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.


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 health, 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, cholesterol, 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 PUFA 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 PUFA as nutraceuticals and as pharmaceuticals. The (ω-3/ω-6) ratio can significantly influence the body’s metabolic function, [12].

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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 has also been observed in tissue culture. The n-6 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 [24].

Effects of the type of dietary fats on inflammation 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’s (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>
<thead>
<tr>
<th>Table (1): proximate analysis of sunflower seeds:</th>
</tr>
</thead>
<tbody>
<tr>
<td>D.M%</td>
</tr>
<tr>
<td>-------</td>
</tr>
<tr>
<td>Sunflower seeds</td>
</tr>
</tbody>
</table>

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Table (2-1): Diets composition:

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

*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 Ca-pantothenate, 2.5mg, niacin acid, 10 mg; pyridoxine, 3mg; biotin, 0.1mg; folic acid, 0.25mg; Vitamin B, 0.005mg. Supplied per kilogram 12b of diets: MnSO. 7H O, 100mg, FeSO. 7H O, 20mg; 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 (2-2): Nutritional values calculated:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME. Kcal/Kg</td>
<td>2729</td>
<td>2821</td>
</tr>
<tr>
<td>C.P%</td>
<td>17.25</td>
<td>18.24</td>
</tr>
<tr>
<td>E.E%</td>
<td>5</td>
<td>6.2</td>
</tr>
<tr>
<td>C.F%</td>
<td>4.2</td>
<td>4.1</td>
</tr>
<tr>
<td>Available phosphorus%</td>
<td>0.52</td>
<td>0.63</td>
</tr>
<tr>
<td>Calcium%</td>
<td>3.9</td>
<td>3.9</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Control group (A)</th>
<th>Sunflower supplemented group (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(g/100 g total fatty acids)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SFA</td>
<td>43.53</td>
<td>9.9754</td>
</tr>
<tr>
<td>MFA</td>
<td>17.68</td>
<td>34.6841</td>
</tr>
<tr>
<td>PUFA</td>
<td>38.38</td>
<td>55.3437A</td>
</tr>
<tr>
<td>C18:3 (n-3)</td>
<td>1.38</td>
<td>10.5616</td>
</tr>
<tr>
<td>Σn-3</td>
<td>1.38</td>
<td>10.5616</td>
</tr>
<tr>
<td>C18:2 (n-6)</td>
<td>36.94</td>
<td>31.0756</td>
</tr>
<tr>
<td>C20:4 (n-6)</td>
<td>0.21</td>
<td>6.5908</td>
</tr>
<tr>
<td>Σn-6</td>
<td>37.15</td>
<td>37.6664</td>
</tr>
<tr>
<td>PUFA/SFA</td>
<td>0.88</td>
<td>5.6</td>
</tr>
<tr>
<td>Σn-3/Σn-6</td>
<td>0.04</td>
<td>0.3</td>
</tr>
</tbody>
</table>

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;
The data were analyzed by using Statistics-10 program to determine the IL-6 concentration using the standard curve equation. The optical density of the sample was read against the blank at 540nm.

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.

VI. Discussion

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).

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).

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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).

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

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Control group (A)</th>
<th>Sunflower seeds supplemented group (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(ml/100 ml total fatty acids)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SFA</td>
<td>33.01±1.6840</td>
<td>10.11±1.6840</td>
</tr>
<tr>
<td>MUFA</td>
<td>24.12±2.1320</td>
<td>33.68±2.1320</td>
</tr>
<tr>
<td>PUFA</td>
<td>42.87±2.3130</td>
<td>55.34±2.3130</td>
</tr>
<tr>
<td>C18:3 (n-3)</td>
<td>1.69±1.7127</td>
<td>8.31±1.7127</td>
</tr>
<tr>
<td>Σn-3</td>
<td>1.695±1.8347</td>
<td>8.31±1.8347</td>
</tr>
<tr>
<td>C18:2 (n-6)</td>
<td>23.8±1.8862</td>
<td>32.27±1.8862</td>
</tr>
<tr>
<td>C20:4 (n-6)</td>
<td>10.97±0.5346</td>
<td>5.89±0.5346</td>
</tr>
<tr>
<td>Σn-6</td>
<td>33.97±1.808</td>
<td>38.16±1.808</td>
</tr>
<tr>
<td>Σn-3/Σn-6</td>
<td>0.05±0.6359</td>
<td>0.33±0.6359</td>
</tr>
</tbody>
</table>

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

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**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):**

![Graph showing plasma concentration of Arachidonic, Linoleic, Linolenic, and Oleic Acid (%)](image)

**Table (5): Plasma lipids profile:**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cholesterol mg/dl</th>
<th>Triglycerides mg/dl</th>
<th>HDL-Cholesterol mg/dl</th>
<th>LDL-Cholesterol mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4th week</td>
<td>8th week</td>
<td>4th week</td>
<td>8th week</td>
</tr>
<tr>
<td>Control (A)</td>
<td>219.20±8.05</td>
<td>221.08±8.17</td>
<td>419.13±24.15</td>
<td>402.18±16.66</td>
</tr>
<tr>
<td>Group (B)</td>
<td>187.13±8.0536</td>
<td>161.08±16.1765</td>
<td>396.79±25.007</td>
<td>317.82±16.669</td>
</tr>
</tbody>
</table>

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):**

![Graph showing plasma concentration of IL-6 ng/L](image)
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.

ACKNOWLEDGEMENT

I would like to thank my supervisor, prof. Mohamed El sheikh Barri for his unlimited patience and help, my thanks are

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REFERENCES


