



UNIVERSITI PUTRA MALAYSIA

**ENHANCING NUTRITIVE VALUE OF PALM KERNEL EXPELLER
THROUGH PHYSICAL TREATMENTS FOR USE AS POULTRY FEED
INGREDIENT**

MUHAMAD AKHMAL HAKIM BIN ROSLAN

IPTSM 2019 10



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By

MUHAMAD AKHMAL HAKIM BIN ROSLAN

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

February 2019

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

ENHANCING NUTRITIVE VALUE OF PALM KERNEL EXPELLER THROUGH PHYSICAL TREATMENTS FOR USE AS POULTRY FEED INGREDIENT

By

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February 2019

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Poultry production in Malaysia is heavily dependent on imported feed ingredients. The palm kernel expeller (PKE) is a potential feed ingredient to sustain the poultry industry. However, PKE had to be treated before it could be incorporated in poultry feed to reduce the anti-nutritive factors like indigestible mannan, lignin and broken kernel shells.. Hence, this study aimed at reducing these factors of PKE by extrusion and static cling-electrostatic separation technique to reduce the mannan and broken shells, respectively, and to produce a poultry feed containing treated PKE with a potential for commercial application. Extrusion was conducted by using a custom made co-rotating twin-screw extruder and static cling–electrostatic separation by using a simple fabricated apparatus. Response surface methodology (RSM) was used to optimize both treatments. Extrusion of PKE at optimized conditions (temperature, 178 °C; screw speed, 100 rpm; hopper speed, 5 Hz and PKE moisture, 75%), significantly ($P<0.05$) reduced non-starch polysaccharides from 63.3% to 57.6%, and crude fibre from 16.7% to 13.5%, and significantly ($P<0.05$) increased mannose, glucose, fructose, 1,4- β -D-mannobiose; 1,4- β -D-mannotriose; 1,4- β -D-mannohexaose and 1,4- β -D-mannopentaose content. The production rate of extruded PKE (EPKE) was 180 kg/h. The static cling–electrostatic method at optimized conditions (13% PKE moisture; <1.5 mm PKE particle size and 110 g/min feed rate), reduced shells, lignin and fibre of PKE from 8.0% to 1.6%; 15.8% to 8.1% and 17.1% to 10.2%, respectively, and increased crude protein from 16.5% to 18.4%. The production rate of less-shell PKE (LSPKE) was 50 kg/h. The study on the effects of control (0% PKE), UPKE (untreated), EPKE, LSPKE and ELSPKE at 10%, 20% and 30% inclusion rates in finisher diets (d 21-42) showed that growth performance of broilers (Cobb 500) fed 10% PKE (irrespective of treatments), 20% LSPKE and ELSPKE, were comparable to control birds. At 30% PKE inclusion, broilers performance was significantly ($P<0.05$) reduced. Birds fed PKE diets, irrespective of treatments and inclusion levels had lower LDL and total cholesterol, and similar villi height and crypt depth compared to control. At 30% PKE inclusion level, although energy metabolism was enhanced, as indicated by the up-regulation of hexokinase I and

phosphofructokinase, but broilers growth performance did not improve. The feeding trial showed that broilers fed 25% or 30% LSPKE (grower feed, d 16-24), followed by 20% LSPKE (finisher feed, d 25-35) containing feed supplements 0.02% commercial enzymes and 0.30% humic acid), had comparable FI, BWG and FCR to broilers fed commercial feeds. Broilers fed 25% or 30% UPKE (grower feed, d 16-24) followed by 20% UPKE (finisher feed, d 25-35) showed significantly higher ($P < 0.05$) FCR compared to birds fed commercial feeds. The cost of feeds for production of birds fed LSPKE feeds (2.27-2.29 RM/kg liveweight), was lower than those fed commercial feeds (2.36 RM/kg liveweight). The study showed that physical treatments enhanced the nutritive value of PKE and feeds containing 25-30% and 20% LSPKE as grower and finisher rations, respectively, could attain broilers growth performance comparable to broilers fed commercial feeds. These diets could be potential feeds for commercial poultry production.



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

MENINGKATKAN NILAI PEMAKANAN HAMPAS ISIRUNG KELAPA SAWIT MELALUI RAWATAN FISIKAL UNTUK KEGUNAAN SEBAGAI BAHAN MAKANAN POLTRI

Oleh

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Produksi ternakan poltri di Malaysia sangat bergantung kepada bahan makanan import. Hampas isirung kelapa sawit (PKE) berpotensi menjadi makanan alternatif untuk mengekalkan industri poltri. Walaubagaimanapun, PKE perlu dirawat sebelum ia digunakan sebagai bahan dalam makanan poltri untuk mengurangkan bahan anti nutrisi seperti mannan, lignin dan cangkerang. Oleh itu penyelidikan ini bermatlamat untuk meningkatkan nutrisi PKE melalui teknik ekstrusi dan pemisahan statik berpaut – elektrostatik untuk mengurangkan serat dan cangkerang dan menghasilkan makanan poltri yang mengandungi PKE terawat untuk aplikasi komersial. Ekstrusi dijalankan menggunakan mesin ekstrusi skru berkembar dan radas pemisahan statik berpaut – elektrostatik yang dibuat khas. Metodologi permukaan sambutan (RSM) telah digunakan untuk mengoptimumkan kedua-dua jenis rawatan. Ekstrusi PKE pada kondisi optimum (suhu, 178 °C; kelajuan skru, 100 rpm; kelajuan corong, 5 Hz dan kelembapan PKE, 75%), signifikan menurunkan ($P < 0.05$) kandungan polisakarida bukan kanji dari 63.3% kepada 57.6% dan serat mentah daripada 16.7% kepada 13.5%, dan signifikan meningkatkan kandungan mannan, glukosa, fruktosa dan mannan oligosakarida (1,4- β -D-mannobiosa; 1,4- β -D-mannotriosa; 1,4- β -D-mannoheksaosa and 1,4- β -D-mannopentaosa). Kadar produksi PKE terekstrusi (EPKE) adalah 180 kg/jam. Teknik pemisahan statik berpaut – elektrostatik pada kondisi optimum, menurunkan cangkerang, lignin dan serat dalam PKE masing-masing daripada 8.0% kepada 1.6%; 15.8% kepada 8.1% dan 17.1% kepada 10.2%, dan meningkatkan protin mentah daripada 16.5% to 18.4%. Kadar produksi PKE kurang cangkerang (LSPKE) adalah 50 kg/jam. Kajian terhadap kesan pemakanan kawalan (0% PKE), UPKE (tidak dirawat), EPKE, LSPKE dan ELSPE pada 10%, 20% dan 30% kadar kemasukan dalam dedak pengakhir (hari 21-42) menunjukkan prestasi tumbesaran ayam pedaging (Cobb 500) diberi makan 10% PKE (tanpa mengira jenis rawatan), 20% LSPKE dan ELSPE, adalah setanding dengan ayam kawalan. Pada kemasukan 30% PKE, prestasi ayam pedaging ($P < 0.05$) menurun dengan signifikan. Ayam diberi makan diet PKE, tanpa mengira jenis rawatan dan kadar tambahan, mempunyai rendah kolesterol lipoprotein densiti rendah (LDL) dan kolesterol keseluruhan dalam serum dan serata tinggi vilus dan kedalaman crypt berbanding ayam

kawalan. Penambahan 30% PKE meningkatkan metabolisma tenaga melalui peningkatan ekspresi heksokinase I dan fosfofruktokinase. Walaubagaimanapun, tumbesaran ayam pedaging tidak bertambah baik. Ujian akhir pemberian makanan terhadap ayam pedaging menunjukkan ayam yang diberi 25% atau 30% LSPKE (dedak pertumbuhan, hari 16-24), diikuti oleh 20% LSPKE (dedak pengakhir, hari 25-35) mengandungi aditif (0.02% enzim komersial dan 0.30% asid humik), mempunyai pengambilan makanan, kenaikan berat badan dan nisbah penukaran makanan yang setanding ayam diberi makan dedak komersial. Ayam diberi 25% atau 30% UPKE (dedak pertumbuhan hari 16-24), diikuti oleh 20% UPKE (dedak pengakhir, hari 25-35) menunjukkan peningkatan nisbah penukaran makanan yang signifikan berbanding ayam yang diberi makan dedak komersial. Kos dedak bagi produksi ayam yang diberi dedak ini (2.27-2.29 RM/kg berat hidup) adalah lebih rendah berbanding ayam diberi dedak komersial (2.36 RM/kg berat hidup). Kajian ini menunjukkan rawatan fizikal menambah baik nutrisi PKE, dan dedak mengandungi 25% dan 20% LSPKE masing-masing sebagai formula dedak pertumbuhan dan pengakhir, boleh mencapai prestasi tumbesaran ayam yang setanding dengan ayam yang diberi dedak komersial. Dedak ini berpotensi menjadi dedak untuk produksi poltri secara komersial.

ACKNOWLEDGEMENTS

I would like to thank Professor Dr. Norhani Abdullah for giving me the opportunity, trust and responsibility to complete my study under her supervision, it is truly an honor. Her wisdom and teaching on responsibility and integrity, will always be my lifetime inspirations. I would like to thank Professor Dr. Zulkifli Idrus, Professor Dr. Shuhaimi Mustafa and Associate Professor Dr. Mohd Yunus Abd Shukor for all their kind supports during my study. I also acknowledged the cooperation and assistance from my colleagues, Norafilah, Intan, Faridah, Mutaz, Elizabeth, Syikin and Dr. Mohammad, and ITAFoS staffs Mr. Faizol, Ms Azilah, Mr. Farhan, Mr. Eddy, Mr. Zahidi, Mr. Zulhelmi, Mr. Fazmen, Dr. Abdoreza and Dr Suriya.

I would also like to thank the Institute of Tropical Agriculture and Food Security (ITAFoS) and Universiti Putra Malaysia for the facilities provided and to the Ministry of Higher Education of Malaysia for the research grant under the Long Term Research Grant Scheme (LRGS).

To my beloved wife Rawdhah, thank you so much for countless love and support given that helped me to get through the challenging period wonderfully. Lastly, very special thanks to my parents Siti Sara and Roslan for their endless love and strong support throughout my life. This study would have been impossible to complete without their pray and support.

My lord, make what you teach us beneficial, teach us what is beneficial, and increase us in knowledge. Amen.

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

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LIST OF ABBREVIATIONS

Adj. R ²	Adjusted coefficient of determination
AIDC	Apparent ileal digestibility coefficient
AJDU	Apple juice depectinizing units
AME	Apparent metabolisable energy
AMEN	Nitrogen-corrected apparent metabolizable energy
ANOVA	Analysis of variance
AOAC	Association official agricultural chemists
ASTM	American society for testing and materials
BBD	Box-behnken design
BGU	Beta-glucanase unit
Bil	Billion
BW	Body weight
BWG	Body weight gain
cDNA	Complementary deoxyribonucleic acid
CI	Confidence interval
Cl	Chloride
cm	Centimeter
CMCU	Carboxymethyl cellulase
CP	Crude protein
C (t)	Effluent concentration–time curve
d	day
DCP	Dicalcium phosphate
DM	Dry matter

DNA	Deoxyribonucleic acid
DP	Degree of polymerization
DPKC	Deproteinated palm kernel cake
ECT	Total extrudate collection time
EDTA	Ethylenediaminetetraacetic acid
ELSPKE	Extruded less shell palm kernel Expeller
EPKE	Extruded palm kernel Expeller
Exp	Experiment
FAU	Fungal amylase unit
FCR	Feed conversion ratio
FI	Feed intake
FPRT	Fastest particle residence time
FTIR	Fourier transform infrared spectroscopy
g	Gram
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
gDNA	Genomic deoxyribonucleic acids
GFSI	Global Food Security Index
h	Hour
HCl	Hydrochloric acid
HDL	High density lipoprotein cholesterol
HK1	Hexokinase I
HPLC	High performance liquid chromatography
HUT	Hemoglobin unit on a tyrosine basis
Hz	Hertz
IVPD	In-vitro protein digestibility
L	Liter

LDL	Low density lipoprotein cholesterol
LSPKE	Less shell palm kernel Expeller
MDCP	Mono dicalcium phosphate
MJ/kg	Megajoules per kilogram
mg	Milligram
min	Minutes
MIU	Milli-international unit
mL	Millilitre
mM	Millimolar
MOS	Mannan oligosaccharide
MUC2	Mucin 2
mRNA	Messenger ribonucleic acid
Na	Sodium
nm	Nanometer
NSP	Non-starch polysaccharide
°C	Degree celcius
PCR	Polymerase chain reaction
PDI	Pellet Durability Index
PFK	Phosphofructokinase
pH	Hydrogen ion concentration
pKa	Acid dissociation constant
PKC	Palm kernel cake
PKE	Palm kernel expeller
PKM	Palm kernel meal
ppm	Parts per million
Pred	Predicted

R ²	Coefficient of determination
rpm	Revolutions per minute
RM	Ringgit Malaysia
RSM	Response surface methodology
RTD	Residence time distribution
SEM	Standard error of mean
s	Seconds
SC	Swelling capacity
SPU	Solid state fermentation phytase unit
T-NSP	Total non-starch polysaccharides
TiO ₂	Titanium dioxide
T _m	Particle mean residence time
μg	Microgram
μl	Microliter
UPKE	Untreated palm kernel Expeller
WRC	Water retention capacity
XU	Xylanase unit
YCW	Yeast cell wall



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CHAPTER 1

INTRODUCTION

Malaysia is currently self-sufficient in poultry meat and eggs, but the industry is not sustainable in the long term due to its heavy dependency on imported feeds such as soybean meal, corn and fish meal. Although some raw materials such as tapioca and fishmeal are produced locally, the amount is not sufficient to meet the requirements of the local feed industry (Raghavan, 2000).

Hence, continued availability of the main conventional feedstuffs is a major concern and this triggers interest by researchers to find alternative feed ingredients available locally. The utilization of agro-industrial wastes as feedstuff is one of the strategies to partially replaced imported feed ingredients in poultry production since it is not competing with human consumption.

Palm kernel cake (PKC) of the oil palm industry is the most suitable by-product to be considered as a source of energy. It is the residue left after the palm kernel is crushed during the oil extraction process (Ab Rahman et al., 2011). Due to the increase in palm oil production in the country, the amount of PKC available is also increased. As one of the world's greatest palm oil producers, the production of PKC in Malaysia, according to the Malaysian Palm Oil Board is about 2 million metric tons per year (MPOB, 2018; Mundi, 2018). Palm kernel cake contains 14 to 21% crude protein, which is adequate for older birds (Sundu et al., 2006; Zahari and Alimon, 2005). However, the high fibre content (about 21%), lignin (14%) and non-starch polysaccharides (60%) (Lawal et al., 2010; Sundu et al., 2006) impose a constraint for it to be utilized as poultry feed at a high inclusion rate.

As the main constraint for PKC to be utilized as poultry feed is the presence of fibre, appropriate strategies should be evaluated to alleviate the high fibre content (Sharmila et al., 2014). Upgrading of low quality agricultural waste into high quality products by microbial treatment has been practiced for a long time. The growth of microbes on agro-wastes is able to furnish almost all the hydrolytic enzymes often added in the preparation of feeds. Hence, many studies have been conducted to improve the nutritive values of PKC through solid-state fermentation (SSF) either by using fungi such as *Trichoderma harzianum* (Ramin et al., 2010), *T. longibrachiatum* and *T. koningii* (Iluyemi et al., 2006), *Rhizopus stolonifer* (Lateef et al., 2008), *Aspergillus niger*, *T. varidae* and *Mucor mucedo* (Lawal et al., 2010) or bacterial species such as *Paenibacillus polymyxa* and *P. curdolanolyticus* (Alshelmani et al., 2014).

Many feeding trial studies have been conducted to evaluate the growth performance of birds fed fermented PKC. Alshelmani et al. (2016) reported that solid-state fermentation process was able to reduce the fibre content of PKC and their feeding trial with broilers showed that PKC fermented by *P. polymyxa* ATCC 842 could be fed to broiler chickens only up to 15% without any adverse effect on nutrient digestibility.

A feeding trial conducted by Lawal et al. (2010), showed that the apparent digestibility of nutrients of broilers fed the fermented PKC was improved compared to untreated PKC. In addition, the performance of the birds on fermented PKC was also improved significantly. Similarly, other studies reported positive results where broilers fed diets containing 30% fermented PKC showed no adverse effect on performance, carcass yield and blood constituents (Bello et al, 2011).

Although fermented PKC shows promising potential as an alternative feed ingredient for poultry, but until today, there is no commercially available fermented PKC with improved nutritive values in the market. The fermentation technologies, some of which have been filed (Jing, 2016; Madsen and Pettersson, 2015; Young and Gondipon, 2006) are unable to penetrate the feed market at commercial production scale probably due to cost of operation and time-consuming process as well as the inconsistency of results in the growth performance of broilers when fed fermented PKC.

As biological treatments have considerable drawbacks like long fermentation time (5-14 days), possible production of mycotoxin by fungal activity and formation of inhibitors (Lim et al., 2001), physical treatments should be considered as alternative methods to improve the nutritive of PKC. Physical treatments (soaking and boiling) have been shown to reduce crude fibre content and increase the metabolisable energy value of cowpea seed hulls (Adebiyi et al., 2010). Other physical methods like extrusion could be a possible technique to improve PKC as extrusion method is based on a combination of severe shear force and hydrothermal process with the ability to disrupt the structure of the non-starch polysaccharides (Kumar et al., 2018; Colovic et al., 2016; de Vries et al., 2012) within a short period of time. The extrusion treatment can increase accessible surface area and size of pores, decrease crystallinity and degrees of polymerization of cellulose and mannan polymers in PKC. Hence, in modern feed milling operations, extrusion process has been considered as one of the best processing methods not only to enhance the nutritional content of the feed ingredients but also the efficiency of the feed (Rahman et al., 2015).

Another physical treatment to reduce the fibre content of PKC is the electrostatic separation method where, the difference in electrostatic properties of materials allow the materials to be separated into different components, like separation of proteins from biomass-containing polysaccharides, lignin, and polyphenols (Barakat et al., 2015) and separation of proteins and lignin (Basset et al., 2016). Other applications of electrostatic separation include separation of reusable plastic material from plastic mixture, minerals or ash contents from coal powder, removal of tea stalks and foreign objects from product in food industry and coal cleaning (Higashiyama and Asano, 1998). This method triggers considerable interest to separate the broken shells contaminants, fibre and lignin from the PKC residue.

Problem Statement

Developing a sustainable poultry production requires abundant supply of locally available feed ingredients like agricultural by-products that do not compete with human

consumption. Utilization of PKE as a major feed ingredient in poultry feed could reduce the dependency on imported feed ingredients. However, PKE had to be treated before it could be incorporated in poultry feed to reduce the indigestible mannan and lignin content that reduce nutrient digestibility.

Hypothesis

Physical treatments like extrusion and static cling-electrostatic separation method could reduce the broken shells, fibre, lignin and NSP content of PKE, and thus enhance the nutritive value of PKE and its level of inclusion in the poultry feed.

Objectives

The general objective of this study was to improve the nutritive value of PKE by physical treatments and to evaluate the performance of broiler chickens fed different levels of treated PKE. The specific objectives were:

- i) To enhance the nutritive value of PKE by optimisation of multiple variables of twin screw extrusion process using response surface methodology (RSM).
- ii) To remove broken shells contaminants in PKE by optimisation of multiple variables of static cling - electrostatic separation process using RSM.
- iii) To evaluate the effects of feeding treated PKE on broiler chickens growth performance, serum biochemistry, intestinal histology and gene expression profiles.
- iv) To develop a suitable feed formula based on PKE for broilers with a potential commercial application.

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BIODATA OF STUDENT

Muhamad Akhmal Hakim bin Roslan is a researcher and technopreneur. He is passionate in helping farmers to reduce costs and increase profits via sustainable farming. He holds a Bachelor of Science Degree in Biochemistry from Faculty of Biotechnology and Biomolecular Science at Universiti Putra Malaysia. Before undertaking his doctoral studies in 2012, Akhmal worked as a consultant at Superior Training & Consultancy; since 2010. He is actively involved with seminars & workshops for science subject among school students. In 2013, he was appointed as the editorial assistant for the scientific journal “Bulletin of Environmental Science and Management” and “Nanobio & Bionano” Hibiscus Publisher. He is a co-founder and managing director of Halways Sdn Bhd, an agriculture technology company that focused on empowering farmers to produce food in a sustainable way. He is currently active doing research with his team on the application of probiotics for aquaculture and poultry industries.

LIST OF PUBLICATIONS

Intellectual Property

An apparatus for removing contaminants from palm kernel cake and a method thereof.
Inventor: Norhani Abdullah, Muhamad Akhmal Hakim Roslan, Mohd. Yunus Abd. Shukor and Faridah Hanim Shakirin
IP type: patent
IP status: Patent pending
Application no.: PI 2014703428
Filing date: 17/11/2014

Journal Publications

- Roslan, M. A. H., Abdullah, N., & Mustafa, S. (2015). Removal of shells in palm kernel cake via static cling and electrostatic separation. *Journal of Biochemistry, Microbiology and Biotechnology*, 3(1), 1-6. (Published)
- Roslan, M. A. H., Abdullah, N., Murad, N. Z. A., Halmi, M. I. E., Idrus, Z., & Mustafa, S. (2017). Optimisation of Extrusion for Enhancing the Nutritive Value of Palm Kernel Cake Using Response Surface Methodology. *BioResources*, 12(3), 6679-6697. (Published)

Conference Proceedings

- Roslan, M. A. H., Abdullah, N., Mustafa, S., Halmi, M. I. E. & Shukor, M.Y.A. (2015). *Proceeding of WVPA (Malaysia Branch) and WPSA (Malaysia Branch) Scientific Conference: Enhancing Innovation in Poultry Health and Production, Kuala Lumpur, 2015*. Serdang, Universiti Putra Malaysia



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