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BLOOD LIPID COMPONENTS IN BROILER CHICKENS FED ON DIETS CONTAINING LIPIDS FROM DIFFERENT SOURCES

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ABSTRACT

In a completely randomized design, 240 male broilers (Ross 308) were divided into five treatment groups with four replicates per each. The impact of lipid addition to diet from different sources on serum lipid profile was measured at days 28 and 42 of age. Chicks were assigned to receive control with no lipid supplementation or one source of lipid either fish oil or corn oil or olive oil or tallow in their grower and finisher diet at 3 and 4% dry matter, respectively. At day 28 of age, there were significant differences among treatments for triglyceride content, with the highest level in chicks fed diet containing starch as control and the lowest level for those fed diet containing corn or olive oils. There were no significant differences among treatments for serum cholesterol and high-density lipoprotein cholesterol (HDL) contents of broiler chicks fed different lipid sources. Broilers fed diet containing corn oil had higher low-density lipoprotein cholesterol (LDL) and lesser very low-density lipoprotein cholesterol (VLDL) as compared to the other groups. The lower LDL was for chicks fed diet containing fish oil and tallow at day 42 of age. The results showed that chicks fed on diets supplemented with fish oil or olive oil had significant decreases in serum triglyceride levels, whereas control groups had significantly higher in triglyceride levels than in the other groups. In this period like day 28 of age, there were no significant differences among treatments for serum cholesterol, HDL and LDL contents of broiler chicks fed different lipid sources. Fed on diets containing fish oil and corn oil caused a significant decreased in VLDL, while fed on control diet caused a significant increased as compared to other groups. Feeding diets containing different lipid sources (fish, corn, olive oils and tallow) has no effect on serum total cholesterol and HDL levels of chickens, but serum levels of triglyceride, LDL and VLDL seems to be related with dietary lipid sources. Fish oil decreased serum triglyceride and LDL and compare to other lipids was better, after that olive oil has beneficial effects and tallow had the worse effects on serum lipid profiles of chicks.

Keywords: *Different Lipid Source, Serum, Lipid Profile, Chicks*

INTRODUCTION

Supplementation of lipids from plant or animal sources in commercial broiler diets, as an economic means of producing energy-rich formulations, has become essential to achieve recommended energy concentrations and essential fatty acids (Newman *et al.*, 2002). In the literature, there is an extent studies concerning the impact of type and quantity of oil to increase the efficiency of performance, feed utilization, carcass quality and meat quality of chickens; however, limited studies exist showing the effect of different lipid source on lipid components of blood. Some of these oil sources are rich in elements such as long chain polyunsaturated fatty acid that can change the proportion of the lipid constituents of the blood in human and animals. It is possible to control fatty acid profile in blood and meat of birds as a result of transferring certain components from the diet. The results in the literature regarding the effect of dietary fatty acids intake on plasma cholesterol concentrations are contradictory. Hollands *et al.*, (1980) and Mori *et al.*, (1999) verified that polyunsaturated fatty acids of dietetic oils decrease both the meat and the plasma cholesterol concentrations. On the other hand, Bartov *et al.*, (1971) and Washburn and Nix (1974) did not observe such effect. The composition of fatty acids in the broiler meat can be changed by including vegetable oil, fish oil or animal fat to diet, because a difference of fatty acids profile and a

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reduction in endogenous produced fatty acids occurs. Poly unsaturated fatty acids (PUFA), in place of Saturated fatty acids (SFA) or carbohydrates, has been shown to lower the plasma low-density lipoprotein cholesterol (LDL) concentration (Kris-Etherton and Yu, 1997). A study was conducted on dietary olive oil and reported that olive oil was associated with significantly raised plasma concentration of LDL, high-density lipoprotein cholesterol (HDL). Another study (Rueda-Clausen *et al.*, 2007) reported that dietary intervention with olive oil in comparison with alternative vegetable oils increased triacylglycerols. Results from different studies on the effect of olive oil consumption on lipid profile are inconsistent. Therefore, this study was conducted to examine the effect of the different dietary source of fat on the triglycerides, total cholesterol, LDL, HDL/VLDL in broiler chickens.

MATERIALS AND METHODS

Animal and diets: Two hundred and forty one-day-old male broiler chicks (Ross 308) were prepared from a commercial hatchery and used in a 42 days feeding trial. In a completely randomized design, chicks were divided into 5 treatments (control and four lipid sources) with 4 replicates and 12 chicks per each.

Table 1: Ingredients and nutrient compositions of experimental diets in grower (day 11 to day 28)

Ingredients (%)	Control	Fish oil	Corn oil	Olive oil	Tallow
Corn	51.70	55.44	55.44	55.44	55.44
Soybean Meal(46% CP)	27.40	31.70	31.70	31.70	31.70
Starch	8.70	0.00	0.00	0.00	0.74
Fish oil	0.00	3.00	0.00	0.00	0.00
Corn oil	0.00	0.00	3.00	0.00	0.00
Olive oil	0.00	0.00	0.00	3.00	0.00
Tallow	0.00	0.00	0.00	0.00	3.00
Corn Gluten	8.00	4.00	4.00	4.00	4.00
Calcium Carbonate	1.10	1.10	1.10	1.10	1.10
Dicalcium Phosphate	1.75	1.75	1.75	1.75	1.75
Sodium Chloride	0.33	0.33	0.33	0.33	0.33
DL-methionine	0.28	0.28	0.28	0.28	0.28
Vitamin and Mineral Premix ¹	0.50	0.50	0.50	0.50	0.50
L-Lysine	0.25	0.25	0.25	0.25	0.25
Zeolite	0.00	1.60	1.60	1.60	0.90
Nutrient Compositions (%)					
AME (kcal/kg)	3,050	3,050	3,050	3,050	3,050
Crude protein	21.01	21.00	21.00	21.00	21.00
Crude Fat	3.91	7.18	7.18	7.18	7.18
Lysine	1.15	1.24	1.24	1.24	1.24
Methionine	0.62	0.60	0.60	0.60	0.60
Methionine plus Cystine	0.98	0.95	0.95	0.95	0.95
Fatty acids (%)					
C18:1 n-9	1.03	1.99	1.99	3.32	2.14
C18:2 n-6	2.05	2.25	3.81	2.46	2.31
C18:3 n-3	0.09	0.15	0.13	0.12	0.13
(n-6)/(n-3)	22.77	15.00	29.30	20.05	17.76

1- premix supplying composition was as follows (amounts in 10 g): 0.5 g Mg, 0.3 g S, 1.0 g Na, 1.6 g Cl, 6.0 mg Cu, 0.2 mg I, 45.0 mg Fe, 59.0 mg Mn, 0.2 mg Se, 29.0 mg Zn, 4000 IU Vitamin A Palmitate, 1000 IU Cholecalciferol, 50 IU Vitamin E acetate, 0.5 mg Menadione sodium bisulfite, 0.2 mg biotin, 10 µg cyanocobalamin, 2 mg folic acid, 30 mg nicotinic acid, 16 mg calcium pantothenate, 7 mg pyridoxine - Hcl, 6.0 mg riboflavin, and 6.0 mg thiamin Hcl.

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Treatments included of free lipid supplemented diet as control group and 4 different levels of dietary lipids comprised of fish oil as a n-3 fatty acid source, corn oil as a n-6 fatty acid source, olive oil as a n-9 fatty acid source and tallow as a saturated fatty acid which were added to diets as 1.5, 3 and 4% in the starter, grower and finisher, respectively. Control diet had no supplemental dietary lipid and energy content was supplied by including pure starch (Tables 1, and 2). Throughout the study, chicks accessed to feed and water ad libitum. Lighting schedule was 23 h light and 1 h dark while the temperature was gradually reduced 3 °C from initially 32 °C in each week.

Blood sampling: At days 28 and 42 of age, 3 ml of blood sample was collected from wing vein of two birds in each replicate (8 birds per each treatment), centrifuged at 1500 × g for 10 min and the serum was separated, then stored at -20°C until analysis. The obtained sera were used also for spectrophotometric analysis of serum triacylglycerol, total cholesterol and HDL, LDL by using of enzymatic method of spin react kits according to the methods of Buccolo *et al.*, (1973), Zak *et al.*, (1954), Naito (1984) and Okata *et al.*, (1998), respectively. Very low density lipoprotein cholesterol (VLDL) was calculated by division of TAG by 5 (mg/dl) while the LDL was calculated (mg/dl) by subtracting the sum of HDL and VLDL from total cholesterol.

Table 2: Ingredients and nutrient composition of experimental diets in finisher (day 29 to day 42)

Ingredients (%)	Control	Fish oil	Corn oil	Olive oil	Tallow
Corn	56.50	59.90	59.90	59.90	59.90
Soybean Meal(46% CP)	19.00	29.20	29.20	29.20	29.20
Starch	9.86	0.00	0.00	0.00	1.00
Fish oil	0.00	4.00	0.00	0.00	0.00
Corn oil	0.00	0.00	4.00	0.00	0.00
Olive oil	0.00	0.00	0.00	4.00	0.00
Tallow	0.00	0.00	0.00	0.00	4.00
Corn Gluten	10.60	1.90	1.90	1.90	1.90
Calcium Carbonate	1.03	1.04	1.04	1.04	1.04
Dicalcium Phosphate	1.70	1.60	1.60	1.60	1.60
Sodium Chloride	0.35	0.33	0.33	0.33	0.33
DL-methionine	0.19	0.26	0.26	0.26	0.26
Vitamin and Mineral Premix ¹	0.50	0.50	0.50	0.50	0.50
L-Lysine	0.41	0.15	0.15	0.15	0.15
Zeolite	0.00	1.10	1.10	1.10	0.12
Nutrient Compositions (%)					
AME (kcal/kg)	3,150	3,150	3,150	3,150	3,150
Crude protein	19.00	19.00	19.00	19.00	19.00
Crude Fat	3.94	8.30	8.30	8.30	8.30
Lysine	1.09	1.09	1.09	1.09	1.09
Methionine	0.53	0.55	0.55	0.55	0.55
Methionine plus Cystine	0.86	0.86	0.86	0.86	0.86
Fatty acids (%)					
C18:1 n-9	1.05	2.34	2.33	4.11	2.53
C18:2 n-6	2.09	2.34	4.41	2.63	2.41
C18:3 n-3	0.07	0.16	0.13	0.12	0.13
(n-6)/(n-3)	29.85	14.62	33.92	21.91	18.53

1- premix supplying composition was as follows (amounts in 10 g): 0.5 g Mg, 0.3 g S, 1.0 g Na, 1.6 g Cl, 6.0 mg Cu, 0.2 mg I, 45.0 mg Fe, 59.0 mg Mn, 0.2 mg Se, 29.0 mg Zn, 4000 IU Vitamin A Palmitate, 1000 IU Cholecalciferol, 50 IU Vitamin E acetate, 0.5 mg Menadione sodium bisulfite, 0.2 mg biotin, 10 µg cyanocobalamin, 2 mg folic acid, 30 mg nicotinic acid, 16 mg calcium pantothenate, 7 mg pyridoxine - Hcl, 6.0 mg riboflavin, and 6.0 mg thiamin Hcl.

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Statistical Analysis: All values were analyzed by one-way ANOVA using the GLM procedure of SAS for Windows version 9.1 (SAS Institute Inc., Cary, NC). When the F-test for treatments was significant at $P \leq 0.05$ in the ANOVA table, means were compared for significant differences using the Tukey test of SAS.

RESULTS AND DISCUSSION

Results presented in Table 3 showed the effect of different lipid sources in diets on serum triglyceride, cholesterol, HDL, LDL and VLDL levels of broilers at day 28 of age. There were significant differences among treatments for triglyceride content, with the highest level in chicks fed diet containing control and the lowest level for those fed diet containing corn or olive oils. There were no significant differences among treatments for serum cholesterol and HDL contents of broiler chicks fed different lipid sources. Broilers fed diet containing corn oil had higher LDL and lower VLDL as compared to the other groups. The lower LDL was for chicks fed diet containing fish oil and tallow.

The results of blood factors in this study agreed with the results of Newman *et al.*, (2002). Many evidence exist (Celebi and Utlu, 2006; Harris, 1989; Sanz *et al.*, 2000) that the triglyceride content of serum reduced as the levels of dietary PUFA increased. Legrand *et al.*, (1987) in broilers and Lochsen *et al.*, (1997) in rats demonstrated that unsaturated fatty acids may inhibit the activity of Δ^9 -desaturase enzyme that resulted in decreases of release of VLDL and triglycerides from the liver to the blood stream. In another experiment on broilers, it was shown that the increase in the proportion of unsaturated fatty acids in blood may be increase beta-oxidation rate that resulted in increase of uptake of fatty acids from blood to the tissues (Sanz *et al.*, 2000). In experiments on rats and humans, the researchers concluded that diets containing different amounts of PUFAs reduce triglycerides, serum cholesterol and LDL, while they increased blood HDL (Sunitha *et al.*, 1997; Oritz-Munoz *et al.*, 2009).

In agreement with the results to our findings, Alparsan *et al.*, (2005) showed that the level of serum cholesterol was not affected noticeably by dietary rich fish oil. The discrepancies between studies on the lipid content of serum maybe attributed to the genetic, sex and dietary factors. In general, the best result for cholesterol values at day 28 of age was seen in the groups that contained fish oil. In agreement to our finding, Fan *et al.*, (1995) reported that diets with different fat sources had no effect on blood cholesterol levels.

Table 3: The effect of different lipid sources in diets on serum lipid profile of broilers at day 28 of age

Treatment	Triglyceride mg/dl	Cholesterol mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl
Control	62.4 ^a	169	97.7	59.2 ^{ab}	12.4 ^a
Fish oil	18.8 ^b	143	83.7	46.2 ^b	6.9 ^{ab}
Corn oil	34.8 ^{ab}	187	106	72.2 ^a	3.7 ^b
Olive oil	19.6 ^b	171	100	54.0 ^{ab}	3.9 ^b
Tallow	36.0 ^{ab}	145	86	45.8 ^b	7.2 ^{ab}
SEM	15.61	20.3	12.5	9.33	3.12

^{ab}Within columns, values with different superscripts differ significantly ($P \leq 0.05$).

The effects of dietary lipid from different sources on serum triglyceride, cholesterol, HDL, LDL and VLDL levels of broilers at day 42 of age are shown in Table 4. The results showed that chicks fed on diets supplemented with fish oil or olive oil had significant decreases in serum triglyceride levels, whereas control groups had significantly higher in triglyceride levels than in the other groups. In this period like day 28 of age, there were no significant differences among treatments for serum cholesterol, HDL and LDL contents of broiler chicks fed different lipid sources. Fed on diets containing fish oil and corn oil caused a significant decreased in VLDL, while fed on control diet caused a significant increased as compared to other groups.

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Our results with regard to addition of fish oil to the diet agreed with previous studies founded that fish oil reduced serum cholesterol and lipoproteins in different age of one stomach animals fed on hypercholesterolemic diets (Choi *et al.*, 1993) and it improves serum lipid profile (Ramadan *et al.*, 2008). These results may be possibly explains on the basis that fishoil is rich in n-3 PUFAs. Polyunsaturated fats stimulate the catabolic rate of LDL, thus resulting in the reduction of serum LDL (Choi *et al.*, 1993).

Concerning addition of olive oil to the diet, our finding are similar to some extent with previous reports showing that chicks or rats fed a olive oil-rich diet had lower values of serum triglyceride (Asadi *et al.*, 2008) and significantly higher lever of serum HDL (Shad *et al.*, 2002). Feeding rats on diet containing olive oil significantly improved lipid profile as it reduced serum triglyceride, cholesterol and lipoproteins (Choi *et al.*, 1993; Oluba *et al.*, 2008) and decreased serum HDL concentrations compared with coconut oil (Scholtz *et al.*, 2004), these results agreed with our findings.

Improvements in lipid profile in mice fed on the diet containing olive oil may be explains on the basis that olive oil is a rich source of MUSFA that improves lipid profile. The primary mono unsaturated fatty acids (MUFAs) in the diet are oleic (C18:1, n-9) and palmitoleic (C16:1, n-9) acids. Olive oil is excellent source of oleic acid. Previous studies demonstrated that olive oils containing a large fraction of MUFAs and a substantial amount of PUFAs promote a better triacylglycerol clearance from the blood (Beynen *et al.*, 1987). In additionally, a diet with olive oil is a good source of monounsaturated fatty reduced serum triglyceride, LDL concentrations with respect to diets rich in SFAs (Hayes *et al.*, 1994). Healthy heart effectsfrom olive oil are attributed to its higher contents of monounsaturated fats and its higher ingredients of antioxidants (including: chlorophyll, carotenoids and the polyphenolic compounds: tyrosol, hydrotyrosol and oleuropein), all of these compounds have free radical scavenging ability and protect vitamin E found in olive oil (Morello *et al.*, 2007; Puela *et al.*, 2004). Diet rich in olive oil, has much more favorable effects on blood lipid profile and plasma lipoproteins compared with coconutoil (Mrroueh *et al.*, 2009). With regard to the effect of sunflower oil, our findings agreed with Kris-Etheton and Yu (1997) who reported that HDL production was greater in young rats fed on safflower than in those fed on palm oil. These results may be related to the type of fatty acids in sunflower oil, which is rich in PUFAs. Polyunsaturated fatty acids are effective in lowering serum cholesterol.

Concerning, the effect of tallow on serum lipid profile, our findings agreed with previous reported showing that, tallow produced a significant rise in serum cholesterol and VLDL (Wardlaw and Snook, 1990) and had no effect on other lipid components. These results may be explained on basis that the high SFAs and low PUFAs contents in tallow, which is an important contributing factor to raising serum cholesterol level.

Table 4: The effect of different lipid sources in diets on serum lipid profile of broilers at day 42 of age

Treatment	Triglyceride mg/dl	Cholesterol mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl
Control	40.0 ^{ab}	115	66.5	36.3	8.0 ^{ab}
Fish oil	22.4 ^b	102	68.9	41.5	4.5 ^b
Corn oil	26.0 ^{ab}	118	69.1	38.2	4.7 ^b
Olive oil	23.7 ^b	123	76.8	36.6	5.2 ^{ab}
Tallow	52.4 ^a	110	59.5	28.5	10.4 ^a
SEM	9.83	11.2	7.42	3.53	1.86

^{ab}Within columns, values with different superscripts differ significantly ($P \leq 0.05$).

It is often underappreciated that, although food sources including dietary oils, may be rich in one type of fatty acid; they are not 100% SFA, MUFA, or PUFA. Thus, it is important to consider the effects of specific food choices, particularly with regard to the effects of substitution of one food or food component for another.

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Conclusion

Feeding diets containing different lipid sources (fish, corn, oliveoils and tallow) has no effect on serum total cholesterol and HDL levels of chickens, but serum levels of triglyceride, LDL and VLDL seems to be related with lipid sources in diet. Fish oil decreased triglyceride and LDL and compare to other lipids was better, after that olive oil has beneficial effects and tallow had the worse effects on lipid profiles of chicks.

REFERENCES

- Alparslan G and Ozdogan M (2006).** The effects of diet containing fish oil on some blood parameters and the performance values of broilers and cost efficiency. *International Journal of Poultry Science* **5**(1) 415-419.
- Asadi F, Shahriari A, Pourkabir M and Maclaren R (2008).** Short and long-term effects of corn oil on serum lipid and lipoprotein and visceral abdominal in rats. *Journal of Food Lipids* **15**(1) 68-80.
- Bartov I, Bornstein S and Budowski P (1971).** Variability of cholesterol concentration in plasma and egg yolk of hens and evaluation of the effects of some dietary oils. *Poultry Science* **50**(6) 1357-1364.
- Beynen AC, Visser JJ, Schouten JA and Katan MB (1987).** Cholesterol metabolism in rabbits fed diets containing either corn oil or olive oil. *Nutrition and Reproduction International* **35**(1) 111-116.
- Buccolo G (1973).** Quantitative determination of serum triglycerides by use of enzymes. *Clinical Chemistry* **19**(5) 476-482.
- Celebi S and Utlu N (2006).** Influence of animal and vegetable oil in layer diets on performance and serum lipid profile. *International Journal of Poultry Science* **5**(1)370-373.
- Choi Y, Ahn C, Rhee H, Choe M, Kim C and Kim J (1993).** Comparative effects of dietary palm oil, perilla oil and soybean oil on lipid profile in different aged rats on hypercholesterolemic diets. *Bioscience, Biotechnology and Biochemistry* **57**(1) 65-68.
- Fan Q, Feng J, Wu S, Specht K and She S (1995).** Nutritional evaluation of rice bran oil and a blend with corn oil. *Nahrung* **39**(1) 490-496.
- Harris WS, Park Y and Isley WL (2003).** Cardiovascular disease and long-chain omega-3 fatty acids. *Current Opinion in Lipidology* **14**(1) 9–14.
- Hayes KC, Pronezuk A, Lindsey S and Diersen-Schade D (1994).** Dietary saturated fatty acids (12:0, 14:0, 16:0) differ in their impact on plasma cholesterol and lipoproteins in non-human primates. *American Journal of Clinical Nutrition* **53**(3) 491-498.
- Holland KG, Grunder AA and Williams CL (1980).** Response to five generations of selection for blood cholesterol levels in white Leghorns. *Poultry Science* **59**(3) 1316-1323.
- Kris-Etherton PM and Yu S (1997).** Individual fatty acid, effect on plasma lipids and lipoproteins: Human studies. *American Journal of Clinical Nutrition* **65**(10) 16255-16265.
- Legrand P, Mallard J, Bernard-Griffiths MA, Douaire M and Lemrchal P (1987).** Hepatic lipogenesis in genetically lean and fat chickens: In vitro studies. *Comparative Biochemistry and Physiology* **87**(3) 789–792.
- Lochsen T, Ormstad H, Braud H, Brodal B, Christiansen EN and Osmundsen H (1997).** Effects of fish oil and n-3 fatty acids on the regulation of Δ^9 fatty acid desaturase mRNA and activity in rat liver. *Journal of Nutrition Biochemistry* **8**(1) 408–413.
- Mori AV, Mendonça CX and Santos COF (1999).** Effect of dietary lipid lowering drugs upon plasma lipids and egg-yolk cholesterol levels of laying hens. *Journal of Agriculture and Food Science* **47**(11) 4731-4735.
- Morello JR, Motilva MJ, Tovar MJ and Romero MP (2007).** Changes in commercial virgin olive oil during storage, with special emphasis on the phenolic fraction. *Journal of Agriculture and Food Chemistry* **85**(2) 357-364.
- Mroueh M, Sakr J and Daher C (2009).** The effects of oil enriched diet on plasma lipid profile, glucose and coagulation time in rats. PAN Arab. *Medical Journal* **1**(4) 25-31.

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Naito HK (1984). HDL Cholesterol. Kaplan A. Clinal Chemistry. The C.V mosby Co. St Louis. Toronto. Prineeton 437 and 1207-1213.

Newman RE, Bryden WL, Fleck E, Ashes JR, Buttemer WA, Storlien LH and Downing JA (2002). Dietary n-3 and n-6 fatty acids alter avian metabolism: metabolism and abdominal fatdeposition. *British Journal of Nutrition* **88**(1) 11–18.

Oluba PE, Osim EE, Ojieh GC, AboluwayeGC and Eidangbe GO (2008). Comparative effect of soybean oil and palm oil on serum lipids and some serum enzymes in cholesterol-fed rats. *European Journal of Science and Research* **32**(3) 559-566.

Ortiz-Munoz G, Houard X, Martin-Ventura JL, Ishida BY, Loyau S, Rossignol P, Moreno JA, Kane JP, Chalkley RJ and Burlingame AL (2009). HDL antielastase activity prevents smooth muscle cell anoikis, a potential new antiatherogenic property. *FASEB Journal* **23**(9) 3129-3139.

Puela C, Quintina A, Agaliasa A, Matheya J, Obleda C and Mazura A (2004). Olive oil and its main phenolic micronutrient (oleuropein) prevent inflammation- induced bone loss in the ovariectomised rat. *British Journal of Nutrition* **92**(1) 119-127.

Ramadan MA, Amer MA and Awad AE (2008). Coriander seed and soybean oils improve plasma lipid profile in rats fed a diet containing cholesterol. *European Food Research and Technology* **227**(10) 1173-1182.

Rueda-Clausen CF, Silva FA, Lindarte MA, Villa-Roel C, Gomez E, Gutierrez R, Cure-Cure C and Lopez-Jaramillo P (2007). Olive, soybean and palm oils intake have a similar acute detrimental effect over the endothelial function in healthy young subjects. *Nutrition and Metabolism Cardiovascular Disease* **17**(1) 50–57.

Sanz M, Flores A and Lopez-Bote CJ (2000). The metabolic use of energy from dietary fat in broilers is affected by fatty acid saturation. *British Poultry Science* **41**(1) 61-66.

Scholtz SC, Pieters M, Oosthuizen W, Jerling JC, Bosman MJ and Vorster HH (2004). The effect of red palm olein and refined palm olein on lipids and haemostatic factors in hyperfibrinogaemic subjects. *Thromboxane Research* **113**(1) 13-25.

Shad MA, Iqbal AT and Tayyabe M (2002). Serum lipid profile: The effects of dietary PUFA and MUFA an experimental animal study. *Professional Medical Journal* **9**(1) 116- 120.

Sunitha T, Manoram A and Rukmini C (1997). Lipid profile of rats fed blends of rice bran oil in combination with sunflower and safflower oil. *Plant Foods and Human Nutrition* **51**(3) 219-230.

Wardlaw GM and Snook JT (1990). Effect of diets high in butter, corn oil, or high-oleic acid sunflower oil on serum lipids and apolipoproteins in men. *American Journal of Clinical Nutrition* **51**(1) 815-821.

Washburn KW and Nix DF (1974). A rapid technique for the extraction of yolk cholesterol. *Poultry Science* **53**(8) 1118-1122.

Zak B, Dickenman RC, White EG, Burnett H and Cherney PJ (1954). Rapid estimation of free and total cholesterol. *American Journal of Clinical Pathology* **24** (5) 1307-1315.