**UNIVERSITI PUTRA MALAYSIA**

# *PHYSICAL TREATMENTS TO ENHANCE NUTRITIVE VALUE OF PALM KERNEL EXPELLER IN FINISHER DIET FOR BROILERS*

**FARIDAH HANIM BINTI SHAKIRIN**

**IPTSM 2021 3**

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By

**FARIDAH HANIM BINTI SHAKIRIN**

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

**December 2020**

# DEDICATION

This thesis is dedicated to my beloved husband, lovely parents, kids and siblings for their endless support and encouragement

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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**Chair : Liang Juan Boo, PhD**
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Annual importation of livestock feed in Malaysia amounting to RM11bil, with corn and soybeans accounted for the bulk of the imported feed costs. Depending on the price of ingredients, by weight, corn makes up 60-65% and soybean 26-32% of the poultry feed in finisher diet. Thus, Malaysia has to cut back on the importation of poultry feed ingredients, particularly corn and soybean, to ensure long–term sustainability of the industry. Higher usage of palm kernel expeller (PKE) in poultry feed could reduce the country dependency on imported feed. Effort to use PKE as feed component in broilers has been constrained by its high fiber content. Biological treatments are the most common methods used to improve the nutritive value of PKE but they achieved limited success. Physical treatments which have been proven to be effective in enhancing the nutritive value of other feed ingredients, but not well tested in PKE, is the theme of this thesis.

Several physical treatments; including grinding, sieving and extrusion, were evaluated for their effects on chemical composition, particularly crude fiber (CF) reduction, and alteration of hydration properties of PKE. From the results, both extrusion and sieving (but not grinding) significantly ($p<0.05$) reduced CF by 1.3 and 1.2 folds, respectively, as compared to the untreated PKE. Also, extrusion significantly ($p<0.05$) increased total reducing sugar, soluble protein and starch contents by 5, 1 and 8.5 times, respectively, as compared to the control; while sieving resulted in no increase ($p>0.05$) in reducing sugar, 1.5 folds increment in soluble protein, however, sieving increased ($p<0.05$) swelling capacity, and water retention capacity by 1.3 and 1.2 folds, respectively.

In the second experiment, 64 male Cobb 500 chicks were used to determine the apparent metabolizable energy (AME), protein and amino acid digestibility of the sieved and extruded PKE prepared according to the protocols of the first experiment. Results showed that extrusion significantly ($p<0.05$) increased the AME of PKE from 13.21 to 14.04 MJ/kg, while sieving has no effect on AME of PKE. Both, extrusion and sieving significantly ($p<0.05$) enhanced protein digestibility by 1.32 and 1.39 folds, respectively.

The primary objective of the feeding trial was to compare the production parameters of broiler fed increasing levels (10, 20 and 30%) of untreated and extruded PKE to determine the maximum inclusion level of PKE in the finisher diet. The trial focused on finisher-diet phase in which diets were formulated to contain up to 30% PKE. Changes in the intestinal morphology and nutrient assimilation in the intestine using the expressions of transporter genes were also monitored. Results showed that birds fed 30% extruded PKE (30EPKE) sustained similar weight gain (WG) and feed conversion ratio (FCR) while those fed 30% untreated PKE (30PKE) had poorer WG and FCR ($p<0.05$) as compared to those fed 10% PKE. The above result indicated that extruded PKE (30EPKE) can be included up to 30% in finisher broiler diet. The better WG and FCR of birds fed 30EPKE ($p<0.05$) over 30PKE were accompanied by up-regulation ($p<0.05$) of sugar (GLUT2, SGLT5) and amino acid (PepT1 and EAAT3) transporters in the former group. Increased villus height and crypt depth ($p<0.05$) were observed in birds fed high (30%) PKE and mainly at the jejunum and ileum sections. Feeding up to 30% PKE (extruded or untreated) did not significantly ($p>0.05$) altered the accumulation of minerals in the liver as there is no changes on the liver and kidney morphology. This indicated that the health and wellbeing of the birds were not compromised. It is concluded that extruded PKE can be included up to 30% in finisher-diet to sustain the normal growth and FCR without affecting the overall health and wellbeing in broilers.

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

**RAWATAN FIZIKAL UNTUK MENINGKATKAN NILAI PEMAKANAN HAMPAS ISIRUNG KELAPA SAWIT DALAM DIET PENGAKHIR AYAM PEDAGING**

Oleh

**FARIDAH HANIM BINTI SHAKIRIN**

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Import tahunan makanan ternakan di Malaysia berjumlah RM11 bilion, dengan jagung dan kacang soya menyumbang sebahagian besar kos makanan yang diimport. Dari segi berat, jagung mewakili 60-65% dan kacang soya 26-32% dari jumlah keseluruhan makanan ayam dalam fasa makanan pengakhir. Oleh itu, Malaysia harus mengurangkan pengimportan bahan makanan ternakan ayam , terutama jagung dan kacang soya, untuk memastikan kelangsungan jangka panjang industri ini. Penggunaan isirung kelapa sawit (PKE) yang lebih tinggi dalam makanan ternakan ayam dapat mengurangkan kebergantungan negara terhadap makanan yang diimport. Usaha menggunakan isirung kelapa sawit (PKE) sebagai komponen makanan pada ayam pedaging telah menghadapi beberapa halangan termasuk kandungan seratnya yang tinggi. Rawatan biologi adalah kaedah yang paling biasa digunakan untuk meningkatkan nilai nutrien PKE tetapi ia tidak berjaya. Langkah untuk menjalankan rawatan fizikal telah terbukti berkesan terhadap ramuan makanan yang lain, tetapi tidak diuji dengan begitu baik terhadap PKE, dan ini merupakan tema kepada tesis ini.

Beberapa rawatan fizikal; termasuk pengisaran, penapisan dan ekstrusi, diukur melalui kesan terhadap komposisi kimia, terutamanya pengurangan kandungan serat kasar (CF), dan perubahan sifat penghidratan PKE. Kedua-dua ekstrusi,  dan penapisan telah dapat mengurangkan CF secara ketara (iaitu sebanyak, 1.3 dan 1.2 kali ganda), tetapi kesan pengurangan CF tidak berlaku terhadap pengisaran, dan ia dibandingkan dengan PKE yang tidak dirawat. Selain itu juga, ekstrusi meningkatkan jumlah gula penurun, protein larut dan kandungan kanji, sebanyak 5 kali ganda, 1 kali ganda dan 8.5 kali ganda, berbanding dengan kawalan; sementara penapisan tidak menghasilkan

peningkatan gula penurun, peningkatan 1.5 kali ganda protein larut dan peningkatan 1.3 kali ganda kesan penghidratan, dan daya penahan air sebanyak 1.2 kali ganda.

Dalam eksperimen kedua, 64 ekor anak ayam Cobb 500 jantan digunakan untuk mengukur tenaga (AME), tahap pencernaan protein dan asid amino PKE yang ditapis dan diekstrusi seperti yang dinyatakan di dalam protokol eksperimen pertama. Hasil kajian menunjukkan bahawa ekstrusi secara signifikan ($p<0.05$) meningkatkan AME terhadap PKE dari 13.21 kepada 14.04 MJ/kg, sementara penapisan tidak memberi kesan kepada AME PKE. Kedua-dua ekstrusi dan penapisan meningkatkan kecernaan protein dengan ketara ($p<0.05$) pada 1.32 dan 1.39 kali ganda.

Objektif utama percubaan makan adalah untuk membandingkan parameter pengeluaran ayam pedaging pada tahap peningkatan PKE (10, 20 dan 30%) yang tidak dirawat dan diekstrusi untuk menentukan tahap penyertaan maksimum PKE dalam diet pengakhir. Percubaan ini difokuskan pada fasa diet pengakhir di mana diet dirumuskan untuk mengandungi hingga 30% PKE. Perubahan morfologi usus dan asimilasi nutrien dalam usus menggunakan ekspresi gen transporter juga dipantau. Hasil kajian menunjukkan bahawa ayam pedaging yang diberi makan 30% PKE ekstrusi (30EPKE) mengalami kenaikan berat badan yang serupa (WG) dan nisbah penukaran makanan (FCR) sementara ayam yang diberi makan 30% PKE yang tidak diberi rawatan (30PKE) mempunyai WG dan FCR yang lebih rendah ($p<0.05$) berbanding dengan mereka diberi makan 10% PKE. Hasil di atas menunjukkan bahawa PKE yang diekstrusi (30EPKE) dapat dimasukkan hingga 30% dalam diet ayam pedaging. Ayam yang diberi makan 30EPKE mempunyai lebih baik WG dan FCR ($p<0.05$) berbanding 30PKE disertai dengan pengatur kenaikan ($p<0.05$) gula (GLUT2, SGLT5) dan pengangkut asid amino (PepT1 dan EAAT3) dalam kumpulan sebelumnya. Peningkatan ketinggian villus dan kedalaman crypt ($p<0.05$) diperhatikan pada ayam yang diberi PKE tinggi (30%) dan terutamanya pada bahagian jejunum dan ileum. Memberi makan hingga 30% PKE (diekstrusi atau tidak dirawat) tidak menyebabkan perubahan secara signifikan ($p>0.05$) pengumpulan mineral di hati kerana tidak ada perubahan pada morfologi hati dan ginjal. Ini menunjukkan bahawa kesihatan dan kesejahteraan ayam tidak terganggu. Disimpulkan bahawa PKE yang diekstrusi dapat dimasukkan hingga 30% dalam diet pengakhir untuk mempertahankan pertumbuhan normal dan FCR tanpa mempengaruhi keseluruhan kesihatan dan kesejahteraan pada ayam pedaging.

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## TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

## LIST OF ABBREVIATIONS

| | |
|---|---|
| AA | Amino acid |
| AAs | Amino acids |
| AATs | Amino acids transporter |
| ADF | Acid detergent fiber |
| ALT | Alanine aminotransaminase |
| AME | Apparent Metabolizable Energy |
| ANOVA | Analysis of Variance |
| AOAC | Association of Official Agricultural Chemists |
| AST | Aspartate aminotransaminase |
| BWG | Body weight gain |
| Ca | Calcium |
| CAID | Coefficient of apparent ileal digestiblity |
| CF | Crude fiber |
| CP | Crude protein |
| Cu | Copper |
| $CuSO_4$ | Copper (II) Sulfate |
| DM | Dry matter |
| DNA | Deoxyribonucleic acid |
| EAAT1 | Excitatory amino acid transporter 1 |
| EAAT3 | Excitatory amino acid transporter 3 |
| EPKE | Extruded PKE |
| FCR | Feed conversion ratio |
| FI | Feed intake |

| | |
|---|---|
| GAPDH | Glyceraldehyde phosphate dehydrogenase |
| gDNA | Genomic deoxyribonucleic acid |
| GE | Gross energy |
| GLUT | Glucose transporter |
| GLUT2 | Na+-independent glucose, galactose, and fructose transporter |
| GLUT3 | Glucose transporter3 |
| GLUT4 | Glucose transporter4 |
| GLUT5 | Na+-independent fructose transporter |
| GLUT8 | Glucose transporter8 |
| H | Hydrogen |
| HE | Hematoxylin Eosin |
| $H_2SO_4$ | Sulfuric acid |
| HCL | Hydrochloric acid |
| HPLC | High-Performance liquid Chromatography |
| Hz | Hertz |
| $LiOH.H_20$ | Lithium hydroxide monohydrate |
| ME | Metabolizable energy |
| mg | milligram |
| min | minute |
| MJ | Mega Joule |
| Mn | Manganase |
| MPOB | Malaysian Palm Oil Board |
| mRNA | Messenger Ribonucleic Acid |
| N | Nitrogen |
| NDF | Neutral detergent fiber |

| | |
|---|---|
| NRC | Nutrient Research Council |
| NSPs | Non starch polysachharides |
| P | Phosphorus |
| PCR | Polymerase chain reaction |
| PepT1 | Peptide transporter 1 |
| PepT2 | Peptide transporter 1 |
| PHT1 | Peptide-histidine transporter 1 |
| PHT2 | Peptide-histidine transporter 2 |
| PKC | Palm kernel cake |
| PKE | Palm kernel expeller |
| RNA | Ribonucleic Acid |
| SGLT1 | Na+-dependent glucose and galactose transporter |
| SGLT5 | sodium/glucose cotransporter 5 |
| sNSP | Soluble non starch polysachharides |
| SSF | Solid state fermentation |
| TME | True Metabolizable energy |
| UPM | Universiti Putra Malaysia |
| WG | Weight gain |
| Zn | Zinc |
| 10EPKE | 10% extruded PKE |
| 10PKE | 10% untreated PKE |
| 20EPKE | 20% extruded PKE |
| 20PKE | 20% untreated PKE |
| 30EPKE | 30% extruded PKE |
| 30PKE | 30% untreated PKE |

# CHAPTER 1

## INTRODUCTION

Malaysia exported a total of 27.88 million tonnes of oil palm related products in 2019, an increase of 12.1% from the 24.88 million tonnes exported the previous year (Kadir *et al.,* 2020). The above statistic shows that the oil palm industry in Malaysia produces large amount of products and by-products; the latter includes empty fruit bunches, palm oil mill sludge and palm kernel expeller (PKE). The production of PKE, the primary high-value by-product from the oil palm industry, increased by 3.3% from 2018 to 2019 indicating that production of PKE may continue to increase over the year (MPOB, 2020).

Palm kernel expeller is the by-product generated after the kernel is crushed during the oil extraction process. It is commonly used as a feed in ruminant production, but its low nutritional value, including high fiber limits its incorporation at high inclusion level in poultry diet (Aguzey *et al.,* 2020). Majority of the fiber in PKE is from cell-wall components, consisted of 35.2% mannose, 2.6% xylose and 1.1% arobinose (Cervero *et al.,* 2010). The protein content in PKE ranges between 14 – 18%, with limiting amount of essential amino acids including lysine, methionine and tryptophan (Aguzey *et al.,* 2020), while the fat content is in the range of 7-9% depending on the efficiency of the kernel oil extraction process.

Malaysia is currently self-sufficient in poultry meat and eggs, but the industry is not sustainable in the long run because of its strong reliance on imported feed. Feed represents a large proportion of the production costs in any livestock production, including the poultry industry. The latter relies on imported feedstuffs ranging from cereal grains, vegetable and animal proteins and various micro-ingredients and feed additives to increase productivity to remain competitive in the market. Continued availability and price stability of the conventional feedstuffs are major concerns which forced feed manufacturers to consider using locally available feed ingredients, including agricultural by products. Palm kernel expeller is one of the most studied by-products for poultry production because of its high energy and moderate protein contents. However, the high fiber content (about 21% of PKE) imposes a constraint for its use in poultry feed, particularly at high inclusion rates. Hence, reduction of fiber by appropriate treatments need to be developed to improve the nutritive value of PKE.

Although commercial enzymes to breakdown the fiber of PKE are available, their applications are limited due to high cost and low efficiency as they were not designed specifically for degradation of the fiber components in PKE. Biological treatments of PKE with various microbial species have been extensively studied. Fungal species such as *Aspergillus niger, Sclerotium* *rolfsii*, *Trichoderma harzianum* (Iyayi *et al.,* 2010; Supriyati *et al.,* 2015) or bacterial species such as *Paenibacillus polymyxa* and *P. curdlanolyticus* (Alshelmani *et al.,* 2016) have been tested with some success in enhancing the nutritional value of PKE by solid-state fermentation (SSF). However, the use of some fungal species in feed treatment has limitation because they produced mycotoxins such as aflatoxin which is among the most carcinogenic substances known. These toxins could be found in animal meat and milk which had been fed fungal contaminated feeds (Olukomaiya *et al.,* 2020). Although bacterial bioprocesses have an edge as they are devoid of producing such toxins, but SSF using bacteria requires much longer time to achieve the needed improvement (Alshelmani *et al.,* 2014).

Physical treatments, such as soaking and boiling, have been reported to reduce crude fiber (CF) content and increase the metabolizable energy (ME) of cowpea seed hulls (Adebiyi *et al.,* 2010). Other physical treatments, including sieving has been reported to increase feed utilization (Amerah, 2015) and extrusion of canola meal increased the apparent metabolizable energy (AME) in the broiler chicken (Ahmed *et al.,* 2014). Positive effects of extrusion in breaking down other dietary fiber in feed ingredients and increased nutrient availability have also been reported (Avazkhanloo *et al.,* 2019; Hejdysz *et al.,* 2017; Zare-Sheibani *et al.,* 2015). However, the efficacy of physical treatments which have shown to enhance the nutritive value and nutrient utilization of a variety of feedstuffs have not been scientifically evaluated in PKE.

## 1.1 Problem Statement

Developing long term sustainable poultry production must cut down the over dependency on imported feedstuffs, particularly corn and soyabean. Increased use of non-conventional feed ingredients available in the country in poultry feed could help the country become less reliant on imported feed. The use of the abundance supply of PKE, provides a realistic option to the above problem. However, the high fiber content in PKE limited its use to only 5-10% in the poultry feed. Although several biological treatments were tested, they offered only limited success. Physical treatments such as extrusion, sieving and grinding which have been shown to be effective in treating other feed ingredients could be an alternative option to improve the nutritive value of PKE.

## 1.2 Hypothesis

Physical treatments can reduce the fiber content of PKE to enhance its nutritive value and thus its inclusion rate in the poultry feed. The research framework are depicted in Figure 1.1.

## 1.3 Objectives

The general objective of this thesis was to enhance the nutritive value of PKE by physical treatments and to evaluate the effects of varying levels of the treated PKE in finisher broiler diet on growth performance and selected physiological parameters. The specific objectives were:

1. To evaluate various potential physical treatments including, extrusion, sieving and grinding on the chemical and hydration properties of PKE.

2. To investigate the effect of selected physical treatments on apparent metabolizable energy (AME) and nutrient digestibility (crude protein and amino acids) of PKE in broiler chickens.

3. To examine the effect of extruded PKE at different inclusion rates in finisher broiler diet on growth performance, intestinal morphology and expression of nutrient transporter genes.

4. To determine organ histopathological changes, mineral accumulation and blood profile related to health and wellbeing of feeding PKE at high inclusion rate in broiler chickens.

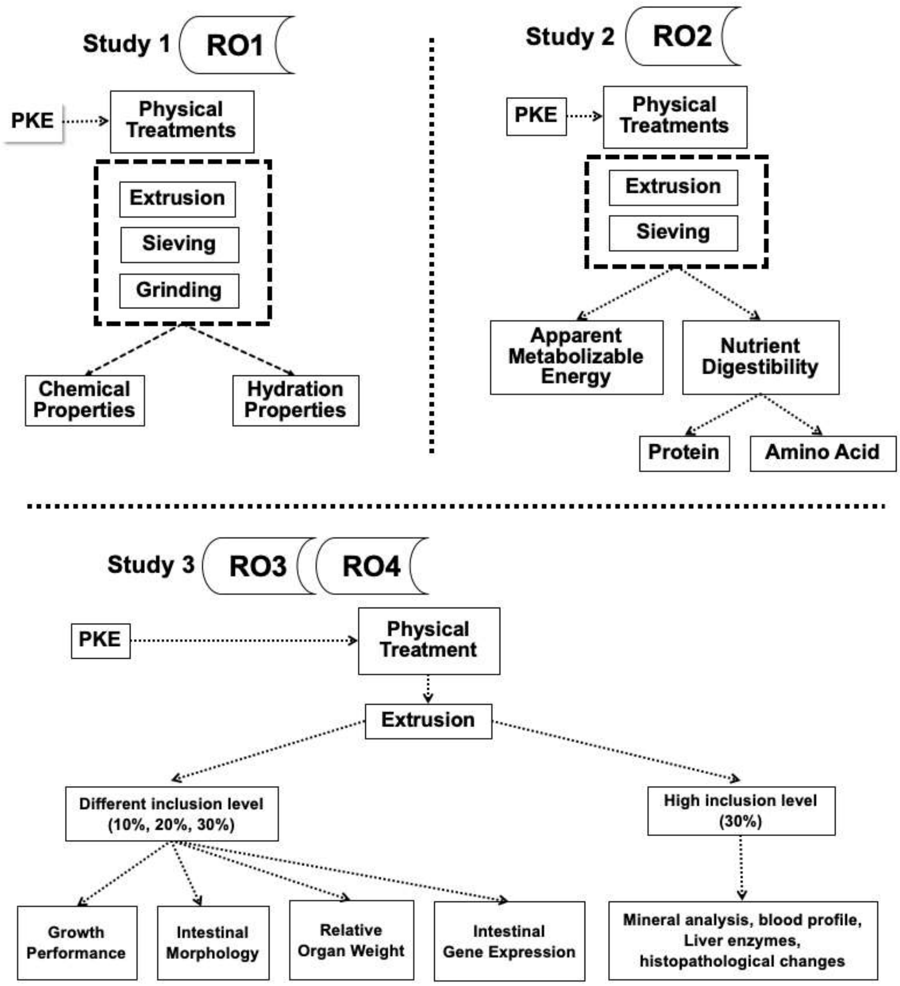


**Figure 1.1 : Research framework**

RO1: Research objectives 1; RO2: Research objective 2; RO3; Research objective RO4; Research objective 4

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## BIODATA OF STUDENT

Faridah Hanim binti Shakirin was born on the 11th August 1985 at Johor Bahru. She received her primary education at SK Bukit Hampar, Segamat, Johor. In 1996, she continued her secondary education at SMK Sains Kota Tinggi. She furthers her study at Matriculation Centre of Kuala Pilah, Negeri Sembilan. She pursued her degree education, majoring in the field of Biochemistry at the Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia (UPM). She graduated with a Bachelor of Science (Hons.) majoring in Biochemistry with Second Class Upper in 2006. In 2007, the author pursued her Master degree in UPM in the field of Nutritional Sciences at the Department of Nutritions and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia. During her Master Degree, she published three papers in ISI indexed journals and received numerous awards on Inventive Silver Award at Invention, Research and Inovation Exhibition, UPM. In the 2012, she pursued her Doctor of Philosophy in Animal Nutrition at the Institute of Tropical Agricultural and Food Security, UPM under the supervision of Prof Dr Norhani binti Abdullah and later (2019) under supervision of Dr Liang Juan Boo. To date she has published one full research paper in international journal and three papers in proceedings.

## LIST OF PUBLICATION

### Journal

**Faridah HS**, Goh YM, Noordin MM, Liang JB. 2020. Extrusion enhances apparent metabolizable energy, ileal protein and amino acid digestibility of palm kernel cake in broilers. Asian-Australasian Journal of Animal Science.

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**Faridah H.S.**, Norhani, A, Liang, J.B. and Noordin M.M. (2016) Extrusion improves the nutritive value of palm kernel cake. In: 37th Annual Conference of the Malaysian Society of Animal Production (MSAP), 1-3 June 2016, Melaka, Malaysia. (pp. 68-69).

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