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CARDIOPROTECTIVE EFFECTS OF YELLOWSTRIPE SCAD COMPARED TO SALMON AMONG HEALTHY OVERWEIGHT ADULTS IN A RANDOMIZED CROSSOVER TRIAL

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By

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CARDIOPROTECTIVE EFFECTS OF YELLOWSTRIPE SCAD COMPARED TO SALMON AMONG HEALTHY OVERWEIGHT ADULTS IN A RANDOMIZED CROSSOVER TRIAL

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January 2019

Chair: Associate Professor Loh Su Peng, PhD
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Fish intake may be cardioprotective, with omega-3 fatty acid as the possible major contributing nutrient. However, intervention study determining the cardioprotective effects of omega-3 rich fish is scarce. It is unclear whether yellowstripe scad (YSS) as compared with salmon, both rich in omega-3 fatty acids, have effects on cardiovascular disease (CVD) risk factors. The objective of this study was to compare the effects of YSS and salmon on selected CVD risk factors among healthy overweight adults. A randomized crossover trial with two diet periods was conducted among healthy overweight (with BMI 23.0-27.4 kg/m2) Malaysian adults aged 21-55 years. Steamed whole YSS fish or salmon fillet was given for eight weeks (3 days per week), retaining approximately 1000 mg eicosapentaenoic acid and docosahexaenoic acid (EPA+DHA) per day. Diets were switched after an 8-week washout period. Fasting blood samples were collected before and after each diet period. Biochemical changes in serum and plasma were analysed. About 98 subjects were screened for eligibility and 49 subjects were participated in the intervention (35% male and 65% female; mean age 29±7 years). Results showed that P-selectin and erythrocyte sedimentation rate (ESR) were significantly reduced from baseline following the consumption of YSS (-13.024% and -14.76% respectively, $p< .05$). Conversely, salmon group had significant improvement on lipid profile [triglycerides (-15.09%), HDL-cholesterol (+3.85%), and VLDL-cholesterol (-14.58%)] and inflammatory cytokines [IL-6 (-5.88%) and TNF-α (-2.19%)] ($p< .05$). Uric acid was increased significantly by salmon diet from 0.32±0.10 mmol/L to 0.35±0.09 mmol/L ($p< .05$). Greater favourable effects on triglycerides (0.09 mmol/L, 95% CI=0.06-0.22, $p= .01$), VLDL-cholesterol (0.04 mmol/L, 95% CI=0.03-0.11, $p= .01$), and IL-6 (0.01 pg/ml, 95% CI=-0.04-0.07, $p= .03$) were noted in salmon group as compared with YSS. These outcomes demonstrated that short-term consumption of salmon showed more pronounced effect on lipid profile and inflammatory factors as compared with YSS. Nevertheless, the favourable changes on haematological factors exerted by YSS group might deserve same attention. The

beneficial effects of YSS and salmon on selected cardiovascular risk factors were unlikely to be similar despite their comparable EPA+DHA content.

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

KESAN KARDIOPROTEKTIF PENGAMBILAN IKAN SELAR KUNING (YSS) BERBANDING SALMON DI KALANGAN SUBJEK BERLEBIHAN BERAT BADAN YANG SIHAT DALAM SUATU PERCUBAAN *CROSSOVER* RAWAK

Oleh

CHANG WEI LIN

Januari 2019

Pengerusi: Profesor Madya Loh Su Peng, PhD
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Pengambilan ikan mungkin bersifat kardioprotektif, dengan asid lemak omega-3 sebagai penyumbang nutrien utama. Namum, kajian intervensi yang menentukan kesan kardioprotektif ikan kaya omega-3 adalah terhad. Ia adalah tidak jelas sama ada ikan selar kuning (YSS) berbanding dengan salmon, kedua-dua ikan yang kaya dengan asid lemak omega-3, mempunyai kesan atas faktor-faktor risiko penyakit kardiovaskular (CVD). Objektif kajian ini adalah membandingkan kesan YSS dan salmon pada faktor-faktor risiko CVD di kalangan orang dewasa berlebihan berat badan yang sihat. Percubaan *crossover* rawak dengan dua fasa diet dijalankan di kalangan orang dewasa Malaysia berusia 21-55 tahun dan berlebihan berat badan yang sihat (BMI 23.0-27.4 kg/m^2). Ikan YSS atau fillet salmon diberikan selama lapan minggu (3 hari seminggu), mengekalkan kira-kira 1000 mg asid eikosapentaenoik dan asid dokosaheksaenoik (EPA+DHA) setiap hari. Diet telah ditukar selepas satu fasa. Terdapat tempoh 8 minggu antara dua fasa tersebut di mana sebarang diet tidak diberikan. Sampel darah puasa telah dikumpulkan sebelum dan selepas setiap fasa diet. Perubahan biokimia dalam serum dan plasma dianalisikan. Sebanyak 98 subjek telah disaring untuk kelayakan dan 49 subjek telah mengambil bahagian dalam intervensi ini (35% lelaki dan 65% wanita; berumur 29±7 tahun). Hasil kajian menunjukan bahawa P-selektin dan kadar endapan eritrosit (ESR) berkurang dengan ketara berbanding dengan tahap asas selepas pengambilan YSS (-13.024% dan -14.76% masing-masing, p <.05). Sebaliknya, kumpulan salmon mempunyai peningkatan ketara pada profil lipid [trigliserida (-15.09%), HDL-kolesterol (+3.85%), dan VLDL-kolesterol (-14.58%)] dan sitokin keradangan [IL-6 (-5.88%) dan TNF-α (-2.19%)] (p< .05). Asid urik meningkat dengan ketara oleh diet salmon dari 0.32±0.10 mmol/L hingga 0.35±0.09 mmol/L (p< .05). Kesan pada trigliserida (0.09 mmol/L, 95% CI=0.06-0.22, p=.01), VLDL-kolesterol (0.04 mmol/L, 95% CI=0.03-0.11, p= .01), dan IL-6 (0.01 pg/ml, 95% CI=-0.04-0.07, p= .03) dicatatkan lebih baik dalam kumpulan salmon berbanding dengan YSS. Hasil ini menunjukkan bahawa pengambilan salmon secara jangka pendek menunjukkan kesan

yang lebih ketara terhadap profil lipid dan faktor hematologi berbanding dengan YSS. Walau bagaimanapun, perubahan yang menggalakkan terhadap faktor hematologi yang ditunjukkan oleh kumpulan YSS patut mendapat perhatian yang sama. Kesan manfaat YSS dan salmon pada faktor-faktor risiko CVD terpilih mungkin tidak sama walaupun kandungan EPA+DHA kedua-dua ikan tersebut adalah setanding.

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This thesis was 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 were as follows:

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LIST OF ABBREVIATIONS

AA	Arachidonic acid
ADHD	Attention-deficit/hyperactivity disorder
AHA	American Heart Association
ALT	Alanine aminotransferase
ALP	Alkaline phosphatase
ANOVA	Analysis of variance
BP	Blood pressure
BMI	Body mass index
CVD	Cardiovascular disease
DHA	Docosahexaenoic acid
eGFT	Estimated glomerular filtration rate
ELISA	Enzyme-linked immunosorbent assay
EPA	Eicosapentaenoic acid
ESR	Erythrocyte sedimentation rate
FAO	Food and Agriculture Organization of the United Nations
GGT	Gamma-glutamyl transferase
HbA1c	Haemoglobin a1c
Hcy	Homocysteine
HDL-C	High density lipoprotein-cholesterol
IFN	Interferon
IHD	Ischaemic heart disease
IHME	Institute for Health Metrics and Evaluation
IL	Interleukin
IPAQ	International Physical Activity Questionnaires
IQR	Interquartile range
LDL-C	Low density lipoprotein-cholesterol
LT	Leukotriene
MDRD	Modification of Diet in Renal Disease
OD	Optical density
OECD/FAO	Organisation for Economic Co-operation and Development/ Food and Agriculture Organization of the United Nations
PAI-1	Plasminogen activator inhibitor-1
PG	Prostaglandin
PUFA	Polyunsaturated fatty acid
SD	Standard deviation
TC	Total cholesterol
TG	Triglycerides
TNF	Tumour necrosis factor
TX	Thromboxane
UNEP-WCMC	United Nations Environment Programme's World Conservation Monitoring Centre
VLDL-C	Very low density lipoprotein-cholesterol
vWF-A2	Von Willebrand factor-A2
WCC	White cell count
WHO	World Health Organization
YSS	Yellowstripe scad
24HR	24-hour dietary recall

CHAPTER 1

INTRODUCTION

1.1 Background

Cardiovascular disease (CVD) is a global health problem with epidemic proportions, accounting for 422.7 million cases (Roth et al., 2017). The high burden of CVD death may be attributable to several risk factors, including raised and abnormal blood lipids (dyslipidaemia), raised blood sugar (diabetes), raised blood pressure (hypertension) and raised body mass index (obesity) (Forouzanfar et al., 2016). Increase in the prevalence of these risk factors is closely related to CVD development. If this is confirmed, nearly half of the Malaysian adults are at high risk of developing CVD (Institute for Public Health, 2015).

CVD is preventable through the improvement of its risk factors. Multiple efforts have been devoted towards CVD prevention. Healthy eating promotion is likely to be most cost-effective than other strategies including use of medication, screening and lifestyle advice, and smoking cessation (Brunner, Cohen, & Toon, 2001). This implies that proper diet is the cornerstone of CVD prevention and the ideal for cardiovascular health. A comprehensive analysis of 20 studies done by Mozaffarian and Rimm (2006) reported that consuming 85 grams of fatty fish once or twice a week may reduce the CVD death risk by 36 %. The findings provided strong support over the role of fish for the prevention of CVD.

The cardioprotective effects of fish are suggested to be the synergistic effects of various nutrients in fish (He, 2009). The nutrients include selenium (Rayman, Stranges, Griffin, Pastor-Barriuso, & Guallar, 2011), vitamin D (Pludowski et al., 2013), amino acids (Ahmadian, Roshan, Aslani, & Stannard, 2017), and omega-3 fatty acids (Gammelmark et al., 2016). Among these nutrients, omega-3 is proposed to be the major contributor to these effects (Gammelmark et al., 2016).

Omega-3 fatty acids are essential polyunsaturated fatty acids that need to be obtained from diet due to the lack of delta-12 and delta-15 desaturases in human (Gropper & Smith, 2016). Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the two main long-chain omega-3 fatty acids commonly found in marine source (Nettleton, 2012). Marine fish, especially salmon, is the principal source of EPA and DHA (United States Department of Agriculture, 2017). However, for tropical countries like Malaysia, salmon is very costly and need to be imported from other cold climacteric countries such as Norway. Recently, yellowstripe scad (YSS, *ikan selar kuning*), a local fish in Malaysia, was identified to be able to provide a comparable EPA+DHA content (Abd Aziz, Azlan, Ismail, Mohd Alinafiah, & Razman, 2012) as salmon sold in supermarket (Blanchet et al., 2005) (879 mg/100 g vs. 947 mg/100 g). YSS is not only cheap but commonly available in Malaysia throughout the year.

According to American Heart Association (Kris-Etherton & Hill, 2008) and World Health Organization (Amine et al., 2002), general adult population is recommended to consume at least twice a week of 100-gram fish (particularly fatty fish) or EPA+DHA supplementation of 200-500 mg daily for CVD prevention. Higher intake may be needed for people with established CVD and for those at high risk of developing CVD. However, a recent study suggested that in order for individuals without CVD to have greatest CVD risk protection, the EPA+DHA intake should be at least 1 g/day of EPA+DHA (Patterson, Chalil, Henao, Streit, & Stark, 2015). Given that Malaysia is a maritime country, it is not surprising that Malaysians achieved the recommended fish intake, by consuming one and one-half medium fish at least once a day (Norimah et al., 2008). Indeed, Malaysia was among the world's biggest consumers of fish, eating at least 56.5 kilograms of fish per person each year (Aruna, 2014). Among the three ethnic groups in Malaysia, Malays had higher fish consumption as compared with others (Chinese and Indians) (Ahmad et al., 2016).

1.2 Problem Statement

Cumulative observational evidence has demonstrated favourable association between fish consumption and CVD risk (Gammelmark et al., 2016; Grung et al., 2015; Kiecolt-Glaser et al., 2012; Kim et al., 2015) although not all agree (Rhee, Kim, Buring, & Kurth, 2017). Unfortunately, data from intervention studies examining the cardioprotective effects of fish is sparse and less conclusive (Grieger, Miller, & Cobiac, 2014; Lindqvist, Langkilde, Undeland, & Sandberg, 2009; Vazquez et al., 2014; Zhang et al., 2012).

Secondly, YSS is one of the most frequently consumed marine fish in Malaysia (Ahmad et al., 2016). Since YSS and salmon have comparably high EPA and DHA content and these nutrients may be the major contributor in cardioprotection (Zhang et al., 2012), it is meaningful to investigate if both fish species could have similar beneficial health properties. However, to date, their cardio-protective effects has not yet been explored and compared.

Moreover, there is limited research trials conducted among overweight population, who is at increased CVD risk independent of the traditional risk factors (Bogers et al., 2007). Therefore, this study will address the research question "What is the difference in the effects between YSS and salmon on CVD risk factors among healthy overweight subjects?"

1.3 Significance of the Study

The study is important as the results could act as the baseline data on YSS as potential source of safe cardio-protective agent with minimal side effects. Favourable findings may also promote YSS as an alternative source of omega-3 fatty acids in healthy eating practice, which is better in terms of cost and availability as compared with salmon. The health benefits towards CVD protection may initiate future research to widen its

utilization as a new source of functional ingredient such as emulsion or micro-encapsulated products that further improve its economic importance.

1.4 Objectives

1.4.1 General Objective

To study the effects of YSS compared with salmon on CVD risk factors among healthy overweight subjects

1.4.2 Specific Objective

1) To study the effects of consuming YSS and salmon respectively on CVD risk factors (including atherosclerotic factors: blood lipid profile, blood glucose profile, blood pressure, and body weight status; inflammatory factors: inflammatory cytokine and homocysteine levels; and haematological factors: haemostatic parameters and complete blood count; other factors: liver and renal functions) among healthy overweight subjects

2) To compare the effects between YSS and salmon on CVD risk factors among healthy overweight subjects

1.5 Hypotheses

1) There are significant effects of consuming YSS and salmon respectively on CVD risk factors among healthy overweight subjects

2) There is no significant difference in the effects between YSS and salmon on CVD risk factors among healthy overweight subjects

1.6 Conceptual Framework

Figure 1.1 shows the conceptual framework of the study. Based on the study objectives, CVD risk factors were studied as dependent variables; whereas, dietary fish intake was included as independent variables.

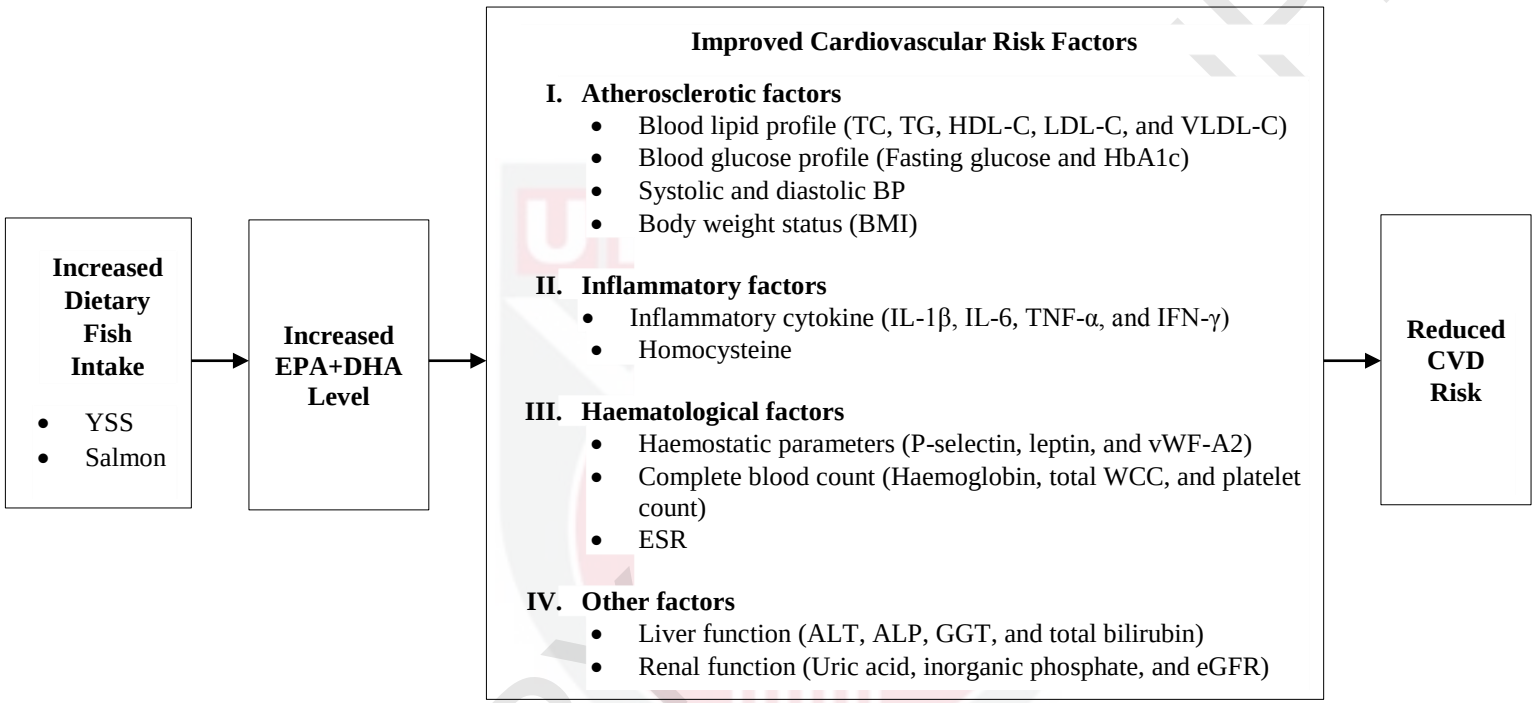

Figure 1.1: Conceptual Framework of the Study

Note. YSS: yellowstripe scad; TC: total cholesterol; TG: triglycerides; HDL-C: high density lipoprotein-cholesterol; LDL-C: low density lipoprotein-cholesterol; VLDL-C: very low density lipoprotein-cholesterol; HbA1c: haemoglobin A1c; BP: blood pressure; BMI: body mass index; IL: interleukin; TNF: tumour necrosis factor; IFN: interferon; vWF-A2: von Willebrand factor-A2; WCC: white cell count; ESR: erythrocyte sedimentation rate; ALT: alanine aminotransferase; ALP: alkaline phosphatase; GGT: gamma-glutamyl transferase; eGFR: estimated glomerular filtration rate

Previous studies have shown that atherosclerotic factors, including high blood lipid, high fasting glucose, high blood pressure, and high body weight status are the traditional risk factors of CVD (Forouzanfar et al., 2016; Mendis, Puska, & Norrving, 2011; Nordestgaard et al., 2012). A study suggested that for every 4 kg/m² increase in body mass index, there was 52% increase in CVD risk (Nordestgaard et al., 2012). Apart from being an indicator of glycaemic control, high haemoglobin A1c (HbA1c) level has shown to be associated with increased risk of future CVD, especially among general population without known diabetes (Khaw et al., 2004; Kim et al., 2008; Rijkelijkhuizen et al., 2012).

Other than the traditional risk factors, inflammation is closely linked with CVD (Arima et al., 2008; Danesh et al., 2004; Kuller, Tracy, Shaten, Meilahn, & Group, 1996; Mendall et al., 1997; Mohamed, Musa, & Ismail, 2015). Pro-inflammatory cytokines such as IL-1β, IL-6, TNF-α, and IFN-γ appear to be strongly associated with CVD (Kaptoge et al., 2013). Similarly, elevated homocysteine level has been documented to be a risk factor of CVD (Sahu, Gupta, Kavishwar, & Singh, 2015).

The role of haematological disorders in the development of CVD has received much attention recently (Folsom, Wu, Rosamond, Sharrett, & Chambless, 1997; Folsom, Wu, Shahar, & Davis, 1993; Smith et al., 2005; Stec et al., 2000; Willeit et al., 2013). P-selectin and leptin, both promote platelet activation (Corsonello et al., 2003; Zee et al., 2006), have emerged as potentially useful predictors of CVD (Palavra, Reis, Marado, & Sena, 2015; Romero-Corral et al., 2008; Vleuten et al., 2005). Raised vWF level, as the marker of hypercoagulability, has been related to the atherothrombotic complications and endothelial damage, leading to the development of CVD (Montoro-García, Shantsila, & Lip, 2014). In terms of complete blood count, a recent study found that haemoglobin disorders were common among patients with cardiovascular disease in South Asians population (Patel et al., 2013). Elevated total white cell and platelet counts are directly associated with increased incidence of CVD (Lee et al., 2001; Madjid & Fatemi, 2013; Park et al., 2010). ESR appears to be a long-term independent predictor of CVD in both men and women (Andresdottir, Sigfusson, Sigvaldason, & Gudnason, 2003; Erikssen et al., 2000; Ingelsson, Årnlöv, Sundström, & Lind, 2005).

Other risk factors include liver function (Schindhelm et al., 2007) and renal function (Forouzanfar et al., 2016). Abnormal levels of liver enzymes including alanine aminotransferase (ALT), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) have shown to be associated with CVD (García-Hermoso, Hackney, & Ramírez-Vélez, 2017; Kim et al., 2012; Kunutsor, Apekey, Seddoh, & Walley, 2014; Kunutsor, Bakker, Gansevoort, Chowdhury, & Dullaart, 2015; Motamed et al., 2017). In contrast, high level of total bilirubin has shown to have protection against CVD (Kim et al., 2012; Kunutsor et al., 2015). In terms of renal function, higher levels of uric acid and phosphate, as well as reduced glomerular filtration rate are independent risk factor of CVD (Kim et al., 2010; Kleber et al., 2015; Manjunath et al., 2003; Qin et al., 2014; Scialla & Wolf, 2014).

All these risk factors could be minimised through the consumption of dietary fish intake (Burr et al., 1989; Dolecek & Grandits, 1991; Erkkilä et al., 2014; He et al., 2002; Hu, Cho, Rexrode, Albert, & Manson, 2003; Levitan, Wolk, & Mittleman, 2010; Yamagishi et al., 2008).

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BIODATA OF STUDENT

Chang Wei Lin is a girl who born in Ipoh, Perak on 2 August 1992. She is graduated with a Bachelor of Science (Nutrition and Community Health) at Universiti Putra Malaysia in 2016. Her ambition is to become a lecturer in Nutrition course. Given that holding a PhD degree serves as an essential qualification for a lecturer at Research University, she thus pursued her master degree under Associate Professor Dr. Loh Su Peng upon graduation. Since she likes to eat fish and has a great interest in doing intervention study, she conducted a fish-related intervention as her master project.

LIST OF PUBLICATIONS

Chang, W. L., Azrina, A., Sabariah, M. N., Irmi Zarina, I., & Loh, S. P. (2017). Effects of consuming yellowstripe scad versus salmon on lipid profile, fasting glucose, body weight status and blood pressure among healthy overweight Malaysian adults. *Malaysian Journal of Nutrition*, 23(3), 343-352.

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