African Journal of Biotechnology Vol. 9(51), pp. 8881-8888, 20 December, 2010
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.1233
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Effects of varying levels of *n-6:n-3* fatty acid ratio on plasma fatty acid composition and prostanoid synthesis in pregnant rats

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Accepted 5 November, 2010

This study investigated the effects of varying dietary levels of *n-6:n-3* fatty acid ratio on plasma fatty acid composition and prostanoid synthesis in pregnant rats. Four groups consisting of seven rats per group of non pregnant rats were fed diets with either a very low *n-6:n-3* ratio of 50% soybean oil (SBO): 50% cod liver oil (CLO) 1:1 group , a low ratio of 84% SBO: 16% CLO 6:1 group, a high ratio of 96% SBO: 4% CLO 30:1 group and control group was given only rat chow diet. Blood samples were taken at day 15 post mating and the plasma was analyzed for fatty acid profile, specifically the *n-6:n-3* fatty acid ratio and prostaglandins F2α and E2. The *n-3* polyunsaturated fatty acids (PUFA) in plasma of group 1:1 were significantly ($P < 0.05$) higher than the other groups, while the *n-6:n-3* fatty acid ratio was significantly lower. The total *n-6* PUFA was significantly higher ($P < 0.05$) in group 30:1 as compared to the control and 1:1 groups. The total PGF2α and PGE2 were significantly higher ($P < 0.05$) in group 30:1 rats fed a diet high in *n-6* or *n-6:n-3* fatty acids. The diet higher in *n-6* fatty acids appear to increase arachidonic acid(AA) and prostaglandins synthesis in plasma of rats. PGE2 productions in plasma were significantly lower in rats fed diets with a lower dietary ratio of *n-6:n-3* fatty acids than in those fed diets with a higher dietary ratio. Regression analysis revealed a significant positive correlation between PGF2α and PGE2 and the ratio of *n-6:n-3*, and significant positive correlation between different ratio *n-6:n-3* on fatty acid plasma compstion and PGF2α and PGE2 concentration on plasma. These results demonstrated that the dietary ratio of *n-6:n-3* modulates PGF2α and PGE2 production. The *n-6:n-3* fatty acid ratio significantly affected plasma fatty acids profile and prostaglandin synthesis in pregnant rat.

key words: *n-6:n-3* fatty acid ratio, plasma fatty acids, prostanoid synthesis, pregnant rat.

INTRODUCTION

Polyunsaturated fatty acids (PUFAs) can be classified as omega 3 (*n-3*), omega 6 (*n-6*) and omega 9 (n-9) fatty acids and are essential in the diet, with the longer chain of *n-3* PUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) readily available from marine sources. The *n-3* PUFAs are incorporated into many cells and have recently been shown to have a significant and far reaching health benefits in a wide range of physiological systems (Bartsch et al., 1999; Simopoulos, 2002; Tapiero et al., 2002). Despite this, the average western diet has a higher ratio of *n-6:n-3* PUFAs (10:1) than is currently recommended (Abayasekara and Wathes, 1999). It is well known that PUFA plays a role in the maintenance of different physiological functions (Aaes-Jorgensen, 1961; Rivers and Frankel, 1981). Burr and Burr (1930) originally reported that certain PUFAs prevent and or cure some of the signs and symptoms

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seen in rats fed diets almost devoid of these lipid constituents. They have assumed three PUFAs: linoleic acid (18:2 *n-6*), arachidonic acid (20:4 *n-6*) and α-linolenic acid (18:3 *n-3*) to be essential for rats.

Linoleic acid (LA, 18:2 *n-6*) is the main component of fatty acids in vegetable oils such as corn, sunflower and soybean oils (Sargent 1997). More than 50% of the total fatty acids of these vegetable oils are LA. Linoleic acid can be desaturated and elongated to arachidonic acid (AA, 20:4 *n-6*) in animal tissues (Kinsella et al. 1990; Bezard et al. 1994). Arachidonic acid works as the main substrate for eicosanoid synthesis via the cyclo-oxygenase (COX) and lipoxygenase pathways (Wolf, 1982; Smith, 1992). Eicosapentaenoic acid (EPA, 20:5 *n-3*) synthesized endogenously or originating primarily from fish and fish oil, reduces the synthesis of eicosanoids through competitive inhibition of COX, as well as competing with AA for the *sn-2* position on phospholipids (Smith 1992). Supplemental dietary PUFAs may alter reproductive performance both by changing the energy status and by providing precursors for the synthesis of prostaglandins (PGs) (Abayasekara, 1999; Mattos, 2000). AA is the precursor for the production of the two series prostaglandins such as PGF2α and PGE2 via the action of cyclo-oxygenase (COX), but COX can also convert dihomo-γ-linolenic acid into 1 series PGs such as PGF1α and PGE1 (Kinsella et al., 1990) and EPA into 3 series PGs such as PGF3α and PGE3 (Sargent 1997). The 1 and 3 series prostaglandins are believed to be less biologically active than the 2 series (Gurr and Harwood, 1991; Lands, 1992) but may be produced at the expense of 2 series PGs, depending on the proportions of the various precursors present in the membrane phospholipids. The synthesis of PGE2 involves either cyclooxygenase-1 or the inducible cyclooxygenase-2, which convert arachidonic acid into PGH_2, followed by the conversion of PGH_2 to PGE_2 by cytosolic (Tanioka et al., 2000; Beuckmann et al., 2000) or membrane-associated PGE synthase (Jakobsson et al., 1999; Murakami et al., 2000).

Many studies indicate that the *n-3*: *n-6* fatty acids ratio in the diet, instead of absolute amount of *n-3* fatty acids, is the significant factor preventing eicosanoid biosynthesis from arachidonic acid. Thus, in evaluating desirable levels of dietary *n-3* fatty acids, the amounts of dietary *n-6* fatty acids may have to be adjusted with amounts of *n-3* fatty acids (Boudreau et al., 1991).

The dietary fatty acid manipulation can change the fatty acid composition of plasma and tissue. The fatty acid change in the body can affect on prostaglandins synthesis in both experimental animals and man (Mathias and Dupont, 1979). By increasing arachidonic acid in the diet, the prostaglandin synthesis increased. Prostaglandin production had been decreased by supplementaion of diet with α-linolenic acid (18:3 *n-3*) (Lokesh and Kinsella, 1985) or marine oils containing eicosapentaenoic acid (20:5 *n-3*), thereby decreasing the PGF2α synthesis (Linska et al., 2003). Blond et al. (1976) showed that linoleic can deplete arachidonic acid by preventing the Δ^6 desaturase thereby inhibiting the first stage of linolenic acid conversion to arachidonic acid. Clup et al. (1979) reported that Eicosapentaenoic acid can be efficiently incorporated into membrane phospholipids to replace arachidonic acid and act as a cyclooxygenase inhibitor, leading to decreased prostaglandin synthesis. On the other hand, increased linoleic acid in the diet, has been shown to lead to increased prostaglandin production (Epstein et al., 1982). Many studies used different levels and different types of fatty acid to evaluate the prostaglandin synthesis (Fine et al., 1981; Dupont et al., 1972). Changes in the amounts of PUFAs or their ratios may affect production of PGs in the reproductive system in both cows (Fine et al., 1981) and humans (Dupont et al., 1972). The aim of this study was two fold: 1) to investigate how the dietary ratio of *n-6:n-3* fatty acids influences the plasma fatty acid profile of regnant rat; 2) to determine correlations between arachidonic acid fatty acid plasma and 2 series of prostaglandins concentration in the plasma.

MATERIALS AND METHODS

Animals and experimental design

Twenty eight (28) female, two month old Sprague-Dawley (240 ± 20 g body weight) rats were used in this experiment. After 2 weeks of adaptation, the rats were randomly divided into four treatment groups consisting seven rats in each group. The cod liver oil was used as the main source of *n-3* PUFA [Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)] and soybean oil is the main source of n-6 fatty acids and linolinic acid (LA).The SBO and CLO were first analysed to determine the fatty acid profile and are shown in Table 1. The treatment groups include rats fed with normal rat chow diet the control (C) group, rats fed chow diet supplemented with 5% (w/w) SBO and 5% (w/w) CLO (group 1:1), rats fed chow diet added with 8.4% (w/w) SBO and 1.6% (w/w) CLO(group 6:1), and rats fed chow diet added with 9.6% (w/w) SBO and 0.4% (w/w) CLO (group 30:1). The rats were fed 7% of body weight daily and water was provided *ad libitum*. The diets were prepared daily to minimize rancidity and oxidative damage, the rats were fed once daily and the left over feeds were collected before new feeding. After 6 weeks of feeding, the stage of estrous cycle of the female rats was determined by vaginal smears taken between 8 a.m to 10 a.m daily (for 2 weeks). Cell types in the smear were subsequently examined microscopically to determine the estrous stage. The consecutive stages of the estrous cycle of cornified cells and the presence of nucleated cells; estrus, presence of only cornified cells; metestrus, presence of leukocytes and fewer cornified cells; and diestrus, mostly polymorphonuclear leukocytes (Turner and Bagnara, 1976). Vaginal smears of 28 female rats were performed every morning during for 2 weeks and unstained native material was observed using the microscope without the aid of the condenser lens. Using the 10x objective lens, it was easier to analyze the proportion among the three cellular types, which were present in the vaginal smear. While using the 40x objective lens, it was easier to recognize each one of these cellular types. Mating was done during proestrus stage by placing one adult male and one female into individual cages for 24 h. The presence of spermatozoa in the vaginal the following morning via vaginal smear was defined

Table 1. Fatty acids profile of SBO and CLO (mean ± SE; n = 3).

Fatty Acids	Code liver oil (CLO)	Soybean oil (SBO)
C14:0	8.88	0.11
C14:1	0.22	0.01
C15:0	0.74	0.02
C15:1	0.30	0.01
C16:0	18.53	11.85
C16:1	0.84	0.12
C18:0	4.22	4.55
C18:1	26.83	31.99
C18:2 *n-6*	5.90	55.40
C18:3 *n-3*	0.56	0.49
C20:5 *n-3*	15.25	nd
C22:6 *n-3*	17.73	nd
Total saturated	32.36	16.53
Total unsaturated	67.64	83.47
Total monoenes	28.20	27.58
Total PUFA *n-3*	33.54	0.49
Total PUFA *n-6*	5.90	55.40
n-6:n-3 Ratio	0.17	112.82
Unsat: Sat	2.12	5.05
Poly: Sat Ratio	1.25	3.38

∑SFA = sum of C10:0, C12:0, C14:0, C15:0, C16:0, C17:0, C18:0. C20:0; ∑MUFA = sum of C14:1, C16:1, C18:1; ∑ PUFA *n-6* = C18:2*n-6*, C20:4*n-6*; ∑PUFA *n-3* = C18:3*n-3*, C22:6*n-3*; *n-6:n-3* = (C18:2*n-6*+ C20:4*n-6*) / (C18:3*n-3*+ C22:6*n-3*).

as day 0 of pregnancy. Pregnant females were separated from male rats on the day of pregnancy. The rat were individually housed in polycarbonate cages (43 × 28 × 16 cm) with sawdust bedding, in controlled room temperature (23 ± 2°C) with 12 h of light and 12 h of darkness. On the day 14 prior to sacrifice,food was withdrawn at 9:00 A.M. and the animals were sacrificed at 9:00 a.m the following morning on the day 15 of pregnancy after 10 weeks (8 weeks + 15 days) of feeding rats were anesthetized with an Intraperitoneal injection of 60 mg/kg body weight ketamine + 8 mg/kg body weight xylazine and blood samples were obtained by cardiac puncture for determining plasma Fatty Acisds Profile and prostaglandin concentrations (PGE2 and PGF2α) . The study was reviewed and approved by the Animal Care and Use Committee, Faculty of Veterinary Medicine, Universiti Putra Malaysia (UPM/FPV/PS/3.2.1.551/AUP-R23).

Feed and plasma fatty acids determination

The total lipids were extracted from feeds and plasma using a chloroform: methanol solvent mixture at 2:1 (v/v) based on the method of Folch et al. (1957). Butylated hydroxytoluene (BHT) was added as antioxidant to prevent oxidation during sample preparation. Briefly 2 ml plasma was used or 0.5 g of ground feed were homogenized in 40 ml chloroform: methanol (2:1 v/v). The mixture containing the extracted fatty acids was filtered through No. 1 Whatman paper (Whatman International Ltd., Maidstone, England) into a 250 ml separating funnel using a funnel. Ten (10) ml of normal saline solution were added to facilitate phase separation. The mixture was then shaken vigorously for one minute and was left to stand for four hours. After complete separation at the end of fourth hour, the upper phase was discarded and the lower phase was collected in a round bottom flask and evaporated by via rotary evaporation (Laborota 4000-efficient, Heidolph, Germany) at 70°C. The total lipid extract was then immediately transferred into a capped methylation tube by rediluting it with 5 ml fresh chloroform-methanol (2:1, v/v). Transmethylation of the extracted fatty acids to their fatty acid methyl esters (FAME) was carried out using 14% methanolic boron trifluoride (BF_3) (Sigma Chemical Co. St. Louis, Missouri, USA) according to methods in AOAC (1990). The internal standard, heneicosanoic acid (21:0) (Sigma Chemical Co., St. Louis, Missouri, USA) was added to each sample prior to trans-methylation to determine the individual fatty acid concentrations within the samples. The methyl esters were quantified by Gas Chromatography (GC) (Agilent 7890 N) using a 30 m x 0.25 mm ID (0.20 µm film thickness) Supelco SP-2330 capillary column (Supelco, Inc., Bellefonte, PA, USA). One microliter of fatty acid methly estar (FAME) was injected by an auto sampler into the chromatograph, equipped with a split/splitless injector and a Flame ionization detector (FID). The injector temperature was programmed at 250°C and the detector temperature was 300°C. The initial column temperature was set at 100°C for 2 min, then increased at 10°C/min to 170°C where it was held for another 2 min. Finally, it was warmed to 220°C at 7.5°C/min to reach a final temperature of 250°C and held for 20 min to facilitate optimal separation. All results of fatty acid presented as the percentage of total fatty acids.

Determination of prostaglandins

Plasma prostaglandin concentrations (PGE2 and PGF2α) were measured using a rat prostaglandin ELISA kit (Cayman Chemicals, Ann Arbor, MI, USA) according to the manufacturer's protocol. Concentrations of PGE2 and PGF2α were measured using plasma samples. All the samples and reagents were brought to room temperature before the assay. The ELISA reagent and plasma samples were reacted according to manufactor's protocol. The plate was then incubated for 60 to 90 min in darkness.Finally, the absorbance was read at 405 nm using a micro plate reader (Bio-Rad).

Data analysis

All data were analysed as a completely randomized design experiment using the General Linear Model of SAS (Statistical Analysis Systems Institute Inc., 1992). The fatty acid profiles of the rat treatment diets and prostaglandin were analysed used the one-way Analysis of varince (ANOVA). The Duncan's multiple range test was used to elucidate differing means. Correlations between selected parameters were tested by Pearson's correlation.

RESULTS

Dietary fatty acids profiles

Table 2 shows the cod liver oil was used as the source source of PUFA *n-3* and soybean oil was used as the source of PUFA *n-6*. Major fatty acid contributors for the total saturated fat content in the diet were palmitic acid (16:0). The total *n-6* PUFA was high in 30:1 group (48.03).Diet 1:1 group had highest amounts of *n-3* PUFA (7.87). The *n-6:n-3* ratio increased progressively from

Table 2. Fatty acids profile of the experimental diets (Mean ± SE; n = 3).

Treatment group	1:1	6:1	30:1	Con
14:00	2.22	1.03	0.41	1.47
16:00	14.55	13.43	12.94	16.14
16:01	2.48	1.12	0.40	0.54
17:00	0.72	1.03	0.53	1.02
18:00	3.99	4.26	4.27	3.73
18:01	25.51	27.08	26.65	26.42
18:2 *n-6*	34.88	43.08	47.70	40.75
18:3 *n-3*	0.70	0.54	0.77	0.68
20:00	3.94	4.87	4.96	3.80
20:4 *n-6*	3.83	1.52	0.32	1.95
20:5 *n-3*	3.41	0.94	0.39	nd
22:6 *n-3*	3.77	1.08	0.65	nd
∑ Saturated	25.42	24.60	23.78	26.15
∑ Unsaturated	74.58	75.40	76.22	73.85
∑ MUFA	27.99	28.40	27.05	30.46
∑ PUFA *n-3*	7.87	2.40	1.15	0.68
∑ PUFA *n-6*	38.72	44.60	48.03	42.70
n-6:n-3 Ratio	4.92	18.20	44.80	64.29
Unsat: Sat.	2.93	3.06	3.21	2.86
Poly: Sat. ratio	1.83	1.92	2.07	1.67

∑SFA = sum of 10:0, 12:0, 14:0, 15:0, 16:0, 17:0, 18:0. 20:0; ∑MUFA = sum of 14:1, 16:1, 18:1; ∑ PUFA *n-6* = 18:2*n-6*, 20:4*n-6*; ∑PUFA *n-3* = 18:3*n-3*, 22:6*n-3*; *n-6:n-3* = (18:2*n-6*+ 20:4*n-6*) / (18:3*n-3*+ 22:6*n-3*). Poly., polyunsaturated; Sat., saturated.

4.92 in 1:1 group to 64.29 in the control group. Group 30:1 contained a high proportion of linoleic acid (18:2 *n-6*), which was the major fatty acid and the only *n-6* fatty acid.

Plasma fatty acids profile

Table 3 shows the plasma fatty acids composition of the rats treated with different diets after 10 weeks of feeding trial. In this study, 17 fatty acids were detected (from lauric, 12:0 to docosahexaenoic acid, DHA, 22:6 *n-3*) in plasma. At the end of feeding period, the plasma fatty acids profiles were markedly different between the treatment groups. The rats for group 1:1 had more *n-3* PUFA ($P < 0.05$) in their plasma as compared to other groups.

Plasma fatty acids profiles of the 6:1 and 30:1 groups showed significantly greater ($P < 0.05$) percentages of total PUFA. Arachidonic acid (AA) was significantly higher ($P < 0.05$) in 30:1and 6:1 diet group as compared to 1:1 diet group. In the 1:1 diet group, decreased level of AA was observed as compared to the 6:1 and 30:1 diet groups. The 1:1 diet group had the highest value of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DPA) in comparison to 30:1 and 6:1 diet groups. The plasma *n-3* fatty acid in 1:1 diet group was the highest at 11.21% ($P < 0.05$) and this clearly reflected the contribution of *n-3* fatty acids from CLO. Linoleic acid was the main unsaturated fatty acid in the plasma of all diet groups. The *n-6:n-3* ratio was significantly lower (3.49) for the 1:1 group ($P < 0.05$) as compared to other groups. Both the 30:1and 6:1 groups had been almost similar *n-6:n-3* ratios at 8.79 and 6.35, respectively. The *n-6:n-3* ratio was always in the increasing order starting from 1:1, 6:1, 30:1 and Control group. At the end of 10 weeks of the feeding trial, the unsaturated: saturated ratios between the four groups were not significantly different ($P > 0.05$).

Effect of different fatty acids on prostaglandins production

Table 4 shows the effect of feeding of different n-6:n-3 fatty acid ratio supplementation on the plasma levels of both PGE2 and PGF2α at 15 days of gestation. On day 15 of gestation in the 30:1 group, the linoleic acid (n-6) supplementation altered significantly the PGE2 level on the plasma ($P < 0.05$), although the PGF2α concentration was slightly lower in plasma treated with the diet 1:1. For both PGE2 and PGF2α, there were significant difference between 30:1 group as compared to 1:1 and 6:1 groups. There were significant difference ($P > 0.05$) in PGF2α concentrations between the group fed with diet supplemented with high PUFA *n-3* in the (1:1) and (6:1) groups. By increasing the arachidonic acid (AA) fatty acids in 30:1 group, the level of PGE2 raised to 2071.76 ng/ml and the level of PGF2α increased to 1110.56 ng/ml respectively when compared to other treatment groups. Table 5 shows the values for plasma PGE2 and PGF2α revealed a significant positive correlation with the AA fatty acids (Figure 1 *Panel A and b*). It is clear that dietary PUFA, specifically the ratio of n6:n3 fatty acids, are a factor in determining blood plasma content of AA , and this in turn determines capacity to synthesize PGE2. It is possible that other eicosanoids are affected, and modulation of biosynthesis can exert a positive or negative influence on physiologic events in pregnancy rat .

DISCUSSION

Many factors like dietary fatty acids, absorption from different part of body and fat storage can affect the amount and composition of plasma and tissue fatty acids (Rise et al., 2007; Mohamed et al., 2002). It is well known that plasma and tissue fatty acids profiles are affected by diet (William, 1996, Mohamed et al., 2002). *n-3* and *n-6* PUFAs interact and compete with each other for incorporation into phospholipids and as substrates for metabolic enzymes (especially desaturase and COX) (Olsen et al., 1992; Abayasekara and Wathes, 1999; Mattos et al., 2000). Changes in the amounts of PUFAs or their ratios may affect production of PGs in the reproductive system

Table 3. The fatty acid profiles of the rat plasma (%) after 10 weeks of feeding (Mean ± SE; n = 7).

Fatty acid composition	1:1	6:1	30:1	Con
C12:0	2.25 ± 0.28^{a}	1.02 ± 0.16^{b}	1.84 ± 0.09^{a}	0.56 ± 0.19^{b}
C14	1.31 ± 0.33^{a}	1.19 ± 0.41^{a}	Nd	0.53 ± 0.11^{b}
C15:0	1.66 ± 0.30^{a}	1.67 ± 0.28^{a}	1.12 ± 0.19^{a}	0.41 ± 0.05^{b}
C15:1	0.86 ± 0.06^{b}	1.34 ± 0.09^{a}	0.65 ± 0.02^{b}	0.75 ± 0.20^{b}
C16:0	15.53 ± 0.80^{b}	12.04 ± 0.83^{b}	13.75 ± 1.07^{b}	21.67 ± 2.11^{a}
C16:1	0.50 ± 0.05^{b}	0.94 ± 0.15^{ab}	0.72 ± 0.14^{ab}	1.27 ± 0.22^{a}
C17:0	0.66 ±0.05^{a}	0.77 ± 0.10^{a}	1.06 ± 0.23^{a}	1.11 ±0.24^{a}
C17:1	1.00 ± 0.15^{bc}	1.28 ± 0.17^{ab}	1.63 ± 0.27^{a}	0.69 ± 0.06^{c}
C18:0	14.55 ± 0.36^{ab}	14.76 ± 0.70^{a}	13.83 ± 0.89^{ab}	12.20 ± 0.90^{b}
C18:1	10.34 ± 0.90^{b}	8.93 ± 1.30^{b}	9.88 ± 1.42^{b}	14.93 ± 1.23^{a}
C18:2 *n-6*	24.85 ± 0.95	23.06 ± 1.45	24.93 ± 2.48	24.21 ± 1.02
C20:0	0.70 ± 0.04	0.77 ± 0.11	0.75 ± 0.10	Nd
C20:4 *n-6*	13.94 ± 1.32^{b}	23.43 ± 2.14^{a}	22.62 ± 1.66^{a}	19.58 ± 2.42^{ab}
C22:0	0.66 ± 0.07^{b}	0.94 ± 0.14^{a}	0.85 ± 0.07^{ab}	Nd
C20:5 *n-3*	3.82 ± 0.72^{a}	3.05 ± 0.34^{ab}	1.73 ± 0.58^{b}	Nd
C22:5 *n-3*	0.90 ± 0.26^{a}	0.59 ± 0.24^{b}	0.32 ± 0.14^{b}	Nd
C22:6 *n-3*	6.49 ±0.47^{a}	4.22 ± 0.57^{b}	4.33 ± 0.64^{b}	2.09 ± 0.15^{c}
Total Saturated	37.32 ± 1.00^{a}	33.16 ± 0.42^{b}	33.19 ± 0.77^{b}	36.48 ± 1.40^{a}
Total Unsaturated	62.68 ± 1.00^{b}	66.84 ± 0.42^{a}	66.81 ± 0.77^{a}	63.52 ± 1.40^{b}
Total Monoenes	12.69 ± 0.80^{b}	12.50 ± 1.51^{b}	12.88 ± 1.40^{b}	17.65 ± 1.14^{a}
Total PUFA *n-3*	11.21 ± 0.49^{a}	7.85 ± 0.54^{b}	6.38 ± 1.00^{b}	2.09 ± 0.15^{c}
Total PUFA *n-6*	38.79 ± 0.42^{bc}	46.49 ± 1.54^{a}	47.55 ± 1.17^{a}	43.79 ± 1.51^{b}
n-6:n-3 Ratio	3.49 ± 0.17^{c}	6.05 ± 0.54^{bc}	8.79 ± 2.16^{b}	21.36 ± 1.44^{a}
Unsat: Sat	1.69 ± 0.07	2.02 ± 0.04	2.02 ± 0.07	1.76 ± 0.10
Poly: Sat Ratio	1.34 ± 0.05^{b}	1.64 ± 0.06^{a}	1.63 ± 0.09^{a}	1.27 ± 0.08^{b}

Values with different superscripts within row differ significantly at $P < 0.05$; ^{ns} No significant difference nd: not detected; ∑SFA = sum of C10:0, C12:0, C14:0, C15:0, C16:0, C17:0, C18:0. C20:0; ∑MUFA = sum of C14:1, C16:1, C18:1; ∑ PUFA *n-6* = C18:2*n-6*, C20:4*n-6*; ∑PUFA *n-3* = C18:3*n-3*, C22:6*n-3*; *n-6:n-3* = (C18:2*n-6*+ C20:4*n-6*) / (C18:3*n-3*+ C22:6*n-3*).

Table 4. The prostaglandin F2α and prostaglandin E2 concentration (ng/ml) after 15 days pregnancy of rats (Mean ± SE, n = 7).

Treatment	PGF2α	PGE2
1:1	469.33 ± 48.08^{c}	700.94 ± 25.34^{c}
6:1	764.49 ± 41.91^{b}	1124.82 ± 67.08 ^{b}
30:1	1110.56 ± 106.23^{a}	1971.76 ± 96.73 ^{a}
con	576.51 ± 22.37^{c}	611.85 ± 16.98^{c}

Values with different superscripts within column differ significantly at $P < 0.05$.

Table 5. Correlations observed between plasma PGE2 and PGF2α concentrations and AA in the plasma (Pearson r values) n = 7.

AA	1:1	6:1	30:1	Con
PGE2	0.10	0.64	0.97*	0.43
PGF2α	0.54	0.78	0.92*	0.48

*$P < 0.05$.

in both cows (Thatcher et al., 1994), humans (Graham et al., 1994) and rats (Leaver et al., 1989).

Our results revealed that the modification of dietary fatty acids resulted in significant changes in plasma fatty acids profile. Changes in both plasma fatty acids profile dependent on the type of fatty acids in the diet. Several studies had reported that dietary fat affects the fatty acids profile of animal and human plasma and tissues (Mohamed et al., 2002; Wickwire et al., 1987;Harris 1989). In this study, the level of long chain *n-3* PUFA was the highest in rats fed with very low ration of n-6:n-3 (1:1), the intermediate in rats fed with low ratio of n-6:n-3 (6:1), and lowest in rats fed with the high ratio of n-6:n-3 (30:1). Supplementation of n-3 PUFA has been shown to inhibit Δ^{6} desaturase activity (Garg et al., 1988), potentially explain the elevation of plasma linoleic acid (LA) levels with elevated *n-3* PUFA previously reported (Hwang et al., 1988; Whelan et al., 1991). The fatty acid composition of plasma lipids reflected the dietary fatty acid intake. Rats on the 1:1 diet showed higher levels of EPA and DHA and lower amounts of arachidonic acid. Those animals fed the 6:1 diet showed a modest rise in DHA

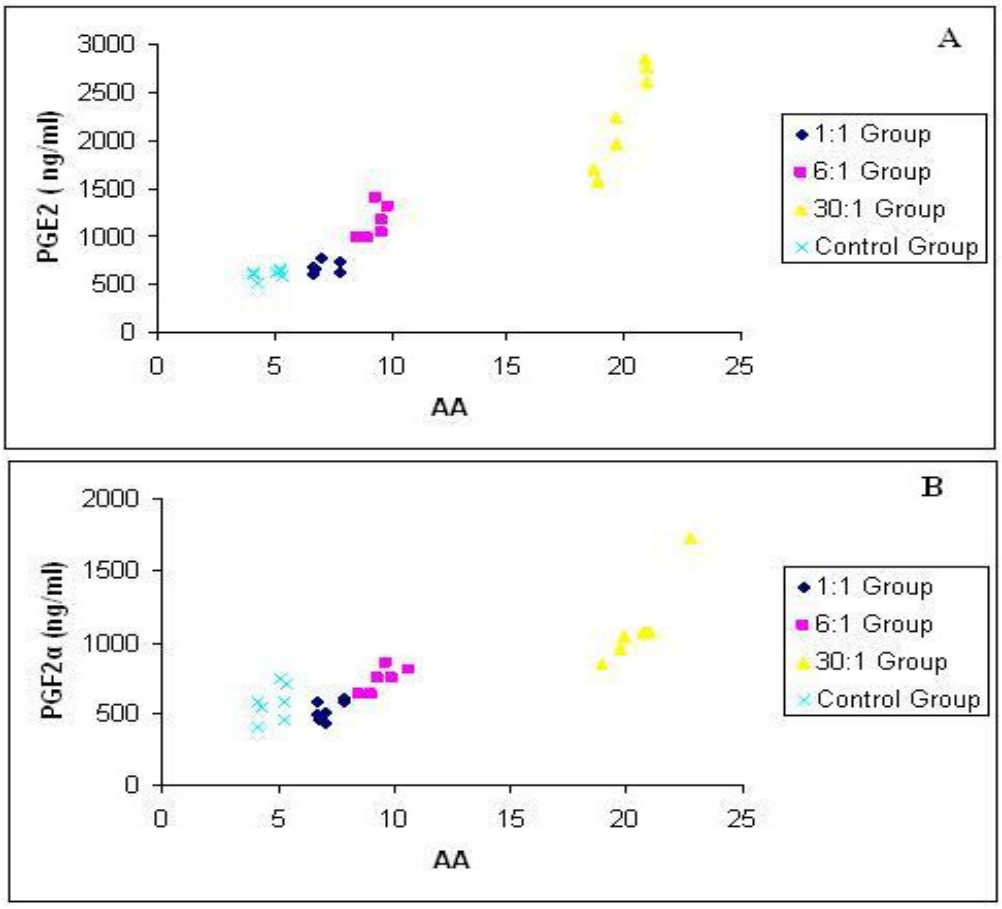

Figure 1. Relationship between PGE2 and PGF2α concentration and the AA plasma in rats fed different proportions of soybean and code liver oils. Panel A (r = 0.95) and Panel B (r = 0.86).

and arachidonic acid but no change in EPA compared to animals fed the 30:1 diet. Presumably the increased levels of arachidonic acid in plasma lipids 30:1 group are a result of the higher level of l inoleic acid in soybean oil compared to the 1:1 or control group. Supplementation of ratio 1:1 group increased levels of EPA and DHA, while no change was detected for arachadonic acid levels. These data are in contrast with previous studies that have indicated supplementation of EPA can decrease synthesis of arachadonic acid metabolites (Barham et al., 2000). In the present study, increasing LA levels of the (30:1) group led to the increase in plasma AA levels. When feeding soybean oil, as a source of the arachidonic acid (AA) precursor, there was a significant increase in plasma AA content. This increament is in agreement with Kevin et al. (1984) who fed rats with high level of safflower oil. In the diet 1:1, supplementation of *n-3* PUFA decreased eicosanoid synthesis, which was more pronounced at lower fat intakes. However, this study also indicated that if *n-6* PUFA remained low in the diet 6:1 group, elevation of total dietary fat is as equally effective in reducing PGE2 and PGF2α production and in some regard may influence the effect of *n-3* PUFA.

There was a greater propensity of *n-6* PUFA to alter eicosanoid production in rats fed on the high ratio of *n-6:n-3* (30:1) rather than the diet 1:1 group. This data was supported by the fact that the highest concentrations of 2-series prostaglandins measured in this experiment was caused by higher conventration of AA in plsma fatty acid (Elmes et al., 2004). Although the effect of feeding 1:1 group the n-*6* PUFA intake in response 2-series prostaglandins level was reversed by increased *n-3* PUFA on the diet. The diet 1:1 data indicate that there was a greater total *n-3* in the plasma induced decrease level in PGE2 and PGF2α in the plasma (Olsen et al., 1992; Mattos et al., 2000; 2002; 2003) compared with the increase in PGE2 and PGF2α in the plasma 30:1 group.

The prostanoid data, particularly for PGE2 and PGF2α production, were lowered in 1:1 group. This result is in agreement with Kevin et al. (1984) who reported that PGF2α was reduced when EPA fish oil was supplemented as a source of the long chain *n-3* PUFA. The arachidonic acid (AA) content of plasma lipids was significantly elevated in animals on the soybean oil 1:1 group . The 1:1 group supplementation diets had high amount *n-3* PUFA in the plasma depressed the AA

content of plasma fatty acid . The AA elevation of plasma is in agreement with Winters et al. (1994) showed that by adding long chain *n-3* PUFA, particularly DHA to diet the AA concentration decreased. In addition, this data is in agreement with Kevin et al. (1984). Linoleic acid (LA) has been reported to stimulate or inhibit the production of prostaglandin (PG) in different studies. In the rat gastric mucosal cells, for example, supplementation with LA stimulated AA and PGE2 production (Nakaya et al. 2001). In this study, the AA in 1:1 group was lower as compared to 30:1 group. The results of 1:1 group confirmed previous studies that *n-3* fatty acids are potent inhibitors of PG secretion in mammalian species (Olsen et al., 1992; Mattos et al., 2000; 2002; 2003) and the PG concentration on plasma increased in 30:1 group as compared 1:1 group. Kevin et al. (1984) noted that safflower oil as a source of *n-6* PUFA when fed to rat, could elevate the PGF2α release. In this study, our findings support a positive role for *n6:n3* fatty acids in plasma modeling in pregnancy rats and provide a therapeutic context for the use of dietary *n6:n3* fatty acids in modulating eicosanoid production to control pregnant rat.

In conclusion, dietary polyunsaturated fatty acid (PUFA) intake can inhibit prostaglandin production in plasma. This study emphasizes that there are changes induced by varying levels of *n-6:n-3* fatty acid contents of the diet with a more pronounced effect following *n-6* rather than *n-3* supplementation. These data suggest that a high *n-6* diet increased the amount of PGE2 and PGF2α production in the rats and may therefore, have implications for the control of luteolysis by actions of PGF2α is to decrease ovarian and other PG-mediated events such as ovulation.

Abbreviations: SO, Soybean oil; **CLO**, code liver oil; **PUFAs**, polyunsatruted fatty acids; **EPA**, eicosapentaenoic acid; **DHA**, docosahexaenoic acid; **LA**, linoleic acid, **AA**, arachidonic acid; **ALA**, α-linolenic acid; **COX**, cyclooxygenase **PGs**, prostaglandins.

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