Turk J Vet Anim Sci
24 (2000) 473-478

Effects of Dietary Oils on Lipoproteins, Lipid Peroxidation and Thromboxane A_2 Production in Chicks

Recep GÖKÇE**, İdris AKKUŞ*, Mustafa YÖNTEM**, Mahmut AY*, Ahmet GÜREL*, Osman ÇAĞLAYAN*, Said BODUR***, Serdar ERGUN**
Departments of Biochemistry* and Public Health***, Faculty of Medicine, University of Selçuk, Konya-TURKEY
Department of Molecular Biology**, Faculty of Science, University of Dumlupınar Kütahya-TURKEY

Received: 24.12.1999

Abstract : The effects of some commonly consumed oils on plasma lipids, lipid peroxidation and thromboxane A_2 (TxA_2) production in chicks were studied. A total of 150 1-day-old chicks were divided into 5 groups and selected as butter, margarine, sunflower oil, olive oil and corn oil groups. The oils were added to their chow as 5% (w/w) at the beginning and 7% at the end. After a period of 45 days, blood samples were obtained from the vena axillaris. Then, plasma levels of total cholesterol, HDL-C, HDL_2-C, HDL_3-C, LDL-C, thiobarbutiric acid reactive substrates (TBARS) as malondialdehyde (MDA) and TxA_2 were measured.

The results indicated that total cholesterol, HDL-C, HDL_3-C and TxA_2 levels were highest in the butter group whereas HDL_2-C was highest in the olive oil group. The lowest HDL_2-C was in the margarine group and the second lowest level was in the butter group. TBARS levels were highest in the corn oil group, lowest in the butter group and increased in the order butter<margarine< olive oil< sunflower oil< corn oil.

Our findings show that unsaturated fats added to the chow of chicks are more peroxidated in their blood than saturated ones. The effect of this peroxidation on the meat of chicks and on those who eat them is not known.

On the other hand, in spite of the fact that the metabolism of lipids, lipoproteins and TxA_2 might be somewhat different from those in humans, these findings can be regarded as a guide for humans. It may be speculated that although unsaturated fats do not contain cholesterol they constitute a risk factor for many diseases including atherosclerosis, due to their low resistance to lipid peroxidation. On the other hand, although saturated fats constitute a risk factor for atherosclerosis due to their high cholesterol and saturated fatty acids contents, they are beneficial for humans due to their high resistance to lipid peroxidation. Therefore, in evaluating the consumption of fats, these 2 points must be taken into account. In addition, olive oil is the most resistant oil to lipid peroxidation among unsaturated oils and the low level of HDL_2-C in the margarine group is interesting with respect to atherosclerosis. Also, our unexpected finding which shows that TxA_2 production is higher in animals fed saturated fats than in those fed unsaturated ones needs to be investigated.

Key Words : saturated oils, unsaturated oils, lipoproteins, lipid peroxidation, thromboxane A2

Diyetteki Yağların Piliçlerde Lipoproteinler, Lipid Peroksidasyonu ve Tromboksan A_2 Üretimi Üzerine Olan Etkilerinin Araştırılması

Özet : Bu çalışma, toplumda en çok tüketilen çeşitli yağların piliçlerde plazma lipid, lipid peroksidasyonu ve tromboksan A_2 (TxA_2) üretimi üzerine olan etkilerini karşılaştırmalı olarak araştırmak amacıyla gerçekleştirildi.. Çalışmaya toplam 150 adet bir günlük civciv alındı ve civcivler tereyağı, margarin, ayçiçek yağı, zeytinyağı ve mısırözü yağı ile beslenmek üzere 5 gruba ayrıldı. Civcivlerin yemlerine başlangıçta % 5 (w/w) sona doğru %7 oranında yağ ilave edildi ve 45 gün süre ile beslendi. Bu süre sonunda piliçlerden vena axillaris'den kan örnekleri alınarak plazmalarında total kolesterol, HDL-C, HDL_2-C, HDL_3-C, LDL-C, tiobarbitürik asit reaktif maddeleri (TBARS) ile TxA2 seviyeleri ölçüldü. TBARS, malondialdehit (MDA) şeklinde ölçüldü. Sonuçta total kolesterol, HDL-C, HDL_3-C ve TxA_2 düzeyleri tereyağı grubunda en yüksek, HDL_2-C düzeyi ise zeytinyağı grubunda en yüksekti. En düşük HDL_2-C düzeyi margarin grubunda ve ikinci olarak da tereyağı grubunda idi. TBARS düzeyleri en yüksek mısırözü yağı grubunda, en düşük tereyağı grubunda olup düşükten yükseğe doğru tereyağı, margarin, zeytin yağı, ayçiçek yağı ve mısırözü yağı şeklindeydi.

Bulgularımız, piliçlerin yemlerine ilave edilen doymamış yağların doymuş yağlara göre hayvanların kanlarında daha yüksek oranda peroksidasyona uğradığını göstermektedir. Bunun, piliçlerin etleri ve onları yiyen insanlar üzerinde nasıl bir etki oluşturduğu bilinmemektedir. Öte yandan, piliçlerle insanların lipid metabolizması ve TxA_2 sentezi biraz farklılık gösterse bile bu bulgular insanlar için bir referans olarak alındıklarında, lipid peroksidasyonuna dirençsiz olan doymamış yağların, kolesterolden fakir olsalar bile, ateroskleroz dahil birçok hastalık için risk oluşturdukları söylenebilir. Yüksek kolesterol ve doymuş yağ asidi içerdikleri için koroner kalp hastalığı bakımından risk oluşturan doymuş yağların ise lipid peroksidasyonuna dirençli olmaları canlının oldukça lehine bir durumdur. Dolayısı ile yağ tüketimi değerlendirilirken bu iki noktanın göz önünde bulundurulması gerekmektedir. Ayrıca, doymamış yağlar içinde lipid peroksidasyonuna en dirençli yağ zeytinyağı iken, margarin grubunda HDL_2-C düzeyinin düşük bulunması aterojenik özellik bakımından ilgi çekicidir. TxA_2 düzeyinin doymamış yağ grubunda doymuş yağ grubundan daha fazla bulunması beklenirken bulgularımız bunun tersini gösterdiğinden, bu bulgunun daha fazla araştırılması gerektiği kanaatindeyiz.

Anahtar Sözcükler : doymuş yağlar, doymamış yağlar, lipoproteinler, lipid peroksidasyonu, tromboksan A_2

Introduction

Saturated oils (especially butter) are known to constitute a risk factor for coronary artery disease (CAD) due to their high cholesterol and saturated fatty acid contents. Unsaturated oils are preferred in this respect. Therefore, the consumption of unsaturated oils in diet is recommended both to decrease high cholesterol intake and to increase the ratio of polyunsaturated to saturated fatty acids to prevent the development of atherosclerosis (1,2).

Although margarines do not contain cholesterol, they contain large amounts of trans-unsaturated fatty acids which occur as by-products during the saturation of them in the process of hydrogenation. The presence of trans-fatty acids in diets raises the question of their safety as food additives. Also, they are metabolized more like saturated than like the cis-unsaturated ones and may have profound significance on the molecular packing in membranes (3).

Polyunsaturated fatty acids (PUFA) are subject to free-radical reactions leading to lipid peroxidation, which is known to play a significant role in the development of cancer, aging, diabetes mellitus, atherosclerosis etc. (3-5).

It was found that the use of monounsaturated oils rather than polyunsaturated ones generates lipoprotein particles markedly resistant to transition-metal-induced oxidative reactions (6,7). Olive oil was reported to offer a good protection against lipid peroxidation because of its high content of monounsaturated fatty acid (oleic acid) and vitamin E. Oleic acid is resistant to lipid peroxidation and inhibits it perhaps by chelating free iron (8,9). Also, olive oil has been reported to decrease serum cholesterol level and provide a protective effect against the development of atherosclerosis (10).

Sunflower oil and corn oil are the most unsaturated oils among widely consumed oils. Both of them are rich in oleic, linoleic and linolenic acids. Therefore, they are easily affected by free radical reactions, which results in the formation of oxidized LDL (o-LDL). This particle has recently been shown to be another significant risk factor for atherosclerosis (11-13).

The unsaturation degree and fatty acid content of oils are also important with respect to platelet aggregation, which is also important in the development of atherosclerosis. TxA_2 is an end product of the arachidonic acid metabolism, and promotes platelet aggregation.

It is therefore apparent that dietary saturated and unsaturated oils have an important role in the development of atherosclerosis, lipid peroxidation and platelet aggregation. Thus, each has some advantages and disadvantages with respect to health. The purpose of the present study was to compare butter, margarine, olive oil, sunflower oil and corn oil in terms of their effects on serum lipids, lipid peroxidation and TxA_2 production in chicks.

Materials and Methods

The study was conducted on 150 healthy 1-day-old Avian Farms chicks. The chicks were divided into 5 groups randomly and housed in separate pathogen-free steel cages. Environmental conditions (humidity, heat, light ventilation, etc.) were kept constant for all cages for 24 hours daily during the course of the study. The light was on throughout the study for 24 hours/day, room temperature started at 30ºC and continued for the first week and gradually fell to 26ºC at the end of the study. A veterinarian experienced in animal research assisted with the details of such technical procedures.

The 5 groups were selected as butter (group 1), margarine (group 2), sunflower oil (group 3), olive oil (group 4) and corn oil (group 5) groups. All chicks were fed basic chow for the first week of the study. Basic chow consisted of 89.2% dry material, 23.44% crude protein, 5.5% cellulose, 5.95% ash, 3.17% crude fat, 0.82% methionine-cystein, 1.13 % lysine, 1.0% calcium, 0.76% phosphorus and 2830 kcal/kg of metabolic energy. Each group received the indicated oils in the percentage of 5% (w/w) for the second and third weeks, 6% for the fourth and fifth weeks and 7% for the seventh and eighth weeks. Feeding was ad libitum. Butter and margarine were slightly melted and then mixed with the chow to obtain a homogeneous mixture. The mixtures were prepared daily and unused chow in a period of 24 hours was discarded in order to prevent spontaneous peroxidation. Oils were reported to have no significant peroxide content at room temperature for 24 hours (14). The feeding continued for 45 days. At the end of that period the chicks were fasted for 16 hours and then blood samples were collected from the vena axillaris. A total of 10 ml of blood was drawn.

Of the blood samples, 6 ml was used for the determination of blood lipoproteins and lipid peroxide determinations. Serum TC, HDL-C and LDL-C levels were determined by commercially available kits. HDL_2 and HDL_3-cholesterol levels were estimated by the method of Warnick et al. (15) based on the dextran sedimentation rate. Lipid peroxidation was estimated by the method of Jain et al. (16) based on thiobarbituric acid (TBA) reactivity on the same day of blood collection without delay.

Malondialdehyde (MDA), an end product of fatty acid peroxidation, reacts with TBA to form a colored complex that has maximum absorbance at 532 nm. BHT, an antioxidant, was added to prevent MDA formation during the assay, which could result in falsely elevated TBA reactivity. The addition of BHT to standard MDA did not affect the color development with TBA. MDA values as nmol/ml were determined by using the absorbance coefficient of the MDA-TBA complex at 532 nm=$1.56X10^5$ cm^{-1} mol^{-1} (16).

The last 4 ml of the blood samples were immediately emptied into tubes containing 100 mg EDTA and 100 µg indomethacine in order to determine thromboxane A_2 (measured as thromboxane B_2). Indomethacine was added to inhibit cyclooxygenase activity (17). Thromboxane A_2 was determined by using a commercially available kit (Amersham International plc., England).

Statistical analysis were performed by the statistical package SPSS for Windows, version 6.0. TxA_2 results were compared by using the Kruskall-Wallis and Bonferroni corrected Mann-Whitney U tests. Multiple comparison of the other data was done by using the Tukey-HSD test after one-way analysis of variance (ANOVA). In these tests $p \leq 0.05$ was considered as statistically significant. The data are presented as mean±SD.

Results

The mean and standard deviations of all the parameters and comparison of the results by ANOVA are given in the Table. The highest total cholesterol, HDL-C, HDL_3 -C and TxA_2 levels were found in the butter group and the highest HDL_2-C level was in the olive oil group. Total cholesterol and HDL-C levels of the butter group were significantly higher than those of the sunflower and the corn oil groups. HDL_3-C and TxA_2 levels of the butter group were higher than those of all the other groups. The HDL_2-C level of the margarine group was lowest among all the groups whereas that group had the second highest levels of HDL_3-C and TxA_2.

Table. Comparison of lipids, lipoproteins, MDA and TxA_2 levels of chicks feds butter, margarine, olive oil, sunflower oil and corn oil.

	Cholesterol (mg/dl)		HDL-C (mg/dl)		HDL_2-C (mg/dl)		HDL_3-C (mg/dl)		LDL-C (mg/dl)		MDA (nmol/ml)		TxA_2 (pmol/ml)	
Group	n	F:4.17 p: 0.014	n	F:4.67 p: 0.001	n	F:7.33 p: 0.001	n	F:24.89 p: 0.001	n	F:1.36 p: 0.24	n	F:8.44 p: 0.001	n	F: 48.9 p:0.0001
Butter (1)	28	161±22.5	24	58±14.5	20	16.0±5.1	24	43.5±10.4	24	99±19.5	26	19.4±8.4	29	42.2±9.4
Margarine (2)	22	145±25.7	20	48±13.1	16	12.7±4.1	18	35.6±18.3™	17	91±23.4	23	21.5±9.1	23	28.9±15.1♠
Sunflower oil (3)	26	141±17.1♠	28	39±10.2♠	19	16.1±4.8	28	26.1±6.5♠♣	27	99±14.8	24	23.7±16.8	24	15.4±12.3♠♣
Olive oil (4)	28	154±15.4	28	50±15.4	28	25.2±10.6♣♠♥	28	25.0±4.9♠♣	28	101±17.2	28	23.0±10.0	23	16.0±12.0♠♣
Corn oil (5)	27	141±18.7♠	28	45.6±13.9♠	24	23.3±12.8♣	26	25.2±6.5♠♣	25	90±19.7	27	32.6±14.6♠♣♦	25	22.4±15.2♠

♠: Significantly different from Group 1 ♣: Significantly different from Group 2 ♥: Significantly different from Group 3 ♦: Significantly different from Group 4

TBARS were highest in the corn oil group and lowest in the butter group. TBARS levels of the groups were increased in the order of butter, margarine, olive oil, sunflower oil and corn oil. The differences between TBARS levels of corn oil and those of the other groups, except that of sunflower oil group, were statistically significant. There was no significant difference between LDL-cholesterol levels of the groups.

Discussion

Our results show that unsaturated fats in the chow of chicks result in a higher level of lipid peroxidation in their blood than saturated ones (18,19). These results are consistent with the findings of Salvati et al. (20), who showed that oils with high unsaturation level induce oxidative stress. However, our study is the first to investigate the effect of commonly consumed oils on many parameters in chicks. In particular, margarine is important in this respect. Although the similarities and differences between the lipoprotein metabolism of chicks and humans are not well known, chicks are widely used for lipid research (28-34) because the changes in the lipoprotein metabolism can be easily shown in them in a short time. Moreover, most of the triacylglycerol in chicken plasma is present in the VLDL fraction and it carries triacylglycerol to tissues as in humans (34). Therefore, our findings can be regarded, at least, as a guide for humans.

Increased lipid peroxidation is a significant risk factor for many diseases and results in increased synthesis of oxidized LDL (o-LDL) and oxidized cholesterol which have recently been shown to be other significant risk factors for CAD (21-23). Hennig et al. (23) showed that lipid hydroperoxide levels were highest in oxidized LDL derived from rabbits fed corn oil. This result supports our findings.

In the present study, among the vegetable oils studied, olive oil seems to be the least oxidized one. Visioli et al. (27) found that natural antioxidants present in olive oil prevent the oxidation of low density lipoprotein. Also, some investigators found that the lowest serum total cholesterol and LDL cholesterol levels were in humans consuming more olive oil than other oils (24-26).

As seen from the Table, although the serum cholesterol level of the chicks fed butter was highest, their HDL-C level was also highest and HDL_2-C level was lowest. It is known that the protective fraction of HDL-C against atherosclerosis is HDL_2-C. This finding shows that subfractions of HDL-C are more informative in evaluating the risk of atherosclerosis.

The lowest level of HDL_2-C found in the margarine group is a new finding which draws attention to the atherosclerotic effect of this oil and needs to be investigated further. Our study is the first in the literature to measure lipid peroxidation, blood lipids and TxA_2 in animals fed margarine. Since margarine contains large amount of trans-fatty acids (3), its effects on lipid peroxidation and blood lipids are important in evaluating the risk for many diseases.

We also studied TxA_2 (as TxB_2) in order to show the thrombogenic effect of the above oils. As seen from the Table, TxA_2 levels were found to be higher in the saturated oil groups than in unsaturated ones. This is a surprising result because TxA_2 is an end product of arachidonic acid, which is mainly present in unsaturated oils or can be more easily produced from them. Also, it is known that TxA_2 is produced after activation of platelets, which may occur during blood collection. However, this effect is common to all the oils studied. Therefore, that effect might be the direct result of the effects of oils on platelets themselves. Therefore, we concluded that saturated oils might activate platelets more than unsaturated ones. This finding also confirms the atherogenic effects of saturated oils in another way and needs to be investigated further.

Unsaturated oils are recommended to be consumed due to their low cholesterol content to reduce the risk of atherosclerosis. However, as seen from our results, they are not very safe in this respect, with the exception of olive oil, due to their very low resistance to lipid peroxidation, which is a significant risk factor for many diseases, including atherosclerosis.

Our findings confirmed on a large animal population that saturated oils are more resistant to lipid peroxidation than unsaturated ones, except olive oil. This is a significant risk factor for many diseases including atherosclerosis because of the formation of o-LDL. Also, our lipoprotein findings, especially that of HDL-C and HDL_2-C found in the butter and margarine groups and the conflicting results of TxA_2, need to be investigated further on a large animal population or in humans if possible.

References

1. Singman, H.S., Berman, S.N., Cowell, C., Maslansky, E., Archer, M. The anti-coronary club. Am. J. Clin. Nutr. 1980; 33:1183-1191.
2. Cutler, R.G. Antioxidants and aging. Am. J. Clin. Nutr. 1991; 56: 373-379.
3. Mayes, P. A. Lipids of phyiological significance and metabolism of unsaturated fatty acids and eicosanoids, In: Harper's Biochemistry, 22nd edition Librarie du Liban, Beirut 1995; pp:137,221.
4. Esterbauer, H., Wag, G., Pulh, H. Lipid peroxidation and its role in atherosclerosis. British Medical Bulletin. 1993; 49: 566-576.
5. Halliwell, B. Free radicals, antioxidants and human disease: curiosity, cause or consequence? Lancet. 1994; 344: 721-724.
6. Reaven, P., Parthasarathy, S., Grasse, B.J., et al. Feasibility of using an oleate-rich diet to reduce the susceptibility of low-density lipoprotein to oxidative modification in humans. Am. J. Clin. Nutr. 1991; 54: 701-706.
7. Balasubramanian, K.A., Nalini, S., Cheeseman, K,H., Slater, T.F. Nonesterified fatty acids inhibit iron-dependent lipid peroxidation. Biochim. Biophys. Acta. 1989; 1003: 232-237.
8. Simpson, R.J., Peters, T.J. Iron binding lipids of rabbit duodenal brush-border membrane. Biochim. Biophys. Acta. 1987; 898: 181-186.
9. Simpson, R.J., Moore, R., Peters, T.J. Significance of nonesterified fatty acids in iron uptake by intestinal brush-border membrane vesicles. Biochim. Biophys. Acta. 1988; 94: 39-47.
10. The Expert Panel. Report of the National Cholesterol Education Program Expert Panel on detection, evaluation and treatment of high blood cholesterol in adults. Arch. Intern. Med. 1988; 148: 36-39.
11. Jayakumari, N., Ambikakumari, V., Balakrishnan, K.G., et al. Antioxidant status in relation to free radical production during stable and unstable anginal syndromes. Atherosclerosis. 1992; 94: 183-190.
12. Kumar, S., Menon, V.P. Changes in levels of lipid peroxides and activity of superoxide dismutases and catalase in diabetes associated with myocardial infarction. Indian. J. Experim. Biol. 1992; 30: 122-127.
13. Stringer, M.D., Görög, H.G., Freeman, A., et al. Lipid peroxides and atherosclerosis. Br. Med. J. 1992; 34: 497-500.
14. Klaus, A.M., Fuhrmann, H., Sallmann, H.P. Peroxidation and antioxidative metabolism of the broiler chicken as influenced by dietary linoleic acid and vitamin E. Arch. Geflügelk. 1995; 59, (2): 135-144.
15. Warnick, G.R., Benderson, J.M., Alber, J.J. Quantitation of high-density lipoprotein subclasses after separation by dextran sulfate and Mg+2 precipitation. Clin. Chem. 1982; 28: 1574 (a).
16. Jain, S.K., McVie, R., Duett, J., et al. Erythrocyte membrane lipid peroxidation and glycosylated hemoglobin in diabetes. Diabetes. 1989; 20: 95-111.
17. Fuhrmann, H., Sallmann, H.P. The influence of dietary fatty acids and vitamin E on plasma prostanoids and liver microsomal alkane production in broiler chickens with regard to nutritional encephalomalacia. J. Nutr. Sci. Vitaminol. 1995; 41: 553-563.
18. Ansari, K.A., Kaplan, E., Shoeman, D. Age-related changes in lipid peroxidation and protective enzymes in the central nervous system. Growth Development and Aging. 1989; 153: 117-121.
19. Wolff, S.P. Diabetes mellitus and free radicals. Br. Med. Bull. 1993; 49: 642-652.
20. Salvati, S., Campeggi, L.M., Benedetti, P.C., et al. Effects of dietary oils on fatty acid composition and lipid peroxidation of brain membranes (myelin and synaptosomes) in rats. J. Nutr. Biochem. 1993; 4 (June): 346-350.
21. Halliwell, B. Free radicals and vascular disease: how much do we know? British. Med. J. 1993; 307: 885-886.
22. Jayakumari, N., Ambikakumari, V., Balakrishnan, K.G., et al. Antioxidants status in relation to free radical production during stable and unstable anginal syndromes. Atherosclerosis. 1992; 94: 183-190.
23. Hennig, B., Toborek, M., Boissonneault, G.A., Shantha, N.C., Decker, E.A., Oeltgen, P.R. Animal and plant fats selectively modulate oxidizability of rabbit LDL and LDL-mediated disruption of endothelial barrier function. J. Nutr. 1995; 125: 2045-54.
24. Nardini, M., Scaccine, Cristina., D'Aquino, M., et al. Lipid peroxidation in liver microsomes of rats fed soybean, olive, and coconut oil. J. Nutr. Biochem. 1993; 4 (January): 39-44.
25. Odeleye, O.E., Watson, R.R., Eskolsen, C.D., Mufti, S.I. Dietary polyunsaturated fatty acid promote peroxidation and possible role in the promotion of cancer. Biological Reactive Intermadiates IV, Edited by C.M. Witmer et al. Plenum Press, New York, 1990.
26. Mahley, R.W., Palaoglu, K.E., Atak, Z., Dawson, Pepin. J., et al. Turkish Heart Study: Lipids, lipoproteins, and apolipoprotein. J. Lipid Research. 1995; 36: 839-859.
27. Visioli, F., Belloma, G., Montedoro, G., Galli, C. Low density lipoprotein oxidation is inhibited in vitro by olive oil constituents. Atherosclerosis. 1995; 117: 25-32.
28. Stein, E.A., Myers, G.L. Lipids, Lipoproteins, and Apolipoproteins. In: Burtis CA., Ashwood ER. eds. Tietz textbook of Clinical Chemistry. Second Edition. Philadelphia: W.B. Saunders. 1994; pp: 1028-1030.
29. Talavera, E.M., Zafra, M.F., Gil, Villarino. A., Perez, M.I., Alvarez Pez, J.M., Garcia, Peregrin. E. Changes in chemical composition and physico-chemical properties of chick low- and high-density lipoproteins induced by supplementation of coconut oil to the diet. Biochimie. 1997; 79: 333-40.
30. Fuhrmann, H., Sallmann, H.P. The influence of dietary fatty acids and vitamin E on plasma prostanoids and liver microsomal alkane production in broiler chickens with regard to nutritional encephalomalacia. J. Nutr. Sci. Vitaminol. 1995; 41: 53-61.

31. Sheehy, P.J., Morrissey, P.A., Flynn, A. Consumption of thermally-oxidized sunflower oil by chicks reduces alpha-tocopherol status and increases susceptibility of tissues to lipid oxidation. Br. J. Nutr. 1994; 71: 53-65.

32. Sheehy, P.J., Morrissey, P.A., Flynn, A. Influence of heated vegetable oils and alpha-tocopheryl acetate supplementation on alpha-tocopherol, fatty acids and lipid peroxidation in chicken muscle. Br. Poult. Sci. 1993; 34: 367-81.

33. Allen, P.C., Wong, H.Y. Effect of atherogenic diet on chicken plasma lipids and lipoproteins Poult. Sci. 1992; 72: 1673-8.

34. Phetteplace, H.W., Watkins, B.A. Effects of various n-3 lipid sources on fatty acid compositions in chicken tissues. J. Food. Comp. Anal. 1989; 2: 104-17.