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

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

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

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DEDICATION

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

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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 diggunakan oleh ayam pedaging. Walaupun pelbagai kajian telah berjaya menunjukkan pengurangkan 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 pengunaan 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, manosaoligosakarida 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. Walaubagaimanapun, tiada perbezaan pada prestasi pertumbuhan ayam antara kumpulan rawatan ezim dan kumpulan tanpa rawatan enzim. Keupayaan 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 penghadaman 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 mannosa adalah cekap.

Penilaian diversiti mikrobiom sekum menunjukkan penggantian filum *Bacteroidetes* oleh filum *Firmicutes* dalam ayam pedaging berumur 14 hari ($P < 0.05$) dan ayam daging 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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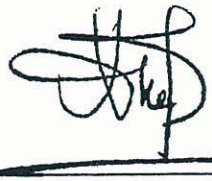

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LIST OF ABBREVIATIONS

°C	Degrees centigrade
×g	Relative centrifugal force
µM	Milimolar
A. terreus	Aspergillus terreus
AA	Amino acids
ADG	Average daily gain
AME	Apparent metabolisable energy
bp	Basepair
BW	Body weight
BWG	Body weight gain
CCD	Central composite design
CF	Crude Fiber
cm	Centimeter
CP	Crude protein
CPT1	Carnitine palmitoyltransferase1,
Cq	Quantitative cycle
DM	Dry matter
DNA	Deoxyribonucleic acid
EAAT	Excitatory amino acid transporter
EE	Ether extract
EPKE	Enzyme-treated PKE
FATP	Fatty acid transport protein
FCR	Feed conversion ratio
FI	feed intake
g	Gram
GAPDH	Glyceraldehyde-3-Phosphate Dehydrogenase
GB	Gigabyte
GE	Gross energy
GLUT	Glucose transporter
h	Hour
HPLC	High performance liquid chromatography
HT-NGS	High throughput next generation sequencing
KEGG	Kyoto Encyclopedia of Genes and Genomes
kg	Kilogram
L	Liter
LAB	*Lactobacillus*, LAB
M	Molar
ME	Metabolisable energy
Mg	Milligram
min	Minutes
MJ	Megajoules
mL	Milliliter
mm	Milimeter
MOS	Mannanoligosaccharides
mRNA	Messenger RNA
NDF	Neutral detergent fiber
NGS	Next-generation sequencing

NSP	Non-starch polysaccharides
NTC	No-template control
OligoPKE	Oligosaccharides from PKE
PANDAseq	PAired-eND Assembler for DNA sequences
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
PEPT	Peptide transporter
PICRUSt	Phylogenetic investigation of communities by reconstruction of unobserved states
PKE	Palm kernel expeller
PPAR	Peroxisome proliferator-activated receptor
PyNAST	Python Nearest Alignment Space Termination
QIIME	Quantitative Insights Into Microbial Ecology
qPCR	Quantitative polymerase chain reaction
R2	Correlation coefficient values
RAM	Random access memory
RNA	Ribonucleic acid
rRNA	Ribosomal RNA
RSM	Response surface methodology
RT-PCT	Real-time PCR
s	Second
SCFA	Short chain fatty acid
SD	Standard deviation
SEM	Standard error of mean
SGLT	Sodium-glucose cotransporter member
SLC	Solute carrier
SPSS	Statistical Package for the Social Science
SSF	Solid state fermentation
STAMP	Statistical Analysis of Metagenomic Profiles
TME	True Metabolisable energy
TZD	Thiazolidinedione
U	Enzyme unit
UPKE	Untreated PKE
µL	Microliter

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*

al., 2016) 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.

REFERENCES

Abdollahi, M.R., Hosking, B.J., Ning, D. and Ravindran, V., 2016. Influence of Palm Kernel Meal Inclusion and Exogenous Enzyme Supplementation on Growth Performance, Energy Utilization, and Nutrient Digestibility in Young Broilers. *Asian-Australasian Journal of Animal Sciences*, 29(4): 539-548.

Ahmadian, M., Suh, J.M., Hah, N., Liddle, C., Atkins, A.R., Downes, M. and Evans, R.M., 2013. PPARγ signaling and metabolism: the good, the bad and the future. *Nature Medicine*, 99(5): 557-566.

Ahmed, A., Zulkifli, I., Farjam, A.S., Abdullah, N. and Liang, J.B., 2014. Extrusion enhances metabolizable energy and ileal amino acids digestibility of canola meal for broiler chickens. *Italian Journal of Animal Science*, 13(1): 3032.

Akpodiete, O.J., 2008. Performance response and egg qualities of laying birds fed enzyme supplemented palm kernel cake, PKC based diets. *Animal Research International*, 5(1): 819-822.

Alimon, A.R., 2004. The nutritive value of palm kernel cake for animal feed. *Palm Oil Developments*. 40(1): 12-14.

Alshelmani, M.I., Loh, T.C., Foo, H.L., Sazili, A.Q. and Lau, W.H., 2016. Effect of feeding different levels of palm kernel cake fermented by *Paenibacillus polymyxa* ATCC 842 on nutrient digestibility, intestinal morphology, and gut microflora in broiler chickens. *Animal Feed Science and Technology*, 216(June): 216-224.

Alton, G., Hasilik, M., Niehues, R., Panneerselvam, K., Etchison, J.R., Fana, F. and Freeze, H.H., 1998. Direct utilization of mannose for mammalian glycoprotein biosynthesis. *Glycobiology*, 8(3): 285-295.

AOAC. 1990. Official Method of Analysis. *Association of Official Analytical Chemists*. Washington, D.C.

Apajalahti, J., Kettunen, A. and Graham, H., 2004. Characteristics of the gastrointestinal microbial communities, with special reference to the chicken. *Would's Poultry Science Journal*. 60(2): 223-232.

Arndt, D., Xia, J., Liu, Y., Zhou, Y., Guo, A.C., Cruz, J.A., Sinelnikov, I., Budwill, K., Nesbe, C.L. and Wishart, D.S., 2012. METAGENassist: a comprehensive web server for comparative metagenomics. *Nucleic Acids Research*, 40: W88-W95.

Atil, O., Roslan, J., Subakir, F.N.M., Razak, R.A.A. and Mohamed, M., 2009. MPOB-Q-PKMTM. *MPOB Information Series*. 440.

Axford, J., 2001. The impact of glycobiology on medicine. *Trends in Immunology*, 22(5): 237-239.

Azari, E.K., Leitner, C., Jaggi, T., Langhans, W. and Mansouri, A., 2013. Possible role of intestinal fatty acid oxidation in the eating-inhibitory effect of the PPAR-α agonist Wy-14643 in high-fat diet fed rats. *PloS one*, 8(9): e74869.

Baurhoo, B., Ferket, P., Ashwell, C.M., de Oliviera, J. and Zhao, X., 2012. Cell walls of Saccharomyces cerevisiae differentially modulated innate immunity and glucose metabolism during late systemic inflammation. *PloS one*, 7(1): e30323.

Bello, K.M., Oyawoye, E.O., Bogoro, S.E. and Dass, U.D., 2011. Performance of broilers fed varying levels of palm kernel cake. *International Journal of Poultry Science*, 10(4): 290-294.

Bervoets, L., Van Hoorenbeeck, K., Kortleven, I., Van Noten, C., Hens, N., Vael, C., Goossens, H., Desager, K.N. and Vankerckhoven, V., 2013. Differences in gut microbiota composition between obese and lean children: a cross-sectional study. *Gut pathogens*, 5(1): 10.

Biddle, A., Stewart, L., Blanchard, J. and Leschine, S., 2013. Untangling the genetic basis of fibrolytic specialization by *Lachnospiraceae* and *Ruminococcaceae* in diverse gut communities. *Diversity*, 5(3): 627-640.

Bjerrum, L., Engberg, R.M., Leser, T.D., Jensen, B.B., Finster, K. and Pedersen, K., 2006. Microbial community composition of the ileum and cecum of broiler chickens as revealed by molecular and culture-based techniques. *Poultry Science*, 85(7): 1151-1164.

Boateng, M., Okai, D.B., Baah, J. and Donkoh, A. 2010. The chemical composition of palm kernel cake, PKC in Ghana as influenced by extraction process. Retrieved 5 March 2017 from http://www.docstoc.com/docs/27151127/37-THE-CHEMICAL-COMPOSITION-OF-PALM-KERNEL-CAKE-,PKC

Boateng, M., Okai, D.B., Baah, J. and Donkoh, A., 2008. Palm kernel cake extraction and utilisation in pig and poultry diets in Ghana. *Livestock Research For Rural Development*, 20(7): 2008.

Bomar, L., Maltz, M., Colston, S. and Graf, J., 2011. Directed culturing of microorganisms using metatranscriptomics. *MBio*, 2(2): e00012-11.

Bonugli-Santos, R.C., Durrant, L.R., da Silva, M. and Sette, L.D., 2010. Production of laccase, manganese peroxidase and lignin peroxidase by Brazilian marine-derived fungi. *Enzyme and Microbial Technology*, 46(1): 32-37.

Bosch, H.M.V., Bunger, M., de Groot, P.J., Bosch-Vermeulen, H., Hooiveld, G.J. and Müller, M., 2008. PPARalpha-mediated effects of dietary lipids on intestinal barrier gene expression. *Bmc Genomics*, 9(1): 231.

Boyaci, I.H., 2005. A new approach for determination of enzyme kinetic constants using response surface methodology. *Biochemical Engineering Journal*, 25(1): 55-62.

Brandt, J.M., Djouadi, F. and Kelly, D.P., 1998. Fatty acids activate transcription of the muscle carnitine palmitoyltransferase I gene in cardiac myocytes via the peroxisome proliferator-activated receptor α. *Journal of Biological Chemistry*, 273(37): 23786-23792.

Buddington, R.K., and Diamond, J.M., 1989. Ontogenetic development of intestinal nutrient transporters. *Annual Review of Physiology*, 51(1): 601-617.

Buddington, R.K., Buddington, K.K. and Sunvold, G.D., 1999. Influence of fermentable fiber on small intestinal dimensions and transport of glucose and proline in dogs. *American journal of veterinary research*, 60(3): 354-358.

Byers, M.S., Howard, C. and Wang, X., 2017. Avian and Mammalian Facilitative Glucose Transporters. *Microarrays*, 6(2): 7.

Cai, L., Ye, L., Tong, A.H.Y., Lok, S. and Zhang, T., 2013. Biased diversity metrics revealed by bacterial 16S pyrotags derived from different primer sets. *PLoS One*, 8(1): e53649.

Canfora, E.E., Jocken, J.W. and Blaak, E.E., 2015. Short-chain fatty acids in control of body weight and insulin sensitivity. *Nature Reviews Endocrinology*, 11(10): 577.

Cano, M., Calonge, M.L., Peral, M.J. and Ilundain, A.A., 2001. A Na+-dependent D-mannose transporter in the apical membrane of chicken small intestine epithelial cells. *Pflügers Archiv European Journal of Physiology*, 441(5): 686-691.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I. and Huttley, G.A., 2010. QIIME allows analysis of high-throughput community sequencing data. *Nature Methods*, 7(5): 335-336.

Cardenas, E. and Tiedje, J.M., 2008. New tools for discovering and characterizing microbial diversity. *Current Opinion in Biotechnology*, 19(6): 544-549.

Carvalho, L.P.F., Melo, D.S.P., Pereira, C.R.M., Rodrigues, M.A.M., Cabrita, A.R.J. and Fonseca, A.J.M., 2005. Chemical composition, in vivo digestibility, N degradability and enzymatic intestinal digestibility of five protein supplements. *Animal Feed Science and Technology*, 119(1): 171-178.

Chen, H., Pan, Y., Wong, E.A. and Webb, K.E., 2005. Dietary protein level and stage of development affect expression of an intestinal peptide transporter, cPepT1 in chickens. *The Journal of Nutrition*, 135(2): 193-198.

Chen, W.L., Liang, J.B., Jahromi, M.F., Abdullah, N., Ho, Y.W. and Tufarelli, V., 2015. Enzyme treatment enhances release of prebiotic oligosaccharides from palm kernel expeller. *BioResources,* 10(1): 196-209.

Chen, W.L., Liang, J.B., Jahromi, M.F., Ho, Y.W. and Abdullah, N., 2013. Optimization of multi-enzyme production by fungi isolated from palm kernel expeller using response surface methodology. *BioResources*, 8(3): 3844-3857.

Chong, C.H., Zulkifli, I., Blair, R. and Jelan, Z.A., 2003. Effects of dietary inclusion of palm kernel cake and enzyme supplementation on performance and metabolisable energy in broiler chickens. *Malaysian Journal of Animal Science* 8(1): 59-69.

Cobb500,2015. *Broiler performance and nutrition supplement*. Cobb-vantress Inc., Arkansas, USA.

Conejos, J.R.V., Acda, S.P., Capitan, S.S., Agbisit, E.M. and Merca, F.E., 2013. Mannan oligosaccharides from Yeast (*Saccharomyces cerevisiae*) cell wall improves nutrient digestibility and intestinal morphology of growing pigs [Sus domesticus (Erxleben)]. *The Philippine Agricultural Scientist*, 95(3): 305-311.

Corrigan, A., de Leeuw, M., Penaud-Frézet, S., Dimova, D. and Murphy, R.A., 2015. Phylogenetic and functional alterations in bacterial community compositions in broiler ceca as a result of mannan oligosaccharide supplementation. *Applied and environmental microbiology*, 81(10): 3460-3470.

Corrigan, A., Horgan, K., Clipson, N. and Murphy, R.A., 2012. Effect of dietary prebiotic, mannan oligosaccharide supplementation on the cecal bacterial community structure of turkeys. *Microbial Ecology* 64 (3): 826-836.

Cox, B., Kislinger, T., Wigle, D.A., Kannan, A., Brown, K., Okubo, T., Hogan, B., Jurisica, I., Frey, B., Rossant, J. and Emili, A., 2007. Integrated proteomic and transcriptomic profiling of mouse lung development and Nmyc target genes. *Molecular Systems Biology,* 3(1): 109.

Crouzoulon, G. and Korieh, A., 1991. Fructose transport by rat intestinal brush border membrane vesicles. Effect of high fructose diet followed by return to standard diet. *Comparative Biochemistry and Physiology Part A: Physiology*, 100(1): 175-182.

Daniel, H. 2004. Molecular and integrative physiology of intestinal peptide transport. *Annual Review of Physiology*. 66: 361-384.

Danzeisen, J.L., Kim, H.B., Isaacson, R.E., Tu, Z.J. and Johnson, T.J., 2011. Modulations of the chicken cecal microbiome and metagenome in response to anticoccidial and growth promoter treatment. *PloS one*, 6(11): e27949.

De Filippo, C., Cavalieri, D., Di Paola, M., Ramazzotti, M., Poullet, J.B., Massart, S., Collini, S., Pieraccini, G. and Lionetti, P., 2010. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proceedings of the National Academy of Sciences of the United States of America*. 107(33): 14691-14696.

Debnam, E.S., Denholm, E.E. and Grimble, G.K., 1998. Acute and chronic exposure of rat intestinal mucosa to dextran promotes SGLTI-mediated glucose transport. *European Journal of Clinical Investigation*, 28(8): 651-658.

Deepak, V., Kalishwaralal, K., Ramkumarpandian, S., Babu, S.V., Senthilkumar, S.R. and Sangiliyandi, G., 2008. Optimization of media composition for Nattokinase production by *Bacillus subtilis* using response surface methodology. *Bioresource Technology*, 99(17): 8170-8174.

Denbow, 2015. Carbohydrates metabolism. In Scanes, C.G.,Ed.,2014. *Sturkie's avian physiology*. Elsevier.

Desvergne, B. and Wahli, W., 1999. Peroxisome proliferator-activated receptors: nuclear control of metabolism 1. *Endocrine Reviews*, 20(5): 649-688.

Deuel Jr, H.J., Hallman, L.F., Murray, S. and Hilllard, J., 1938. Studies on ketosis. 15. The comparative metabolism of d-mannose and d-glucose. *Journal of Biological Chemistry*, 125: 79-84.

De La Horra, M.C., Cano, M., Peral, M.J., Calonge, M.L. and Ilundáin, A.A., 2001. Hormonal regulation of chicken intestinal NHE and SGLT-1 activities. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 280(3): R655-R660.

Douard, V. and Ferraris, R.P., 2008. Regulation of the fructose transporter GLUT5 in health and disease. *American Journal of Physiology-Endocrinology and Metabolism*, 295(2): E227-E237.

Dowd, S.E., Callaway, T.R., Wolcott, R.D., Sun, Y., McKeehan, T., Hagevoort, R.G. and Edrington, T.S., 2008. Evaluation of the bacterial diversity in the feces of cattle using 16S rDNA bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP). *BMC microbiology*, 8(1): 125.

Durán, J.M., Cano, M., Peral, M.J. and Ilundáin, A.A., 2004. D-mannose transport and metabolism in isolated enterocytes. *Glycobiology*, 14(6): 495-500.

Dusterhoft, E.M. and Voragen, A.G.J., 1991. Non-starch polysaccharides from sunflower, *Helianthus annuus* meal and palm kernel, *Elaeis guineenis* meal-preparation of cell wall material and extraction of polysaccharide fractions. *Journal of the Science of Food and Agriculture*. 55(3): 411-422.

Dusterhoft, E.M., Posthumus, M.A. and Voragen, A.G.J., 1992. Non-starch polysaccharides from sunflower, *Helianthus annuus* meal and palm-kernel, *Elaeis guineenis* meal-investigation of the structure of major polysaccharides. *Journal of the Science of Food and Agriculture*. 59(2): 151-160.

Ebrahimi, R., Faseleh Jahromi, M., Liang, J.B., Soleimani Farjam, A., Shokryazdan, P. and Idrus, Z., 2015. Effect of dietary lead on intestinal nutrient transporters mRNA expression in broiler chickens. *BioMed Research International*, 2015, Article ID 149745.

Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics*, 26(19): 2460-2461.

Edvardsson, U., Bergström, M., Alexandersson, M., Bamberg, K., Ljung, B. and Dahllöf, B., 1999. Rosiglitazone, BRL49653, a PPARγ-selective agonist, causes peroxisome proliferator-like liver effects in obese mice. *Journal of Lipid Research*, 40(7): 1177-1184.

Engberg, R.M., Hedemann, M.S., Steenfeldt, S. and Jensen, B.B., 2004. Influence of whole wheat and xylanase on broiler performance and microbial composition and activity in the digestive tract. *Poultry Science*, 83(6): 925-938.

Esuga, P.M., Sekoni, A.A., Omage, J.J. and Bawa, G.S., 2008. Evaluation of enzyme, Maxigrain® supplementation of graded levels of palm kernel meal, PKM on the performance of broiler chickens. *Pakistan Journal of Nutrition*, 7*(*7): 607-613.

Fadrosh, D.W., Ma, B., Gajer, P., Sengamalay, N., Ott, S., Brotman, R.M. and Ravel, J., 2014.An improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the Illumina MiSeq platform. *Microbiome*, 2(1): 6.

Fernandez, F., Hinton, M. and Van Gils, B., 2002. Dietary mannan-oligosaccharides and their effect on chicken caecal microflora in relation to *Salmonella enteritidis* colonization. *Avian Pathology*. 31(1): 49-58.

Ferraris, R. P. 2001. Dietary and developmental regulation of intestinal sugar transport. *Biochemical Journal,* 360(2): 265–276.

Ferraris, R. P., Casirola, D. M. and Vinnakota, R. R., 1993. Dietary carbohydrate enhances intestinal sugar transport in diabetic mice. *Diabetes*, 42(11): 1579–1587

Ferraris, R.P. and Diamond, J.M., 1989. Specific regulation of intestinal nutrient transporters by their dietary substrates. *Annual Review of Physiology*, 51(1): 125-141.

Ferraris, R.P., Diamond, J. and Kwan, W.W., 1988. Dietary regulation of intestinal transport of the dipeptide carnosine. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 255(2): G143-G150.

Forder, R.E.A., Howarth, G.S., Tivey, D.R. and Hughes, R.J., 2007. Bacterial modulation of small intestinal goblet cells and mucin composition during early posthatch development of poultry. *Poultry Science*, 86(11): 2396-2403.

Fukasawa, T., Kamei, A., Watanabe, Y., Koga, J. and Abe, K., 2010. Short-chain fructooligosaccharide regulates hepatic peroxisome proliferator-activated receptor α and farnesoid X receptor target gene expression in rats. *Journal of Agricultural and Food Chemistry*, 58(11): 7007-7012.

Garcia, Y. and Diaz-Castro, J., 2013. Advantages and disadvantages of the animal models v. in vitro studies in iron metabolism: a review. *Animal*, 7(10): 1651-1658.

Germain, P., Staels, B., Dacquet, C., Spedding, M. and Laudet, V., 2006. Overview of nomenclature of nuclear receptors. *Pharmacological Reviews*, 58(4): 685-704.

Gibson, G.R., Probert, H.M., Van Loo, J.A.E. and Roberfroid, M.B., 2004. Dietary modulation of the human colonic microflora: updating the concept of prebiotics. *Nutrition Research Reviews,* 17(2): 257-259.

Gilbert, E.R., Li, H., Emmerson, D.A., Webb Jr., K.E. and Wong, E.A., 2007. Developmental regulation of nutrient transporter and enzyme mRNA abundance in the small intestine of broilers. *Poultry Science*, 86(8): 1739–1753.

Goda, T., 2000. Regulation of the expression of carbohydrate digestion/absorption-related genes. *British Journal of Nutrition*, 84(S2): S245-S248.

Gong, J., Yu, H., Liu, T., Gill, J.J., Chambers, J.R., Wheatcroft, R. and Sabour, P.M., 2008. Effects of zinc bacitracin, bird age and access to range on bacterial microbiota in the ileum and caeca of broiler chickens. *Journal of Applied Microbiology,* 104(5): 1372-1382.

Gourbeyre, P., Denery, S. and Bodinier, M., 2011. Probiotics, prebiotics, and synbiotics: impact on the gut immune system and allergic reactions. *Journal of Leukocyte Biology*, 89(5): 685-695.

Guerre-Millo, M., Gervois, P., Raspé, E., Madsen, L., Poulain, P., Derudas, B., Herbert, J.M., Winegar, D.A., Willson, T.M., Fruchart, J.C. and Berge, R.K., 2000. Peroxisome proliferator-activated receptor α activators improve insulin sensitivity and reduce adiposity. *Journal of Biological Chemistry*, 275(22): 16638-16642.

Guilloteau, P., Martin, L., Eeckhaut, V., Ducatelle, R., Zabielski, R. and Van Immerseel, F., 2010. From the gut to the peripheral tissues: the multiple effects of butyrate. *Nutrition Research Reviews*, 23(02): 366-384.

Hamilton, K.L. and Butt, A.G., 2013. Glucose transport into everted sacs of the small intestine of mice. *Advances in Physiology Education*, 37(4): 415-426.

Hanafiah, H.A., Zulkifli, I., Soleimani, A.F. and Awad, E.A., 2017. Apparent metabolisable energy and ileal crude protein digestability of various treated palm kernel cake based diets for heat-stressed broiler chickens. *European Poultry Science*, 81.

Harismendy, O., Ng, P.C., Strausberg, R.L., Wang, X., Stockwell, T.B., Beeson, K.Y., Schork, N.J., Murray, S.S., Topol, E.J., Levy, S. and Frazer, K.A., 2009. Evaluation of next generation sequencing platforms for population targeted sequencing studies. *Genome Biology*, 10(3): R32.

Healy, B., Cooney, S., O'Brien, S., Iversen, C., Whyte, P., Nally, J., Callanan, J.J. and Fanning, S., 2010. Cronobacter (Enterobacter sakazakii): an opportunistic foodborne pathogen. *Foodborne Pathogens and Disease*, 7(4): 339-350.

Hermans, D., Van Deun, K., Martel, A., Van Immerseel, F., Messens, W., Heyndrickx, M., Haesebrouck, F. and Pasmans, F., 2011. Colonization factors of Campylobacter jejuni in the chicken gut. *Veterinary Research*, 42(1), p.82.

Hirai, T., Fukui, Y. and Motojima, K., 2007. PPARα agonists positively and negatively regulate the expression of several nutrient/drug transporters in mouse small intestine. *Biological and Pharmaceutical Bulletin*, 30(11): 2185-2190.

Hojo, M., Takada, I., Kimura, W., Fukuda, K. and Yasugi, S., 2006. Expression patterns of the chicken peroxisome proliferator-activated receptors (PPARs) during the development of the digestive organs. *Gene Expression Patterns*, 6(2): 171-179.

Hooge, D.M., Pierce, J.L., McBride, K.W. and Rigolin, P.J., 2010. Meta-analysis of Broiler Chicken Trials Using Diets With or Without Allzyme® SSF Enzyme Complex®. *International Journal of Poultry Science*, 9(9): 819-823.

Howard, A., Goodlad, R.A., Walters, J.R., Ford, D. and Hirst, B.H., 2004. Increased expression of specific intestinal amino acid and peptide transporter mRNA in rats fed by TPN is reversed by GLP-2. *The Journal of Nutrition*, 134(11): 2957-2964.

Hu, Y., Smith, D.E., Ma, K., Jappar, D., Thomas, W. and Hillgren, K.M., 2008. Targeted disruption of peptide transporter Pept1 gene in mice significantly reduces dipeptide absorption in intestine. *Molecular Pharmaceutics*, 5(6): 1122-1130.

Hueck, C.J., 1998. Type III protein secretion systems in bacterial pathogens of animals and plants. *Microbiology and Molecular Biology Reviews*, 62(2): 379-433.

Huff, G.R., Huff, W.E., Farnell, M.B., Rath, N.C., de Los Santos, F.S. and Donoghue, A.M., 2010. Bacterial clearance, heterophil function, and hematological parameters of transport-stressed turkey poults supplemented with dietary yeast extract. *Poultry Science*, 89(3): 447-456.

Humphrey, B.D., Stephensen, C.B., Calvert, C.C. and Klasing, K.C., 2004. Glucose and cationic amino acid transporter expression in growing chickens (Gallus gallus domesticus). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 138(4): 515-525.

Hussar, P., Kärner, M., Järveots, T., Pendovski, L., Duritis, I. and Popovska-Percinic, F., 2016. Comparative Study of Glucose Transporters GLUT-2 and GLUT-5 in Ostriches Gastrointestinal Tract. *Macedonian Veterinary Review*, 39(2): 225-231.

Ibuki, M., Kovacs-Nolan, J., Fukui, K., Kanatani, H. and Mine, Y., 2010. Analysis of gut immune-modulating activity of β-1,4-mannobiose using microarray and real-

time reverse transcription polymerase chain reaction. *Poultry Science*. 89(9): 1894-1904.

Iqbal, M., Philbin, V.J. and Smith, A.L., 2005. Expression patterns of chicken Toll-like receptor mRNA in tissues, immune cell subsets and cell lines. *Veterinary Immunology and Immunopathology*, 104(1): 117-127.

Jahanian, R. and Ashnagar, M., 2015. Effect of dietary supplementation of mannan-oligosaccharides on performance, blood metabolites, ileal nutrient digestibility, and gut microflora in *Escherichia coli*-challenged laying hens. *Poultry Science*, 94(9): 2165-2172.

Jahromi, M.F., Liang, J.B., Abdullah, N., Goh, Y.M., Ebrahimi, R. and Shokryazdan, P., 2015. Extraction and characterization of oligosaccharides from palm kernel cake as prebiotic. *BioResources*, 11(1): 674-695.

Jamroz, D., Jakobsen, K., Knudsen, K.E.B., Wiliczkiewicz, A. and Orda, J., 2002. Digestibility and energy value of non-starch polysaccharides in young chickens, ducks and geese, fed diets containing high amounts of barley. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 131(3): 657-668.

Jappar, D., Wu, S.P., Hu, Y. and Smith, D.E., 2010. Significance and regional dependency of peptide transporter (PEPT) 1 in the intestinal permeability of glycylsarcosine: in situ single-pass perfusion studies in wild-type and Pept1 knockout mice. *Drug Metabolism and Disposition*, 38(10): 1740-1746.

Jiao, N., Wu, Z., Ji, Y., Wang, B., Dai, Z. and Wu, G., 2015. L-glutamate enhances barrier and antioxidative functions in intestinal porcine epithelial cells. *The Journal of Nutrition*, 145(10): 2258-2264.

Jones, P.E., 1951. The absorption of glucose and xylose by adult and newborn rats. *The Journal of Physiology*, 113(2-3), p.276

Joost, H.G. and Thorens, B., 2001. The extended GLUT-family of sugar/polyol transport facilitators: nomenclature, sequence characteristics, and potential function of its novel members. *Molecular Membrane Biology*, 18(4): 247-256.

Józefiak, D., Rutkowski, A. and Martin, S.A., 2004. Carbohydrate fermentation in the avian ceca: a review. *Animal Feed Science and Technology*, 113(1): 1-15.

Jumpertz, R., Le, D.S., Turnbaugh, P.J., Trinidad, C., Bogardus, C., Gordon, J.I. and Krakoff, J., 2011. Energy-balance studies reveal associations between gut microbes, caloric load, and nutrient absorption in humans. *The American Journal of Clinical Nutrition*, 94(1): 58-65.

Kadowaki, T., Hara, K., Kubota, N., Tobe, K., Terauchi, Y., Yamauchi, T., Eto, K., Kadowaki, H., Noda, M., Hagura, R. and Akanuma, Y., 2002. The role of PPARγ in high-fat diet-induced obesity and insulin resistance. *Journal of Diabetes and Its Complications*, 16(1): 41-45.

Kalavathy, R., Abdullah, N., Jalaludin, S. and Ho, Y.W., 2003. Effects of Lactobacillus cultures on growth performance, abdominal fat deposition, serum lipids and weights of organs of broiler chickens. *British Poultry Science*, 44: 139-144.

Kanai, Y. and Hediger, M.A., 2004. The glutamate/neutral amino acid transporter family SLC1: molecular, physiological and pharmacological aspects. *Pflügers Archiv*, 447(5): 469-479.

Khattak, F.M., Pasha, T.N., Hayat, Z. and Mahmud, A., 2006. Enzymes in poultry nutrition. *Journal of Animal and Plant Sciences*, 16(1-2): 1-7.

Khuri, A.I. and Mukhopadhyay, S., 2010. Response surface methodology. Wiley Interdisciplinary Reviews: *Computational Statistics,* 2(2): 128-149.

Kim, G.B., Seo, Y.M., Kim, C.H. and Paik, I.K., 2011. Effect of dietary prebiotic supplementation on the performance, intestinal microflora, and immune response of broilers. *Poultry Science*, 90(1): 75-82.

Kishi, K., Tanaka, T., Igawa, M., Takase, S. and Goda, T., 1999. Sucrase-isomaltase and hexose transporter gene expressions are coordinately enhanced by dietary fructose in rat jejunum. *The Journal of Nutrition*, 129(5): 953-956.

Klasing, K.C., 2007. Nutrition and the immune system. *British Poultry Science*, 48(5): 525-537.

Knight, R., Jansson, J., Field, D., Fierer, N., Desai, N., Fuhrman, J.A., Hugenholtz, P., Van Der Lelie, D., Meyer, F., Stevens, R. and Bailey, M.J., 2012. Unlocking the potential of metagenomics through replicated experimental design. *Nature Biotechnology*, 30(6): 513-520.

Kodera, T. and Nio, N., 2006. Identification of an Angiotensin I-converting Enzyme Inhibitory Peptides from Protein Hydrolysates by a Soybean Protease and the Antihypertensive Effects of Hydrolysates in 4 Spontaneously Hypertensive Model Rats. *Journal of Food Science*, 71(3): C164–C173.

Konikoff, T. and Gophna, U., 2016. Oscillospira: a central, enigmatic component of the human gut microbiota. *Trends in Microbiology*, 24(7): 523-524.

Kotrba, P., Inui, M. and Yukawa, H., 2001. Bacterial phosphotransferase system (PTS) in carbohydrate uptake and control of carbon metabolism. *Journal of Bioscience and Bioengineering*, 92(6): 502-517.

Krieg N. R., Staley J. T., Brown D. R., Hedlund B. P., Paster B. J., Ward N. L., Ludwig W., Whitman W. B. (2010). Family III. Rikenellaceae fam. nov. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 4, p. 54. Edited by Krieg N. R., Staley J. T., Brown D. R., Hedlund B. P., Paster B. J., Ward N. L., Ludwig W., Whitman W. B.. New York: Springer. Kris, E.S. and Marlett, J.A., 1990. Measuring lignin and neutral sugars in neutral detergent soluble and

insoluble fractions of human and rat feces. *Journal of Agricultural and Food Chemistry*, 38(5): 1218-1223.

Kuczynski, J., Lauber, C.L., Walters, W.A., Parfrey, L.W., Clemente, J.C., Gevers, D. and Knight, R., 2012. Experimental and analytical tools for studying the human microbiome. *Nature Reviews Genetics*, 13(1): 47-58.

Langille, M.G., Zaneveld, J., Caporaso, J.G., McDonald, D., Knights, D., Reyes, J.A., Clemente, J.C., Burkepile, D.E., Thurber, R.L.V., Knight, R. and Beiko, R.G., 2013. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nature Biotechnology*, 31(9): 814-821.

Larsen, P.J., Jensen, P.B., Sørensen, R.V., Larsen, L.K., Vrang, N., Wulff, E.M. and Wassermann, K., 2003. Differential influences of peroxisome proliferator–activated receptors γ and α on food intake and energy homeostasis. *Diabetes*, 52(9): 2249-2259.

Laverty, G., Bjarnadóttir, S., Elbrønd, V.S. and Árnason, S.S., 2001. Aldosterone suppresses expression of an avian colonic sodium-glucose cotransporter. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 281(4): R1041-R1050.

Lawal, T.E., Iyayi, E.A., Adeniyi, B.A. and Adaramoye, O.A., 2010. Biodegradation of palm kernel cake with multienzyme complexes from fungi and its feeding value for broilers. *International Journal of Poultry Science,* 9(7): 695-701.

Levin, L., Herrmann, C. and Papinutti, V.L., 2008. Optimization of lignocellulolytic enzyme production by the white-rot fungus *Trametes trogii* in solid-state fermentation using response surface methodology. *Biochemical Engineering Journal*, 39(1): 207-214.

Li, L.L., Hou, Z.P., Li, T.J., Wu, G.Y., Huang, R.L., Tang, Z.R., Yang, C.B., Gong, J., Yu, H., Kong, X.F. and Pan, E., 2008. Effects of dietary probiotic supplementation on ileal digestibility of nutrients and growth performance in 1-to 42-day-old broilers. *Journal of the Science of Food and Agriculture*, 88(1): 35-42.

Lin, T.C. and Chen, C., 2004. Enhanced mannanase production by submerged culture of Aspergillus niger NCH-189 using defatted copra based media. *Process Biochemistry*, 39(9): 1103-1109.

Lis, M.T., Crampton, R.F. and Matthews, D.M., 1971. Rates of absorption of a dipeptide and the equivalent free amino acid in various mammalian species. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, 233(2): 453-455.

Liu, X., Yan, H., Lv, L., Xu, Q., Yin, C., Zhang, K., Wang, P. and Hu, J., 2012. Growth performance and meat quality of broiler chickens supplemented with *Bacillus licheniformis* in drinking water. *Asian-Australasian Journal of Animal Sciences*, 25(5): 682-689.

Livak, K.J. and Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. *Methods*, 25(4): 402-408.

Loman, N.J., Constantinidou, C., Chan, J.Z., Halachev, M., Sergeant, M., Penn, C.W., Robinson, E.R. and Pallen, M.J., 2012. High-throughput bacterial genome sequencing: an embarrassment of choice, a world of opportunity. *Nature Reviews Microbiology*, 10(9): 599-606.

Lopetuso, L.R., Scaldaferri, F., Petito, V. and Gasbarrini, A., 2013. Commensal Clostridia: leading players in the maintenance of gut homeostasis. *Gut Pathogens*, 5(1), p.23.

Lu, J., Idris, U., Harmon, B., Hofacre, C., Maurer, J.J. and Lee, M.D., 2003. Diversity and succession of the intestinal bacterial community of the maturing broiler chicken. *Applied and Environmental Microbiology*, 69(11): 6816-6824.

Luo, Z. et al., 2013. Journal of Pharmacological and Toxicological Methods Ex vivo and in situ approaches used to study intestinal absorption. *Journal of Pharmacological and Toxicological Methods*, 68(2): 208–216.

Macfarlane, S., Woodmansey, E.J. and Macfarlane, G.T., 2005. Colonization of mucin by human intestinal bacteria and establishment of biofilm communities in a two-stage continuous culture system. *Applied and Environmental Microbiology*, 71(11): 7483-7492.

Mackie, R.I., Aminov, R.I., Hu, W., Klieve, A.V., Ouwerkerk, D., Sundset, M.A. and Kamagata, Y., 2003. Ecology of uncultivated Oscillospira species in the rumen of cattle, sheep, and reindeer as assessed by microscopy and molecular approaches. *Applied and Environmental Microbiology*, 69(11): 6808-6815.

Madsen, S.L. and Wong, E.A., 2011. Expression of the chicken peptide transporter 1 and the peroxisome proliferator-activated receptor α following feed restriction and subsequent refeeding. *Poultry Science*, 90(10): 2295-2300.

Mahraoui, L., Rodolosse, A., Barbat, A., Dussaulx, E., Zweibaum, A., Rousset, M. and Brot-Laroche, E., 1994. Presence and differential expression of SGLT1, GLUT1, GLUT2, GLUT3 and GLUT5 hexose-transporter mRNAs in Caco-2 cell clones in relation to cell growth and glucose consumption. *Biochemical Journal*, 298(3): 629-633.

Mariat, D., Firmesse, O., Levenez, F., Guimarães, V.D., Sokol, H., Doré, J., Corthier, G. and Furet, J.P., 2009. The Firmicutes/Bacteroidetes ratio of the human microbiota changes with age. *BMC microbiology*, 9(1), p.123.

Martin, G., Schoonjans, K., Lefebvre, A.M., Staels, B. and Auwerx, J., 1997. Coordinate regulation of the expression of the fatty acid transport protein and acyl-CoA synthetase genes by PPARα and PPARγ activators. *Journal of Biological Chemistry*, 272(45): 28210-28217.

Masella, A.P., Bartram, A.K., Truszkowski, J.M., Brown, D.G. and Neufeld, J.D., 2012. PANDAseq: paired-end assembler for illumina sequences. *BMC Bioinformatics*, 13(1), p.31.

Meng, H., Li, H., Zhao, J.G. and Gu, Z.L., 2005. Differential expression of peroxisome proliferator-activated receptors alpha and gamma gene in various chicken tissues. *Domestic Animal Endocrinology*, 28(1): 105-110.

Merat, A. and Sahmani, M., 2003. Effect of acarbose on in vitro intestinal absorption of monosaccharides in diabetic rats. *Archives of Iranian Medicine*, 6(1): 40-43.

Mesonero, J., Matosin, M., Cambier, D., Rodriguez-Yoldi, M.J. and Brot-Laroche, E., 1995. Sugar-dependent expression of the fructose transporter GLUT5 in Caco-2 cells. *Biochemical Journal*, 312(3): 757-762.

Metzker, M.L., 2010. Sequencing technologies-the next generation. *Nature Reviews Genetics*, 11(1): 31-46.

Mikkelsen, H., Sivaneson, M. and Filloux, A., 2011. Key two-component regulatory systems that control biofilm formation in *Pseudomonas aeruginosa*. *Environmental Microbiology*, 13(7): 1666-1681.

Mirnawati, Y.R., Marlida, Y. and Kompiang, I.P., 2011. Evaluation of palm kernel cake fermented by *Aspergillus niger* as substitute for soybean meal protein in the diet of broiler. *International Journal of Poultry Science*, 10(7): 537-541.

Miron, J., Yosef, E., Ben-Ghedalia, D., Chase, L.E., Bauman, D.E. and Solomon, R., 2002. Digestibility by dairy cows of monosaccharide constituents in total mixed rations containing citrus pulp. *Journal of Dairy Science*, 85(1): 89-94.

Montgomery, D.C. and Myers, R.H., 1995. Response surface methodology: process and product optimization using designed experiments. *Raymond H. Meyers and Douglas C. Montgomery.A Wiley-Interscience Publications*.

Mookiah, S., Sieo, C.C., Ramasamy, K., Abdullah, N. and Ho, Y.W., 2014. Effects of dietary prebiotics, probiotic and synbiotics on performance, caecal bacterial populations and caecal fermentation concentrations of broiler chickens. *Journal of the Science of Food and Agriculture*, 94(2): 341-348.

Morgan, E., Campbell, J.D., Rowe, S.C., Bispham, J., Stevens, M.P., Bowen, A.J., Barrow, P.A., Maskell, D.J. and Wallis, T.S., 2004. Identification of host-specific colonization factors of *Salmonella enterica serovar Typhimurium*. *Molecular Microbiology*, 54(4): 994-1010.

Mott, C.R., Siegel, P.B., Webb, K.E. and Wong, E.A., 2008. Gene expression of nutrient transporters in the small intestine of chickens from lines divergently selected for high or low juvenile body weight. *Poultry Science*, 87(11): 2215-2224.

Mountzouris, K.C., Tsitrsikos, P., Palamidi, I., Arvaniti, A., Mohnl, M., Schatzmayr, G. and Fegeros, K., 2010. Effects of probiotic inclusion levels in broiler nutrition on growth performance, nutrient digestibility, plasma immunoglobulins, and cecal microflora composition. *Poultry Science*, 89(1): 58-67.

MPOB, 2016. Production of palm kernel cake. Retrieved on 24 December 2016 from http://bepi.mpob.gov.my/index.php/en/statistics/production/168-production-2016/743-production-of-palm-kernel-cake-2016.html

Mtui, G.Y., 2012. Lignocellulolytic enzymes from tropical fungi: Types, substrates and applications. *Scientific Research and Essays*, 7(15): 1544-1555.

Muangkeow, N. and Chinajariyawong, C., 2011. Determination of true amino acid digestibility and metabolizable energy in fermented palm kernel meal with Aspergillus wentii TISTR 3075 for chickens. *Walailak Journal of Science and Technology (WJST)*, 6(2): 231-241.

Munro, S. and Freeman, M., 2000. The notch signalling regulator fringe acts in the Golgi apparatus and requires the glycosyltransferase signature motif DXD. *Current Biology*, 10(14): 813-820.

Murphy, E.F., Cotter, P.D., Healy, S., Marques, T.M., O'sullivan, O., Fouhy, F., Clarke, S.F., O'toole, P.W., Quigley, E.M., Stanton, C. and Ross, P.R., 2010. Composition and energy harvesting capacity of the gut microbiota: relationship to diet, obesity and time in mouse models. *Gut*, 59(12): 1635-1642.

Myers, W.D., Ludden, P.A., Nayigihugu, V. and Hess, B.W., 2004. Technical note: A procedure for the preparation and quantitative analysis of samples for titanium dioxide. *Journal of Animal Science*, 82(1): 179-183.

Navidshad, B., Liang, J.B., Jahromi, M.F., Akhlaghi, A. and Abdullah, N., 2015. A comparison between a yeast cell wall extract (Bio-Mos®) and palm kernel expeller as mannan-oligosac-charides sources on the performance and ileal microbial population of broiler chickens. *Italian Journal of Animal Science*, 14(1): 3452.

Navidshad, B., Liang, J.B., Jahromi, M.F., Akhlaghi, A. and Abdullah, N., 2016. Effects of enzymatic treatment and shell content of palm kernel expeller meal on performance, nutrient digestibility, and ileal bacterial population in broiler chickens. *The Journal of Applied Poultry Research*, 25(4): 474-482.

Ng, W.K., Lim, H.A., Lim, S.L. and Ibrahim, C.O., 2002. Nutritive value of palm kernel meal pretreated with enzyme or fermented with Trichoderma koningii (Oudemans) as a dietary ingredient for red hybrid tilapia (Oreochromis sp.). *Aquaculture Research*, 33(15): 1199-1207.

Nir, I., Nitsan, Z., Dror, Y. and Shapira, N., 1978. Influence of overfeeding on growth, obesity and intestinal tract in young chicks of light and heavy breeds. *British Journal of Nutrition*, 39(01): 27-35.

Nkukwana, T.T., Muchenje, V., Masika, P.J. and Mushonga, B., 2015. Intestinal morphology, digestive organ size and digesta pH of broiler chickens fed diets supplemented with or without Moringa oleifera leaf meal. *South African Journal of Animal Science*, 45(4): 362-370.

O'Croinin, T. and Backert, S., 2012. Host epithelial cell invasion by Campylobacter jejuni: trigger or zipper mechanism?. *Frontiers in Cellular and Infection Microbiology,* 2: 25.

Obst, B.S. and Diamond, J., 1992. Ontogenesis of intestinal nutrient transport in domestic chickens (*Gallus gallus*) and its relation to growth. *The Auk* 109(3): 451-464.

Oehlert, G.W., 2010. *A first course in design and analysis of experiments*. WH Freeman, New York, New York, USA.

Oku, T. and Nakamura, S., 2003. Comparison of digestibility and breath hydrogen gas excretion of fructo-oligosaccharide, galactosyl-sucrose, and isomalto-oligosaccharide in healthy human subjects. *European Journal of Clinical Nutrition*, 57(9): 1150-1156.

Olukosi, O.A., Bolarinwa, O.A., Cowieson, A.J., and Adeola, O., 2012. Marker type but not concentration influenced apparent ileal amino acid digestibility in phytase-supplemented diets for broiler chickens and pigs. *Journal of Animal Science*, 90(12), 4414-4420.

Ondov, B.D., Bergman, N.H. and Phillippy, A.M., 2011. Interactive metagenomic visualization in a Web browser. *BMC Bioinformatics*, 12: 385.

Onifade, A.A. and Babatunde, G.M., 1998. Comparison of the utilisation of palm kernel meal, brewers' dried grains and maize offal by broiler chicks. *British Poultry Science*, 39(2): 245-250.

Oskoueian, E., Abdullah, N., Idrus, Z., Ebrahimi, M., Goh, Y.M., Shakeri, M. and Oskoueian, A., 2014. Palm kernel cake extract exerts hepatoprotective activity in heat-induced oxidative stress in chicken hepatocytes. *BMC Complementary and Alternative Medicine*, 14(1), p.368.

Palander, S., Nasi, M., and Palander, P., 2010. Digestibility and energy value of cereal-based diets in relation to digesta viscosity and retention time in turkeys and chickens at different ages estimated with different markers. *Archives of Animal Nutrition*, 64(3), 238-253.

Park, S.H., Lee, S.I. and Ricke, S.C., 2016. Microbial populations in naked neck chicken Ceca raised on pasture flock fed with commercial yeast cell wall prebiotics via an illumina MiSeq platform. *PloS one*, 11(3), p.e0151944.

Park, S.H., Perrotta, A., Hanning, I., Diaz-Sanchez, S., Pendleton, S., Alm, E. and Ricke, S.C., 2017. Pasture flock chicken cecal microbiome responses to prebiotics and plum fiber feed amendments. *Poultry Science*, 96(6): 1820-1830.

Parks, D.H., Tyson, G.W., Hugenholtz, P. and Beiko, R.G., 2014. STAMP: statistical analysis of taxonomic and functional profiles. *Bioinformatics*, 30(21): 3123-3124.

Patterson, J.A. and Burkholder, K.M., 2003. Application of prebiotics and probiotics in poultry production. *Poultry Science*, 82(4): 627-631.

Peng, Q., Zeng, X.F., Zhu, J.L., Wang, S., Liu, X.T., Hou, C.L., Thacker, P.A. and Qiao, S.Y., 2016. Effects of dietary Lactobacillus plantarum B1 on growth performance, intestinal microbiota, and short chain fatty acid profiles in broiler chickens. *Poultry Science*, 95(4): 893-900.

Perland, E. and Fredriksson, R., 2016. Classification Systems of Secondary Active Transporters. *Trends in Pharmacological Sciences*, 38(3): 305-315.

Pourabedin, M., Guan, L. and Zhao, X., 2015. Xylo-oligosaccharides and virginiamycin differentially modulate gut microbial composition in chickens. *Microbiome*, 3: 15.

Pourabedin, M., Xu, Z., Baurhoo, B., Chevaux, E. and Zhao, X., 2014. Effects of mannan oligosaccharide and virginiamycin on the cecal microbial community and intestinal morphology of chickens raised under suboptimal conditions. *Canadian Journal of Microbiology*, 60 (5): 255-266.

Quail, M.A., Smith, M., Coupland, P., Otto, T.D., Harris, S.R., Connor, T.R., Bertoni, A., Swerdlow, H.P. and Gu, Y., 2012. A tale of three next generation sequencing platforms: comparison of Ion Torrent, Pacific Biosciences and Illumina MiSeq sequencers. *BMC Genomics*, 13: 341.

Ravindran, V., Abdollahi, M.R. and Bootwalla, S.M., 2014. Nutrient analysis, metabolizable energy, and digestible amino acids of soybean meals of different origins for broilers. *Poultry Science*, 93(10): 2567-2577.

Rezaei, K., Jenab, E. and Temelli, F., 2007. Effects of water on enzyme performance with an emphasis on the reactions in supercritical fluids. *Critical Reviews in Biotechnology*, 27(4): 183-195.

Rezaei, S., Jahromi, M.F., Liang, J.B., Zulkifli, I., Farjam, A.S., Laudadio, V. and Tufarelli, V., 2015. Effect of oligosaccharides extract from palm kernel expeller on growth performance, gut microbiota and immune response in broiler chickens. *Poultry science*, 94(10): 2414-2420.

Rosen, E.D., Sarraf, P., Troy, A.E., Bradwin, G., Moore, K., Milstone, D.S., Spiegelman, B.M. and Mortensen, R.M., 1999. PPARγ is required for the differentiation of adipose tissue *in vivo* and *in vitro*. *Molecular cell*, 4(4): 611-617.

Ruas-Madiedo, P., Gueimonde, M., Fernández-García, M., Clara, G. and Margolles, A.,2008. Mucin degradation by Bifidobacterium strains isolated from the human

intestinal microbiota. *Applied and Environmental Microbiology*, 74(6): 1936-1940.

Saenphoom, P., Liang, J.B., Ho, Y.W., Loh, T.C. and Rosfarizan, M., 2013. Effects of enzyme treated palm kernel expeller on metabolizable energy, growth performance, villus height and digesta viscosity in broiler chickens. *Asian-Australasian Journal of Animal Sciences*, 26(4): 537-544.

Saenphoom, P., Liang, J.H. and Loh, T.R., 2011. Effect of enzyme treatment on chemical composition and production of reducing sugars in palm (*Elaeis guineenis*) kernel expeller. *African Journal of Biotechnology*, 10(68):15372-15377.

Sampath, H. and Ntambi, J.M., 2005. Polyunsaturated fatty acid regulation of genes of lipid metabolism. *Annual Review of Nutrition*, 25: 317-340.

Sato, K., Abe, H., Kono, T., Yamazaki, M., Nakashima, K., Kamada, T. and Akiba, Y., 2009. Changes in peroxisome proliferator-activated receptor gamma gene expression of chicken abdominal adipose tissue with different age, sex and genotype. *Animal Science Journal*, 80(3): 322-327.

Saulnier, D.M., Spinler, J.K., Gibson, G.R. and Versalovic, J., 2009. Mechanisms of probiosis and prebiosis: considerations for enhanced functional foods. *Current Opinion in Biotechnology*, 20(2): 135-141.

Scheepers, A., Joost, H.G. and Schurmann, A., 2004. The glucose transporter families SGLT and GLUT: molecular basis of normal and aberrant function. *Journal of Parenteral and Enteral Nutrition*, 28(5): 364-371.

Seki, Y., Sato, K., Kono, T., Abe, H. and Akiba, Y., 2003. Broiler chickens (Ross strain) lack insulin-responsive glucose transporter GLUT4 and have GLUT8 cDNA. *General and Comparative Endocrinology*, 133(1): 80-87.

Servin, A.L. and Coconnier, M.H., 2003. Adhesion of probiotic strains to the intestinal mucosa and interaction with pathogens. *Best Practice & Research Clinical Gastroenterology*, 17(5): 741-754.

Shakila, S., Sudhakara Reddy, P., Reddy, P.V.V.S., Ramana, J.V. and Ravi, A., 2012. Effect of palm kernel meal on the performance of broilers. *Tamilnadu Journal of Veterinary and Animal Sciences*, 8(4): 227-234.

Shang, H.M., Hu, T.M., Lu, Y.J. and Wu, H.X., 2010. Effects of inulin on performance, egg quality, gut microflora and serum and yolk cholesterol in laying hens. *British Poultry Science*, 51(6): 791-796.

Shanmugasundaram, R., Sifri, M. and Selvaraj, R.K., 2013. Effect of yeast cell product supplementation on broiler cecal microflora species and immune responses during an experimental coccidial infection. *Poultry Science*, 92(5): 1195-1201.

Sharma, K.K., Shrivastava, B., Sastry, V.R.B., Sehgal, N. and Kuhad, R.C., 2013. Middle-redox potential laccase from Ganoderma sp.: its application in improvement of feed for monogastric animals. *Scientific Reports*, 3: 1299.

Shaufi, M.A.M., Sieo, C.C., Chong, C.W., Gan, H.M. and Ho, Y.W., 2015. Deciphering chicken gut microbial dynamics based on high-throughput 16S rRNA metagenomics analyses. *Gut Pathogens*, 7: 4.

Shimakura, J., Terada, T., Saito, H., Katsura, T. and Inui, K.I., 2006. Induction of intestinal peptide transporter 1 expression during fasting is mediated via peroxisome proliferator-activated receptor α. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 291(5): G851-G856.

Shiraga, T., Miyamoto, K.I., Tanaka, H., Yamamoto, H., Taketani, Y., Morita, K., Tamai, I., Tsuji, A. and Takeda, E., 1999. Cellular and molecular mechanisms of dietary regulation on rat intestinal H+/peptide transporter PepT1. *Gastroenterology*, 116(2): 354-362.

Shirazi-Beechey, S.P., Gribble, S.M., Wood, I.S., Tarpey, P.S., Beechey, R.B., Dyer, J., Scott, D. and Barker, P.J., 1994. Dietary regulation of the intestinal sodium-dependent glucose cotransporter (SGLTI). *Biochemical Society Transactions*. 22(3): 655–658

Short, F.J., Gorton, P., Wiseman, J., anf Boorman, K.N., 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. *Animal Feed Science and Technology*, 59(4), 215-221.

Silva, G.L., Lee, I.S. and Kinghorn, A.D., 1998. Special problems with the extraction of plants. *Natural Products Isolation, pp.* 343-363.

Singh, K M., Shah, T., Deshpande, S., Jakhesara, S.J., Koringa, P.G., Rank, D.N., and Joshi, C.G., 2012. High through put 16S rRNA gene-based pyrosequencing analysis of the fecal microbiota of high FCR and low FCR broiler growers. *Molecular biology reports*, 39(12): 10595-10602.

Singh, K.M., Shah, T.M., Reddy, B., Deshpande, S., Rank, D.N. and Joshi, C.G., 2014. Taxonomic and gene-centric metagenomics of the fecal microbiome of low and high feed conversion ratio (FCR) broilers. *Journal of Applied Genetics*, 55(1): 145-154.

Sinurat, A.P., Purwadaria, T., Ketaren, P.P. and Pasaribu, T., 2014. Substitutions of soybean meal with enriched palm kernel meal in laying hens diet. *Jurnal Ilmu Ternak dan Veteriner*, 19(3): 184-192.

Sohail, M.U. et al., 2015. Molecular analysis of the caecal and tracheal microbiome of heat-stressed broilers supplemented with prebiotic and probiotic Molecular analysis of the caecal and tracheal microbiome of heat-stressed broilers supplemented with prebiotic and probiotic. *Avian Pathology*, 44(2): 67–74.

Sohail, M.U., Hume, M.E., Anwar, H., Hussain, G., Shabbir, M.Z., Nisbet, D.J., Ahmad, S. and Kamran, Z., 2014. Metagenomics of prebiotic and probiotic supplemented broilers gastrointestinal tract microbiome. *Indo American Journal of Pharmaceutical Research*, 4(9): 3780-3784.

Soltan, M.A., 2009. Growth performance, immune response and carcass traits of broiler chicks fed on graded level of palm kernel cake without or with enzyme supplementation. *Livestock Research for Rural Development*, 21(3), p.37.

Sonnenburg, J.L., Xu, J., Leip, D.D., Chen, C.H., Westover, B.P., Weatherford, J., Buhler, J.D. and Gordon, J.I., 2005. Glycan foraging in vivo by an intestine-adapted bacterial symbiont. *Science*, 307 (5717): 1955-1959.

Spring, P., Wenk, C., Dawson, K.A. and Newman, K.E., 2000. The effects of dietary mannaoligosaccharides on cecal parameters and the concentrations of enteric bacteria in the ceca of salmonella-challenged broiler chicks. *Poultry Science*, 79 (2): 205-211.

SPSS Inc. (1999). SPSS for window (Release 16.0), Standard Version. SPSS Inc. Headquaters, 233 S. Wacker Drive, 11 th floor Chicago, Illinois 60606, USA.

Stanley, D., Denman, S.E., Hughes, R.J., Geier, M.S., Crowley, T.M., Chen, H., Haring, V.R. and Moore, R.J., 2012. Intestinal microbiota associated with differential feed conversion efficiency in chickens. *Applied Microbiology and Biotechnology*, 96(5): 1361-1369.

Steel, A., Nussberger, S., Romero, M.F., Boron, W.F., Boyd, C.A. and Hediger, M.A., 1997. Stoichiometry and pH dependence of the rabbit proton-dependent oligopeptide transporter PepT1. *The Journal of Physiology*, 498(3): 563-569.

Su, C., Lei, L., Duan, Y., Zhang, K.Q. and Yang, J., 2012. Culture-independent methods for studying environmental microorganisms: methods, application, and perspective. *Applied Microbiology and Biotechnology*, 93(3): 993-1003.

Su, S., Miska, K.B., Fetterer, R.H., Jenkins, M.C. and Wong, E.A., 2015. Expression of digestive enzymes and nutrient transporters in *Eimeria*-challenged broilers. *Experimental Parasitology*, 150: 13-21.

Su, Y., Zhang, X., Hou, Z., Zhu, X., Guo, X. and Ling, P., 2011. Improvement of xylanase production by thermophilic fungus *Thermomyces lanuginosus* SDYKY-1 using response surface methodology. *New Biotechnology*, 28(1): 40-46.

Sundu, B. and Dingle, J.G., 2003. Use of enzymes to improve the nutritional value of palm kernel meal and copra meal. In *Queensland Poultry Science Symposium* 2003(Vol. 11: 1-15). World's Poultry Science Association.

Sundu, B., Bahry, S. and Dien, R., 2015. Palm Kernel Polysaccharides as a Feed Additive for Broiler Chickens. *International Journal of Poultry Science*, 14(7): 394-397.

Sundu, B., Kumar, A. and Dingle, J., 2006. Palm kernel meal in broiler diets: effect on chicken performance and health. *World's Poultry Science Journal*, 62(2): 316-325.

Tanaka, H., Miyamoto, K.I., Morita, K., Haga, H., Segawa, H., Shiraga, T., Fujioka, A., Kouda, T., Taketani, Y., Hisano, S. and Fukui, Y., 1998. Regulation of the PepT1 peptide transporter in the rat small intestine in response to 5-fluorouracil–induced injury. *Gastroenterology*, 114(4): 714-723.

Tazawa, S., Yamato, T., Fujikura, H., Hiratochi, M., Itoh, F., Tomae, M., Takemura, Y., Maruyama, H., Sugiyama, T., Wakamatsu, A. and Isogai, T., 2005. SLC5A9/SGLT4, a new Na+-dependent glucose transporter, is an essential transporter for mannose, 1, 5-anhydro-D-glucitol, and fructose. *Life Sciences*, 76(9): 1039-1050.

Teo, A.Y. and Tan, H.M., 2007. Evaluation of the performance and intestinal gut microflora of broilers fed on corn-soy diets supplemented with Bacillus subtilis PB6 (CloSTAT). *The Journal of Applied Poultry Research*, 16(3): 296-303.

Toloza, E.M. and Diamond, J., 1992. Ontogenetic development of nutrient transporters in rat intestine. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 263(5): G593-G604.

Tontonoz, P. and Spiegelman, B.M., 2008. Fat and beyond: the diverse biology of PPARγ. *Annual Review of Biochemistry*, 77: 289-312.

Torok, V.A., Allison, G.E., Percy, N.J., Ophel-Keller, K. and Hughes, R.J., 2011. Influence of antimicrobial feed additives on broiler commensal posthatch gut microbiota development and performance. *Applied and Environmental Microbiology*, 77(10): 3380-3390.

Tremaroli, V. and Bäckhed, F., 2012. Functional interactions between the gut microbiota and host metabolism. *Nature*, 489(7415): 242-249.

Turnbaugh, P.J., Ley, R.E., Mahowald, M.A., Magrini, V., Mardis, E.R. and Gordon, J.I., 2006. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature*, 444(7122): 1027-1031.

Uldry, M. and Thorens, B., 2004. The SLC2 family of facilitated hexose and polyol transporters. *Pflügers Archiv*, 447(5): 480-489.

Van der Wielen, P.W., Biesterveld, S., Notermans, S., Hofstra, H., Urlings, B.A. and van Knapen, F., 2000. Role of volatile fatty acids in development of the cecal microflora in broiler chickens during growth. *Applied and Environmental Microbiology*, 66(6): 2536-2540.

Van Soest, P.V., Robertson, J.B. and Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science*, 74(10): 3583-3597.

Vasta, V., Nuddha, A., Cannas, A., Lanza, M. and Priolo, A., 2008. Alternative feed resources and their effects on the quality of meat and milk from small ruminants. *Animal Feed Science and Technology* 147(1): 223-246.

Vázquez-Baeza, Y., Pirrung, M., Gonzalez, A. and Knight, R., 2013. EMPeror: a tool for visualizing high-throughput microbial community data. *Gigascience*, 2(1): 16.

Wei, S., Morrison, M. and Yu, Z., 2013. Bacterial census of poultry intestinal microbiome. *Poultry science*, 92(3): 671-683.

Wilkens, S., 2015. Structure and mechanism of ABC transporters. *F*1000*prime reports*, 7.

Wilson, T.H. and Vincent, T.N., 1955. Absorption of sugars in vitro by the intestine of the golden hamster. *Journal of Biological Chemistry*, 216(2): 851-866.

Wösten, M.M., van Dijk, L., Parker, C.T., Guilhabert, M.R., van der Meer-Janssen, Y.P., Wagenaar, J.A. and van Putten, J.P., 2010. Growth phase-dependent activation of the DccRS regulon of *Campylobacter jejuni*. *Journal of Bacteriology*, 192(11): 2729-2736.

Wright, E.M. and Turk, E., 2004. The sodium/glucose cotransport family SLC5. *Pflügers Archiv*, 447(5): 510-518.

Wright, E.M., Martín, M.G. and Turk, E., 2003. Intestinal absorption in health and disease-sugars. *Best Practice &Research Clinical gastroenterology*, 17(6): 943-956.

Ximenes, E.A., Chen, H., Kataeva, I.A., Cotta, M.A., Felix, C.R., Ljungdahl, L.G. and Li, X.L., 2005. A mannanase, ManA, of the polycentric anaerobic fungus Orpinomyces sp. strain PC-2 has carbohydrate binding and docking modules. *Canadian Journal of Microbiology*, 51(7): 559-568.

Yan, F., 2016. The effects of dietary probiotic inclusion on skeletal health of poultry and its possible mechanisms, PhD Thesis, Purdue University

Yanagita, K., Manome, A., Meng, X.Y., Hanada, S., Kanagawa, T., Tsuchida, T., Mackie, R.I. and Kamagata, Y., 2003. Flow cytometric sorting, phylogenetic analysis and in situ detection of Oscillospira guillermondii, a large, morphologically conspicuous but uncultured ruminal bacterium. *International Journal of Systematic and Evolutionary Microbiology*, 53(5): 1609-1614.

Yang, J., Martínez, I., Walter, J., Keshavarzian, A. and Rose, D.J., 2013. *In vitro* characterization of the impact of selected dietary fibers on fecal microbiota composition and short chain fatty acid production. *Anaerobe*, 23: 74-81.

Ye, J.L., Gao, C.Q., Li, X.G., Jin, C.L., Wang, D., Shu, G., Wang, W.C., Kong, X.F., Yao, K., Yan, H.C. and Wang, X.Q., 2016. EAAT3 promotes amino acid transport and proliferation of porcine intestinal epithelial cells. *Oncotarget*, 7(25): 38681-38692.

Yegani, M. and Korver, D.R., 2013. Effects of corn source and exogenous enzymes on growth performance and nutrient digestibility in broiler chickens. *Poultry Science*, 92(5): 1208-1220.

Yitbarek, A., Echeverry, H., Brady, J., Hernandez-Doria, J., Camelo-Jaimes, G., Sharif, S., Guenter, W., House, J.D. and Rodriguez-Lecompte, J.C., 2012. Innate immune response to yeast-derived carbohydrates in broiler chickens fed organic diets and challenged with Clostridium perfringens. *Poultry Science*, 91(5): 1105-1112.

Yoshida, A., Takata, K., Kasahara, T., Aoyagi, T., Saito, S. and Hirano, H., 1995. Immunohistochemical localization of Na+-dependent glucose transporter in the rat digestive tract. *The Histochemical Journal*, 27(5): 420-426.

Yuzbashian, E., Zarkesh, M., Asghari, G., Zadeh-Vakili, A., Mahmoodi, B., Daneshafrooz, A., Hedayati, M. and Mirmiran, P., 2017. Dietary intake of linoleic, linolenic, oleic, and arachidonic acid PPAR-gamma gene expression in visceral and subcutaneous adipose tissue among healthy subjects. *Endocrine Abstracts,* 49:642.

Zhang, H.J., Guo, Y.M., Yang, Y. and Yuan, J.M., 2006. Dietary conjugated linoleic acid enhances spleen PPAR-γ mRNA expression in broiler chicks. *British Poultry Science*, 47(6): 726-733.

Zhang, J., Jia, S., Liu, Y., Wu, S. and Ran, J., 2011. Optimization of enzyme-assisted extraction of the Lycium barbarum polysaccharides using response surface methodology. *Carbohydrate Polymers*, 86(2): 1089-1092.

Zhang, J., Liu, Y., Tian, L., Yang, H., Liang, G. and Xu, D., 2012. Effects of dietary mannan oligosaccharide on growth performance, gut morphology and stress tolerance of juvenile Pacific white shrimp, Litopenaeus vannamei. *Fish and Shellfish Immunology*, 33(4): 1027-1032.

Zhang, X., Zhou, M., Guo, Y., Song, Z. and Liu, B., 2015. 1,25-Dihydroxyvitamin D3 Promotes High Glucose-Induced M1 Macrophage Switching to M2 via the VDR-PPAR Signaling Pathway. *BioMed Research International*, 2015: 157834.

Zhang, Z.F. and Kim, I.H., 2013. Effects of probiotic supplementation in different energy and nutrient density diets on performance, egg quality, excreta microflora, excreta noxious gas emission, and serum cholesterol concentrations in laying hens. *Journal of Animal Science*, 91(10): 4781-4787.

Zhou, M. and Hernandez-Sanabria, E., 2010. Characterization of variation in rumen methanogenic communities under different dietary and host feed efficiency conditions, as determined by PCR-denaturing gradient gel electrophoresis analysis. *Applied and Environmental Microbiology*, 76(12): 3776-3786.

Zwarycz, B. and Wong, E.A., 2013. Expression of the peptide transporters PepT1, PepT2, and PHT1 in the embryonic and posthatch chick. *Poultry Science*, 92(5): 1314-1321.