UNIVERSITI PUTRA MALAYSIA

EFFECTS OF CONJUGATED LINOLEIC ACIDS, FISH OIL AND SOYBEAN OIL ON THE GROWTH PERFORMANCE, CARCASS TRAITS, LIPID CHARACTERISTIC AND PPARs mRNA EXPRESSION IN BROILER CHICKENS

MARYAM ROYAN

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By

MARYAM ROYAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in fulfillment of the Requirement for the Degree of Doctor of Philosophy

June 2012

DEDICATION

I dedicate this thesis to

my husband Dr Bahman Navidshad, without whom I'd never have started and finished. I give my deepest expression of love and appreciation for the sacrifices you made during this PhD program;

and to our little angle Kasra.

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

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June 2012

Chairman: Goh Yong Meng, PhD
Faculty: Veterinary Medicine

The conjugated linoleic acids (CLA) are known to decrease hepatic lipid deposition and overall lipid metabolism in selected animal models. These properties can be employed to reduce body fat accumulation in food animals, as in broiler chicken. In fact, every 1 % decrease in the chicken's body fat would result in the average Malaysian eating 300 g less fat for a year from chicken meat, based on per capita chicken meat consumption figures for 2010. However, the anti-lipogenic effects of CLA are variable and complicated because of the differences in animal species, diet composition and possibly their interactions with other dietary fatty acids. We hypothesized that CLA effects on fat metabolism in broiler chickens could be altered by the presence of dietary n-3 and n-6 fatty acids. Hence, fish oil (rich source of n-3 fatty acids) and soybean oil (rich source of n-6 fatty acids) were used in this trial.

This study aimed to investigate the effects of dietary conjugated linoleic acids, fish oil, soybean oil and their mixtures, as well as palm oil on broiler chickens. A total

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of 560 day-old Ross 308 male broiler chickens allotted randomly into 7 equal treatment groups and used in 42 days experimental period. All chicks were fed a starter corn-soybean meal-based diet for 10 days. The treatment diets used were adjusted to be isocaloric and isonitrogenous comprising the corn-soybean meal plus specific dietary fat supplements consisting of conjugated linoleic acid (CLA), fish oil, soybean oil, palm oil or their combinations. The dietary fats were included in the experimental diets at 7% for single fats and 3.5% + 3.5% for dual mixes, and because of the lower metabolisable energy of palm oil as compared to PUFAs, its inclusion rate was about 12%. The conjugated linoleic acids supplement used in this study was LUTA-CLA 60, containing 60% conjugated linoleic acid. Therefore the dietary inclusions of 7 and 3.5% LUTA-CLA 60 were effectively supplying 4.2 and 2.1% CLA, respectively. The treatment groups were 7% soybean oil (SO), 7% LUTA-CLA 60 (CL), 7% fish oil (FO), 3.5% LUTA-CLA 60+ 3.5% soybean oil (CLSO), 3.5% fish oil + 3.5% soybean oil (FOSO), 3.5% LUTA-CLA 60 + 3.5% fish oil (CLFO) and 12% palm oil (PO). The experimental diets were used at grower (11-28 d) and finisher (29-42 d) phases and performance data were collected for each period. Tissue and serum sampling was carried out at the end of experiment (42 d).

The high level of palm oil inclusion in the diet did not adversely affect chicken's feed intake or growth rate. The lowest weight gains were recorded for birds from FO and CL groups during the grower (24.2 g/b/d) and finisher (50.9 g/b/d) phases, respectively. The FO diet also reduced the feed intake of chickens (34.5 g/b/d) while dietary conjugated linoleic acids had no effect on feed intake regardless of its dosage and combinations with other fats. It was also found that palm oil supplemented at about 12 % (w/w) increased the weight of abdominal fat pads significantly (%2.4 of live weight), while higher conjugated linoleic acids

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content of the CL group increased post slaughter liver weights (%3.3 of live weight) (P<0.05). Lipid contents of the breast tissues were higher in the PO (%2.46), SO (%2.02) and FO (%2.02) groups (P<0.05) versus others (%1.11-1.55). Birds from the FO group had the highest amount of fats in their thigh muscles (%3.94) (P<0.05).

Fish oil was more effective in reducing serum undesired lipoproteins (comparable effect with soybean oil), and CL diet enhanced serum favourite HDL fraction. It was also evident that conjugated linoleic acids in combination with soybean oil or fish oil resulted in less fat accumulation in both thigh and breast tissues, as compared to birds treated with soybean oil or fish oil only. Deposition and enrichment of longer chain n-3 fatty acids were also higher in the breast tissue of birds treated with conjugated linoleic acid in combination with fish oil (275 mg/100 g meat) as compared to the fish oil only treatment (254 mg/100 g meat).

The treatment oils demonstrated different effects on PPAR genes. The PPARγ gene was up-regulated significantly in the PO group, whereas the levels of adipose PPARγ gene expression were no different across treatments containing conjugated linoleic acids, fish oil, soybean oil or the mixture of these fats. On the other hand PPARα gene expression in the liver tissue was up-regulated in response to dietary fish oil inclusion and the differences were significant for both FO and CLFO treatments compared to PO, SO and CL treatments.

In conclusion, the results of the present study showed that the dietary 7% fish oil or 4.2% CLA supplements reduced broiler chicken performance. This combination resulted in the enrichment of n-3 in chicken meat. The combination of CLA with soybean oil on the other hand increases the CLA levels in the

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chicken meat. The PPARα gene demonstrated anti-lipogenic effects when it is upregulated in the presence of CLA. Changes in the abdominal fat deposition in broiler chickens could be attributed to both PPARα (in hepatocytes), and PPARγ in adipocytes. Lower abdominal fat deposition was achieved by up-regulating the PPARα (of hepatocytes), in tandem with the down-regulation of PPARγ in the adipocytes.

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Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

KESAN ASID LINOLEIK TERKONJUGAT, MINYAK IKAN DAN MINYAK SOYA PADA PRESTASI, CIRI KARKAS DAN LIPID SERTA EKSPRESI mRNA PPARs AYAM PEDAGING

Oleh

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Jon 2012

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Fakulti: Perubatan Veterinar

Asid lemak terkonjugat (CLA) berupaya mengurangkan pengumpulan lipid hepar serta merendahkan metabolisme am haiwan. Kesan ini boleh digunapakai untuk mengurangkan pengumpulan lemak badan pada hawian ternakan, seperti ayam pedaging. Pengurangan sebanyak 1 % pada lemak badan ayam pedaging bakal mengurangkan sebanyak 300 g lemak setahun daripada daging ayam, berdasarkan data pengambilan daging ayam per kapita tahun 2010. Walaubagaimanapun, kesan anti-lipogenik CLA adalah berbeza dan kompleks mengikut spesis haiwan, kandungan diet, dan kemungkinan interaksi CLA dengan asid lemak yang lain. Hipotesis kajian ini berdasarkan andaian bahawa kesan CLA ke atas metabolisme lemak boleh diaruh oleh kehadiran asid lemak n-3 dan n-6. Sehubungan itu, minyak ikan (sumber asid lemak n-3) serta minyak kacang soya (sumber asid lemak n-6) digunakan dalam eksperimen ini.

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Penyelidikan ini mengkaji kesan asid lemak terkonjugat, minyak ikan, minyak soya dan campurannya, serta minyak kelapa sawit ke atas ayam pedaging. Sejumlah 560 ayam pedaging Ross 308 jantan digunakan dalam kajian ini. Ayam tersebut dibahagikan secara rawak ke dalam 7 kumpulan dan diberi makan diet asas berteraskan jagung dan kacang soya selama 42 hari. Diet rawatan yang digunakan adalah isonitrogen dan isokalori setelah disesuaikan mengikut tahap dan jenis lemak suplemen yang digunakan. Suplemen lemak ditambah pada kadar 7% untuk lemak tunggal atau pada kadar 3.5% + 3.5% untuk campuran 2 lemak. Minyak kelapa sawit ditambah pada kadar yang lebih tinggi (12 %) memandangkan ia mempunyai tenaga metabolism yang rendah berbanding asid lemak politaktepu yang lain.

Diet rawatan telah dirumus dan digunakan semasa peringkat tumbesaran (hari 11-28) dan peringkat penamat (hari 29-42). Data prestasi dikumpul untuk kedua-dua peringkat tersebut, sementara pensampelan tisu dan serum dilakukan di akhir eksperimen pada hari ke-42.

Suplemen asid lemak terkonjugat yang digunakan adalah LUTA-CLA 60 yang mengandungi 60% asid lemak terkonjugat. Sehubungan itu, kadar suplemen 7 % sebenarnya hanya membekalkan 4.2 % aisd lemak terkonjugat, dan 3.5 % LUTA-CLA 60 hanya memberikan 2.1 % asid lemak terkonjugat. Berdasarkan maklumat ini, diet kumpulan rawatan adalah 7 % minyak soya (SO), 7 % LUTA-CLA 60 (CL), 7 % minyak ikan (FO), 3.5 % LUTA-CLA 60 + 3.5 % minyak soya (CLSO), 3.5 % minyak ikan + 3.5 % minyak soya (FOSO), 3.5 % LUTA-CLA 60 + 3.5 % minyak ikan (CLFO), dan 12 % minyak sawit (PO) ditambah kepada diet asas.

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Penggunaan minyak sawit yang banyak dalam rangsum tidak menjejaskan kadar pengambilan makan dan tumbesaran ayam. Penambahan berat badan yang terendah dicatatkan pada ayam daripada kumpulan FO sewaktu tempoh tumbesaran dan kumpulan CLA untuk tempoh penyudahan. Diet FO juga mengurangkan tahap pengambilan makanan ayam, sementara asid lemak terkonjugat tidak menunjukkan kesan sedemikian apabila digunakan secara rawatan tunggal atau secara campuran dengan lemak lain. Minyak kelapa sawit yang diberikan pada tahap 12 % telah meningkatkan berat tisu lemak abdomen secara signifikan ($P<0.05$), sementara tahap asid lemak terkonjugat dari kumpulan CLA telah menyebabkan berat hati yang lebih tinggi ($P<0.05$). Kandungan lemak isi dada adalah tinggi di kalangan kumpulan PO, SO dan FO ($P<0.05$) berbanding kumpulan lain. Ayam FO mencatatkan tahap lemak yang tertinggi untuk isi peha ($P<0.05$). Secara amnya, kombinasi asid lemak terkonjugat dengan minyak soya atau minyak ikan telah menyebabkan pengumpulan lemak tisu yang lebih rendah pada isi dada dan peha, berbanding kumpulan ayam yang dirawat secara tunggal dengan minyak soya atau minyak ikan sahaja. Malah, ayam yang dirawat dengan kombinasi asid lemak terkonjugat dan minyak soya atau minyak ikan mencatatkan pengayaan tahap asid lemak n-3 yang lebih tinggi berbanding rawatan dengan minyak ikan atau minyak soya sahaja.

Keputusan kajian juga menunjukkan tiap jenis lemak/minyak mempunyai kesan yang berbeza ke atas gen PPAR. Aktiviti gen PPARγ telah menunjukkan kenaikan dalam kumpulan PO, sementara minyak dan kombinasinya lain tidak menunjukkan sebarang kesan ke atas gen yang sama. Sementara itu, ekspresi gen PPARα pada tisu hati telah meningkat

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selepas dirawat dengan minyak ikan dan kombinasinya. Perbezaan ini adalah signifikan (P<0.05) jika dibandingkan dengan PO, SO dan CLA.

Kesimpulannya, keputusan kajian ini menunjukkan bahawa penambahan 7 % minyak ikan dan 4.2 % CLA mengurangkan prestasi ayam pedaging. Walaubagaimanapun, kombinasi ini breupaya meningkatkan kandungan asid lemak n-3 dalam daging ayam. Gabungan CLA dan n-6 pula meningkatkan kandungan CLA dalam daging ayam. Kesan anti-lipogenik PPARa ketara dengan kehadiran CLA. Perubahan kadar pengumpulan lemak abdomen bergantung kepada kesan PPARα dan PPARγ. Pengurangan lemak abdomen tercapai dengan peningkatan kawalaturan PPARα (pada hepotosit), dan pengurangan kawalaturan PPARγ pada sel adiposit.

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I certify that a Thesis Examination Committee has met on 2012 to conduct the final examination of **MARYAM ROYAN** on her Doctor of Philosophy thesis entitled "**EFFECTS OF CONJUGATED LINOLEIC ACIDS, FISH OIL AND SOYBEAN OIL ON THE GROWTH PERFORMANCE, CARCASS TRAITS, LIPID CHARACTERISTIC AND PPARs mRNA EXPRESSION IN BROILER CHICKENS "**

" in accordance with the Universities Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the student be awarded the Doctor of Philosophy.
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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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DECLARATION

I hereby declare that the thesis is my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously and is not correctly submitted for any other degree at Universiti Putra Malaysia or other institutions.

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Date: 14 June 2012

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LIST OF ABBREVIATIONS

AQP7	Aquaporin 7
°C	degrees Celsius
µg	micro gram
AA	Arachidonic Acid
ACC	Acetyl CoA carboxylase
ACO	Acyl-CoA oxidase
ACS	Acyl CoA synthetase
ANOVA	Analysis of variance
aP2	Adipocyte fatty acid binding protein
ATP	Adenosine triphosphate
Ava P	Available Phosphor
Ca	Calcium
cAMP	cyclic AMP
CD-36/FAT	Fatty acid translocase
CFIA	Canadian Food Inspection Agency
cGPDH	cytosolic glycerol phosphate dehydrogenase
CLA	Conjugated linoleic acid
CL	Diet containing 4.2% CLA
CLFO	Diet containing 2.1%CLA and 3.5% fish oil
CLSO	Diet containing 2.1%CLA and 3.5% soybean oil
CoA	Acyl coenzyme A
COX	Cyclooxygenase
CP	Crude Protein
CPT-I	Carnitine palmitoyl transferase I
Ct	Threshold cycle
Cys	Cysteine
d	Day
DAG	Diacylglycerols
DCP	Dicalcium phosphate
DHA	Docosahexaenoic acid
DM	Dry Matter
DNA	Deoxyribonucleic acid
EFA	Essential fatty acids
EFA	Essential Fatty Acid
FABP	Fatty Acid Binding Protein
FACoA	Fatty acyl CoA
FAS	Fatty acid synthase
FAT	Fatty acid transporter CD36
FATP	Fatty acid transport protein
FCR	Feed Conversion Ratio
FFAs	Free Fatty Acids
FO	Diet containing 7% fish oil
FOSO	Diet containing 3.5% fish oil and 3.5% soybean oil
FXR	Farnesoid X receptor
g	Gram
GLUT4	Glucose transporter 4
HDL	High Density Lipoproten
HNF	Hepatic nuclear factors
IP3	Inositol triphosphate

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Kd	Dissociation constant
kg	Kilo Gram
KGF	Keratinocyte Growth Factor
LA	Linoleic Acid
LCFAs	Long Chain Faty Acids
LCPUFAs	Long Chain Poly Unsaturated Fatty Acids
LDL	Low Density Lipo Protein
LNA	Linilenic Cid
LOX	Lipoxygenase
LPL	Lipoprotein Lipase
LXR	liver X receptor
Lys	Lysine
ME	Metabolisable Energy
Met	Methionine
mg	Milligram
min	Minute
mL	Milliliter
mM	Millimole
mRNA	Messenger RNA
MUFAs	Monounsaturated fatty acids
Na	Sodium
NEFA	Non-esterified fatty acids
NFjB	Nuclear factor j B
NR	Nuclear receptor
NRC	Nutrient Requirements committee
P	Phosphor
PO	Diet containing about 12% palm oil
PPARS	Peroxisome proliferator-activated receptors
P/S	Polyunsaturated to saturated fatty acids ratio
PGE3	Prostaglandin E3
PGI3	Prostaglandin I3
PI	Peroxidizability index
PL	Phospholipids
pM/µL	Pico mole / macro liter
PO2	Oxygen partial pressure
PUFAs	Poly Unsaturated Fatty Acids
RNA	Ribonucleic acid
RQ	Respiratory quotient
RXR	Retinoid X receptor
RXRa	Retinoid X receptor
S.D	Standard Deviation
SAS	Statistical Analysis Software
SCD1	stearyl CoA desaturase-1
SFA	Saturated Fatty Acids
SO	Diet containing 7% soybean oil
SREBP-1c	Sterol regulatory element binding protein 1c
T3	Triiodothyronine
TCA cycle	Tricarboxylic acid cycle
TG	Triglycerides
TNF-α	Tumor necrosis factor-α
TXA3	Thromboxane A3
TZDs	Thiazolidinediones
UCPs	Uncoupling proteins
USFA	Unsaturated Fatty Acid
Vit-Min	Vitamin-Mineral Premix
VLDL	Very Low Density Lipoprotein

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CHAPTER 1

GENERAL INTRODUCTION

1.1 Chicken meat as a functional food

Traditionally, poultry farmers aimed to produce birds with superior body weight and feed conversion ratio. However, in line with current developments, there are other traits that must be taken into account as well. These include producing poultry meat with lower body fat content, as well as improving the nutritiveness of the poultry meat to appeal to consumer requirements. The interrelated importance of human diet and health status is an ancient subject. In 500 B.C., Hippocrates was quoted to say that ‘‘Food is medicine, and medicine is food’’. Human foods as a lifestyle factor, is involved in the incidence of many types of diseases such as cardiovascular diseases and cancer (Russo, 2009). Recent reports suggested that not only the quantity but also the composition of dietary fat is an important factor to prevent these metabolic diseases in human populations (Sealls *et al*., 2008).

Nowadays, extra focus has been given to the production of functional foods, with components that have beneficial effects on human well-being. In definition, functional foods contain particular nutrients and (or) non-nutrients that have an effect on human health, beyond what is usually known as nutritional effects (Pisulewski, 2005). There are several compounds in foods that improve the

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consumer's health status (Zanini *et al*., 2006), among them the polyunsaturated fatty acids (PUFA, n-6 and n-3 series) and conjugated linoleic acid (CLA) (Pisulewski, 2005) have been more attractive. The fatty acid profile of chicken meat can be modified by feeding. This in turn results in chicken products that could offer a more economical source of health-effective fatty acids (Phetteplace and Watkins, 1989; Pisulewski *et al.,* 2002; Pisulewski and Kostogrys 2003; Pisulewski, 2005).

In humans, the n-3 fatty acids are important anti-inflammatory factors or precursors, with key roles in mitigating effects accompanying proinflammatory cytokines in coronary heart disease, depression, aging, cancer and autoimmune disorders such as Crohn's disease, ulcerative colitis, and lupus erythematosus (Simopoulos, 2002). CLA exerts anti-obesity, anti-carcinogenic, anti-atherogenic, and immuno-modulatory effects in experimental animals and humans (Fritsche and Steinhart 1998a; Roche *et al*., 2001). These specific fatty acids could affect chicken production efficiency too. High fat deposition in the chicken body reduces the effective energy use and can lead to lower feed efficiency (Grashorn, 2007). There are reports that PUFA and CLA could reduce fat deposition, but saturated fatty acids (SFA) from animal origin tend to result in higher fat deposition in the chicken carcass (Crespo and Esteve-Garcia, 2002; Zanini *et al.,* 2006). These reports obviously showed that dietary fats are important factors that influence both the functional properties of chicken meat and the efficiency of chicken production.

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1.2 General objectives and organization of the study

The thesis consisted of two distinct sections. The literature review section briefly presents the highlights of fatty acid metabolism in birds. The later section of the literature review paid attention to the properties of PUFA and CLA, and their effects on body metabolism. In the current work, broiler chickens were fed dietary fats rich in n-3 or n-6 PUFA (fish oil and soybean oil, respectively), and CLA as the sole dietary fat source, or in dual mixes as well in order to modify the chicken's fat metabolism and meat fatty acid composition. Description of the general approach and methodology for this study is given in Chapter III.

Chapter IV presents the effects of different dietary fat types on the performance and carcass characteristics of broilers and reports on productive trait changes as a result of the dietary fat inclusion, while the experiment in chapter V investigated the effects of dietary fats on serum lipid fraction as an index of fat metabolism. The results reported in chapter VI demonstrated the effects of including different dietary fatty acid profiles on the enrichment of chicken's meat with n-3 PUFA and CLA.

In Chapter VII, Peroxisome proliferator-activated receptors (PPARs) gene expression in liver and adipose tissues as a consequence of feeding different dietary fatty acids was reported. This chapter is meant to illustrate the probable evidences that would help to clarify results obtained in Chapters IV, V and VI. At

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the end of the thesis, a general discussion integrates the results of the whole research and the areas requiring further research.

It was hypothesized that the dietary n-3 fatty acids and CLA would induce changes in different aspects of lipid metabolism in broiler chickens. These fatty acids would alter both the fatty acid profile of the meat as well as the whole body fat deposition. When the mixtures of different fats were fed to broiler chickens, it was hypothesized that the fatty acids will interact to modify lipid metabolism. Based on these hypotheses, the general objectives of the present study were:

1- To determine the effects of soybean oil, fish oil, conjugated linoleic acid and palm oil on performance, serum lipids, chicken meat fat content and fatty acid profile and PPARs gene expression in broiler chickens.

2- To determine the likely synergistic effects between dietary fats on performance, serum parameters and tissue fatty acid composition of broiler chickens, focusing on anti-lipogenic effects.

3- To investigate the relationship between dietary n-6 : n-3 ratio and CLA concentrations on fat deposition.

4- To determine possible interactions between CLA and n-3 and n-6 PUFA on PPARs gene expression.

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