



UNIVERSITI PUTRA MALAYSIA

***EFFECT OF FEEDING PALM KERNEL CAKE FERMENTED BY FIBER
DEGRADING BACTERIA ON PERFORMANCE OF BROILER CHICKEN***

MOHAMED M IDRIS ALSHELMANI

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By

MOHAMED M IDRIS ALSHELMANI

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
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Philosophy**

February 2015

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DEDICATION

I would like to dedicate this work to those who taught, motivated and helped me throughout my study. This work is also dedicated to my lovely mother, 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 requirement for the Degree of Doctor of Philosophy

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By

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February 2015

Chairman: Professor Loh Teck Chwen, PhD
Faculty: Agriculture

Malaysia has an abundant amount of palm kernel cake (PKC), which is considered to be an agro-industrial waste after the extraction process of oil from palm fruits. The challenge of using the PKC in the poultry diet is the presence of the high levels of fibers. were conducted to investigate the effect of palm kernel cake fermented Based on the limitation and importance of using the PKC in broiler diets, it appears to be necessary to improve the nutritive value of this by-product by solid state fermentation (SSF) using fiber degrading bacteria. Therefore, four experiments by cellulolytic bacteria (FPKC) on the performance of broiler. The objectives of the first two experiments were to characterize the cellulolytic bacteria in terms of their properties of producing cellulolytic enzymes; in addition, to improve the nutritive value of the PKC via SSF. In addition, a digestibility trial was conducted to investigate the effect of FPKC on crude protein (CP) and amino acids (AA) digestibility on broiler chickens. The fourth experiment was carried out to study the effect of FPKC on broiler growth performance, carcass and meat quality, nutrient digestibility, villi height, gut microflora and blood biochemistry. In the first experiment, nine cellulolytic bacteria were characterized in different substrates; carboxymethyl cellulose (CMC) or xylan from birchwood or locus bean gum (LBG) galactomannan. Results showed that *Bacillus amyloliquefaciens* DSMZ 1067, *Bacillus megaterium* ATCC 9885, *Paenibacillus curdolanolyticus* DSMZ 10248, and *Paenibacillus polymyxa* ATCC 842 produced higher enzymes activities compared to the other bacterial cultures grown in different substrates. In the

second experiment, the PKC was undergone SSF by the four cellulolytic bacteria mentioned earlier. The findings observed that bacterial cultures produced high enzymes activities at the 4th day of SSF, and decreased to zero at the 8th day of SSF. Moreover, the fiber contents were significantly decreased ($P < 0.05$) and the reducing sugars were significantly increased ($P < 0.05$) in FPKC compared with untreated PKC. In the third experiment, a total of 24 broiler chickens were randomly distributed into three treatments: untreated PKC, FPKCa by *P. polymyxa* ATCC 842 and FPKCb by *P. curdolanolyticus* DSMZ 10248. The findings showed that of CP was increased from 16.47% in the untreated PKC to 16.68% and 16.80% in FPKCa and FPKCb, respectively. In addition, the apparent ileal digestibility (AID) of CP and glutamate were significantly ($P < 0.05$) increased in FPKCa compared to the untreated PKC. The rest of AA digestibility were increased in FPKC but not significantly different ($P > 0.05$). In the fourth experiment, a total of 245 one-day-old broiler chicks were randomly distributed to seven dietary treatments containing 0, 5, 10 and 15% PKC and 5, 10 15% FPKC by *P. polymyxa* ATCC 842. The results showed that the addition of 10 or 15% PKC in broiler diets led to a significant ($P < 0.05$) decrease in the growth performance and nutrient digestibility. However, it was significantly ($P < 0.05$) improved in groups of chickens fed with 10 or 15% FPKC compared with those fed with 0 or 15% PKC. The relative weight of the gizzard was significantly ($P < 0.05$) higher for the broiler group fed with 15% PKC compared to those birds fed with the negative control or FPKC at three weeks of age. No significant ($P > 0.05$) differences were observed between the dietary treatments in terms of meat quality, blood biochemistry, villi height and internal organs. Nevertheless, gut microflora were significantly improved ($P < 0.05$) in birds fed with 15% FPKC compared to the other dietary treatments. In conclusion, PKC fermented by *P. polymyxa* ATCC 842 could be fed to broiler chickens up to 15% in their rations without any adverse effects on their growth performance.

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

**KESAN PEMAKANAN ISIRUNG KELAPA SAWIT TERTAPAI OLEH
BAKTERIA PENGURAI SERAT TERHADAP PRESTASI AYAM
PEDAGING**

Oleh

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Malaysia mempunyai sejumlah besar isirung kelapa sawit (IKS) yang dikategorikan sebagai sisa buangan industri sejeurus selesai proses pengekstrakan minyak sawit. Penghalang penggunaan IKS dalam diet ternakan ialah tahap seratnya yang tinggi. Berdasarkan kepada keterbatasan dari segi pengambilan dan kepentingan IKS dalam diet ayam pedaging, maka adalah wajar untuk meningkatkan kandungan nutrisi pada produk sampingan fermentasi pepejal ini dengan menggunakan bakteria pengurai serat. Untuk tujuan tersebut, empat eksperimen telah dijalankan untuk mengenal pasti kesan IKS tertapai (fermented) oleh bakteria cellulolytic ke atas prestasi ayam pedaging. Objektif eksperimen pertama dan kedua adalah untuk mengenal pasti sifat atau ciri-ciri pada bakteria cellulolytic dalam penghasilan enzim. Matlamat lain adalah untuk menambah baik nilai nutrisi pada IKS melalui kaedah fermentasi pepejal. Ujian ketercernaan turut dijalankan bagi mengkaji kesan IKS tertapai terhadap protein kasar (crude protein) dan ketercernaan asid amino terhadap ayam pedaging. Eksperimen keempat dijalankan untuk menganalisis kesan IKS tertapai terhadap prestasi pertumbuhan ayam pedaging, karkas dan kualiti daging, ketercernaan nutrien, ketinggian vilus, mikroflora usus dan biokimia darah. Dalam eksperimen yang pertama, Sembilan bakteria cellulolytic telah dikelaskan kepada beberapa substrat yang berbeza, iaitu carboxymethyl cellulose (CMC) atau xylan daripada birchwood atau locus bean gum (LBG) galactomannan. Dapatan menunjukkan bahawa *Bacillus amyloliquefaciens* DSMZ 1067, *Bacillus megaterium* ATCC 9885, *Paenibacillus curdolanolyticus* DSMZ 10248, dan *Paenibacillus polymyxa* ATCC 842

menghasilkan aktiviti enzim yang lebih tinggi daripada kultur bakteria daripada substrat yang lain. Dalam eksperimen yang kedua, IKS telah melalui proses fermentasi pepejal oleh bakteria cellulolytic. Penemuan daripada eksperimen ini menunjukkan bahawa kultur bakteria menghasilkan aktiviti enzim yang tinggi pada hari keempat proses fermentasi dan menurun pada nilai sifar pada hari kelapan. Tambahan pula, nilai kandungan serat menurun ($P < 0.05$) dan nilai gula meningkat ($P < 0.05$) dalam IKS tertapai berbanding IKS yang tidak dirawat. Dalam eksperimen ketiga, sebanyak 24 ekor ayam pedaging dibahagikan kepada tiga rawatan, iaitu IKS tidak dirawat, IKSa oleh *P. polymyxa* ATCC 842 dan IKSb oleh *P. curdlanolyticus* DSMZ 10248. Hasilnya, protein kasar meningkat daripada 16.47% kepada 16.68% dalam IKS tidak dirawat dan meningkat kepada 16.80% dalam IKSa dan IKSb. Nilai ketercernaan ileum semu (apparent ileal digestibility) pada protein kasar dan glutamate bertambah ($P < 0.05$) dalam IKS tanpa perbezaan yang ketara ($P > 0.05$). Dalam eksperimen keempat, sebanyak 245 ekor ayam pedaging berusia satu hari dibahagikan secara rawak kepada tujuh rawatan diet yang mengandungi 0, 5, 10 dan 15% IKS dan 5, 10 dan 10% IKS tertapai oleh *P. polymyxa* ATCC 842. Dapatan menunjukkan bahawa penambahan 10 atau 15% IKS ke dalam diet ayam pedaging menurunkan ($P < 0.05$) prestasi pertumbuhan dan ketercernaan nutrien. Walau bagaimanapun, nilai ini diperbaiki ($P < 0.05$) dalam kumpulan ayam yang menerima 10 atau 15% IKS tertapai. Berat relatif hempedal ($P < 0.05$) adalah lebih tinggi pada ayam pedaging yang menerima 15% IKS berbanding kumpulan yang mengambil IKS tertapai atau pemakanan tidak terkawal pada usia tiga minggu. Tidak terdapat perbezaan ketara ($P > 0.05$) dikenal pasti pada rawatan pemakanan dari segi kualiti daging, biokimia darah, ketinggian vilus dan organ dalaman, melainkan mikroflora usus yang mengalami perubahan ketara ($P < 0.05$) pada ayam yang menerima 15% IKS tertapai. Kesimpulannya, IKS yang difermentasi oleh *P. polymyxa* ATCC 842 boleh diberikan kepada ayam pedaging sehingga kadar nisbah 15% tanpa sebarang kesan sampingan terhadap prestasi pertumbuhannya.

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I certify that a Thesis Examination Committee has met on 12 February 2015 to conduct the final examination of Mohamed M. Idris Ali Alshelmani on his thesis entitled "Effect of Feeding Palm Kernel Cake Fermented by Fiber Degrading Bacteria on Performance of Broiler Chicken" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the degree of Doctor of Philosophy.

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LIST OF ABBREVIATIONS

PKC	Palm kernel cake
FPKC	Fermented palm kernel cake
ATCC	American Type Culture Collection
DSMZ	Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Culture
SSF	Solid state fermentation
CF	Crude fiber
DM	Dry matter
NDF	Neutral detergent fiber
ADF	Acid detergent fiber
ADL	Acid detergent lignin
EE	Ether extract
CP	Crude protein
NFE	Nitrogen free extract
GE	Gross energy
ME	Metabolizable energy
AME	Apparent metabolizable energy
TME _n	True metabolizable energy nitrogen corrected
NSPs	Non-starch polysaccharides
PKM	Palm kernel meal
MOS	Mannan oligosaccharide
FAO	Food and Agriculture Organization
Glu	Glucose
Gal	Galactose
Man	Mannan
LAB	Lactic acid bacteria
ENT	Enterobacteriaceae
CFU	Colony forming unit
CMC	Carboxymethyl cellulose
LBG	Locust bean gum
RTU	Ready to use
DNS	3,5 dinitrocalysilic acid
dH ₂ O	Distilled water
OD	Optical density
BSA	Bovine serum albumin
CRD	Complete randomized design
GLM	General linear model
SAS	Statistical analysis system
ANOVA	Analysis of variance
CTAB	Acetyl methyl ammonium bromide
OPA	O-phthalaldehyde reagent
FMOC	Fluorenyl methyl chloroformate reagent
GIT	Gastrointestinal tract
HPLC	High performance liquid chromatography
BW	Body weight

BWG	Body weight gain
FI	Feed intake
FCR	Feed conversion ratio
CIE	Commission on illumination
WHC	Water holding capacity
EDTA	Ethelenedimethyl tetra acetic acid
BCG	Bromcresol green
LPL	Lipoprotein lipase
TAG	Triacylglycerol
MRS	De Man ROGOSA and SHAPE
H ₂ SO ₄	Sulfuric acid
HBr	Hydrogen bromide
NaOH	Sodium hydroxide
Na ₂ HPO ₄	Di sodium hydrogen phosphate anhydrous
NaH ₂ PO ₄	Sodium dihydrogen phosphate
Kg	Kilo gram
g	Gram
M	Molar
N	Normal
H ₂ O ₂	Hydrogen peroxide
L	Liter
ml	Milli liter
μl	Micro liter
AID	Apparent ileal digestibility
ISR	Internal standard ratio
SDW	Sample dry weight
VFA	Volatile fatty acids

CHAPTER I

GENERAL INTRODUCTION

The global consumption of poultry products such as meat or eggs, nowadays, is showing an increased trend in the developing countries. On the other hand, the global demand for the main poultry feedstuffs will be increased, especially the protein and energy resources such as soybean meal and yellow corn, respectively. Therefore, the global price of these feedstuffs will be increased. Thus, the cost of poultry diets will definitely be increased as a result of the global demand. The availability of feedstuffs for poultry nutrition nowadays is becoming more competitive. The world's population is expected to increase to 9.1 billion in 2050 (FAO, 2009). Therefore, for human consumption demands for poultry products such as meat and eggs will be increased. In addition, there is an increasing trend to produce biofuel from feedstuffs, especially corn, to meet the deficiency of energy sources all over the world. Thus, the food security, especially in the developing countries will be threatened.

Currently, efforts are being made world over to use alternative sources of protein and energy to be substituted for soybean meal and yellow corn in monogastric animals such as poultry and swine. It is known that some developing countries produce a huge amount of alternative feedstuffs, considered as agro waste by-products such as wheat bran, rice bran, cotton seed meal, copra meal and palm kernel cake. However, many of these agro waste by-products are featuring on presence of non-starch polysaccharides (NSPs) such as xylan and mannan as well as anti-nutritional factors.

The NSPs found to be the main reason for increasing the viscosity in the small intestine of the birds, and hence lead to increased moisture content of the excreta. Thus, the productivity and health status of the chickens could be affected. Therefore, the inclusion of these agro waste by-products in poultry feed are limited.

The global production of palm kernel cake (PKC) has been increased in several tropical countries. It is known that Malaysia is one of the largest worldwide producers of palm oil, and therefore, it produces a huge amount of PKC which is considered as an agro waste by-product.

PKC is the by-product of oil extraction from palm fruits, and it has been used in poultry diets as an alternative of soybean meal. However, the use of PKC is limited in poultry nutrition because of its high content of fibers, coarse texture and gritty appearance (McDonald *et al.*, 1995; O'Mara *et al.*, 1999; Sundu and Dingle, 2002). This is considered as a big problem facing the nutritionists, and has restricted the use of PKC for manipulation of feed formulation. Hence,

there is a need to use other techniques to improve the quality of PKC either with supplementation of exogenous enzymes, or through fermentation of the PKC by cellulolytic microorganisms expecting that these treatments would improve the digestibility and nutritional value. Not only does the fermentation of PKC by microorganisms lead to decrease the crude fiber content, but it would also increase the crude protein as well as amino acids.

The PKC itself may be beneficial to poultry health and production as this by-product contains mannanoligosaccharide (MOS) (Anaeto *et al.*, 2009). The MOS is considered to be a prebiotic that can reduce the pathogenic bacteria as well as increase the health-positive bacteria in the hind gut of the broiler chickens.

Recently, more emphasis is being given to use cellulolytic microorganisms such as fungus or bacteria to produce fermented PKC rather than the supplementation of exogenous enzymes in poultry diets. This technique allows the nutritionists to increase the PKC inclusion levels in poultry diets to improve their performance, and decrease the cost of diets as a result of substitute PKC for soybean meal and yellow corn. This technique is known as solid state (substrate) fermentation (SSF) which enables the cellulolytic microorganisms via their enzymes to breakdown the β -glycosidic bonds that link the cellulose, xylan and mannan in the PKC.

It is known that nutrition account for about 60% to 70% of the total cost in poultry production. The nutritionists attempt to substitute local feedstuffs for soybean meal which is considered to be the main source of protein in feed formulation. Nevertheless, some of local feedstuffs are not acceptable for monogastric animals and therefore used in small proportions in their diets. Malaysia produces abundant amount of PKC, and it could constitute an important by-product feed if it could be successfully exploited by supplementation of exogenous enzymes, or inoculation with cellulolytic microorganisms such as fungus or bacteria to modify the high levels of fibers as well as improving the crude protein. However, the use of fungus in SSF could produce mycotoxins during the fermentation process. Therefore, using cellulolytic bacteria might be more effective to avoid the production of mycotoxins during the fermentation of the PKC.

Objectives

The main objective of this research was to study the effect of feeding fermented PKC (FPKC) produced using cellulolytic bacteria on the performance of broiler chickens.

The specific objectives of this study were:

- To characterize the cellulolytic bacteria on different substrates, and to determine the optimum PKC - moisture ratio during solid state fermentation (SSF).
- To improve the nutritive quality to the PKC by SSF using cellulolytic bacteria.
- To study the effect of FPKC on crude protein and amino acid digestibility.
- To investigate the effect of FPKC on nutrient digestibility, broiler performance, meat quality, abdominal fat, internal organs, blood biochemistry, gut microflora and villi height and crypt depth.

Malaysia has an abundant amount of PKC, which is considered to be an agro-industrial waste after the extraction process of oil from palm fruits. The challenge of using the PKC in the poultry diet is the presence of the high levels of fibers. Based on the limitation and importance of using the PKC in broiler diets, it appears to be necessary to improve the nutritive value of this by-product. Several attempts have been made to degrade the PKC by using solid state fermentation (SSF) in order to improve the nutritive quality.

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