RELEASING ENERGY FROM RICE BRAN
BY ENZYME SUPPLEMENTATION AND
MICROWAVE TREATMENT

by
N.G.A. Mulyantini

A thesis submitted in partial fulfilment of the requirements for the degree of
Master of Science in Agriculture of the University of New England

July, 1997

Department of Animal Science
University of New England
AUSTRALIA
DECLARATION

I certify that the substance of this thesis has not already been submitted for any degree and is not being submitted currently for any other degree.

I certify that, to the best of my knowledge, all the work for this project was carried out solely by the candidate. All assistance received in the preparation of the thesis and all resources used have been acknowledged herein.

July, 1997

N.G.A. Mulyantini
ACKNOWLEDGMENTS

I wish to express my sincerest thanks to my supervisors, Dr. Mingan Choc and Associate Professor John Thwaites, for their patience, guidance, and encouragement during the period of my studies at the University of New England.

Thanks are extended to Mr. Peter Bevon of Wool CRC, University of New England, and Primary Technologies Pty. Ltd. for arranging the microwave treatment of samples used in experiments 2 and 3.

Special thanks to Ms. Kylie Day who shared her expertise and valuable time in the carbohydrate analysis, and to Mr. Evan Thomson who assisted in bomb calorimetry and various aspects of computing.

My appreciation is also extended to Mr. John Smallshaw and all staff of the Animal House complex for their assistance during the experimental work. The help of Ms. Maria Hyland during the animal experiments is much appreciated and Mr. Robert Taylor’s help in the mixing of experimental diets is also acknowledged.

I am greatly indebted to the Australian Agency for International Development (AUSAID) for the scholarship award and the Ministry of Education of Indonesia for allowing me to undertake this course of study.

The friendship and moral support given by fellow post-graduate students, especially Freweini Tedla and Ann Tukei, were invaluable to me and thanks go also to all Indonesian friends in Armidale for their kindness and encouragement. Finally I wish to dedicate this thesis to my mother and father, the prayers and blessings of them were the invisible help for me.
Summary

Three experiments were conducted to determine whether various exogenous enzymes (xylanase, lipase, and phytase) would enhance the nutritive value of rice bran for poultry. Water soaking and microwave treatment of rice bran, in combination with xylanase supplementation, were also examined both in vitro and in vivo.

Experiment 1 was conducted in two parts. First, the ability of commercial glycanases to release sugars from rice bran in vitro was examined. Full-fat or defatted rice bran (50 g) was incubated with 100 mL of water containing either a multi-activity glycanase (enzymes A; recommended dosage rate 800 mL/kg or 2 x 800 mL/kg) or a xylanase-based glycanase (Enzyme B; recommended dosage rate 500 mL/kg or 2 x 500 mL/kg). The mixtures were continuously shaken at 40°C for 24h. Negligible amounts of sugars were released from both full-fat rice bran and defatted rice bran by both enzymes at two dosage rates. Second, specific frequencies of microwave energy (68°C) were applied to a mixture (50:50) of rice bran and Enzyme A for 5, 10, 20 or 30 min. Five minutes of microwave treatment released 3.5% free sugars from rice bran. Longer periods of treatment did not lead to increased release of sugars.

Experiment 2 was a 2 x 3 factorial design with two factors (with or without water soaking for 48h) and three treatments (control diet containing 30% rice bran, control diet + enzyme (xylanase) supplementation, and control diet + enzyme (Xylanase) + microwave). Soaking had a negative effect on AME, but was positive on feed intake, weight gain and feed conversion. Supplementation of the diet with a xylanase significantly (P<0.01) increased AME, but had little or no effects on bird performance. Application of specific frequencies of microwave energy to enzyme-rice bran mix, further improved AME. There was no effect of this treatment on bird performance.
Experiment 3 was conducted to examine the effect of supplementation of xylanase (Enzyme B), phytase, and lipase on the performance of broilers fed a diet containing 30% rice bran. There were eight diets in a 2 x 2 x 2 factorial design: 1) Control diet; 2) Control + xylanase; 3) Control + lipase; 4) Control + phytase; 5) Control + xylanase + lipase; 6) Control + xylanase + phytase; 7) Control + lipase + phytase; and 8) Control + lipase + xylanase + phytase. Enzyme products (xylanase, phytase and lipase) did not have a significant effect (P>0.05) on weekly feed intake and weight gain of birds. But feed intake tended to drop and weight gain tended to increase in response to supplementation of all three types of enzymes. Thus, weight gain of the birds was increased by 0.6% with lipase, 3.7% with phytase and 2.4% with xylanase. There were no interaction of the enzymes on these two parameters. Xylanase had a marked effect (P<0.01) on both FCR and AME, decreasing FCR from 1.52 to 1.44 and increasing AME from 12.29 to 12.60 MJ/kg. There was also a highly significant (P<0.01) interaction between the three enzymes on AME. The lipase and phytase both improved FCR but had no effect on AME. The improvement in FCR by lipase, phytase and xylanase was 2.1, 2.6 and 5.4%, respectively.

It is concluded that (a) the release of free sugars from rice bran in vitro by the current commercial glycanases is minimal; (b) AME of broiler diets containing a high level (30%) of rice bran may be improved by xylanase supplementation, and (c) application of appropriate microwave energy to enzyme-supplemented rice bran can not only increase NSP degradation in vitro, but also increase the AME of the diet.
## List of Tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1.</td>
<td>Estimated rice bran production in the world from the rice paddy harvest reported by FAO in 1997</td>
<td>5</td>
</tr>
<tr>
<td>2.2.</td>
<td>The composition (g/kg) of the water soluble and alkali soluble fractions of rice bran hemicellulose</td>
<td>7</td>
</tr>
<tr>
<td>2.3.</td>
<td>Average nutrient composition and metabolisable energy (ME) values of some cereals and by-products, with particular reference to Asia and the Pacific area</td>
<td>12</td>
</tr>
<tr>
<td>2.4.</td>
<td>Metabolisable energy (ME, dry basis) of rice bran of different quality from different sources, determined by conventional method</td>
<td>13</td>
</tr>
<tr>
<td>2.5.</td>
<td>Apparent digestibility of some amino acids in full-fat (FFRB) and defatted rice bran (DFRB) in adults cockerels with ileal cannulas</td>
<td>14</td>
</tr>
<tr>
<td>2.6.</td>
<td>The amounts (g/kg DM) and apparent digestibilities (%) of essential amino acids in full-fat Australian rice bran</td>
<td>15</td>
</tr>
<tr>
<td>2.7.</td>
<td>Range of some essential minerals in rice bran</td>
<td>16</td>
</tr>
<tr>
<td>2.8.</td>
<td>Composition of rice bran obtained from different types of mills commonly used in Asian countries (% dry matter basis)</td>
<td>18</td>
</tr>
<tr>
<td>2.9.</td>
<td>Free fatty acid development in rice bran during storage</td>
<td>23</td>
</tr>
<tr>
<td>2.10.</td>
<td>Estimated potential and actual rice bran oil production in major rice growing countries in 1996 (1000 tonnes) (FAO, 1997)</td>
<td>25</td>
</tr>
<tr>
<td>4.1.</td>
<td>The NSP composition of defatted rice bran</td>
<td>39</td>
</tr>
<tr>
<td>4.2.</td>
<td>Chemical composition of the full-fat rice bran and defatted rice bran used in the current experiment</td>
<td>41</td>
</tr>
<tr>
<td>4.3.</td>
<td>The effect of the enzymes on releasing total free sugars in</td>
<td>43</td>
</tr>
</tbody>
</table>
4.4. The effect of different duration of microwave heating on free sugar release from rice bran

5.1. The composition of the basal diet used in the experiment

5.2. Effects of water soaking, enzyme supplementation and microwave treatment on the AME, FCR, WG, FI and excreta moisture of broiler chickens fed diets containing rice bran

6.1. Ingredient composition of the basal diet

6.2. Feed intake (FI; g/bird/week), weight gain (WG; g/bird/week), feed conversion ratio (FCR), apparent metabolisable energy (AME; MJ/kg) and excreta moisture content (EM;%) of chicks fed a high rice bran diet with or without supplementation of lipase, phytase and xylanase
List of Figures

5.1. Effects of enzyme supplementation and microwave treatment on the AME of a high rice bran diet in broilers 49
# List of Plates

<table>
<thead>
<tr>
<th>Plate</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1.</td>
<td>A brooding cage for chicks used prior to the commencement of experiments</td>
<td>33</td>
</tr>
<tr>
<td>3.2.</td>
<td>Metabolism cages used for determination of AME</td>
<td>34</td>
</tr>
</tbody>
</table>
# Table of Contents

Summary i
List of Tables iii
List of Figures v
List of Plates vi

## Chapter 1  General Introduction 1

## Chapter 2  Literature Review 4

2.1. General Description of Rice Bran 4
2.2. Utilisation of Rice Bran 5
2.3. Limitation to the Use of Rice Bran in Poultry Diets 6
   Physical and Chemical Composition 6
   Anti-Nutritive Factors 8
   Phytic Acid 9
   Rancidity 10
   Microbial Toxins 11
2.4. Proximate Composition of Rice Bran 11
2.5. Quantity and Quality of Nutritive Elements 12
   Energy 12
   Carbohydrate 13
   Protein and Amino Acids 14
   Oil and Fatty Acids 15
   Minerals 16
   Vitamins 17
2.6. Processing of Rice Bran 17
2.7. Feeding Rice Bran to Poultry 18
2.8. The Effect of Rice Bran on Poultry Performance 19
Chapter 3 General Materials and Methods

3.1. Animals

3.2. Housing
  Cages for Chicks
  Growing Cages

3.3. Feeding and Provision of Drinking Water

3.4. Feed Formulation and Mixing
  Feed Formulation
  Feed Mixing

3.5. Selection and Allocation of Test Birds

3.6. Data Collection and Calculations
  Weight Gain
  Feed Intake
  Feed Conversion Ratio
  AME Determinations

3.7. Analytical Techniques and Related Procedures
  Preparation and Storage of Feed and Excreta Samples
  Gross Energy
  Moisture Content of Excreta (%)

3.8. Statistical Analysis
Chapter 4  Release of Sugars from Full-fat and De-fatted Rice Bran by Enzyme Supplementation and Microwave Treatment in vitro

4.1. Introduction

4.2. Materials and Methods
   Preparation of Defatted Rice Bran
   Experimental Protocol
   Experiment 1a. Release of free sugars from rice bran by commercial glycanases in vitro
   Experiment 1b. Increasing the activity of enzymes by microwave manipulation
   Determination of free sugars

4.3. Result
   Experiment 1a.
   Experiment 1b.

4.4. Discussion

Chapter 5  The Effects of Water Soaking, Enzyme Supplementation and Microwave Treatment on the Nutritive Value of Rice Bran for Broiler Chickens

5.1. Introduction

5.2. Materials and Methods
   Experimental Diets
   Diet Mixing
   The AME Trial

5.3. Results

5.4. Discussion
Chapter 6  The Effect of Xylanase, Phytase and Lipase Supplementation on the Performance of Broiler Chickens fed a Diet with a High Level of Rice Bran

6.1. Introduction  51

6.2. Materials and Methods  52
   The Enzyme Cocktails  52
   Chicks and Diets  52
   AME Trial  53

6.3. Results  53

6.4. Discussion  55

Chapter 7. General Discussion  58

References  62