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Full Length Research Paper

Effect of *n-3* and *n-6* fatty acid supplementation on fetal, gestation and parturition in pregnant Sprague Dawley rats

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The aim of this study was to evaluate the effect of different ratio of *n-6:n-3* on gestation and parturition as well as to determine the hormone concentration on plasma of the pregnant rats. At the end of the trial period, blood plasma was collected and analysed for progesterone, estradiol and prostaglandin F2α hormone (PGF2α) concentrations, and fatty acids profile. The results indicated that the group with a very low ratio of *n-6:n3* fatty acids (diet 1) showed increased concentration of progesterone in the plasma (P<0.05), while the group with high ratio of *n6:n3* fatty acids (diet 3) with high arachidonic acid (AA) showed significantly (P<0.05) increased concentration of estradiol and PGF2α in the plasma. The *n-3* polyunsaturated fatty acids (PUFA) in plasma of diet 1 group were significantly (P<0.05) higher than the other treatment diet groups. The total *n-6* PUFA was significantly higher (P<0.05) in diet 3 group as compared to diet 1 group. In contrast, the number of implanted embryos was significantly lower (P<0.05) in the diet 3 group at 15 days of gestation, while, the litter size were significantly lower in the diet 2 group and diet 3 group by 4.57 and 1.00 folds respectively, as compared to the control group (diet 4). In conclusion, for the rat with very low ratio on diet 1, the *n-6:n-3* ratio satisfied the requirement for the growth of mother and fetuses but was inadequate for the normal process of parturition, probably through inadequate production of the prostaglandins involved.

Key words: *n-6:n-3*, plasma fatty acids, progesterone, estradiol, prostaglandin production, pregnant rat.

INTRODUCTION

It is well known that polyunsaturated fatty acids (PUFA) play role in the maintenance of different physiological Essential fatty acids (EFAs) include two series of prostaglandin synthesis (Leat and Northrop, 1981). Unsaturated fatty acids that are involved in the maintenance of membrane function and precursors for functions (Monis et al., 1981; Rivers and Frankel, 1981).

The parent acid of the first series (*n-6*) is linoleic acid (*cis-9 cis-12*) which is converted to α-linolenic acid (*n-3*). Essential fatty acids (EFAs) include two series of unsaturated fatty acids that are involved in the maintenance of membrane function and are precursors for prostaglandin; a precursor of prostaglandin E1 and F1, and then arachidonic acid (C20: 4 *n-6*), a precursor of prostaglandin E2 and F2α (Mead and Fulco, 1976). The prostaglandin PGF2α is implicated in reproductive functions such as ovulation, luteolysis and parturition. Actions of PGF2α are mediated by the prostaglandin F receptor (FP) (Sugimoto et al., 1994).

Previous studies have shown that dietary *n-3* increased

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

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

Fatty acid	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 and C20:0; ∑MUFA = sum of C14:1, C16:1 and 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*).

gestation length and differences in onset of labor (Baguma-Nibasheka et al., 1999), altered fatty acid profiles of ovarian follicle components, and increased follicle number and size in cattle (Robinson et al., 2002; Zeron et al., 2002), as well as increased ovulation rate in mice (Trujillo and Broughton, 1995) although exogenous PGF2α induces a decline in the progesterone concentration in some species and mimics the parturition process (Alexandrova and Soloff, 1980). Furthermore, evidence of a direct beneficial effect of linoleic acid during early embryo development has been observed in more developed human embryos that incorporated more linoleic acid compared with their undeveloped counterparts (Haggarty et al., 2006).

Progesterone not only prepares the uterus for implantation of the embryo but also helps maintain pregnancy by providing nourishment to the conceptus. Between 25 and 55% of mammalian embryos die in early gestation, many of these losses are due to inadequate function of luteal cells (Niswender and Nett, 1994). Furthermore, progesterone has a major role in the endometrium in preparation for implantation of a fertilized ovum, and in many species a decrease in circulating progesterone at the time of fertilization is sufficient to delay implantation (Rothchild, 1983). Estrogen stimulates uterine secretion of PGF2α (Knickerbocker et al., 1986; Thatcher et al., 1994). Furthermore, estrogen can increase the sensitivity of the corpus luteum CL to PGF2α (Howard et al., 1990), thus causing a more complete regression of the CL; lowered estradiol-17b may prevent premature regression of the CL and prevent early embryonic death. The aim of this study was to determine the effect of feeding diets rich in omega 6 (linoleic acid) and omega 3 (eicosapentaenoic and docosahexaenoic acids) fatty acid on progesterone concentration and to determine the relation between intake of soybean oil in pregnancy and risk of preterm delivery and low birth weight in rat.

MATERIALS AND METHODS

Rats and diets

A total of 56 virgin, female Sprague-Dawley rats, aged 2 month-old and weighing 240 ± 20 g were used in this study. The rats were kept at the animal unit of the Faculty of Veterinary Medicine, University Putra Malaysia. After two weeks of adaptation to the experimental diets, the rats were randomly divided into four treatment groups of 14 rats each. All the rats were given standard chow pellet containing 19.5% protein, 6.8% fat, 4.5% fiber and 1.7% moisture. The four treatment groups included the rats fed with standard chow diet supplemented with 50% w/w soybean oil and 50% w/w cod liver oil (1:1) (diet 1), rats fed with standard chow diet supplemented with 84% w/w soybean oil and 16% w/w cod liver oil (6:1) (diet 2), rats fed with standard chow diet supplemented with 96% w/w soybean oil and 4% w/w cod liver oil (30:1) (diet 3), and rats fed with only standard rat chow diet and acted as the control (diet 4). The fatty acids profile of soybean oil (SBO) and cod liver oil (CLO) shown in fatty acids profile of the treatment diets are shown in Tables 1 and 2 (Amira et al., 2010). 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). After two months of feeding trial, daily vaginal smears were taken to determine the estrous cycle of each rat. The pregnancy was induced by overnight caging of a proestrous female with a male of proven fertility. The next day, the presence of a vaginal plug or spermatozoa in the vaginal smear was termed as day 0 of pregnancy. Pregnant females were separated from the male rats after confirmation of pregnancy. 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, the rats were anesthetized with an intraperitoneal injection of 60 mg/kg body weight ketamine + 8 mg/kg body weight xylazine. The blood were collected into EDTA coated tube and kept at 4°C and centrifuged at 3000 g for 10 min for plasma collection and stored at -80°C until future analysis. The other 28 pregnant rats were left until parturition and the length of pregnancy and litter size were recorded.

Fatty acids analysis

The total fatty acids were extracted from feeds and plasma using chloroform: methanol 2:1 (v/v) based on the method of Folch et al.

Table 2. Fatty acids profile of the treatment diets.

Fatty acid composition (g/100 g) of feed sample	Group			
	Diet 1	Diet 2	Diet 3	Diet 4
C14:0 Myristic acid	2.22	1.03	0.41	1.47
C16:0 Palmitic acid	14.55	13.43	12.94	16.14
C16:1 Palmitoleic acid	2.48	1.12	0.40	0.54
C17:0 Hepatadecanoic acid	0.72	1.03	0.53	1.02
C18:0 Stearic acid	3.99	4.26	4.27	3.73
C18:1 Oleic acid	25.51	27.08	26.65	26.42
C18:2 *n-6* Linoleic acid (LA)	34.88	43.08	47.70	40.75
C18:3 *n-3* Linolenic acid (ALA)	0.70	0.54	0.77	0.68
C20:0 Arachidic acid	3.94	4.87	4.96	3.80
C20:4 *n-6* Arachidonic acid	3.83	1.52	0.32	1.95
C20:5 *n-3* Ecosapentaenoic acid	3.41	0.94	0.39	Nd
C22:6 n-3 Docosahexaenoic acid	3.77	1.08	0.65	Nd
Total Saturated fatty acid	25.42	24.60	23.78	26.15
Total Unsaturated fatty acid	74.58	75.40	76.22	73.85
Total MUFA fatty acid	27.99	28.40	27.05	30.46
Total PUFA n-3	7.87	2.40	1.15	0.68
Total 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 C10:0, C12:0, C14:0, C15:0, C16:0, C17:0, C18:0 and C20:0, ∑MUFA = sum of C14:1, C16:1 and 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*).

(1957) and modified by Rajion et al. (1985), with butylated hydroxytoluene (BHT) as an antioxidant for preventing the oxidation during sample preparation. Briefly, 2 ml plasma was used or 0.5 g of ground feed was homogenized in 40 ml chloroform: methanol (2:1 v/v). The mixture containing the extracted fatty acids was filtered through a No. 1 Whatman paper (Whatman International Ltd., Maidstone, England) into a 250 ml separating funnel using a filter funnel. 10 ml of normal saline solution were added to facilitate the phase separation. The mixture was then shaken vigorously for 1 min and was left to stand for 4 h. After complete separation at the end of 4 h, the upper phase was discarded and the lower phase was collected in a round bottom flask and evaporated by Heidolph vacuum 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) were 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 transmethylation to determine the individual fatty acid concentrations within the samples. The methyl esters were quantified by gas chromatography (GC) (Agilent 7890N) using a 30 m x 0.25 mm ID (0.20 µm film thickness) Supelco SP-2330 capillary column (Supelco, Inc., Bellefonte, PA, USA). 1µl 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 20 min and held for 20 min to facilitate optimal separation. All results of the fatty acid were presented as the percentage of total fatty acid.

Biochemical analysis

Plasma progesterone and estrodial was assayed by radio-immunoassay (RIA, Perkin-Elmer® 1470 Wizard Automatic Gamma Counter, and Waltham, MA, USA) using commercial radio-immunoassay kit (Coat-A-Count, Siemens Medical Solution, Los Angeles, CA).

Determination of prostaglandins

Plasma prostaglandin concentrations (PGF2α) were measured using a rat prostaglandin ELISA kit (Cayman Chemicals, Ann Arbor, MI, USA) according to the manufacturer's protocol. All the samples and reagents were brought to room temperature before the assay. The ELISA reagent and plasma samples were reacted according to

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

Fatty acid composition	Diet 1	Diet 2	Diet 3	Diet 4
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 differenc; 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*).

manufactory protocol. The plate was then incubated for 60 to 90 min in darkness. The absorbance was read at 405 nm using a micro plate reader

Data analysis

All data were analyzed as a completely randomized design using the General Linear Model of SAS (Statistical Analysis Systems Institute Inc., 1992). The fatty acid profiles of the rat treatment diets, progesterone, estradiol and prostaglandin were analyzed with the one-way ANOVA. The Duncan's multiple range tests were used to elucidate differing means.

RESULTS

Plasma fatty acids profile

Table 3 shows the plasma fatty acids composition of the rats supplemented with oil after 10 weeks of the feeding trial (Amira et al., 2010). In this study, 17 fatty acids were detected from lauric (C12:0) to docosahexaenoic acid (DHA) (22:6 *n-3*) in the plasma. At the end of the feeding period, the plasma fatty acids profiles were markedly different between all the treatment groups. The rats of diet 1 group had more *n-3* PUFA ($P<0.05$) in their plasma compared to other groups. Plasma fatty acids profiles in diet 2 group and diet 3 group showed significantly greater ($P<0.05$) percentage of total PUFA in plasma at the end of the trial. Arachidonic acid was significantly higher ($P<0.05$) in diet 2 and diet 3 groups as compared to diet 1 group. In the diet 1 group, the arachidonic acid (AA) was decreased significantly ($P<0.05$) as compared to the diet 2 and diet 3 groups. In diet 1 group, the eicosapentaenoic acid (EPA) and docosahexaenoic acid (DPA) was significantly ($P<0.05$) higher as compared to diet 2 and diet 3 groups. The plasma *n-3* fatty acid in diet 1 group (11.21%) was the highest ($P<0.05$) and this clearly reflected the contribution of *n-3* fatty acids from fish oil. Linoleic acid was the main unsaturated fatty acid in the plasma of all the experimental diet. The *n-6:n-3* ratio was significantly lower for the diet 1 group ($P<0.05$) as compared to diet 3 group. At the end of 10 weeks of the feeding trial, the unsaturated: saturated ratios between the four groups were not significantly different ($P>0.05$).

Table 4. The estradiol and progesterone concentration (pg/ml) at 15 days of pregnancy in rats (Mean ± SE, n = 7).

Parameter	Diet 1	Diet 2	Diet 3	Diet 4
Estradiol	24.08±1.36^{c}	34.25±0.84^{b}	49.12±2.12^{a}	15.52±0.53^{d}
Progesterone	3.57±0.38^{a}	2.46±0.12^{b}	1.36±0.13^{c}	3.38±0.27^{a}

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

Table 5. The Prostaglandin F2α concentration (ng/ml) on 15 days of pregnancy in rats (Mean ± SE, n = 7).

Treatment	PGF2α
Diet 1	469.33 ± 48.08^{c}
Diet 2	764.49 ± 41.91^{b}
Diet 3	1110.56 ± 106.23^{a}
Diet 4	576.51 ± 22.37^{c}

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

Estradiol and progesterone production

Table 4 shows the effect of feeding different *n-6:n-3* fatty acid ratio on the plasma levels of both progesterone and estradiol in the pregnant rats. After 15 days of gestation period, progesterone was still significantly (P<0.05) lower in the rat fed diet supplemented with high *n-6* PUFA (Diet 3) as compared to diet 4, but there was no difference in the progesterone level between the control and rat fed with diet supplemented with a high level of *n-3* PUFA (diet 1 groups). Estradiol concentration was significantly higher (P< 0.05) in the rat fed diet supplemented with high level of *n-6* PUFA (diet 3) as compared to diet 4 groups.

Prostaglandins F2α production

There were no significant differences (P>0.05) in PGF2α concentrations between diet 1 group and diet 4 group (Table 5). Increasing the AA in diet 3 group led to elevation of PGF2α as compared to diet 1 group. In diet 3 group, the high level of linoleic acid led to significantly (P<0.05) higher synthesis of PGF2α and it was slightly lower in the plasma of diet 1 group. The PGF2α production followed the trend of diet 3 > diet 2 > diet 4 > diet 1 groups.

Fetal number, implantations and litter size

Table 6 shows the fetal number at 15 days of gestation. The results showed no significant different between the diet 4 and diet 1 groups, but showed significant different (P<0.05) between the diet 4, diet 2 and diet 3 groups. Similarly, the fetal implantations at day 15 of gestation were observed to be non-significantly different (P>0.05) in the diet 1 and diet 4 groups. However, the number of implanted embryos was significantly lower in the diet 3 group at 15 days of gestation. In contrast, litter sizes were significantly lower in the diet 2 and diet 3 groups as compared to the diet 4 group (Amira et al., 2011).

The length of gestation and number of pups

The number of fetuses at 15 days of gestation and the length of gestation are shown in Table 7. The diet 3 group had fewer fetuses at 15 days of gestation, but was significantly decreased in the length of gestation as compared to the diet 4 group. Consequently, at birth, the litter size of diet 3 group was significantly (P<0.05) smaller as compared to the control group by 1.00 and 11.14 pups, respectively. The diet 3 groups also showed the shortest length of gestation compared to diet 1 and diet 4 groups. The numbers of pups were highest in the diet 4 and diet 1 group and were significantly higher when compared with the diet 3 group.

DISCUSSION

This study demonstrated that manipulation of *n-6*:*n-3* fatty acids ratio in diet, can change plasma fatty acid profile and reproductive hormone synthesis in pregnant rats. Our results revealed that the modification of dietary fatty acid resulted in significant changes in plasma fatty acid profile. Changes in the plasma fatty acid profile were dependent on the type of fatty acids in the diet. Several studies have reported the effects of dietary fat on the fatty acid profile of animal and human plasma and tissues (Harris, 1989; Kevin et al., 1984). They showed that the level on long chain *n-3* PUFA was highest in the rats fed with cod liver oil supplementation, intermediate in rats fed with cod liver oil mixed with soybean oil supplementation, and lowest in rats fed with soybean oil supplementation.

The results of this experiment showed that dietary supplementation can modify the plasma fatty acid profile and prostanoid synthesis. The significant of these modifications vary according to the type of polyunsaturated fat and the ratio of *n-6: n-3* fatty acid. The *n-3* fatty acids in cod liver oil were incorporated into plasma, and were reported by Bang et al. (1980).

Table 6. Effect of different ratio of *n-6:n-3* fatty acid supplementation on fetal number, fetal implantations and litter size at 15 days of gestation in rat.

Parameter	Diet 1	Diet 2	Diet 3	Diet 4
Fetal number	10.57±0.30[a]	5.14±0.26[b]	1.14±0.46[c]	11.00±0.44[a]
Fetal implantations	10.57±0.30[a]	10.29±0.18[a]	3.86±0.55[b]	11.00±0.44[a]
Litter size (At birth)	10.43±0.37[a]	4.57±0.48[b]	1.00±0.38[c]	11.14±0.46[a]

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

Table 7. Effect of different ratio of *n-6:n-3* fatty acid supplementation on the length of gestation and number of pups/fetuses.

Parameter	Diet 1	Diet 2	Diet 3	Diet 4
Length of gestation	23.57±0.20[a]	19.14±0.34[a]	17.50±0.22[b]	22.71±0.18[a]
Number of pups/fetuses	10.43±0.53[a]	4.86±0.40[b]	1.86±0.70[c]	11.00±0.65[a]

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

In addition, they also reported that the PGF2α release was reduced when EPA fish oil was supplemented as a source of long chain *n-3* PUFA (Kevin et al., 1984). Thus, the low level of PGF2α in diet 1 group in this study agreed with that of the previous study and feeding that safflower oil as a source of *n-6* PUFA when fed to rat caused the elevation of PGF2α (Kevin et al., 1984).

Supplementation of high ratio of *n6:n3* in diet 3 group increased the concentration of circulating progesterone (Milvae et al., 1977). Secretion of progesterone is the main function of the corpus luteum (CL). Progesterone not only prepares the uterus for implantation of the embryo but also helps maintain pregnancy by providing nourishment to the conceptus. Between 25 and 55% of mammalian embryos die in early gestation, many of these losses are due to inadequate function of luteal cells (Niswender and Nett, 1994).

The plasma progesterone and estradiol decreased significantly ($P<0.05$) in diet 3 and diet 2 groups as compared to diet 1 group. This result is in agreement with Baguma-Nibasheka et al. (1999) who fed sheep with high level of *n-3* PUFA and observed the rise in estradiol levels in control ewes but not in the *n-3* feeding group.

The prostaglandin F2α content of the rat plasma was low at 15 days of gestation in diet 3 and diet 1 groups coinciding with an increase in progesterone (Flower, 1977). On the other hand, PGF2α reduced the production of progesterone because *n-6* PUFA caused an increased production of prostaglandins in the plasma with a reduction of the progesterone synthesis. The progesterone production is reduced when PGs are administered to luteinizing hormone (LH) rats (Kato, 1982). In this study, the group with the lowest total PGF2α production was the diet 4 and diet 1 group had the highest progesterone production, whereas rats in the diet 3 groups displayed the highest PGF2α concentrations with lower progesterone production (Dukwes et al., 1974).

During an investigation of the possible role of linolenic acid in the nutrition of the rat, it was noted that pregnant rats fed with linolenic acid had an impaired parturition (Leat and Northrop, 1979) which precluded the investigation of the second generation animals. In the case of pregnant rats, diets containing high *n-6* (Diet 3) resulted in an abnormal development of fetuses, as evidenced by the smaller fetuses plus reduction in the length of gestation and number of pups and by the significantly diminutive embryo after 15 days of gestation. This consequently, resulted in a reduction of the litter size (number of newborns) (1.86 number of pups/fetus) and the length of gestation (17.50 days). These abnormal fetal development and fetal desorption in the diet 3 group might have been the result of high AA in the plasma as a disturbance of the endometrial lining system and interferes with fetal development.

The rats fed with the *n-6* PUFA supplementation resulted in normal parturition but with the *n-3* PUFA, the results were only slightly better than those obtained with the basal diet alone (Quackenbush et al., 1942).

These results are explained by the relationship between the different ratios of *n-6*:*n-3* and prostaglandins production. Linoleic acid (*n-6*) is the precursor of prostaglandins (PGE2 and PGF2α) whereas linolenic acid (*n-3*) is the precursor of PGE3. The prostaglandins of the *n-6* series, particularly PGF2α are important for the normal process of parturition (Cheng et al., 2005).

It is well known that there is competition between the various families of unsaturated fatty acids for the enzymes which elongate and desaturate the precursor of fatty acid; the affinity been in the order of *n-3* > *n-6* > *n*-9 (Mohrhauer and Holman, 1963). On the basis of preliminary observations, the adverse effect of *n-3* on the process of parturition might be explained by competition between the *n-6* and *n-3* in the diet. However, supplementation of this Diet 1 group with comparatively large amounts of *n-3* could have suppressed the production of PGF2α (Quackenbush et al., 1942).

However this hypothesis is not adequate as it stands to explain the impaired parturition in one rat fed the basal diet alone, and carrying 11 fetuses. This observation indicated that the feeding of *n-6* PUFA was related to decrease progesterone concentration and increased PGF2α concentration in the plasma. These results suggested that the impaired parturition might be related to the number of fetuses present. A closer examination of all the results indicated that the rats supplemented with very low ratio of *n-6*: *n-3* had increased the number of fetuses (10.43) in diet 1 group as compared to those fed with high ratio *n-6: n-3* which had an apparently normal parturition (1.86) (Diet 3 group). It appears that, when rats are fed a diet high ratio of *n-6*: *n-3* fatty acid, the critical number of pups is low as a result of impaired parturition. The prostaglandins produced from *n-3* appear are inadequate as far as smooth muscle contraction and parturition are concerned (Niswender and Nett, 1994).

The adverse effect of *n-3* PUFA on parturition can be overcome by substituting *n-3* PUFA with *n-6* PUFA during the last three to six days of gestation; in diet 1 group and in those rats there was presumably adequate *n-6* PUFA for the formation of the necessary prostaglandins at parturition. It appears that the balance of *n-6* and *n-3* fatty acids in late gestation is more important in parturition as compared to the long term EFA status of the animal.

Conclusions

This study investigated whether increased linoleic acid (*n-6*) during pregnancy may prolong gestation or decrease fetal growth and modify PG synthesis. These findings support ideas that a high *n-6* PUFA supplemented diet may influence fetal and placental development and may also play an important role in determining the length of gestation. Dietary *n-3* fatty acid supplementation suppresses PGF2α plasma levels in the plasma. These in turn minimized the adverse effect of abundant *n-6* fatty acids on the rats reproductive system.

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