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EFFECT OF LONG TERM SUPPLEMENTATION WITH DIETARY LIPIDS ON GROWTH, FATTY ACID COMPOSITION AND HISTOPATHOLOGY IN RABBITS LIVER

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ABSTRACT

The purpose of this study was to investigate the effect of long-term supplementation with dietary lipids on growth, fatty acid composition and histopathology of male and female rabbit's liver**.** Soybean oil (SBO), fish oil (FO), sesame oil (SO), docosahexaenoic acid (DHA) and docosahexaenoic acid (DHA) / arachidonic acid (ARA) were used as source of dietary lipids (treatments) in this study. The experimental diets were prepared by adding the oil blend to the basal diet (70 g/kg diet). The experiment was conducted on forty-five weanling (25 male (5 in each treatment) and 20 female (4 in each treatment)) New Zealand white rabbits. Dietary lipids had a distinct effect on growth rate particularly in males. Male rabbits fed the fish oil diet showed the highest total omega-3 (ω-3) fatty acids, lowest ω-6/ω-3 and ARA/EPA ratios. Rabbits fed the DHA diet had highest total saturated fatty acids and lowest values of total ω-6 in both genders. DHA/ARA diet supported highest 20:4 (ARA), 22:6 (DHA) in both genders and least total saturated fatty acids in males. Effect of treatment of dietary lipid was not significant among different gender and ω-6/ω-3 ratios of the rabbits treated with FO, DHA and DHA/ARA were within the recommended range of 4. ALP activity was significantly lowest in fish oil group. Liver treated with DHA and DHA/ARA showed very mild inflammation, and those with sesame oil showed patchy moderate micro vesicular steatosis. The rabbit livers treated with soya bean oil and fish oil showed no significant abnormality. However, granuloma, hepatocellular necrosis or fibrosis was absent in all groups. In summary, evidence of pathological damage in the rabbit livers was very limited to mild patchy micro vesicular steatosis, mild patchy chronic inflammation and few scattered eosinophil. This study shows that long-term supplementation of dietary lipids especially fish oil has beneficial effect on the fatty acid profile, ALP activity and histopathology of liver of rabbits.

Keywords: Lipids; fatty acids;ω-6/ω-3 ratios; arachidonic acid; histology; fish oil.).

INTRODUCTION

Dietary lipids are considered as macronutrients that provide energy, facilitate the absorption of fat soluble vitamins, involved in the storage and transport of energy for insulation and for mechanical protection (Manchila-Caravalho and Crews, 1990). Lipids consist of three groups of fatty acids which include saturated, monounsaturated and polyunsaturated. Saturated fatty acid contains no double bond, monounsaturated fatty acid contains 1double bond and polyunsaturated fatty acids (PUFA) contain 2 to 6 double bonds. Long chain polyunsaturated fatty acids (LCPUFAs) of ω-6 arachidonic acid (ARA, 20:4) and ω-3 eicosapentaenoic acid (EPA, 20:5) and docosahexaenoic acid (DHA, 22:6) families are synthesized in the liver, brain and retina from their respective PUFAs, linoleic acid (LA, 18:2, ω-6) and α-linolenic acid (ALA, 18:3, ω-3) via a series of alternating desaturation and elongation steps (Sauerwald *et al*., 1997). The modern western diet tends to contain too high ω-6 / ω-3 ratios. Increasing the amount of ω-3 or reducing ω-6 in diets will lead to more favorable ω-6 to ω-3 ratios. EPA, DHA and ARA are biologically very significant as they are elements of structural lipids that affect the activities of membrane linked functional molecules such as receptors, enzymes and transporters (Fernstorm, 2000) and signal transduction mechanisms (Decsi and Koletzko, 1994). Linoleic acid (LA) and ARA are important for growth, protection against infection and liver and kidney function (Carlson, 1997). Linoleic acid and α-linolenic acid (essential fatty acids) acts as precursors of LCPUFAs and as a substrate for production of prostaglandin. They are either oxidized or stored in the adipose tissue as such or were converted into LCPUFA at the microsomal level (Mohrauer and Holman, 1963). LA and ALA (essential fatty acids) acts as precursors of LCPUFAs and act as a substrate for production of prostaglandin. Biosynthesis of fatty acid occurs in all animals tissues but the major sites are generally considered to be adipose tissue, liver and lactating mammary glands (Volp and Vagelos, 1976).

Rabbit's meat has great nutritional value and is highly valuable because of its dietary properties such as low fat content, less saturated fatty acids and cholesterol

than other meats (Hernandez, 2008) and other characteristics such as rapid growth rate, high reproductive potential, short generation interval (Amaravadhi *et al.,* 2012). Feeding of dietary lipids has very important impact on quality of meat and body composition of non-ruminants species including rabbits and is influenced by the composition of diet (Oliver *et al.,* 1997). Studies have shown that when rabbits are fed with different fatty acid supplements for a month, the fatty acid profile of rabbit muscles was effectively modified (Hernandez *et al*., 2000). To our knowledge hardly any comparative study is available which shows effect of dietary consumption of vegetable, fish and microalgae oils on the fatty acid profile and histopathology of rabbit's liver. The purpose of this study is to investigate the long term effect of consumption of different dietary oil (vegetable, fish and microalgae oils) sources with varying ω-6/ω-3 PUFAs on the fatty acid profile and histopathology of rabbits liver and to determine the role of gender to respond to the variations.

MATERIALS AND METHODS

Animals and Care: The experiment was conducted on forty-five weanling (25 male and 20 female) New Zealand white rabbits (6-wk-old, weighing 500–1000 g), obtained from Experimental Animal Care and Experimental Surgery Center at the Faculty of Medicine, King Saud University, Riyadh, Saudi Arabia. All procedures for the use and care of animals for laboratory research were in accordance with the University Committee for Animal Use and Care at King Saud University. They were randomly divided by weight into five groups (5 males and 4 females in each treatment). The weight difference between the rabbits of each group did not exceed ± 100 g. All rabbits were individually housed in stainless steel cages under controlled temperature (25 ± 2 °C) and relative humidity (50±5%), with a 12-h light/dark cycle. Food and drinking tap water were offered *ad libitum* throughout the experimental period of 100 days. Food cups were refilled every two days, and food provided and the left over were weighed to calculate daily food consumption.

Diets Formulation and Preparation: Basal diet was obtained from the Arabian Agricultural Services Company (ARASCO), Riyadh, Saudi Arabia; which was prepared in accordance with its specification for rabbit feed (47152- Rabbit 18/14 Pellet, without fat). Five oils, namely soy bean oil (SBO, from a local market in Cairo, Egypt), fish oil (FO, from Huatai Biopharm, Inc., Deyang, China), Docosahexaenoic acid (DHA) 40% + eicosapentaenoic acid (EPA) 30%, sesame oil (SO, from Horse Factory for Food Products, Riyadh, Saudi Arabia), and two types of marine brown microalgae oils of the genus *Crypthecodinium cohnii*; and that is 40% DHA (from Huatai Biopharm, Inc., Deyang, China,) and 40% ARA (from Nutrakey Industries, Inc., Qingdao, China) were used in this study. All oils were kept refrigerated at 4°C until used in the preparation of diets. The experimental diets were prepared by adding the oil blend to the basal diet (70 g/kg diet) as follows:

SBO diet--- 70 g soy bean oil/kg diet
FO diet--- 50 g fish oil + 20 g soy bean oil
SO diet--- 50 g sesame oil + 20 g soy bean oil
DHA diet--- 50 g DHA oil + 20 g soy bean oil
DHA/ARA diet--- 25 g DHA oil + 25 g ARA oil + 20 g soy bean oil.

Oils were added into the basal diet by spraying under pressure with continuous mixing during the spraying. Fresh diets were mixed weekly to avoid oil oxidation and kept refrigerated at 4 °C until fed. The diets provided 7% fat (70 g/kg diet) which is adequate for growing rabbits (Reeves, 1997). The feed composition data are given as footnote to Table 1.

Growth: Body weight of the rabbits was recorded in the non-fed state at the beginning of the study (initial weight) and at time before slaughter (final weight). Weight gain (final body weight (g) − initial body weight (g)) and growth rate (total weight gain (g) /100 days study period) were calculated.

Sampling and sample storage method: After 100 days, rabbits were food deprived over-night; 10 ml of blood was collected from non-anesthetized rabbit via cardiac puncture in vacutainer heparinized tube and centrifuged at 1753*g* for 8 min at 4 °C to obtain the plasma. Plasma was stored in an eppendorf tube at 4 °C until analyzed for alkaline phosphatase (ALP). After blood was collected, the rabbits were immediately slaughtered according to the procedures of the Experimental Animal Care and Experimental Surgery Center at the Faculty of Medicine, King Saud University, Riyadh, Saudi Arabia).

Fatty acid analysis: Liver samples were homogenized at 4 °C (Bench top Homogenizer 300 DS PRO Scientific, Inc., Oxford, CT, USA). Total lipids from 1 g of the homogenized tissue were extracted according to Folch, *et al.*, (1957). Extracted lipids and/or oil blend samples were trans methylated to obtain fatty acid methyl esters (FAMEs), using 14% boron trifluoride in methanol Bligh and Dyer (1959). FAMEs were separated by gas chromatography (GC Clarus 500, Perkin Elmer, Shelton, WA, USA), using an Omegawax™ 320 capillary column (30 m × 0.32 mm i.d × 0.25 µm film thickness, Supelco, Inc., Bellefonte, PA, USA) at oven temperature, 200 °C; carrier gas, helium 25 cm/s at 200 °C; detector, flame ionization (FID) 260 °C; injection, 1 µl split 100:1 at 250 °C. FAMEs (C14–C22) from liver samples and oil blends were identified by comparison with retention times of standard fatty acids (Supelco, Inc., Bellefonte, PA, USA) PUFA-2, animal source, and FAME Mix RM-

1, Oil Reference(Sigma–Aldrich, St. Louis, MO, USA), respectively; and fatty acid concentration (w/w) was calculated as percent in total fatty acids.

ALP activity was measured (in duplicate) according to Bowers and McComb (1966) using commercial colorimetric/kinetic method kits (United Diagnostics Industry (UDI), Dammam, Saudi Arabia).

Histological studies: At the end of the experiment, randomly the liver of three rabbits from each treatment (irrespective of gender) was removed and rinsed with normal saline and dried using lint free paper towel. A section was taken and fixed in 10% formalin, then embedded in paraffin and sectioned at 5 µm. Sections were stained with Hematoxylin-eosin and evaluated under a light microscope (Olympus, Tokyo, Japan). The liver tissue was examined under the light microscope for acute and chronic portal tract inflammation, acute and chronic lobular inflammation, presence of eosinophil's, foreign body multinucleated giant cells, granulomas, necrosis, steatosis (macro vesicular and micro vesicular) and fibrosis. All the mentioned parameters were scored in a range from 0 to 4 (0= negative, 1= focal mild, 2= focal moderate, 3= diffuse moderate and 4= severe).

Statistical analysis: Data were analyzed using SPSS statistical software package (version 22) and expressed as mean ±standard deviation. The differences among the dietary treatment groups were analyzed by ANOVA at a significance level of $p \leqslant 0.05$; if significant differences were found, a Post-hoc analysis using Duncan's multiple range tests was performed.

RESULTS AND DISCUSSION

Fatty acid profile of the formulated diet: The fatty acid profile of the formulated diet is shown in Table 1. Total saturated fatty acid contents of the formulated diet were highest for DHA (23.59g/ 100 g total fatty acid) and lowest for FO group (3.80 g/100g total fatty acid). The SO diet had the highest ω-6/ω-3 ratio (21.75 g/100g total fatty acid) and FO diet had lowest (0.39 g/100g total fatty acid) and contained highest amount of EPA and DHA (20.4 and 29.8 g/100g of total fatty acid, respectively). Whereas the SBO diet had an intermediate ω-6/ω-3 ratio and contained highest amounts of LA and ALA (61.6 and 7.1 g/100 g of total fatty acid respectively). Total MUFA was highest in SO diet and lowest in DHA (8.09g/100g of total fatty acid). The amount of LA and ALA ranged from 17-61.6 and 2.1-7.1 g/100 g of total fatty acid respectively. ARA, EPA and DHA ranged from 0.9-1.5, 0.6-20.4 and 22.8-29.8 g/100 g of total fatty acids respectively. SBO, SO and FO diets were devoid of GLA and DTA was not observed in any treatment. Similarly SBO and SO diets were devoid of ARA (ω-6 PUFA) and EPA and DHA (ω-3 PUFA), respectively.

Growth: The growth of male rabbits was significantly ($p \leqslant 0.05$) affected by the dietary treatment. Long term dietary supplementation with various dietary lipids had a distinct effect on final body weight; weight gain and growth rate (Fig. 1. A and B). These growth indicators were significantly higher in SBO and SO groups and significantly lower in DHA group. Decreased food intake might be the reason for the reduction in growth indicator of rabbits fed with DHA diet. Whether the decrease in food intake was because of decreased palatability of the algae oils diet or intake of particular fatty acids remain unknown, further studies in this area is required. Previously dietary lipids was found to be ineffective on growth indicators in male rabbits (Guillevic *et al*., 2009, Lobo *et al*., 2009; Sirosis *et al*., 2003) which might be related to short duration (15-63 days) of the study period. Other reasons might be related to the type, amount and variations in oil blends used in various studies. Wainwright *et al*. (1997) observed reduced growth of mouse pups after dietary supplementation with up to 9% DHA (w/w), regardless of dietary ARA or total ω-6/ω-3 ratios. In the present study, diets showed no effect on growth of female rabbits which is in agreement with findings of Sirosis *et al*., (2003). Significant differences were observed in growth indicators of male rabbit but not in female rabbits although both of them were using similar diet. Further studies are required to know the mechanism behind this difference.

Effect of treatment on the fatty acid profile of rabbit's liver: Effect of dietary treatments on the fatty acid profile of rabbit's liver has been shown in Table 2. Compared to SBO (control group), rabbits fed the FO diet had highest concentration of EPA (20:5, ω-3) in both males and females. Lowest value of DHA (22:6, ω-3), total saturated fatty acids, ω-6/ω-3, and ARA/EPA in liver samples from female rabbits were recorded. Male rabbits fed the FO diet showed the highest total ω-3 fatty acids and lowest ω-6/ω-3 and ARA/EPA ratios. Diets containing SO resulted in lowest total ω-3 fatty acids in both males and females. It showed lowest value of total PUFA, C14:0, C16:0, C18:3 (ω-6), C20:4 (ω-6), total saturated fatty acids in females and highest values of total MUFA, C16:1, C18:1 (ω-9) and ω-6/ω-3 in males. Rabbits fed the DHA diet had highest values of C16:0, total saturated fatty acids and lowest values of C14, C18:1(ω-9), C18:2 (ω-6), C18:3(ω-3), total MUFA, total ω-6 in both males and females. The results revealed highest C14:0 and lowest C16:1 in females and lowest total PUFA, C20:4 (ω-6) in males. Rabbits fed the DHA/ARA diet had highest 20:4 (ω-6), 22:6 (ω-3) in both males and females and highest 18:0, total ω-3 in females and lowest C16:1, total saturated fatty acids in males. However, the rabbits fed the control diet had highest total PUFA, C18:2, C18:3 (ω-6), C18:3 (ω-3), total ω-6 in both males and females, highest C14:0,

C18:0, C22:4 (ω-6) in males, C16:1, C18:1 (ω-9), total MUFA in females and lowest C16:0 in males.

Palmitic acid contributed to the major part of saturated fatty acid and myristic acid showed the least concentration with all treatments. Palmitic acid is most abundant saturated fatty acids in human diet. Potency of myristic acid which is the most cholesterolaemic fatty acids (Hayes and Khosla, 1992) has been expected to be 4 times that of palmitic acid. In a previous study it has been shown that myristic and palmitic acid were less influenced by the diet than the oleic acid (Peiretti, 2012). The present study showed that various dietary oil sources differing in their ω-6/ω-3 ratios significantly altered the fatty acids level of liver. Moreover liver fatty acid profile reflected the dietary level of ω-6 and ω-3 fatty acids fed to rabbits. PUFA intake has a greater influence on liver function. PUFA decreases expression of hepatic genes encoding glycolytic and lipogenic regulatory enzymes involved in flux of glucose to fatty acids (Giudetti *et al*., 2003). Saturated fatty acids and MUFAs are synthesized in vivo and as a result are not as much influenced by the diet than PUFA such as linoleic acid (18:2 ω-6) and alpha linoleic acid (18:3, ω-3) which to some extent reflect the dietary profile of oil (Enser *et al*., 2000). Fatty acid composition including chain length, extent of unsaturation, and relative concentration of the specific lipid moieties determines the extent to which dietary lipids affect cell structure and metabolism (Bajranti *et al*., 1994).

Rabbits fed the FO diet (lower in ω-6/ω-3 ratio and higher in EPA) maintained lower ω-6/ω-3 ratio and higher EPA concentrations in their liver. Rabbits fed the SO diet (higher in ω-6/ω-3 ratio) maintained higher ω-6/ω-3 ratio. Those fed the SBO control diet (higher in LA and ALA) maintained higher LA and ALA concentrations in liver. Furthermore this study showed that liver fatty acid profile in rabbits was influenced by lipids source in the diet. However there was no effect of different dietary ω-6/ω-3 ratios on liver LA, ALA or DHA concentrations. An inverse relationship was found between ω-6/ω-3 ratios and EPAs concentrations. No significant difference ($p \leqslant 0.05$) was found between males and females rabbits (Table 2). It is known that high ω-6/ω-3 ratio is a risk factor of cancer and coronary heart disease (Assmann *et al*., 1999). In the present study the ω-6/ω-3 ratio of soybean and sesame oil was markedly higher than the recommended value of 4, and studies shows that higher ω-6/ω-3 ratio have bad effect on human health (Wood *et al.,* 2003).The ω-6/ω-3 ratio of FO, DHA and DHA/ ARA in the experimental diets was found to be within the recommended range. The increase in dietary long chain ω-3 PUFA, achieved for instance by adding marine fat sources to the diet, has been reported to be more effective (Wood and Enser, 1997). The composition of biological tissues can be altered through diet, either by direct incorporation of the absorbed compounds or by their interactions with anabolic and catabolic pathways. If ω-6/ω-3 ratio of diet is reduced, this generates eicosanoids with more beneficial effects in some chronic diseases than those derived from ω-6 fatty acids (Simopoulos, 1999; Siddiqui *et al*., 2008). Different dietary ω-6/ω-3 ratios had pronounced effect on the liver EPA, total ω-3, ω-6/ω-3, ARA/EPA and ARA/DHA ratios in the present study. As the dietary ω-6/ω-3 ratio increased from 0.4 to 21.8, the concentration of EPA and total ω-3 declined. This study confirmed that different dietary oil sources with varying ω-6/ω-3 ratios significantly altered the fatty acid profile of liver. Oliver *et al*.,(1997) showed the effect of the composition of the diet on the fatty acid composition of rabbit fat. Consumption of EPA and DHA could have different effect on liver. In a study by Giudetti *et al*.,(2003) the supplementation of Wistar rats with EPA or DHA for 1-4 weeks, EPA treatment increased EPA and DPA content in plasma, while DHA treatment mainly increased plasma DHA concentration. But the same treatment decreased arachidonic acid percentage and ω-6/ω-3 PUFA ratio. Rabbits fed SBO or SO diets containing LA and ALA with no ARA, EPA and DHA had higher levels of LA and ALA, yet a lower level of ARA and no EPA and DHA in liver in contrast those fed FO, DHA or DHA/ARA diets supplemented with ARA, EPA and DHA had higher level of these fatty acids in liver compared to SBO and SO groups. LA and ALA in meat are mostly affected by diet because other fatty acids can be synthesized in the body of rabbits. The concentrations of ARA, EPA and DHA found in tissue phospholipids are the net result of the rates of endogenous synthesis from LA and ALA and the amount preformed ARA, EPA and DHA in the diet (Innis, 2000). Furthermore there were preferential uptake of preformed ARA, EPA and DHA compared with the biosynthesis route (Crawford, 2000). Therefore the absence of EPA and DHA in liver of rabbits fed SBO and SO diets and depletion in ARA was presumably due to low and poor efficiency of elongation and desaturating enzymes in the growing rabbits. Thus it can be concluded that feeding of the diet with preformed ARA, EPA is more effective to enrich liver tissues than to supplements with precursors in rabbits. The effect of dietary lipids on fatty acid synthesis in hepatic and adipose tissue appears to depend upon the composition of the lipids. In a previous study by Mitchaothai, (2007) it was found that the concentration of alpha- linolenic acid in the diet and adipose tissue may not be strongly correlated especially when the level in the diet is very low. Results from the present study showed that supplementation with high concentration of DHA from fish oil resulted in increased DHA in rabbit's liver.

In this study the concentration of ARA was negatively correlated with EPA (r^2 =-0.013), but positively correlated with DHA (r^2 = 0.664). ARA and EPA compete for the same biosynthetic enzyme systems

(Whelan, 1996) with substrate preference of ARA over EPA (Lands, 1992). However, Croft *et al*.,(1998) reported antagonistic effect of EPA on ARA in leukocytes of rats. Molnar, (2012) suggested that the ratio of ARA to ω-3 PUFAs (EPA and DHA) in human diets is also an important factor. Nevertheless the issue whether dietary ARA is harmful or not is unequivocal since ARA has both pro and antithrombotic and inflammatory effects (Netleton, 2008).

Alkaline phosphatase (ALP) is present in a number of tissues including liver, placenta, bone, and intestine. A rise in ALP activity occurs with all forms of cholestasis. The response of the liver to any form of biliary tree obstruction is to synthesize more ALP. ALP activity was significantly highest in ARA/ DHA group and lowest in FO group (Fig.2). In a study treatment with lead acetate significantly ($p \leqslant 0.05$) increased serum AST, ALT, ALP compared to the control but the concentration was normalized after treatment with either of the two doses of Omega-3 (125 mg/kg or 260 mg/kg body weight) in combination with lead acetate (Abdou and Hassan, 2014).

Effect of treatment on the histopathology of rabbit's liver: Lipid metabolism is mainly regulated by the liver, including both the synthesis and degradation of fatty acids, and several enzymes regulating these pathways show varying affinities for the different fatty acids available in the organ (Henderson 1996), thus imbalances in the dietary fatty acids could modify the functioning and morphology of this organ. Rabbit liver is divided into hepatic lobules formed of radially arranged strands of hepatocytes (polygonal in shape with eosinophilic granular cytoplasm and vesicular basophilic nuclei) that extend from the central vein to periphery of the lobule and hepatocytes are bi nucleated. The overall histopathological study of the treated rabbit livers showed very few pathological findings, ranging from (1) minimal patchy chronic inflammatory infiltrate of mainly lymphocytes in the portal tracts and the hepatic lobules, (2) focal micro vesicular steatosis, (3) occasional samples showed eosinophil's and (4) foreign body giant cells. Foreign body giant cells are multinucleated giant macrophages which form in response to any foreign material/drug introduced in the tissue. The eosinophil too is most likely in response to the foreign material/ drug. The rabbit livers treated with soya bean oil showed no abnormality (Fig. 3A). Liver tissue treated with DHA algae oil showed mild chronic lymphocytic inflammation in the portal tracts and hepatic lobules with few scattered eosinophil's (Fig. 3B and 3C). Liver treated with sesame oil showed patchy moderate micro vesicular steatosis (Fig. 3D) with scattered rare eosinophil and lymphocytes. The DHA/ARA treated liver showed a mild patchy chronic inflammatory infiltrate in the portal tracts and in the hepatic lobules and few eosinophil (Fig. 3E). Liver of rabbits fed diet supplemented with fish oil also did not show any abnormality. In summary, evidence of pathological damage in the rabbit livers was minor and limited to mild patchy micro vesicular steatosis and mild patchy chronic inflammation, few scattered eosinophil's and few foreign body multinucleated giant cells. There was no granuloma, hepatocellular necrosis or fibrosis noted in any of the sampled liver tissue. Morphological studies have revealed that EPA increases the area fraction of both mitochondria and peroxisomes, reducing the number of lipid droplets in the liver (Caballero *et al*., 2004). Chen *et al*., (2003) demonstrated that emulsions that were derived from fish oil and were high in omega-3 led to a lower hepatic fat content in rats that received total parenteral nutrition than emulsions that were derived from olive and safflower oils. A 12-week feeding trial was conducted to evaluate the effects of fish oil replacement by soybean oil, on lipid distribution and liver histology of two commercially important finfish species. These data suggest that both sea bass and trout can be fed diets containing up to 50% soybean oil without adverse effects on tissue lipid composition or liver histology (Silva *et al.*, 2005). Hassan *et al*., (2012), recorded that fish oil supplementation reduced mortality rate, hematological changes, hepatic biochemical alterations, and improved immune status of guinea pigs. In sea bream, steatosis has been observed as a result of the inclusion of vegetable oils (Alexis, 1997), although its effect in the correct functioning of the liver and its possible reversibility is not well understood. If the majority of the fat droplets that are visible in formaldehyde fixed and paraffin embedded section is smaller or bigger than the nucleus of hepatocytes, this liver is said to be micro or macro steatotic respectively (Crowley *et al*., 2000). In this study it has been suspected that low dietary PUFA might be responsible for little abnormality found in the histology of the liver. A beneficial effect of omega 3 PUFA has been observed in this study as liver of rabbits fed diet supplemented with fish oil (higher in omega 3 and lower in ω-6/ω-3 ratios) did not show any abnormality.

Table 1. Fatty acid composition (g/100 g total fatty acids) of the experimental diets.

Fatty acids[2]	Experimental Diets[1]				
	SBO	**FO**	**SO**	**DHA**	**DHA/ARA**
Saturated fatty acids (SAT)					
C14:0	0.05	0.09	0.03	2.51	22.910
C16:0	5.87	1.96	10.16	18.94	8.370
C18:0	2.79	1.75	6.04	1.95	1.150
C20:0	0.38	-	0.59	0.19	0.240
Monounsaturated fatty acids (MUFA)					
C16:1 ω-7	0.02	0.12	0.12	0.67	0.550
C18:1 ω-7	-	1.04	-	7.33	5.060
C18:1 ω-9	24.33	11.35	35.40	0.02	4.990
C20:1 ω-9	0.23	1.31	0.15	0.07	ND
ω-3 Polyunsaturated fatty acids (ω-3 PUFA)					
C18:3 ALA	7.09	2.41	2.07	2.14	3.020
C20:5 EPA	-	20.44	-	0.67	0.520
C22:6 DHA	-	29.77	-	28.31	22.80
ω-6 Polyunsaturated fatty acids (ω-6 PUFA)					
C18:2 LA	61.55	18.89	45.02	18.24	17.00
C18:3 GLA	-	-	-	0.14	0.100
C20:4 ARA	-	1.49	-	1.16	0.890
C22:4 DTA	-	-	-	-	-
Total					
Total SAT	9.09	3.80	16.82	23.59	12.67
Total MUFA	24.58	13.82	35.67	8.09	10.60
Total ω-3	7.09	52.62	2.07	31.12	26.34
Total ω-6	61.55	20.38	45.02	19.54	17.99
Ratios					
ω-6/ ω-3	8.68	0.39	21.75	0.63	0.680
ARA/DHA	-	0.05	-	0.04	0.040

[1]The basal diet contained the following (g/kg): Corn-150; barley-106; wheat bran-200; soy meal-162; limestone-2.8; alfalfa- 370.5; cholinechloride60%,-0.6%, methionine powder-1; dicalcium phosphate 18%-5.1;vitamin and mineral mix-2. The experimental diet included SBO-soybean oil (control), FO-fish oil; SO- sesame oil; DHA- DHA algae oil; DHA/ARA-(DHA+ARA algae oils, 1:1 ratio).
[2] SAT: saturated fatty acids: MUFA- monounsaturated fatty acid; PUFA-polyunsaturated fatty acids; ALA-alpha liniolenic acid; EPA- eicosapentaenoic acid, DHA- docosahexaenoic acid; LA- linoleic acid; GLA- gamma linolenic acid; ARA- arachidonic acid, DTA-docosatetraenoic acid.

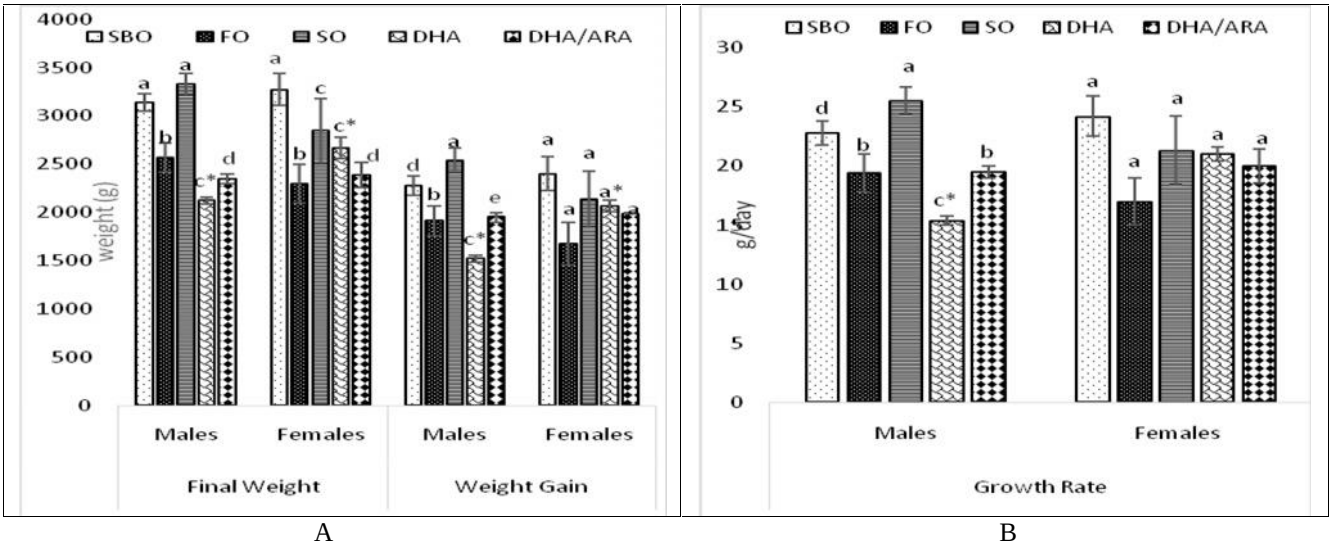
Table 2. Effect of treatments on the fatty acid profile of liver of rabbits.

Fatty acids[2]	Dietary treatment[1]									
	SB0		**FO**		**SO**		**DHA**		**DHA/ARA**	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
C14:0	0.657± 1.24^{a}	0.48± 0.49^{a}	0.23± 0.08^{a}	0.28± 0.170^{a}	0.42± 0.33^{a}	0.19± 0.25^{a}	0.14± 0.18^{a}	0.68± 0.64^{a}	0.19± 0.04^{a}	0.33± 0.07^{a}
C16:0	12.51± 2.78^{a}	15.97± 3.16^{a}	28.66± 9.50^{c}	17.06± 10.19^{a}	12.82 ±7.39^{a}	9.82± 7.66^{a}	33.35± 25.74^{b}	47.96± 25.91^{b}	20.07± 4.70^{c}	25.1± 3.42^{a}
C16:1 (ω -7)	0.31± 0.24^{a}	0.60± 0.49^{a}	0.29± 0.20^{a}	0.38± 0.30^{a}	0.63± 0.49^{a}	0.08± 0.13^{a}	0.41± 0.87^{a}	0.08± 0.13^{a}	0.14± 0.12^{a}	0.21± 0.04^{a}
C18:0	20.73± 2.23^{a}	19.13± 2.72^{a}	18.59± 3.69^{a}	12.46± 7.40^{a}	19.39± 7.462^{a}	13.79± 10.39^{a}	20.51± 12.63^{a}	15.72± 8.08^{a}	11.57± 7.01^{a}	20.81± 3.15^{a}
C18:1 (ω -9)	9.39± 1.76^{e}	10.90± 4.25^{b}	5.20± 1.58^{d}	4.72± 3.15^{ab}	13.75± 6.36^{c}	7.31± 5.72^{ab}	1.47± 2.11^{a}	2.40± 1.44^{a}	1.83± 0.14^{b}	2.73± 0.45^{a}
C18:1 (ω -7)	0.88± 0.71^{b}	0.95± 0.40^{a}	2.24± 0.47^{c}	1.69± 0.99^{a}	0.43± 0.61^{b}	0.23± 0.35^{a}	0.06± 0.12^{a}	0.27± 0.42^{a}	1.12± 0.50^{b}	1.95± 3.77^{a}

C18:2	35.10±	34.31±	10.13±	8.42±	31.72±	17.18±	4.23±	5.63±	4.57±	5.96±
(ω -6)	3.94^{b}	5.47^{b}	2.17^{a}	5.01^{a}	12.367^{b}	17.26^{a}	4.29^{a}	3.20^{a}	1.43^{a}	0.51^{a}
C18:3	0.20±	0.17±	0.13	0.11±	0.18±	0.03±	0.01±	0.04±	0.11±	0.17±
(ω -6)	0.11^{a}	0.11^{a}	±0.18^{a}	0.22^{a}	0.20^{a}	0.08^{a}	0.02^{a}	0.10^{a}	0.09^{a}	0.08^{a}
C18:3	1.28±	1.142±	0.82±	1.12±	0.7±	0.87±	0.07±	0.11±	0.34±	0.38±
(ω-3)	0.22^{c}	0.19^{b}	0.43^{d}	0.87^{b}	0.80^{d}	1.16^{ab}	0.14^{a}	0.17^{a}	0.25^{ab}	0.19^{ab}
20:1	0.02±	0.05±	0±	0±	0.06±	0±	0±	0±	0±	0±
(ω -9)	0.03^{a}	0.11^{a}	0^{a}	0^{a}	0.12^{a}	0	0^{a}	0	0^{a}	0^{a}
20:4	6.62±	5.58±	7.78±	5.52±	4.19±	3.68±	2.93±	7.18±	20.52±	18.33±
(ω -6)	2.26^{d}	2.18	3.30^{b}	3.24^{a}	3.13^{d}	3.67^{a}	3.23^{a}	3.86^{a}	2.78^{c}	2.69^{b}
20:5	0±	0±	4.71±	5.75±	0±	0±	0.25±	0.022±	0.09±	0.16±
(ω -3)	0^{a}	0^{a}	4.25^{b}	6.26^{b}	0^{a}	0^{a}	0.56^{a}	0.04^{a}	0.17^{a}	0.23
22:4	0.22±	0.15±	0±	0±	0.09±	0±	0±	0±	0.16±	0±
(ω -6)	0.32^{a}	0.30^{a}	0^{a}	0^{a}	0.26^{a}	0^{a}	0^{a}	0^{a}	0.31^{a}	0^{a}
22:6	0±	0±	8.96±	5.73±	0±	0±	5.07±	11.37±	11.64±	15.63±
(ω -3)	0^{a}	0^{a}	8.30^{d}	6.29^{ab}	0^{a}	0^{a}	9.05^{ab}	11.60^{d}	12.25^{c}	9.19^{c}
	33.897±	35.58±	47.48±	29.8±	32.63±	29.8±	54.00±	64.36±	31.83±	46.24±
Total Sat	10.09^{a}	9.98^{a}	14.41^{a}	8.67^{a}	9.63^{a}	9.80^{a}	16.74^{a}	24.16^{b}	9.97^{a}	13.23^{c}
Total	10.6±	12.5±	7.73±	6.79±	14.81±	7.62±	1.94±	2.75±	3.09±	4.89±
MUFA	4.50^{e}	5.19^{b}	2.39^{c}	2.14^{c}	6.70^{d}	3.60^{c}	0.68^{a}	1.14^{a}	0.86^{b}	1.33^{c}
Total	43.42±	41.35±	32.53±	26.64±	36.88±	21.76±	12.55±	24.35±	37.43±	40.64±
PUFA	29.89^{b}	27.62^{c}	2.51^{b}	1.02^{c}	25.09^{b}	14.15^{c}	1.25^{a}	0.95^{c}	9.39^{b}	5.85^{c}
Total	42.14±	40.21±	18.04±	14.04±	36.18±	20.89±	7.16±	12.85±	25.36±	24.46±
ω -6	16.65^{c}	16.37^{b}	5.22^{b}	4.16^{a}	15.23^{c}	8.15^{a}	2.13^{a}	3.74^{a}	25.36^{b}	24.46^{c}
Total	1.28±	1.14±	14.49±	12.6±	0.7±	0.87±	5.39±	11.50±	12.07±	16.18±
ω -3	0.20^{a}	0.20^{a}	1.38^{b}	0.19^{b}	0.65^{a}	0.70^{a}	6.43^{a}	7.87^{b}	1.54^{b}	3.95^{b}
	32.92 ±	35.21±	1.24±	1.12±	51.68±	24.01±	1.33±	1.12±	2.1±	1.51±
ω-6/ω-3	3.70^{a}	3.87^{b}	0.32^{a}	0.37^{a}	83.86^{b}	16.18^{b}	15.69^{a}	1.43^{a}	0.15^{a}	0.04^{a}
			1.65±	0.96±			11.72±	326.36±	228±	114.56±
ARA/EPA	-	-	0.58^{a}	0.50^{a}	-	--	39.76^{b}	187.50^{b}	61.61^{c}	54.11^{c}
			0.86±	0.96±			0.577±	0.631±	1.762±	1.172±^{a}
ARA/DHA	-	-	0.37^{a}	0.34^{a}	-	-	5.97^{a}	0.355^{a}	0.07^{a}	0.05^{a}

[1]Data are expressed as mean± standard deviation. SBO-soybean oil (control); FO-fish oil; SO- sesame oil; DHA-DHA algae oil; DHA/ARA- (DHA+ARA algae oils, 1:1 ratio). $^{a-e}$ small letters indicate significant difference among dietary treatment groups for each gender separately as indicated by ANOVA followed by Duncan's multiple range test (a>b>c>d>e).

[2]Total sat-total saturated fatty acids, MUFA-Monounsaturated fatty acids, PUFA-Polyunsaturated fatty acids, ARA- Arachidonic acid, EPA-Eicosapentaenoic acid, DHA-Docosahexaenoic acid.

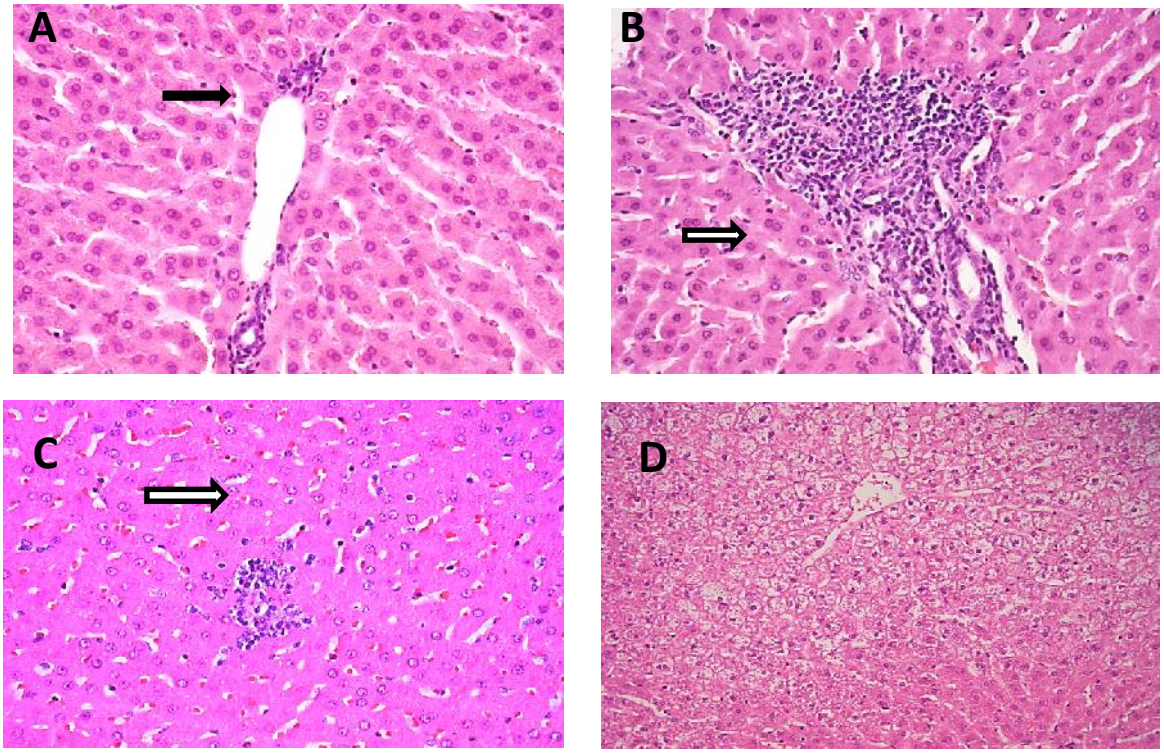


A B

Fig. 1. Growth indicators of males and females rabbits fed with different dietary oil sources .

Data are expressed as mean± standard deviation. SBO-soybean oil (control); FO-fish oil; SO- sesame oil; DHA- DHA algae oil; DHA/ARA-(DHA+ARA algae oils, 1:1 ratio). [a-e] small letters indicate significant difference among dietary treatment groups for each gender separately as indicated by ANOVA followed by Duncan's multiple range test (a>b>c>d>e).

Fig. 2. ALP activity of males and females raabits fed with different dietary oil sources .
Data are expressed as mean± standard deviation. SBO-soybean oil (control); FO-fish oil; SO- sesame oil; DHA- DHA algae oil; DHA/ARA-(DHA+ARA algae oils, 1:1 ratio). [a-e] small letters indicate significant difference among dietary treatment groups as indicated by ANOVA followed by Duncan's multiple range test (a>b>c>d>e).



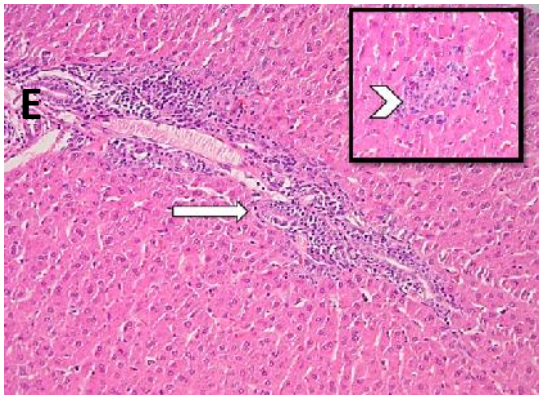


Fig.3. Photomicrograph of rabbit liver treated with different dietary lipids

A: Photomicrograph of rabbit liver treated with soya bean oil (control) with no pathological changes. The portal tract (arrow) and the surrounding hepatocytes of the hepatic lobule appear unremarkable. **B**: Photomicrograph of rabbit liver treated with DHA algae oil shows mild chronic lymphocytic inflammation of the portal tract (arrow). **C:** Photomicrograph of rabbit liver treated with DHA algae oil shows a mild chronic inflammation of the hepatic lobules (arrow). **D**: Photomicrograph of rabbit liver treated with sesame oil shows patchy areas of moderate micro vesicular steatosis. Normal looking hepatocytes can be seen at the lower right portion of the image.**E**: Photomicrograph of rabbit liver treated with DHA and ARA algae oil shows mild chronic lymphocytic inflammation in the portal tract (arrow) and in the hepatic lobules (arrow head in the inset).

Conclusion: The current study clearly demonstrated that long term supplementation of dietary lipids has tremendous effect on the fatty acid profile of liver in rabbit irrespective of gender. FO, DHA and DHA/ARA groups supported the ω-6/ω-3 ratios in liver within the recommended range. The concentration of ARA was negatively correlated with EPA and positively correlated with DHA. Highest ALP activity was found in DHA/ ARA group and lowest in FO group. On histology, evidence of pathological damage in the rabbit livers was very limited to mild patchy micro vesicular steatosis and mild patchy chronic inflammation and few scattered eosinophil. This study shows that long term supplementation of dietary lipids especially fish oil has very good effect on the fatty acid profile, ALP activity and histopathology of liver of rabbits. Algae oil supplementation might also be considered beneficial for liver function.

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