UNIVERSITI PUTRA MALAYSIA

LIPID SIGNALING PATHWAYS GENES EXPRESSION LEVEL IN HEPG2 CELLS SUPPLEMENTED WITH EXOGENOUS LIPID & EDIBLE BIRD NEST (EBN) EXTRACT

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A project paper submitted to the
Faculty of Veterinary Medicine, Universiti Putra Malaysia
In partial fulfilment of the requirement for the
DEGREE OF DOCTOR OF VETERINARY MEDICINE
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It is hereby certified that we have read this project paper entitled "Evaluation of Lipid Signalling Pathways Genes Expression in HepG2 Cells Supplemented with Exogenous Lipid & Edible Bird Nest (EBN) Extract", by Darreen A/P Tawai and in our opinion it is satisfactory in term of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999 Final Year Project.

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DEDICATIONS

To my pillars of strength, my rocks,my backbones, my muse, and my believers…It has been a rough ride, but I am here today because of all of you.

-Darreen-

If you look at what you have in life, you'll always have more. If you look at what you don't have in life, you'll never have enough.

-Oprah Winfrey-

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LIST OF ABBREVIATIONS

EBN Edible-bird‟s nests

EGF Epidermal Growth Factor

LDL Low Density Lipoprotein

LDs Lipid Droplets

ACAT2 Acetyl-CoA acetyltransferase 2

DGAT2 Diacylglycerol O-acyltransferase 2

LDLR Low density lipoprotein receptor

PCSK9 Proprotein Convertase Subtilisin/kexin type 9

SREBP2 Sterol regulatory element binding transcription factor 2

HMGCR 3-hydroxy-3-methylglutaryl-CoA reductase

TAGs Triacylglycerols/Triglycerides

ER Endoplasmic reticulum

PBS Phosphate Buffer Saline

ATCC AmericanTypeCulture Collection

AMEM Advanced Modified Eagle‟s Medium

FBS Fetal Bovine Serum

CC Cholesterol Concentrate

BC Base Control

NC Negative Control

PC Positive Control

TX1 Treatment 1

TX2 Treatment 2

TX3 Treatment 3

VLDL Very Low Density Lipoprotein

PCR Polymerase Chain Reaction

mAbs Monoclonal antibodies

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD4999 – Projek Tahun Akhir

PENENTUAN EKSPRESI GEN-GEN YANG TERLIBAT DALAM LALUAN LIPID DI DALAM SEL HEPG2 DENGAN LIPID TAMBAHAN DAN EKTRAK SARANG BURUNG

Oleh

Darreen A/P Tawai

2018

Penyelia: Dr Mohd Mokrish Md Ajat

Penyelia bersama: Dr Rozaihan Mansor, Dr Intan Shameha Abdul Razak

"Kaviar dari Timur" ataupun lebih dikenali sebagai sarang burung walit adalah produk yang sangat bermanfaat dan berharga di mana ianya terdiri daripada rembesan air liur burung dari beberapa jenis burung walit pemakan serangga. Burung walit ini tergolong dalam family Apodidae dan dua genera yang mempunyai tahap komersial yang paling tinggi adalah Aerodramus (burung walit bergemea) dan Collocalia (burung walit tidak bergema). Tujuan utama kajian ini dijalankan adalah untuk menentukan tahap kualitatif gen-gen yang terlibat dalam laluan lipid seperti Acetyl-CoA acetyltransferase 2 (ACAT2), Diacylglycerol O-acyltransferase 2 (DGAT2), Low density lipoprotein receptor (LDLR), Sterol regulatory element binding transcription factor 2 (SREBP2), 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), Proprotein Convertase Subtilisin/kexin type 9 (PCSK9) di dalam sel mammalia HepG2 dengan atau pun tanpa lipid tambahan dan ekstrak sarang burung. Sarang burung

dibersihkan, dikeringkan di dalam incubator pada suhu 60°C dan dibiarkan semalaman. Dalam projek ini, supernatant sarang burung ditambahkan dengan asseon dengan menggunakan pada kadar nisbah 1:2 dan di simpan pada suhu -80°C selamas atu jam. Sel mammalia HepG2 dikultur dan diberi rawatan mengikut Kawalan Asas, Kawalan Positif, Kawalan Negatif, Rawatan 1, Rawatan 2 dan Rawatan 3. Berdasarkan kajian ini, terdapat perubahan ketara dalama spekkualitatif gen-gen setelah diberi ekstrak sarang burung dan lipid tambahan terutamanya terhadap gen ACAT2 dan LDLR.

Kata kunci: Sarangburung, Aerodramus spp., metabolisme lipid, ekspresi gen, PCR kualitatif

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4999 Final Year Project

LIPID SIGNALLING PATHWAYS GENES EXPRESSION LEVEL IN HEPG2 CELLS SUPPLEMENTED WITH EXOGENOUS LIPID AND EDIBLE BIRD NEST (EBN) EXTRACT

By

Darreen A/P Tawai

2018

Supervisor: Dr. MohdMokrish Md. Ajat

Co-Supervisors: Dr Rozaihan Mansor, Dr IntanShameha Abdul Razak

"Caviar of the East" or better known as Edible-Bird Nest (EBN) is a highly valuable product composed from solidified salivary secretion of a few insectivorous swiftlet species. Swiftlets falls under the family Apodidae and the two main genera with the highest commercial values are Aerodramus (echolating swiftlets) and Collocalia (non-echolating swiftlets). The objective of this study is to determine the qualitative genes expression associated in lipid signalling pathways such as Acetyl-CoA acetyltransferase 2 (ACAT2), Diacylglycerol O-acyltransferase 2 (DGAT2), Low density lipoprotein receptor (LDLR), Sterol regulatory element binding transcription factor 2 (SREBP2), 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR),

Proprotein Convertase Subtilisin/kexin type 9 (PCSK9) in HepG2 mammalian cell with or without supplementation of EBN extract and exogenous lipid. Raw EBN were manually cleaned, dried in 60°C oven and left overnight. In this project, EBN supernatant were added with acetone using 1:2 ratio and kept in -80°C for an hour. Hep G2 cells were cultured in six wells plates and each wells were treated according to Base Control, Positive Control, Negative Control, Treatment 1, Treatment 2 and Treatment 3. In this study, there are significant changes in term of genes expression with supplementation of EBN extract especially in ACAT2 and LDLR genes.

Keywords: Edible bird nest, Aerodramus spp., lipid metabolism, genes expression, qualitative PCR

CHAPTER 1

INTRODUCTION

"Caviar of the East" or better known as Edible Bird Nest (EBN) is a highly valuable product composed from solidified salivary secretion of a few insectivorous swiftlet species. These swiftlets fall under the family Apodidae and the two main genera with the highest commercial values are Aerodramus (echolating swiftlets) and Collocalia (non-echolating swiftlets) (Wong, 2013). Swiftlets are distributed nearly worldwide except for coldest and treeless areas of the northen hemisphere with the highest distribution in warmer tropical regions.

According to Wong (2013), *Aerodramusfuciphagus* is the most commonly found swiftlet species in Malaysia, producing the premium grade white nest due to its composition: purely solidified salivary secretion with high concentrations of N-acetylneuraminic acid (sialic acid) and epidermal growth factor (EGF) (Looi et al., 2017). It is believed that EBN offers abundant of medicinal and health-boosting properties however there are not much scientific reports to prove this.

The objective of this study is to determine the expression level of lipid signalling pathways genes such as Acetyl-CoA Acetyltransferase (ACAT), Diacylglycerol O-acyltransferase 2 (DGAT2), Sterol regulatory element binding transcription factor 2 (SREBP2), 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR),Low density lipoprotein receptor (LDLR), Proprotein Convertase Subtilisin/kexintype 9 (PCSK9) in HepG2 mammalian cells after supplemented with exogenous lipid and treated with or without Edible Bird Nest extract.

Hypothesis

H_o: There are no significant changes in the expression of lipid signalling genes after supplementation with exogenous lipid & EBN extract

H_A: There are significant changes in expression of lipid signalling pathway genes in the mammalian cells after supplementation with exogenous lipid & EBN extract

questionable due to its clarity, EBN extract supplementation might have a positive effect in reducing PCSK9 expression. Further research can be done to look into deeper the significant of EBN extract towards genes expression.

6.2 RECOMMENDATIONS

I would like to propose a recommendation to extend/provide a longer period for final Year Project. Research-based projects as such in this project require a longer period of time in order to produce a more reliable and satisfactory result and at the same time minimizing technical error. As for recommendation related to my project, a higher cell confluency, approximately 80-100%, is needed in order to achieve a higher RNA concentration for PCR study.

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