Chapter 1

General Introduction

Continued increase in poultry production is an important contributor to the supply of protein to the expanding human population of the world. In Asia and the Pacific region, from 1976 to 1996, the poultry meat production increased annually by 20.6% and egg production by 15.3% (FAO, 1997). This rapid growth was due to the transformation of poultry farming to a more commercial enterprise with improvements in genetics, disease control, management and nutrition (Ravindran and Blair, 1991).

In the case of nutrition, the broiler industry is continually looking for new dietary ingredients. By-products of cereal milling processes are appealing as potential feedstuffs because they often contain considerable amounts of protein, starch and fat. Their use, however, may lead to production problems due to presence of components which possess anti-nutritive properties.

Rice bran, a by-product of the rice milling industry, is available in many parts of the world and is used mainly in monogastric diets. Hull-free rice bran contains many nutrients, and typically has a composition of 20% oil, 15% protein, 45% nitrogen free extract, plus vitamin and minerals (Juliano, 1985; Sayre *et al.*, 1987; Warren and Farrell, 1990a). It, however, has high levels of phytate phosphorus that is largely indigestible, and high levels of non-starch polysaccharides (NSP, ie 25%), which cause a general inhibition of absorption of macronutrients and probably micronutrients (Farrell *et al.*, 1993; Annison *et al.*, 1995). Rice bran

also lacks stability due to its high oil content, especially in a warm and humid environment (Farrell, 1994).

Generally, several methods of improving the nutritional value of rice bran have been developed, either by markedly reducing the anti-nutritive properties or by reducing the unfavourable effects of the anti-nutritive factors on nutrient absorption (Reddy, 1993). Feed enzyme technology may offer possible solutions to the problems of feeding rice bran to poultry by allowing enhanced utilisation of the nutrients in rice bran, reducing the deleterious effects of the anti-nutritive factors and facilitating access of endogenous enzymes to the feed (Donkers, 1989; Hotten, 1992). Production of efficacious enzyme could allow the NSP in rice bran to be a potential source of energy for poultry. Furthermore, if enzymes successfully enhance the utilisation of P and N in rice bran, they will not only lessen the need for supplementation of these nutrients but will also decrease pollution of the environment. This is of particular significance in regions such as Asia where high levels of cereal products are included in poultry diets and the human population is dense.

Another method to stabilise rice bran is dry heating, which is effective but requires a long period of heating. Also, severe processing conditions are likely to damage the components of rice bran. Recently, microwave heat treatment has been suggested to inactive lipoxygenase and trypsin inhibitors (Vetrimani *et al.*, 1992). In addition, application of microwave energy could increase the activity of supplementary enzymes which in turn accelerate the breakdown of NSP.

In the literature review which follows the theory and application of enzyme supplementation and microwave treatment to rice bran will be examined from the viewpoint of improvement of its nutritive value and to increase its metabolisable energy content for poultry. The efficacy of different commercial glycanases in improving the performance of broiler chickens will also be reviewed.

The objectives of the current project were to examine: (1) the possibility of releasing utilisable carbohydrate from rice bran by enzyme supplementation and microwave treatment, and (2) the effects of phytase, xylanase and lipase on the nutritive value of rice bran for poultry.

Chapter 2

Literature Review

2.1. General Description of Rice Bran

Rice is the principal cereal in many parts of the world and the by-products from its milling are important livestock feed resources (Kratzer *et al.,* 1974). At present, it is intensively used for animal and poultry feeding throughout the rice-producing areas of the world (Sayre *et al.*, 1988; Singh *et al.*, 1995). Additionally, Barber and De Barber (1980) defined rice (Oriza sativa) as the principal food cereal in the tropics, where it contains varying amounts of rice hulls which determine its feed grade. Rice grains in their hulls are known as paddy or rough rice. It is often available during the harvest season at a favourable price. It is very hard and abrasive, and grinding is recommended before paddy rice is used as poultry feed.

Large quantities of rice bran are produced annually, and the latest world paddy rice harvest reported by the Food and Agriculture Organisation (FAO, 1997) was 562 million metric tons (MT) per year (Table 2.1). Approximately 91% of all rice bran is produced in Asia, and this is greater than the production of any other single crop. In Australia rice bran production totals about 86,000 MT per year (Sayre *et al.* 1988; Warren and Farrell, 1990b). Ravindran and Blair (1991) stated that rice polishings, which come from the inner layer of the kernel, have a wider use than bran due to their higher ME contents. However, in Asian countries their availability is low because only a small proportion of the rice mills employ multiple stage milling that separates polishings from the bran.

Table 2.1. Estimated rice bran production in the world from the rice paddy harvest reported by FAO in 1997.

	Rice bran[1]	
	(MT)	(%)
World	562,259,500	100.00
Africa	16,028,780	2.85
Asia	512,944,800	91.23
Eastern Europe	70,674	0.01
Western Europe	2,713,600	0.48
Russian Federation	500,000	0.09
Latin America		
Carrabean	20,389,950	3.63
North and Central America	9,878,816	1.76
Oceania	19,236	0.00
South America	18,282,130	3.25
Europe	2,784,274	0.49

[1]Estimated as 10% of paddy rice (Shaheen *et al*, 1975).

2.2. Utilisation of Rice Bran

Rice bran is used both as a food and as a component of food products. It is used as a snack food ingredient and a breakfast cereal; in making bread, cakes and cookies; and as a raw material for hydrolysed vegetable proteins (Saunders, 1986). Tomlin and Read (1988) recommended rice bran as a component of human diets in Western countries for increasing faecal bulk and bowel regularity. In addition, the intake of rice bran decreases the incidence of arteriosclerosis and diverticular diseases (Singh *et al.*, 1995). Rice bran can be also used as a source of dietary fibre when prepared by the so-called "stabilisation treatment" (Aoe *et al.*, 1993).

Currently, rice bran has been well documented for its effectiveness in lowering blood cholesterol in both animals and humans (Diment, 1984; Singh *et al.*, 1995). Neutral detergent fibre (NDF) and the unsaponifable fraction of the oil have been shown useful for this purpose (Topping *et al.*, 1990; Kahlon *et al.*, 1991). Furthermore, Aoe *et al.* (1993) and Singh *et al.* (1995) stated that cholesterol

lowering activity was demonstrated by a number of components of rice bran, including a water soluble polysaccharide fraction (Aoe *et al.*, 1993).

Also, a variety of pharmaceutical products can be obtained from rice bran, for example vitamin B-complex, lecithin, fatty acids, and phytin. In addition, its oil is used as a substitute for wax for various industrial applications, and could provide a valuable new source of edible oil in many countries (Buvanendran, 1961; Juliano, 1985).

Rice bran is mainly used as an animal feedstuff, normally as a partial substitute for maize, in pig and poultry diets in Asian countries (Creswell, 1988). In Australia, rice bran has special use in layer diets to increase egg size due to its high linoleic acid content (Balnave, 1982; Karunajeewa and Tham, 1984).

2.3. Limitations to the Use of Rice Bran in Poultry Diets

The main problem which prevents rice bran from reaching its full commercial potential as a human food is the instability of the oil and of fractions containing high levels of oil during storage, especially in warm and humid environments (Donkers, 1989; Graham, 1992; Hotten, 1992; Tao *et al.*, 1993). Another major limitation to the use of rice bran in livestock feed is the high variability of its chemical composition. It is known to contain a number of anti-nutritional factors and, due to inherent biological constrains, also suffers from an insufficiency of endogenous enzymes.

Physical and Chemical Composition

Rice bran is classified as a fibrous feed as a consequence of its high NDF and ADF (acid detergent fibre) values (van Soest, 1985). Harland (1989) defined dietary fibre as the portion of the carbohydrate fraction which cannot be digested by enzymes in the small intestines. The type of linkage of molecules in the NDF and ADF fractions, coupled with a lack of endogenous enzymes in monogastrics renders these compounds of little nutritional value (Farrell and Hutton, 1990).

There have been many studies and reviews of the consequences of high dietary fibre in animals (van Soest, 1985; Harland, 1989; Jansen and Carre, 1989), and the general conclusion is that high fibre levels are associated with reduced energy intake. Inclusion of more than 30% rice bran in the diet resulted in poor performance in broilers (Zablan *et al.*, 1963; Martin, 1995). Moreover, Thomson and Weber (1981) explained that fibre affects the availability of dietary minerals and other nutrients. This is illustrated by the work of Halpin and Baker (1986) and Warren and Farrell (1991) which showed that the NDF fraction of rice bran caused a reduction in tissue Mn levels, and in the retention of Ca and a lower utilisation of several other minerals. In addition, Watts (1960) reported that an increase in the bulk of a diet is another factor associated with high levels of fibre in the diets, and that as a consequence the growth and feed conversion of birds declined when rice bran was incorporated in the diet.

There are a number of reviews which consider the chemical properties of the fibre complex of rice bran (Juliano, 1985; Saunders, 1986). Mod *et al.* (1978) characterised the fibre components of rice bran from four Australian cultivars as presented in Table 2.2.

Table 2.2. The composition (g/kg) of the water soluble and alkali soluble fractions of rice bran hemicellulose

Components	Solubility fractions	
	Water soluble	Alkali soluble
Rhamnose	2.0	19.0
Arabinose	394.0	260.0
Xylose	102.5	300.0
Mannose	9.5	8.7
Galactose	302.8	8.0
Glucose	22.5	33.3
Protein (N x 6.25)	49.0	174.8
Uronic acid	100.0	107.5

Source : (Mod *et al.*, 1978).

Monosaccharide units in cellulose would be excellent sources of energy for chickens if they could be released from the lignified or partially lignified β-1-4-

linked glucose units of cellulose (Annison and Johnson, 1989). Maynard *et al.* (1980) stated that in ruminants, both hemicellulose and cellulose can supply a substantial part of energy requirements through fermentation by gastrointestinal microorganisms; hexose and pentose sugars that are released by the microorganisms can be used as sources of potential energy. In birds, on the other hand, fermentation does not occur at a sufficient rate to allow significant use of these compounds as energy sources.

Water soluble fibre increases digesta viscosity, decreases mixing of digestive enzymes with their substitutes and changes gut physiology. The net effect is for reduced nutrient digestion and/or absorption (Choct *et al.*, 1996). Poultry can only partly digest the water soluble fraction of NSP (Mod *et al.*, 1981; Carre *et al.*, 1990). Xylan has no value for poultry but they can partly utilise xylose (Norman and Ory, 1984; Schutte *et al.*, 1991).

Anti-Nutritive Factors

Bersch *et al.* (1989) stated that rice bran contains anti-nutrients such as trypsin inhibitors and lectins, which can be deactivated by autoclaving at 121°C (see Section 2.9). Furthermore, rice bran contains approximately 10% of pentosans which can adversely affect the performance of poultry (Houston, 1972; Warren and Farrell, 1990b; Adrizal *et al.*, 1996). The anti-nutrients, except pentosans, have minimal impact in practice because of their denaturation by heat treatment and gastric digestion prior to enzyme access in the small intestine (Marsono and Topping, 1993).

Tashiro and Maki (1991) reported that trypsin inhibitors in rice are localised in the embryo or germ, and thus their activity decreases with germination time. Bradbury *et al.* (1992) reported a trypsin inhibitor in the germ fraction of rice at a level of 220 units. Rice bran trypsin inhibitors belong to the family of *Browman-Birk* protease inhibitors (Norton, 1991). Trypsin inhibitors are also present in raw soybeans and cause growth depression when soybeans, without adequate heat treatment, are given to poultry (Xian and Farrell, 1991).

One of the effects of trypsin inhibitors is enlargement of the pancreas, an effect that is activated through a negative feedback mechanism (Nitzan *et al.*, 1991; Norton, 1991). Sayre *et al.* (1987) observed reduced growth and increased weight of the pancreas in broilers grown to 25 d of age with a diet containing 60% rice bran. Bradbury *et al.* (1992) also reported anti-trypsin factors to be possible causes of growth depression in birds fed diets containing rice bran. Kratzer and Payne (1977) stated that heat treatment caused a small reduction in the trypsin inhibitor content of rice bran. Work with Wistar rats fed a casein based diet containing 0.2% and 0.4% trypsin inhibitor from rice bran decreased body weight gain at one week of age but not at two weeks of age (Tashiro and Maki, 1991).

Creswell (1988) indicated that the anti-nutritional factors in rice bran limit the inclusion of the feedstuff to less than 200 g/kg in broiler and layer diets. Diment (1984), on the other hand, reported that the adverse effects of rice bran are not manifested until an inclusion rate of above 300 g/kg. Due to the potential for edible oil production from rice bran, a process was developed by Sayre *et al.* (1987) to stabilise the bran and prevent enzymatic hydrolysis of the oil.

Phytic Acid

One of the factors contributing to a depression in the performance of birds fed high levels of rice bran is the presence of relatively high concentrations of phytic acid, which interacts with endogenous enzymes. Belly *et al.* (1991) reported that a large proportion of the phytate P in rice bran may not be hydrolysed by birds and that partly hydrolysed phytic acid in the intestinal tract may cause nutritional problems. Phytic acid is a very strong chelating agent and several compounds can readily complex with the molecule (Graf, 1986; Kratzer and Vhora, 1986). Generally, phytic acid causes reduced availability of some minerals, such as Mg, Mn, Ca, and Zn (Roberts and Yudkin, 1960; Deolankar and Singh, 1979; Soares, 1990; Robertson and Edwards, 1994). Phytic acid reduced the availability of Mn for chicks and rats (Reddy *et al.*, 1982; Morris, 1986; Baker and Halpin, 1991). Deolankar and Singh (1979) suggested that the reduction in Ca availability due to

dietary rice bran was not only due to phytate but also to the formation of Ca-fatty acid soaps in the gut. Robertson and Edwards (1994) reported that phytic acid present in isolated soybean meal has been shown to reduce zinc-65 availability in chickens, and its bioavailability is increased by dietary zeolite due to the effect on phytate phosphorus metabolism.

The ability of poultry to utilise phytate P depends on the phytate first being hydrolysed to inositol and orthophosphoric acid and/or its salts (Nelson, 1976). Phytate is catalysed by any endogenous phytase from the birds, from the bacteria in their gut as well as by the intrinsic phytase in some feed ingredients (Ravindran, 1995).

Rancidity

The nutritional quality of rice bran deteriorates rapidly as the oil undergoes hydrolytic and oxidative rancidity (Houston, 1972; Sayre *et al.,* 1988; Adrizal *et al.,* 1996). Deterioration commences when hydrolytic enzymes are mixed with bran lipids during the milling of rough rice (Tao *et al.,* 1993).

In general, lipase activity is present in different parts of cereal grains. In the case of rice, most lipase activity is present in the outer layers (bran) (Rajeshwara and Prakash, 1995). Bersch *et al.* (1989) reported that lipase activity results in the release of free fatty acids and the development of rancidity unless the bran is stored in a freezer. NSP in rice bran bind bile acids readily and thus decrease lipase activity (Norman and Ory, 1984). Kirby and Nelson (1988) and Annison (1993) proposed that NSP might inhibit the digestion of fat by stabilising oil/water emulsions and thereby limiting lipase action. There are several methods available for arresting the lipase enzyme activity in rice bran so that the stabilised bran can be stored for a longer period (see Section 2.9).

Hussein and Kratzer (1982) concluded that the rancidity of rice bran had no direct effect on the rancidity of tissue fat in chickens or on the flavour of chicken meat. Rancidity also has no effect on the energy content of the rice bran, and it does not interfere with its nutritional value (Scott *et al.,* 1976; Ravindran, 1995). Storage

temperature and humidity are important factors in determining the rate of hydrolysis of the oil in rice bran (Farrell, 1994).

Microbial Toxins

Mycotoxins can also be a problem in full-fat rice bran, particularly if the bran has a high moisture content and is improperly stored. However, the nature of the solvent defatting process makes it unlikely that mycotoxins will be a problem in defatted rice bran (Warren and Farrell, 1990a). Furthermore, Scott and Dean (1991) stated that aflatoxin is one of the growth inhibitors in rice bran, especially if the rice bran is kept under poor storage conditions. It has been reported by Bradbury *et al.* (1992) that even if no aflatoxin had been formed in rough rice before milling, the bran after milling could produce aflatoxin during storage. The levels of aflatoxin are significantly increased during the wet season. In some countries, such as Indonesia, there is currently no limit for the maximum tolerated aflatoxin level, whereas in Belgium, The Netherlands, and Denmark the maximum level allowed is 20 μg/kg (Purwoko *et al.*, 1991).

2.4. Proximate Composition of Rice Bran

Rice bran may vary in chemical composition. The composition (g/kg DM) of several brans ranged from 134-173 crude protein (with a good amino acid balance), 204-234 lipid (as ether extract), 105 ash, and 256 NDF (Warren and Farrell, 1990b). Majun and Payne (1977) and Bersch *et al.* (1989) stated that rice bran is rich in B vitamins, trace minerals and contains 13 MJ/kg apparent metabolizable energy (AME). In the Asia Pacific area the ME value of rough rice is only 75% of that of maize due to its high fibre and ash contents (Table 2.3).

Table 2.3. Average nutrient composition and metabolisable energy (ME) values of some cereals and by-products, with particular reference to Asia and the Pacific area

Feedstuff	Crude Protein (%)	Crude Fat (%)	Crude F bre (%)	Ash (%)	NFE (%)	Ca (%)	P (%)	ME (MJ/kg)
Maize,grain,meal	9.0	4.0	2.5	1.5	83.0	0.02	0.25	14.0
Rice,unmilled grain with husk (paddy)	8.0	2.0	0.0	6.5	73.5	0.04	0.20	12.5
Rice, bran with germ with polishing with broken grain, full-fat	13.0	13.0	3.0	12.0	49.0	0.07	1.30	13.5
Rice, bran with germ with polishing with broken grain, solvent extracted	18.0	1.0	19.0	14.0	48.0	0.10	1.70	12.0

Source : Ravindran and Blair (1991).

Moreover, Balnave (1982) stated that in Australia rice pollard contains approximately 13.4 MJ/kg of metabolisable energy (ME) and 130 g/kg of crude protein, and is relatively low in fibre (90 g/kg). In addition, it contains high concentrations of oil and linoleic acids (50-80 g/kg). McCall *et al.* (1953) and Houston (1972) reported that variations in nutritive value are due to inherent factors such as the rice cultivar and agronomic practices applied during rice production.

2.5. Quantity and Quality of the Nutritive Elements

Energy

Several studies have determined the metabolizable energy (ME) value of rice bran (Table 2.4). In Australia, there have been reports of both good and poor bird performance when rice bran has been fed to poultry. It is rich in oil, which is a valuable commodity when extracted from the bran (Warren *et al.*, 1985).

Table 2.4. Metabolizable energy (ME, dry matter basis) of rice brans of different qualities from different sources, determined by conventional methods.

Country source	Quality of bran	ME (MJ/kg)	Test birds	Excreta collection (d)	Rice bran (g/kg)	Reference
USA	Good	12.68	Chicks	3	400	Maust *et al.* (1972)
India	Poor	9.12	Broiler chicks	-	-	Poddar and Biswas (1992)
India	Good	13.89	Broiler chicks	-	-	Poddar and Biswas (1992)
Australia	Good	13.03	Adult cockerels	5	400	Sotelo *et al.* (1990)
Australia	Good	9.22	Broiler chicken	5	400	Warren (1985)
Australia	Poor	11.78	Chicks	7	400	Nitis (1973)

Source : Martin (1995).

From experiments using both broiler chickens and adult cockerels, Warren and Farrell (1990a) estimated the AME of rice bran in broiler chickens to be 14.7-15.0 MJ/kg DM, while in adult cockerels a lower range of values was recorded (9.6-10.9 MJ/kg DM). The plant cell wall represents the least digested component of commercial chicken diets. This explains why cell-wall related parameters are efficient predictors of the energy value of chicken diets (Carre *et al.*, 1990).

Carbohydrate

The largest part of practical poultry feed is comprised of carbohydrates which are classified into two major types, starch and fibre. In rice bran the carbohydrate content ranges from 396 to 608 g/kg DM (Diment and Kompiang, 1983). Under most circumstances, starch is the dominant carbohydrate (Juliano and Bechtel, 1985). Saunders (1986) has stated that the content of starch in rice bran is affected by cultivar, post harvest handling of paddy rice and degree of milling. The fibre of rice bran consist primarily of cellulose and hemicellulose (Choct, 1997). Cellulose and hemicellulose are present at levels of 96-128 and 87-114 g/kg, respectively (Morita, 1979). The bran fraction of paddy rice contains the greatest amounts of cellulose and hemicellulose (Juliano, 1972), but the presence of high levels of hemicellulose and cellulose in poultry diets causes some nutritional problems (Prawirokusumo *et al.*, 1977; Annison, 1992).

Protein and Amino Acids

Rice bran contains good quality protein as gauged by a high level of albumin (370 g/kg) relative to total protein and a good amino acid balance (Saunders, 1986). In addition, other soluble proteins such as globulin (370 g/kg), glutelin (220 g/kg) and prolamine (50 g/kg) are also present in significant amounts (Houston, 1972; Bedford and Classen, 1993).

The apparent digestibilities in adult cockerels of some of the amino acids in rice brans are shown in Table 2.5. Values of lysine (0.87) and methionine (0.93) were similar for all brans, while threonine was much lower. Serine is a notable non-essential amino acid that was also lower (Warren, 1985). The inadequate amino acid availability in rice bran does not appear to be a problem when it is used in poultry diet (Creswell, 1988).

Table 2.5. Apparent digestibility coefficients of some amino acids in full-fat (FFRB) and defatted (DFRB) rice brans in adults cockerels with ileal cannulas

Harvest	DFRB 1981	DFRB 1982	FFRB 1982	FFRB Starbonnet
Essential				
Lysine	0.896	0.866	0.857	0.863
Methionine	0.939	0.864	0.939	0.987
Threonine	0.785	0.649	0.655	0.747
Valine	0.862	0.755	0.782	0.780
Mean	0.872	0.794	0.808	0.854
Non Essential				
Alanine	0.837	0.769	0.800	0.841
Aspartic	0.827	0.718	0.758	0.806
Serine	0.800	0.671	0.671	0.807
Tyrosine	0.880	0.820	0.805	0.846
Mean	0.825	0.747	0.775	0.830

Source : Warren (1985).

Warren and Farrell (1991) observed that the amino acid digestibility of Australian rice bran was relatively high for both essential and non essential amino acids. However, the digestibility of most essential amino acids, including the limiting amino acids, was lower for chickens than for cockerels (Table 2.6).

Table 2.6. The amounts (g/kg DM) and apparent digestibilities (%) of essential amino acids in full-fat Australian rice bran

Essential amino acids	Amount	Apparent digestibility	
		Chickens	Cockerels
Arginine	11.6 - 13.8	65	92
Aspartic acid	10.2 - 13.4	-	-
Histidine	3.7 - 5.2	71	90
Isoleucine	4.4 - 6.2	67	74
Leucine	9.0 -11.1	73	82
Lysine	7.3 - 7.6	71	86
Methionine	2.6 - 3.0	71	96
Phenylalanine	6.2 - 7.6	72	80
Serine	4.4 - 6.7	-	-
Threonine	4.4 - 6.0	66	70
Tyrosine	4.6 - 7.8	-	-
Valine	6.8 - 9.3	64	78

Source : Warren and Farrell (1991).

Lysine, valine, isoleucine, threonine and methionine are the most limiting amino acids for poultry in Australian rice bran based on plasma concentrations. Lysine and methionine are equally limiting, based on chick growth rate (Nitis, 1973).

Oil and Fatty Acids

Among cereal brans, full-fat rice bran contains the highest quantity of oil (crude fat) and that the oil is rich in linoleic acid (Farrell and Hutton, 1990). Oakenfull (1989) reported that rice bran oil contains the highest level of phytosterol (3.6 g/100 g oil) among the cereal bran oils. This compound, however, makes no contribution to the ME of the oil.

The chick has a poor capacity to digest dietary fat (Leeson, 1993) and this may explain some of the problems associated with rice bran utilisation by young birds. At 1-3 days of age, chicks digest only 61% of rice bran oil, while at 5-7 days of

age, they digest 73.5%; at 16 weeks of age, 85.6% of the oil is digested (Askbrant and Farrell, 1987). Krogdahl (1985) suggested that the poor ability of young birds to digest fat is due to their limited secretion of bile salts. It would appear

that the main disadvantage of full-fat rice bran is the inability of growing chicks to utilize fully the oil, while for defatted rice bran it is its low ME (Warren *et al.*, 1985; Warren and Farrell, 1990b).

Minerals

The concentration of specific minerals in rice bran is affected by the degree of milling (Tanaka *et al.*, 1973). The wide range of values recorded for some minerals is shown in Table 2.7. The high level of total P is one of the nutritionally important features of the minerals present in rice bran, in contrast with low amounts of Ca. The ratio of these minerals for Australian rice bran is 1 : 44, which is higher than is nutritionally desirable (Hutton, 1984).

Table 2.7. Range of some essential minerals in rice bran

Source of bran	Basis of reporting	Ca (mg/g)	P (mg/g)	Mg (mg/g)	Zn (µg/g)	Fe (µ/g)	Reference
Australia	Dry basis	0.3-0.5	16.2-17	6.1 - 7.7	44.2-53.9	37.9-48.1	Farrell & Hutton (1990)
Not specified	-	0.3-1.2	11.0-25	5.0-13.0	43.0-258	86.0 -430	Juliano (1985)
Not specified	-	0.1-1.3	14.8-17	8.6-12.3	80.0	190 - 530	Saunders (1986)

Source : Martin (1995).

Moreover, much of the phosphorus in rice bran is present as phytate P. The effect of these factors on the overall nutrition of birds when a high level of rice bran is used in feed formulation has not been well explored. It is not only important with respect to production performance but also to bone strength which affects handling and processing of finished birds. Utilisation of phytate P by the chick is poor (Corley *et al.*, 1980; Belly *et al.*, 1991).

Ravindran and Blair (1991) have explained that the availability of P in complete diets can be increased by steam pelleting. This improvement has been attributed to activation of the natural phytase in the feed ingredients. Much of the phytate P in rice is concentrated in the bran, principally in the aluerone layer.

Vitamins

Rice bran is a relatively rich source of thiamine, biotin, pantothenic acid and niacin (Hutton, 1984). These vitamins, particularly the water soluble vitamins, are concentrated in the outer layer of the endosperm, which largely ends up in the bran and polishings (Houston, 1972).

2.6. Processing of Rice Bran

Conventionally, paddy rice is separated into edible rice (69%) and its by-products (31%) during the milling process. These by-products are polished rice, 50-66%; broken rice, 1-17%; polishings, 2-3%. bran, 6-8%; and hulls 20% (Din *et al.*, 1979; Cornelius, 1980; Juliano, 1985). Furthermore, the milling process that separates the germ and bran layers from the endosperm, also serves to concentrate the fat in the milling residue commercially known as bran (Pilliang *et al.*, 1982). The oil content of the isolated bran thus rises to between 14 and 18% (Sotelo *et al.*, 1990; Annison, 1991).

Cornelius (1980) observed that more than half of the rice crop in several Asian countries, and about one third of that in India, was hand pounded, a process which is gradually being replaced by mechanical dehullers in many countries. Countries, such as Sri Lanka, the Philippines, Nepal and India, use a large number of dehullers with capacities ranging from 250-600 kg/hour. The bran produced is a mixture of husk and bran since there is no device to separate them. Singh *et al.* (1995) reported that in some countries rice is milled in different types of mills and under variable conditions. As a result, the bran obtained has a large variation in composition and quality. The variability is largely related to the level of hull contamination, which in turn is governed by the type of machinery used for milling (Table 2.8).

Table 2.8. Composition of rice bran obtained from different types of mills commonly used in Asian countries (% dry matter basis)

	Type of mill		
	Modern	Semi modern	Traditional
Crude protein	10.0	9.0-11.0	7.3 - 7.5
Crude fibre	12.6-12.9	15.4-15.9	29.3 -30.9
Fat	23.4-31.6	19.2-19.7	6.6 - 8.6
Ash	12.8-14.3	14.1-15.0	15.5 -20.5

Source : (Diment and Kompiang, 1983).

2.7. Feeding Rice Bran to Poultry

The optimum levels of rice bran in poultry rations vary considerably. This could be due to factors such as the chemical composition of the bran and to variations in the conditions adopted in different feeding trials. Anti-nutritional factors in rice bran also limit its inclusion in diets (Creswell, 1988). Variations in the gastrointestinal tract may also be a factor for inter-species differences in feeding levels. At a given age, ducks have a higher capacity to utilise rice bran than chickens (Moran, 1985). Farrell (1994) stated that inclusion of rice bran in chicken diets in excess of 20% frequently depresses growth, but higher level can be tolerated by ducklings. Laying birds can tolerate high levels of rice bran. Although some reports indicate successful inclusion of well above 600 g/kg, a practical upper limit of 450 g/kg is generally accepted.

The negative effects of high levels of rice bran on production vary with age, species and type of poultry (Warren, 1985). Generally, young birds are most affected and the inclusion of rice bran at above 200 g/kg has been observed to have adverse effects on feed consumption. Rizk *et al.* (1994) explained that older chickens, in contrast, are able to maintain feed intake even at high levels of rice bran inclusion and thus maintain an acceptable level of production.

2.8. The Effect of Rice Bran on Poultry Performance

The Effect of Rice Bran on Poultry Products

The beneficial effects of the high level of linoleic acid in full-fat rice bran in terms of egg size are well known (Balnave, 1982). The pelleting of high rice bran containing diets increases egg production significantly (Buvanendran, 1961; Warren *et al.,* 1985). Several investigators have successfully used diets containing 20 to 45% of rice bran or polishings for layers (Barber, 1978; Pilliang *et al.,* 1982; Bedford, 1993).

Majun and Payne (1977) and Oakenful (1989) concluded from their experiments that laying birds can be fed up to 33% rice bran on low energy diets (10.1 MJ/kg) with a minimum protein level of 15% to support an egg production of 70.7% and a feed conversion efficiency of 0.362. In contrast, Din *et al.* (1979) reported that factors such as low methionine and cystine intake could have been causes of the depression in feed intake, egg production and feed conversion efficiency. Oakenful (1989) reported that high levels of rice bran (38.5%) resulted in low egg production (65.7%) and feed conversion efficiency (0.326). Nitis (1973) analysed the effects of level of rice bran (0-700 g/kg) inclusion in the diet of broilers, and found that the crude protein and ash contents of the carcasses were not affected at four weeks of age. Also the fat content of carcasses did not differ between the groups fed rice bran but was significantly lower in the control group fed a diet without rice bran. Skin and yolk pigmentation, however, may be adversely effected by the inclusion of rice bran in the diet as this feedstuff is devoid of carotenoid pigments. This problem can be prevented by adding natural or synthetic pigments in the diet (Balnave, 1982).

The Effect of Rice Bran on Growth of Chickens

The growth of pullets fed diets containing rice bran and alfalfa meal was significantly better than that of birds fed other by-products, such as wheat bran, dried molasses, and wheat middlings (Classen *et al.*, 1991). However, Warren and Farrell (1991) showed that the addition of 10 g/kg of rice bran above 200 g/kg inclusion in the diet resulted in a decrease in the growth of broilers (21-49 days of age) by 0.51 g/d. Furthermore, Kratzer *et al.* (1974) and Zombade and Ichponani (1983) reported that the growth rate of broiler chickens was reduced by 20% with the inclusion of 40%, and by approximately 30% with the inclusion of 60% rice bran in their diet. Growth was depressed by 33% when broilers were offered diets containing 60% rice bran (Majun and Payne, 1977).

Addition of rice bran-NSP (RB-NSP) to a broiler diet based on sorghum and casein had no detrimental effects on bird performance nor on the nutrient (starch and protein) digestibilities in the small intestine. Farrell (1978a) reported that the increase in dietary AME values with increasing amounts of RB-NSP may be attributed to one of two effects : a systematic effect may have reduced the amount of nitrogen excreted in the urine, and alternatively, a more likely effect is that the digestion of normally indigestible material was promoted by increased hindgut fermentation of fibre and protein from RB-NSP. Farrell (1978b) and Annison (1993) confirmed that RB-NSP are highly fermentable substrates in birds and may thus have promoted a hindgut microflora more able to degrade sorghum NSP and possibly protein. These effects would increase the AME of the diets.

The nutritive value of rice bran differs between cultivars, but in general, it is tolerated well by broilers at dietary concentrations of up to 200 g/kg, above which growth is depressed. Such depression was not associated with the lipid fraction of rice bran nor with its water soluble components (Warren and Farrell, 1990a).

2.9. Improving the Feeding Value of Rice Bran

There are several potential treatments for increasing the nutritional value of rice bran. The potential problem of instability of the oil can be avoided by either removing bran oil or by inactivating detrimental lipase enzymes. The potential treatments can be categorised into three areas, namely: reducing anti-nutritional effects, prolonging shelf-life and increasing the availability of nutrients. These are covered in the following sections of the review.

Heat Treatment

Heating the bran in the presence of moisture permanently denatures hydrolytic enzymes, and can thus extend the shelf life. Principally, subjecting rice bran to heat treatment results in destruction of the anti-nutritive agents, microorganisms and insects (Kratzer and Payne, 1977).

Parboiling rice bran before milling destroyed the lipase activity that otherwise initiates hydrolytic rancidity (Shaheen *et al.*, 1975; Gunawan and Tangendjaja, 1988). However, the destruction of lipase and delaying the development of rancidity is not the major cause of the growth response to the autoclaving of raw rice bran, because a similar response is also obtained with fat-extracted bran diets (Hussein and Kratzer, 1982).

Tangendjaja (1985) reported that hot water treatment of rice bran reduced the phytic acid level by about 80%. Infra red exposure has also suggested for enzyme inactivation (Vali and Singh, 1989; Vetrimani *et al.*, 1992). However Tao *et al.* (1993) suggested that rice bran should not be exposed to high temperatures otherwise, oxidative spoilage may occur. Takemasa and Hijikuro (1992) suggested that heating bran to 110°C for ten minutes and cooling it to 0°C is a practical method that could be adopted.

Inclusion of 60% of non-autoclaved rice bran in the diet depresses performance of birds, and decreases shell thickness compared with autoclaved rice bran. Non-autoclaved rice bran also contains some factors which cause changes in yolk

colour. Majun and Payne (1977) reported that autoclaving at 120°C for 20 min prevented the expected depression in egg production in birds fed diets containing 60% rice bran. Similarly, Din *et al.* (1979) reported a significant improvement in egg production when an autoclaved rice bran (30 min at 100.5 kPa) was used in a layer diet. The growth depressing effect of raw rice bran was removed by autoclaving at 121°C for 15 min (Kratzer *et al.*, 1974 ; Kratzer and Payne, 1977). Sayre *et al.* (1987) observed that a meat strain of chicken fed on a diet containing extrusion cooked (130°C) rice bran (600 g/kg) to 25 days of age made greater weight gains than chickens fed a raw rice bran diet. In another report, Sayre *et al.* (1988) claimed a positive effect compared with raw rice bran on growth and FCR in chickens for the first two weeks of feeding extrusion-cooked rice bran (600 g/kg), but this advantage was lost by the end of the feeding period as birds reached maturity (Kratzer and Payne, 1977; Takemasa and Hijikuro, 1992). On the other hand, Majun and Payne (1977) found that autoclaving rice bran for chicken did not affect the liveweight of birds. Marsono and Topping (1993) further explained that heat treatment fails to degrade the polysaccharides, and is probably not economical, although extrusion cooking will stabilize the oil before extraction (Farrell, 1994).

Microwave Treatment

One of the serious drawbacks of rice bran as a source of oil is the presence of a high level of lipolytic enzymes. Stabilisation of rice bran by heat treatment is time consuming, and is also likely to damage some of its components (Vetrimani *et al.* 1992). Moreover, it is difficult to achieve a uniform temperature within rice bran during heat treatment and that treatment for a long period may decrease its nutritional value (Tao *et al.*, 1993). Therefore, microwave treatment was suggested for arresting lipase enzyme activity in the bran so that the stabilised bran can be stored for longer periods (Thomas *et al.*, 1991). Yoshida *et al.* (1975) stated that the advantages of microwave energy are its depth of penetration and rapid rate of heating, which are functions of the frequency and dielectric properties of the system used. Microwave energy is potentially an important heating method because it is commercially available and has been used by the food industry for

warming, drying, thawing, pasteurisation, sterilisation and blanching (Marsono and Topping, 1993).

During a few days of storage the free fatty acid (FFA) level in rice bran can increase. The time gap between bran production and oil extraction is large at present so that the FFA content of the bran oil increases to very high levels (Gunawan and Tangendjaja, 1988; Tao *et al.*, 1993). When rice bran is exposed to microwaves for progressively longer periods, FFA formation steadily decreases. Reduction of the activity was achieved when the exposure time was increased to 1 min, and the highest reduction value was 2.8% in a sample stored for 21 days after microwave treatment for 3 min (Thomas *et al.*, 1991; see Table 2.9).

Table 2.9. Free fatty acid development in rice bran during storage

Time of microwave exposure (min)	FFA development (% oil wt basis)		
	2 days	8 days	21 days
0	8.3	17.0	47.2
1.0	4.0	10.0	25.2
2.0	3.0	5.4	5.9
2.5	0.8	1.3	4.3
3.0	0.1	0.6	2.8

Source : Thomas *et al.* (1991).

Chemical Treatment

Anti-oxidant and chelating agents, such as ethylene diaminetetraacetate (EDTA 1000 mg/kg) or ethoxyquin (250 g/kg), inhibit lipase activity and retard the development of rancidity in rice bran (Kratzer *et al.*, 1974; Shastry and Rao, 1975). This may be specifically related to improvement in the growth of chicks as a result of inactivation of pro-oxidant metal ions (Cabel and Waldroup, 1989). Hussein and Kratzer (1982) noted that after three months of storage the content of FFA in rice bran treated with EDTA was lower than in rice bran without EDTA.

Furthermore, they observed that the performance of birds fed rice bran that had been stored for three months with EDTA (600 g/kg) was comparable to that in

birds fed fresh rice bran, but was significantly better than in birds fed untreated, stored rice bran. Another chemical treatment involves the use of HCl (1 litre HCl/25 kg rice bran) to inactive lipase enzymes and improve the feeding value (Bersch *et al.*, 1989). However, Thomas *et al.* (1991) reported that the use of HCl at the suggested level of 40 mL per kg of bran creates chemical hazards and handling problems. Deolankar and Singh (1979) and Sayre *et al.* (1988) attempted to inactive trypsin inhibitors in rice bran using 0.10% H_2SO_4, 0.1% NaOH and 0.1% NaCl, but found these treatments to be ineffective.

Defatted Rice Bran (DFRB)

The practice of extracting oil from full-fat rice bran has resulted in increasing amounts of defatted rice bran (DFRB) being available for animal feeding. In order to utilise the high quality of rice bran oil and to overcome some of the problems associated with storage of full-fat rice bran, considerable quantities of bran are now being defatted (Cornelius, 1980). However, the residual DFRB may be more difficult to utilise as a feedstuff compared with full-fat rice bran since it is dusty, lower in bulk density, higher in fibre and protein and has a lower ME than full-fat rice bran (Warren and Farrell, 1990a). Extracting the oil from rice bran has an additional advantage in that it yields a foodstuff with high protein and vitamin contents. Rice bran oil already makes a significant contribution to the edible oil supply in some countries. Table 2.10 shows that less than 10% of the potential production was realised in 1996 in major rice growing countries.

Aoe *et al.* (1993) reported that calcium hydroxide is appropriate for the extraction of soluble fibre from defatted rice bran because it produces the least colour change, gives a desirable composition and yield, and retains the hypocholesterolemic activity. Solvent extraction is the most efficient method to recover oil when content is as low as 10-20%; hydraulic pressing or screw expelling has resulted in rather poor recoveries under such circumstances.

Table 2.10. Estimated potential and actual rice bran oil production in major rice growing countries in 1996 (1000 tonnes) (FAO, 1997)

Country	Paddy	Rice	Rice Bran	Rice Bran oil Potential
China	141,400	94,172	7,534	1,130
India	69,000	45,954	3,676	551
Indonesia	26,317	17,527	1,402	210
Bangladesh	19,355	12,890	1,031	155
Thailand	17,500	11,655	932	140
Japan	15,600	10,390	831	125
Vietnam	10,500	6,993	559	84
Burma	9,500	6,327	506	76
Korea	8,300	5,528	442	66
The Philippines	7,000	4,662	373	56
Pakistan	4,500	2,997	240	36
World	377,769	251,594	20,128	3,019

Modern solvent extraction plants also give a more stable defatted bran. Hexane is used in commercial solvent extraction; other solvents have been examined on an experimental basis in order to isolate other useful constituents from rice bran, but are not used commercially (Cornelius, 1980). Vali and Singh (1989) stated that the stabilized full-fat or solvent extracted bran was found to be superior to raw rice bran when included in poultry diets.

Research by Adrizal *et al.* (1996) indicated that the performance of broiler chicks fed defatted rice bran diets was not significantly different from that of chickens fed a conventional corn-soybean diet. Similarly, Zombade and Ichponani (1983) reported that there were no differences in weight gain between broilers fed a corn-groundnut cake basal diet and others fed a 20% DFRB basal diet.

Defatted rice bran contains a higher concentration of crude protein and minerals than full-fat rice bran (Houston, 1972); it also contains a greater concentration of fibrous substances (NDF, ADF and lignin), phytate, and a smaller concentration of fat. The ME value of DFRB is low due to the decrease in oil content and the increase in fibre (Warren and Farrell, 1990b). Therefore, the relatively low ME_n content and the possibility of poorly available P in DFRB may limit its usefulness as a feedstuff for poultry (Annison, 1993). Ravindran and Blair (1991) reported that the ME_n value of DFRB was 75% of that of full-fat rice bran. The lower fat

content of DFRB would contribute considerably to this difference, but the presence of a substantial amount of NSP, in rice bran may also reduce the ME_n value of diets in which it is used (Baker, 1977; Singh *et al.,* 1995).

There was a significant depression in ME for DFRB compared to full-fat rice bran for both adult cockerels and growing chickens. In addition, adult cockerels were able to metabolise significantly more energy from full-fat rice bran than were growing chickens, due to an inability of the latter to digest the lipids in the bran. Digestibility of starch and neutral detergent fibre did not differ with the age of the bird (Farrell, 1978b).

Enzyme Supplementation

Enzymes can be used to improve the nutritional value of rice by-products and perhaps also to reduce the variability commonly associated with them. Enzymes have also been shown to largely eliminate fermentation in the small intestine and improve nutrient digestibility and the well being of the birds (Choct *et al.,* 1996)

Biological Considerations

Poultry normally digest their feed with the aid of enzymes secreted into their gastrointestinal tract. However, there are inherent biological constraints to the utilisation of feeds and thus to feed conversion efficiently (Moran, 1985). Poultry are devoid of the endogenous enzymes required for the breakdown of complex polysaccharides (Maynard *et al.,* 1980), and this represents a major constraint to the utilisation of fibrous feeds such as rice bran.

Moran (1985) explained that the digestive capacity of fowl for starch develops during the embryonic stage and birds are competent in this respect after hatch. Recently, Nitzan *et al.* (1991) reported that amylase secretion by the chick's pancreas is low at hatch and attains a maximum value eight days afterwards. In contrast, Moran (1982) pointed out that pancreatic amylase secretion at hatch is commensurate with the chick's requirement for starch digestion at that early stage.

According to Moran (1985), poultry are capable of adjusting to changes in dietary starch by altering the amount of amylase released, their intestinal surface area and the carbohydrate concentration in enterocytes. Starch digestion by poultry is almost exclusively accomplished by pancreatic α-amylase, as there is no salivary amylase (Moran, 1982).

Spring *et al.* (1996) stated that to reach their potential in improving the nutritive value of feedstuffs, enzymes have to be biologically active when they reach the gastrointestinal tract. Birds have a very short intestinal tract compared with mammals, and food passage through the gut is thus much faster than in mammals (Baker, 1991). Enzymes currently used as commercial feed additives are relatively crude preparations often containing many different activities, and they may be affected by treatment: steam pelleting at above 60°C, for example, strongly reduced phytase activity (Nunes, 1993). Cellulase, amylase and pentosanase can be pelleted up to at least 80°C without a considerable loss in activity. However, stability of some NSP-degrading enzymes might even be higher as its addition decreased the viscosity of the diet even after being pelleted at 100°C (Marsono and Topping, 1993; Spring *et al.*, 1996).

General Description of Enzymes

Enzymes are special proteins that catalyse or accelerate the rate of specific chemical reactions. Enzyme activity may be dependent on the substrate in a random manner or it may be through very specific sites on substrates such as starch, protein and fat. Some lytic enzymes hydrolyse chemical bonds within several different types of molecules that have common chemical characteristics (Ferket, 1993).

There are effectively two general categories of feed enzymes: 1) enzymes which are added to feed to enhance the utilisation of raw materials, primarily the cereals, and 2) those which can be added to feeds to enhance the utilisation of specific

nutrients. An obvious example of the second category is phytase, a microbial enzyme which enhances phosphorus utilisation (Belyavin, 1994).

It is reasonable to predict that, by the year 2000, the majority of animal feeds will contain only basic ingredients and their by-products which have been processed within environmentally accepted guidelines. Feed enzymes hold great promise to impact positively on the feed industry if their use is managed in accordance with environmental parameters (Campbell and Bedford, 1992; Sifri, 1993; Lavie, 1994). As environmental concern about animal waste disposal increases, enzymes are more frequently being considered as a means of reducing manure output and nutrient excretion, particularly excesses of phosphorus, nitrogen, copper and zinc (Friesen *et al.,* 1991; Ferket, 1993).

The potential of enzymes to improve feed utilisation is now considered by many as the most exciting development in poultry nutrition for many years (Belyavin, 1994). It has been claimed that while feed enzymes have optimal activity under normal physiological conditions in digestive tracts of the animals (Cowan, 1993) they also have the capacity to maintain potency under conditions of feed manufacturing and storage (Classen *et al.,* 1991; Inborr, 1993).

Types of Feed Enzymes and Their General Application

Feed enzymes are mostly hydrolytic in nature, and are produced from suitable microbial strains selected to produce predominantly one activity (Classen and Campbell, 1990) and a number of side activities (Classen *et al.* 1991). Enzyme preparations currently available are mostly derived from fungi such as *Trichoderma reesei*, *Aspergillus niger* or *Bacillus subtilis* (Broz, 1991), and are classified into phytases, lipases, xylanase and proteases (Hotten, 1992).

Phytase. Phytase has become widely available as a feed additive in recent years. Phytase is a microbial enzyme preparation designed to enhance phosphorus utilisation (Simons *et al.*, 1990; Belyavin, 1994; Robertson and Edwards, 1994). A purified preparation of phytase derived from *Aspergillus ficuum*, was effective in

hydrolysing phytate when added to a maize-soybean diet for chickens (Nelson *et al.,* 1971). Yi *et al.* (1996) reported that phytate has potential which allows it to form a wide variety of insoluble salts with cations such as Ca, Mg, Zn and Cu. Moreover, Perney *et al.* (1991) and Belyavin (1994) stated that the addition of phytase to poultry diets may decrease the amount of P excreted and thus reduce the nutrient load placed on the land as a result of spreading animal manure. They found, for example, that there was a 34% reduction in P excretion in broiler chicks fed diets containing phytase. Some of the inorganic P supplements currently added to poultry diets can be replaced by dietary phytase, with a consequent improvement in the utilisation of the P present in the basal diet (Perney *et al.,* 1991).

Lipase. Lipases are an important class of fat degrading enzymes present in mammals, microbes and plants. These enzymes can be defined as long chain fatty acid ester hydrolases. Lipases from plant sources have received less attention as compared to mammalian lipases, which are important in the field of medical biochemistry (Rajeshwara and Prakash, 1995). In the case of rice, most of the lipase activities are present in the outer bran layers of the grain (Rotter *et al.,* 1989). Lipase was successfully used in poultry diets containing tallow, but its beneficial effect declined with age of the birds (Polin *et al.,* 1980).

Xylanase. Xylanase is of fungal or bacterial origin (Biswas *et al.,* 1990) and is not an enzyme endogenous to avian or mammalian species. Xylan is a major component of hemicellulose and can be enzymatically hydrolysed to xylose by xylanase (Baker, 1977). Xylanase should be a supplement in the diet of the animal if any nutritional benefit from xylan degradation is to be derived (Ritz *et al.,* 1995). A commercially prepared enzyme cocktail that includes a xylanase component which is commonly utilized in supplements formulated for use with high fibre diets, was utilized in the experiments reported in this thesis to determine whether xylanase supplementation would benefit a rice bran-diet.

Effect of Enzyme Supplementation on Poultry Performance

In the early days of their use, feed enzyme preparations were usually added to a standard feed formulation at inclusion rates of 0.5 to 1.0 kg/tonne of feed. Generally, this approach gave satisfactory results in terms of improved animal performance (Bedford and Morgan, 1996). Thus, Brenes *et al.* (1993) concluded that enzyme supplementation of highly viscous barley diets significantly improved the nutritional value of the diet and chick performance but also reduced the size of the pancreas and the gastrointestinal tract. This latter effect is presumably the result of an adaptive response to increased nutrient digestibility and availability. The decrease in gut size may also contribute to an increase in carcass yield at the processing plant.

In birds fed wheat-based diets, the use of enzymes to reduce intestinal viscosity is likely to be the most effective method for improving performance (Bedford and Morgan, 1996). Enzymes could also be used to maximise the efficiency of feed utilisation by reducing the effects of anti-nutritional factors, manufacturing costs, and the variability of nutrient bioavailability in feed (Annison and Choct, 1991).

The addition of relatively small amounts of a commercial enzyme preparation to a wheat-based diet greatly improved chick performance, yielding a feed to gain ratio that was less than 2.0 : 1. The reason for such an unexpected response to enzymes in birds fed a wheat based diet could be the presence of a relatively high content of water soluble pentosans (Brenes *et al.*, 1993; Guenter, 1993; Lavie, 1994). Diets containing 60% rice pollard supplemented with either 0.5% pepsin or 0.5% amylase or a combination of these two enzymes had no effect on chick performance (Nitis, 1973). More recently, Donkers (1989) reported that enzyme preparations or combinations may promote the hydrolysis of specific substrates in rice bran and thus overcome the effects of anti-nutritional compounds. Feed enzymes thus appear to have the potential to increase the nutritional value of rice bran, particularly when their potency and stability are improved (Belly, 1991).

Even though the response to enzyme supplementation has been variable, enzymes have become a permanent part of poultry feed formulation, especially for broilers,

today. In layers, enzyme supplementation may induce a particularly positive response during peak production when there is an extremely high demand for nutrients to maintain growth and high egg production (Petterson and Aman, 1989; Rotter *et al.,* 1989; Wyat and Goodman, 1993). But other positive effects of using enzymes in monogastric diets, such as reduced excreta output, decreased excreta moisture and improved well-being of birds, are being increasingly recognised throughout the world.

Chapter 3

General Materials and Methods

All experimental applications were approved by the Animal Care and Ethics Committee (ACEC) of the University of New England. Health and husbandry practices complied with the "Code of Practice for the Welfare of the Domestic Fowl", Australian Bureau of Animal Health (1983).

3.1. Animals

The birds were obtained from the Baiada commercial hatchery in Tamworth, N.S.W., and were transported by car from the hatchery at day old to the animal house at the University of New England.

3.2. Housing

Cages for Chicks

Brooding cages were used for all chick experiments. The chicks were housed from day 1 to day 21 in standard four-tiered electrically-heated brooding cages (Plate 3.1). These cages had wire mesh floors and were equipped with external metal feeders and waterers. Each cage allowed six chicks to be grown to 21 d of age. The experimental room, cages and other facilities were washed and disinfected before the commencement of each experiment. The thermostats of the brooders were adjusted to provide mean temperatures of 35°C and 27°C, for the first and second weeks of the experimental period respectively, and the settings were then gradually decreased as the birds aged. Dead birds were removed immediately, and excreta was removed and the trays replaced twice a week.

Plate 3.1. Brooder cages

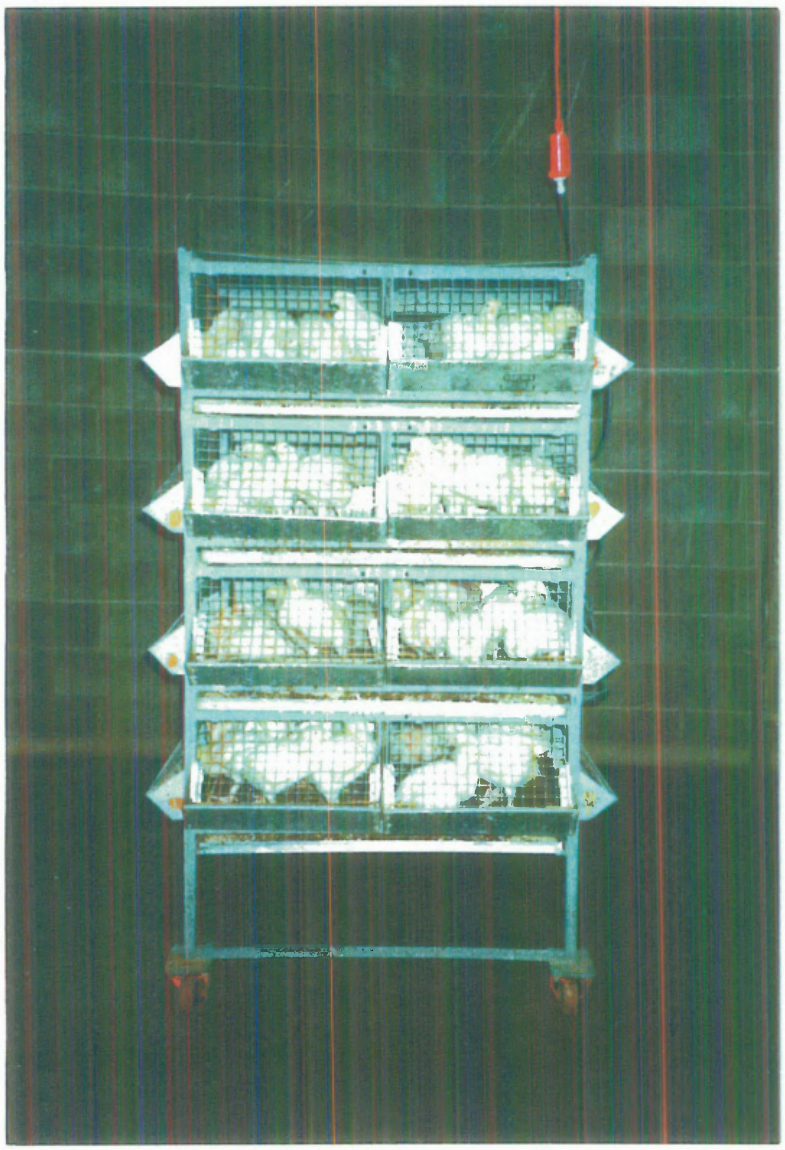

Plate 3.2. Metabolism cages used for determination of AME

Growing Cages

On day 21, birds were transferred to ME cages (Plate 3.2) with 4 birds in each cage. The experimental room had brick walls and concrete floors and adjustable glass windows which provided adequate ventilation. Continuous illumination was provided by fluorescent lamps and the room temperature was maintained between 18 and 20°C. The growing cages had wire mesh floors, and external galvanised feed troughs located at the front, and water troughs at the side. Excreta was collected from a tray beneath each cage, and plastic sheeting was used to ensure that all excreta was collected.

3.3. Feeding and Provision of Drinking Water

The chicks were fed *ad libitum* on commercial starter crumbles (Millmaster Feeds, Tamworth, NSW) prior to the commencement of each experiment (from day 1 to

day 21). The diet provided 21% crude protein, 3% crude fat, a maximum of 4% crude fibre, 0.35% salt and a maximum of 0.035% fluorine. Feeding troughs were filled to a level which minimized feed wastage. Fresh, clean drinking water was provided at all times; drinkers were cleaned and refilled every second day.

3.4. Feed Formulation and Mixing

Feed Formulation

The "Feedmania" package (Mania software Pty. Ltd. A.B.R.I., University of New England) was used to formulate the experimental diets, using the recommended levels of nutrients (NRC, 1993) for optimum performance.

Feed Mixing

Mixing feed was done one day before the commencement of experimental period. First, each ingredient was accurately weighed and mixed thoroughly. An amount proportional to the concentration required in each dietary treatment was then weighed out from this batch, added to the mixer and mixed for 30 minutes. All diets were cold-pelleted.

3.5. Selection and Allocation of Test Birds

At the start of each experiment (21 days of age) birds were weighed, and they were then randomly allocated to treatments and replicates on the basis of liveweight. Sick, very large or small birds were excluded from the experiment.

3.6. Data Collection and Calculations

Weight Gain

The weight of birds allocated to each pen was recorded as the initial group weight. At the end of each trial the weight of the birds was also recorded, and the average daily gain of the birds was then computed as follows :

$$\text{Average Daily Gain (g/bird/d)} = \frac{\text{Final group weight (g) - Initial group weight (g)}}{\text{Total number of birds x length of trial (days)}}$$

Feed Intake

Feed was weighed into labelled plastic bags for each pen at the start of each experiment. Feed was given in the same amount, and extra feed was added as needed during the experiment. At the end of trial the total amount of feed remaining was weighed. Feed intakes were measured over the final 7-day period. The amount of feed consumed during the excreta collection period was also recorded. Feed consumption per bird was expressed on a daily basis and calculated using the formula:

$$\text{Daily Feed Intake (g/bird/d)} = \frac{\text{Feed consumed (g) - Feed residue (g)}}{\text{Total number of birds x length of trial (days)}}$$

Feed Conversion Ratio

Feed conversion ratio (FCR) was determined by dividing feed intake by the body weight gain of the birds over the same period.

AME Determinations

Determination of AME was done over a 7-day period as indicated in each experiment. On day 21, the birds were weighed and transferred to ME cages in a temperature controlled room. The first three days enabled the chickens to adapt to the feeds and served as an adaptation period. During the last four days the excreta

were collected daily. Spilled feeds and feathers were manually removed from excreta before drying in a forced-draft oven (80°C) overnight. Dried excreta from quantitative collection were weighed after allowing it to equilibrate with atmospheric conditions for one day, and were then pooled for gross energy determination. The energy metabolised from the gross energy (GE) intake of the birds in a given period was determined using the formula :

$$\text{AME (MJ/kg)} = \frac{[\text{GE diet x feed intake (kg)}] - [\text{GE excreta x amount of excreta (kg)}]}{\text{Feed intake (kg)}}$$

3.7. Analytical Techniques and Related Procedures

Preparation and Storage of Feed and Excreta Samples

Bulked excreta samples were ground to pass a 1 mm sieve, and thoroughly mixed before sub samples of 200 g were taken and stored in plastic bags at room temperature. A sample of about 500 g of each diet obtained at the time of feed mixing was also ground to pass a 1 mm sieve, and a 200 g sub sample of the well-mixed and ground feed was stored in a screw-capped plastic container at room temperature.

Gross Energy

GE of feed and excreta samples was determined using a CP 500 automatic calorimetric processor (Digital Data Systems Pty. Ltd, Northcehff, South Africa). Benzoic acid was used to standardise the bomb calorimeter.

Moisture Content of Excreta (%)

Fresh excreta were dried in a force-drought oven at 80°C for 24 hour. Moisture content of excreta was calculated as follows :

$$\frac{\text{(Weight of wet excreta (g) - Weight of dry excreta (g)}}{\text{Weight of excreta (g)}} \times 100$$

3.8. Statistical Analysis

Data were assessed using analysis of variance (ANOVA). Statgraphics (STSC, Inc, Rockville, MD) was used to perform the analyses. Multiple comparisons were made using Duncan's multiple range test.

Superscripts in tables were used to indicate statistical differences between means. The conventions detailed below have been used to indicate statistical significance throughout the text and tables:

NS	non significant ; $P > 0.05$
*	$0.01 < P \leq 0.05$
**	$0.001 < P \leq 0.01$
***	$P \leq 0.001$

Chapter 4

Release of Sugars from Full Fat and De-fatted Rice Bran by Enzyme Supplementation and Microwave Treatment *in vitro*

4.1. Introduction

Cereal by-products contain high levels of cell wall components and are therefore usually rich in NSP and low in nutritive quality. The current world production of rice, for example, is approximately 562 million tonnes per annum (FAO, 1997), of which 91% is produced in Asia. Assuming rice contains 10% bran, this means Asia alone produces some 51 million tonnes of rice bran a year. Rice bran contains approximately 20-25% NSP which consist of approximately equal amounts of arabinoxylans and cellulose. The NSP composition of defatted rice bran is shown below:

Table 4.1. The NSP composition of defatted rice bran (%)

	Arabinoxylan	ß-Glucan	Cellulose	Mannose	Galactose	Uronic Acid	Total
Soluble	0.2	t	-	t	0.2	t	0.5
Insoluble	8.3	-	11.2	0.4	1.0	0.4	21.3

Source : Choct (1997). t = trace; - not applicable

Although all NSP represent potential sources of energy for poultry, the commercial enzymes available to date are not capable of totally depolymerising these NSP within the normal digesta transit time of the chicken. Also, the fermentative capacity of the chicken is limited, thus its ability to turn NSP to VFA

is not substantial. The digestibilities of NSP are very low in poultry and large amounts of NSP are wasted. The enzyme technology is widely available for a complete breakdown of some of the well-characterised NSP such as cellulose, ß-glucans and arabinoxylans. But utilisation of arabinoxylan as an energy source by monogastrics is doubtful since the 5-carbon sugars are not used efficiently in either pigs or poultry (Schutte, 1991). A large bulk of the NSP, however, are polymers of 6-carbon sugars (glucose, galactose, mannose) which can be used efficiently as energy sources. Cellulose is a straight chain 1-4 ß-glucan and requires a combination of cellobiohydrolase, endoglucanase and ß-glucosidase for complete breakdown to glucose. The (1-3), (1-4) - ß-glucan of barley and oats can be rapidly broken down to glucose with the combination of endoglucanase and β-glucosidase. Half of the rice bran NSP is cellulose (Saunders, 1986). A long period of incubation time or an increased activity of enzymes may lead to increased release of sugars from rice bran.

This experiment was conducted to examine whether soaking rice bran with one or other of two commercial xylanase preparation (referred to as Enzymes A and B) and or microwave treatment would increase the release of sugars from rice bran.

4.2. Materials and Methods

Preparation of Defatted Rice Bran (DFRB)

Rice bran was mixed with hexane and continuously stirred at room temperature for 1h. The hexane layer was then decanted and discarded. The extracted rice bran was dried at 45°C for 16h. Chemical composition of the resultant defatted rice bran is given in Table 4.2.

Table 4.2. Chemical composition of the full fat rice bran and defatted rice bran used in the current experiment (%).

Sample name	Fat	Crude Protein	Ash
Full fat rice bran	15.4	13.5	9.5
Defatted rice bran	3.9	16.5	9.7

Experimental Protocol

Experiment 1a. Release of free sugars from rice bran by commercial glycanases *in vitro*.

Ten lots of 50 g rice bran were weighed in 500-mL beakers and two enzyme preparations, Enzymes A and B, were added. Enzyme A was a multi-activity glycanase product and Enzyme B was a xylanase based product. The recommended dosage rate for Enzyme A was 800 mL/kg and for Enzyme B was 500 mL/kg. The samples were then incubated at 40°C with continuous shaking for 48h. The following are the treatment details:

1. Full fat rice bran + 100 mL water - **control**
2. Full fat rice bran + (Enzyme A; recommended dose) + 100 mL water
3. Full fat rice bran + (Enzyme A; recommended dose x 2) + 100 mL water
4. Full fat rice bran + (Enzyme B; recommended dose) + 100 mL water
5. Full fat rice bran + (Enzyme B; recommended dose x 2) + 100 mL water
6. Defatted rice bran + 100mL water - **control**
7. Defatted rice bran + (Enzyme A; recommended dose) + 100 mL water
8. Defatted rice bran + (Enzyme A; recommended dose x 2) + 100 mL water
9. Defatted rice bran + (Enzyme B; recommended dose) + 100 mL water
10. Defatted rice bran + (Enzyme B; recommended dose x 2) + 100 mL water

Experiment 1b. Increasing the activity of enzymes by microwave manipulation

To 50 g of rice bran, 50 mL of water containing the recommended dosage of Enzyme A were evenly sprayed using a pressure-spray bottle. It was then subjected to specific microwave energy for 5, 10, 20 and 30 min, respectively. At the end of treatment, the microwave temperature was taken up to 100°C for 30 seconds to deactivate enzyme. Then the samples were dried at 40°C in a force-draught oven. Each sample was replicated 8 times.

Determination of free sugars

Extraction of free sugars. The sample of rice bran was ground to pass through a 0.5 mm screen, and a sample weighing approximately 200 mg was placed in a 30-mL screw-capped culture tube. Five mL of 80% ethanol were then added to the residue and heated to 80°C for 10 min. The tube was then centrifuged at 2000 g for 10 min. The supernatant containing the free sugars was transferred to an 8 mL vial and dried under nitrogen at 40°C.

Hydrolysis. The residue was hydrolysed for 2h at 100°C using 3 mL of 1M H_2SO_4. A 0.4 mL aliquot of hydrolysate was transferred to a 30-mL culture tube to which 0.10 mL of 28% NH_3 was added. The 50 µL of Inositol (4 mg/mL) and 50 µL of allose (4 mg/mL) were added as internal standards. The mixture was dried under nitrogen at 40°C.

Reduction. The monosaccharides were reduced using sodium borohydride as follows : to the mixture of sugar hydrolysate and internal standards, water (0.2 mL), absolute ethanol (0.2 mL), and 3M ammonia (1 drop) were added. After thoroughly mixing, freshly prepared $NaBH_4$ (prepared by dissolving 50 mg sodium per ml 3M NH_4OH) (0.3 mL) was added. The tubes were then capped and incubated in a water bath at 40°C for 1 h.

Acetylation. To the reduced mixture, 5-7 drops of glacial acetic acid were added to decompose the excess of $NaBH_4$. Then 0.5 mL 1-methylimidazole and 5 mL acetic anhydride were added and mixed, and left for 10 min at room temperature. Absolute ethanol (0.8 mL) was added, mixed and left for 10 min at room temperature to effect acetylation of the sugars. The samples were then placed in an ice bath, and to each tube 5 mL H_2O was added to decompose any excess of acetic anhydride. Five mL of 7.5M KOH were added, the tubes were capped and gently mixed 6 times by inversion. Another 5 mL of 7.5M KOH were added, capped and mixed again. At this stage a clear ethyl acetate top layer was visible; this layer was then transferred into a 4-mL vial by using a Pasteur pipette and was evaporated to dryness under N_2. It was then re-dissolved in 0.4 mL ethyl acetate and the sugars were quantified with a Varian 3400CX gas chromatographic instrument.

4.3. Results

Experiment 1a. Both Enzyme A and enzyme B released negligible amounts of sugars from rice bran after 24 h of incubation at 40°C (Table 4.3).

Table 4.3. The effect of the enzymes on releasing total free sugars in rice bran

Sample ID	Sugars released (g/kg)	Sample ID	Sugars released (g/kg)
Full Fat Rice Bran		**Defatted Rice Bran**	
1	7.19	6	6.05
2	8.15	7	4.43
3	8.13	8	9.50
4	6.65	9	10.10
5	5.76	10	5.76

Experiment 1b. The amounts of sugars released by 5 minutes of microwave treatment were higher than 24 h incubation with the enzyme at 40°C.

Longer periods (up to 30 min) of treatment, however, did not lead to more sugars being released (Table 4.4).

Table 4.4. The effect of different duration of microwave heating on free sugar release from rice bran

Microwave Time (min)	Sugars released (g/kg)	**Microwave Time (min)**	Sugars released (g/kg)
5	34.80	**20**	30.62
10	26.72	**30**	27.72

4.4. Discussion

The current study demonstrates that depolymerisation of NSP present in rice bran (mainly arabinoxylans and cellulose) by commercial glycanases is not extensive, which confirms the view that the benefits of using enzymes in monogastric diets are not due to a complete breakdown of the NSP and subsequent absorption of the released sugars, but it is rather due to the ability of the enzymes to partially cleave the soluble NSP, thereby removing their anti-nutritive effects on nutrient digestion and absorption (Choct, 1996).

All enzymes require certain amounts of energy in order to act on the substrates. Appropriate frequencies of microwave treatment appear to "energise" the enzymes, the net effect of which is an increased activity of the enzymes. Thus, microwave treatment, released markedly higher amounts of sugars than normal treatment in the current experiment. Given the very short treatment duration (5-30 min), the effect observed was of great significance. Whether this is manifested in bird performance will be examined in Chapter 5 of this thesis.