



**UNIVERSITI PUTRA MALAYSIA**

***Kluyveromyces marxianus FROM FERMENTED RICE AND IRANIAN WINE AS MICROBIAL FEED ADDITIVE FOR RUMINANTS***

**SEYED EEMAN NOORAE**

**ITA 2013 7**



***Kluyveromyces marxianus* FROM FERMENTED RICE AND IRANIAN WINE  
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By

**SEYED EEMAN NOORAE**

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

**June 2013**

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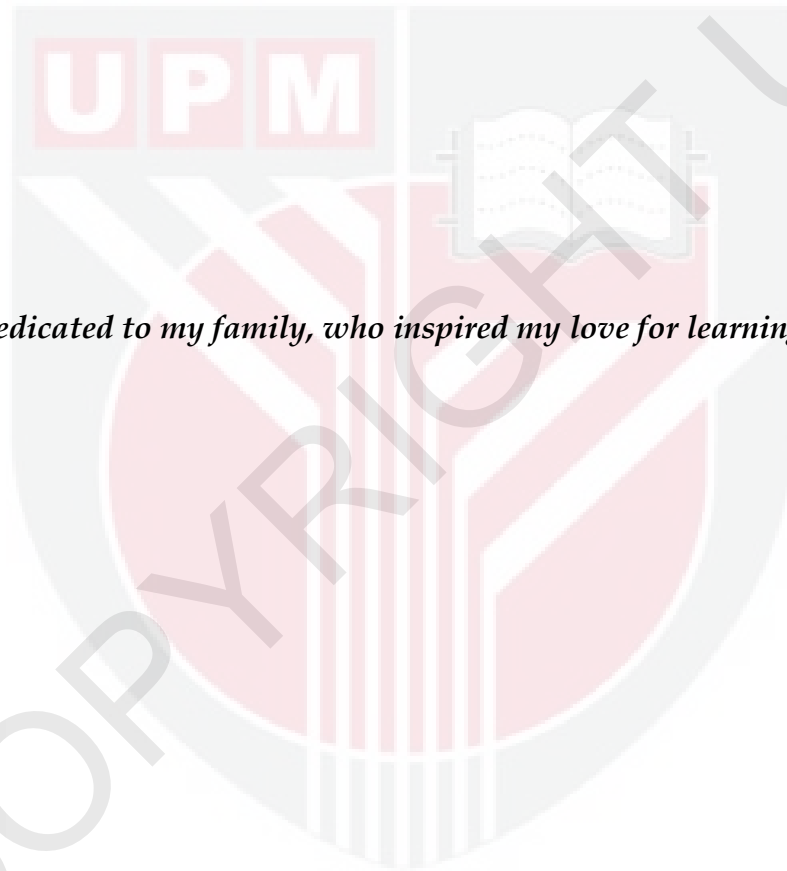
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## DEDICATION

*Dedicated to my family, who inspired my love for learning*



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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy.

***Kluyveromyces marxianus* FROM FERMENTED RICE AND IRANIAN WINE  
AS MICROBIAL FEED ADDITIVE FOR RUMINANTS**

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**SEYED EEMAN NOORAE**

**June 2013**

**Chair: Professor Abdul Razak Alimon, PhD**

**Institute: Institute of Tropical Agriculture**

Feeding to manipulate rumen microbial ecosystem and to enhance overall rumen fermentation in order to improve production efficiency of ruminant animals is a main concern for animal nutritionists and rumen microbiologists. Microbial feed additive would be a possible solution.

A series of experiments was conducted to isolate and characterize a suitable yeast from traditional fermented rice (Tapai) and home-made wine as a microbial feed additive for ruminants.

Yeast isolates were selected based on their tolerance to volatile fatty acids (VFA) mixture of acetic, propionic and butyric acids and to pH and temperature according to the rumen condition. The ability to grow and produce ethanol was determined in yeast extract peptone glucose broth supplemented with a VFA mixture. Fifty-five

isolates showed optical density,  $OD_{660nm}$ , values between 0.35-0.60 and 27 isolates showed ethanol production in the range of 0.17-0.30 (%v/v). All 27 isolates from Tapai and wine were identified as *Kluyveromyces marxianus* based on biochemical tests or molecular identification using the rDNA nontranscribed spacer 2 region. The best isolate, *Kluyveromyces marxianus* WJ1, in terms of ethanol production and co-utilization of different carbon source with D-xylose was selected for further evaluations.

In a 4×4 factorial experiment the effect of alfalfa hay and corn grain ratios (100:0; 70:30; 50:50 and 30:70, respectively) and optical density (OD) of *Kluyveromyces marxianus* WJ1 cell suspension (OD:0.0, OD: 0.1, OD: 0.2 and OD:0.3) as a microbial feed additive on *in vitro* gas production were examined. Gas production was affected by *K. marxianus* WJ1 (+11.7%;  $P<0.01$ ) and substrate significantly ( $P<0.01$ ). Interaction of *K. marxianus* WJ1 by substrate was significant ( $P<0.01$ ). Diphasic model was applied to explain the interaction between *K. marxianus* WJ1 and substrate.

In a completely randomized design, five plant materials, i.e. alfalfa (*Medicago sativa*), guinea grass (*Panicum maximum*), bermuda grass (*Cynodon dactylon*), rice straw (*Oryza sativa*) and timothy hay (*Phleum pretense*) were used to evaluate the effect of different *K. marxianus* WJ1 cells suspensions ( OD: 0.0, OD: 0.1, OD: 0.2 and OD:0.3) on *in vitro* apparent dry matter digestibility. The *in vitro* apparent dry matter digestibility of alfalfa (55.0% vs 51.3%), guinea grass (61.0% vs 52.1%) and timothy hay (72.2% vs 62.1%) was improved significantly ( $P<0.01$ ).

The effect of *K. marxianus* WJ1 on nutrient digestibility, ruminal fermentation and rumen microbial population in goats was evaluated. Treatments were: control (basal

diet), basal diet plus 1g freeze dried *K. marxianus* WJ1 and basal diet plus 5g freeze dried *K. marxianus* WJ1. Total volatile fatty acid production (92.2 mM vs 82.8 mM;  $P<0.01$ ) and acetic acid production (66.8% vs 63.8%;  $P<0.05$ ) were increased when *K. marxianus* WJ1 (1g) and (5g) was included in basal diet respectively. Ruminal ammonia nitrogen and total protozoa count were reduced significantly ( $P<0.05$ ) when experimental diet was supplemented by *K. marxianus* WJ1.

A production experiment was conducted to investigate the effect of *K. marxianus* WJ1 on nutrient digestibility and daily weight gain in goats. Treatments were: basal diet (control) and basal diet plus 1g freeze dried *K. marxianus* WJ1. Organic matter digestibility was increased significantly ( $P<0.05$ ) compared with control. However, daily weight gain was not significantly affected by treatment.

It can be concluded that based on *in vitro* and *in vivo* studies, *K. marxianus* WJ1 could be considered as a potential microbial feed additive for ruminants.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

***Kluyveromycesmarxianus* DARIPADA BERAS DIPERAM DAN WAIN IRAN  
SEBAGAI BAHAN ADITIF MAKANAN MIKROB UNTUK RUMINAN**

Oleh

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Pemberian makanan untuk memanipulasi ekosistem mikrob rumen bagi meningkatkan penapaian rumen keseluruhan dan memaksimumkan kecekapan pengeluaran haiwan ruminan sering menjadi fokus kajian utama bagi ahli pemakanan haiwan dan mikrobiologi rumen. Bahan aditif makanan mikrob boleh menjadi salah satu penyelesaiannya.

Satu siri eksperimen telah dijalankan untuk mengasingkan ciri-ciri yang sesuai dari yis penapaian beras tradisional (Tapai) dan wain buatan sendiri sebagai bahan tambahan makanan mikrob untuk ruminan.

Isolat yis yang telah dipilih berdasarkan toleransi kepada campuran asid lemak meruap (VFA) asid asetik, propionik dan butyric, pH dan suhu mengikut keadaan rumen. Keupayaan untuk berkembang dan menghasilkan etanol telah



ditentukan dalam ekstrak yis pepton broth glukosa ditambah dengan campuran VFA. Lima puluh lima isolat telah menunjukkan ketumpatan optik, nilai OD<sub>660nm</sub>, antara 0.35-0.60 dan 27 isolat menunjukkan pengeluaran etanol dalam julat 0.17-0.30 (%v/v). Kesemua 27 isolat dari tapai dan wain telah dikenal pasti sebagai *Kluyveromyces marxianus* berdasarkan ujian biokimia atau pengenalan molekul menggunakan rDNA spacer non-transcribed2 rantau. Isolat yang terbaik, *Kluyveromyces marxianus* WJ1, dari segi pengeluaran etanol dan penggunaan bersama sumber karbon yang berbeza dengan D-xylose telah dipilih untuk penilaian selanjutnya.

Dalam ujikaji 4 × 4 faktorial kesan nisbah hay alfalfa dan bijirin jagung (100:0; 70:30; 50:50 dan 30:70, masing-masing) dan ketumpatan optik suspensi (OD) *Kluyveromyces marxianus* WJ1 (OD: 0.0, OD: 0.1, OD: 0.2 dan OD: 0.3) sebagai bahan tambahan makanan mikroba ke atas pengeluaran gas *in vitro* telah diteliti. Pengeluaran gas telah meningkat dengan ketara disebabkan oleh *K. marxianus* WJ1 (+11.7%; P <0.01) dan substrat (P <0.01). Interaksi *K. marxianus* WJ1 dengan substrat adalah signifikan (P <0.01). Model Di-phasic telah digunakan untuk menerangkan interaksi antara *K. marxianus* WJ1 dan substrat.

Dalam ujikaji reka bentuk rawak, limabahan tumbuhan, iaitu alfalfa (*Medicago sativa*), rumput guinea (*Panicum maximum*), rumput bermuda (*Cynodon dactylon*), beras jerami (*Oryza sativa*) dan timothy hay (*Phleum pratense*) telah digunakan untuk menilai kesan yang berbeza *K. marxianus* WJ1 suspensi sel (OD: 0.0, OD: 0.1, OD: 0.2 dan OD: 0.3) di dalam *in vitro* jelas perkara penghadaman kering. Dalam *in vitro* jelas penghadaman bahan kering alfalfa (55.0% vs 51.3%), rumput guinea (61.0% vs 52.1%) dan timothy hay (72.2% vs 62.1%) telah meningkat dengan ketara (P <0.01).

Kesan *K. marxianus* WJ1 pada pencernaan nutrien, penapaian rumen dan populasi mikrob rumen di kambing telah dinilai. Rawatanyang digunakan adalah; 1) kawalan (diet basal), 2) diet basal ditambah 1g pengeringan beku *K. marxianus* WJ1 dan pemakanan basal ditambah 5g *K. marxianus* WJ1 dibeku kering. Jumlah menentu pengeluaran asid lemak (92.2 mM vs 82.8mM; P <0.01) dan pengeluaran asid asetik (66.8% vs 63.8%; P <0.05) telah meningkat apabila *K. marxianus* WJ1 (1g) dan (5g) telah dimasukkan ke dalam diet basal masing-masing. Kandungan nitrogen ammonia rumen dan kiraan jumlah protozoa telah menurun dengan ketara (P <0.05) apabila makanan eksperimen telah ditambah dengan *K. marxianus* WJ1.

Seterusnya, satu eksperimen pengeluaran telah dijalankan untuk mengkaji kesan *K.marxianus* WJ1 pada pencernaan nutrien dan peningkatan berat badan harian kambing. Rawatan yang digunakan adalah:diet basal (kawalan) dan diet basal ditambah 1g *K. marxianus* WJ1 dibeku kering. Pencernaan bahan organik telah meningkat dengan ketara (P <0.05) berbanding dengan kawalan. Walau bagaimanapun, peningkatan berat badan harian tidak terjejas oleh diet yang diberikan.

Kesimpulannya, berdasarkan kajian *in vitro* dan *in vivo*, *K. Marxianus* WJ1 boleh dianggap sebagai mikrob berpotensi untuk bahan aditif makanan untuk ruminan.

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I certify that a Thesis Examination Committee has met on 14 June 2013 to conduct the final examination of Seyed Eeman Noorae on his thesis entitled “*Kluyveromyces marxianus* from Fermented Rice and Iranian Wine as Microbial Feed Additive for Ruminants” in accordance with 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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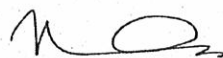
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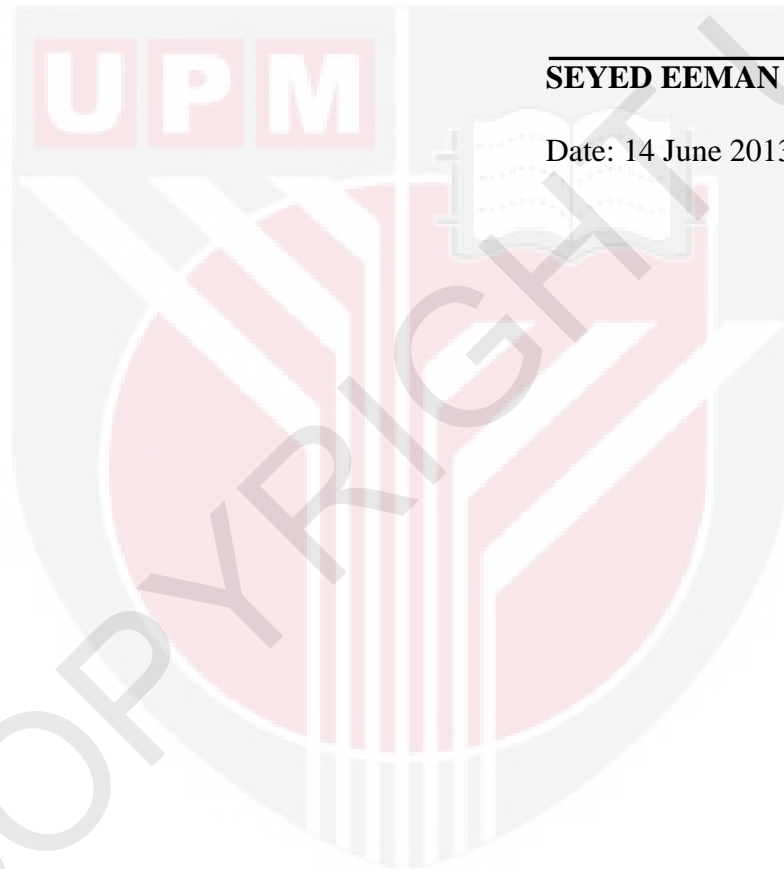
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## DECLARATION

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



**SEYED EEMAN NOORAE**

Date: 14 June 2013

## TABLE OF CONTENTS

	<b>Page</b>
<b>ABSTRACT</b>	iv
<b>ABSTRAK</b>	vii
<b>ACKNOWLEDGEMENTS</b>	x
<b>APPROVAL</b>	xii
<b>DECLARATION</b>	xiv
<b>LIST OF TABLES</b>	xx
<b>LIST OF FIGURES</b>	xxii
<b>LIST OF ABBREVIATIONS</b>	xxiii
<b>CHAPTER</b>	
<b>1 INTRODUCTION</b>	1
<b>2 LITERATURE REVIEW</b>	5
2.1 Introduction	5
2.2 Definition of probiotic and direct-fed-microbial	5
2.3 Earliest studies	7
2.4 The yeast flora of the rumen	8
2.5 The effects of yeast culture ( <i>Saccharomyces cerevisiae</i> ) on nutritional parameters	9
2.5.1 Dry matter intake (DMI)	10
2.5.2 Rumen pH	11
2.5.3 Volatile fatty acids, NH <sub>3</sub> , CH <sub>4</sub>	12
2.6 The effects of yeast culture on nutrient digestibility and degradability	16
2.7 The effects of yeast culture on ruminal microbial population	19
2.8 The effect of yeast culture on production and efficiency in ruminant	20
2.9 Mode of action of <i>Saccharomyces cerevisiae</i>	22
2.10 Taxonomy of <i>Kluyveromyces marxianus</i>	26
2.11 Potential applications of <i>Kluyveromyces marxianus</i> in biotechnology	27
2.12 Conclusion	29
<b>3 CHARACTERIZATION AND IDENTIFICATION OF YEAST ISOLATES FROM TRADITIONAL FERMENTED RICE (TAPAI) AND HOME-MADE WINE</b>	30
3.1 Introduction	30
3.2 Materials and Methods	32
3.2.1 Sources of yeasts	32
3.2.2 Enrichment procedure	32
3.2.3 Isolation of yeasts	32
3.2.4 VFA-supplemented medium	34
3.2.5 Growth of isolates in VFA supplemented medium	34
3.2.6 Ethanol production of isolates in VFA supplemented medium	34
3.2.7 Identification of isolates:	35
3.2.8 Statistical analysis	36
3.3 Results and Discussion	36
3.4 Conclusion	44
	xv



<b>4</b>	<b>EFFECT OF <i>Kluyveromyces marxianus</i> WJ1 ON FERMENTABILITY AND APPARENT DRY MATTER DIGESTIBILITY OF DIFFERENT SUBSTRATES <i>IN VITRO</i></b>	<b>45</b>
4.1	Introduction	45
4.2	Materials and Methods	46
4.2.1	Experiment I: <i>In vitro</i> gas production technique	46
4.2.2	Experiment II: Two-stage <i>in vitro</i> digestion technique	49
4.2.3	Statistical analysis	54
4.3	Results and Discussions	57
4.3.1	Experiment I	57
4.3.2	Experiment II	69
4.4	Conclusion	71
<b>5</b>	<b>EFFECT OF <i>Kluyveromyces marxianus</i> WJ1 ON RUMEN FERMENTATION, NUTRIENT DIGESTIBILITY, RUMEN MICROBIAL POPULATION AND BODY WEIGHT GAIN IN GOATS</b>	<b>72</b>
5.1	Introduction	72
5.2	Materials and Methods	73
5.2.1	Experiment I: fermentation and digestibility experiment	73
5.2.2	Experiment II: Production experiment on goats	80
5.3	Results and discussion	82
5.3.1	Experiment I	82
5.3.2	Experiment II	95
5.4	Conclusion	99
<b>6</b>	<b>GENERAL DISCUSSION AND RECOMMENDATION</b>	<b>100</b>
	<b>REFERENCES</b>	<b>105</b>
	<b>APPENDICES</b>	<b>118</b>
	<b>BIODATA OF STUDENT</b>	<b>162</b>
	<b>LIST OF PUBLICATIONS</b>	<b>163</b>