



UNIVERSITI PUTRA MALAYSIA

***ENZYMATIC TREATMENT IN IMPROVING NUTRITIVE
VALUE OF LOCAL BROWN RICE FOR POULTRY FEED***

NURUL ASYIFAH MUSTAPHA

FBSB 2012 49

**ENZYMATIC TREATMENT IN IMPROVING NUTRITIVE
VALUE OF LOCAL BROWN RICE FOR POULTRY FEED**

The logo of Universiti Putra Malaysia (UPM) is a shield-shaped emblem. It features a central vertical element with a book at the top, flanked by two stylized wings or leaves. The shield is divided into several sections with different colors and patterns. The letters 'UPM' are prominently displayed in a red box at the top left of the shield.

NURUL ASYIFAH MUSTAPHA

MASTER OF SCIENCE

UNIVERSITI PUTRA MALAYSIA

2012

**ENZYMATIC TREATMENT IN IMPROVING NUTRITIVE VALUE
OF LOCAL BROWN RICE FOR POULTRY FEED**

By

NURUL ASYIFAH MUSTAPHA

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

November 2012

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in
fulfilment of the requirement for the degree of Master of Science

**ENZYMATIC TREATMENT IN IMPROVING NUTRITIVE VALUE
OF LOCAL BROWN RICE FOR POULTRY FEED**

By

NURUL ASYIFAH MUSTAPHA

November 2012

Chairman : Professor Suraini Abd Aziz, PhD

Faculty : Biotechnology and Biomolecular Sciences

Malaysian poultry industry depends heavily on expensive imported feed materials like soybean and maize. Local brown rice is a potential feed ingredient to replace these imported feeds. It is produced locally, easily available and low in production cost. Brown rice composed of nutrients required by poultry such as protein, carbohydrates and fats. However, it may also contain anti-nutrients that cause adverse effects to poultry performance. In this study, brown rice varieties MR239 and MR257 were investigated for their nutrients, anti-nutritional factors mainly non-starch polysaccharides (NSP) and its true metabolisable energy (TME) value. The enzymatic hydrolysis was carried out with the aim of eliminating the NSP and further optimize for production of hydrolyzed brown rice. The effects of enzyme supplementation were evaluated through true metabolisable energy (TME) value of poultry. The composition of both varieties

showed that they contained nutrient as required by the poultry such as protein content of 9.0% and 8.8%, carbohydrates contents of 87.6% and 87.7% for MR239 and MR257, respectively and balanced amino acids. The energy value of both varieties were also high represent by gross energy of 16.0 MJ/kg and 15.9 MJ/kg and also TME value of 12.2 MJ/kg and 15.5 MJ/kg for MR239 and MR257, respectively. The NSP was present in small amount as compared to other types of feedstuffs such as wheat and barley which represent by cellulose 1.6% and 0.1%, hemicelluloses 4.3% and 2.0%, arabinoxylan 0.042% and 0.03% and also β -glucan 0.2% and 0.3% for MR239 and MR257, respectively. The application of enzymes by enzymatic hydrolysis showed that xylanase, cellulase and β -glucanase were able to degrade the fibrous fraction of NSP in brown rice. The optimization of enzymatic hydrolysis condition was carried out using these three enzymes with activity range from 1 – 3 U/mL, temperature from 30 - 50°C, pH of 4 - 6 and substrate concentration of 5 – 15% w/v. The range of each factor was set based on preliminary experiment. The significant factors screened using two-level factorial design was temperature, pH, substrate and cellulase activity. These parameters were optimized by central composite design and produced optimal value of pH 4.5, temperature of 40°C, 12.5% w/v of substrate concentration and 2 U/mL of cellulase activity. Hydrolyzed brown rice was produced based on these optimized conditions. The effectiveness of enzymes supplementation was evaluated by determination of chemical composition and conducted the force feeding experiment for TME value. The composition of hydrolyzed brown rice has shown the improvement in the nutrients content by

increase in protein content as 11.2%, fat as 2.7% and also the reduction in NSP components to 3.0% cellulose, 1.3% hemicelluloses, 0.4% β -glucan and 0.05% arabinoxylan after enzymatic hydrolysis process. The energy value has been increased for hydrolyzed brown rice. The TME value of hydrolyzed substrate was 16.1 MJ/kg as compared to TME of untreated brown rice which was 13.9 MJ/kg. Local brown rice MR239 and MR257 that were not suitable for human consumption was selected for poultry feed as it has potential to replace the imported feed. The results showed the nutritive values of brown rice were improved with the treatment of enzymes.



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

**RAWATAN BERENZIM UNTUK PENINGKATAN NILAI NUTRIEN
BERAS PERANG TEMPATAN SEBAGAI MAKANAN AYAM**

Oleh

NURUL ASYIFAH MUSTAPHA

November 2012

Pengerusi : Profesor Suraini Abd Aziz, PhD

Fakulti : Bioteknologi dan Sains Biomolekul

Industri ayam di Malaysia sangat bergantung kepada sumber makanan import yang mahal seperti soya dan jagung. Beras perang tempatan adalah sumber makanan yang berpotensi untuk menggantikan makanan ayam yang diimport ini. Ia dihasilkan dalam negara, mudah didapati dan kos pengeluaran yang rendah. Beras perang mengandungi nutrien yang diperlukan oleh ayam seperti protin, karbohidrat dan lemak. Walau bagaimanapun, ia juga mungkin mengandungi anti-nutrien yang menyebabkan kesan buruk terhadap prestasi ayam. Dalam kajian ini, beras perang MR239 dan MR257 dikaji dari segi nutrien dan anti-nutrien terutamanya polisakarida bukan kanji (PBK) dan nilai tenaga metabolisma sebenar (TME) sebagai makanan ayam. Hidrolisis berenzim telah

dijalankan dengan tujuan menghapuskan PBK dan diteruskan dengan proses pengoptimuman untuk pengeluaran beras perang terhidrolisis. Kesan daripada penambahan enzim dinilai melalui nilai tenaga metabolisme sebenar (TME) ayam. Komposisi kedua-dua varieti menunjukkan bahawa ia mengandungi nutrien yang diperlukan oleh ayam seperti kandungan protein 8.96% dan 8.79%, kandungan karbohidrat 87.58% dan 87.71% masing-masing untuk MR239 dan MR257 dan asid amino yang seimbang. Nilai tenaga bagi kedua-dua varieti juga tinggi yang diwakili oleh tenaga kasar 16.03 MJ/kg dan 15.89 MJ/kg serta nilai TME 12.15 MJ/kg and 15.45 MJ/kg masing-masing untuk MR239 dan MR257. PBK hadir dalam jumlah yang kecil berbanding dengan jenis makanan lain yang digunakan untuk ayam seperti gandum dan barli yang ditunjukkan oleh selulose 1.63% dan 0.08%, hemiselulose 4.25% dan 1.97%, arabinoxylan 0.042% dan 0.03% serta β -glucan 0.17% and 0.29% masing-masing untuk MR239 and MR257. Penambahan enzim kepada beras perang melalui hidrolisis berenzim menunjukkan bahawa xilanase, selulase dan β -glukanase dapat merendahkan pecahan berserabut PBK dalam beras perang. Pengoptimuman keadaan hidrolisis berenzim telah dijalankan menggunakan tiga enzim ini dengan aktiviti berbeza daripada 1 - 3 U/mL bersama-sama dengan julat suhu 30-50°C, pH 4 - 6 dan kepekatan substrat daripada 5 - 15% w/v. Julat setiap faktor ditentukan melalui eksperimen awal. Faktor penting yang disaring menggunakan reka bentuk faktorial dua peringkat adalah suhu, pH, kepekatan substrat dan aktiviti selulase. Parameter ini dioptimumkan oleh reka bentuk komposit pusat dan menghasilkan nilai optimum pH 4.5, suhu 40°C, kepekatan substrat 12.5% w/v

dan 2 U/mL aktiviti selulase. Beras perang terhidrolisis dihasilkan berdasarkan syarat optimum melalui hidrolisis berenzim. Keberkesanan penambahan enzim telah dinilai oleh penentuan komposisi kimia dan melalui kajian suapan paksa untuk nilai TME. Komposisi beras perang terhidrolisis menunjukkan peningkatan dalam kandungan nutrien dengan peningkatan protein menjadi 11.2%, lemak 2.65% dan juga penurunan dalam komponen PBK menjadi 2.99% selulose, 1.3% hemiselulose, 0.4% β -glucan dan 0.05% arabinoxylan selepas proses hidrolisis berenzim. Nilai tenaga telah meningkat dalam beras perang terhidrolisis. Nilai TME substrat terhidrolisis adalah 16.1 MJ/kg berbanding dengan TME beras perang yang tidak dirawat adalah 13.9 MJ/kg. Beras perang tempatan variasi MR239 dan MR257 yang tidak sesuai untuk kegunaan manusia telah dipilih sebagai makanan ayam kerana ia mempunyai potensi untuk menggantikan makanan import. Keputusan menunjukkan nilai nutrient beras perang telah dipertingkatkan dengan rawatan berenzim.

ACKNOWLEDGEMENTS

Alhamdulillah, I am very grateful to Allah S.W.T with His permission and Blessing, I have completed my master project entitled “Enzymatic Improvement of Nutritional Value of Brown Rice for Poultry”. I would like to express gratitude to my supervisor, Prof. Dr. Suraini Abd Aziz for her suggestions and guidance, advices and approval for this project. My most sincere thank to my co-supervisors, Dr. Phang Lai Yee and Puan Azlian Mohamad Nazri for their guidance and supports.

A special thank also to all members of Laboratory of Livestock Feed Bioprocess, Strategic Livestock Research Centre MARDI, laboratory at Biotech 3 and MTDC, UPM for their guidance, help and encouragement. I wish to thank my course mate and all master and PhD students for their support and motivation throughout this project. Also a million thank to my late parents, my beloved family, and husband for always believing in me and encouraging me in preceding my dream and to all individual who have contributed in this project.

I certify that a Thesis Examination Committee has met on 9 November 2012 to conduct the final examination of Nurul Asyifah Mustapha on her thesis entitled “Enzymatic Treatment in Improving Nutritive Value of Local Brown Rice for Poultry Feed” 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.

Members of the Thesis Examination Committee were as follows:

Rosfarizan Mohamad, PhD

Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Norhani Abdullah, PhD

Professor
Institute of Tropical Agriculture
Universiti Putra Malaysia
(Internal Examiner)

Norhafizah Abdullah, PhD

Associate Professor
Faculty of Engineering
Universiti Putra Malaysia
(Internal Examiner)

Amirul Al-Ashraf Abdullah, PhD

Associate Professor
School of Biological Sciences
Universiti Sains Malaysia
(External Examiner)

SEOW HENG FONG, PhD

Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as the fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Suraini Abd. Aziz, PhD

Professor,
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Chairman)

Phang Lai Yee, PhD

Senior Lecturer,
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

Azlian Mohamad Nazri,

Strategic Livestock Research Centre
Malaysian Agriculture of Research and Development Institute (MARDI)
(Member)

BUJANG BIN KIM HUAT

Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledge. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Putra Malaysia or other institution.



NURUL ASYIFAH MUSTAPHA

Date: 9 November 2012

TABLE OF CONTENTS

	Page
ABSTRACT	ii
ABSTRAK	v
ACKNOWLEDGEMENTS	viii
APPROVAL	ix
DECLARATION	xi
LIST OF TABLES	xvi
LIST OF FIGURES	xvii
LIST OF ABBREVIATIONS	xviii
CHAPTER	
1 INTRODUCTION	1
2 LITERATURE REVIEW	
2.1 Poultry feedstuffs	6
2.2 Paddy rice	9
2.2.1 White rice	12
2.2.2 Brown rice	13
2.2.3 Rice by-products	14
2.3 Composition of feed	15
2.3.1 Nutrients requirement in poultry	15
2.3.2 Nutrient limitation by non-starch polysaccharides (NSP) to the poultry	17
2.4 Methods to reduce anti-nutritive components	20
2.4.1 Physical methods	20
2.4.2 Chemical methods	22
2.4.3 Biological methods	23
2.5 Application of supplemental enzymes to improve nutrient digestibility and utilization	26
2.5.1 Feed enzymes	26
2.5.2 Common exogenous enzymes used in poultry feed	28
2.6 Enzymatic hydrolysis	32

2.7	Statistical analysis	37
2.7.1	Experimental factorial designs	37
2.7.2	Response Surface Methodology (RSM)	38
2.7.3	Evaluation of the fitted model	41
2.7.4	Comparison of response surface methodology and conventional methods	42
2.8	Energy evaluation in poultry	43
2.8.1	Apparent metabolisable energy (AME)	43
2.8.2	True metabolisable energy (TME)	45
3	MATERIALS AND METHODS	
3.1	Substrate	47
3.2	Enzymes	48
3.3	Brown rice compositions determination	48
3.3.1	Proximate composition	49
3.3.1.1	Dry matter determination	49
3.3.1.2	Ash determination	50
3.3.1.3	Fat determination	50
3.3.1.4	Crude protein determination	51
3.3.2	Amino acid composition	52
3.3.2.1	Determination of all amino acids except cystine, methionine and tryptophan	52
3.3.2.2	Determination of cystine and methionine	53
3.3.2.3	Determination of tryptophan	53
3.3.3	True metabolisable energy (TME)	54
3.3.3.1	Experimental procedures	55
3.3.3.2	Endogenous energy	55
3.3.3.3	Forced feeding technique	56
3.3.3.4	Output energy	56
3.3.3.5	Intake energy	56
3.3.3.6	Gross energy assay	57
3.3.3.7	TME calculation	57
3.3.4	Non-starch polysaccharides determination	58
3.3.4.1	Crude fibre	58
3.3.4.2	Neutral detergent fibre (NDF)	60
3.3.4.3	Acid detergent fibre (ADF)	61
3.3.4.4	Acid detergent lignin	63
3.3.4.5	Arabinoxylan	64
3.3.4.6	β -glucan	65

3.4	Optimization of enzymatic hydrolysis conditions of brown rice	66
3.4.1	Enzymatic hydrolysis	66
3.4.2	Screening of parameters using two-level factorial design	67
3.4.3	Central composite design (CCD)	68
3.4.4	Statistical analysis and modeling	70
3.4.5	Validation of optimized parameters	70
3.5	Analytical assays	71
3.5.1	Cellulase assay	71
3.5.2	Xylanase assay	72
3.5.3	β -glucanase assay	73
3.5.4	Reducing sugar analysis	74
3.6	Evaluation of hydrolyzed brown rice on true metabolisable energy (TME) of poultry	75
3.6.1	Preparation of hydrolyzed brown rice	75
3.6.2	Compositions analysis	75
3.6.3	True metabolisable energy (TME) value	76
3.6.4	Statistical analysis	76
4	RESULTS AND DISCUSSION	
4.1	Nutrients composition of local brown rice MR239 and MR257	77
4.1.1	Proximate composition	77
4.1.2	True Metabolisable Energy	83
4.1.3	Amino acids composition	85
4.2	Non-starch polysaccharides	88
4.3	Preliminary enzymatic hydrolysis	92
4.3.1	Effect of enzymes addition on enzymatic hydrolysis	93
4.3.2	Effect of substrate on enzymatic hydrolysis	96
4.3.3	Effect of temperature on enzymatic hydrolysis	98
4.3.4	Effect of pH on enzymatic hydrolysis	99
4.4	Optimization of parameters for enzymatic hydrolysis of brown rice	101
4.4.1	Two-level factorial design	101
4.4.2	Central composite design	105
4.4.3	Validation experiment of optimized parameters	114
4.5	Evaluation of hydrolyzed brown rice	115
4.5.1	Composition of hydrolyzed brown rice	115
4.5.2	TME of raw brown rice and hydrolyzed brown rice	122

5	SUMMARY, GENERAL CONCLUSION AND RECOMMENDATION FOR FUTURE RESEARCH	127
5.1	Summary	127
5.2	Conclusion	129
5.3	Recommendations for future works	131
	REFERENCES	132
	APPENDICES	148
	BIODATA OF STUDENT	154
	LIST OF PUBLICATIONS	155

