

CONTENTS

	Page
Acknowledgement	d
Abstract in Thai	e
Abstract in English	g
Lists of Tables	l
Lists of Figures	m
List of Abbreviations	o
List of Symbols	s
Chapter 1 Introduction	1
1.1 Principle, theory and rational/Hypothesis	1
1.2 Literature review	3
1.2.1 Obesity and insulin resistance	3
1.2.2 Obese-insulin resistance and the heart	5
1.2.3 Insulin resistance and mitochondrial function	5
1.2.4 Obese-insulin resistance and gut microbiota	6
1.2.5 The probiotics	9
1.2.6 Effects of probiotics on obesity and metabolic syndrome	10
1.2.7 Effects of probiotics on the inflammatory process and oxidative stress	11
1.2.8 Effects of probiotics on cardiovascular disease	12
1.3 Purposes of the study	12
1.4 Hypothesis of the study	13
Chapter 2 Materials and methods	14
2.1 Research design, scope, and methods	14
2.1.1 Study protocol	14
2.2.2 General methods	15
1) Animal preparation	15
2) High-fat diet preparation	17
3) Probiotics preparation	17

4) Echocardiography protocol	18
5) Heart rate variability (HRV) protocol	18
6) Blood pressure (BP) protocol	19
7) Left ventricle pressure –volume loop (P-V loop) protocol	19
8) Cardiac mitochondrial function protocol	19
9) Western blot analysis for Bax and Bcl-2	22
10) Determination of serum lipopolysaccharide (LPS)	23
11) HPLC based assay of malondialdehyde (MDA) concentration	23
2.1.3 Chemical analysis	24
1) Determination of plasma insulin level	24
2) Determination of plasma glucose level	25
3) Determination of total cholesterol (TC) level	25
4) Determination of plasma triglyceride (TG) level	26
5) Determination of high density lipoprotein (HDL) and low density lipoprotein (LDL) level	27
6) Oral glucose tolerance test (OGTT) protocol	27
7) Determination of insulin resistance (HOMA index)	27
2.2 Statistical analysis	28
Chapter 3 Results	29
3.1 Effects of probiotics improved inflammation and lipid profile in HFD-induced obese-insulin resistant rats.	30
3.2 Effects of probiotics attenuated blood pressure in HFD-induced obese-insulin resistant rats.	32
3.3 Effects of probiotics improved heart rate variability in HFD-induced obese-insulin resistant rats.	35
3.4 Effects of probiotics improved cardiac function in HFD-induced obese-insulin resistant rats.	36
3.5 Effects of probiotics attenuated cardiac mitochondrial dysfunction in HFD-induced obese-insulin resistant rats.	38
3.6 Effects of probiotics improved oxidative stress but did not change in cardiac apoptosis in HFD-induced obese-insulin resistant rats.	39

Chapter 4 Discussion and conclusion	41
References	46
Appendix	65
Appendix A	66
Appendix B	70
Appendix C	72
Appendix D	79
Curriculum vitae	83



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

LIST OF TABLES

	Page
Table 2.1 The contents of the normal diet (ND)	16
Table 2.2 The contents of the high-fat diet (HFD)	17
Tables 3.1 The metabolic parameters at baseline and after 12-weeks of either ND or HFD consumption.	30
Tables 3.2 The metabolic parameters after 12-weeks of vehicle and probiotics administration in ND-fed rats and HFD-fed rats.	31
Tables 3.3 The pressure-volume loop parameters after 12-weeks of vehicle and probiotics administration in ND-fed rats and HFD-fed rats.	38

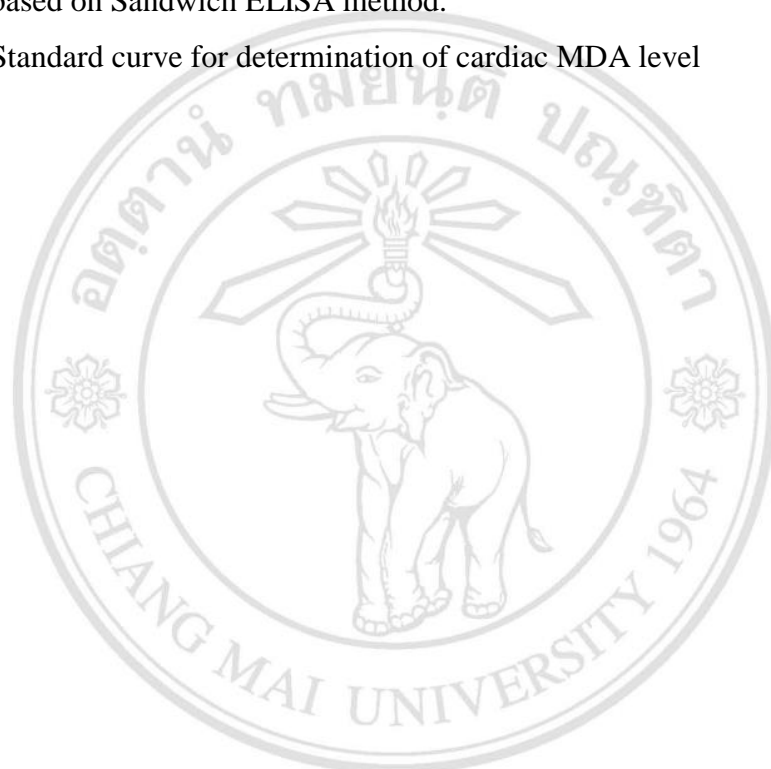


ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

LIST OF FIGURES

	Page
Figure 1.1 Insulin signaling pathway modulating glucose uptake in skeletal muscle cells.	4
Figure 1.2 Composition of dominant microbial species in the human gastrointestinal tract.	8
Figure 1.3 A diagram summarizing the association of long-term HFD, obese-insulin resistance and cardiac dysfunction.	8
Figure 1.4 Potential direct effects of probiotics	10
Figure 2.1 A diagram to demonstrate the study protocol.	15
Figure 2.2 Mechanism of 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA) de-esterification to 2,7-dichlorodihydrofluorescein (DCFH), and further oxidation to fluorescent 2,7-dichlorofluorescein (DCF) by ROS and RNS	21
Figure 3.1 The effects of HFD consumption on metabolic endotoxemia at 12 weeks of HFD consumption and post-treatment.	32
Figure 3.2 The effects of HFD consumption on blood pressure, heart rate variability and echocardiographic parameters at the baseline and after 12 weeks of HFD consumption.	33
Figure 3.3 The effects of probiotics on blood pressure in ND and HFD-fed rats at 4, 8 and 12 weeks of the intervention period.	34
Figure 3.4 The effects of probiotics on LF/HF ratio in ND and HFD-fed rats at 4, 8 and 12 weeks of the intervention period.	35
Figure 3.5 The effects of probiotics on echocardiography parameters in ND and HFD-fed rats at 4, 8 and 12 weeks of the intervention period.	37
Figure 3.6 The effects of probiotics on cardiac mitochondria function in ND and HFD-fed rats.	39
Figure 3.7 The effects of probiotics on oxidative stress and apoptotic makers in ND and HFD-fed rats.	40

Figure 4.1	A diagram summarizing the effects of chronic HFD consumption on cardiac function in HFD-induced obese-insulin resistant rat model.	45
Figure 4.2	A diagram summarizing the effects of probiotics <i>Lactobacillus paracasei</i> ST11 HP4 on cardiac function in HFD-induced obese-insulin resistant rats model.	45
Figure A-1	Standard curve for determination of insulin concentration based on Sandwich ELISA method.	68
Figure A-2	Standard curve for determination of cardiac MDA level	69



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
 Copyright© by Chiang Mai University
 All rights reserved

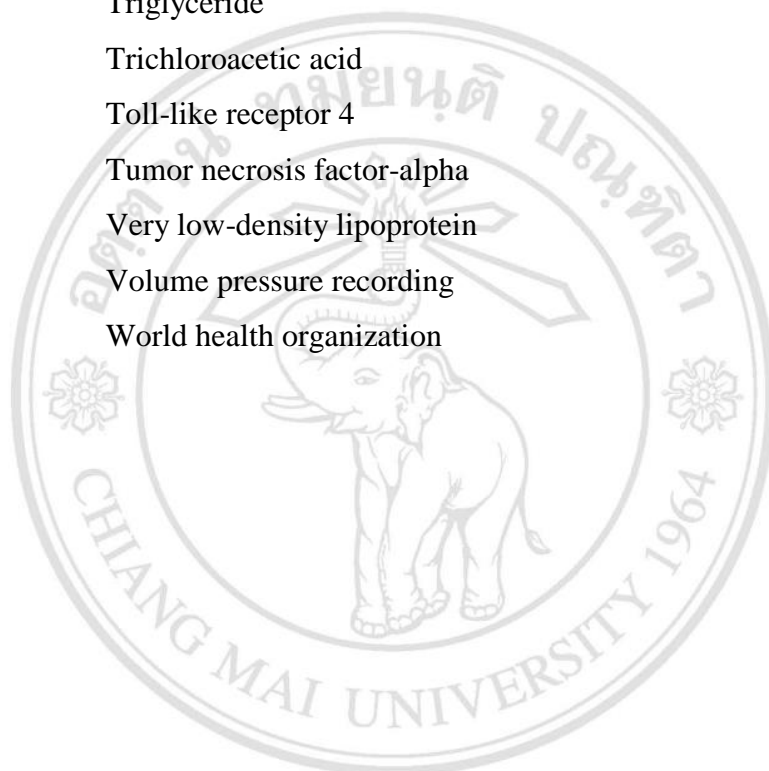
LIST OF ABBREVIATIONS

+ dP/dt	Maximum slope of the systolic pressure increment
- dP/dt	Minimum slope of the systolic pressure increment
Akt	Serine/threonine-specific protein kinase
ATP	Adenosine triphosphate
Bax	BCL2 Associated X Protein
BCA	Bicinchoninic acid
BHT	Butyrate hydroxytoluene
BP	Blood pressure
BSA	Bovine serum albumin
CFU	Colony forming unit
CHE	Cholesterol esterase
CHO	Cholesterol oxidase
CVD	Cardiovascular disease
CRP	C-reactive protein
DBP	Diastolic blood pressure
DCF	Fluorescent 2,7-dichlorofluorescein
DCFDA	Dichlorohydrofluorescein diacetate
DCFH	De-esterification to 2,7-dichlorodihydrofluorescein
DM	Diabetes mellitus
FFA	Free fatty acid
ECG	Electrocardiogram
EGTA	Ethylene glycol bis (2-amino ethylether)-N,N,N,N-tetraacetic acid
ETC	Electron transport chain
g	Gram
GOD	Glucose oxidase
H₂O₂	Hydrogen peroxide
H₃PO₄	H ₃ PO ₄

HDL	High-density lipoprotein
HF	High frequency
HFD	High-fat diet
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HOMA	Homeostasis model assessment
HPLC	High-performance liquid chromatography
HRV	Heart rate variability
IL-6	Interleukin-six
IL-10	Interleukin-ten
IL-12	Interleukin-twelve
IL-17	Interleukin-seventeen
I/R	Ischemia/reperfusion
IRS	Insulin receptor substrate
JC-1	The dye 5, 5', 6, 6'-tetrachloro-1, 1', 3, 3'-tetraethylbenzimidazolcarbocyanine iodide
KCl	Potassium chloride
KH₂PO₄	Potassium dihydrogen phosphate
L	Liter
LAL	Limulus ameocyte lysate
LDL	Low-density lipoprotein cholesterol
LF	Low frequency
LPS	Lipopolysaccharides
LV	Left ventricular
LVEDP	Left ventricular end-diastolic pressure
LVESP	Left ventricular end-systolic pressure
LVIDd	Left ventricular internal diameter during diastole
LVIDs	Left ventricular internal diameter during systole
MAP	Mean arterial pressure
MAPK	Mitogen-activated protein kinase
MDA	Malondialdehyde
Mets	Metabolic syndrome

mg	Milligram
min	Minute
ml	Milliliter
mm	Millimeter
mM	Milimolar
mmHg	Millimeter of mercury
mmol	Millimole
NaCl	Sodium chloride
Na₂CO₃.H₂O	Sodium carbonate monohydrate
NaF	Sodium fluoride
NAFLD	Non-alcoholic fatty liver disease
NaHCO₃	Sodium bicarbonate
Na₂HPO₄	Di-sodium hydrogen phosphate
NaOH	Sodium hydroxide
NaHCO₃	Sodium bicarbonate
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
ND	Normal diet
nm	Nanometer
O-cuff	Occlusion cuff
OGTT	Oral glucose tolerance test
ox-LDL	Oxidized low density lipoprotein
p38	tumor protein 38
PAI-1	Plasminogen activator inhibitor-1
PBS	Phosphate Buffer Saline
PI3K	Phosphatidylinositol 3-kinase
PKB	Protein kinase B
PV-loop	Pressure-volume loop
R/O	Reverse osmosis drinking water
ROS	Reactive oxygen species
SDS	Sodium dodecyl sulfate
SBP	Systolic blood pressure

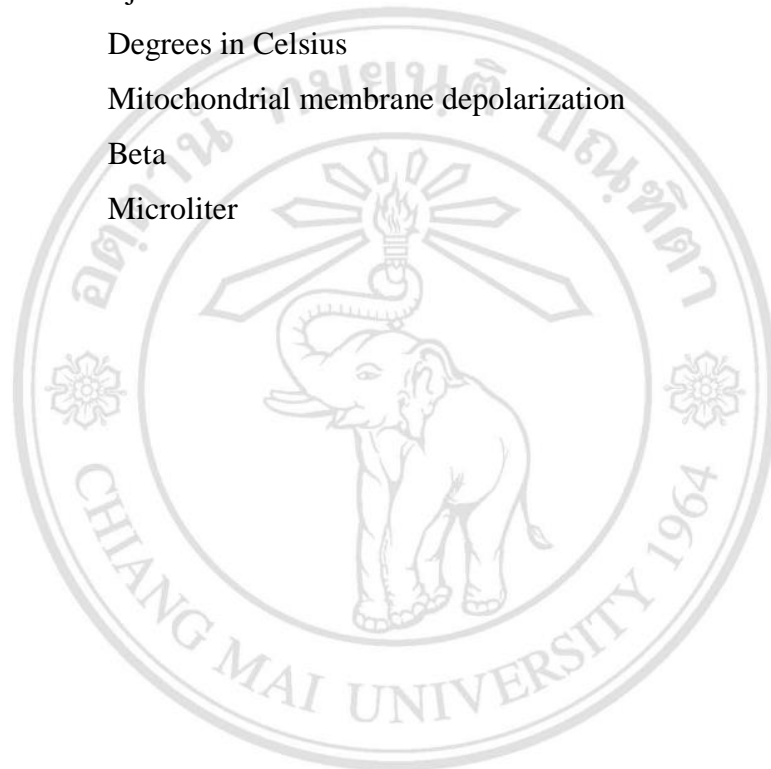
SOD	Superoxide dismutase
SV	Stroke volume
SW	Stroke work
SWR	Standard working reagent
TBA	Thiobabituric acid
TC	Total cholesterol
TG	Triglyceride
TCA	Trichloroacetic acid
TLR-4	Toll-like receptor 4
TNF-α	Tumor necrosis factor-alpha
VLDL	Very low-density lipoprotein
VPR	Volume pressure recording
WHO	World health organization



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
 Copyright© by Chiang Mai University
 All rights reserved

LIST OF SYMBOLS

%	Percentage
%FS	Fractional shortening
%E	Percent of total energy
%EF	Ejection fraction
°C	Degrees in Celsius
$\Delta\Psi$	Mitochondrial membrane depolarization
β	Beta
μl	Microliter



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved