UNIVERSITI PUTRA MALAYSIA

EFFECTS OF PALM KERNEL EXPELLER AND ITS Oligosaccharides ON NUTRIENT ASSIMILATION AND CECAL MICROBIOTA IN BROILER CHICKENS

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By

CHEN WEI LI

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

October 2017
This thesis is dedicated to my lovely parents, and my 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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Chairman: Associate Professor Liang Juan Boo, PhD
Institute: Institute of Tropical Agriculture and Food Security

The attempt to use palm kernel expeller (PKE) as an alternative feed ingredient in broiler chickens has encountered several constraints, including the adverse anti-nutritional effects of PKE on chicken performance caused by its high fiber content. One of the methods proposed to overcome this problem is the use of appropriate enzymes to hydrolyze the fiber component into more soluble sugars which presumably can then be used by the birds. However, studies have shown that enzyme treatment could successfully reduce the fiber contents but when the enzyme treated PKE was fed to the birds, results of birds’ performance were generally not encouraging and inconsistent. It has been hypothesized that the lack of improvement in animal performance when fed with enzyme-treated PKE is due to the poor assimilation of its enzymatic products, which are mainly consisted of mannose monomers.

Apart from direct utilization of nutrient by the animal, gut microbiota were also known to help in harvesting nutrient from the feed and making them available to the host animal. In addition, the mannanoligosaccharides extracted from PKE (OligoPKE) have been reported to be a potential prebiotics owing to its ability in decreasing pathogenic bacteria and improving animal immune system. To date, the effects of PKE on mannose and other nutrient assimilation, changes in microbial diversities and functional properties that could possibly led to the lack of improvement in growth performance despite the increase in the nutritional value of PKE after enzyme treatment remain unclear. Thus, this thesis investigated the effects of supplementing enzyme-treated PKE on growth performance, specifically from the aspects of nutrient assimilation and the diversity and functional potential of cecal microbiome to fill the knowledge gap in this area. Concurrently, the prebiotic potential of OligoPKE was also evaluated.
Results of this study showed that fermenting PKE at 60% initial moisture with 9.0 U/g PKE mannanase at 51°C for 18 h resulted in approximately 40% reduction in the crude fiber, 57% and 22% increase in monosaccharides and oligosaccharides, respectively. Broiler chickens fed with 5% and 20% enzyme-treated PKE, respectively, for starter and finisher periods had no detrimental effect (P>0.05) on the body weight gain, feed intake, and feed conversion ratio (FCR) as compared to control. However, no advantage in term of the above animal performance data was noted between enzyme treated and non-treated PKE.

The capability of broilers to assimilate PKE-derived mannose and other nutrient was evaluated based on the disappearance of mannose from the intestinal tract (digestibility trial) and the expression of mannose absorption transporter (SGLT4). Results indicated that enzyme-treated PKE had significantly higher (P<0.05) apparent mannose digestibility as compared to untreated PKE. In addition, the expression of mannose transporter (SGLT4) was significantly increased (P < 0.05) in all the PKE-based diets, further suggesting that assimilation of mannose were efficient.

Evaluation of the cecal microbiota using the 16S rRNA deep-sequencing analysis showed the replacement of the phylum Bacteroidetes by the phylum Firmicutes in Day-14 broiler and Day-28 broiler fed with treatment diets (untreated PKE, enzyme-treated PKE and OligoPKE). At both ages, the relative abundance of phylum Firmicutes were higher in the untreated PKE as compared to enzyme-treated PKE group, suggesting the higher efficiency of untreated PKE in enhancing cecal microbiota involved in increasing nutrient intake or nutrient absorption as compared to enzyme-treated PKE. In addition, the supplementation of OligoPKE showed to increase (P<0.05) the abundance of Lactobacillus, especially in the 14 day-old broiler chicks. In conclusion, results of the present study showed that despite the positive effect of such pre-treatment on reduction in fiber content, the non-effectiveness of enzyme-treated PKE in improving the growth performance of broiler chickens was not due to the poor assimilation of mannose sugars. Instead, it is possible that the above observation was a result of changes in the cecal microbiota, in which untreated PKE was able to support bacteria involved in increasing nutrient intake, whilst enzyme-treated PKE stimulated growth of group of bacteria that often relates to decrease in nutrient intake.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KESAN ISIRUNG KELAPA SAWIT DAN OLIGOSAKARIDA DARIPADA PKE KEATAS ASIMILASI NUTRISI MAKANAN DAN DIVERSITI MICROBIOTA SEKUM AYAM DAGING

Oleh

CHEN WEI LI

Oktober 2017

Pengerusi: Profesor Madya Liang Juan Boo, PhD
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Usaha dalam menggunakan isirung kelapa sawit (PKE) sebagai makanan alternatif bagi ayam pedaging telah menghadapi beberapa halangan, termasuklah kesan anti-nutrisi terhadap prestasi pertumbuhan ayam yang disebabkan oleh kandungan serat yang tinggi. Salah satu kaedah yang dicadangkan untuk mengatasi masalah tersebut adalah dengan penggunaan enzim yang bersesuaian untuk mengurai serat tersebut kepada gula terlarut yang mungkin boleh digunakan oleh ayam pedaging. Walaupun pelbagai kajian telah berjaya menunjukkan pengurangan kandungan polisakarida bukan kanji (NSP) hasil daripada penggunaan enzim, namun pemberian PKE yang telah dirawat dengan enzim kepada ayam tidak menunjukkan prestasi pertumbuhan yang memberansangkan serta tidak konsisten. Ia telah dicadangkan bahawa pertumbuhan ayam yang kurang memuaskan tersebut mungkin diesebabkan oleh kurangnya asimilasi ayam kepada kebanyakan produk pengsakaridaan PKE oleh enzim, yang kebanyakkannya terdiri daripada monomer manosa.

Selain daripada penggunaan nutrisi makanan secara langsung oleh haiwan, mikrobiom usus juga diketahui umum dalam membantu penuaian nutrisi daripada makanan, lalu menjadikan nutrisi tersebut lebih tersedia kepada haiwan. Sebagai tambahan, manosäoligosakarida yang diekstrak daripada PKE (OligoPKE) telah dilaporkan sebagai prebiotik yang berpotensi dan mempunyai kebolehan untuk mengurangkan bakteria patogenik serta meningkatkan sistem imunisasi. Sehingga kini, kesan asimilasi manosa daripada PKE dan juga nutrisi-nutrisi lain, serta perubahan dalam diversiti mikrobiom dan sifat-sifat fungsi yang mungkin menjejaskan prestasi pertumbuhan ayam walaupun dengan penambahan kandungan nutrisi pada PKE yang dirawat dengan enzim, masih tidak dapat dijelaskan. Dengan itu, tesis ini menyiasat kesan pemberian PKE yang dirawat dengan enzim pada prestasi pertumbuhan, terutamanya dari segi asimilasi nutrisi, diversiti serta sifat-sifat fungsi mikrobiom sekum ayam pedaging, untuk menyumbang ilmu baru dalam bidang ini. Di samping itu, potensi OligoPKE sebagai prebiotik juga dinilai.
Kajian ini menunjukkan bahawa penapaian PKE dengan kelembapan permulaan sebanyak 60%, dengan 9.0 U/g PKE mannanase pada suhu 51 °C selama 18 h boleh mengurangkan lebih kurang 40% serat dalam PKE, serta peningkatan dalam monosakarida dan oligosakarida, masing-masing pada 57% dan 22%. Pemberian PKE yang dirawat dengan enzim pada kadar 5% dalam diet pemulaan dan 20% dalam diet penamatan pada ayam pedaging tidak memberi kesan negatif (P > 0.05) terhadap berat badan, pengambilan makanan, dan nisbah penukaran makanan (FCR) berbanding dengan kumpulan kawalan. Walaupun demikian, ayam pedaging dalam mengasimilasikan manosa yang terhasil daripada PKE, telah dinilai berdasarkan kehilangan manosa dari saluran usus dan juga ekspresi relatif gen translokasi manosa (SGLT4). Hasil kajian menunjukkan bahawa PKE yang dirawat dengan enzim mempunyai penghada manosa yang lebih tinggi (P < 0.05) berbanding dengan kumpulan yang diberi PKE mentah. Sebagai tambahan, ekspresi relatif gen translokasi manosa (SGLT4) telah meningkat secara ketara (P < 0.05) dalam semua kumpulan ayam yang diberi makan berasaskan PKE, lalu mencadangkan bahawa asimilasi manosa adalah cekap. 

Penilaian diversiti mikrobiom sekum menunjukkan penggantian filum Bacteroidetes oleh filum Firmicutes dalam ayam pedaging berumur 14 hari (P < 0.05) dan ayam pedaging berumur 28 hari (P > 0.05). Dalam kedua-dua peringkat umur, populasi filum Firmicutes adalah lebih tinggi dalam kumpulan PKE mentah berbanding kumpulan PKE dengan rawatan enzim, mencadangkan kecekapan yang lebih tinggi pada PKE mentah dalam meningkatkan mikrobiota sekum yang terlibat dalam pengambilan atau penyerapan nutrisi. Di samping itu, pemberian OligoPKE kepada ayam pedaging menunjukkan peningkatan (P < 0.05) dalam populasi Lactobacillus, terutamanya pada ayam pedaging berumur 14 hari. Sebagai konklusi, hasil kajian penyelidikan ini menunjukkan bahawa ketidak-berkesanan PKE dirawat dengan enzim dalam meningkatkan prestasi pertumbuhan ayam pedaging bukanlah disebabkan oleh kurangnya asimilasi gula manosa, walaupun kesan positif dapat dilihat seperti pengurangan kandungan serat dalam PKE hasil rawatan enzim. Sebaliknya, keadaan tersebut mungkin disebabkan oleh perubahan dalam diversiti mikrobiota sekum, di mana PKE mentah dapat menampung bakteria yang terlibat dalam pengambilan nutrisi, manakala PKE dirawat dengan enzim pula merangsang pertumbuhan bakteria yang sering dikaitkan dengan penurunan dalam pengambilan nutrisi.
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I certify that a Thesis Examination Committee has met on 31 October 2017 to conduct the final examination of Chen Wei Li on her thesis entitled "Effects of Palm Kernel Expeller and its Oligosaccharides on Nutrient Assimilation and Cecal Microbiota in Broiler Chickens" 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 Doctor of Philosophy.

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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>i</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>iii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>v</td>
</tr>
<tr>
<td>APPROVALS</td>
<td>vi</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>viii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xiii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xiv</td>
</tr>
<tr>
<td>LIST OF APPENDICES</td>
<td>xy</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xvi</td>
</tr>
<tr>
<td>1  INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>2  LITERATURE REVIEW</td>
<td></td>
</tr>
<tr>
<td>2.1 Palm kernel expeller</td>
<td>3</td>
</tr>
<tr>
<td>2.1.1 PKE in chickens growth</td>
<td>3</td>
</tr>
<tr>
<td>2.1.2 Enzymes used to enhance PKE in poultry diets</td>
<td>3</td>
</tr>
<tr>
<td>2.1.2.1 Type of enzymes used</td>
<td>3</td>
</tr>
<tr>
<td>2.2 Response surface methodology</td>
<td>6</td>
</tr>
<tr>
<td>2.3 Effectiveness of enzyme treatment on PKE</td>
<td>6</td>
</tr>
<tr>
<td>2.4 Intestinal nutrient absorption</td>
<td>7</td>
</tr>
<tr>
<td>2.4.1 Molecular approach in evaluating nutrient absorption</td>
<td>8</td>
</tr>
<tr>
<td>2.4.2 Monosaccharide assimilation transporters</td>
<td>8</td>
</tr>
<tr>
<td>2.4.2.1 Sodium/glucose cotransporter family (SGLT)</td>
<td>8</td>
</tr>
<tr>
<td>2.4.2.2 Glucose transporter family (GLUT)</td>
<td>9</td>
</tr>
<tr>
<td>2.4.3 Protein assimilation transporters</td>
<td>10</td>
</tr>
<tr>
<td>2.4.3.1 Peptide transporter 1 (PepT1)</td>
<td>10</td>
</tr>
<tr>
<td>2.4.3.2 Excitatory amino acid transporter 3 (EAAT3)</td>
<td>11</td>
</tr>
<tr>
<td>2.4.3.3 Peptide uptake versus free amino acids</td>
<td>11</td>
</tr>
<tr>
<td>2.4.4 Peroxisome proliferator-activated receptor (PPAR)</td>
<td>11</td>
</tr>
<tr>
<td>2.5 Prebiotic</td>
<td>12</td>
</tr>
<tr>
<td>2.5.1 Mannanoligosaccharides (MOS)</td>
<td>13</td>
</tr>
<tr>
<td>2.5.2 PKE as source of MOS</td>
<td>14</td>
</tr>
<tr>
<td>2.6 Next-generation sequencing (NGS) technology</td>
<td>14</td>
</tr>
<tr>
<td>2.6.1 Predictive functional profiling of microbial communities</td>
<td>15</td>
</tr>
<tr>
<td>2.7 Summary</td>
<td>16</td>
</tr>
</tbody>
</table>
### OPTIMIZATION OF ENZYMATIC HYDROLYSIS OF PALM KERNEL EXPELLER

3.1 Introduction 17
3.2 Materials and methods 18
3.2.1 Source of Enzyme 18
3.2.2 Production of crude enzyme by *Aspergillus terreus* K1 18
3.2.3 Measurement of mannanase activity 19
3.2.4 Optimization of enzymatic hydrolysis of PKE 19
3.2.4.1 Experimental design 19
3.2.4.2 Enzymatic hydrolysis of PKE 20
3.2.4.3 Determination of monosaccharide and oligosaccharides production 22
3.2.4.4 Determination of crude fiber reduction 22
3.2.4.5 Verification of predicted condition 22
3.2.5 Determination of PKE inclusion level in broiler chickens 23
3.2.5.1 Chemical compositions of PKE and enzyme-treated PKE 23
3.2.5.2 Experimental animals and management 23
3.2.5.3 Statistical analysis 23
3.3 Results 25
3.3.1 Optimization of enzymatic hydrolysis 25
3.3.2 Effect of different PKE inclusion levels on performance of broiler chickens 30
3.4 Discussion 32
3.5 Conclusion 34

### INTESTINAL DIGESTIBILITY AND ABSORPTION OF FUNCTIONAL SUGARS FROM PALM KERNEL EXPELLER

4.1 Introduction 35
4.2 Materials and methods 36
4.2.1 Preparation of enzyme-treated PKE and OligoPKE 36
4.2.2 Monosaccharides and Oligosaccharides of PKE 37
4.2.3 Chickens and their management 37
4.2.4 Sample collections 37
4.2.5 Determination of functional sugar digestibility 39
4.2.5.1 Determination of titanium dioxide concentration 39
4.2.5.2 NDF preparation 39
4.2.5.3 Determination of monosaccharides 39
4.2.6 Nutrient absorption gene expression 40
4.2.6.1 Isolation of intestinal tissues total RNA 40
4.2.6.2 Qualitative real-time PCR of samples 40
4.2.7 Statistical Analysis 42
4.3 Results 42
4.3.1 Growth performance 42
4.3.2 Effect of PKE on digestibility of NDF and functional sugars 44
4.3.3 Effect of PKE on nutrient assimilation genes 45
4.3.3.1 Monosaccharides transporter genes 45
4.3.3.2 Protein assimilation transporter 48
4.3.3.3 Peroxisome Proliferator Activated Receptors, PPAR 49
4.4 Discussion 50
4.5 Conclusion 54
5 EVALUATION OF CECAL MICROBIAL COMMUNITY IN RESPONSE TO OIL PALM BY-PRODUCTS BASED ON HIGH-THROUGHPUT SEQUENCING OF 16S rRNA GENE AMPLICON

5.1 Introduction 56
5.2 Material and methods 57
  5.2.1 Cecal content collection 57
  5.2.2 Isolation of bacterial genomic DNA 57
  5.2.3 PCR amplification of V3-V4 region of 16S rRNA gene 58
  5.2.4 Bioinformatics analysis 58
  5.2.5 Quality trimming and merging of Illumina pair-end reads 58
  5.2.6 OTU picking and taxonomy assignment 58
  5.2.7 Statistical analysis 59
  5.2.8 Nucleotide sequence accession numbers 59
  5.2.9 Metagenome prediction 59
5.3 Results 60
  5.3.1 Sequencing data analysis 60
  5.3.2 Dietary effect on cecal microbial diversity 60
  5.3.3 Dietary alteration on cecal bacterial diversity 63
    5.3.3.1 Bacterial communities in control group 64
    5.3.3.2 Comparison of bacterial communities between treatments 66
  5.3.4 Predictive functional metagenome of broiler cecal microbiota 67
5.4 Discussion 69
5.5 Conclusion 74

6 GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS FOR FUTURE RESEARCH
6.1 General Discussion 76
6.2 Conclusion 78
6.3 Recommendations for future research 79

REFERENCES 80
APPENDICES 102
BIODATA OF STUDENT 107
LIST OF PUBLICATION 108
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1 Chemical and nutrient compositions of PKE</td>
<td>4</td>
</tr>
<tr>
<td>2.2 Research using different approaches to improve PKE utilization in broilers</td>
<td>5</td>
</tr>
<tr>
<td>3.1 Coded values of variables used in central composite design</td>
<td>20</td>
</tr>
<tr>
<td>3.2 Experimental design for central composite design</td>
<td>21</td>
</tr>
<tr>
<td>3.3 Compositions of the dietary treatments</td>
<td>24</td>
</tr>
<tr>
<td>3.4 Central composite design with experimental and predicted values of enzymatic treatment of PKE</td>
<td>26</td>
</tr>
<tr>
<td>3.5 Analysis of variance (ANOVA) table for monosaccharides production</td>
<td>27</td>
</tr>
<tr>
<td>3.6 Analysis of variance (ANOVA) table for oligosaccharides production</td>
<td>27</td>
</tr>
<tr>
<td>3.7 Analysis of variance (ANOVA) table for crude fiber reduction</td>
<td>28</td>
</tr>
<tr>
<td>3.8 Predicted optimal conditions for monosaccharides production, oligosaccharides production and crude fiber (CF) reduction</td>
<td>29</td>
</tr>
<tr>
<td>3.9 Proximate analysis of untreated PKE and enzyme treated PKE</td>
<td>30</td>
</tr>
<tr>
<td>3.10 Growth performance of chicken fed with different dietary combination treatments</td>
<td>31</td>
</tr>
<tr>
<td>4.1 Compositions of the dietary treatments</td>
<td>38</td>
</tr>
<tr>
<td>4.2 Primer sequences (5’→3’) for nutrient absorption genes</td>
<td>41</td>
</tr>
<tr>
<td>4.3 Growth performance of chicken fed with different dietary combination treatments</td>
<td>43</td>
</tr>
<tr>
<td>4.4 Composition of monosaccharides and oligosaccharides of different PKE products</td>
<td>44</td>
</tr>
<tr>
<td>4.5 Analyzed digestibility of NDF and constituent monosaccharides in starter and grower diets (g/kg)</td>
<td>45</td>
</tr>
<tr>
<td>5.1 Analysis of bacterial diversity in broiler’s cecal exposed to different dietary treatment at 14 and 28 day-old broiler</td>
<td>62</td>
</tr>
</tbody>
</table>
### LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Contour plot showing the interaction between moisture and enzyme concentration when other two variables are hold at center point.</td>
<td>29</td>
</tr>
<tr>
<td>4.1</td>
<td>The effect of different dietary treatments on the hexose transporter gene expression in broiler chickens.</td>
<td>47</td>
</tr>
<tr>
<td>4.2</td>
<td>The effect of different dietary treatments on the protein transporter gene expression in broiler chickens.</td>
<td>48</td>
</tr>
<tr>
<td>4.3</td>
<td>The effect of different dietary treatments on the PPARs gene expression in broiler chickens.</td>
<td>49</td>
</tr>
<tr>
<td>5.1</td>
<td>Rarefaction curves of cecal bacteria communities based on the V3-V4 16S rRNA gene sequences of different treatment groups.</td>
<td>61</td>
</tr>
<tr>
<td>5.2</td>
<td>Principal Coordinate Analysis (PCoA) plots of beta diversity.</td>
<td>63</td>
</tr>
<tr>
<td>5.3</td>
<td>Relative abundance of cecal bacteria at phylum level.</td>
<td>64</td>
</tr>
<tr>
<td>5.4</td>
<td>Relative abundance of cecal bacteria at class, order and family level.</td>
<td>65</td>
</tr>
<tr>
<td>5.5</td>
<td>Relative abundance of cecal bacteria at genus level.</td>
<td>66</td>
</tr>
<tr>
<td>5.6</td>
<td>Differences in predicted functional metagenomes classified into first level of KEGG orthologs (KO).</td>
<td>67</td>
</tr>
<tr>
<td>5.7</td>
<td>Predicted functional metagenomes within the metabolism category in cecal sample of D14 and D28 broilers fed with different dietary treatment.</td>
<td>68</td>
</tr>
</tbody>
</table>
LIST OF APPENDICES

<table>
<thead>
<tr>
<th>Appendix</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Standard curve of Mannanase</td>
<td>102</td>
</tr>
<tr>
<td>2 Relative gene expression of nutrient assimilation genes normalized to control group chickens fed different dietary treatments</td>
<td>103</td>
</tr>
<tr>
<td>3 Illustration of the cecal bacterial communities of broiler chickens reported based on the relative abundance of control treatment (corn-soybean based diets) visualized using the Krona Interactive Hierarchical Browser</td>
<td>105</td>
</tr>
<tr>
<td>4 PCOA plots for KEGG Orthologs</td>
<td>106</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>°C</td>
<td>Degrees centigrade</td>
</tr>
<tr>
<td>×g</td>
<td>Relative centrifugal force</td>
</tr>
<tr>
<td>µM</td>
<td>Milimolar</td>
</tr>
<tr>
<td>A. terreus</td>
<td>Aspergillus terreus</td>
</tr>
<tr>
<td>AA</td>
<td>Amino acids</td>
</tr>
<tr>
<td>ADG</td>
<td>Average daily gain</td>
</tr>
<tr>
<td>AME</td>
<td>Apparent metabolisable energy</td>
</tr>
<tr>
<td>bp</td>
<td>Basepair</td>
</tr>
<tr>
<td>BW</td>
<td>Body weight</td>
</tr>
<tr>
<td>BWG</td>
<td>Body weight gain</td>
</tr>
<tr>
<td>CCD</td>
<td>Central composite design</td>
</tr>
<tr>
<td>CF</td>
<td>Crude Fiber</td>
</tr>
<tr>
<td>cm</td>
<td>Centimeter</td>
</tr>
<tr>
<td>CP</td>
<td>Crude protein</td>
</tr>
<tr>
<td>CPT1</td>
<td>Carnitine palmitoyltransferase1,</td>
</tr>
<tr>
<td>Cq</td>
<td>Quantitative cycle</td>
</tr>
<tr>
<td>DM</td>
<td>Dry matter</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>EAA</td>
<td>Excitatory amino acid transporter</td>
</tr>
<tr>
<td>EE</td>
<td>Ether extract</td>
</tr>
<tr>
<td>EPKE</td>
<td>Enzyme-treated PKE</td>
</tr>
<tr>
<td>FATP</td>
<td>Fatty acid transport protein</td>
</tr>
<tr>
<td>FCR</td>
<td>Feed conversion ratio</td>
</tr>
<tr>
<td>FI</td>
<td>Feed intake</td>
</tr>
<tr>
<td>g</td>
<td>Gram</td>
</tr>
<tr>
<td>GAPDH</td>
<td>Glyceraldehyde-3-Phosphate Dehydrogenase</td>
</tr>
<tr>
<td>GB</td>
<td>Gigabyte</td>
</tr>
<tr>
<td>GE</td>
<td>Gross energy</td>
</tr>
<tr>
<td>GLUT</td>
<td>Glucose transporter</td>
</tr>
<tr>
<td>h</td>
<td>Hour</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>HT-NGS</td>
<td>High throughput next generation sequencing</td>
</tr>
<tr>
<td>KEGG</td>
<td>Kyoto Encyclopedia of Genes and Genomes</td>
</tr>
<tr>
<td>kg</td>
<td>Kilogram</td>
</tr>
<tr>
<td>L</td>
<td>Liter</td>
</tr>
<tr>
<td>LAB</td>
<td><em>Lactobacillus</em>, LAB</td>
</tr>
<tr>
<td>M</td>
<td>Molar</td>
</tr>
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<td>ME</td>
<td>Metabolisable energy</td>
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</tr>
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<td>Minutes</td>
</tr>
<tr>
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<td>Megajoules</td>
</tr>
<tr>
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<td>Milliliter</td>
</tr>
<tr>
<td>mm</td>
<td>Milimeter</td>
</tr>
<tr>
<td>MOS</td>
<td>Mannanoligosaccharides</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger RNA</td>
</tr>
<tr>
<td>NDF</td>
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</tr>
<tr>
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<td>Next-generation sequencing</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>-------------</td>
</tr>
<tr>
<td>NSP</td>
<td>Non-starch polysaccharides</td>
</tr>
<tr>
<td>NTC</td>
<td>No-template control</td>
</tr>
<tr>
<td>OligoPKE</td>
<td>Oligosaccharides from PKE</td>
</tr>
<tr>
<td>PANDAseq</td>
<td>PAired-eND Assembler for DNA sequences</td>
</tr>
<tr>
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<td>Polymerase chain reaction</td>
</tr>
<tr>
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<td>Potato dextrose agar</td>
</tr>
<tr>
<td>PEPT</td>
<td>Peptide transporter</td>
</tr>
<tr>
<td>PICRUSt</td>
<td>Phylogenetic investigation of communities by reconstruction of unobserved states</td>
</tr>
<tr>
<td>PKE</td>
<td>Palm kernel expeller</td>
</tr>
<tr>
<td>PPAR</td>
<td>Peroxisome proliferator-activated receptor</td>
</tr>
<tr>
<td>PyNAST</td>
<td>Python Nearest Alignment Space Termination</td>
</tr>
<tr>
<td>QIIME</td>
<td>Quantitative Insights Into Microbial Ecology</td>
</tr>
<tr>
<td>qPCR</td>
<td>Quantitative polymerase chain reaction</td>
</tr>
<tr>
<td>R2</td>
<td>Correlation coefficient values</td>
</tr>
<tr>
<td>RAM</td>
<td>Random access memory</td>
</tr>
<tr>
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<td>Ribonucleic acid</td>
</tr>
<tr>
<td>rRNA</td>
<td>Ribosomal RNA</td>
</tr>
<tr>
<td>RSM</td>
<td>Response surface methodology</td>
</tr>
<tr>
<td>RT-PCT</td>
<td>Real-time PCR</td>
</tr>
<tr>
<td>s</td>
<td>Second</td>
</tr>
<tr>
<td>SCFA</td>
<td>Short chain fatty acid</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error of mean</td>
</tr>
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<td>Sodium-glucose cotransporter member</td>
</tr>
<tr>
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<td>Solute carrier</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical Package for the Social Science</td>
</tr>
<tr>
<td>SSF</td>
<td>Solid state fermentation</td>
</tr>
<tr>
<td>STAMP</td>
<td>Statistical Analysis of Metagenomic Profiles</td>
</tr>
<tr>
<td>TME</td>
<td>True Metabolisable energy</td>
</tr>
<tr>
<td>TZD</td>
<td>Thiazolidinedione</td>
</tr>
<tr>
<td>U</td>
<td>Enzyme unit</td>
</tr>
<tr>
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<td>Untreated PKE</td>
</tr>
<tr>
<td>μL</td>
<td>Microliter</td>
</tr>
</tbody>
</table>
CHAPTER 1

INTRODUCTION

Rapid expansion in the livestock industry to meet the rising demand for meat and animal products have led to direct competition in the use of traditional feed grains such as soybean and corn in animal feed and human nutrition (Vasta et al., 2008). This has led to the attempt to use various agricultural by-products as replacement to conventional feed resource. One of the by-products is palm kernel expeller (PKE), which is being produced massively (2.2 million tons annually) in Malaysia. However, the use of PKE in broilers production is often limited by its high fiber content, mainly in the form of highly crystalline and insoluble mannan hemicellulose. The above problem had led to various researches, most of which involve the use of fungal culture and/or commercial enzymes, focusing on the alleviation of the undesirable properties of PKE (Iyayi and Davies, 2005; Saenphoom et al., 2011; Saenphoom et al., 2013). Despite the nutritional improvement of PKE as the results of enzyme treatment (Saenphoom et al., 2011, Saenphoom et al., 2013, Hanafiah et al., 2017), no enhancement in growth performance of broiler chickens fed enzyme-treated PKE as compared to those fed untreated PKE was observed, and the maximum inclusion level of both treated and non-treated PKE was capped at 20% (Saenphoom et al., 2013). The authors suggested that although enzyme treatment was able to hydrolyze the NSP into its simpler sugar derivatives, the chickens were not able to utilize them presumably because these sugars were in the form of mannose monomers or mannose polymers, which have been shown to be absorb at a slower rate compared to glucose, galactose, fructose and xylose (Wilson and Vincent, 1955; Denbow, 2000).

To date, there is little study conducted on the mannose assimilation capability in broiler chickens. Using insoluble marker, Jamroz et al. (2002) reported that chickens fed high amount of barley have low apparent mannose digestibility (10%), with high amount of soluble mannose remain unabsorbed and retained in the intestinal tracts. In contrast, gene expression studies of mannose transporter bound to avian enterocytes (Cano et al., 2002; Duran et al., 2004), and in vivo studies using anesthetized rats (Alton et al., 1997) indicated otherwise. These authors showed that mannose was being absorbed rapidly in the intestine through two mannose transport systems, namely the passive and sodium-dependent active transports.

Apart from the direct assimilation of dietary nutrient by the host, gut microbiota have been shown to affect nutrient digestion, absorption, and energy metabolism (Forder et al., 2007; Klasing, 2007). Microbial fermentation by-products such as the short chain fatty acid (SCFA) have been estimated to contribute at least 3.5 to 10 % of metabolizable energy (ME) in poultry (Jamroz et al., 2002; Jozefiak et al., 2004). In addition to their contribution towards energy metabolism, PKE also represents a potential source for prebiotics production. The earlier report of lower mortality rates in broiler chickens fed with enzyme-treated PKE (Saenphoom et al., 2013) led to further studies and the development of the PKE-extract called OligoPKE (Jahromi et
which has been shown to support growth of specific strain of *Lactobacillus* (Chen *et al.*, 2015; Jahromi *et al.*, 2016) in *in vitro* study. However, results of *in vivo* feeding of OligoPKE showed a decrease in pathogenic bacteria (Chen *et al.*, 2015; Jahromi *et al.*, 2016) with no significant increase in beneficial bacterial.

Up to now, mannose and other nutrient assimilation, as well as the changes in microbial diversities that could possibly led to the lack of improvement in growth performance despite the increase in the nutritional value of PKE after enzyme treatment remain unclear. It is hypothesized that this were due to poor assimilation of mannose, and the effect of nutrient assimilation caused by changes in microbial diversity in broilers fed with untreated PKE and enzyme-treated PKE.

Therefore, this thesis aims to investigate the effects of inclusion of untreated- and enzyme-treated PKE on mannose and nutrient assimilation and changes in cecal microbiota. The specific objectives of this study were:

1. To optimize the enzymatic hydrolysis of PKE using a crude enzyme produced by *Aspergillus terreus* K1 by using response surface methodology,

2. To determine the optimal inclusion level of enzyme-treated PKE in broiler’s diet,

3. To determine the effect of feeding enzyme-treated PKE and PKE-extract (OligoPKE) on growth performance of broilers,

4. To evaluate the effect of feeding untreated PKE, enzyme-treated PKE and OligoPKE on the apparent functional sugars digestibility, and expression of nutrient assimilation genes in broiler chickens,

5. To investigate the effects of untreated PKE, enzyme-treated PKE and OligoPKE on cecal microbial community as well as the functional potential of these bacteria.
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