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EFFECTS OF XYLANASE AND CELLULASE SUPPLEMENTATION ON EARLY GROWING STAGE, CAECAL BACTERIA POPULATION AND FERMENTATION PROFILES OF BROILER FED WITH PALM KERNEL MEAL

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By

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EFFECTS OF XYLANASE AND CELLULASE SUPPLEMENTATION ON EARLY GROWING STAGE, CAECAL BACTERIA POPULATION AND FERMENTATION PROFILES OF BROILER FED WITH PALM KERNEL MEAL

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Faculty: Agriculture

The optimum inclusion of palm kernel meal in broiler diets is reported about 20% without having any detrimental effect on the performance and health of broilers. The addition of enzyme in palm kernel mealdiet for broilers isnecessary in promoting growth performances of the broilers through increase in feed efficiency and digestibility. Since, previous study only focusing on their effect on growth performance; therefore, the present study was conducted to study the effect of enzyme supplementation in palm kernel mealdiet on caecal fermentation profiles, early growth performances and caecal bacteria population of broilers. The *in-vitro* analysis was carried out by incubating the formulated diets with caecal digesta of broilers for 72 hours at 39±5^{o}C. The gas production and end products of the fermentation were examined. The gas production rate of unsupplemented and enzyme-supplemented palm kernel meal diet observed was similar in trends. However, it was found that the amount of acetate was significantly ($P<0.05$) reduced with enzyme supplementation, whilst the *in-vitro* dry matter degradability of both, starter and finisher diet were improved ($P<0.05$), with more outstanding result observedfollowing xylanase supplementation at 11.09 and 17.59%, respectively.In the feeding trial, 75 broiler chicks were randomly allocated to three dietary treatment groups receiving diet containing 20% of palm kernel meal with or without xylanase and cellulase supplementation. The individual body weight and feed intakewere recorded weekly, and at day 35, broilers were slaughtered for ceacal digesta collection. Both enzyme addition did not improve the average daily gain of broiler at starter phase (1- 21 days), but had improved at grower phase (22-35 days), where broilers fed with xylanase supplementation had improved their body weight by 7% compared to control diet (61.98 g/bird). The similar result was also observed in the final body weight of broilers, where xylanase-supplemented group was the highest followed by control and cellulase-supplemented group which values is 1495.2, 1436.8 and 1367.8 kg, respectively. In the enumeration of caecal bacteria population, lactic acid bacteria and *Salmonella* s*pp*. population were reduced, by 4.1 and 1.9% in

xylanase supplemented group, and 8.3 and 7.9%in cellulase supplemented group, respectively. In contrast, the caecal bacteria population quantified using real-time PCR analysis revealed that enzyme supplementation had reduced the number of total bacteria, lactic acid bacteria, enterococcus, enterobacteria, *Escherichia coli*, bifidobacteria and *Salmonella*spp., respectively compared to unsupplemented palm kernel meal diet. Apart from that, significant negative correlation ($P<0.01$) was observed between the population of coliform bacteria (-0.97) and *Salmonella* spp. (-0.84) with n-butyrate in caecal digesta of broilers. The current data show that n-butyrate have a toxic effect on coliform bacteria and *Salmonella* spp. In conclusion, enzyme supplementation in 20% palm kernel meal diet was only effective in improving the body weight of broilers at growing phase but not in the starter phase. So, reducing the amount of palm kernel meal in the starter broiler diet is recommended; since, body weight of broilers fed 20% palm kernel meal diet was not improved, even with enzyme addition.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Sarjana Sains

KESAN SUPLEMENTASI XILANASE DAN SELULASE TERHADAP PERINGKAT AWAL TUMBESARAN, POPULASI BAKTERIA DAN PROFIL FERMENTASI SEKUM AYAM PEDAGING YANG DIBERI MIL ISIRONG SAWIT

Oleh

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Tahap kemasukan optimum mil isirong sawit dalam diet ayam pedaging dilaporkan sebanyak 20% tanpa memberi kesan mudarat terhadap prestasi tumbesaran dan kesihatan ayam pedaging. Penambahan enzim dalam diet mil isirong sawit adalah penting bagi meningkatkan prestasi tumbesaran ayam pedaging melalui peningkatan kecekapan dan pencernaan makanan. Oleh kerana kajian sebelum ini hanya memberi tumpuan kesannya terhadap prestasi tumbesaran ayam pedaging, maka kajian ini dijalankan untuk mengkaji kesan suplemen enzim dalam diet mil isirong sawit terhadap profil fermentasi sekum, peringkat awal tumbesaran dan populasi bakteria sekum ayam pedaging. Analisis *in-vitro* telah dijalankan dengan mengikubasi rumusan diet berserta digesta sekum ayam pedaging selama 72 jam pada suhu 39±5^{o}C. Kadar pengeluaran gas dan produk akhir fermentasi diperiksa. Kadar pengeluaran gas diet mil isirong sawit dengan suplemen enzim atau tidak adalah sama. Walau bagaimanapun, didapati jumlah asid asetik telah berkurang secara nyata ($P<0.05$) oleh diet dengan suplemen enzim, manakala kadar penguraian bahan kering diet bagi ransum pemula dan pembesar telah meningkat secara ketara ($P<0.05$), dengan kadar penguraian yang paling tinggi oleh diet dengan suplemen xilanase pada kadar 11.09 dan 17.59%. Dalam ujian perbandingan makanan, sebanyak 75 ekor anak ayam pedaging secara rawaknya dibahagikan kepada tiga kumpulan diet menerima diet 20% mil isirong sawit tanpa atau dengan suplemen enzim xilanase dan selulase. Berat badan dan pengambilan makanan individu telah direkodkan setiap minggu, dan pada hari ke-35, ayam disembelih bagi pengumpulan digesta sekum. Penambahan enzim tidak meningkatkan purata berat badan harian ayam pedaging pada fasa permulaan (1-21 hari), tetapi telah meningkat pada fasa pembesaran (22-35 hari) oleh ayam pedaging yang diberi makan diet dengan suplemen enzim xilanase sebanyak 7% berbanding dengan diet tanpa enzim (61.98 g/ayam). Di akhir eksperimen,berat badan ayam pedaging yang diberi makan diet dengan suplemen xilanase adalah yang tertinggi, diikuti oleh ayam pedaging yang diberi diet tanpa enzim, seterusnya dengan suplemen selulase, masing-masing sebanyak 1495.2,

1436.8 dan 1367.8 kg. Dalam penghitungan populasi bakteria sekum, bakteria asid laktik dan *Salmonella* spp. telah berkurang sebanyak 4.1 dan 1.9% dengan suplemen xilanase manakala berkurang sebanyak 8.3 dan 7.9% dengan suplemen selulase.Sebaliknya analisa populasi bakteria sekum melalui kaedah 'PCR' menunjukkan bahawa diet dengan suplemen enzim telah mengurangkan bilangan populasi bakteria total, bakteria asid laktik, enterokokus, enterobakteria, *Escherichia coli*, bifidobakteria dan *Salmonella* spp. berbanding diet tanpa suplemen enzim. Selain itu, korelasi negatif yang signifikan ($P<0.01$) didapati antara populasi bakteria koliform (-0,97) dan S*almonella* spp. (-0,84) dengan asid n-butirik dalam digesta sekum ayampedaging.Data semasa menunjukkan bahawa asid n-butirikmempunyai kesan toksik terhadap bakteria koliform dan *Salmonella* spp. Kesimpulannya, suplemen enzim dalam 20% diet mil isirong sawit hanya dapat meningkatkan berat badan ayam pedaging pada fasa pembesaran tetapi tidak pada fasa permulaan.Oleh itu, adalah disyorkan untuk mengurangkan jumlah mil isirong sawit dalam diet fasa awal ayam pedaging, kerana berat badan ayam pedaging diberi makan 20% mil isirong sawit tidak meningkat walaupun dengan suplemen enzim.

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I certify that a Thesis Examination Committee has met on 22 December 2014 to conduct the final examination of Sharmila binti Ahmadon her thesis entitled " Effect of Xylanase and Cellulase Supplementation on Early Growing Stage, Caecal Bacterial Population and Fermentation Profiles of Broiler Fed with Palm Kernel Meal " 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 Master of Science.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ADF	Acid detergent fiber
ADFCR	Average daily feed conversion ratio
ADFI	Average daily feed intake
ADG	Average daily gain
ADL	Acid detergent lignin
AME	Apparent metabolizable energy
CF	Crude fiber
CFCR	Cumulative feed conversion ratio
CFI	Cumulative feed intake
CFU	Colony forming unit
CP	Crude protein
CPKO	Crude palm kernel oil
CPO	Crude palm oil
Cq	Quantification cycle
CWG	Cumulative weight gain
DDGE	Denaturing gradient gel electrophoresis
DF	Dietary fiber
DNA	Deoxyribonucleic acid
E	Amplification efficiency
EE	Ether extract
g	Gram
GIT	Gastrointestinal tract
HCl	Hydrochloric acid
HFFS	High fiber feedstuff
hr	Hour
IVDMD	*In-vitro* dry matter degradability
LAB	Lactic acid bacteria
MPOB	Malaysian palm oil board
MU	Million units
NaOH	Sodium hydroxide
NDF	Neutral detergent fiber
NFE	Nitrogen free extract
NH_3	Ammonia
NSP	Non-starch polysaccharide
NTC	No-template control
PKM	Palm kernel meal
POS	Palm oil sludge
PPF	Palm press fiber
ppm	Part per million
qPCR	Quantitative real-time polymerase chain reaction
rRNA	Ribosomal ribonucleic acid
SBM	Soybean meal
spp	Subspecies
SSF	Solid state fermentation
TGGE	Temperature gradient gel electrophoresis
T-RFLP	Terminal restriction fragment length polymorphism
VFA	Volatile fatty acid

wt	Weight
X.L.D	Xylose-lysine-Deoxycholate

CHAPTER 1

INTRODUCTION

The used of agriculture by-products such as palm kernel meal (PKM) in broilers diet has been widely practiced worldwide. It could spare conventional feed ingredients such as maize and soybeans because it is relatively cheap, easily available (Onuh *et al*., 2010) and virtually has no competition between man and farm animals (Kperegbeyi and Ikperite, 2011). The PKM contains moderate amount of protein and energy (Alimon, 2004); however, the nutrient value of PKM is considered very low, due to poor amino acid contents particularly the essential amino acids such as lysine and methionine (Sundu *et al*., 2006) besides the high fiber content in the form of non-starch polysaccharides (NSPs) (Sekoni *et al*., 2008) contributed to its low inclusion in the broiler diet.

It has been reported that, the optimum inclusion of PKM in broilers diet varied from one study to another. Sekoni *et al*. (2008) reported that PKM can be added in the diet of broilers up to 20%, without having any detrimental effect on their growth performances. In contrast, in a study conducted by Adrizal *et al*.(2011) found that the inclusion can be varied between 20 to 30%. In another study, Saenphoom *et al*.(2013) reported that 20% PKM can only be included in the grower diet of broilers, whilst they observed that 5% PKM can be added in the starter diet of broilers without impairing their growth performances. However, there is still lack of information on the optimum utilization of PKM in thestarter diet of broilers.

The used of high fiber feedstuff such PKM in the broiler diets can impair the digestibility of the diet due to fact that broilers have a limited capacity to assimilate dietary fiber because they lack of fiber digestive enzymic activity in their gastro-intestinal tract. The digestibility of feed, particularly apparent nitrogen and calcium retention is found to decrease with the inclusion of PKM, although the feed intake is increased (Sundu *et al*., 2005). Reducing in apparent nitrogen digestibility for example, indicates that the protein of PKM is entrapped in the cell walls, making it unavailable and difficult for digestive enzymes to digest it and to be absorbed by broilers. Furthermore, contamination of PKM with the nut shell also contributed to the poor digestibility of PKM by broilers. Since the digestibility of PKM is very low, efforts are made to improve the digestibility and utilization of PKM by broilers. Previously, several studies have shown that the supplementation of exogenous enzyme in the PKM diet for example, could improve its nutritive quality and make it more available to the broilers through the enhance in the digestibility of nutrients entrapped in the cell wall of PKM (Chong *et al*., 2008; Sekoni *et al*., 2008).

The research on feeding of high fiber feedstuffs to broilers is typically focusing on their detrimental effect on the digestibility of feed and performance of broilers. However, there is still lack of understanding on how fiber is actually degraded in the intestinal tract, particularly in the caeca, and the potential of feeding high fiber

feedstuffs such as PKM to broilers. It has been reported that, dietary fibers are fermented in the caeca by caecal microorganisms which produces end products such as volatile fatty acids (VFAs) that play an important role in controlling the proliferation of pathogenic bacteria such as *Salmonella* spp. (van der Weilan, 2000). The assessment of high fiber feedstuffs fermentability by caecal and/or faecal microorganism can be measured by measuring the gas production (Menke *et al*., 1979), and a study conducted by McLean *et al*. (2004) showed that supplementation of enzymes in the high fiber diet had influenced the fermentation characteristic of the diet tested. Thus, it is also important to examine the extent of fiber fermentation in the caeca of broilers in response to enzyme supplementation, and it effects on microbial population in the caeca.

In general, the basis of inclusion of enzyme in PKM diet of broilers is to reduce the effect of non-starch polysaccharides in PKM especially to promote their growth performance through the increases in broilers' feed efficiency and digestibility. However, there is no report on their effect on the fermentation profiles of PKM diet in the caeca of broilers and on caecal bacteria population. The use of exogenous enzymes could improved the nutritional value of broilers diet, facilitate the growth of beneficial gut microflora of broilers and consequently, improve the utilization of feeds by maximizing the conversion of dietary nutrients into broilers meat (Bedford and Coweison, 2012). Thus, this study was conducted to study the effect of dietary enzymes supplementation on early growth performance, caecal fermentation profiles and caecal bacteria population of broilers fed with PKM diet.

1.1 RESEARCH HYPOTHESIS

The supplementation of xylanase and cellulase in PKM diet fed to broilers will influence the caecal fermentation characteristics of PKM diet, will improve the early growth performances of broilers, and will also reduce the population of pathogenic bacteria in the caecum.

1.2 OBJECTIVES

i. To examine the fermentation profiles of caeca in response to xylanase and cellulase supplementation.

ii. To determine the effect of xylanase and cellulase supplementation in PKMdiet on the early growthperformance of the broilers.

iii. To determine the effect of xylanase and cellulasesupplementation in PKMdiet on caecal bacteria counts and population through culture method and real-time PCR analysis.

iv. To determine the effect of xylanase and cellulase supplementation in PKMdiet on the VFAs production of caecal digesta.

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