ASSESSMENT OF ALTERNATIVE FEED SOURCES WITH DIFFERENT LEVELS OF ENERGY TO PROTEIN RATIO TOWARDS ENHANCEMENT OF LAYER AND BROILER PERFORMANCE

MOHAMMED FAROOQ ABDULHAMEED

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MOHAMMED FAROOQ ABDULHAMEED

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

January 2019

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DEDICATION

THIS WORK IS DEDICATED TO MY FATHER SOUL, MY DEAREST MOTHER, MY LOVELY CHILDREN AND SIBLING.

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

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MOHAMMED FAROOQ ABDULHAMEED

February 2019

Chairman : Henny Akit, PhD
Faculty : Agriculture

Formulation of poultry diets plays a role in decrease cost of the production. Poultry producers face a challenge to decrease cost of poultry products after competition between the human and animal on some ingredients is raised. There is a little information about using rice waste, meat and bone waste, and black soldier fly larvae meal as sources of energy and protein on layer and broiler performance. The objective of this study was to determine the effect of different levels of energy to protein ratio using rice waste as source of energy, and meat and bone waste as well as black soldier fly larvae meal as sources of protein in the diet on egg quality, productivity, and egg sensory characteristics of laying hens. Also, on growth performance, nutrient digestibility, microbial population, meat quality, gut histology, and sensory evaluation, and hepatic IGF-I and GHR mRNA gene expression of the broiler.

In the first experiment, a total of 54 Arabic strain hens 36 weeks old were divided to three treatments, each 6 hens mixed with one cock. The energy to protein ratio of control treatment was 155, which is recommended by Nutrition Research Council. Low and high energy to protein ratio were used for second and third treatments, which were 144, and 170 respectively. Black soldier fly larvae were included in the diets at levels of 0, 5 and 1 % respectively. The results showed that a low energy to protein ratio diet increased hen day and hen house egg production ($P<0.05$), egg mass ($P<0.001$), feed conversion ratio (feed/kg egg) ($P<0.01$), sensory characteristics of eggs ($P<0.05$), and fertility rate($P<0.05$). However, negative effects such as decrease of egg weight ($P<0.001$), and egg shell characteristics ($P<0.05$) accompanied with low energy protein ratio diet.

In the second experiment, a total of 160 Cobb 500 one-day old chicks were divided randomly into four treatments. Conventional feed ingredients were formulated to form a control treatment. The normal energy to protein ratio of control treatment which is recommended in the guide of Cobb 500 was154 for the starter period and 167 for the finisher period. Rice waste, meat and bone waste as well as black soldier fly larvae meal were formulated to form the other three dietary treatments. The normal, low, and high energy to protein ratios of alternative feed diets were 154,143, and 166 for the starter period as well as 167, 155, and 177 for the finisher period respectively. The results showed that normal, low, and high levels of energy to protein ratio of alternative feed diets decreased feed intake ($P<0.001$), body weight gain ($P<0.01$), feed conversion ratio ($P<0.001$), carcass weight ($P<0.01$), crude protein digestibility ($P<0.05$), colour a* and b* values ($P<0.001$), villus height and crypt depth in duodenum ($P<0.05$), population of *Lactobacillus spp* ($P<0.001$) in ileum and cecum, population of *Escherichia coli spp* ($P<0.001$) in ileum, and population of *Enterococcus spp* ($P<0.001$) in cecum, but increased villus height and crypt depth ($P<0.01$) in jejunum, colour L* value of meat ($P<0.05$) compared with control diet. Normal and high energy to protein ratio of alternative feed diets decreased ($P<0.05$) ether extract digestibility, and population of *Salmonella spp* in ileum. Highest ash digestibility ($P<0.001$) and lowest cooking loss ($P<0.01$) values were observed in birds fed low energy to protein ratio. Low and high energy to protein ratio diets decreased population of *Bifidobacterium spp* in the cecum($P<0.05$), pH of meat ($P<0.05$), and TBARS value ($P<0.001$) after 5 days of storage. Normal and low energy to protein ratio of alternative feed diets decreased ($P<0.05$) population of *Enterococcus spp* population in the ileum.

In the third experiment, a total of 120 Cobb 500 one-day-old broiler chicks were divided randomly into four treatments. Conventional feed ingredients (corn, soy bean, and fish meal) were replaced with food waste (rice waste, meat and bone waste), and black soldier fly larvae at 10%, 30%, and 50%. The energy to protein ratio of all treatments was 154 for starter period and 166 for finisher period. The results showed that body weight gain ($P<0.01$), feed conversion ratio ($P<0.05$), the relative weight of abdominal fat ($P<0.05$), GHR and IGF1 mRNA gene concentrations ($P<0.01$) were increased when conventional feed ingredients were replaced with alternative feed ingredients at 30% and 50%. *Lactobacillus spp* population ($P<0.01$) in ileum and cecum, *Escherichia coli* population ($P<0.001$) in ileum, digestibility of ($P<0.001$) dry matter, crude protein, ether extract and ash, were increased when replacement of conventional feed ingredients with alternative feed ingredients at 10, 30, and 50%. However, *Escherichia coli* population were decreased ($P<0.01$) in cecum of birds fed diets of 10, 30, and 50% replacement. *Enterococcus spp* population was highest ($P<0.01$) in in ileum and cecum of birds fed diet containing 50% alternative feed ingredients.

The present findings found that low energy to protein ratio diet increased hen day and hen house egg production of laying hens. Normal, low, and high energy to protein ratio of rice waste, larvae meal, and meat and bone waste of diet decreased growth performance and nutrient digestibility of broiler. Also, replacing of corn, soybean, fish

meal with rice waste, larvae meal, and meat and bone waste up to 50% in the diet improved growth performance and nutrient digestibility of broiler.

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

PENILAIAN SUMBER MAKANAN ALTERNATIF DENGAN NISBAH TENAGA KEPADA PROTIN BERBEZA TERHADAP PENINGKATAN PRESTASI AYAM PENELUR DAN PEDAGING

Oleh

MOHAMMED FAROOQ ABDULHAMEED

Februari 2019

Pengerusi : Henny Akit, PhD
Fakulti : Pertanian

Formulasi diet poltri memainkan peranan dalam mengurangkan kos pengeluaran. Pengeluar poltri menghadapi cabaran untuk mengurangkan kos hasil poltri selepas persaingan antara manusia dan haiwan untuk beberapa bahan makanan. Terdapat kekurangan maklumat mengenai penggunaan sisa nasi, daging dan sisa tulang, dan larva lalat *black soldier* sebagai sumber tenaga dan protein terhadap prestasi ayam penelur dan pedaging. Objektif kajian ini adalah untuk menentukan kesan perbezaan kadar nisbah tenaga kepada protin menggunakan sisa nasi sebagai sumber tenaga, daging dan sisa tulang dan juga larva lalat black soldier sebagai sumber protin utama terhadap kualiti telur, daya pengeluaran, dan penilaian deria telur ayam penelur. Juga, prestasi pertumbuhan, kebolehcernaan nutrisi, populasi mikroorganisma, kualiti daging, morfologi perut, dan penilaian deria, dan ekspresi gen mRNA IGF-1 dan GHR hati ayam pedaging.

Dalam kajian pertama, sejumlah 54 ekor ayam betina baka Arab berusia 9 bulan telah dibahagikan kepada tiga rawatan, setiap 6 ekor ayam betina dicampurkan dengan seekor ayam jantan. Nisbah tenaga kepada protein untuk rawatan kawalan adalah 155, iaitu yang disarankan oleh *Nutrition Research Council*. Nisbah tenaga kepada protin rendah dan tinggi telah digunakan untuk rawatan kedua dan ketiga, iaitu masing-masing 144 dan 170. Larva lalat black soldier telah dimasukkan ke dalam diet pada kadar 0, 5 dan 1 % masing-masing. Keputusan menunjukan bahawa nisbah tenaga kepada protein yang rendah meningkatkan produksi *hen day* dan *hen house* telur ($P<0.05$), jisim telur ($P<0.001$), nisbah penukaran makanan (makanan/kg telur) ($P<0.01$), penilaian ciri-ciri telur ($P<0.05$), dan kadar kesuburan ($P<0.05$). Walau bagaimanapun, kesan negatif seperti penurunan berat telur ($P<0.001$), dan ciri-ciri kulit telur ($P<0.05$) disertai dengan nisbah tenaga protin yang rendah dalam diet.

Dalam kajian kedua, sejumlah 160 ekor anak ayam Cobb 500 berumur sehari dibahagikan secara rawak kepada empat rawatan. Bahan makanan tradisi diformulasikan untuk membentuk satu rawatan kawalan. Nisbah biasa tenaga kepada protein untuk rawatan kawalan yang dicadangkan dalam bimbingan Cobb 500 ialah 154 untuk tempoh masa permulaan dan 167 untuk tempoh masa pengakhiran. Sisa nasi, daging dan sisa tulang, dan larva lalat *black soldier* telah diformulasikan untuk membentuk tiga kumpulan diet rawatan yang lain. Nisbah biasa, rendah dan tinggi tenaga kepada protein diet makanan alternatif adalah 154, 143, dan 166 untuk tempoh masa permulaan dan juga 167, 155, dan 177 masing-masing untuk tempoh masa pengakhiran. Keputusan menunjukkan yang kadar biasa, rendah dan tinggi tenaga kepada protein diet makanan alternatif mengurangkan pengambilan makanan ($P<0.001$), peningkatan berat badan ($P<0.01$), nisbah penukaran makanan ($P<0.001$), berat karkas ($P<0.01$), kebolehcernaan protein kasar ($P<0.05$), nilai warna a* dan b* ($P<0.001$), ketinggian vilus dan kedalaman krip di dalam duodenum ($P<0.05$), populasi *Lactobacillus spp* ($P<0.001$) di dalam ileum dan cecum, populasi *Escherichia coli spp* ($P<0.001$) di dalam ileum, dan populasi *Enterococcus spp* ($P<0.001$) di dalam cecum, tetapi meningkatkan ketinggian vilus dan kedalam krip ($P<0.001$) di dalam jejunum, nilai warna L* daging ($P<0.05$) dibandingkan dengan diet kawalan. Nisbah biasa dan tinggi tenaga kepada protin diet makanan alternatif berkurang ($P<0.05$), kebolehcernaan ekstrak ether, dan populasi *Salmonella spp.* di dalam ileum. Kebolehcernaan abu tertinggi ($P<0.001$) dan nilai kehilangan air melalui memasak ($P<0.01$) telah diperhatikan pada ayam diberi makan nisbah tenaga kepada protin yang rendah. Nisbah tenaga kepada protin yang rendah dan tinggi mengurangkan populasi *Bifidobacterium spp* di dalam cecum ($P<0.05$), pH daging ($P<0.05$), dan nilai TBARS ($P<0.001$) selepas 5 hari penyimpanan. Nisbah tenaga kepada protin diet makanan alternatif biasa dan rendah mengurangkan ($P<0.05$) populasi *Enterococcus spp* populasi di dalam ileum.

Di dalam kajian ketiga, sejumlah 120 ekor anak ayam pedaging Cobb 500 berumur sehari telah dibahagikan secara rawak kepada empat rawatan. Bahan-bahan makanan konvensional (jagung, soya, dan ikan kisar) telah digantikan dengan sisa makanan (sisa nasi, daging dan sisa tulang), dan larva lalat *black soldier* sebanyak 10%, 30% dan 50%. Nisbah tenaga kepada protin untuk semua rawatan adalah 154 untuk jangka masa permulaan dan 166 untuk jangka masa pengakhiran. Keputusan menunjukkan peningkatan berat badan ($P<0.01$), nisbah penukaran makanan ($P<0.05$), dan berat relative lemak abdomen ($P<0.05$), kepekatan gen mRNA GHR dan IGF1 ($P<0.01$) telah meningkat apabila bahan makanan konvensional digantiklan dengan bahan makanan alternatif pada 30% dan 50%. Populasi *Lactobacillus spp.* ($P<0.01$) di dalam ileum dan cecum, populasi *Escherichia coli* ($P<0.001$) di dalam ileum, kebolehcernaan ($P<0.001$) bahan kering, protin kasar, ekstrak ether dan abu telah meningkat setelah bahan-bahan makanan konvensional digantikan dengan bahan-bahan makanan alternatif pada 10, 30 dan 50%. Walau bagaimanpun, populasi *Escherichia coli* telah menurun ($P<0.01$) di dalam cecum ayam yang telah diberi makan 10, 30 dan 50% diet gantian. Populasi *Enterococcus spp* tertinggi ($P<0.01$) di dalam ileum dan cecum ayam diberi makan 50% bahan-bahan makanan alternatif.

Dapatan kajian menjelaskan bahawa diet nisbah rendah tenaga kepada protein meningkatkan *hen day* dan *hen house* penghasilan telur ayam penelur. Nisbah tenaga kepada protin biasa, rendah dan tinggi diet sisa nasi, larva dan sisa daging dan tulang mengurangkan prestasi pertumbuhan dan kebolehcernaan nutrisi ayam pedaging. Selain itu, penggantian jagung, soya, ikan kisar dengan sisa nasi, larva, daging dan sisa tulang setinggi 50% di dalam diet meningkatkan prestasi pertumbuhan dan kebolehcernaan nutrisi ayam pedaging.

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

Henny Akit, PhD
Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Chairman)

Dahlan Ismail, PhD
Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Anjas Asmara @ Ab. Hadi Samsudin, PhD
Associate Professor
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Lokman Hakim Idris, PhD
Senior Lecturer
Faculty of Veterinary
Universiti Putra Malaysia
(Member)

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School of Graduate Studies
Universiti Putra Malaysia

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Name of Member of Supervisory Committee: Professor Dr. Dahlan Ismail

Signature: _____
Name of Member of Supervisory Committee: Associate Professor Dr. Anjas Asmara @ Ab. Hadi Samsudin

Signature: _____
Name of Member of Supervisory Committee: Dr. Lokman Hakim Idris

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LIST OF ABBREVIATIONS

FW	Food waste
GHG	Greenhouse gas
MSW	Municipal solid waste
EU	European Union
MBM	Meat and bone meal
BSF	Black soldier fly
FSCW	Food supply chain waste
DM	Dry matter
CP	Crude protein
EE	Ether Extract
BVWM	Blood vegetable waste meal
CWM	Cassava waste meal
FLFI	Food leftover feed ingredient
DFWP	Dehydrated food waste product
DLF	Dried leftover food
DPFW	Dehydrated processed food waste
L*	Lightness
a*	Yellowness
b*	Redness
DFWP1	Dehydrated food waste with feed stock (soy hulls and wheat flour)
DFWP2	Dehydrated food waste with feed stock (soy hulls and ground corn)
Kg	Kilogram
%	Percent
˚C	Celsius
H	Hour
HP	High protein

BSF	Black soldier fly
BSFL	Black soldier fly larvae
GNC	Groundnut cake
WWLM	West wood larva meal
EPR	Energy to protein ratio
MBW	Meat bone waste
RW	Rice waste
AOAC	Association of official agricultural chemists
ME	Metabolizable energy
CF	Crude fiber
HDEP	Hen day egg production
HHEP	Hen house egg production
NRC	National research council
FCR	Feed conversion ratio
SEM1	Pooled standard error
FWBSFL	Food waste (rice waste, meat and bone waste) and black soldier fly larvae
IACUC	Institutional Animal Care and Use Committee
CEPI	Conventional energy and protein ingredients
µL	Microliter
ml	Millilitre
ng	Nanogram
mM	Millimolar
mm	Millimetre
TBARS	Thiobarbituric acid reactive substances
g	Gram
MDA	Malondialdehyde
PBS	Phosphate buffer saline
µm	Micrometre
FI	Feed intake

BWG	Body weight gain
LW	Live weight
GH	Growth hormone
IGF-1	Insulin-like growth factor 1
CSFM	Corn, soybean, and fish meal
TiO_2	Titanium oxide
RMB	RW, MBW and BSFL
rpm	Revolutions per minute
GHR	Growth hormone receptor
GFR	Glomerular filtration rate

CHAPTER 1

INTRODUCTION

According to United National, the current world population is growing rapidly and is forecast to rise from its current total of 7.35 billion to 8.50 billion by 2030 and 9 billion by 2050 (Steinfeld et al., 2006). At the same time, levels of heart disease and arteriosclerosis, as well as environmental pollution, are also increasing. Chicken is considered a good type of meat for those seeking healthy food due to its low cholesterol content, high percentage of proteins, good ratio of unsaturated to saturated fatty acids, and the presence of essential vitamins and minerals. Thus, an increase in poultry production has followed an increase in demand for poultry diets. Grains constitute most of the diets of poultry. Therefore, there is competition between people and animals for grains such as soybean, and an increase in demand for corn has meant an increase in the prices of ingredients. Similarly, the price of fish meal, a conventional source of protein in poultry diets, has also risen (Deutsch et al., 2007; Tacon and Metian, 2008; Owen et al., 2009; Ani and Omeje, 2011). The elevated prices of ingredient negatively have a negative effect on poultry prices because the cost of feed constitutes around 70% of the production costs. Producers try to reduce the cost of feed by replacing feed ingredients with cheap alternatives that are safe enough to avoid any health problems and contain a good nutritional profile (FAO, 2004).

Globally, around 1.3 billion tons of food waste (FW) is generated per year (FAO, 2011). In Malaysia, more than 15000 thousand tons of FW is produced daily (Malaysiakini, 2016). Atwater (1895) was one of the first to highlight FW from households as a nutritional concern in the scientific literature at the end of the 19th century. Scientific information about FW was then provided through published research. For example, Quested, and Johnson (2009) reported that there were three types of FW: (i) needless losses- meaning items of food and drink that are thrown away because they have exceeded their expiry date such as meat and bread. (ii) possibly needless losses- inedible items of food and drink such as banana peels. (iii) indispensable losses- all items thrown away during consumption such as egg peels, bones, and skins. FAO (2011) recorded five sources of FW based on their generation: agricultural output, postharvest handling and storage, treatment, allocation, and consumption. Parfitt et al. (2010) defined FW as "leftovers that take place at the end of the food chain due to behaviour of retailers and consumers". Lipinski et al. (2013), on the other hand, define it as good food that can be consumed but declines before or after it becomes spoilt. Östergren et al. (2014) describe FW as edible or inedible food that has declined in the food supply chain and is then disposed of. FW is generated during retail and consumption stages due to either negligence or a conscious decision to throw food away. Developing countries face greater difficulties in managing FW than developed countries as many leftovers and waste items at the end of food chain have been observed (Parfitt et al., 2010; Gustavsson et al., 2011). Recently, greenhouse gas (GHG) emissions in landfills have also increased as FW is not easily separated from Municipal Solid Waste (MSW). A third of all greenhouse gas emissions in the European Union (EU) are produced from food waste (Garnett, 2011). Climate change is then affected by the emission of greenhouse gases. Therefore, in

some countries, FW has been subjected to a series of processing and recycling techniques. In some developed countries, laws have been enacted to use FW in animal feed (Ishoka, 2006; Kim et al., 2011). Most FW consists of meat and bone meal (MBM) and rice waste, plate waste, vegetable, blood, heads, trimmings, undigested feed, and grease, all of which are considered unfit for human consumption. The microbial decomposition that occurs during the disposal of FW may have a passive impact on human health and is an additional cost of FW treatment. Although the nutritional composition of FW is not constant, it plays a vital role in decreasing the cost of poultry production when it is handled properly and used as a safe alternative feed source in poultry diets (Viana and Schulz, 2003; Hossein and Dahlan, 2015).

Furthermore, insect meal can be an alternate protein source in poultry diets because it has high nutritional value, most notably in terms of protein, fat, minerals, and vitamins (Ojewola and Udom, 2005; Ojewola and Annah, 2006; Ijaiya and Eko, 2009). Lycaon conducted the first study on the effect of using insect meal in aquaculture diets in 1974 (Bondari and Sheppard, 1981). Newton et al. (1977b) then studied the formulation of swine diets with dried black soldier fly larvae (*H. illucens*). At a later stage, researchers replaced larvae meal in aquaculture (Ogunji et al., 2008) and poultry diets (Pretorius, 2011) with fish meal. Most insects contain a better balance of essential amino acids (methionine and lysine) than grains (Ravindran and Blair, 1993; Rumpold and Schluter, 2013; Makkar and Ankers, 2014). However, in the EU, using insect meal as a source of protein in animal diets is not permitted for animals raised for human consumption under Regulation EC 999/ 2001 (Regulation, 2001), which prohibits all processed animal protein (Papargyropoulou et al., 2014) except for hydrolyzed proteins (Regulation, 2004; Van Huis, 2013; Nadia et al., 2016). However, using processed animal protein in feed aquaculture became legal in June 2013. Additionally, the EU encourages free-range farming of animals, where the intake of invertebrates is the correct method in terms of animal welfare and feed intake. However, "natural feed" is not processed or checked for pollutants such as heavy metals, toxins, or pesticides (Radu-Rusu et al., 2013; Mbilu and Lyimo, 2015). It is unlikely that insects will be permitted in animal diets unless overall consideration is given to the safety of their use. Many animals already consume insects as part of their natural diet. Consequently, many insect species such as grasshoppers, crickets, Musca domestics, black soldier fly (BSF) larvae, and earthworms, have been used as a source of protein in animal diets (Wang et al., 2005; Prayogi, 2011; Choi et al., 2013).

Despite some research on feed alternatives in poultry diets, there is a paucity of information on the effect of using black soldier fly less than one week of age in layer diets on the quality and productivity of laying hens. Similarly, there is little information on the effect of combining black soldier fly larvae meal with food waste (rice waste and meat and bone waste) in broiler diets on growth performance, meat quality, nutrient digestibility, gut histology, sensory evaluation, IGF1 and GHR mRNA expression, and microbial population.

The hypotheses for this study are therefore as follows:

1. Different levels of energy to protein ratio using black soldier fly larvae will affect production and quality eggs of laying hens.
2. Different energy-to-protein ratios using a combined black soldier fly larvae meal and food waste (rice waste, meat and bone waste) diet will alter growth performance, nutrient digestibility, meat quality, histology, sensory evaluation, and the microbial population of broilers.
3. The replacement of black soldier fly larvae with food waste (rice waste, meat and bone waste) will affect growth performance, nutrient digestibility, meat quality, and hepatic IGF-I and GHR mRNA gene expression of broilers.

1.1 Objectives

- To determine the effect of different levels of energy-to-protein ratios using black soldier fly larvae on the quality and productivity of laying hens.
- To determine the effect of different levels of energy-to-protein ratios using food waste (rice waste, and meat and bone waste) and black soldier fly larvae on growth performance, nutrient digestibility, meat quality, histology, sensory evaluation, and microbial population of broilers.
- To determine the effect of replacing conventional feed ingredients (corn, soybean, fish meal) with food waste (rice waste, meat and bone waste) and black soldier fly larvae meal on growth performance, nutrient digestibility, meat quality, and hepatic IGF-I and GHR mRNA gene expression.

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