent, was added thereto, and the mixture was heated for 15

min again. After cooling the mixture to room temperature, 1 ml of heptane and 2 ml of NaCl saturated solution were added to it, which was then mixed for 1 min. This mixture was left for 30 min at room temperature. Supernanant (1~2 µl) was taken, and it was injected to GLC (ACEM 6000 model, Young In Scientific Co., Ltd., Korea) for analysis of fatty acids. PUFA No. 2, animal source (Supelco, USA) as a standard solution and FFAP capillary column (30 m × 0.25 mm I.D., 0.25 µm film thickness) as a column were used for fatty acid analysis. Nitrogen (1 ml min^{-1}) was used as a carrier gas. Injection port temperature, detector temperature and oven temperature were set up at 240 ºC, 250 ºC and 160 ºC, respectively. The split ratio was 10:1.

Statistical analysis : ANOVA was performed by means of the statistical software SAS using the GLM procedure (SAS, 2000). Statistical significance between the average values of the treatment groups was tested at 95% using Duncan's multiple range test ($p<0.05$).

Results and Discussion

Feed intake and changes in egg production during the study are shown in Table 1. There was no statistical significance in feed intake between the treatment groups. On the other hand, the egg production of the control group was significantly lower than that of other groups ($p<0.05$). Haugh unit, eggshell thickness, eggshell breaking strength and yolk color are shown in Table 2. Haugh unit was significantly higher in CO, HSO and EPO groups than control group ($p<0.05$). There were no statistically significant differences noted in Haugh unit among CO, HSO and EPO groups, and also control, CO and HSO groups. There were no statistically significant differences in eggshell thickness and eggshell breaking strength among the treatment groups. Yolk colors were classified into 14 grades according to its light and shade. As compared to control group, egg yolk color was significantly higher in CO, HSO and EPO groups ($p<0.05$), while there were no statistically significant differences observed among CO, HSO and EPO groups

The treatment group fed with diet containing HSO showed least changes in plasma total cholesterol (Fig. 1) and triglyceride (Fig. 2). There was a significant difference found in total cholesterol and triglyceride between control and other treatment groups ($p<0.05$). It was estimated that this resulted due to synergistic effect between n-3 fatty acid and GLA in HSO, which would have consequently reduced the cholesterol level of the eggs (not analyzed) (Leicer *et al.,* 2000; Özpinar *et al.,* 2003).

Changes in the blood fatty acid composition of laying hens are shown in Table 3. GLA was not detected in the blood samples of control group and treatment group fed with the diet containing CO. In contrast, a significantly higher level of GLA was found in the treatment group fed with the diet containing HSO and the treatment group fed with the diet containing EPO, compared with the control group ($p<0.05$). This shows that GFA, which was taken through the experimental diet, was absorbed into the blood.

Table 1: Feed intake and egg production for laying hens fed with experimental diets[1)]

Item	Control	CO	HSO (1.68% GLA)	EPO (8.03% GLA)	SEM[2)]
Feed intake (g d^{-1} hen^{-1})	121.59	121.87	121.23	121.60	2.8867
Egg production (%)	96.07[b]	97.54[a]	97.86[a]	97.75[a]	0.2501

[1)]CO: corn oil, HSO: hemp seed oil, EPO: evening primrose oil; GLA; gammo-linolenic acid; [2)]SEM : standard error of means; [a,b]Values with different superscripts within a row differ significantly ($p<0.05$)

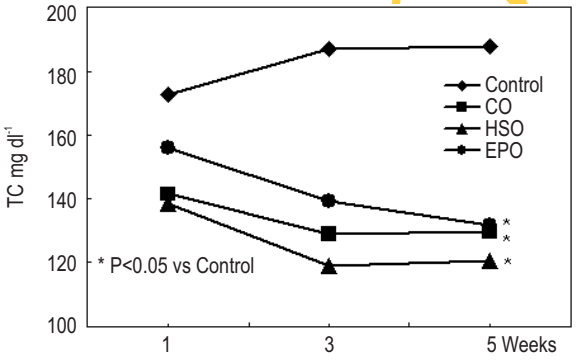

Fig. 1 : Changes in total cholesterol(TC) contents of plasma from laying hens fed with experimental diets[1)]. [1)]Refer to Table 1

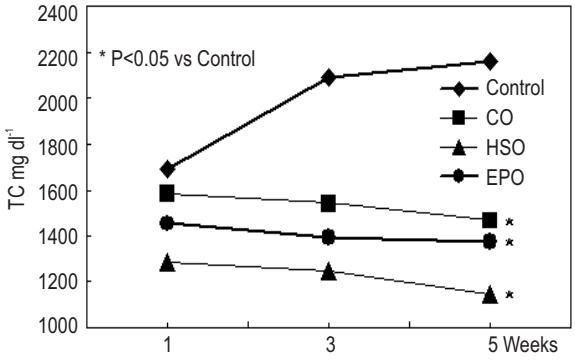

Fig. 2: Changes in triacylglyceride(TAG) contents of plasma from laying hens fed with experimental diets[1)]. [1)]Refer to Table 1

Table 2 : Changes in the egg quality for laying hens fed with experimental diets[1)]

Item	Control	CO	HSO (1.68% GLA)	EPO (8.03% GLA)	SEM[2)]
Haugh unit	97.77[b]	98.85[ab]	98.98[ab]	100.37[a]	0.4179
Egg shell thickness (mm)	0.36	0.35	0.35	0.33	0.0065
Egg shell breaking strength (kg cm^{-2})	3.55	3.70	4.25	3.65	0.1266
Egg yolk color (RCF)	7.88[b]	8.66[a]	8.44[ab]	8.66[a]	0.1305

[1)]Refer to Table 1. [2)]SEM : standard error of means; [a,b]Values with different superscripts within a row differ significantly($p<0.05$)

Table 3 : Changes in the fatty acid composition of plasma from laying hens fed with experimental diets[1)]

	(% of total fatty acids)				
Fatty acids	**Control**	**CO**	**HSO**	**EPO**	**SEM[2)]**
14:0	0.89[a]	0.33[b]	0.21[b]	0.21[b]	0.0981
16:0	25.84[a]	23.66[b]	21.08[c]	21.56[c]	0.5947
16:1n-7	4.65[a]	0.36[b]	0.26[b]	0.12[b]	0.5798
18:0	1.58[a]	0.21[b]	0.54[b]	0.33[b]	0.1795
18:1n-9	49.82[a]	41.54[c]	40.95[c]	45.36[b]	1.0880
18:2n-6	18.22[d]	31.51[a]	29.25[b]	26.35[c]	1.5265
18:3n-6	-	-	1.86[b]	4.57[a]	0.6328
18:3n-3	-	-	4.56	-	0.2886
18:1n-9	-	-	-	-	-
22:4n-6	-	-	1.29	1.50	0.1885
20:5n-3	-	-	-	-	-
22:6n-3	-	-	-	-	-
Total	100	100	100	100	-

[1)]Refer to Table 1. [2)]SEM : standard error of means; [a,b,c,d]Values with different superscripts within a row differ significantly ($p<0.05$)

Table 4 : Changes in the fatty acid composition for eggs from laying hens fed with experimental diets[1)]

	(% of total fatty acids)				
Fatty acids	**Control**	**CO**	**HSO**	**EPO**	**SEM[2)]**
14:0	1.02[a]	0.51[b]	0.41[b]	0.42[b]	0.0850
16:0	4.55[d]	24.01[a]	22.09[b]	21.44[c]	2.3625
16:1n-7	0.74[a]	0.35[b]	0.25[c]	0.32[b]	0.0578
18:0	0.88[a]	0.61[b]	0.29[d]	0.41[c]	0.0675
18:1n-9	64.26[a]	40.24[c]	38.99[d]	40.56[b]	3.1814
18:2n-6	28.55[c]	32.07[b]	32.65[a]	29.04[c]	0.5807
18:3n-6	-	-	1.09[b]	4.87[a]	0.7267
18:3n-3	-	-	3.23	-	0.0173
20:4n-6	-	2.21	-	-	0.0173
20:5n-3	-	-	1.00[b]	4.00[a]	0.6708
22:6n-3	-	-	-	-	-
Total	100	100	100	100	-

[1)]Refer to Table 1. [2)]SEM : standard error of means; [a,b,c,d]Values with different superscripts within a row differ significantly($p<0.05$)

Changes in the fatty acid compositions of eggs produced by the laying hens are shown in Table 4. GLA in the eggs had a cumulative effect as observed in the blood samples of the treatment groups fed with the diet containing HSO and the diet containing EPO. GLA retention significantly increased the eggs produced by laying hens having ingested lipids containing GLA ($p<0.05$).This means that the ingested GLA was absorbed into the blood and transmigrated to the eggs and accumulated in them. Although it is widely known that GLA is not accumulated in animals, fatty acid compositions of chicken eggs can be easily

changed by supplying the feed containing a certain amount of specific unsaturated fatty acids because monogastric animals such as chickens don't have any microorganisms in their stomach (Fruse *et al*., 1992; Özpinar *et al.,* 2003). The most significant finding of this study was that the GLA content of egg yolk could be increased by adding HSO or EPO as a GLA source to the feed. This result is supported by Fruse *et al*. (1992) who reported that fatty acid compositions can be changed if the lipid feed source for monogastric animals is changed. Gamma fatty acids are synthesized from linoleic acid; linoleic acid is first converted into GLA and then DGLA by Δ-6 desaturase, one of the rate-limiting enzymes, and is also converted into arachidonic acid by Δ5-desaturase at a speedy rate; therefore, accumulation of GLA in human or animal bodies cannot be obtained naturally (Park, 1997; Duche and Rkste, 2001; Kim *et al*., 2012) but can be obtained by GLA supply. GLA is insufficient in animals and humans due to lack of Δ6-desaturase activity and various factors suppressing Δ6-desaturase activity although GLA is normally produced, and gamma fatty acids are rapidly converted into arachidonic acid in human bodies (Park, 2008; Johnson *et al*., 2013; Sato *et al*., 2013). For this reason GLA was not accumulated in the eggs produced by the laying hens fed with diet containing CO, even though CO contained linolenic acid.

In conclusion, researchers could identify the new fact that supplementing the layer diets with hemp seed oil or evening primrose oil resulted in egg production enriched with gamma-linolenic acids.

Acknowledgments

This study was supported by Korea Institute od Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (Project No.: C1006638-01-02), 2009 and 2013 Post Doc Grant, Kangwon National University, Republic of Korea.

References

Anderassi, M., P. Foreo., A.D. Lori., S. Masci., G. Abate and P. Amerio: Efficacy of gamma linolenic acid in the treatment of patients with atopic dermatitis. *J. Int. Med. Res.,* **25,** 286-301 (1997).

Barre, D.E.: Potential of evening primrose, borage, black currant, and fungal oils in human health. *Ann. Nutr. Metab*., **45,** 47-57 (2001).

Demmelmair, H., M. Baumheuer and B. Koletzko: Investigation of long-chain polyunsaturated fatty acid metabolism in lactating women by means of stable isotope techniques. *Adv. Exp. Med. Biol.* **501,** 169-177 (2001).

Duché, N. and K.B. Rksté: Polyunsaturated n-3 fatty acids and the development of atopic disease. *Lipids*., **36,** 1033-1042 (2001).

Fan, Y.Y. and R.S. Chapkin: Importance of dietary γ-linolenic acid in human health and nutrition. *J. Nut.,* **128,** 1411-1414 (1998).

Foster, R. H., G. Hardy and R. G. Alany: Borage oil in the treatment of atopic dermatitis. *Nutrition*. **26,** 708-718 (2010).

Furuse, M., R. Okada., K. Kita., K. Asakura and J. Okumura: Effect of gamma-linolenic acid on lipid metabolism in laying hens. *Comp. Biochem. Physiol. A Comp. Physiol.,* **101,** 167-169 (1992).

Hwangbo, J., E.C. Hong, A. Jang, H.K. Kang, J.S. Oh., B.W. Kim and B.S. Park: Utilization of house fly-maggots, a feed supplement in the production of broiler chickens. *J. Environ. Biol.,* **30,** 609-614 (2009).

Johnson, M.M., D.D. Swan, M.E. Surette, J. Stegner, T. Chilton, A.N. Fontech and F.H. Chilton: Dietary supplementation with γ -linolenic acid alters fatty acid content and eicosanoid production in healthy human. *Nutr.,* **127,** 1435 (1997).

Kim, O.Y., H.H. Lim and M.J. Park: Association of fatty acid composition in serum phospholipids with metabolic syndrome and arterial stiffness. *Nut., Metabol. Cardiovas. Dise*., **23,** 366-374 (2012).

Leicer, C., D. Ribnicky and A. Pouler: The composition of hempseed oil and its potential as important source of nutrition. *J. Nutra. Func. Med. Food,* **2,** 35-54 (2000).

Menendez, J.A., L. Vellon, R. Colomer and R. Lupu: Effect of gamma-linolenic acid on the transcriptional activity of the Her-2/neu (erbB-2) oncogene. *J. Natl. Cancer Inst*., **97,** 1611-1615 (2005).

Miles, E.A., T. Banerjee and M.M. Dooper: The influence of different combinations of gamma-linolenic acid, stearidonic acid and EPA on immune function in healthy young male subjects. *Brit. J. Nutr.,* **91,** 893-903 (2004).

Miles, E.A., T. Banerjee and P.C. Calder: The influence of different combinations of gamma-linolenic, stearidonic and eicosapentaenoic acids on the fatty acid composition of blood lipids and mononuclear cells in human volunteers. *Prostagland. Leukot. Essen. Fatty Acids.,***70,** 529-538 (2004).

NRC: National Research Council, Nutrients requirements of poultry. 9th rev. National Academy Press, Washington DC. (1994).

Nykiforuk, C.L., C. Shewmaker and I. Harry: High level accumulation of gamma linolenic acid (C18:3&Dgr; 6.9,12 cis) in transgenic safflower (*Carthamus tinctorius*) seeds. *Transgenic Res.,* **21,** 367-381 (2012).

Özpinar, H., R. Kahraman, I. Abas, H.C. Kutay, H. Eseceli and M.A. Grashorn: Effect of dietary fat source on n-3 fatty acid enrichment of broiler meat. *Poult. Sci.,* **67,** 57-64 (2003).

Park, B.S. and A.V. Zammit: Effect of dietary oil containing γ-linolenic acid on the plasma lipid levels and thrombotic activity in rats. *Korean J. Nutr.,* **36,** 889-897 (2003).

Park, B.S.: Effects of pitamin on growth performance, carcass characteristics and cecal microflora of broiler chicken. *J. Environ. Biol.,* **32,** 585-590 (2011).

Park, B.S.: Effect of dietary γ-linolenic acid on plasma lipid metabolism in rats. *J. Korean Oil Chemist's Soc*., **19,** 181-188 (2002).

Park, B.S.: Evening primrose oil and hemp seed oil as an γ-linolenic acid source for broiler: Influence of fatty acid composition of chicken skin, thigh and breast muscle. *J. Korean Oil Chemist's Soc*., **24,** 196-201 (2007).

Park, B.S.: Lipids as new sources for production of animal novel foods. *J. Food Sci. Ani. Resour.,* **17,** 118-130 (1997).

Park, B.S.: Gamma fatty acid: A review. *J. Korean Oil Chem.,* **25**, 446-458 (2008).

SAS Institute, SAS® User's guide: Statistics. Version 8 edition SAS Institute Inc., Cary, NC, USA. (2000).

Sato, A., K. Dodo and M. Makishima: Synthesis and evaluation of 2,3-dinorprostaglandins: Dinor-PGD1 and 13-epi-dinor-PGD1 are peroxisome proliferator-activated receptor α/& gamma; dual agonists. *Bioorganic. Med. Chem. Letters.,* **23,** 3013-3017 (2013).

Thijs, C., A. van. Houwelingen., I. Poorterman, A. Mordant and P. van

Den Brandt: Essential fatty acids in breast milk of atopic mothers: Comparison with non-atopic mothers, and effect of borage oil supplementation. *Eur. J. Clin. Nut.* **54,** 234-238 (2000).

Tso, P., J. Caldwell and D. Lee: Comparison of growth, serum biochemistries and n-6 fatty acid metabolism in rats fed diets supplemented with high-gamma-linolenic acid safflower oil or borage oil for 90days. *Food Chem. Toxicol.,* **50,** 1911–1919 (2012).

Yasumoto, R., H. Fujita, T. Yamamoto, S. Tokura and M. Takikawa: The effectiveness, safety and usefulness of borage oil on dermatitis. *Acta. Dermatologic.,* **92,** 249-256 (1997).