UNIVERSITI PUTRA MALAYSIA

EFFECTS OF LONG CHAIN N-3 POLYUNSATURATED FATTY ACIDS SUPPLEMENTATION ON DEVELOPMENT OF COGNITIVE FUNCTION IN RATS

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By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Master of Science

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Dedicated to

My beloved family

Abstract of thesis presented to the Senate of Universiti Putra Malaysia
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Chairman : Goh Yong Meng, PhD

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The effects of long-chain polyunsaturated fatty acid (LC-PUFA) supplementation on cognitive function development in rats were evaluated in three interlinked experiments. In Experiment I, twenty eight three-month old female Sprague-Dawley rats were allocated equally into four treatment groups. Rats were fed either rat chow only (Control group, n=7), rat chow containing 10% (w/w) butter (BT group, n=7), rat chow added with 6.66% (w/w) menhaden oil and 3.34% (w/w) soybean oil (M3 group, n=7) and rat chow added with 3.34% (w/w) menhaden oil and 6.66% (w/w) soybean oil (S6 group, n=7) diets. All rats were fed for six weeks and the individual body weights were recorded weekly to obtain their body weight gain between week-0 to week-6. Baseline observations for the spatial mental ability were acquired using the Morris Water Maze (MWM) test. Plasma fatty acid (FA) profiles were also assayed using chromatographic techniques. Results showed that after six weeks of treatments, there were statistically significant improvements in path length and time to reach the hidden platform. Rats in the M3 group showed a shorter path length and duration ($P<0.05$) to swim and reach the platform than those in the S6, BT and

Control groups. The M3 group which was fed with a high level of menhaden oil rich in docosahexaenoic acid (DHA) performed better on path length and time to find the hidden platform. Blood plasma FA profiles mirrored the dietary FA composition. The plasma and tissues of the M3 animals also had significantly increased ($P<0.05$) levels of polyunsaturated fatty acids (PUFAs) at the end of the trial, compared to both BT and Control groups. The n-6:n-3 PUFA ratio was 0.9 in the M3 group and was lower than the other groups. Results indicated that the dietary n-3 PUFA supplementation produced a better performance in the MWM test and tissue FA profile as well.

Experiment II was designed to determine the effects of maternal n-3 supplementation on plasma FA profile and mental ability performance in the second generation offsprings. All rats (n=28) from Experiment I were given the same treatment diet throughout the entire experiment period. This included two weeks of acclimatization period, three weeks of gestation and throughout the entire lactation period of six weeks. After parturition, pups were separated on day 21 according to their sex. On postnatal day four, blood plasma was collected from all pups (n= 20) via cardiac puncture. Milk samples were obtained from dams for FA profile determination. All pups were than treated with the same treatment diet as their dams upon weaning. On postnatal day 21, all pups (n=20) were tested for mental ability using the MWM test and animal behavior scores. Results showed that the n-3 supplemented M3 pups had significantly better performances in the MWM test ($P<0.05$). The M3 pups also had significantly better animal behavior scores such as open field activity and maternal behavior. Fatty acid profiles were closely related to the maternal plasma FA profiles. High maternal dietary intake of n-3 PUFA such as

menhaden oil increased the level of DHA in milk and plasma, particularly in the M3 group. The n-6:n-3 ratio was lower in the M3's milk at 4.7 compared to the other groups. This indicated that blood plasma played a major role in the transport of DHA from the placenta to the fetus. In addition, results showed that the rats (n=7) on the M3 group had significantly higher levels of docosahexaenoic acid at 6.1% (341.2±86.4 mg/100ml) and total n-3 PUFA at 30.0% in their plasma after week-15.

Experiment III attempted to determine the effects of n-3 PUFA supplementation on distribution and neuronal histology. After one year of induction period, all rats from first and second experiment (Dams, n=28 and Pups, n=20) were sacrificed and the whole brain were removed and weighed. Brain tissues were fixed for histological examinations. The brain weight of the M3 and S6 groups were significantly lower than the butter-supplemented BT group ($P<0.05$). However, neuronal density was highest among the n-3 supplemented M3 dams and pups. These results reaffirmed that n-3 PUFA supplementation affects neuronal and brain development associated with learning performance in rats.

In summary, dietary n-3 PUFA supplementation promoted the development of cognitive function or mental ability in rats. This was achieved via structural and functional changes in neurons involved in learning and spatial memory in the experimental subjects.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

KESAN SUPLEMENTASI ASID LEMAK POLITAKTEPU BERANTAI PANJANG KE ATAS PERKEMBANGAN FUNGSI KOGNITIF DALAM TIKUS

Oleh

HAFANDI AHMAD

Januari 2007

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Kesan suplementasi asid lemak politaktepu berantai panjang (LC-PUFA) ke atas perkembangan fungsi kognitif tikus telah dikaji dalam tiga eksperimen yang berangkai. Di dalam Eksperimen I, dua puluh lapan ekor tikus betina baka Sprague-Dawley berumur tiga bulan diagihkan kepada empat kumpulan rawatan. Tikus diberi makan pelet tikus sahaja (Kumpulan kawalan, n=7), pelet tikus ditambah dengan 10% (w/w) mentega (Kumpulan BT, n=7), pelet tikus ditambah dengan 6.66% (w/w) minyak menhaden dan 3.34% (w/w) minyak kacang soya (Kumpulan M3, n=7) dan pelet tikus ditambah dengan 3.34% (w/w) minyak menhaden dan 6.66% (w/w) minyak kacang soya (Kumpulan S6, n=7). Semua tikus menerima rawatan pemakanan masing-masing selama 6 minggu dan berat badan dicatat secara individu sekali seminggu. Kemampuan ingatan berasaskan ruang (spatial mental ability) telah dinilai dengan menggunakan ujian Morris Water Maze (MWM). Kandungan asid lemak di dalam plasma juga ditentukan dengan menggunakan teknik kromatografi. Selepas rawatan selama enam minggu, keputusan menunjukkan perbezaan bererti pada jarak laluan dan masa yang diambil untuk sampai ke platform. Subjek daripada kumpulan M3 yang diberi makan pelet tikus yang mempunyai kandungan minyak menhaden yang tinggi dan kaya dengan asid docosahexaenoic (DHA) mencatatkan jarak laluan dan masa yang lebih baik untuk menuju ke platform. Profil asid lemak di dalam plasma mencerminkan komposisi asid lemak di dalam makanan yang diberikan. Plasma dan tisu di dalam haiwan M3 juga menunjukkan peningkatan tahap asid lemak politaktepu yang nyata ($P<0.05$) di akhir eksperimen, dan berbanding kumpulan BT dan Kawalan. Nisbah n-6:n-3 PUFA adalah 0.9 di dalam kumpulan M3 dan berbeza daripada kumpulan yang lain. Keputusan juga menunjukkan supplementasi n-3 PUFA terbukti mempengaruhi prestasi tikus di dalam ujian MWM dan profil asid lemak tisu.

Eksperimen II bertujuan untuk menyelidik kesan suplementasi asid lemak politaktepu n-3 kepada ibu tikus dan kesannya terhadap kemampuan mental pada anak tikus dalam generasi kedua. Semua tikus (n=28) daripada Eksperimen I diberi makanan yang sama semasa kajian dijalankan. Ini termasuklah dua minggu untuk penyesuaian, tiga minggu semasa bunting dan seterusnya 6 minggu sepanjang tempoh penyusuan. Anak tikus diasingkan pada hari ke-21 lepas lahir mengikut jantina. Pada hari ke-4 selepas lahir, sampel darah dikumpulkan daripada semua anak tikus (n=20) melalui cucukan ke jantung. Sampel susu diperolehi daripada ibu tikus untuk penentuan profil asid lemak. Semua anak tikus diberi rawatan makanan yang sama seperti ibu tikus sehingga cerai susu. Pada hari ke-21 selepas lahir, semua anak tikus (n=20) diuji untuk kemampuan mental mereka dengan menggunakan kaedah MWM dan skor tingkahlaku haiwan. Keputusan menunjukkan suplementasi n-3 di dalam susu dan diet anak tikus M3 menunjukkan pencapaian lebih baik di dalam ujian MWM ($P<0.05$). Anak tikus M3 juga menunjukkan prestasi yang nyata lebih baik untuk ujian tingkahlaku haiwan, seperti ujian ruang terbuka (open field

test) dan perilaku keibuan (maternal behavior). Profil asid lemak anak tikus mirip dengan profil asid lemak plasma ibu. Pengambilan diet ibu yang tinngi dengan n-3 PUFA seperti minyak menhaden meningkatkan tahap DHA di dalam susu dan plasma, terutamanya bagi Kumpulan M3. Nisbah n-6:n-3 juga menunjukkan tahap rendah di dalam susu M3 pada 4.7 dan ini berbanding kumpulan yang lain. Ini menunjukkan peranan penting plasma darah dalam membawa DHA ke uri dan seterusnya ke janin tikus. Selain daripada itu, keputusan menunjukkan tikus (n=7) pada kumpulan M3 mempunyai tahap asid docosahexaenoic yang tinggi pada 6.1% (341.2±86.4 mg/100ml) dan jumlah n-3 PUFA pada 30.0% di dalam plasma selepas minggu ke-15.

Eksperimen III bertujuan untuk mengkaji kesan suplementasi n-3 PUFA pada taburan dan histologi neuron. Di akhir eksperimen, semua tikus daripada Eksperimen I dan II (Tikus, n=28 and Anak tikus, n=20) dimatikan dan sampel otak ditimbang sebelum diproses untuk kajian histologi. Keputusan menunjukkan kumpulan M3 dan S6 mempunyai berat tisu otak yang rendah berbanding dengan kumpulan BT yang diberi makan mentega ($P<0.05$). Walau bagaimanapun, kepadatan neuron paling tinggi di kalangan ibu dan anak tikus daripada kumpulan M3 yang menerima suplementasi n-3 PUFA. Keputusan ini mengesahkan bahawa suplementasi n-3 PUFA memberi kesan kepada perkembangan neuron dan otak yang berkaitan dengan proses kognitif pada tikus.

Kesimpulannya, suplementasi diet n-3 PUFA meningkatkan perkembangan fungsi kognitif atau mental di dalam tikus. Peningkatan ini dicapai melalui perubahan struktur dan fungsi yang berlaku ke atas neuron yang terlibat dengan proses pembelajaran dan kemampuan ingatan berasaskan ruang pada subjek eksperimen.

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I certify that an Examination Committee has met on ___________ to conduct the final examination of Hafandi Ahmad on his Master of Science thesis entitled "Effects of Long Chain n-3 Polyunsaturated Fatty Acid Supplementation on Cognitive Function Development in Rats" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

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Date : 12 APRIL 2007

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

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HAFANDI AHMAD

Date : 18 MARCH 2007

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LIST OF ABBREVIATIONS

ALA	alpha linolenic acid
ANOVA	analysis of variance
ARA	Arachidonic acid
ATP	adenosine triphosphate
^{o}C	degrees Celsius
^{o}C/min	degrees Celsius per minute
cal	calorie
CA	*Cornu ammonis*
cm	centimeter
CNS	Central nervous system
COX	cyclooxygenase
DHA	Docosahexaenoic acid
DPA	Docosapentaenoic acid
DM	dry matter
EFA	Essential fatty acids
EPA	Eicosapentaenoic acid
FA	Fatty acid
g	gram
G	gravity force
h	hour
HDL	High Density Lipoprotein
H & E	Haematoxylin and Eosin
IL	interleukin
kcal	kilo calories

kg	kilogram
L	litre
LA	linoleic acid
LDL	Low Density Lipoprotein
M	Molar
MJ/kg	Megajoules per kilogram
m	metre
mm	millimeter
min	minute
μm	micrometer
mg	milligram
mg/L	milligrams per litre
mL	millilitre
mL/min	millilitres per minute
MUFA	Monounsaturated fatty acids / Monoenoic fatty acids
MWM	Morris Water Maze
N	Normal
n-3:n-6 ratio	Total n-3 PUFA to Total n-6 PUFA ratio
P:S ratio	Total PUFA to Total SFA ratio
PUFA	Polyunsaturated fatty acids
SD	standard deviation
SE	standard error
sec	second
SFA	Saturated fatty acids

TAG	Triacylglycerol
TNF	Tumor Necrosis Factor
UFA	Unsaturated fatty acids
U:S ratio	Total UFA to Total SFA ratio
VLDL	Very Low Density Lipoprotein
wk	week
w	weight
v	volume

CHAPTER I

INTRODUCTION

Fatty acids are essential components of the diet and sources of food energy (Whalley *et al.,* 2004; Rudin and Felix, 1996). The n-3 and n-6 fatty acids are essential for the growth and functional development of the infant and early life (Horrocks and Yeo, 1999). Previous studies have shown that fatty acids had a significant impact in early life on CNS, growth development and learning ability (Yamamoto *et al.,* 1987). A study carried out by Rajion (1985), reported Burr and Burr (1930) first acknowledged that specific components of fatty acids may be necessary for the proper growth and development of animals and possibly humans. In 1960s, the essentiality of fatty acids thought to be of nutritional importance in human and animals was proposed with clinical signs of EFA deficiency such as cardiovascular disease, immune system and neurologic as well as brain development (Uauy and Dangour, 2006). Thus, the dietary fatty acids are vitally important for mammalian development in particular the brain function and the associated cognitive performance.

In mammals, the fatty acids have been recognized as important nutrition factors for cognitive performance (Whalley *et al.,* 2004). Nutrient supplementation of macronutrients such as n-3 PUFA are widely accepted to improve diets, and contribute to the maintenance of good health, especially on the cardiovascular system and other aspects of health, such as cognition throughout life (Bakker *et al.,* 2003; Youdim *et al.,* 2000). Fish oil from menhaden, salmon and sardines are the