The implications of sunflower seeds on plasma lipids profile and fatty acids profile-IL-6 correlation in laying hens

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Abstract- The present study was done to examine the ascendency of incorporating plant source of Omega-6 fatty acids into laying hens diet, on the plasma lipids profile and plasma fatty acids profile-IL-6 correlation.

Fifty birds (Hisex-breed), 20 week old, were brought from animal production research center (Kuku), they were halved into two groups, group (A) was signed as control group, subjected to a diet based on corn, group (B), was under basic diet supplemented with 10% sunflower seeds, the two formulae were designed to sustain the requirements of laying hens as recommended by (NRC).

The trial extended for eight weeks, blood samples were collected once per month (week 4 and 8), in EDTA coated vials, immediately placed into iced-container, centrifuged at 3000rpm/20 min, samples were separated in aliquot, and stored at -20°C and -80°C until analysis.

Saturated fatty acids and Arachidonic acid levels were significantly ($p<0.05$), high in control group (A), while the summation of omega-3 fatty acids was significantly ($p<0.01$) high in sunflower seeds treated group.

The control group (A), revealed significant high concentration of plasma cholesterol, triglycerides and LDL-Cholesterol, while the HDL-Cholesterol level was enhanced by the addition of sunflower seeds.

Adding 10% of sunflower seeds to the basic diet of laying hens subsequently reduced the level of plasma IL-6 significantly compared to the control group (A).

Index Terms- Omega-6, Arachidonic Acid, HDL-Cholesterol, IL-6-Laying hens.

Abbreviations: **LDL**: Low Density Lipoprotein, **HDL**: High Density Lipoprotein, **EFA**: Essential Fatty Acids, **PUFA**: Poly Unsaturated Fatty Acids, **NFE**: Nitrogen Free Extract, **FID**: Flame Ionization Detector, **LA**: Linoleic Acid, **AA**: Arachidonic Acid, **LC-PUFA:** long chain-PUFA, **GLA**: Gamma Linolenic Acid, **SCD**: Stearoyl Co-A Desaturase.

I. Objectives

To investigate the influence of adding sunflower seeds to laying hen diets on lipids metabolism, which critically related to the incidence of inflammation via the connection between the concentration of arachidonic acid and the presence of IL-6 as an example of inflammation markers.

- The evaluation of plasma lipids and fatty acids profile, could give a strong clue about the potent capability of laying hens to deposit different types of fatty acids into their egg yolk, since the egg yolk content of poly unsaturated fatty acids is crucial in improvement of human heath, this trial was held as step towards that ultimate goal.

II. Introduction

The unsaturated fatty acids are classified into three main families; n-3, n-6 and n-9, due to which carbon atom from the methyl end where the first double bond is attached.

Due to lack of enzymes essential for desaturation at carbon atoms 3 and 6, the n-3 and n-6 families cannot be produced in the body. Thus, the parent fatty acids in these families (linolenic acid [18:3 n-3] and linoleic acid [18:2 n-6], respectively) are essential and can only be derived from the diet.

Essential fatty acids (EFA) and their derivatives played the major role in lipid metabolism, platelet functions, immune system, inflammatory response, and epidermal functions, it is important to assure optimal EFA intakes, **[1].** Dietary essential fatty acids (EFA), linoleic acid [18: 2(n-6), LA] and alpha-linolenic acid [18:3(n-3), ALA] are converted to long-chain polyunsaturated fatty acids (LCPUFAs) by desaturase and chain-elongation enzyme systems **[2].** Phospholipids, cholesterols, saturated fatty acids and monounsaturated fatty acids can be synthesized de novo within the human body. Because mammals cannot introduce a double bond beyond the delta-9 position in the fatty acid chain, however, linoleic (n-6) and linolenic acid (n-3) must be ingested in the diet [3]. Each of these EFA is in turn the substrate for further desaturation and elongation.

The ω-3 and ω-6 PUFAs play an important role in health and treatment of diseases. They can act as antibacterial agents, **[3]; [4]** and **[5],** anti-inflammatory agents, **[6]; [7],** antioxidants **[8]** in the treatment of cardiovascular diseases, **[9]**, and cancer cell proliferation, **[10]** and **[11]**, such properties are indicative of the potential for PUFAs as nutraceuticals and as pharmaceuticals. The (ω-3/ω-6) ratio can significantly influence the body's metabolic function, **[12].**

Linoleic acid (LA) is an N-6 fatty acid, is thought to be pro-inflammatory and it can be converted into arachidonic acid (AA), **[13].** AA is the major substrate for eicosanoids production, which plays an important role in regulating inflammatory and immune responses **[14].**

There is one omega-6, however, called gamma linolenic acid (GLA) with an impressive set of disease-fighting powers. New research reveals this nutrient's power to combat chronic inflammation, eczema, dermatitis, asthma, rheumatoid arthritis, atherosclerosis, diabetes, obesity even cancer **[15]; [16]; [17]; [18]; [19]; [20]; and [21].**

GLA also shows promise in lowering low-density lipoprotein (LDL) and triglyceride levels, while increasing high-density lipoprotein (HDL) concentration [18]; and **[22].**

The regulation of gene expression by n-3 and n-6 PUFA's also occurs in adipose tissue. For example, **[23],** have demonstrated adipocyte-specific gene regulation by linolenic acid (LNA 18:3n-3). Stearoyl-CoA desaturase 1 (SCD1) mRNA activity in rodent adipose tissue is reduced when fed a high n-6 PUFA diet **[24].** Modulation of SCD1 activity by n-3 and n-6 PUFA's has also been observed in tissue culture. The n-6 PUFA's AA (20:4n-6) and LA (18:2n-6) as well as the n-3 PUFA's LNA (18:3n-3) and EPA (20:5n-3) depress SCD1 mRNA stability in a dose dependent manner in 3T3-L1 adipocyte cell lines **[25].**

Effects of the type of dietary fats on inflammation n-6 PUFA (particularly of linoleic acid), is negatively viewed as pro-inflammatory considering the fact that it can be metabolized into AA and subsequent undesirable metabolites. Furthermore, n-6 PUFA may compete for cyclooxygenase thus reducing the formation of anti-inflammatory mediators from n-3 PUFA, [26]. Nevertheless, a rigorously well conducted systematic review reported that no clinical evidence to-date were able to suggest the pro-inflammatory effects of linoleic acid in healthy, non-infant individuals, **[27].**

Sunflower oil has gained importance due to increased content of oleic and linoleic acid that may help diminishing the cholesterol leading to reduction in heart diseases, [28]. Also, cholesterol content and cancer risk is controlled by the presence of high content of phytosterols in sunflower seeds, *i.e.*, approximately 280 mg per 100 gm. Tocopherols in sunflower oil protect body from inflammation and tumors by neutralizing free radicals and avoiding oxidative injury to cells, thus helpful in diseases like rheumatoid arthritis and bronchial asthma, **[29].** Vitamin E has a positive effect on coronary system of the body and hence reduces stroke and atherosclerosis, [30]. Sunflower oil contains magnesium that is supportive for curing migraine, hypertension, and bronchial asthma along with maintaining muscular tone of the body. Folic acid in seeds helps in the formation of blood and nucleic acids. Mental stress and uneasiness can be resolved by choline and tryptophan in sunflower seeds. Minerals like zinc improve the immune system, while selenium protects from the prostate cancer due to the antioxidant action. Occurrence of several components in sunflower oil make it curative as anti-inflammatory, anti-bacterial, anti-fungal, anti-cancer, cardio protective and dermoprotective in human beings, **[31].**

III. Material and Methods

The experiment was held in the Veterinary Research Institute (VRI), from January to march 2014. The duration of the experiment was eight weeks.

Four, full wire cages were made, each cage was (2X 1.5X 1 meter), and the capacity of each cage was 15 birds. The cages were placed at an open poultry house.

Fifty laying hens (Hisex) breed, 20 week old, obtained from animal production research center (kuku), were utilized in this study. The birds were divided into two groups, 25 birds per group.

Experimental Diets: The diets were formulated to meet the requirements of egg production according to the directions of the national research council, **[32].** Two formulae of diets were prepared, 10% of sunflower seeds was inserted into the experimental one.

The supplementary source was subjected to proximate analysis, to determine its content of protein, fat, fiber, N.F.E and energy.

- **Management:** Each group received its diet from day one. Drinking system contained two tanks for each cage, the tanks were cleaned, and the water was changed twice daily. Birds received 24 hour light/day throughout the experiment.

Three ml of blood was collected from twenty bird of each group, the blood was taken using a three ml syringe, and received into EDTA coated vials, and immediately were kept in iced container, the samples were centrifuged at 3000 **rpm** for 20 minutes, and plasma was separated in aliquot and transferred into plane vials. Plasma samples were stored at -20 and -80°C until analysis.

- **Fatty Acids Analysis:**

- Lipids were extracted in chloroform-methanol (2:1 v/v), according to the method of **[33]**.
- Methyl esters of the lipid extract were prepared according to [34].

Table (1): proximate analysis of sunflower seeds:

	D.M%	Moisture %	Protein %	Fat %	Fiber%	Ash %	N.F.E %	Energy%
Sunflower seeds	98	2	26.00	36.49	11	6	18.5	3054.07

Table (2-1): Diets composition:

Group	A	B
Raw Materials%		
Corn %	70	59.0
Wheat hull %	0	5.4
Groundnut cake %	14.3	10
Concentrate %	5	5
Calcium Carbonate%	10	10
Salt (Nacl) %	0.125	0.125
Methionine %	0.34	0.31
Lysine %	0.15	0.11
Mycofix %	0.1	0.1
Sunflower seeds%	----	10
*Premix**	0.1	0.1

Table (2-2): Nutritional values calculated:

Parameter	A	B
ME. Kcal/Kg	2729	2821
C.P%	17.25	18.24
E.E%	5	6.2
C.F%	4.2	4.1
Available phosphorus%	0.52	0.63
Calcium%	3.9	3.9

**Supplied per kilogram of diets: Vitamin A, 5000 IU; Vitamin D ,500 IU; Vitamin E, 5 IU; Vitamin K, 1 IU; Vitamin B , 1.5 mg, Vitamin B , 2.5mg, 1 2 Ca-pantothenate, 2.5mg, niacin acid, 10 mg; pyridoxine,3mg; biotin, 0.1mg; folic acid, 0.25mg; Vitamin B , 0.005mg. Supplied per kilogram 12 b of diets: MnSO. 7H O100mg, FeSO. 7H O, 220mg; ZnSO . 7H O, 150mg; CuSO. 7H O, 20mg; KI, 2mg; Na SeO, 0.4 mg.*

- ***A**=Control group, **B**=10% sunflower seeds supplemented group.*

Table (3): Fatty acids profile of control and experimental group diets

Fatty acid	Control group (A)	Sunflower supplemented group (B)
(g/100 g total fatty acids)		
SFA	43.53	9.9754
MFA	17.68	34.6841
PUFA	38.38	55.3437[A]
C18:3 (*n*-3)	1.38	10.5616
Σ*n*-3	1.38	10.5616
C18:2 (*n*-6)	36.94	31.0756
C20:4 (*n*-6)	0.21	6.5908
Σ*n*-6	37.15	37.6664
PUFA/SFA	0.88	5.6
Σ*n*-3/Σ*n*-6	0.04	0.3

***SFA**= Saturated fatty acids, **MFA**=Mono unsaturated fatty acids, **PUFA**=Poly unsaturated fatty acids, **C18:3 (n-3)** =Linolenic acid (omega-3), **C18:2 (n-6)** =Linoleic acid (omega-6),* **C20:4 (*n*-6) =** Arachidonic acid (omega-6).

- ***A**=Control group, **B**=10% sunflower seeds supplemented group.*

❖ **Gas Chromatograph Analysis:**

Fatty acid composition was determined using (Shimadzu-2010) gas chromatograph, fitted with Flame ionization detector (FID). Separation of fatty acids was achieved using DB-WAX column, serial number (us6551263 H), of 0.25um film thickness, 30 meter length and 0.25 mm inner diameter.

Fatty acids methyl esters were identified by comparison of retention times with standards, and expressed as percentage of methyl esters.

❖ **Determination of lipids profile:**

Cholesterol was measured using commercial kits (Spectrum, Cairo, Egypt), using spectrophotometric method described by **[35]**, [36], [37], and [38]. The intensity of the colored complex is proportional to the cholesterol concentration. The absorbance was measured at a wave length 500 nm.

Triglycerides were measured using commercial kits (Spectrum, Cairo, Egypt), using spectrophotometric method described by [39], [40], and [41]. The intensity of the colored complex is proportional to the triglycerides concentration. The absorbance was measured at a wave length 500nm.

HDL-Cholesterol was measured using kits (Spectrum, Cairo, Egypt). The method used is a spectrophotometric method;

described by [42], and [43].The optical density was read against the blank at 546nm.

LDL-Cholesterol was measured using kits (Bio-system, reagent and instruments, Barcelona, Spain). The method used is a spectrophotometric method described by **[44]**. The optical density of the sample was read against the blank at 500 nm.

For the quantitative measurement of chicken Interleukin-6 (IL-6), a sandwich enzyme-linked immunosorbent assay (ELISA) kit (Assay Company), was used. The principle of the test is that the IL-6 is measured using a sandwich monoclonal antibodies in a biotin system ELISA.

Results are calculated by using curve-fitting of standards of known concentration. The IL-6 amount in each sample is determined by interpolating from the absorbance value (Y axis) to IL-6 concentration (X axis) using the standard curve equation.

IV. Statistical Analysis

The data were analyzed by using Statistics-10 program designed for Windows. Differences between obtained values were carried out by analysis of variance (ANOVA) the LCD test was used for determining the significance level of at least $p<0.05$.

V. Results

Saturated fatty acids level was significantly ($p<0.05$), high in control group (A), compared to the treated group, while plasma level of unsaturated fatty acids in group (B) was significantly ($p<0.05$), high compared to the control group (A).

The total detected omega-6 fatty acids, was significantly high ($p<0.05$), in plasma of group (B), compared to group (A).

Group (B), demonstrated high concentration of poly unsaturated fatty acids, the difference was significant at ($p<0.05$), compared to the control group (A).

The plasma concentration of Oleic acid was not significantly different between the control and the treated group (B).

The control group (A), recorded significant ($p<0.05$), high concentration of plasma Arachidonic acid compared to the treated group.

At the fourth week, the control group (A); recorded significant ($p<0.01$) high level of plasma cholesterol compared to group (B), similar result was observed by the end of week 8.

There was no significant different levels of plasma triglycerides observed between the control group (A) and the treated one (B) at the 4th week, while the last week, revealed significant ($p<0.01$) high concentration of plasma triglycerides in group (A) compared to group (B).

There was no significant different concentration of plasma LDL-Cholesterol detected between the control group (A) and the treated one (B). A different result was observed by the end of week 8, thus the control group (A), showed significant ($p<0.05$), high concentration of plasma LDL, compared to the treated one (B).

HDL-Cholesterol level at week 4 was not significantly different between the control group (A), and sunflower supplemented group (B), by the end of the last week of the trial plasma concentration HDL-Cholesterol was significantly ($p<0.05$) elevated in the treated group in comparison with the control one.

At the fourth week group (B), demonstrated no significant different level of plasma IL-6 compared to the control group (A), while the level was noticeably reduced in the treated group by the end of week 8, compared to the control group and the difference between the two levels was significant at ($p<0.05$).

VI. Discussion

There was no significant different level of plasma cholesterol noticed between the control group (A) and the treated one (B), by the end of the first month, this may be due to the slightly high either extract percentage of the treated group's diets, sunflower seeds, is considered as one of highly oil content seeds 36.49% (Table-1).

Compared to the fourth week, week 8 recorded significant low levels of plasma cholesterol in treated group (B) compared to the control group (A), the current study result is corroborated by what was reported by **[45],** that sunflower ethanolic extract, had lowering effect on plasma total cholesterol in diabetic rats, compared to control diabetic rats, and normalize effect compared to the normal control rats.

Table (4): Plasma Fatty acids profile of control and experimental group

Fatty acid	Control group (A)	Sunflower seeds supplemented group (B)
(ml/100 ml total fatty acids)		
SFA	33.01^{A}±1.6840	10.11^{B}±1.6840
MUFA	24.1^{B}±2.1320	33.68^{A}±2.1320
PUFA	42.87^{B}±2.3130	55.34^{A}±2.3130
C18:3 (*n*-3)	1.69^{B}±1.7127	8.31^{A}±1.7127
Σ*n*-3	1.695^{B}±1.8347	8.31^{A}±1.8347
C18:2 (*n*-6)	23^{B}±1.1862	32.27^{A}±1.1862
C20:4 (*n*-6)	10.97^{A}±0.5346	5.89^{B}±0.5346
Σ*n*-6	33.97^{B}±1.1808	38.16^{A}±1.1808
Σ*n*-3/Σ*n*-6	0.05^{A}±0.6359	0.3^{A}±0.6359

Data are means ± standard error. Means in the same raw followed by the same letters are not significantly different at ($p<0.05$).

***SFA**= Saturated fatty acids, **MUFA**=Mono unsaturated fatty acids, **PUFA**=Poly unsaturated fatty acids, **C18:3 (n-3)** =Linolenic acid (omega-3), **C18:2 (n-6)** =Linoleic acid (omega-6),* **C20:4 (*n*-6) =** Arachidonic acid (omega-6).

- ***A**=Control group, **B**=10% sunflower seeds supplemented group.*

Figure (1):

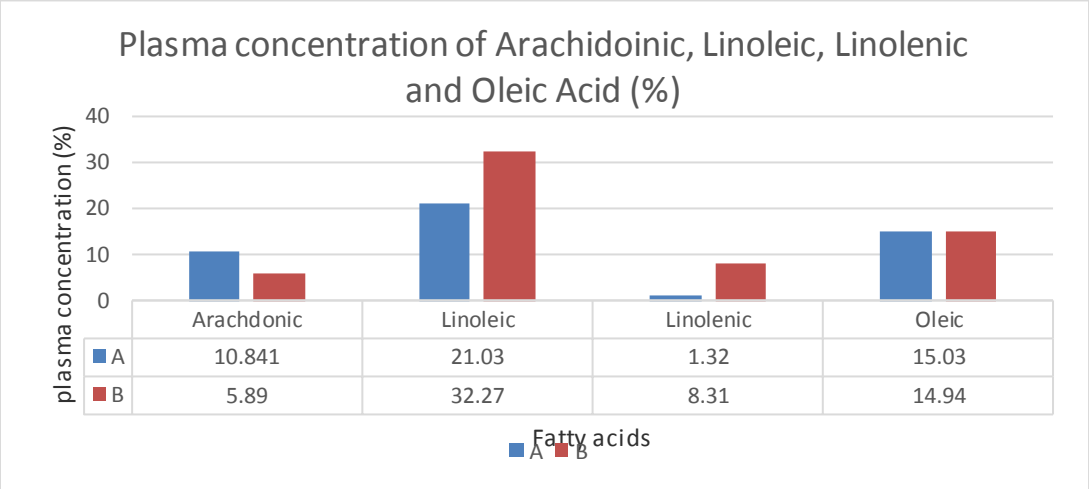


Table (5): Plasma lipids profile:

Parameter	Cholesterol mg/dl		Triglycerides mg/dl		HDL-Cholesterol mg/dl		LDL-Cholesterol mg/dl	
Group	**4th week**	**8th week**	**4th week**	**8th week**	**4th week**	**8th week**	**4th week**	**8th week**
Control (A)	219.20^{A}± 8.05	221.08^{A}±8 .17	419.13^{A}± 24.15	402.18^{A}± 16.66	11.726^{B}± 0.68	10.970^{A}± 0.4987	78.740^{A}± 5.81	80.920^{A}± 4.81
Group (B)	187.13^{B}± 8.0536	161.08^{B}±8 .1765	396.79^{A}± 25.007	317.82^{B}± 16.669	12.706^{AB} ±0.6835	12.676^{B}± 0.4987	67.074^{AB} ±5.8106	65.320^{B}± 4.8108

Data are means ± standard error. Means in the same columns followed by the same letters are not significantly different at ($p<0.05$).

Figure (2):

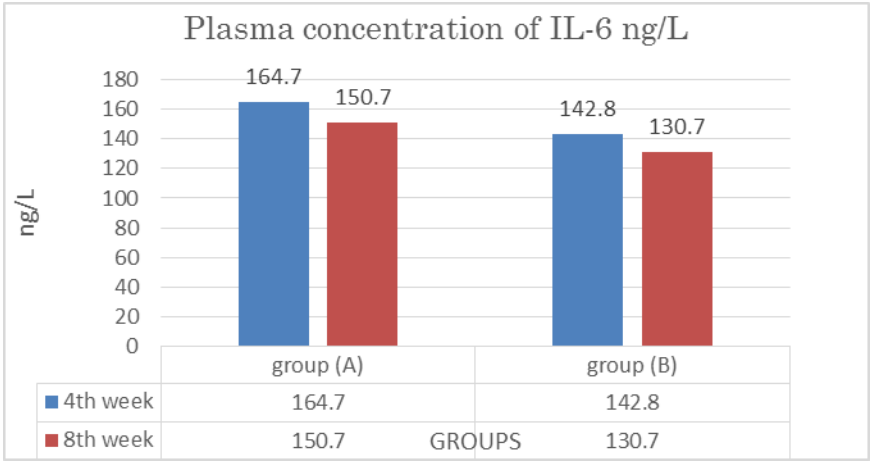
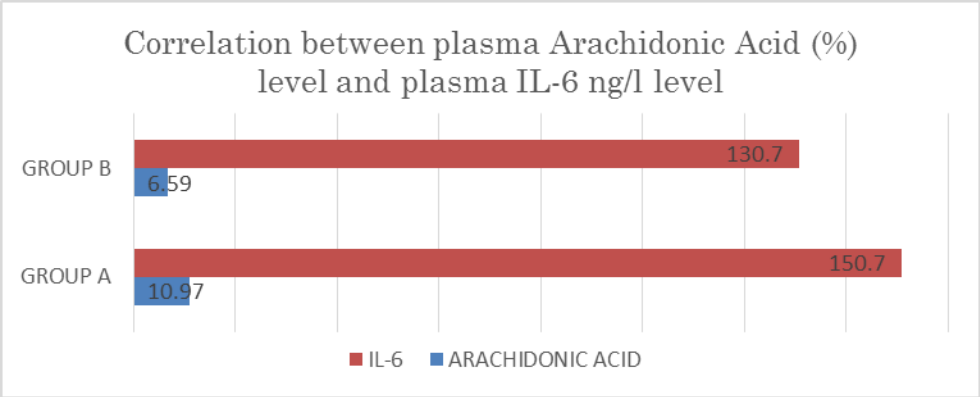


Figure (3):



Another supportive result was stated by [46], who reported that total cholesterol levels were significantly low in ducks received diets included sunflower oil.

A trial conducted by [47], showed that dietary PUFA, reduced plasma cholesterol in broiler chickens, when compared to groups fed saturated fatty acids SFA. Omega-3 fatty acids, suppress the synthesis of triglycerides and Apo lipoprotein B, increase the removal of VLDL, by peripheral tissue or the liver, and increase the excretion of bile in feces **[48]**, which can also reduce the serum concentration of cholesterol and triglycerides.

The fourth week, showed no significant different levels of plasma triglycerides between group (A) and (B), this may be due to the slightly high either extract percentage content of the treated group diets, sunflower seeds, are classified as oil seeds (36.49%) , (table-1). This result agrees with what was reported by [49], that inclusion of sunflower meal into broiler diets increase the plasma content of triglycerides.

The 8th revealed, significant high level of plasma triglycerides in the control group (A) compared to the treated group (B), The results of the current study are in agreement with [50], who reported that, the inclusion of fish oil (omega-3 source), and sunflower oil (omega-6 source), had significantly reduced the serum triglycerides level, these results could be attributed to the high content of PUFA in diets, which had been reported as triglycerides and cholesterol reduction factor. The diet poly unsaturated fatty acids content of the current study confirms these results.

the dietary intake of omega-3 and omega-6, is effective in lowering serum lipids concentration, nevertheless, N-6 and N-3 fatty acids differ in their effect on serum lipids concentration, meaning that N-6 fatty acids, lower serum cholesterol but not triglycerides, while N-3 fatty acids, lower serum cholesterol and triglycerides, [51], in regard to N-6 fatty acids lowering effect on triglycerides in this study, it could be attributed to low lipogenesis capacity of the Hisex strain, and consequently low serum triglycerides concentration.

The high concentration of IL-6, in group C, which was recorded at the first month could be attributed to the inflammatory eicosanoids produced from Arachidonic acid, which derived from linoleic acid.

By the end of the trial, group C, which was supplemented by 10% sunflower seeds, showed significant low level of IL-6 compared to control group, the possible anti-inflammatory effect of omega-6 fatty acids, (Das, 2007**)**, could be an explanation of this result, the mechanism involved in this reduction is within the metabolic pathway of linoleic acid, gamma linoleic acid, which derived from linoleic acid, reduces inflammation by activating the powerful peroxisome proliferator-activated receptors (PPAR), which are intracellular receptors that modulate cell metabolism and response to inflammation, also GLA, has impeding the ability of arachidonic acid to convert to detrimental inflammatory molecules, **[52]**, also the plasma arachidonic acid concentration in the control group A, justify the high level of plasma IL-6 in the plasma of group A compared to group C, the concentration was 10 and 6% of the total detected fatty acids respectively.

The concentration of LDL-Cholesterol, was significantly low in the treated group compared to the control one, while the concentration of plasma HDL-Cholesterol was significantly ($p<0.05$) high in the treated group (B), by the end of the final week, this result could be justified by the modulation of dietary fat composition which affects serum lipid concentrations, replacing SFA and *trans* fatty acids (TFA) with *cis*-PUFA lowers serum LDL-cholesterol concentration, [53].

VII. Conclusion

Treated group (B), showed significant high level of plasma poly unsaturated fatty acids content, whereas the concentration of saturated fatty acids was noticeably lowered.

High concentration of Arachidonic acid was recorded by the control, while the plasma content of omega-6 fatty acids was significantly higher in the treated group.

Feeding laying hens 10% sunflower seeds for 8 weeks, remarkably, reduced plasma concentration of cholesterol, LDL-Cholesterol and triglycerides, while there was pronounced elevation of HDL-Cholesterol level.

The current study revealed a possible anti-inflammatory effect of omega-6 fatty acids, which was also reported by some previous studies.

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REFERENCES

[1] Sardesai, V.M. (1992). Nutritional role of polyunsaturated fatty acids. Journal of nutrition and biochemistry, 3:154–166.

[2] Wayne, S., Fenton, J.H., and Michael K. (2000). Essential Fatty Acids, Lipid Membrane Abnormalities, and the Diagnosis and Treatment of Schizophrenia. Biological Psychiatry journal, 47: 8–21.

[3] Ward, O. P.; Singh, A. (2005). Omega-3/6 fatty acids: Alternative sources of production. Process Biochemistry Journal, 40: 3627-3652.

[4] Guedes, A.C.; Amaro, H.M.; Malcata, F. X. (2011). Microalgae as sources of high added-value compounds--a brief review of recent work. Biotechnology Progress Journal, 27: 597-613.

[5] Huang, C. B.; Ebersole, J. L. (2010). A novel bioactivity of omega-3 polyunsaturated fatty acids and their ester derivatives. Molecular Oral Microbiology, 25: 75-80.

[6] Pulz, O.; Gross, W. (2004). Valuable products from biotechnology of microalgae. Appl. Microbiology and Biotechnology Journal, 65: 635-648.

[7] Schmitz, G.; Ecker. (2008).The opposing effects of n-3 and n-6 fatty acids. Journal of Progress in Lipid Research, 47: 147-155.

[8] Plaza, M.; Herrero, M.; Cifuentes, A.; Ibanez, E. (2009). Innovative natural functional ingredients from Microalgae. Journal of Agriculture and Food Chemistry, 57: 7159- 7170.

[9] Mozaffarian, D.; Wu, J. H. (2011). Omega-3 fatty acids and cardiovascular disease: effects on risk factors, molecular pathways, and clinical events. Journal of American College of cardiology, 58: 2047-2067.

[10] Field, C.J.; Schley, P. D. (2004). Evidence for potential mechanisms for the effect of conjugated linoleic acid on tumor metabolism and immune function: lessons from n-3 fatty acids. American Journal of Clinical Nutrition, 79: 1190-1198.

[11] Das, M.; Zuniga, E.; Ojima, I. (2009). Novel Toxoid-Based Tumor-Targeting Drug Conjugates. Chimica Oggi journal, 27: 54-56.

[12] Candela, C. G.; Lopez, B. L. M.; Kohen. (2011). Importance of a balanced omega 6/omega 3 ratio for the maintenance of health: Nutritional recommendations. Nutrition in Hospitals Journal, 26: 323-329.

[13] Myers JL, Allen JC. (2012). Nutrition and Inflammation: Insight on dietary pattern, obesity and asthma. American Journal of Lipid Metabolism, 6:14–17.

[14] Calder PC. (2006). N-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. American Journal of Clinical Nutrition, 83:1505–1519.

[15] Bordoni A, Biagi PL, Masi Ml. (1988). Evening primrose oil (Efamol) in the treatment of children with atopic eczema. Drugs under Experimental and Clinical Research, 14(4):291-7.

[16] Surette ME, Stull D, Lindemann J. (2008).The impact of a medical food containing gammalinolenic and eicosapentaenoic acids on asthma management and the quality of life of adult asthma patients. Current Medical Research and Opinion, 24(2):559-67.

[17] Boyce, E. G, Leventhal, L.J., and Zurier, R. B. (1993). Treatment of rheumatoid arthiritis with gamma linolenic acid. Annals of Internal Medicine journal, 119:867-873.

[18] Ishikawa T, Fujiyama Y, Igarashi O. (1989). Effects of gammalinolenic acid on plasma lipoproteins and Apo lipoproteins. Atherosclerosis, 75(2-3):95-104.

[19] Schirmer MA, Phinney SD. (2007). Gamma-linolenate reduces weight regain in formerly obese humans. Journal of Nutrition, 137(6):1430-5.

[20] Das UN. (2007). A defect in the activity of delta-6 and delta-5. Desaturases may be a factor in the initiation and progression of atherosclerosis. Prostaglandins, Leukotrienes and Essential Fatty Acids, 76: 251-68.

[21] Fearon KC, Falconer JS, Ross JA. (1996). an open-label phase I/II dose escalation study of the treatment of pancreatic cancer using lithium gammalinolenate. Anticancer Research, 16 (2):867-74.

[22] Guivernau M, Meza N, Barja P, Roman O. (1994). Clinical and experimental study on the long-term effect of dietary gamma-linolenic acid on plasma lipids, platelet aggregation, thromboxane formation, and prostacyclin production. Prostaglandins Leukotriens and Essential Fatty Acids, 51(5):311-6.

[23] Amri, E., Bertrand, B., Ailhaud, G. & Grimaldi, P. (1991). Regulation of adipose cell Differentiation: fatty acids are inducers of the aP2 gene expression. Journal of Lipid Research, 32: 1449-1456.

[24] Jones, B., Maher, M.A., Banz, W.J., Zemel, M.B., Whelan, J., Smith, P. & Moustaid, N. (1996). Adipose tissue stearoyl-CoA desaturase mRNA is increased by obesity and decreased by polyunsaturated fatty acids. American Journal of Physiology, 34: 44-E49.

[25] Sessler, A.M., Kaur, N., Palta, J.P. & Ntambi, J.M. (1996). Regulation of stearoyl-CoA Desaturase 1 mRNA stability by polyunsaturated fatty acids in 3T3-L1 adipocytes. Journal of Biology and Chemistry, 271: 29854-29858.

[26] Sacks FM, Campos H. (2006). Polyunsaturated fatty acids, inflammation, and cardiovascular disease: time to widen our view of the mechanisms. Journal of Clinical Endocrinology and Metabolism, 91:398–400.

[27] Johnson GH, Fritsche K. (2012). Effect of dietary linoleic acid on markers of inflammation in healthy persons: a systematic review of randomized controlled trials. Journal of Academy of Nutrition and Dietetics, 112:1029–1041.

[28] Chowdhury, L.A. Banu, S. Khan and Latif A. (2007). Studies on the Fatty Acid Composition of Edible Oil. Bangladesh Journal of Science and Industrial Research, 42: 311-316.

[29] Dutta and S.K. Dutta. (2003). Vitamin E and its role in the prevention of atherosclerosis and carcinogenesis: a review. Journal of American College of Nutrition, 22: 258-268.

[30] Singh, S. Devaraj and I. Jialal. (2005). Vitamin E, oxidative stress, and inflammation. Annual Review of Nutrition, 25:151-174.

[31] Nandha, H. Singh, K. Garg and S. Rani. (2014). Therapeutic potential of Sunflower seeds. International Journal of Research and Development of Pharmacy, 3: 967-972.

[32] National Research Council (NRC), (1994). Nutrient requirements of poultry, Ninth revised edition.

[33] Folch, J., Lees, M and Sloane-Stanely, G. H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biology and Chemistry, 226:497–507.

[34] Wang, Y., Sunwoo, H., Cherian, G., and Sim, J.S. (2000). Fatty acid determination in chicken egg yolk: a comparison of different methods. Poultry Science, 79: 1168-1171.

[35] Ellefson, RD, Caraway, WT. and Tietz NW. (1976). Fundamentals of clinical chemistry, Ed; P506.

[36] Flegg, H. M. (1973). An investigation of the determination of serum cholesterol by an enzymatic method. Annals of Clinical Biochemistry, 10:79-84.

[37] Richmond, W. (1973). Preparation and properties of a cholesterol oxidase from Nocardia sp. and its application to the enzymatic assay for total cholesterol in serum. Clinical Chemistry, 19:1350-1356.

[38] Roesclau P, Bernt E, Gruber W. (1974). Enzymatic determination of total cholesterol in serum. Z kiln Chem Klin Biochem journal, 12:226-229.

[39] Bucolo, G., and David, H. (1973). Quantitative determination of serum triglycerides by the use of enzymes. Clinical Chemistry, 19:476-482.

[40] Chowdhury, R. F., Rodman, H., Bleicher. (1971). glycerol-like contamination of commercial blood sampling tubes. J. lipid Res. 12, 116.

[41] Tietz, N. M., Borden, T., Stepleton, J. D. (1959). An improved method for the determination of lipase in serum. American Journal of Clinical Pathology, 31: 148-154.

[42] Friedewald, WT Levy, RI. (1972). Estimation of the Concentration of Low-Density Lipoprotein Cholesterol in Plasma, Without Use of the Preparative Ultracentrifuge. Clinical Chemistry, 18, No. 6.

[43] Lopez-Virella, M.F. (1977). High density lipoprotein cholesterol by selective precipitin. Clinical chemistry, 23: 882.

[44] Assmann, G; Jabs, HU; Kohnert, U; Nolte. W and Schriewer, H. (1984). LDL- cholesterol determination in blood serum following precipitation of LDL with polyvinyl sulfate. Clinical Chemistry Acta, 140: 77-83.

[45] Shivani Saini and Sunil Sharma. (2013). Antidiabetic effect of Hellanthus Annus l., Seeds ethanolic extract in Streptozotocin- Nicotinamide induced type 2 Diabetes Mellitus, International Journal of Pharmacy and Pharmaceutical, 5 (2): 0975-1491.

[46] Wei-meng LIU, Shu-jing LA, Li-zhi LU, Fang-xiong SHI, Jing ZHANG1, Yu LIU, Bo YU5, Zheng-rong TAO, Jun-da SHEN, Guo-qin L, De-qian WANG1, Jin-jun L, Yong TIAN. (2012). Effect of dietary fatty acids on serum parameters, fatty acid compositions, and liver histology in Shaoxing laying ducks. Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology), 1673-158.

[47] Newman, R. E., Bryden, W. L., Fleck, E., Ashes, J. R., Buttemer, W. A., Storlien, L. H., Downing, J. A. (2002). Dietary n-3 and n-6 fatty acids alter avian metabolism: metabolism and abdominal fat deposition. British Journal of Nutrition, 88: 11–18.

[48] Leaf A, Weber PC. (1988). Cardiovascular effects of n-3 fatty acids. New England Journal of Medicine, 318:549–557.

[49] Nassiri Moghaddam, H S. Salari, J. Arshami, A. Golian, and M. Maleki. (2012). Evaluation of the nutritional value of sunflower meal and its effect on performance, digestive enzyme activity, organ weight, and histological alterations of the intestinal villi of broiler chickens, Journal of Applied Poultry Research, 21:293–304.

[50] Tejaswi Chavan, Suresh khadke, Shubhangi Harke, Abhijit Ghadge, Manjiri Karandikar, Vtjaya Pandit, Prabhakar Ranjekar, Omkar Kuikarni and Aniket Kuvalekar. (2013). hepatoprotective effect of poly unsaturated fatty acids against repeated subacute Acetaminophen dosing in rats, International Journal of Pharma and Bio Sciences, 4(2): (P) 286 – 295.

[51] Kristina S; Tomisla M; Diana B; Natalija F; and Zvonko S. (2014). Growth performance, serum lipids and fatty acids profile of different tissues in chicken broilers fed a diet supplemented with linseed oil during a prolonged fattening period. Veterinarski Arhiv, 84(1):75-84.

[52] Hontecillas R, O'Shea M, Einerhand A, Diguardo M, Bassaganya-Riera J. (2009). Activation of PPAR gamma and alpha by punicic acid ameliorates glucose tolerance and suppresses obesity-related inflammation. Journal of American College of Nutrition, 28(2):184-95.

[53] NDA (Scientific Panel on Dietetic Products, Nutrition and Allergies). (2004). Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to the presence of Trans fatty acids in foods and the effect on human health of the consumption of Trans fatty acids. The European Food Safety Journal, 81: 1-49.

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