



UNIVERSITI PUTRA MALAYSIA

***EFFECTS OF POSTBIOTIC METABOLITES PRODUCED BY
Lactobacillus plantarum AND ACIDIFIER ON WEIGHT
AND MEAT QUALITY OF BROILER CHICKENS***

ROSYIDAH MOHD RADZI

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By

ROSYIDAH MOHD RADZI

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

November 2015

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of
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Faculty : Agriculture

Postbiotic metabolite produced by *Lactobacillus plantarum* is intermediate and final metabolic product of bacteria. There have antibacterial effect from organic acids and bacteriocin. Acidifier known as feed additive to improve performance of broiler chicken. The studies investigate the synergistic effect of combination of postbiotic metabolite produced from *L. plantarum* strains with acidifier on broiler chicken. Three experiments were conducted to achieve the objectives. Experiment I was conducted to select the best combinations by determination of inhibitory activity assessment. Six strains of *Lactobacillus plantarum* (UL4, TL1, RS5, RI11, RG11 and RG14) and 0.1% until 0.5% concentrations of acidifiers (malic acid and citric acid) were used in this study. Seventy six of the postbiotic metabolite and acidifier treatment were produced. The inhibitory activity against *Pediococcus acidilactici*, *Salmonella enterica*, *Escherichia coli* and *Listeria monocytogenes* of UL4 combine with 0.5% malic acid, RG14 combine with 0.5% malic acid and TL1 combine with 0.4% citric acid showed higher score than the other treatments. Therefore, these treatments were selected for further studies. Experiment II was carried out to determine the effects of combinations postbiotic metabolite with acidifier on the growth performance, fecal pH, microflora counts, villi height and crypt depth. A total of 150 male Cobb broilers were raised for 42 days. The birds were randomly divided into 5 groups and fed with various treatment diets: (i) basal diet (control); (ii) basal diet supplemented with antibiotic (positive control) (iii) basal diet supplemented with 0.3% of UL4 + 0.5% malic acid (iv) basal diet supplemented with 0.3% of RG14 + 0.5% malic acid and (v) basal diet supplemented with 0.3% of TL1 + 0.4% citric acid. Feeding of combination postbiotic metabolites with acidifier and control group did not show significant different ($p>0.05$) for growth performance and lactic acid bacteria counts. *Enterobacteriaceae* (ENT) count result showed that UL4 + 0.5% MA and RG14+0.5%MA did not show significant different ($p>0.05$) with positive control. Combination of RG14 + 0.5% MA showed better effect than control group for histopathology of small intestine. The results indicate that, the inclusion combination of postbiotic metabolite and acidifiers have shown potential effect similar to antibiotic diet group for growth performance, faecal pH, and lactic acid bacteria population. Besides that, it also reduced the population of *Enterobacteriaceae* and increase villus height of small intestine. Overall, combination of RG14 + 0.5% malic acid prominently shows improved effect as compared to other

treated diets. Experiment III was carried out to determine the carcass composition and meat quality of chickens fed with combination of postbiotic metabolite with acidifier. This experiment was continued from second experiment. This study was conducted to examine the effects of feeding postbiotic metabolites which produced from *L. plantarum* and acidifier in the diets of broilers chickens on carcass composition and meat quality. Generally, combination UL4 + 0.5% MA and RG14 + 0.5% MA had shown similar effect as control group for carcass weight, meat percentage and meat quality traits. Nevertheless, the supplementation of combination postbiotic metabolites and acidifier significantly reduced ($p<0.05$) abdominal fat and increased ($p<0.05$) percentage of bone. Thus, these findings suggested that postbiotic metabolite produced by locally isolated *L. plantarum* combine with acidifier is potential alternative feed additives in poultry production.

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

KESAN PEMBERIAN KOMBINASI POSTBIOTIK METABOLIT DARI STRAIN *Lactobacillus plantarum* DAN PENGASID TERHADAP BERAT DAN KUALITI DAGING AYAM PEDAGING

Oleh

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Postbiotik metabolit yang dihasilkan daripada *Lactobacillus plantarum* merupakan produk pertengahan dan produk akhir ketika metabolisme bakteria. Ia mempunyai kesan yang bermanfaat seperti asid organik dan bakteriosin. Pengasid merupakan makanan tambahan untuk meningkatkan prestasi ayam pedaging. Kajian ini adalah untuk mengkaji kesan kesinambungan diantara kombinasi postbiotik metabolit yang dihasilkan daripada strain *L. plantarum* dengan pengasid terhadap ayam pedaging. Tiga eksperimen dilakukan bagi mencapai objektif ini. Eksperimen I dijalankan bagi memilih kombinasi yang terbaik dengan penentuan penilaian aktiviti perencutan. Enam strain daripada *Lactobacillus plantarum* (UL4, TL1, RS5, RI11, RG11 and RG14) dan 0.1% sehingga 0.5% kepekatan pengasid (asid malik dan asid sitrik) digunakan di dalam kajian ini. Tujuh puluh enam rawatan postbiotik metabolit dan pengasid dihasilkan. Aktiviti perencutan terhadap *Pediococcus acidilactici*, *Salmonella enterica*, *Escherichia coli* dan *Listeria monocytogenes* oleh kombinasi UL4 dengan 0.5% asid malik, kombinasi RG14 dengan 0.5% asid malik dan kombinasi TL1 dengan 0.4% asid sitrik menunjukkan jumlah skor tertinggi berbanding rawatan yang lain. Maka, rawatan(rawatan ini dipilih untuk kajian yang seterusnya. Eksperimen II dijalankan untuk menentukan kesan kombinasi postbiotik metabolit dengan pengasid terhadap prestasi pertumbuhan, pH dan bilangan mikroflora tinja, dan ketinggian villus dan kedalaman krip. Sejumlah 150 ayam pedaging jantan baka Cobb dipelihara untuk tempoh 42 hari. Ayam dipilih secara rawak dan dibahagikan kepada 5 kumpulan rawatan makanan seperti berikut; (i) diet asas (kawalan); (ii) diet asas ditambah dengan antibiotik (kawalan positif) (iii) diet asas ditambah dengan 0.3% kombinasi UL4 + 0.5% asid malik (iv) diet asas ditambah dengan 0.3% kombinasi RG14 + 0.5% asid malik dan (v) diet asas ditambah dengan 0.3% kombinasi TL1 + 0.4% asid sitrik. Pemberian makanan kombinasi postbiotik metabolit dengan pengasid dan kumpulan kawalan tidak menunjukkan perbezaan yang ketara ($p>0.05$) untuk prestasi tumbesaran dan bilangan bakteria asid laktik. Keputusan bilangan *Enterobacteriaceae* (ENT) menunjukkan UL4 + 0.5% MA dan RG14 + 0.5% MA tidak menunjukkan perbezaan yang ketara ($p>0.05$) dengan kawalan positif. Kombinasi RG14 + 0.5% MA menunjukkan kesan yang lebih baik daripada kumpulan kawalan untuk histopatologi usus kecil. Keputusan ini menunjukkan bahawa pemberian kombinasi postbiotik metabolit dan pengasid mempunyai kesan potensi yang sama

seperti kumpulan diet antibiotik untuk prestasi tumbesaran, pH tinja dan populasi bacteria asid laktik. Disamping itu, ia juga mengurangkan populasi *Enterobacteriaceae* (ENT) dan meningkatkan ketinggian villus usus kecil. Secara keseluruhannya, kombinasi RG14 + 0.5% MA menunjukkan kesan peningkatan berbanding diet yang lain. Eksperimen III dilakukan bagi penentuan komposisi karkas dan kualiti terhadap daging ayam pedaging yang diberi makan dengan kombinasi postbiotik metabolit dan pengasid. Kajian ini dilakukan berterusan daripada eksperimen kedua. Eksperimen ini dilakukan bagi mengkaji kesan kombinasi postbiotik metabolit yang dihasilkan daripada *L. plantarum* dan pengasid di dalam pemberian makanan ayam pedaging terhadap komposisi karkas dan cirri- cirri kualiti daging. Secara amnya, kombinasi UL4 + 0.5% MA dan RG14 + 0.5% MA menunjukkan kesan yang sama seperti kumpulan kawalan untuk berat karkas, peratus daging dan ciri-ciri kualiti daging. Walaubagaimanapun, penambahan kombinasi postbiotik metabolit dan pengasid menunjukkan pengurangan yang ketara ($p<0.05$) terhadap lemak abdomen dan peningkatan ($p<0.05$) peratus tulang. Oleh itu, penemuan ini, mencadangkan postbiotik metabolit yang dihasilkan dari pengasingan setempat *L. plantarum* dan dikombinasikan dengan pengasid berpotensi sebagai alternatif makanan tambahan di dalam produksi poltri.

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I certify that a Thesis Examination Committee has met on (9 November 2015) to conduct the final examination of Rosyidah Bt Mohd Radzi on her thesis entitled "Effect of Postbiotic Metabolite Produced by *Lactobacillus plantarum* and Acidifier on Weight and Meat Quality of 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 Degree of Master.

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LIST OF ABBREVIATIONS

°C	Degree Celsius
µL	Microliter
a*	Redness
ADG	Average daily gain
AGP	Antibiotic growth promoter
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
AU	Arbitrary unit
b*	Yellowness
BW	Body weight
CFLX	Colorflex
CFS	Cell free supernatant
CFU	Colony forming unit
CL	Cooking loss
cm	Centimeter
CO ₂	Carbon Dioxide
d	Day
DFD	Dark-Firm-Dry
DL	Drip loss
ENT	Enterobacteriaceae
FCR	Feed conversion ratio
Fe	Ferum
FI	Feed intake
g	Gram

GIT	Gastrointestinal tract
GLM	General linear model
GRAS	Generally recognize as safe
h	Hour
IB	Infectious bronchitis
IBD	Infectious bursal disease
kcal	Kilocalories
kg	kilogram
L	Liter
L*	Lightness
LAB	Lactic acid bacteria
M	Molar
mg	Milligram
mL	Mililiter
mM	Milimolar
MRS	DE Man, Rogosa and Shape
ND	Newcastle Disease
nm	Nanometer
OD	optical density
ppm	Part per milion
PSE	Pale-Soft-Exudative
rpm	Rounds per minute
SAS	Statistical analysis software
SEM	Standard Error Mean
TA-HD	Texture Analyser High density

USA	United States of America
VFA	Volatile Fatty Acid
VRE	Vancomycin-resistant enterococci
w/v	Weight versus volume
w/w	Weight versus weight
W1	Weight before
W2	Weight after
WG	Weight gain
WHC	Water Holding capacity

CHAPTER I

INTRODUCTION

Antibiotic is widely used as a promoter since a long time ago and is considered as an important feed additive due to their property as growth promoters and in livestock health. However, some issues have arisen with the use of antibiotics as growth promoters that were previously used to treat or prevent diseases. The issues are about antibiotic developing resistance to many pathogenic diseases and also the presence antibiotic residue. It has been shown that faecal *E. coli* isolated from pigs was resistant to antibiotics such as neomycin, oxytetracycline, chloramphenicol and nalidixic acid (Loh *et al.*, 2006; van den Bogaard *et al.*, 2001). Furthermore, the residue of antibiotics is frequently present in the end products of animal production (van den Bogaard *et al.*, 2001). This has led many countries to ban the use of antibiotics.

In view of the total ban in the use of antibiotics as growth promoters in livestock and poultry production, many parts of the world are experimenting alternative feed additive that may be used to alleviate the problems associated with the withdrawal of antibiotics from feed. Alternative strategies that had been proposed should have a favourable effect on livestock production, without endangering animal or human health, as well as nature. The level must be controllable, the levels used for the purpose of preventing and treating disease should be different, as well as for medical and veterinary purposes (Butaye *et al.*, 2003).

Among the alternatives that has been used are acidifiers. Several organic acids, including citric acid, fumaric acid, formic acid, and propionic acids have a positive influence on growth performance (Partanen and Mroz, 1999). However, the result obtained of acids used in poultry is not consistent (Thompson and Hinton, 1997).

The final or intermediate fermented products produced by *L. plantarum* also known as postbiotic metabolite have natural antibacterial properties. These postbiotic metabolites contains bacteriocin, organic acids and commonly used as food preservatives. Therefore, it has been reported that the postbiotic metabolites can be used as feed additive to replace in-feed antibiotics in the poultry industry (Thanh *et al.*, 2009; Loh *et al.*, 2009).

Many enquiries are raised due to the use of acidifier and postbiotic metabolite as feed additive in the farm. However, the information about the combination of acidifier and postbiotic metabolites produced by 6 available strains locally isolated *L. plantarum* is still unknown. Furthermore, their ability in against pathogenic bacteria such as *Salmonella* spp., *E.coli*, *Listeria* spp. and *Pediococcus* spp. have not been investigated. Similarly, their impact on growth performance, gut microflora, small intestine morphology, and carcass and meat quality also need to be studied. The 6 strains of postbiotic metabolites were obtained by Foo *et al.* (2001) and patented by the Faculty of Biotechnology and Biomolecular Science, Universiti Putra Malaysia.

The hypothesis of this study were; combination of acidifier and postbiotic metabolite produced by *L.plantarum* strains have inhibitory activity against certain pathogens, which can improve growth performance, reduce intestinal *Enterobacteriaceae* (ENT) and increase intestinal lactic acid bacteria (LAB) population, increase villi height, improve carcass composition and meat quality of broilers. Thus, the objectives of this study were;

- i) To quantify the inhibitory activity of acidifier and postbiotic metabolites produced by *Lactobacillus plantarum* (*L. plantarum*) on *E. coli*, *L. monocytogenes*, *S. enterica* and *P. acidilactici* indicators and selection of the highest inhibitory activity.
- ii) To determine the effect of feeding a combination of acidifiers and postbiotic metabolites on growth performance and faecal microbial population, small intestine morphology of broiler chickens.
- iii) To evaluate the effect of feeding a combination of acidifiers and postbiotic metabolites on carcass composition and meat quality of broiler chickens.

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