UNIVERSITI PUTRA MALAYSIA

GROWTH PERFORMANCE AND MEAT QUALITY OF BROILER CHICKENS SUPPLEMENTED WITH DIFFERENT OIL SOURCES

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By

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

August 2016

DEDICATION

I Would Like To Dedicate This Thesis To My Beloved Parents, My Dearest Wife And My Cute Children As Well As My Brothers And Sisters

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

GROWTH PERFORMANCE AND MEAT QUALITY OF BROILER CHICKENS SUPPLEMENTED WITH DIFFERENT OIL SOURCES

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August 2016

Chairman : Professor Loh Teck Chwen, PhD
Faculty : Agriculture

Poultry meat is an indispensable source of animal protein in human diets. In recent time, meat quality perception by consumers is negatively affected due to its implication in the incidence of chronic diseases due to the imbalance in its fatty acid composition. Hence, manipulating the fatty acid profile of poultry meat without compromising growth performance, immune system, meat quality and shelf life need more investigation. Dietary fat is an important source of energy and can be used to modify the fatty acid composition of animal products. Thus, the current study investigated the effects of different oils sources on fatty acid composition, growth performance, immune response, gene expression and meat quality of broiler chickens. The study was conducted in four phases.

The first phase examined the apparent metabolizable energy (AME) of palm oil (PO), soybean oil (SO) and linseed oil (LO) in broiler chickens. The AME assay was conducted using the classical total excreta collection between days 21 and 29 post hatch. A corn-soy based diet was formulated and the test diets, each containing either PO, LO or SO were formulated by replacing (w/w) 60 g/kg of the basal diet with PO, SO or LO. The results showed that the AME did not differ ($P > 0.05$) between the test diets.

The second phase investigated the effects of different oil sources on growth performance, apparent nutrient digestibility, intestinal morphology and meat quality in broiler chickens. A total of 126, one-day old birds were fed either PO, SO or LO at 6% for 42 d. Birds fed diet supplemented with SO and PO had higher ($P < 0.05$) average daily gain, abdominal fat and villi height compared with those fed LO. Birds fed diet supplemented with LO, SO and PO had higher ($P < 0.05$) proportions of α-linolenic, linoleic and oleic acids in breast muscle, respectively. The LO diet increased ($P < 0.05$) the total n-3 fatty acids and decreased the n-6: n-3 compared with the PO and SO diets. Birds fed the PO diet had higher oxidative stability, abdominal fat and cholesterol compared with those fed the SO and LO diets. However, feed efficiency, apparent digestibility of organic matter, ether extract, crude protein and ash were similar between all the treatments.

The third phase assessed the effects of modifying dietary n-6: n-3 fatty acid ratio (FAR) in palm oil -based diet on growth performance, carcass quality, immune response and expression of peroxisome proliferator-activated receptor (PPAR) α, PPAR-γ, and stearoyl-CoA desaturase (SCD) in the liver and breast muscle tissues of broiler chicken. A total of 180 broiler chickens were randomly assigned into five treatment groups as follows: (1) basal diet containing 6 % PO (CON); (2) basal diet containing 4% PO + 1% SO + 1% LO; (3) basal diet containing 3% PO + 2% PO + 1% LO; (4) basal diet containing 3% PO + 1% SO + 2% LO; (5) basal diet containing 2% PO + 2% SO + 2% LO, with the different n-6: n-3 FAR (17.68, 3.70, 3.67, 2.18 and 2.05) and (19.02, 3.28, 3.82, 2.28, 2.23) in the starter and finisher diets respectively. The results showed that decreasing n-6: n-3 FAR in broiler diets led to a significant ($P<0.05$) decrease in the n-6: n-3 FAR in the liver and breast muscle tissues and lower cholesterol level in the serum and breast muscle. In addition, decreasing n-6: n-3 FAR in broiler diets up-regulated the expression of PPARα and PPARγ ($P<0.05$) but down-regulated the expression of SCD ($P<0.05$) in the breast muscle and liver. Decreasing n-6: n-3 FAR in broiler diet increased the plasma immunoglobulin M (IgM) linearly. However, no significant ($P> 0.05$) differences were observed between the dietary treatments for growth performance, dressing percentage, meat quality, antioxidant activity, villi height and plasma immunoglobulin G (IgG).

The fourth phase assessed the effect of graded levels of blend of PO, SO and LO in the ratio of 4:1:1 on growth performance, carcass characteristics, serum lipid profile, immune response, tissue fatty acid profiles and gene experission in the liver and breast mucle tissues in broiler chicken. A total of 216 one-day-old chicks were randomly assigned into six dietary treatments of six replicates (6 chicks per replicate). The birds received any of the six levels of oil blend (0, 2, 4, 6, 8, 10% of diet). Results showed that increasing dietary oil blend up to 8% improved ($P< 0.01$) growth performance, carcass weight, breast yield, villi height and plasma immunoglobulin (IgG and IgM) and decreased abdominal fat and breast fat content. Increasing dietary level of oil blend increased ($P< 0.01$) n-3 FA, decreased n-6: n-3 FAR, up-regulated the expression of PPARα and PPARγ genes and down regulated the expression of SCD in the liver and breast tissues and lowered cholesterol level in the breast muscle and serum. However, no significant ($P> 0.05$) differences were observed between the dietary treatments for dressing percentage, lipid oxidation and proximate composition of breast meat except for fat content.

It can be concluded that dietary oils differ in their effects on growth performance and meat quality of broiler chickens. Modifying the n-6: n-3 FAR via oil blends is an effective strategy for modifying the fatty acid profile of broiler meat without compromising growth performance, immune response, meat quality and shelf life in broiler chickens. Moreover, oil blend can be included up to 8% in the broiler diet to improve growth performance, gut morphology, reduce breast meat cholesterol and total serum cholesterol and production of lean meat.

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

PRESTASI PERTUMBUHAN DAN KUALITI DAGING DALAM AYAM PEDAGING DIBERI MAKAN DIET YANG DITAMBAH DENGAN SUMBER MINYAK YANG BERBEZA

Oleh

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Daging poltri adalah sumber protein haiwan yang sangat diperlukan dalam diet manusia. Komposisinya yang padat dengan nutrien menjadikannya sebahagian daripada diet yang sihat dan seimbang. Kebelakangan ini, persepsi pengguna terhadap kualiti daging telah terjejas disebabkan kesannya dalam insiden penyakit kronik yang disebabkan oleh ketidakseimbangan komposisi asid lemak. Oleh itu, banyak kajian perlu dilakukan bagi memanipulasi profil asid lemak daging poltri tanpa menjejaskan prestasi pertumbuhan, sistem imun, kualiti daging dan penyiasatan merit jangka hayat. Lemak adalah sumber tenaga yang penting dan boleh digunakan untuk mengubahsuai komposisi asid lemak dalam produk haiwan. Oleh itu, kajian semasa adalah mengkaji kesan minyak yang berbeza dari segi komposisi lemak asid terhadap prestasi pertumbuhan, tindak balas imun, ekspresi gen dan kualiti daging dalam ayam pedaging. Kajian ini telah dijalankan dalam empat peringkat.

Fasa pertama ialah mengkaji tenaga metabolisme kasar (AME) minyak sawit (PO), minyak kacang soya (SO) dan minyak biji rami (LO) dalam ayam pedaging. Cerakin AME dijalankan dengan menggunakan jumlah kutipan najis klasik antara hari ke 21 dan 29 pasca menetas. Diet basal jagung soya telah digubal dan diet ujian, setiap satu mengandungi sama ada PO, LO atau SO yang telah dirangka dengan menggantikan (w/w) 60 g/kg diet basal dengan PO, PP atau LO. Hasil kajian menunjukkan bahawa AME antara diet ujian tidak berbeza ($P > 0.05$).

Kajian kedua mengenai kesan sumber minyak yang berbeza terhadap prestasi pertumbuhan, penghadaman nutrient kasar, morfologi usus dan kualiti daging dalam ayam pedaging. Sebanyak 126 ayam berusia satu hari diberi makan sama ada 6% PO, PP atau LO selama 42 hari. Ayam yang diberi makan diet yang ditambah dengan SO dan PO mempunyai purata kenaikan berat harian, lemak di bahagian abdomen dan ketinggian villi yang lebih tinggi ($P<0.05$) berbanding dengan mereka yang diberi makan LO. Ayam yang diberi makan diet yang ditambah dengan LO, SO dan PO masing-masing mempunyai lebih tinggi ($P <0.05$) perkadaran α-linolenik, linoleik dan asid oleik, berbanding dengan rawatan lain. Diet LO meningkatkan ($P <0.05$) jumlah

asid lemak n-3 dan menurun n-6: n-3 berbanding dengan diet PO dan SO. Ayam yang diberi makan diet PO mempunyai kestabilan oksidatif dan kolestrol yang lebih tinggi berbanding dengan mereka yang diberi makan diet SO dan LO. Walaubagaimanapun, kecekapan makanan, penghadaman kasar bahan organik, ekstrak eter, protin kasar dan abu antara rawatan adalah sama.

Eksperimen ketiga menilai kesan mengubahsuai makanan n-6: n-3 FAR dalam diet berasaskan minyak sawit terhadap prestasi pertumbuhan, kualiti karkas, tindak balas imun dan ungkapan reseptor peroksisom proliferator-diaktifkan (PPAR) ɑ, PPAR-ƴ, dan stearoyl-CoA desaturase (SCD) dalam tisu otot dada ayam pedaging. Sebanyak 180 ekor ayam pedaging telah dibahagikan secara rawak kepada lima kumpulan rawatan seperti berikut: (1) diet basal mengandungi 6% PO (CON); (2) diet basal yang mengandungi 4% PO + 1% SO + 1% LO; (3) diet basal mengandungi 3% PO + 2% PO + 1% LO; (4) diet basal mengandungi 3% PO + 1% SO + 2% LO; (5) diet basal yang mengandungi 2% PO + 2% SO + 2% LO, dengan n-6 yang berbeza: n-3 nisbah asid lemak (FAR) (17.68, 3.70, 3.67, 2.18 dan 2.05) dan (19.02, 3.28, 3.82, 2.28, 2.23) dalam setiap diet pemula dan diet penamat. Hasil kajian menunjukkan bahawa pengurangan n-6: n-3 FAR dalam diet ayam pedaging membawa kepada penurunan signifikan ($P < 0.05$) n-6: n-3 FAR dalam hati dan tisu otot dada dan tahap kolesterol yang lebih rendah dalam serum dan otot dada. Di samping itu, pengurangan n-6: n-3 FAR dalam diet ayam pedaging dapat meningkatkan kawalan ungkapan PPARα dan PPARγ ($P < 0.05$) tetapi mengurangkan kawalan ungkapan SCD ($P < 0.05$) dalam otot dada dan hati. Pengurangan n-6: n-3 FAR dalam diet daging meningkatkan immunoglobulin plasma M (IgM) secara terus. Walaubagaimanapun, tiada perbezaan signifikan ($P > 0.05$) diperhatikan antara rawatan pemakanan untuk prestasi pertumbuhan, peratusan pemotongan, kualiti daging, aktiviti antioksidan, ketinggian villi dan immunoglobulin plasma G (IgG).

Eksperimen keempat menilai kesan tahap gred campuran minyak kelapa, minyak kacang soya dan minyak biji rami dalam nisbah 4: 1: 1 terhadap prestasi pertumbuhan, ciri-ciri karkas, profil lipid serum, tindakbalas imun, profil tisu asid lemak dan ekspresi gen dalam hati dan tisu otot dada dalam ayam pedaging. Sejumlah 216 ekor anak ayam berusia sehari telah dibahagikan secara rawak kepada enam rawatan makanan mengandungi enam replikat (6 anak ayam setiap replikat). Ayam-ayam menerima salah satu daripada enam tahap campuran minyak (0, 2, 4, 6, 8, 10% daripada diet). Hasil kajian menunjukkan bahawa peningkatan sehingga 8% campuran minyak pemakanan meningkatkan ($P < 0.01$) prestasi pertumbuhan, berat karkas, hasil dada, ketinggian villi dan immunoglobulin plasma (IgG dan IgM) dan menurunkan kandungan lemak abdomen dan lemak dada. Peningkatan tahap pemakanan campuran minyak meningkatkan ($P < 0.01$) n-3 FA, mengurangkan n-6: n-3 FAR, meningkatkan kawalan ungkapan gen PPARα dan PPARγ dan mengurangkan kawalan ungkapan SCD dalam hati dan tisu dada serta menurunkan tahap kolesterol dalam otot dada dan serum. Walaubagaimanapun, tiada perbezaan signifikan ($P > 0.05$) diperhatikan antara rawatan pemakanan untuk peratus pemotongan, pengoksidaan lipid dan komposisi proksimat daging dada kecuali kandungan lemak.

Ia boleh disimpulkan bahawa minyak makanan mempunyai kesan yang berbeza terhadap prestasi pertumbuhan dan kualiti daging ayam pedaging. Mengubahsuai n-6: n-3 FAR melalui campuran minyak adalah satu strategi yang berkesan untuk mengubahsuai profil asid lemak daging ayam pedaging tanpa menjejaskan prestasi tumbesaran, tindak balas imun, kualiti daging dan jangka hayat dalam ayam pedaging. Lagipun, sehingga 8% minyak boleh diadun dalam diet ayam bagi meningkatkan prestasi pertumbuhan, menambahbaik morfologi usus, menurunkan kolesterol dalam daging bahagian dada serta mengurangkan jumlah kolesterol dalam serum dan pengeluaran daging tanpa kulit.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirements for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

a*	Redness
ACS	acyl-CoA synthase
ADG	Average daily gain
AID	apparent ileal digestibility
AME	apparent metabolizable energy
ANOVA	analysis of variance
b*	Yellowness
BW	body weight
BWG	body weight gain
Ca	calcium
cal	Calorie
cm	Centimeter
CP	crude protein
CRD	complete randomized design
d	day
DHA	Docosahexaenoic acid
dH_2O	distilled water
DM	dry matter
DPA	docosapentaenoic acid
EDTA	ethelenedimethyl tetra acetic acid
EE	ether extract
EPA	eicosapentaenoic
FAME	fatty acid methyl esters
FAO	Food and Agriculture Organization
FAR	Fatty acid ratio
FATP	fatty acid transport protein
FCR	feed conversion ratio
FI	feed intake
FTTP	fatty acid transport protein
g	Gram
GE	gross energy
GLM	general linear model
h	Hour
H_2O_2	hydrogen peroxide
H_2SO_4	Sulphuric acid
HDL	high density lipoprotein
Ig	immunoglobulin

kcal	kilo calories
Kg	kilogram
L	liter
L*	Lightness
LDL	low density lipoprotein
LO	Linseed oil
LPL	lipoprotein lipase
m	meter
MDA	malondialdehyde
ME	metabolizable energy
mg	milligram
mg/L	milligrams per liter
min	minute
mL	milliliter
mm	millimeter
mmol/L	millimoles per liter
µg	micro gram
µl	micro liter
µmol/L	micromoles per liter
MUFA	monounsaturated fatty acids
n-6: n-3	total n-6 PUFA to total n-3 PUFA ratio
NaOH	sodium hydroxide
^{o}C	degrees centigrade
OD	optical density
OPA	o-phthalaldehyde reagent
%	Percentage
pH0	pre-rigor pH
pHu	ultimate pH
PO	Palm oil
PGE	prostaglandin E
PPAR	Peroxisome proliferator-activated receptor
PUFA	Polyunsaturated fatty acids
s	Second
SAS	statistical analysis system
SCD	stearoyl-CoA desaturase
SEM	standard error of mean
SFA	saturated fatty acids
SO	Soybean oil
SREBP	Sterol regulatory element binding protein
TG	Triacylglycerol and triglycerides

TBARS	thiobarbituric acid-reactive substances
TiO_2	titanium dioxide
UFA	unsaturated fatty acids
UFA: SFA	ratio total UFA to total SFA ratio
v/v	volume per volume
VLDL	very low density lipoprotein
WBSF	Warner-Bratzler shear force
WHC	water holding capacity

CHAPTER 1

GENERAL INTRODUCTION

Chicken meat is an important source of animal protein in human diets and it is valued in many cultural culinary traditions. Its nutrient-dense composition makes it an integral part to healthy and balanced diets. The low saturated fatty acids (SFA) and high polyunsaturated fat acids (PUFA) content of chicken meat have made it a sought after meat by health conscious individuals (Vilarrasa *et al.*, 2015b).

Malaysia is self-sufficient in poultry meat production, and output is expanding slowly, in line with expectations for domestic demand growth (United States Department of Agriculture, 2014). Poultry meat production, consisting almost entirely of broiler meat, is expected to grow at a moderate rate of 2% in 2014, with production forecast at 1.44 million tons (United States Department of Agriculture, 2014).

Although the industry has the capacity to grow further, rising costs of production are limiting growth. A reduction in fuel subsidies, depreciation of Malaysian ringgit, and implementation of minimum wages in 2013 are all factors that have led to higher production costs in 2014. Average cost of production has increased from $1.45 per kg in 2012 to $1.60 per kg in 2013, to a forecast $1.68 in 2014. During this period, the production of broiler chicken met the prevailing consumer demands (Majid and Hassan, 2014). This is due to the improvement in mass production techniques, genetics and selective breeding, control and/or prevention of diseases, and enhanced nutrition, management and processing techniques (Tapsir *et al.*, 2011).

The rapid growth rate of broiler chickens necessitates the supply of high energy diets to support their growth. The supply of energy represents a substantial part of feed cost. Cereals are the major source of energy in poultry feed. However, the recent competition between man and farm animals for cereals has soared the prices of cereals (Corzo *et al.*, 2005a). This scenario gives impetus to the utilization of cheaper and readily available alternative feedstuffs in broiler diet in order to maintain productivity at a lower cost.

Dietary fat is an important source of energy whose utilization portends great benefits to the livestock industry because it is relatively cheap, readily available and virtually not competed for by man and farm animals. Fat is usually included in diet formulations to meet the high energy requirements of broiler chickens, as the energy value of fat is at least twice as high as those of carbohydrates and protein (NRC, 1994). In addition, fat enhances feed palatability and the absorption of fat-soluble vitamins. Fat slows down the rate of food passage through the digestive tract, allowing more time for better digestion and absorption of nutrients (NRC, 1994). Fat also control dust in feed mills and poultry houses.

The efficacy and value of dietary fats in poultry nutrition depends on their digestibility and apparent metabolizable energy (AME) (Vilarrasa *et al.*, 2015a). Most data available on AME and digestion of fats in poultry about 20 to 40 years old are based on studies conducted with slow-growing strain of broiler chickens (Lessire *et al.*, 1982;

NRC, 1994; Wiseman *et al.*, 1986; Young *et al.*, 1963). Thus, it is reasonable to presume that the genetic improvement of broiler chickens may have enhanced the capacity of the birds to digest and utilize nutrients. Thus, current trials are needed to determine the digestibility and AME for the fast growing strains of broiler chickens. Thus, it was proposed that dietary oils (linseed oil (LO), palm oil (PO) and soybean oil (SO)) differing in fatty acid composition will exhibit different digestibility and AME in broiler chickens.

Dietary fat can be used to alter fat deposition and the fatty acid profile of broiler chickens meat (Crespo and Esteve-Garcia, 2002a). This is imperative given the fact that in recent time, the quality perception of chicken meat is grossly affected due to its high n-6: n-3 fatty acid ratio (FAR), which has been implicated in the incidence of various chronic diseases (Simopoulos, 2000). Thus, modifying the fatty acid composition of chicken meat to meet the prevailing health demands of consumers is warranted.

Dietary fats differ in their effects on fat deposition in broiler chickens. Saturated fats especially those from animal origin increase fat deposition in broiler chicken while the PUFA can reduce fat deposition (Crespo and Esteve-Garcia, 2002c; Zanini *et al.*, 2006). High fat deposition in broilers reduces the effective energy use and can lead to lower feed efficiency (Grashorn, 2007). The reduction in fat deposition by PUFA was attributed to their ability to alter the synthesis of fatty acid in the liver and other lipogenic enzyme regulating mRNA synthesis (Blake and Clarke, 1990). These fatty acids decrease lipogenesis by suppressing gene expression in the liver, including that of fatty acid synthase and stearoyl-CoA desaturase (Clarke and Jump, 1994). Thus, it was proposed that oils (PO, SO and LO) differing in fatty acid composition will alter gene expression and fatty acid synthesis in broilers differently. A synergistic effect of these oils on gene expression in broiler chickens is also proposed.

The manipulation of the fatty acid profile of meat could alter its oxidative stability (Adeyemi *et al.*, 2015a). For instance lowering the n-6: n-3 FAR could increase the susceptibility of meat to lipid oxidation. Lipid oxidation could affect quality attributes of meat. Therefore, the nutritive value and safety of meat are compromised when lipid oxidation takes place (Adeyemi *et al.*, 2015a; 2015b). In addition, consumption of peroxidized lipids can affect genomic or cellular stability, modulate gene expression and cell signalling pathways, and promote atypical cell proliferation and inflammation thus contributing to disease pathogenesis in humans (West and Marnett, 2005; Feng *et al.*, 2006).

Dietary incorporation of antioxidant-rich vegetable oils in animal diets has been suggested as an economical and effective way of curbing oxidative deterioration and an alternative way of enhancing these beneficial nutrients in human diets (Kang *et al.*, 2001; Adeyemi *et al.*, 2015a). Thus, it was proposed that oils (PO, LO and SO) differing in n-6: n-3 FAR and antioxidant contents will differ in their effects on the oxidative stability of broiler meat.

In cognizance of the need to enhance bioactive lipids in broiler meat and to optimize animal performance and meat quality, this study was initiated to appraise the efficacy of LO, SO, and PO and their mix on nutrient digestibility, growth performance, meat quality, tissue lipids and gene expression in broiler chickens. This study was conducted with the following hypothesis and objectives.

Hypotheses of the study

1. Linseed oil, PO and SO would exhibit different digestibility and AME in broiler chickens.
2. Palm oil, SO and LO would alter growth performance, immune response, gene expression and fatty acid synthesis in broiler chickens differently.
3. Palm oil, LO and SO would differ in their effects on the physicochemical properties and oxidative stability of broiler meat.

The objectives of the study were:

1. To determine the effect of PO, SO and LO on the apparent metabolizable energy in broiler chickens.
2. To determine the effect of LO, PO and SO on growth performance, apparent nutrient digestibility, intestinal morphology and meat quality in broiler chickens.
3. To determine the effects of modifying dietary n-6: n-3 ratio in PO -based diet on growth performance, carcass quality, immune response and expression of peroxisome proliferator-activated receptor (PPAR) α, PPAR-γ, and stearoyl-CoA desaturase (SCD) in the breast muscle and liver tissue of broiler chicken.
4. To determine the effect of different levels of blend of PO, SO and LO on growth performance, carcass characteristics, meat quality, serum lipid profile, immune response and tissue fatty acid profiles in broiler chicken.

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