INCREASING THE NUTRITIVE VALUE OF FULL-FAT RICE BRAN FOR BROILER CHICKENS

A Thesis Presented in Partial Fulfilment of the Requirements for the Degree of Master of Science in Nutritional Science at Massey University

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ABSTRACT

In the series of experiments presented in this thesis, two hypotheses were examined: 1) Use of a lipase preparation in diets containing full-fat rice bran (FFRB) would increase AME content by increasing lipid digestibility; 2) Use of lipase and phytase enzyme preparations would increase production of broiler chickens when FFRB was added to diets at 9 and 18%. Two experiments were conducted to study the effects of the enzyme preparations and FFRB from two sources (Australia and Thailand) on the performance of male broiler chickens when substituted in a maize-soyabean meal basal diet (Experiments 1 and 2). The third experiment was conducted to examine the effect of lipase and(or) phytase enzyme preparations on the performance of male broiler chickens fed diets that were formulated with FFRB (9 and 18%) as an ingredient in the diet formulation. In the first experiment, using Australian FFRB, the lipase preparation was added to a basal diet and the basal diet substituted with 9% FFRB. Birds were fed pelleted feed *ad libitum* from day 0-35. Excreta were collected over three periods, from day 4-7, day 18-21 and day 32-35, for determination of AME content and lipid digestibility. On day 36, eight randomly-selected birds from each treatment were euthanased to measure digestive organ weights. The lipase preparation did not improve ($P > 0.05$) lipid digestibility of both the basal and 9% FFRB diets. However, the AME content of enzyme-supplemented basal diet was significantly improved between days 18-21 ($P < 0.05$). The AME content was significantly higher in the 9% FFRB diet with enzyme supplementation between days 32-35 ($P < 0.05$). Only the performance of birds fed the basal diet with the lipase preparation was improved numerically from days 8-21 ($P = 0.08$). The lipase preparation reduced ($P < 0.05$) relative caecal weight of the birds fed basal diet, and relative small intestinal weight of birds fed 9% FFRB diet. In Experiment 2, in which a sample of FFRB from Thailand was used, six diets were prepared, being basal, basal + lipase, 9% FFRB, 9% FFRB + lipase, 18% FFRB and 18% FFRB + lipase. Birds were fed pelleted feed *ad libitum* from day 0-14. Excreta were collected over two periods of time, from day 4-7 and day 11-14, for subsequent AME content and lipid digestibility determination. On day 14, six birds from each treatment were randomly selected and euthanased. Lipid digestibility was not improved ($P > 0.05$) by the

addition of the lipase preparation. Performance of birds fed basal diet was improved ($P < 0.05$) by the use of the lipase preparation. Bird performance (growth rate and feed conversion ratio) deteriorated ($P = 0.066$) with increasing level of FFRB in the diets, but feed intake was not affected. The AME contents of the basal and FFRB diets were higher in diets containing the lipase preparation ($P = 0.080$). In Experiment 3, the lipase and/or phytase preparations were used in association with nine diets of similar crude protein (21%) and AME content (3050kJ/kg), these being: a control diet, 9% FFRB + no enzyme, 9% FFRB + lipase, 9% FFRB + phytase, 9% FFRB + lipase + phytase, 18% FFRB + no enzyme, 18% FFRB + lipase, 18% FFRB + phytase and 18% FFRB + lipase + phytase. Birds were fed pelleted feed *ad libitum* from day 0-35. No improvement was found in bird performance when enzyme preparations were added to the FFRB diets. All birds except those on 18% FFRB + lipase + phytase ate significantly less feed than controls ($P < 0.05$). All birds on FFRB-based diets had better feed efficiency value than control. However, only Diet 18% FFRB + no enzyme, 9%FFRB + lipase and 18% FFRB + lipase were significantly better than control ($P < 0.05$).

These studies indicated that:

- The use of a lipase preparation added to diets containing either Australian or Thai FFRB generally improved AME content of both diets and FFRB. However, this was not associated with an improvement in lipid digestibility, suggesting that other enzyme activities in the preparation contributed to the improvement in AME content.
- When added to diets containing FFRB, the lipase preparation produced equivocal results in chicks, as it improved performance with Thai FFRB but not the Australian FFRB. This may be due to the level of FFRB included and varietal and(or) environmental differences during growth.
- Enzyme preparations did not improve performance of birds at low nutrient specification, such as in Experiment 3. This is because a sub-optimal nutrient specification may suppress the feed intake of the birds, thus affecting mean retention time and nutrient digestibility.

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AME	Apparent metabolisable energy
BW	Body weight
Ca	Calcium
Cu	Copper
EDTA	Ethylene-diaminetetraacetate
FCR	Feed conversion ratio
Fe	Iron
Fe^{3+}	Ferric ions
FFRB	Full-fat rice bran
FI	Feed intake
GR	Growth rate
HCL	Hydrochloric acid
K	Potassium
Mg	Magnesium
Mn	Manganase
Ni^{2+}	Nickelous ions
NSP	Non-starch polysaccharides
P	Phosphorus
RB	Rice bran
Si	Silicon
Zn	Zinc

GENERAL INTRODUCTION

Owing to a shortage of locally produced conventional feedstuffs in some developing countries, up to 90% of feed ingredients such as maize, has to be imported from overseas. Trading of imported ingredients in major currencies such as U.S. dollars and Sterling pounds, currency exchange charges, import duties, shipping costs and insurance charges have increased the prices of the imported ingredients. Neglecting capital investment, the daily cost of feeding poultry is about 60-70% of the operation. This directly has made the cost of production of poultry meat and eggs relatively more expensive than developed regions where an ample supply of conventional feed ingredients are available. This has also made those countries become more competitive in the global market for the poultry products.

In many Asian countries, the poultry industry must integrate to become more economically efficient and sustainable. Increased use of unconventional feed ingredients of both vegetable and animal by-products such as rice bran, oil cake meal, insect, snails, earthworms, rumen by-products and poultry by-products, may be one option as they are cheaper and available locally. Some of them (e.g. insect, snails, earthworms, rumen by-product and poultry by-product) may be used as protein sources to animals and some (e.g. rice bran) as an energy source. However, the extent of inclusions of such ingredients is limited as some of them have been found to contain variable quantities of either deleterious agents or antinutritional factors such as non-starch polysaccharides (NSP) and phytate that bring adverse effects to growth of animals.

In order to improve the use of conventional feedstuffs, and encourage the use of unconventional feedstuffs, measures must be taken. Use of exogenous enzymes may improve nutrient utilization, and improve the digestibility of substrates that the bird is inherently less capable of digesting. Full-fat rice bran (FFRB), the by-product of white rice milling, is rich in energy and fat content and can be used as an energy source in broiler rations. However, its use is limited as young chickens are less able to digest FFRB compared to cockerels. Warren and Farrell (1990c) reported a lower ME value of FFRB for chickens compared to cockerels, an effect attributable in part to low

secretion of pancreatic lipase. Therefore, more studies have to be done before this product can be widely used.

The main objective of this thesis is to increase the nutritive value of FFRB fed to broiler chickens by the use of targeted enzymes. The general hypothesis tested was that exogenous enzyme preparations targeted specifically at the lipid and phytate components of FFRB would improve its nutritive value when added at 9% and 18% in broiler chicken diets. More specifically, I hypothesised that the addition of a lipase (enzyme) preparation would enhance lipid digestibility and AME content in broiler chickens.

Chapter 1: Literature Review

1.1 Introduction

Rice bran is available abundantly and cheaply in the Asian region, as about 90% of the world's rice (about 550 million tonnes, FAO, 1995) is produced and consumed in Asia (Marshall and Wadsworth, 1994). Rice bran is relatively high in energy compared to other cereal brans. This is because rice bran has a higher fat content. However, protein, fibre and ash content are comparable to other brans. Rice bran is also high in group B vitamins. Rice bran may be used to replace part of the conventional ration in order to lower the cost of production.

However, high levels of rice bran (i.e. 40%) inclusion in some studies have shown some detrimental effect on broiler production. This may due to some antinutritional factors in rice bran or an inability of broiler chickens to utilize the nutrient in FFRB. Insufficient digestive enzyme production in young chicks may be one of the reasons. Secretion of digestive enzyme is very low at the early life compared to adult cockerels (Nitsan, Ben-Avraham, Zoref and Zir, 1990). Enzyme supplementation in diets may improve the digestibility of the relatively non-digestible portion of the diet and hence improve the efficiency of feed.

1.2 Rice Bran

Rice bran, the by-product of rice milling, consists of both bran and polish fractions. Rice bran is about 10% of rough rice weight (Figure 1.1). When looking separately at the two fractions, bran is relatively more nutritious than polish. Bran, which is made up of pericarp, seed coat (tegmen), nucellus, and aleurone, is about 5-8% of the rough rice weight (Figure 1.2). The aleurone layer consists of many inclusions called protein bodies and lipid bodies. Most of the bran protein and oils are stored in these structures. Bran contains protein, fat, carbohydrate and dietary fibre. Table 1.1 shows the proximate comparison of rice bran to other parts of paddy rice (rough rice) or brown rice as a whole. In addition, the bran contains many vitamins and minerals.

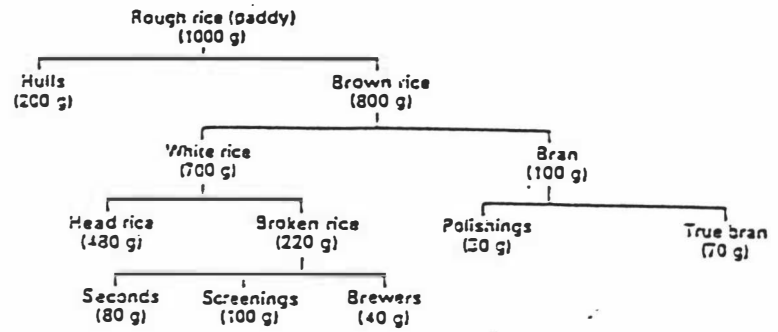


Figure 1.1: The different fractions of paddy rice as a result of milling (adapted from Warren, 1985; cited by Farrell, 1994).

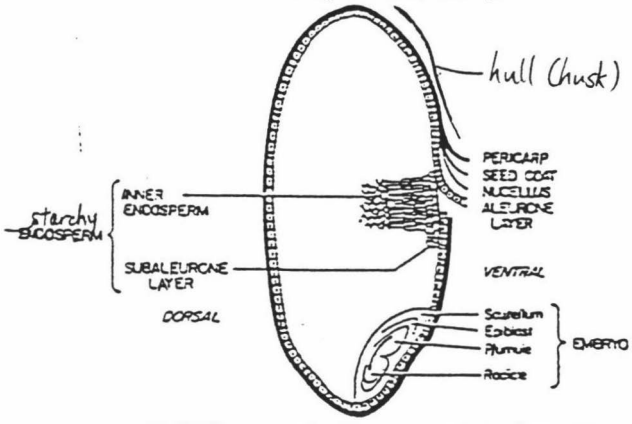


Figure 1.2: Diagramatic representation of rice caryopsis.

Table 1.1 Range of proximate composition of rough rice, brown rice, milled rice, rice hulls, rice bran, rice embryo, and rice polish (% dry basis)

Constituent	Rough	Brown	Milled	Hulls	Bran	Embryo	Polish
Protein (N 5.95)	6.7.-8.3	8.3-9.6	7.3-8.3	2.3-3.2	13.2-17.3	17.7-23.9	13.0-14.4
Crude fat	2.1-2.7	2.1-3.3	0.4-0.6	0.4-0.7	17.0-22.9	19.3-23.8	11.7-14.4
Crude fibre	8.4-12.1	0.7-1.2	0.3-0.6	40.1-53.4	9.5-13.2	2.8-4.1	2.7-3.7
Crude ash	3.4-6.6	1.2-1.8	0.4-0.9	15.3-24.4	9.2-11.5	6.8-10.1	6.1-8.5
Starch	62.1	77.2	90.2	1.8	16.1	2.4	48.3-55.4
Dietary fibre	19.1	4.5	2.7	77.3	27.6-33.3	-	-

source: Pomeranz and Ory (1982), cited in Marshall and Wadswoth (1994).

The polish fraction, which is made up of the subaleurone layer and the starchy endosperm, accounts for 2-3% of the rough rice weight (Juliano, 1985; Marshall and Wadsworth, 1994). The subaleurone layer is rich in protein bodies, it has fewer lipid bodies than the aleurone layer, but contains only a small number of starch grannules. The starchy endosperm is rich in starch granules, contain some protein bodies, especially in outer endosperm layers, and almost no lipid bodies. Polish contains slightly less protein and lipid but considerably more starch than bran (Table 1.1). The polish is only slightly less nutritious than the bran fraction, primarily because it contains lower levels of minerals and vitamins than the bran.

1.2.1 Chemical Composition

Rice bran, like other feed ingredients, contains protein, lipids, fibre, carbohydrate, ash and vitamins. Some component of rice bran may be beneficial to poultry when used as part of the ration (e.g. lipids and vitamin B), but some components of rice bran may be detrimental to poultry production (e.g. silica and phytate).

1.2.1.1 Carbohydrates

1.2.1.1.1 Starch and Free Sugars

Starch is not botanically present in the outer pericarp layers, but because of endosperm breakage during milling appears in the bran. The quantity varies according to the amount of breakage and degree of milling. Values of 5-35% could be expected (Saunders, 1985). However, in an efficient two stages milling operation, values of 5-15% can be expected. Amylose and amylopectin components in the starch depend on rice variety (Saunders, 1985).

Free sugars in rice bran are concentrated in the aleurone layer and are reported to range from 3-5%. Glucose, fructose, sucrose and raffinose have been reported (William and Bevenne, 1953). Non-reducing sugars are more abundant than reducing sugars. Saunders (1974; cited in Saunders, 1985) found 8% sugar in bran on a dry matter basis, of which 80% was sucrose, 5% raffinose, 15% higher oligosaccharides, and only a trace of glucose and fructose.

1.2.1.1.2 Non-starch Polysaccharides

Rice bran is high in dietary fibre, about 30% on a dry matter basis (Table 1.1). Warren and Farrell (1990a) reported a mean neutral detergent fibre (NDF) level of 256 g/kg and an acid detergent fibre (ADF) level of 122 g/kg. NDF of rice bran is more than double the ADF suggesting a high content of hemicellulose (Ali and Leeson, 1995). This may have a negative effect on nutrient utilization particularly by young non-ruminant animals. Nwokoto and Bragg (1977) reported that high fibre levels in diet can depress the availability of some minerals such as Ca, Cu, Mg, Mn, P and Zn. In a study by Normand, Ory and Mod (1979), it showed that crude hemicelluloses from rice bran and whole rice bound bile acids. This means rice bran hemicelluloses have a

good affinity for bile acids and that there is a gradual increase in the amount of bile acids bound as the concentration of hemicellulose increases. Besides the high lignocellulose content, high silica content may also contributes toward the depresses of the digestibility by as much as 7% (Maust, Scott and Pond, 1971). However, Annison, Moughan and Thomas (1995) showed that NSP isolated from rice bran did not depress the AME content of rice bran fed to broiler chicks.

The hemicellulose are a complex fraction not readily digested by non-ruminant animals. The hemicellulose B fraction of bran has been reported to contain 67.9% reducing sugars, primarily pentoses (59.6%). Xylose, arabinose, galactose, and uronic acid were identified as major components, with the first two predominanting. Similar results were found by Annison et al. (1995) in that the soluble NSP consists mainly of arabinose and xylose (0.4 and 0.32 mol % respectively) with appreciable concentrations of galactose (0.17 mol %) and glucose (0.08 mol %) and a trace of mannose (0.03 mol %). The hemicelluloses of bran polish contain a 0.1% water-soluble fraction and a 1% 0.5 mol nitrogen sodium hydroxide extractable fraction. The former had an arabinose : xylose ratio of 1.8 (which is higher than the value of 1.33 in Annison et al., 1995) and contained galactose and protein. The latter contained arabinose and xylose in a 1:1 ratio as a major components, as well as galactose and minor amount of glucose (Luh, Barber and Benedito de Barber, 1991). The 4 and 24% KOH-soluble fractions of hemicellulose, as well as the $\propto$-cellulose fraction (residue from alkaline extraction) of bran (including germ) and polish, have a similar qualitative sugar pattern. They contain galactose, glucose, mannose, arabinose and xylose (Luh et al., 1991).

Cellulose is the most abundant single polymer in the plant kingdom, forming the fundamental structure of plant cell wall. Cellulose in rice bran is reported to range from 9.6-12.8% (Leonizio, 1967; cited in Juliano, 1985). Pure cellulose is a homoglycan of high molecular weight in which the repeating unit is cellobiose. Here the β-glucose residues are 1,4-linked (Figure 1.3).

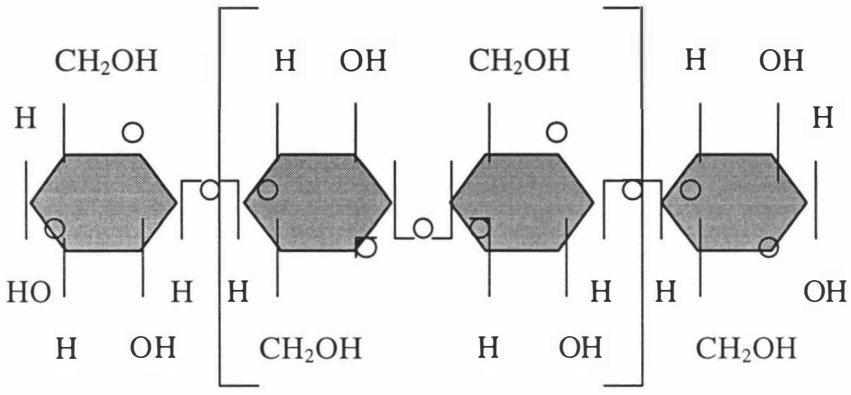


Figure 1.3: Chemical structure of cellulose

1.2.1.2 Lignin

Lignin is not a carbohydrate. It is closely associated with structural carbohydrate (mainly hemicellulose), thus making the carbohydrate less digestible to the enzymes that normally digest them. Strictly speaking, the term 'lignin' does not refer to one, well-defined, individual compound, but is a collective term which embraces a whole series of closely related compound. Lignin is a polymer that originates from three derivatives of phenylpropane: coumaryl alcohol, coniferyl alcohol and sinapyl alcohol. The lignin molecule is made up of many phenylpropanoid units associated in a complex cross-linked struture. The lignin content ranges from 7.7 to 13.11% in the bran, and from 2.01 to 4.42% in the polish (Juliano, 1985).

1.2.1.3 Nitrogen

The nitrogen content of bran varies from 1 to 3% on a dry matter basis. The largest part of rice-bran nitrogen is protein. Non-protein nitrogen accounts for about 16% of the total nitrogen and for about 11% of rice polish nitrogen (Juliano, 1985). Major free amino acids in bran are glutamic acid (7-31%), alanine (11-16%), and serine (5-15%).

The protein (N * 5.95) content of rice bran ranges between 9 and 17%, although it can be closer to 20% in defatted bran. Protein content is influenced by variety, environment, and nitrogen fertilization (Saunders, 1985).

1.2.1.4 Lipids

Rice bran contains 10-23% oil. The main component of crude rice bran oil, the triglycerides, make up approximately 80% of the oil. The triglyceride content varies but is primarily dependent on the extent of hydrolysis that occurs prior to stabilization of the bran. Partial esters present as mono- and diglycerides and free fatty acids are a reflection of the hydrolysis that has occurred. The typical fatty acid composition of rice bran oil is oleic acid (Table 1.2).

Table 1.2 Composition of rice bran oil

Fatty acid	Chain length: No. of double bonds	Percent
Myristic	14:0	0.1-1.0
Palmitic	16:0	12.0-18.0
Palmitoleic	16:1	0.2-0.6
Stearic	18:0	1.0-3.0
Oleic	18:1	40.0-50.0
Linoleic	18:2	20.0-42.0
Linolenic	18:3	0.0-1.0
Arachidic	20:0	0.0-1.0

Source: Nicolosi, Roger, Ausman and Orthoefer (1994).

The minor constituents of an oil consist of phospholipids, glycolipids, sterols, waxes and tocopherols (Table 1.3). The phospholipids present are phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol. The glycolipids are mainly galactose and glucose derivatives (Nicolosi et al., 1994).

Table 1.3 Composition of crude rice bran oil

Triglycerides	80%
Phosphorus	2%
Glycolipids	1%
Sterols	5%
Waxes	2-5%

Source: Nicolosi et al. (1994)

The broad class of unsaponifiable matter of rice bran oil consists of approximately 42% sterols, 24% higher alcohols, 20% ferulic acid esters, 10% hydrocarbons and 2% unknown. The unsaponifiables are generally represented by the sterol fraction of the oils. These include free sterols, sterol esters, sterylglycosides, and acylsteryl glycosides. β-sitosterol is the most abundant sterol present. Although feeding of high

levels of unsaponifiable fractions from natural fats and oils are not deleterious to the performance of chicks, unsaponifiable matter from certain manufacturing processes may contain deleterious materials and therefore should be avoided (Scott, Nesheim and Young, 1982).

Wax concentration in the crude oil is dependent on the extraction method and origin of the bran. Wax may comprise 3-9% of rice bran oil. Generally, the higher the extraction temperature, the greater the quantity of wax removed from the bran when hexane is used as the extraction solvent. Waxes have low iodine values, and high melting points (82-84°C) (Nicolosi et al., 1994).

1.2.1.5 Minerals

Table 1.4 Inorganic constituents of rice bran

Constituent	Content, ppm (dry basis)
Aluminium	53.5-369
Calcium	140-1,310
Chlorine	510-970
Copper	0.37
Iodine	5
Iron	130-530
Magnesium	8,650-12,300
Manganese	110-877
Mercury	0.3
Phosphorus	14,800-28,680
Potassium	13,650-23,960
Selenium	0.170
Silicon	1,700-16,300
Sodium	0-290
Sulfur	80
Tin	17.6-41.3
Titanium	26
Zinc	80

Source: Barber and Benedito de Barber, 1980 (cited in Luh et al., 1991)

Phosphorus is one of the major mineral constituents of bran (see Table 1.4 for mineral content of rice bran). Also present in decreasing order are K, Mg, and Si. The concentration of mineral elements in bran varies with the degree of milling and the growing environment. Some elements (P, K, Mg) increase initially and decrease with deeper milling. Others (Ca, Mn, Fe) exhibit an early sharp decrease with milling. A decreasing concentration gradient occurs in subaleurone layers. The distribution of phytate and mineral elements in endosperm, germ, and pericarp plus aleurone in rice

has been reported by O'Dell et al. (1972; cited Luh et al., 1991). Phosphorus in bran occurs as phytic acid, nucleic acid, inorganic phosphate, carbohydrate, and phosphatide. The reported values, calculated as percentages of the total phosphorus, are 89.9, 4.4, 2.5, 2.3 and 1%, respectively (Luh et al. 1991).

1.2.1.6 Vitamins

Bran is abundant in vitamins of the B group and tocopherol and is poor in vitamin A and C (Table 1.5). Vitamins are not uniformly distributed within the grain. The greatest concentration is found in bran, where the major part of the B vitamins in the grain is located. The pattern of distribution is not identical for all the vitamins. For instance, nearly 80% of the nicotinic acid and only 35% of the thiamine has been found to occur in the pericarp plus aleurone layer. In the germ (scutellum plus embryo), about 2.5% of the nicotinic acid and over 55% of the thiamine are located. Concentration levels of some vitamins, like pantothenic acid and folic acid, in polish may be higher than in bran (Juliano, 1985).

Vitamins occur in bran in free or combined form: 75% of riboflavin was reported to be in esterified form, with 89% of the folic acid, 49% of the pantothenic acid, and 86% of the niacin in the bound form (Juliano, 1985). The vitamin content differs among rice varieties and, to a lesser extent, with location of growth, major causes of variations are the uncontrolled proportion of germ and polish, the differences in the degree of milling and possible hull contamination (Juliano, 1985).

Table 1.5 Vitamin content of rice bran

Constituent, γ/g, dry basis	Content
Vitamin A (carotenes)	4.2
Thiamine	10.1-27.9
Riboflavin	1.7-3.4
Niacin (nicotinic acid)	236-590
Pyridoxine	10.3-32.1
Pantothenic acid	27.7-71.3
Biotin	0.16-0.60
Inositol	4627-9270
Choline	1279-1700
p-Aminobenzoic acid	0.75
Folic acid	0.5-1.46
Vitamin B_{12}	0.005
Vitamin E (tocopherols)	149

Source: Juliano, 1972 (cited in Luh et al., 1991)

1.2.2 Factors Affecting Rice Bran (Bran-Polish) Composition

Other than variety, environment and fertilizer used, the quantity of starch and other nutrients in the bran are a function of the degree of milling and extent of endosperm breakage during the process of milling (Prakash, 1996). Bran contains varying amounts of hull and, in the huller type of mills where dehulling and milling proceed in a single step, bran is actually a mixture of bran and hull. In the case of parboiled rice bran, the paddy undergoes soaking and steaming prior to milling. The process of soaking and steaming hardens the rice kernel and prevents endosperm breakage during milling. This results in a lowering of the starch content in parboiled rice bran. As a result, parboiled rice bran shows a concomitant increase in other nutrients (Prakash, 1996).

Table 1.6 Comparative composition of bran from parboiled and raw rice bran in cone-type mills and from raw rice in huller mills

Constituent	Content (% at 14% moisture)		
	Raw rice bran (cone)	Parboiled rice bran (cone)	Raw rice bran (huller)
Crude fat, %	17.1	19.9	4[b]
Crude Protein, %	14.8	15.9	5.8[b]
Crude fibre, %	10.1	10.7	23.1[b]
Thiamine, µg/g	17.7	6.3	7.0[c]
Riboflavin, µg/g	2.2	1.3	1.2[c]
Niacin, µg/g	215.3	177.6	95.6[c]

Source:

[a] Data from Benedito de Barber et el. (1970; cited in Luh et al. (1991), Kik and Williams (1945; cited in Luh et al. (1991), and Barber and Benedito de Barber (1980; cited in Luh et al. (1991).

[b] From Barber and Benedito de Barber (1980; cited in Luh et al. (1991); minimum values for crude fat and protein, maximum value for fibre.

[c] Calculated from bran-polish and hull data of Kik and Williams (1945; cited in Luh et al. (1991) based on 8% bran and 24% hull.

In addition, bran from parboiled rice is richer in oil (20-25%) but poorer in B vitamins than is bran from raw rice. This is because parboiling results in inward diffusion of B vitamins into the endosperm, with some loss due to heat decomposition. Thus, parboiled milled rice is richer in B vitamins than raw milled rice. It has been shown that higher fat and protein contents of bran from parboiled rice and the dilution of bran by hull in huller mills, causes low fat and protein, but higher fibre levels (Table 1.6). The B vitamins content is higher in raw bran from cone mills than in parboiled rice bran or in huller bran (Juliano, 1985).

1.2.3 Factors Affecting Rice Bran Quality

One problem in the use of rice bran in practical rations is the tendency for the fat to go rancid very rapidly after milling, especially in hot (Sidhom, El Tabey Shetata and Mahasseb, 1975; cited in Hussein and Kratzer, 1982) and humid (Loeb and Mayne, 1952) conditions.

Enzymes, micro-organisms, and insects are major causes of deterioration of rice bran. Lipases, from rice bran itself and molds which include heat resistant spores and, to a lesser extent, oxidases, are responsible for these changes. Insects, whether adults, larvae, or eggs that can cause spoilage, are usually contaminants of commercial rice bran. The oxidative rancidity caused by active lipase and lipoxygenase in rice bran produces breakdown products which are suspected of being toxic, or at least aromatic and thus cause reduced acceptability (Hussein and Kratzer, 1982). Some of these products are suspected to have toxic properties, which may lead locally to damage of the brush border membrance (Kimura, Iida and Taki, 1984; cited in Engberg, Lauridsen, Jensen and Jakobsen, 1996) or, following absorption, to liver damage (Kanazawa, Kanazawa and Nataka, 1985; Kanazawa, Ashida, Minamoto and Nataka, 1986).

Lipases promote the hydrolysis of the bran oil into glycerol and free fatty acids. The rate of free fatty acid formation in rice bran is very high, up to 5-10% in a day and about 70% in a month under condition of high humidity (Desikachar, 1977; cited in Luh, 1991). Poovarodom (1982) reported a rapid rate of free fatty acid formation at 30°C, up to 35% free fatty acid in 23 days, followed by a slower rate. The unsaturated free fatty acids formed are the substrate of lipoxygenase action. Peroxide causes oxidative spoilage of bran components (oil, tocopherols) at low moisture heat stable levels (Luh et al. 1991). Peroxidase appears to be the most heat stable enzyme and is more resistant to heat than lipase and other enzymes in bran.

Methods of stabilizing rice bran can be based on altering the moisture content, temperature, pH, or adding an antioxidant or metal ions to destroy or inactivate the activity of the enzyme lipase. These include processes involving heat treatments, low-temperature storage, chemical treatment and control of relative humidity during storage (Prakash, 1996).

Bran from parboiled paddy is more stable and can stay up to 15 days without any stabilization treatment. It is due to soaking and steaming done prior to milling. Shaheen, El-Dash and El-Shirbeeny (1975) found that parboiling rice bran before milling destroyed lipase activity and prevented hydrolytic rancidity. However, Godber, Martin, Shin, Setlhako, Tricon and Gervais (1993) indicated that although parboiling minimized hydrolytic rancidity, oxidative degradation was still high after milling and during the storage period. Godber et al. (1993) suggested that the high temperature of the parboiling process led to the losses of antioxidant such as Vitamin E and oryzanol, which may be better preserved by extrusion cooking methods. Oxyzanol is a ferulic acid ester of triterpenoid alcohols present at 0.96-2.9% of bran oil. Oxyzanol is reported to have antioxidant properties similar to that of vitamin E (Nicolosi et al., 1994). Nevertheless, a high temperature used in the extrusion cooking processes can also increase the loss of endogenous antioxidant compounds. Martin, Godber, Setlhako, Verma and Wells (1993) showed that an extrusion temperature of 120°C or above provided adequate protection against the development of hydrolytic rancidity. However, Godber et al. (1993) showed that antioxidant compounds were lost at a much faster rate in rice bran extruded at 120°C than 110°C. Martin et al. (1993) indicated that at a temperature of 110°C, the level of free fatty acids was not above 4% until 210 days of storage. Thus, extrusion cooking temperature of 110°C may well be suitable to ensure the destruction of lipases and minimize the loss of antioxidant. The discussion above may support the addition of extra antioxidants (Vitamin E and oryzanol) to reduce the oxidative degradation during storage period.

The optimum temperature of lipase in rice bran is about 37°C, and the optimum pH is 7.5-8.0. Sidhom et al. (1975; cited in Hussein and Kratzer, 1982) observed that the development of free fatty acids in rice bran was much slower when the bran was stored at a cooler temperature. Prabhakar and Venkatesh (1986) showed that lowering the pH of rice bran from about 7 to 4 by using hydrochloric acid at 40 l/ton of bran substantially decrease the activity of lipases. In addition, growth of larvae and mold during the storage period was also depressed using this method compared with heat-treated rice bran (Prabhakar and Venkatesh, 1986).

Hussein and Kratzer (1982) found that ethylene-diaminetetraacetate (EDTA) inhibited the lipase activity in rice bran. Experiments carried out by Hussein and Kratzer (1982) showed that rancid rice bran gave poorer growth than fresh rice bran. When rancidity was prevented by the addition of EDTA, growth of chicks was significantly improved, being essentially equal to that of chicks fed the fresh rice bran. The effect of EDTA was specifically related to delaying rancidity, because its addition to already rancid bran caused no effect on growth.

Lipases are activated by a low concentration of calcium ions and inhibited by heavy metals (Poovarodom, 1982). Mushi, Bhatia, Sekhon and Sukhija (1993) showed that the addition of Fe^{3+} at 100 and 200 μg/g and Ni^{2+} at 200 μg/g in the presence of HCl (2% v/w) reduced the activity of lipases up to 7-10 days of storge.

Extracting the soluble protein fraction from rice bran may remove some of the suspected protease inhibitors and also solubilise some of the rice bran-phytate. However, the protein must be replaced with a trouble-free alternative. Extracted bran would give greater growth rates than that not extracted. There was some indication of this in that extracted bran gave a better response in chickens, and the extract, when added to a bran, slightly reduced performance (Warren and Farrell, 1990b).

Kratzer and Earl (1978; cited in Warren and Farrell, 1990b) showed that pelleting failed to improve the feeding value of diets containing FFRB. Kratzer and Payne (1977) showed that although ethoxyquin was found to slightly reduce the lipases activity, it was less effective than autoclaving or parboiling in preventing the development of rancidity.

1.2.4 Antinutritional Factors in Bran

Antinutritional factors are concentrated in the bran fraction (Juliano, 1985). Barber et al. (1978; cited in Warren and Farrell, 1991) reported trypsin, chymotrypsin and pepsin inhibitors in rice bran extracts. Most of the antinutritional factors are protein in nature and thus heat labile, except for phytin. The phytate content of rice bran is high and varies from 20 to 70 g/kg of the bran; this may also have an effect on mineral nutrition (Warren and Farrell, 1991).

1.2.4.1 Phytin

Rice bran has high phosphorus content (Table 1.4), of which 90% is phytate phosphorus, the highest among cereal brans (Juliano, 1985). Phytin is phosphorus linked to inositol as the Ca-mg salt of myoinositol hexaphosphate. Phytin is located in globoids in the aleurone protein bodies as potassium magnesium salt. Its phosphate groups can readily complex with cations such as calcium, zinc, and iron, and with protein (Juliano, 1985). Oberleas (1973; cited in Ravindran, Bryden and Kornegay, 1995) suggested that phytic acid derived from plants forms complexes with dietary essential elements such as Ca, Zn, Cu and Mg, and make them biologically unavailable. Rice bran has a higher phytate content than wheat bran, corn bran and oat hulls. When these cereal fractions were included at 6% in a chick diet, only rice bran reduced body growth and deposition of zinc, iron and manganese in tibias, which may be explained by the higher phytate levels in the rice bran diets (1.3%) as compared to that in the other diets (0.4%).

1.2.4.2 Trypsin Inhibitor

Tashiro and Maki (1978) and Maki et al. (1980; cited in Juliano, 1985) isolated and characterized trypsin inhibitor from rice bran. It is rich in basic amino acids (lysine, arginine and tryptophan), aspartic acid, glutamic acid, proline and cystine. It is an albumin not a proline. Rice bran trypsin inhibitor differs from soybean inhibitor in not being water-soluble, not being readily destroyed by dry heat, and having a broad spectrum of antiprotease inhibition besides trypsin (Juliano, 1985). However, trypsin inhibitor can be effectively inactivated by boiling or autoclaving the bran at 120°C or removing the trypsin inhibitor with 1% acetic acid for 3 hour compared to dry heating the bran for 15 min and 30 min at 100°C. Nevertheless, trypsin inhibitors in rice bran does not contribute to reduce growth in broilers (Kratzer and Payne, 1977; Deolankar and Singh, 1979).

1.3 Lipid Digestion and Absorption in the Chicken

1.3.1 Digestion and Absorption of Lipid

The digestion and absorption of lipids in the chicken occurs solely in the small intestine. Pancreatic lipase and biliary secretion play important roles in lipid digestion. Both bile and pancreatic secretions enter the small intestine at a more distal site than in other monogastric animals. The most important constituents of bile, from the point of view of lipid digestion, are the conjugated bile salts, although phospholipids, present in only small quantities (less than 4% of total solids) nevertheless have a significant role. The predominant bile acid of the chick, chenodeoxycholic acid, and the minor component, cholic acid, are exclusively conjugated with taurine. The composition of chick bile acids differs from that of both the human (Wooton and Wiggins, 1953) and the pig (Haslewood and Stovall, 1954), the bile salts of these species being conjugated with glycine.

1.3.1.1 Mechanism of Digestion and Absorption

During digestion, large particles of lipid entering the duodenum from the gizzard are subdivided by the emulsifying action of the conjugated bile salts. In this finely divided state, the lipid particles become more susceptible to the action of pancreatic lipase. Co-lipase is required for the attachment and functions of lipase at interfaces, and biliary phospholipids are required for the formation of an active complex of the enzyme at the water-substrate interface (Freeman, 1976).

Sklan, Shachaf, Baron and Hurwitz (1978) showed that there is considerable reflux of digesta between the duodenum and the gizzard. Concentration of bile salts and pancreatic lipase in the gizzard were 10-20% of those found in the duodenum. About 30% of dietary triglycerides appeared to be hydrolysed in the gizzard, as compared to 50-60% in the duodenum (Sklan et al., 1978). This retrograde movement of digesta in the duodenum aids lipid digestion by ensuring that bile salts and pancreatic lipase are distributed throughout the digesta. This processes overcome any disadvantages that arise from bile and pancreatic secretion entering at the more distal part of the duodenum in the chickens, compared to other monogastric species.

The primary products of lipolysis of dietary triglycerides are 2-monoglyceride and free fatty acids, with 1,2-diglycerides as intermediary products. Bile acids play an important role in the transfer of lipolytic products from the oil/water interface to the micellar phase (Freeman, 1976).

Under the conditions existing in the small intestine, bile salts, at a concentration well in excess of their critical micellar concentration and at a temperature above the critical micellar temperature, or "Krafft point", spontaneously aggregate to form polymolecular aggregates, i.e. micelles. The essential, and physiologically important, feature of micellar solutions is their ability to dissolve water-soluble materials, by reason of the facility of the micelle to incorporate appropriately shaped additives either into its core or by interdigitation between the molecules comprising the micelle matrix. This is the principal way by which the products of lipolysis are solubilised into the bile micelle *in vivo* (Freeman, 1976).

Other lipids of physiological significance, e.g. sterols, phospholipids and fat-soluble vitamins, may be solubilised in the liquid paraffinic interior of the mixed micelle. In the case of the various non-polar lipids such as cholesterol, bile salts are considered obligatory for their absorption, since they can only reach quantitatively significant concentration in the aqueous phase in micellar form (Freeman, 1976).

Triglycerides and diglycerides, because of their low or negligible solubilities in bile acid solution, remain as oil droplets. In intestine contents therefore there exists a bulk micellar phase, a particular oil phase, consisting of intact and partial glycerides. Free fatty acids are partitioned between the two phases in a distribution that is dependent both upon pH and, to some extent, the composition of the oil phase (Freeman, 1976).

1.3.1.2 Mode of Uptake and Site of Absorption

It is suggested that the major route of lipid absorption in the chicken is via micelles. However, other minor routes of fat absorption that do not require present of bile also exist (i.e. particulate lipids absorption, diffusion). Although it is known that the major route of lipid absorption is via micelles, but the mechanism by which lipolytic products

pass from the micellar phase into the brush border of intestinal epithelium is largely unknown (Annison, 1983). The jejunum is the major site of lipid absorption in both the chick and laying hen, although some absorption of linoleic, stearic and palmitic acids also takes place in the ileum (Hurwitz, Bar, Katz, Sklan and Budowski, 1973). Following the uptake, triglycerides are resynthesized in the mucosal cells of the small intestine (Freeman, 1976).

1.3.1.3 Transport of Lipids

In mammals, lipids secreted by the microsal cells are almost exclusively transported from the intestine as chylomicrons via lymph (Senior, 1964). In the fowl the lymphatic system is poorly developed, the villus core being uniformly, rather than peripherally, occupied with a capillary network and containing no control lacteal (Kiyasu, 1955; cited in Freeman, 1976). Such a structural design was considered to favour the absorption of all nutrients via the mesenteric portal system. Noyan, Lossow, Brot and Chaikoff (1964) indicated a considerable absorption of fatty acid via the portal route.

1.3.1.4 Factors Affecting the Digestion and Absorption of Lipids

There is a large divergence in the efficiencies with which various lipids and lipid-products are digested by the fowl. Although it is generally recognised that the more unsaturated the fat the higher its digestibility, it has also been suggested that digestibility is influenced by both the melting point of the lipid (Duckworth, Naftalin and Dalgarna, 1950; March and Biely, 1957) and by the chain-length of the constituent fatty acids (Calloway, Kurtz, McMullen and Thomas, 1956; Renner and Hill, 1961a,b). In addition to those above, terminal fatty acid specificity of pancreatic lipase may play important roles in fat digestion. There has been controversy over whether an intramolecular specificity (i.e., a specificity for particular fatty acids at the terminal position) or intermolecular (i.e., a specificity for particular triglyceride species) specificity exists.

It has been shown by Desnuelle and Savary (1963) and Entressangles et al. (1961; cited in Freeman, 1976) that unsaturated fatty acids of chain length C_{12} or greater are hydrolysed from the 1- or 3-position of triglycerides at the same rate as their saturated

counterparts, although short chain fatty acids are hydrolysed from these positions more rapidly. Therefore, intramolecular specificity is confined mainly to the C_4 and C_{12} group of short- and medium-chain fatty acids.

In general, triglycerides containing short-chain or unsaturated fatty acids, irrespective of their intramolecular position, are hydrolysed more rapidly than their long-chain, saturated counterparts (Desnuelle and Savary, 1963; Jenson, Sampugna and Pereira, 1964). This result elaborates the finding of Langworthy and Holmes (1915; cited in Freeman, 1976) that the rate of lipolysis of triglycerides is directly related to their melting point. Undoubtedly the physical properties of the lipids, as influenced by fatty acid composition and to some extent fatty acid configuration of the constituent triglycerides, are important in determining the degree of emulsification of the lipids and consequently its rate of lipolysis.

In addition to the factors affecting lipid digestion above, factors affecting lipid absorption are also important in digestibility of fats. The rate of lipid absorption is limited by the ability and capacity of fatty acids and monoglycerides to form micelles, as fatty acids are the major componet of the micellar phase and principal form in which lipid is absorbed. The equilibria governing the digestion, absorption and transport of lipids is illustrated diagrammatically in Figure 1.4.

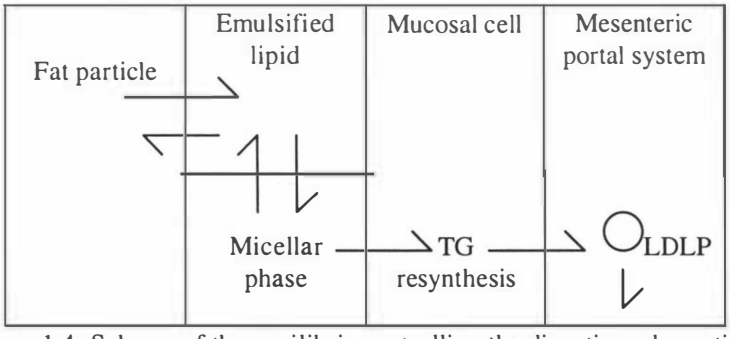


Figure 1.4: Scheme of the equilibria controlling the digestion, absorption and transport of lipid. TG = triglyceride; LDLP = low density lipoprotein (Freeman, 1976)

There is evidence that the partition of fatty acids between the bulk oil and micellar phase is also influenced by the composition of the oil (Freeman, 1969). The fatty acid liberated during fat digestion differ in their capacity to form mixed micelles (Freeman, 1969). Long chain saturated fatty acids (e.g., stearic and palmatic acids) form mixed

micelles less readily than unsaturated fatty acids. The presence of one double bond is sufficient to affect markedly the ease with which micelles are formed, and oleic, linoleic and linolenic acids show similar properties. An important feature of these unsaturated fatty acids is their ability to form mixed micelles with saturated fatty acids (Freeman, 1969). Thus stearic and palmitic acids with their very low solubility in bile salt solution and its greater affinity for the oil phase is heavily discriminated against in entry into the micellar phase. This is result in the almost negligible digestibility of the acid in the free state in chick (Freeman, 1969). Duckworth et al. (1950) showed that mutton fat which has lower unsaturated to saturated fatty acids ratio than linseed oil, has a lower digestibility than linseed oil.

However, within the duodonum, and produced in situ as a result of the digestion of natural fats, is a combination of monoglyceride and unsaturated fatty acids which interact in a manner described above to increase the micellar capacity of the normal-limiting long-chain saturated fatty acids such as stearic and palmitic (Young, Garrett and Griffith, 1963). These interrelationships contribute to the internal synergisms between unsaturated or medium-chain fatty acids and long-chain saturated fatty acids which affect the overall digestibility of more saturated fats. Nevertheless, the use of high levels of long-chain saturated fats in the diet, even in the presence of other oil sources, causes a lower digestibility than if vegetable oil is fed alone. Zollitsch, Knaus, Aichinger and Lettner (1997) showed that the digestibility of animal and vegetable blend oil was less digestible than vegetable oil.

Some lipid is non-polar and has an extremely low solubility in bile salt solutions. These lipid needs higher concentration of bile salt to dissolve (Freeman, 1976). Examples of them include long-chain saturated fatty acids, triglycerides and sterols. This non-polar solute needs the presence of polar solutes to enhance their solubility by expending the outer dimensions of the micelle with a resultant increase in the capacity of its inner core. Thus, monoglycerides, unsaturated and medium-chain fatty acids act synergistically to increase the solubility of their long-chain saturated counterparts in bile salt solutions.

1.3.2 Growth and Development of Digestive Organs

Nitsan et al. (1990) stated that digestive processes are not fully developed in newly-hatched chicks, and that the pancreas is very small. However, the weights of the pancreas, small intestine, small intestinal contents and liver of broiler chicks increases rapidly, at a faster rate than body weight, in early life (Nitsan et al., 1990; Murakami, Akiba and Horiguchi, 1992). This is to accomodate in increase in food intake and storage when digestive system transit from embryonic absorption of yolk to digestion of exogenous feed (see Figure 1.5 and 1.6).

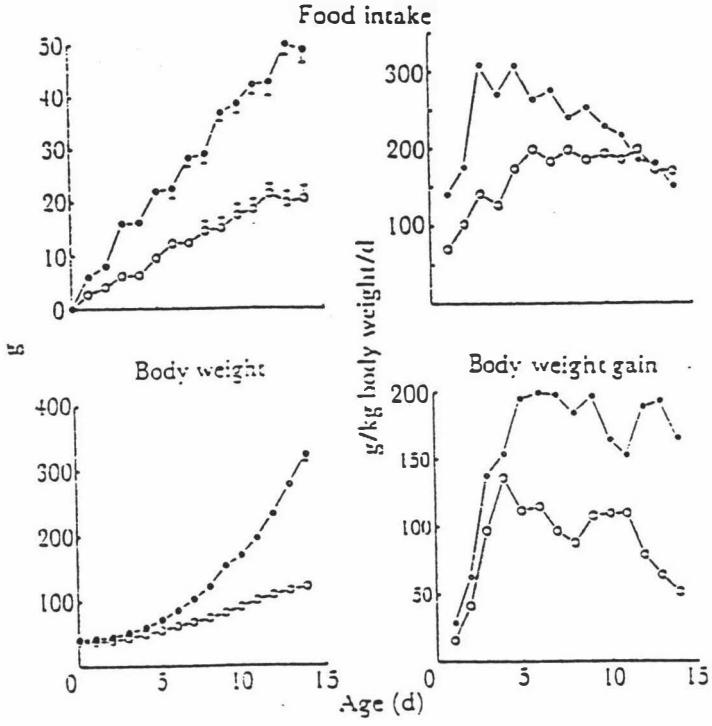
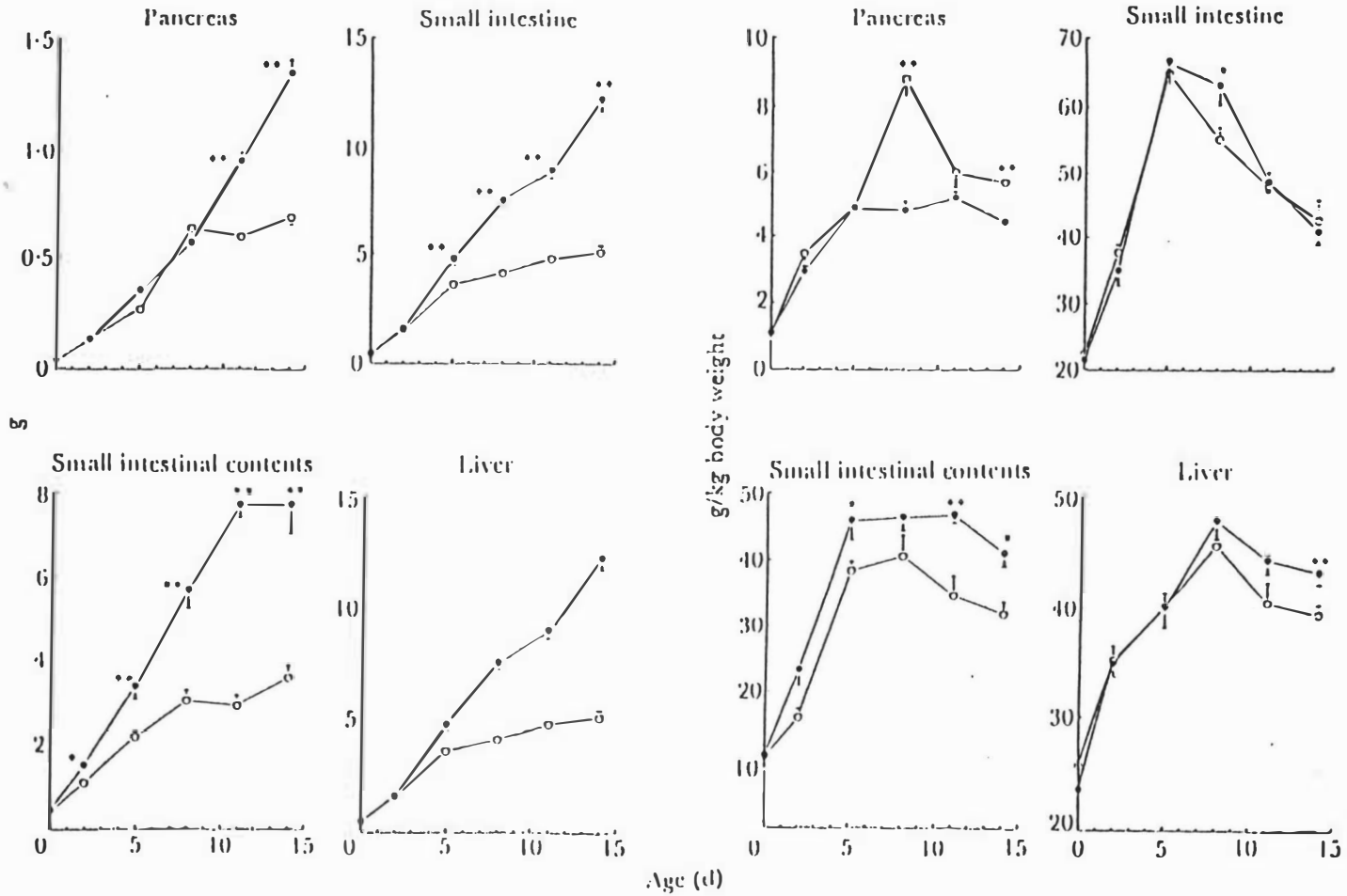


Figure 1.5: Absolute and relative food intake and growth. Broiler, -•-; egg type, -o-. Vertical bars represent the SE; when not shown, the SE is smaller than the symbol (Nir et al., 1993).

Nitsan et al. (1990) indicated that the body weight of the chicks at hatching (42g) doubled by 6 days of age, with another two-fold increase by 11 days. At 23 days of age the average weight (544g) was approximately 13 times that at hatching. In contrast, the weight of the liver increased from approximately 1g on the day of hatching to 15g by day 23. The weight of the pancreas while only 0.006g at hatching increased 10-fold by the 8th day and 30-fold by day 23. The weight of the small intestine was 0.84g at hatching, and increased 10-fold by day 8 and 20-fold by day 23.

Figure 1.6: Absolute (left) and relative (right) weight of the pancreas, small intestine, small intestinal contents and liver of broiler (-•-) or egg type (-o) chicks. Vertical bars represent the SE; when not shown, the SE is smaller than the symbol. Values between breeds differ significantly, *P < 0.05, **P < 0.01 (Nir et al., 1993).



Besides the increase in size and weight of the small intestine, the morphology of the intestine also changes with age. Uni, Noy and Sklan (1995) and Uni, Ganot and Sklan (1997) stated that the intestinal surface changes considerably and the rate of enterocyte proliferation increase posthatch. Bayer, Chawan, Bird and Musgrave (1975) and Uni et al. (1997) indicated that from day-1 to 14 villus area was increased and microvillus development occured throughout the intestine.

In the duodenum, the major increase in villus height (Baranyiova and Holman, 1976) and volume (Noy and Sklan, 1997 and Uni et al., 1997) occurred at/or before four day of ages and the rate of growth then decreased. In contrast, in the jejunum and ileum the increase in villus volume was maintained until 10 days of age, after which time the rate of growth diminished (Noy and Sklan, 1997; Uni et al., 1997). Uni et al. (1995) and Uni et al. (1997) showed that jejunal villus volume was initially lower than duodenal villus volume but became greater after day 10, whereas ileal villus volume was lower throughout. The ileum has the lowest increase in villus volume with age. Other than the villus height and volume, the villus diameter were also found to change significantly at the early life of the chickens (Baranyiova and Holman, 1976). In newly-hatched chickens, the diameter between duodenum, jejunum and ileum were significantly different, with the diameter of the duodenum being the largest, followed by jejunum. However, the differences between them became unsignificant when all of them almost doubled by day five (Baranyiova and Holman, 1976). Crypt depth, which reflects enterocyte differentiating activity, increased linearly in both duodenum and jejunum until 10 to 12 days (Noy and Sklan, 1997; Uni et al. 1997). Noy and Sklan (1997) stated that the increase in crypt depth in jejunum was greater than in the duodenum, however, Uni et al. (1997) indicated otherwise.

Intestinal development, however, can be delayed due to absence of food at an early life. Baranyiova and Holman (1976) and Uni et al. (1997) indicated that villus volume, height and diameter were significantly much smaller in fasted-chickens than fed-chickens.

The caecal tubes constitute a region of the digestive tract where microbial breakdown, particularly of cellulose-containing materials, takes place. Radeff (1928) and Henning (1928; cited in Annison, Hill and Kenworthy, 1968) indicated that fibre digestion

occurs mainly in the caecum and little or none occurs in the crop. Volatile fatty acids are the major end-products of microbial fermentation in the digestive tract of the fowl. As such, their role in birds fed diets containing little fibre is a minor one, even though fermentation of other dietary components, which escape enzymic digestion, also occurs in the caeca. The contribution of the absorbed fermentation products as an energy source to the fowl is small (Annison et al., 1968). As in the tract of other species, e.g., the rumen of ruminant and the pig caecum, acetic acid is produced in the greatest quantity, with lesser amounts of propionic and butyric acid and trace amounts of other acids, the pattern being characteristic of fermentative activity under strictly anaerobiosis (Annison et al., 1968). In addition to being a chief site of fibre digestion, Thornburn and Willcox (1964) indicated that caeca are also active in absorption of water from the digesta. Another possible role for fermentation reactions within the caeca is vitamin synthesis. Although this has not been adequately evaluated, as caecectomy has negligible effect on performance, it would appear that caecal function is of little significance in the modern fowl. Fibre, in addition to its role in fermentation in caeca, also plays an important role in caeca development. Wang, Marquardt, Guenter, Zhang and Han (1997) indicated that rice bran, which has much higher fibre than maize, caused an increase in caecal size (longer and heavier caecal weight).

1.3.3 Digestive Enzyme Activity in the Pancreas

Digestion and absorption of macromolecules requires sufficient enzymatic hydrolysis at, or before, the sites of uptake. When feed intake increases, as in the posthatch chick, greater enzyme activity or longer retention time may be necessary for hydrolysis in the small intestine. Lack of pancreatic enzymic hydrolysis in the intestinal lumen decreases the apparent digestibility of the dietary components and reduces growth (Nitsan et al., 1990). Thus, it is essential that the secretory activity of the pancreas achieve maximal growth in early life.

At hatch and during early growth, the synthesis of digestive enzymes in the pancreas is limited. They then increase to maximum values around day 10, when relative growth rate was maximal, indicating a possible association between these two traits (Nitsan et al., 1990). It was also shown by Nitsan et al. (1990) that in broiler chicks, maximal

relative growth of body weight of 20% was attained at five days of age and was maintained for at least 5 days with some fluctuation later. Chicks hatch with some reserves of pancreatic enzymes that are produced during embryonic growth. These reserves decrease rapidly, because synthesis during this period are less than those required for secretion to the intestine and for maintaining the initial concentration (Nitsan et al., 1990).

Nitsan et al. (1990) showed that specific activities of trypsin, amylase and lipase in the pancreas decreased during the first 3 to 6 d after hatching. They then increased afterwards to between 10 and 20% higher than those at hatching, on days 14, 11 and 21 for trypsin, amylase and lipase, respectively. Similar results (Figure 1.7) were found by Nir, Nitsan and Mahagna (1993), where specific activity of amylase was highest on the day of hatching and decreased up to the day 8. Lipase specific acivity increased gradually from very low post-hatch values to about 40-fold on day 14. Trypsin specific activity increased gradually to attain a peak at 11d, while chymotrypsin declined during the first 8 d and incresaed markedly thereafter. Krogdahl, A. and Sell, J. L. (unpublished observations cited in Krogdahl, 1985) showed that the lipase concentration of pancreas was low at hatching and increased slowly during the first week. It appeared that the lipase concentration was still increasing at eight-weeks of age when the experiment was terminated.

Enzyme activity levels in the pancreas increased with age for relative amylase, total trypsin, total and relative amylase, total trypsin, total and relative chymotrypsin, and total and relative lipase (O'Sullivan, Dunnington, Larsen and Siegel, 1991). Nitsan et al. (1990) found that the increase in lipase activity was gradual during the experimental period and attained about 2.5-fold that at hatching on day 23. When expressed as units of activity per kg body weight, the activities of all enzymes increased with age, reaching maxima on days 8 (amylase and lipase) or 11 (trypsin and chymotrypsin). Lipase activity increased rapidly up to 4 d (Nitsan et al., 1990). Therefore the ability of young bird to digest food is not fully developed, especially the ability to digest lipids as lipase activity has the slowest increase posthatch amongst the major enzymes.

Generally it is agreed that the enzyme activity of the major enzymes increase posthatch. However, their content in pancreas will adapt to diet, and changes in proportion to the dietary content of protein, carbohydrate and fat (Brannon, 1990). A main point in digestive enzyme adaptation is the need for a minimum protein supply. During prolonged starvation (Siddons, 1972) or short-term dietary protein deficiency (Le-Thanh Uyen, 1969; Nicholson, McCarthy and Kim, 1974; Corring and Sancier, 1972; cited in Corring, 1980), all digestive enzyme activity is reduced. When the animal receives enough protein, the presence or absence of other dietary substrates affects the specific hydrolysis of the enzymes involved.

1.3.4 Digestive Enzyme Activities in the Intestinal Content

Specific activities (u/g) of amylase, lipase, trypsin and chymotrypsin are very low after hatch and increase with age. Nir et al. (1993) found that the specific activity of all enzymes was generally superior in layer chick than in broiler chick when these two breeds were compared. However, the difference were less pronounced when expressed in units per kg body weight (Figure 2.8). Nir et al. (1993) indicated that the specific activities (u/g) of all the digestive enzymes in the pancreas of both layer and broiler chick were quite similar. However, the total activities relative to body weight (u/kg BW) showed that they may be limiting in the broiler chicks while in the layer ones they may exceed what is needed to digest the amount of food consumed (Figure 1.7).

Nir et al. (1993) assumed that the broiler chicks ate at a rate approaching gut capacity. This is because during the first week of life, they consumed a daily quantity of food reaching 25 to 30% of their body weight, an amount double that consumed by the layer chicks. This large intake was reflected in the amount of intestinal contents, which was not compensated either by increase in the size of the pancreas and small intestine, or by the activity of the digestive enzymes which were lower in the broiler chick than in the layer chicks (Figure 1.7). Dunnington and Seigel (1995) showed that high body-weight had proportionally smaller gastro-intestinal tracts than low body weight chicks, but feed was digested and cleared at a faster rate in high body-weight chicks. From the above it may be concluded that in broiler chicks, a similar secretion of digestive enzymes had to cope with a higher amount of chyme than in layer chicks, especially for lipase, as its increase in duodenum secretion is the slowest among the enzymes.

Figure 1.7: Activities of amylase, lipase, trypsin and chymotrypsin in the pancreas of broiler (-•-) or egg type (-o) chicks, expressed as units/g (left) and units/kg body weight (right). Vertical bars represent the SE; when SE not shown, the SE is smaller than the symbol. Values between breeds differ significantly, *P < 0.05, **P < 0.01 (Nir et al., 1993).

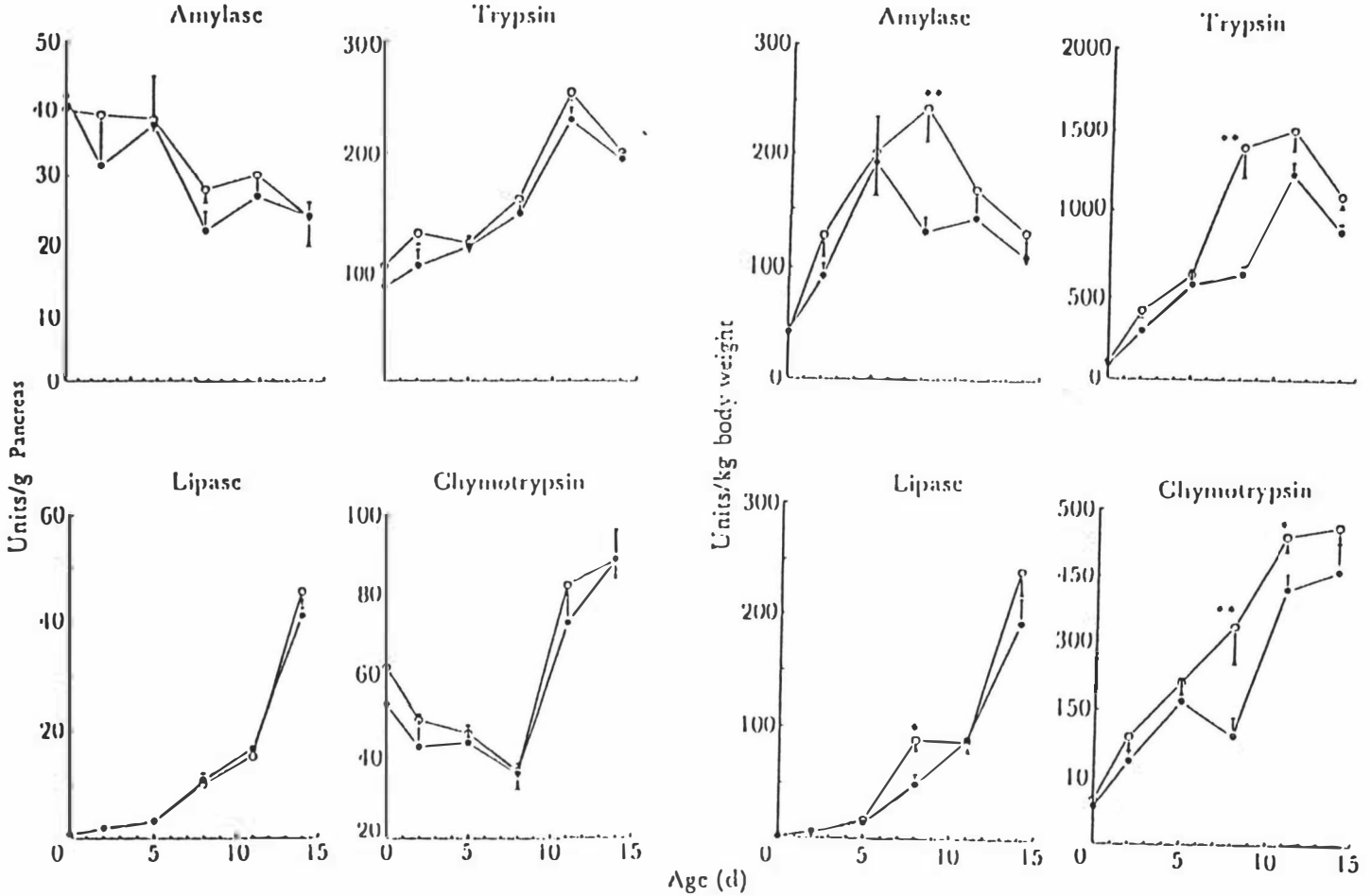
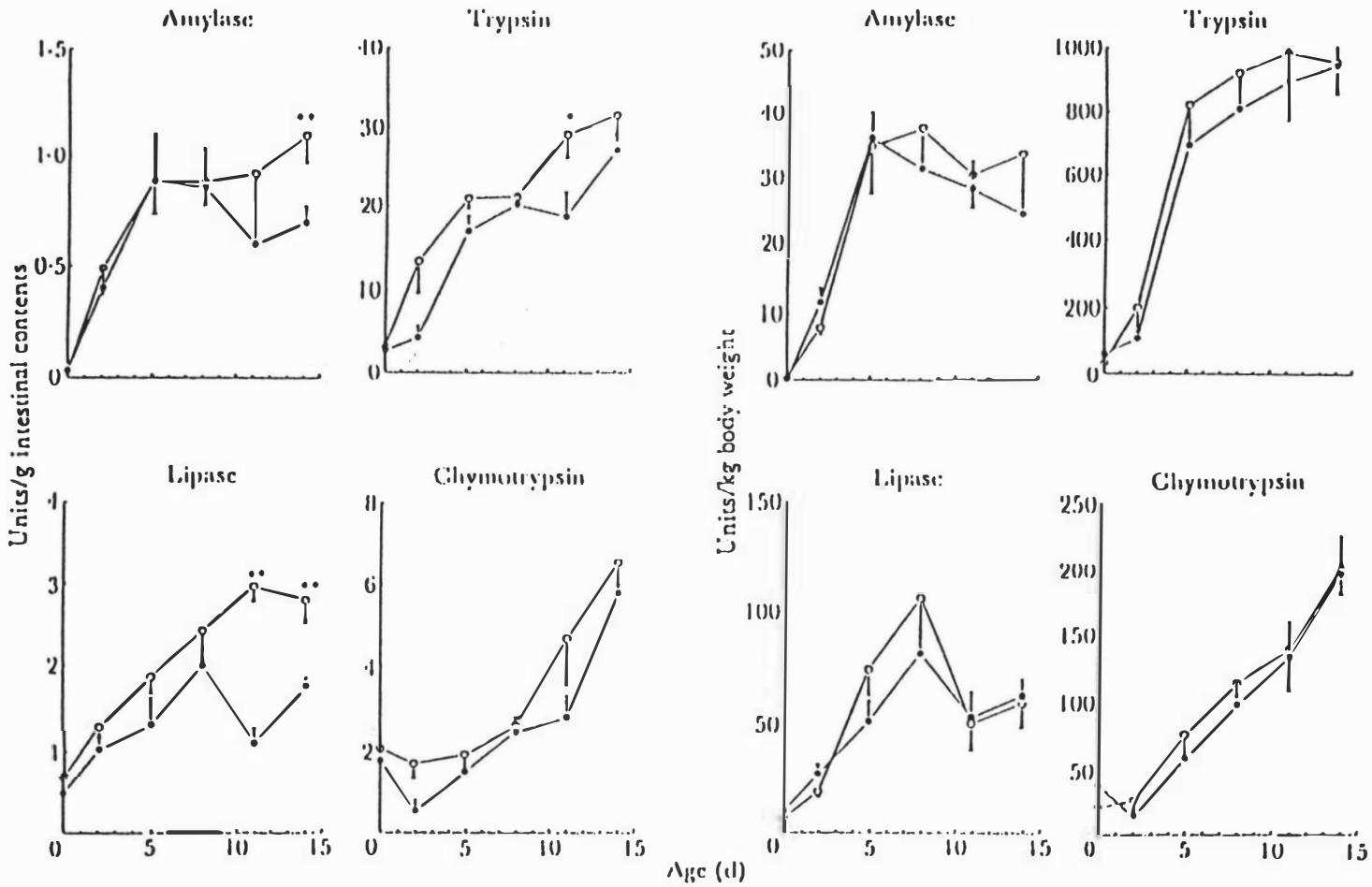


Figure 1.8: Activities of amylase, lipase, trypsin and chymotrypsin in the contents of the small intestine of broiler (-•-) or egg type (-o-) chicks, expressed as units/g (left) and units/kg body weight (right). Vertical bars represent the SE; when not shown, the SE is smaller than the symbol. Values between breeds differ significantly, *P< 0.05, **P < 0.01 (Nir et al., 1993).



1.3.5 Digestion and Absorption of Lipid in Young Broiler Chick

In the newly-hatched fowl, yolk lipids are the major source of energy. Digestion of these lipids appears to be catalyzed by lipases secreted from the internal surface of the yolk sac (Krogdahl, 1985). As the chicks change from an endogenous to exogenous food supply, secretion of pancreatic enzymes is stimulated. The secretion of pancreatic lipase to the duodenum increase less and somewhat more slowly than the other enzymes, especially between 4 and 21 day (where the increase in lipase secretion was lowest, and highest for amylase). However, Krogdahl (1985) found that the extent of increase in lipase secretion and lipase activity of intestinal contents are dependent on the amount of lipid presence in diet. Krogdahl (1985) found that in poults fed high fat diets, the lipase activity per gram dry matter increased about 10-fold from 2 to 56-day of age. In contrast, in chicks fed low fat diets the increase in lipase activity of intestinal contents was negligible.

It was found that not only is secretion of lipase to the duodenum low in the early life of chicks, but the biliary secretion is also low and increases with age. Young birds are unable to replenish bile salts lost by excretion as readily as older birds (Serafin and Nesheim, 1967), perhaps because young chicks have a limited ability to synthesize bile acids. Duodenal bile acid secretion increased more than 2-fold between 4 and 7 day and between 7 and 10 day, and thereafter the rate of secretion increase by 80% between 10 and 21 day (Noy and Sklan, 1995). It was found that supplemental bile salts enhanced fat digestion in 7-day-old chicks (Noy and Sklan, 1995). Kussaibati, Guillaume and Leclercq (1982) also indicated that addition of bile salts mainly improved the digestibility of saturated fatty acids except in the case of oleic and linoleic acid (in maize oil). Similar results were also found by Gomez and Polin (1974, 1976), who found that the addition of cholic acid significantly improved the apparent digestibility and absorbility of all lipid (tallow, lard, hydrogenated soybean oil), and increased metabolizable energy value of the diets. However, the extent of improvement in supplementation of bile acid would be different for different bile acids (Gomez and Polin, 1976; Polin,Wing, Ki and Pell, 1980). Gomez and Polin (1976) showed that cholic acid and chenodeoxycholic acid produced a larger improvement in tallow absorption than taurocholate. Polin et al. (1980) showed that deoxycholic acid

and sodium taurocholate did not improve weight gain and feed efficiency of the birds over the 20 days of the experiment, however cholic acid, chenodeoxycholic acid and dehydrocholic acid did. However, the latter two bile acids seemed to lower dry matter and lipid retention during the third week of the chicks' life. Therefore the lack of an effect by taurocholate and deoxycholic acid indicated that there is a molecular specificity for the improved absorption of tallow.

1.3.6 Broiler Digestion in Feeding of Rice Bran

In general, inclusion of more than 200 g/kg of rice bran in diets for broiler chickens depresses performance, and the higher the amount of rice bran in the diet, the more pronounce the negative effect on performance. However, the magnitute of the effect varies considerably. For example, Farrell (1994) and Warren and Farrell (1991) observed no change in FCR when 400 g/kg rice bran was included in diets in some experiments, while in others a poor feed conversion was observed at 200 g/kg. Clearly other factors like variation in the fibre, protein and oil content, rancidity of the lipid fraction and other constituent in rice bran may be effect chick performance in an unknown manner.

Annison et al. (1995) demonstrated that NSP component in RB had no detrimental effects on growth of chickens. All birds receiving the RB-NSP grew at similar rates to the birds fed sorgum-casein based control diet. In addition, Aboosadi, Scaife, Murray and Bedford (1996) found no increase in AME content or performance when cell-wall degrading enzymes were added to the diets of broiler chickens. Kratzer, Earl and Chlaravanont (1974) indicated that protein did not attribute to the growth depression when 60% of rice bran was fed to chicks of day-old to 28-day-old. In contrast to the finding on NSP and protein above, Warren and Farrell (1990c) found significant reductions in the utilization of dry matter, energy, ether extract and nitrogen in young broiler chickens compared to adult cockerels. Consequently, the AME content of rice bran was lower (by 4.6 MJ/kg on average) in broiler chicken than with adult cockerels, caused predominantly by a reduced apparent digestibility of lipid in rice bran. Similar results were reported by Askbrant and Farrell (1987), where the AME content of oil in rice bran increased significantly with the age of the bird.

However, the low AME content of rice bran in early life may be overcome by incorporating an exogenous lipase preparation in diets containing rice bran to raise the AME, as was demonstrated by Pluske, Moughan, Thomas, Kumar and Dingle (1997). Some improvements on growth rate and FCR in the inclusion of lipase in the diets were also noted. However, the work by Pluske et al. (1997) only showed the response of the broiler chickens up to 14 day of age, therefore the response of the chickens on the lipase preparation after that is not known.

There is still uncertainty in the literature regarding the nutritional value of rice bran. Even in brans of similar chemical composition there may be nutritional differences, as indicated by the general superiority of 'Starbonnet' rice bran by Warren and Farrell (1990b). Warren and Farrell (1990b) suggested that fibre *per se* and the lipid component may not be prime determinants of the nutritional worth of rice bran, as age of bird, availability of mineral and vitamin, and processing methods may influence the nutritive value of rice bran. The effect of variation in nutritive value of rice bran is more pronouced for the diet that contained the high concentration of rice bran (e.g. 50%). It has been indicated that there is usually a decrease in the weight of broiler chicks as the content of full-fat rice bran in the diet is increased (Wang et al., 1997). The magnitute of the effect varies considerably among experiments and in part, depend upon the effect that the diet has on feed intake. Similarly, the FCR is usually poorer with increasing dietary inclusion of rice bran. The point at which this occurs, however, varies.

In addition to affecting the performance of broiler chickens, rice bran substantially increases the size of the gastrointestinal tract (Wang et al., 1997). Similar increases have been reported in chickens fed a diet high in soluble (Scout barley) or insoluble fibre (Bedford barley) (Wang et al., 1997). Part of the effect in the current study may be attributed to difference in mature size of the broiler in the different treatment groups as younger less-developed birds tend to have larger relative sizes of intestines than more mature birds. These observations, nevertheless, suggest that the high content of NDF in rice bran may have been responsible in the current study for the

increased size of the gastrointestinal tract. The arabinoxylan is probably mainly responsible for this effect as they comprise the bulk of the hemicellulose in rice bran.

Rice bran from different regions and harvest years sometimes gives different production responses. Wang et al. (1997) showed that rice bran from Malaysia and China gave different results when fed to chicks with the inclusion of a crude enzyme that contained xylanase, β-glucanase, pectinase, CMC-cellulase, acetyl esterase and β-xylosidase. In contrast to Malaysian rice bran, enzyme addition to diets containing Chinese rice bran did not affect chick performance. In these experiments, the performance of chicks fed the Malaysian rice bran without enzyme and the Chinese rice bran with or without enzyme were generally the same as for chicks fed the control wheat-soybean meal containing diets. In contrast, the weight gain of chicks that were fed the diet containing the Malaysian rice bran that was supplemented with 1 or 10 g/kg enzyme were 6.9% and 10.9% higher than for the chicks fed the control wheat-soy diet.

The high concentration of enzyme added to broiler chicks diets in the study of Wang et al. (1997) containing the Malaysian rice bran not only improved the feed to gain ratio, but also reduced the incidence of vent pasting and decreased the size of the gastrointestinal tract. These result collectively indicate that enzyme suppplementation of chickens containing rice bran can improve chick performance in some cases. The reason for the different response to enzyme treatment of the rice brans obtained from two different regions in Asia was not established. Part of this difference may be caused by differences in processing methods and(or) to varietal or environmental differences during growth. The limited proximate analysis carried out on the rice bran would indicate that it was not caused by a difference in the content of crude protein and fat.

1.4 Nutritional Features of Phytate

1.4.1 Digestion of Phytate and Its Bioavailability

Owing to insufficient or lack of endogenous phytase, which hydrolyses phytic acid, phytate phosphorus is biologically less available to poultry. Bitar and Reinhold (1972) found that phytase activity is present in the mucosa of the small intestine of poultry, and Davies and Motzok (1972) reported that a homogenate of chick intestinal mucosa hydrolysed sodium phytate. Maenz, Engele-Schaan and Classen (1995) showed that the intestinal brush border membrance contains phytase activity that is independent of non-specific phosphatase enzymes. They also reported that, at optimum pH (pH 6), at least two distinct enzymes contribute to the total phytase activity in the brush border. However, the endogenous phytase activity in the intestinal secretions of poultry is extremely low, at least in young birds (Maenz et al., 1995).

1.4.2 Factors Influencing Phytate Phosphorus Utilization

1.4.2.1 Dietary Calcium and Phosphorus Levels

Phytate phosphorus utilization by poultry has been shown to be influenced by both dietary calcium and phosphorus concentrations (Mohammed, Gibney and Taylor, 1991). At very high calcium concentrations phytate conditions, where the dietary calcium and phosphorus concentrations are formulated to result in maximum performance and bone calcification, phytate phosphorus is utilized very little by poultry. Nott, Morris and Taylor (1967) found that, when calcium intake was adequate for optimum shell quality, the birds did not utilize phytate phosphorus, and excretion of phytate phosphorus increased with dietary levels. Ballam, Nelson and Kirby (1984) found that chicks fed on diets containing 1.0% calcium hydrolysed less phytate than those fed diets with 0.85% calcium. Similarly, Mohammed et al. (1991) reported that phytate phosphorus utilization was increased by 15% when the dietary calcium content was reduced from 1.0% to 0.5%.

Vandepopuliere, Ammerman and Harms (1961) found that chicks fed on a diet with a calcium:phosphorus ratio of 1:1 performed better than those fed on a diet where the ratio was 2:1. In addition to mortality decreasing as Ca:P ratio narrowed down from 8:1 to 1:1, less rickets developed. Harms, Waldroup, Shirley and Anderson (1962)

showed that widening the calcium:phosphorus ration in the diets from 1:1 to 2:1 decreased the availability of the phosphorus from phytic acid to a greater extent than that from inorganic supplements such as dicalcium phosphorus.

1.4.2.2 Dietary Vitamin D_3 Level

Ewing (1963) showed that phytate phosphorus utilization was depressed by feeding diets marginal or deficient in vitamin D_3. Edwards (1993) and Mohammed et al. (1991) indicated that the addition of vitaminD_3 enhanced the amount of phytate phosphorus retained by chickens. In these studies the utilization of phytate phosphorus increased from 31-50% to 68-87%. Maddaiah, Kumick, Hulett and Reid (1964) found that the activity of phytase increase when Vitamin D_3 level was raised. Nott et al. (1967) and Mohammed et al. (1991) indicated that addition of vitamin D_3 lower the incidence of hypophosphataemia and hypercalcaemia.

1.4.2.3 Age of Birds

Edwards, Polo, Soonchaerenying and Elliot (1989) reported that the ability of poultry to utilize phytate phosphorus increased with age. They found that 21-day-old broilers utilized phytate phosphorus better than 14- and 7-day-old broilers. In this study a significant effect of sex was also observed, with males retaining more phytate phosphorus than females. Nelson (1976) observed only a slight increase in phosphorus utilization by the older birds. Interestingly, the ability of layers to utilize phytate phosphorus appears to decline with advancing age. Scheideler and Sell (1987; cited in Ravindran et al., 1995) reported that the retention of phytate phosphorus was quite high at 34 weeks of age, averaging 46.7%, but decreased to 9.1% and 16.5%, repectively, at 50 and 72 weeks of age. The reasons for this are unclear. In contrast, Maddaiah et al. (1964) found no significant differences in intestinal phytase activities of chickens and mature hens.

1.4.2.4 Type of Dietary Ingredients

Differences in the solubility of phytate from different sources have been reported by de Boland, Garner and O'Dell (1975), who found that the phytate in soybean meal was more soluble than that in sesame meal. This suggests that the variation in phytate

solubility may be responsible for the differences in the extent of hydrolysis of phytate from different feedstuffs, if one assumes that soluble phytate is a better substrate for enzymatic degradation. Simons, Versteegh, Jongbloed, Kemme, Stump, Bos, Wolters, Beudeker and Verschoor (1990) showed that percentage of phytate in maize and soya bean meal being degraded are different when incubated with phytase for one hour at 40°C.

1.4.3 Effect of Phytate on Bioavailability of Other Nutrients

1.4.3.1 Effects on the Mineral Bioavailability

Phytic acid in foods derived from plants forms complexes with dietary essential elements such as calcium, zinc, copper, iron, and magnesium, and makes them biologically unavailable. Vohra, Gray and Kratzer (1965) reported that phytate forms complexes with cations in the following descending order of strength: $Cu^{2+} > Zn^{2+} > Ni^{2+} > Co^{2+} > Mn^{2+} > Fe^{3+} > Ca^{2+}$.

Although Ca has the lowest binding affinity, the greatest impact of phytate on mineral nutrition (other than phosphorus) is on calcium biovailability. Nelson, Shieh, Wodzinski and Ware (1968) found that the calcium requirement of White Leghorn chicks fed a purified diet containing no phytate was 0.5%, but this was increased to 0.95% with a practical diet containing 12.5% phytic acid. Therefore, Nelson (1984; cited in Ravindran et al., 1995) suggested that the dietary calcium requirement of poultry must be expressed in terms of available rather than total calcium. Thus, if diets were to contain ingredients high in phytate, more calcium would be required to offset the portion that was unavailable as insoluble calcium phytate. The ARC (1975; cited in Warren and Farrell, 1991) suggested that for every 1g phytate phosphorus above 2g/kg in mixed diets for poultry, calcium levels should be increased by 1.3g/kg above those recommended as adequate.

The effect of phytate of sesame meal on zinc availability for chicks has been demonstrated by Lease (1966). O'Dell (1962) reported that growth rate was reduced when phytic acid was added to chicks diets, an effect that could be counteracted by zinc supplementation. Davis, Norris and Kratzer (1961) showed that diets containing

isolated phytate reduced copper and manganese availability for chicks. Deolankar and Singh (1979) showed a depression in calcium and iron availability to chickens of mixed diets containing rice bran relative to a maize-based control. They assumed that the reduction in both calcium and iron availability was the result of the phytate content of rice bran, and postulated the formation of a calcium-fatty acid soap in the intestine which may have reduced the absorption of calcium. Nwokoto and Bragg (1977) found significant inverse relationships between the availability of minerals (Ca, Mg, Zn and P) and phytate levels. In addition to that it has been shown by Warren and Farrell (1991) where the NDF levels were the same for all diets, that calcium and phosphorus retention values were both lower in the diets with higher phytate levels.

Tao, Fox, Phillippy, Fry, Johnson and Johnston (1986) indicated that the lower phosphates of inositol (inositol mono-, bi-, tri- and tetra-phosphate), which are formed during the stepwise dephosphorylation of phytate, may have nutritionally less effect on mineral availability than inositol phosphates with five or more phosphate groups. It has been suggested that at least five of the six possible sites on inositol need to be phosphorylated in order to exert an inhibitory effect on the intestinal absorption of zinc and calcium. It would appear, therefore, that the influence of phytate on mineral utilization may also vary, depending on the degree of phytate degradation and the proportion of lower phosphates of inositol present in the gut.

1.4.3.2 Effect on Protein Availability

Barre et al. (1956; cited in Ravindran et al., 1995) showed that phytate-protein complexes are more resistant to proteolytic digestion than the protein alone. Camovale, Lugaro, and Lombardi-Boccia (1988) reported that a phytate-protein interaction reduced the availability of legume protein, and that the protein source is an important factor. This is because the solubility of phytic acid and protein is different for different protein sources and at different pH levels. Phytic acid can form complexes with protein at both acidic and alkaline pH. Cheryan (1980) offered the following explanation: at low pH (about pH 2), phytic acid is strongly negatively charged while proteins are strongly positively charged, thus phytate-protein complexes may be formed. At high pH both phytate and protein are negatively charged so that

multivalent cations such as calcium are thought to mediate such phytate-protein complexes. This interaction between phytic acid and protein leads to decreased protein solubility (Saio et al. 1967; cited in Ravindran et al., 1995). In turn, certain functional properties of the protein can be adversely affected because they depend on hydration and solubility (Cheryan, 1980). Knuckles, Kuzmicky and Betschart (1985) found improvements in protein utilization with decreased dietary concentrations of phytate. In contrast, Thompson and Serraino (1986) suggested that phytic acid does not affect protein digestibility.

Phytate is known to inhibit a number of digestive enzymes such as pepsin, α-amylase (Deshpande and Cheryan, 1984) and trypsin (Singh and Krikorian, 1982; Caldwell, 1992). Liener (1989; cited in Ravindran et al., 1995) suggested that inhibition may result from the chelation of calcium ions which are essential for the activity of trypsin and α-amylase, or possibly from an interaction with the substrate for these enzymes. These negative influences may also partly explain the effects of phytate on protein utilization. However, the extent to which the inhibition of enzyme activity by phytate contributes to its overall anti-nutritional effect remains uncertain.

1.4.3.3 Effect on Starch Digestibility

Caldwell and Kung (1953) suggested that calcium ions are required for the activity and stability of ∝-amylase. Binding of calcium ions to phytate render them unavailable reduces α-amylase activity. Cawley and Mitchell (1968; cited in Sebastian, Touchburn and Chavez, 1998) reported phytate suppressed the α-amylase activity in sprouted wheat meal by complexing with the calcium ion necessary for enzyme activity. Thomson and Yoon (1984) reported that the addition of phytate to wheat reduced starch digestibility by 60% compared with that of the control treatment. Knuckles and Betschart (1987) showed that phytate decreased strach digestion by enzyme inhibition.

1.4.4 Effects of Microbial Phytase on Phytate Phosphorus Availability

Nelson et al. (1968) incubated soybean meal with a crude phytase preparation before feeding and found that chicks utilized the hydrolysed phytate phosphorus as efficiently as the phosphorus from inorganic sources. Phytate phosphorus in untreated soybean

meal was not utilized by chicks. Nelson, Shieh, Wodzinski and Ware (1971) showed that when fungal phytase was added directly to a maize-soybean meal diet low in phosphorus, an increased bone ash content was observed, indicating hydrolysis and utilization of phytate phosphorus. Similarly, Rojas and Scott (1969) reported almost complete hydrolysis of phytate in cottonseed meals following treatment with a phytase from *Aspergillus Ficuum.* In a study with broiler chickens, Simons et al. (1990) showed that microbial phytase supplementation of a low phosphorus maize-soybean diet increased the availability of phosphorus to over 60% and decreased the amount of phosphorus in the droppings by 50%. They also showed that the GR and FCR of the birds on low-phosphorus diets containing microbial phytase were comparable to, or even better than, those obtained by birds fed on the control diet.

The amount of phytate phosphorus released by microbial phytase depends on the concentration (Kornegay, Denbow, Yi and Ravindran 1996) and source (Eeckhout and de Paepe, 1991; cited in Ravindran et al., 1995) of the added phytase and the dietary phytate (Yi, Kornegay and Denbow, 1995), calcium (Schoner et al. 1996; cited in Ravindran, 1995) and vitamin D3 contents (Edwards, 1993; Qian, Kornegay and Denbow, 1995) and the calcium:phosphorus ratio (Qian et al., 1995). In poultry, phytate hydrolysis occurs mainly within the crop (pH 5-6), the proventriculus and gizzard (pH 2-4). Eeckhout and de Paepe (1991; cited in Ravindran et al., 1995) indicated that microbial phytase has a broader pH activity range than plant phytase. Therefore, microbial phytase action is more consistent compared with that of plant origin.

1.4.5 Effect of Microbial Phytase on the Availability of Cations

As previously discussed, phytate has strong chelating potential and forms a variety of complexes with cations and proteins, rendering these nutrients biologically unavailable. Theoretically, when phytate is hydrolysed by microbial phytase, all minerals bound to it will be released. There is evidence to indicate that microbial phytase supplementation improves the availability of calcium to broiler chickens (Broz, Oldale, Perrin-Voltz, Rychen, Schulze and Simoes Nunes, 1994; Kornegay et al., 1996; Sebastian et al., 1996a). Pallauf et al. (1992; cited in Ravindran et al., 1995) and Sebastian et al.

(1996a) found that phytase addition increased the apparent absorption of Mg, Zn, Cu and Fe in pigs and poultry, respectively. The addition of 800U of phytase/kg to a diet containing 27mg zinc/kg increased zinc retention by chicks (Thiel and Weigand, 1992).

1.4.6 Effects of Microbial Phytase on the Availability of Protein and Amino Acids

Sebastian, Touchburn, Chavez and Lague (1997) found that the optimum growth performance and amino acid digestibility were obtained in a low-phosphorus and low calcium diet supplemented with microbial phytase. In the 28-day period, Sebastian et al. (1997) also found that, in males, phytase supplementation increased the apparent ileal digestibility of CP but had no influence on the apparent ileal digestibility of any amino acids except methionine and phenylalanine. In females the addition of phytase increased the apparent ileal digestibility of all amino acids except lysine, methionine, phenylalanine and proline. In contrast to these various positive responses, Newkirk and Classen (1995), working with canola meal, showed that supplementation of broiler diets with either semi-purified phytase or crude phytase had no significant effect on crude protein digestibility.

1.4.7 Effect of Microbial Phytase on the Performance of Broiler Chickens

Studies by Simons et al. (1990); Broz et al. (1994); Denbow, Ravindran, Kornegay, Yi and Hulet (1995); Michell and Edwards (1996); Kornegay et al. (1996); Sebastian et al. (1996a) indicated that microbial phytase supplementation increases body weight gain and feed intake in broiler chickens. As a result of simultaneous increases in body weight gain and feed intake, Broz et al. (1994), Denbow et al. (1995) and Sebastian et al. (1996a) indicated that microbial phytase supplementation had no significant effect on feed to gain ratio in broiler chickens. In contrast, Simons et al. (1990) reported improved gain : feed with supplemental phytase.

1.4.8 Effect of Dietary Calcium and Calcium:Phosphorus Ratio on the Efficacy of Microbial Phytase.

Only a few studies have investigated the influence of calcium (calcium:phosphorus ratio) on the efficacy of phytase in broiler diets. Sebastian, Touchburn, Chavez and Lague (1996b) reported that phytase supplementation of a diet low (0.6%) in calcium and based on maize-soybean meal resulted in superior broiler growth and mineral utilization compared with those fed on a diet with normal (1.0%) or high (1.25%) calcium content. Schoner et al. (1993; cited in Ravindran et al., 1995) showed that a diet high (0.9%) in dietary calcium and supplemented with microbial phytase resulted in reduced body weight gain, feed intake and phosphorus calcium retention compared with a diet containing low dietary calcium (0.6%) plus microbial phytase.

1.5 Summary

Rice bran is available abundantly and relatively cheaply in the Asian region. Rice bran is relatively high in energy compared to other cereal brans, due to its high fat content. However, this high fat characteristic increases the tendency of rice bran to go rancid rapidly, especially under hot and humid conditions. Rice bran is also high in phosphorus, however 90% of the phosphorus is in the form of phytate which is unavailable to animal.

Studies with young broiler chickens have shown that high levels of rice bran inclusion in diets depressed bird performance. Some authors suggested that this is due to the relataively undeveloped digestive system in young birds, hence they are unable to utilize the high fat content in FFRB efficiently. However, others have suggested that the high phytate content of rice bran makes nutrient such as amino acids, starch and minerals unavailable to chicks, thus reducing their performance. Studies have shown that birds supplemented with a lipase preparation in diets containing rice bran (Pluske et al., 1997), or phytase in diet containing phytate improved performance of birds (Simons et al., 1990).

In order to improve the nutritive value of rice bran, problems of low lipid digestibility at a young age and high phytate level in bran should be overcome. The objective of this thesis is to increase the nutritive value of FFRB fed to broiler chickens. The general hypothesis tested here is that the use of exogenous enzyme preparations based on lipase and phytase would improve the nutritive value of FFRB added in broiler chicken diets, and thus improve bird performance. The experiments were conducted to (a) to confirm mode(s) of action of a lipase preparation in male birds fed diets containing 9 and 18% level FFRB by determining Apparent Metabolisable Energy (AME) and lipid digestibility of the diets, (b) to determine weekly production indices [Growth Rate (GR), Food Intake (FI) and Feed Conversion Ratio (FCR)] throughout the entire growing period to slaughter of birds fed diets containing 9 and 18% FFRB in the presence/absence of a lipase preparation and/or phytase preparation, and (c) to examine if supplementation of lipase and/or phytase preparation in FFRB diets will increase the nutritive value of FFRB. These diets were then compared to a standard maize-soybean diet. The specific objectives of various experiments are outlined in the respective experiments.

CHAPTER 2: General Materials and Methods

2.1 Introduction

The experiments described in this thesis were conducted at the Poultry Research Unit, Turitea Campus, Massey University, Palmerston North. The following sections outline animal housing, animals used, and experimental techniques that were common to the studies conducted. Experimental details peculiar to a particular study are described in each chapter or experiment.

2.2 Animal Housing

2.2.1 Brooder Shed

The brooder shed was used to rear birds during their first and second weeks of life. The room temperature was controlled at ≈28°C from day-old to 14 days of age. The room consisted of six, three-tier blocks of cages, with four pens per tier (two on each side). This give a total of (6*3*4) 72 pens. Each pen has its own feeding trough, but shared a drinking trough with another pen. The pens were electrically heated up to 36°C from day-old to day-4, 34°C from day-5 to day-10, then 30°C up to day-14. On day-14, all birds were transferred to a grower shed. Lighting of 23 hour dark and 1 hour light was provided. The room temperature was regulated and maintained by using radiated heaters and a heat sensored ventilation fan.

2.2.2 Grower Shed

The grower shed consisted of 72 cages, each of which had an individual cup drinker. Three cages shared a long feeding trough, of which different diets fed to different cages were seperated by a metal partition. The shed temperature was gradually reduced by about 0.5°C per day from 27°C at day 14 to 21°C on day 36. Lighting of 23 hour dark and 1 hour light was provided.

2.3 Animals

Day-old male broiler chickens of a commercial Ross strain were obtained from Tegel Hatchery, Levin, New Zealand. The chickens were hatched in the morning and

delivered to the research unit at noon. On the day of arrival, all birds were weighed and placed into narrow weight classes. Birds of relatively low or high bodyweight were omitted. A specific number of birds (according to the experimental design) were then assigned to pens such that all pens had similar weights. All the pens used were randomly assigned to different treatment groups. The birds were then raised in the brooder shed for the first two weeks, and then transferred to the grower shed from 14-day-old to 36-day-old.

2.4 Experimental Diet Preparation

2.4.1 Ingredients Used

The fishmeal, soyabean meal, salt, methionine and lysine used in diets were bought from Hill Feeds North Island, Auckland, New Zealand. The Chilean fishmeal was steam-dried, and contained a minimum of 65% protein. The American soyabean meal contained a minimum of 47.5% protein. The 48% meat and bone used was purchased from Lake View Farm Fresh Ltd., Levin, New Zealand. The maize was bought from Wrightson Ltd., New Zealand, and was ground using a hammer mill with screen size of 4mm before mixing. The Australian rice bran was obtained from Australia Rice Growers Cooperative, Leeton, NSW, Australia (kindly donated by Dr. Keith Hutton). The Thai rice bran was obtained from CP Group, Bangkok, Thailand. The vitamin and minerals used were purchased from Tegel Food Ltd., Auckland, New Zealand. The composition of the vitamin and mineral premix is shown below:

Vitamin (as per kg finished feed)		**Mineral (as per finished feed)**	
Vitamin A I.U.	11.1	Mn ppm	125.0
Vitamin D I.U.	2.4	Zn ppm	60.0
Vitamin E ppm	60.0	Cu ppm	5.0
Vitamin K ppm	4.0	Co ppm	0.3
B1 Thiamine ppm	3.0	Fe ppm	25.0
B2 Riboflavin ppm	12.0	I ppm	1.0
B3 Nicotinic ppm	35.0	Mo ppm	0.5
B5 Pantothenic ppm	12.8	Se ppm	0.2
B6 Pyridoxine ppm	10.0	Choline ppm	637.5
B12 ppm	0.017		
Folic ppm	5.2		
Biotin ppm	0.2		
Antioxidant ppm	100.0		

2.4.2 Method of Mixing Ingredients and Pelleting Diet

All the micro-ingredients and macro-ingredients that were less than 10 kg were mixed thoroughly in a Hobart mixer before putting into the ingredient bin, where further mixing with the rest of the macro-ingredients took place. All the micro-ingredients were weighed on the Mettler AE 163 balance. Diets were mixed, then pelleted with steam (≈60°C) through a 3 mm dye.

2.5 Enzyme Preparations

The lipase-based and phytase-based enzyme preparations were obtained from Alltech Inc. (Nicholasville, KY, U.S.A.) The inclusion level of the lipase-based enzyme preparation was 1kg/tonne of finished feed, and of the phytase-based enzyme preparation was 0.5kg/tonne of finished feed. The lipase-based enzyme preparation contained cellulase and xylanase, produced by *Trichoderma longibrachiatum*, and lipase and protease, produced by *Aspergillus niger*. The activities of enzyme preparation is as below:

- cellulase activity of 19 CMCU/g
- protease activity of 425 HUT/g
- xylanase activity of 400 XU/g
-lipase activity of 900 LU/g.

The phytase-based enzyme preparation contained cellulase and xylanase, produced by *Trichoderma longibrachiatum*, and phytase, glucoamylase and protease, produced by *Aspergillus niger*. The activities of enzyme preparation is as below:

- protease activity of 1,200 HUT/g
- xylanase activity of 300 XU/g
- cellulase activity of 250 CMCU/g
- glucoamylase activity of 25 NG/g
- phytase activity of 250 PU/g.

2.6 Experimental Procedures

2.6.1 Growth Rate (GR) and Feed Intake (FI)

The total weight of birds and feed from each cage were weighed at the beginning and the end of the experiment, at the beginning and end of each excreta collection period, and on day-14 when the birds were transferred to grower cages. Therefore GR, FI and feed conversion ratio (FCR) were determined at specific periods.

2.6.2 Faecal Sample Collection

Total daily excreta were collected on a plastic sheet laid under each cage during the collection period (see experimental chapters for details of collection periods). The plastic sheets put underneath the cages were cut according to the size or area of the cage, weighed, and labelled before placing underneath the cages. The plastic sheets were then collected, weighed, and frozen at -20°C after each day's collection. Upon the completion of each excreta collection period, faeces collected over the four days were pooled and a representative subsample was taken for each cage. The representative samples of all pens were then freeze-dried, lyophilised to equilibrate with atmospheric conditions, and then ground through a 1mm sieve grinder for laboratory analysis of gross energy (GE), dry matter (DM) and crude fat. Samples of each diet were also ground for analysis of GE, DM and crude fat. From the laboratory analysis and freeze-drying, the amount of DM excreted by each pen on each day could be calculated.

2.6.3 Euthanasia of Birds and Weighing of Digestive Organs

At the end of the experiment, specific numbers of birds from each treatment group were randomly selected and euthanased by a lethal intracardiac injection of sodium pentabarbitone (Pentovarb 300 - P.A.R. Class II, Chemstock Animal Health Ltd., Christchurch, New Zealand). The weights of the birds were recorded, as well as the weights of pancreas, liver plus gall bladder, full and empty small intestine, and full and empty caeca.

2.7 Chemical Analysis of Diets and Faecal Samples

All samples were conducted in duplicate. Samples were only re-analysed when it was known or suspected that a fault had occured during analysis. All samples were weighed to four decimal places on an electronic balance (Mettler AE 163).

2.7.1 Determination of Dry Matter

Samples of approximately 1g were accurately weighed into pre-weighed 30ml beakers and dried to a constant weight for approximately 18 hours in a Watvic Oven (Watson Victor Ltd.) at 105°C. Samples and beakers were then cooled in a desiccator, and reweighed.

2.7.2 Determination of Gross Energy

Gross energy was determined in a Gallenkamp Autobomb bomb calorimeter. Samples of approximately 1 g were pelleted and weighed accurately before being ignited with oxygen. The energy content of the samples was estimated according to the formula below:

[(Final Temp. - Initial Temp.) * 10.6595] / pellet weight,

where 10.6595 is the hydrothermal equavalent.

2.7.3 Determination of Crude Fat

The Soxhlet fat extraction method was used for crude fat determination in diets and faecal samples. Samples of 5-6 g were accurately weighed into a soxhlet extraction thimble. The samples and thimbles were then dried at 60°C overnight in an forced-air oven. The samples were then washed with petroleum-ether by refluxing in a soxhlet apparatus to dissolve the fat. The solubilized fat was then collected in the pre-weighed distillation flask, and the increase in weight of the flask represents the dissolved fat.

$$\% \text{ Fat} = \frac{(\text{Flask wt. after extraction}) - (\text{Flask wt. before extraction}) * 100}{\text{Sample wt. (g)}}$$

2.8 Determination of AME Content

2.8.1 Reasons for Using Ad-libitum Feeding and Total Collection Method

Different feeding methods have been adopted for AME measurement. Farrell, Thomson, du Preez and Hayes (1991) measured the ME in poultry feedstuffs using four feeding systems: dual semi-quick (DCQ), conventional, a true metabolisable energy (ATM) and Farrell's rapid method. Farrell et al. (1991) found no significant difference for conventional, DSQ and Farrell's methods. However, with ATM method, AME values declined as food intake declined. In this thesis, the conventional method of ad-libitum feeding is used, as it does not only give an indication of AME content of different diets, but also FI and FCR of the birds consuming treatment diets.

The AME content is evaluated directly by a feed assay but estimates are also obtainable from equations relating chemical composition to coefficient of combustion and digestibility. Direct assays require the heat of combustion of representative samples of the food and excreta, and methods of determining the weight of excreta eliminated per unit of food intake (Miller, 1974). The quantitative determination of excreta voided is obtained either by recovering the total excreta voided or by estimating the total recovery by testing representative samples of the excreta for the presence of an inert indicator fed at a known concentration in the test diets (Miller, 1974).

Both methods are subject to systematic errors. The total collection procedure requires the collection and weighing of all excreta and the quantitative measurement of food intake over a suitable period. It is assumed that the excreta collected are all derived from the food eaten during the corresponding period. This will clearly not be the case at the beginning and the end of each collection (Miller, 1974). Consequent errors, however, are likely to be negligible if collection extends over several days. Incomplete collection of excreta and food wastage are likely to cause more serious errors while the contamination of exreta with spilt food and feathers can lead to erroneous results (Miller, 1974).

If an indigestible substance in the food is used as a marker, the quantitative measurement of food intake and excreta output is unnecessary and ME content can be assessed from relatively straightforward laboratory measurements on samples of food and excreta alone (Miller, 1974). This is an important consideration when measurements are made on groups as opposed to individual chickens, and allows those excreta contaminated with foreign matter to be discarded.

The use of an indicator as a quantitative measure of faeces output to food intake depends on the principle that the total amount of the substance excreted equals the amount ingested during a time period. Thus:

$$C_I I = C_F F$$

$$\frac{C_I}{C_F} = \frac{F}{I}$$

where I and F are the total food intake and faeces output during the collection period and C_I and C_F are the concentrations of the indicator subatance in the food and faeces respectively (Miller, 1974).

The indicator method, however, gave consistantly higher estimates of the excreted per gram food intake and hence lower estimates of ME content (Miller, 1974). On average the values for excreted energy measured by the indicator method were 10% higher than by the total collection procedure. It was suggested that the discrepancies were more likely to be due to incomplete collection of excreta rather than to errors of the indicator procedure (cannot achieve full recovery of indicator).

2.8.2 Derivation of AME Calculation Equation

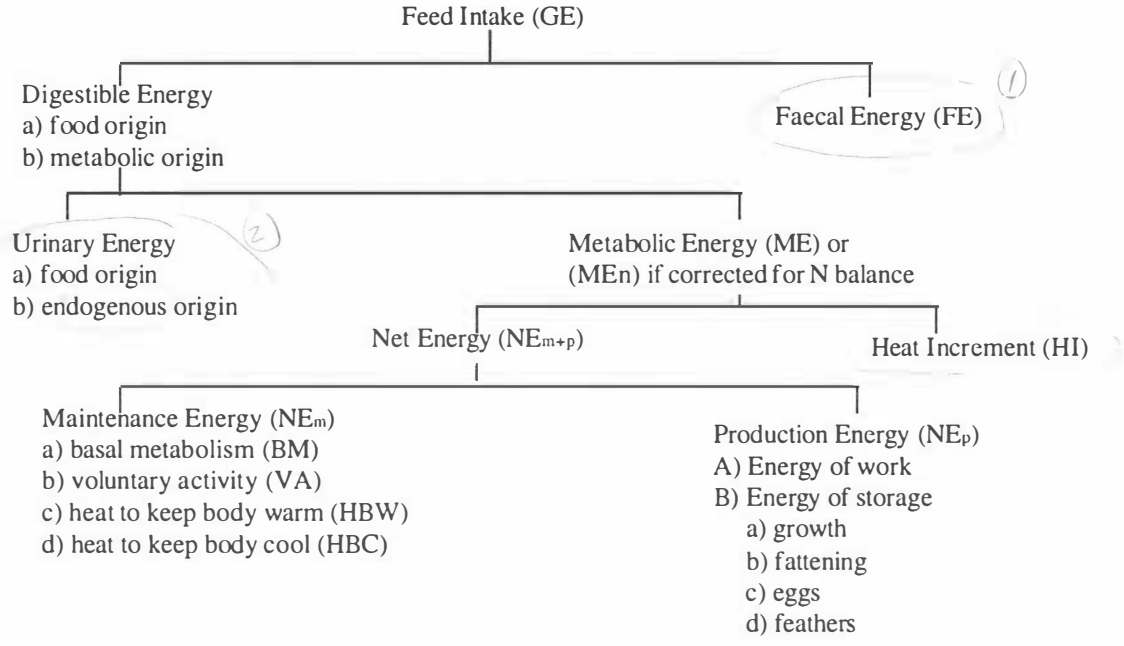


Figure 2.1 The partition of gross energy of food (Vohra, 1972)

From the diagram above, AME content is the gross energy of the food less the energy as faeces, urine and combustible gases. Conversely, it is the energy value of food remaining after allowance for secretion product that is partitionable into heat increment, maintenance and production functions.

$$\text{AME content} = GE_{Diet} - (E_{faeces} + E_{urine} + E_{gas}) = E_{HI} + E_{maintenance} + E_{production}$$

Wolynetz and Sibbald (1984) contend that the contribution of combustible gas loss to excreta energy, for most products, is small and can be ignored. Given this, for most products AME of $GE-E_{faeces}+E_{urine}$ is a near estimate of AME of $GE-E_{faeces}+E_{urine}+E_{gas}$.

Therefore,

$$\text{AME content} = GE_{Diet} - (E_{faeces} + E_{urine})$$

As faeces and urine are voided together by poultry (Vohra, 1972),

$$\text{AME content} = GE_{Diet} - E_{excreta} \text{ ; where } E_{excreta} = E_{faeces} + E_{urine}$$

The unit of measure of AME content is megajoule (MJ),

$$\text{AME content (MJ)} = \text{GE (MJ)} - E_{excreta}\text{ (MJ)}$$

In DM basis,

$$\text{AME content (MJ)} = \text{DM intake} * GE_{Diet}\text{ (MJ/kgDM)} - \text{DM excreted} * E_{excreta}\text{ (MJ/kg DM)}$$

Dividing throughout by kg DM intake,

$$\text{AME (MJ)} = \frac{GE_{Diet}\text{ (MJ/kg DM)} - \text{DM excreted} * E_{excreta}\text{ (MJ/kg DM)}}{\text{kg DM intake}}$$

From the equation, the DM excreted (total excreta weight * DM of excreta) by each pen each day is needed for the AME calculation of the diets fed to the birds, as well as energy per unit weight of diets, energy per unit weight of excreta, total food intake weight and DM of food.

2.8.3 Derivation of Test Ingredient AME Calculation Formula

In Experiments 1 and 2, the test ingredient (RB) was added in replacement of a representative portion of the whole diet. The value of the test ingredient was calculated from the measured AME values of the basal diet and test diets (basal with 9% or 18% RB with or without enzyme). The calculation assumes a linear relation between the AME value of the diet and the proportion of the test ingredient included in it. The AME value of the diet may not change uniformly with increasing levels of inclusion of the test ingredient. The assumption made herein, therefore is that the AME value is constant for all levels of inclusion, and that the AME value of the diet is the sum of the values of its proportion parts.

When a test diet ingredient is added at the expense of a representative portion of the whole diet, the calculation is based on the following equation (Miller, 1974):

$$AME_{test} = (AME_{basal} * P) + \{AME_{Ing} * (1-P)\}$$

subsequently,

$$AME_{Ing} = \frac{AME_{test} - AME_{basal} * P}{1-P}$$

where P = proportion of the basal diet in the test diet, and Ing = test ingredient

CHAPTER 3: Experiment 1

Increasing the Nutritive Value of Australian Full-fat Rice Bran (FFRB) for Broiler Chickens Throughout the Entire Growing Period

3.1 Introduction

In recent years, increasing interest has been expressed in the use of enzymes in improving digestibility and metabolisable energy of foods fed to poultry. Use of biotechnology in this respect has not only improved the efficiency of use of existing or conventional ingredients, but has also allowed the nutritionist to use unconventional feed ingredients that are often cheaper and available in relative abundance. Pluske et al. (1997) showed that lipase and mannanase preparations improved performance of birds fed FFRB and copra meal diets, respectively. However, some precautions should be taken when using unconventional ingredients as some of them may adversely affect the performance of birds. Full-fat rice bran (FFRB) is a by-product available for use in the broiler industry. It has a high gross energy (≈ 21MJ/kg DM) and crude fat (≈ 20g/kg DM) content. A lower ME value of FFRB for chickens compared to cockerels was reported by Warren and Farrell (1990c), an effect thought attributable, in part, to low secretion of pancreatic lipase. Addition of an exogenous source of lipase may therefore improve the nutritive value of FFRB and enhance broiler performance.

The objective of this experiment was to investigate the response of broiler chickens fed a maize-soyabean meal basal diet substituted with 9% Australian FFRB, with or without the addition of a lipase preparation. The hypothesis tested was that the addition of a lipase (enzyme) preparation would enhance AME content by increasing lipid digestibility in broiler chickens. The AME content and lipid digestibility of both basal and treatment diets were determined to confirm mode(s) of action of the lipase preparation at different stages of life. Weekly production indices [Growth Rate (GR), Food Intake (FI) and Feed Conversion Ratio (FCR)] of birds fed different diets throughout the entire growing period of 35-day were also determined.

3.2 Materials and Methods

3.2.1 Animals

A total of 260 day-old male broiler chicks of a commercial Ross strain was used. On the day of arrival, each bird was weighed and placed into a narrow weight class. Birds of relatively low or high body weight were discarded. Three birds were then assigned to each of 72 pens such that all pens had nearly a similar average weight. The 72 pens were randomly assigned to four different treatment groups assigned as, treatments B+, B-, 9+ and 9- (see 3.2.3 below), of which each treatment had 12 replicates.

3.2.2 Experimental Procedures

All birds were given *ad libitum* access to feed and water. The birds from each cage and the feed were weighed at day 0 (the beginning of the experiment), day 14 (when birds were shifted to grower cages), day 36 (at the end of the experiment), and at the beginning and the end of the three excreta collection periods (day 4 to 7, day 18 to 21 and day 32 to 35). Therefore growth rate and feed intake between day 0 to 3, day 4 to 7, day 8 to 13, day 14 to 17, day 18 to 21, day 22 to 31 and day 32 to 35 was measured. However, the measurements above have been presented on a weekly basis.

Procedures for total daily excreta collection procedures, taking and processing of subsamples, and laboratory analysis of GE, DM and crude fat are similar to those described in section 2.6.2 and 2.7. The equations in section 2.7.2 and 2.7.3 were used in the determination of AME content of the diets and test ingredients (FFRB).

On day 36, eight randomly-selected birds from each treatment group were euthanased by lethal intracardial injection with sodium pentobarbitione (see section 2.6.3). The weight of the bird, pancreas, and liver plus gall bladder, full and empty small intestine, and full and empty caecum, were weighed as described in section 2.6.3. All the weights measured were then resolved on a per body weight basis.

3.2.3 Diets

Two basal diets (B+ and B-) were formulated according to the Standard Ross Poultry Finisher 1 Formulation recommendation. Two treatment diets (9+ and 9-), substituted with 9% of Australian FFRB to the basal diet, were also prepared. Diet B+ was the basal diet plus the lipase preparation, whereas Diet B- was the basal diet without the enzyme preparation. Diet 9+ and 9- each contained 9% Australian FFRB and 91% of the basal diet. Diet 9+ was supplemented with 0.1% of enzyme. The composition of the experimental diets are presented in Table 3.1.

Table 3.1 Composition of experimental diets (air-dry basis)

Ingredients (%)	**B+**	**B-**	**9+**	**9-**
Maize	58.7	58.8	53.408	53.508
Soyabean Meal	23	23	20.93	20.93
Soyabean Oil	2	2	1.82	1.82
Meat and Bone Meal	15	15	13.65	13.65
Full-fat Rice Bran	-	-	9	9
L-lysine	0.25	0.25	0.2275	0.2275
DL-Methionine	0.35	0.35	0.319	0.319
Vitamin mixture	0.05	0.05	0.046	0.046
NaCl	0.4	0.4	0.36	0.36
Mineral mixture	0.15	0.15	0.14	0.14
Enzyme	0.1	-	0.1	-
Analysis Value (as is basis)				
GE (MJ/g)	17.10	16.74	16.97	16.98
Fat (%)	5.36	5.27	6.38	6.38
Dry Matter (%)	90.49	90.35	89.06	90.01
Calculated Value (as is basis)				
AME (Kcal/kg)	3110	3110	3095	3095
Crude Protein (%)	23	23	22	22
Crude Fat (%)	6.4	6.4	7.17	7.17
Lysine (%)	1.4	1.4	1.35	1.35
Meth + Cyst (%)	0.97	0.97	0.93	0.93
Total Phosphorus (%)	1.1	1.1	1.2	1.2
Calcium (%)	1.7	1.7	1.6	1.6

3.2.4 Statistical Analyses

Two one-way ANOVA's were conducted. The first analysis examined the effect of enzyme supplementation in the basal diet, and the second analysis examined the effect of enzyme in the 9% FFRB-containing diet. These two analysis were conducted separately to overcome any potentially confounding effects of substitution of FFRB into the basal diet on the parameter measured.

3.3 Results

3.3.1 Effects of Enzyme Supplementation on AME content

The AME content of the diets and FFRB estimated between days 4-7, 18-21 and 32-35 are presented in Table 3.2.

(i) Analysis of basal diet

The AME content was numerically higher in the basal diet with enzyme addition between days 4-7 and 32-35 (P = 0.198 and 0.162, respectively). A significant improvement with enzyme supplementation on the AME content of the basal diet was observed on days 18-21 (14.57 vs 14.32, P = 0.024).

(ii) Analysis of FFRB-containing diet

The AME content was numerically higher in the 9% FFRB diet with enzyme supplementation between days 4-7 and 18-21 (P = 0.215 and 0.218, respectively). A significant improvement with enzyme addition on the AME content was seen between days 32-35 (14.72 vs 14.38, P = 0.008). Enzyme addition increased the AME content of FFRB between days 4-7, 18-21 and 32-35, however, the improvement was not significant between days 4-7 (P = 0.213), 18-21 (P = 0.348) and 32-35 (P = 0.065).

Table 3.2 The effects of Australian FFRB diets supplemented with enzyme on AME content

	Basal Diet		SE	Basal + 9 % FFRB		SE
	+ Enz	-Enz		+Enz	-Enz	
AME (MJ/ kg DM)						
AME_{Diet} d 4-7	14.24	14.03	0.078	14.58	14.38	0.134
AME_{Diet} d 18-21	14.57[a]	14.32[b]	0.075	14.65	14.52	0.069
AME_{Diet} d 32-35	14.41	14.22	0.067	14.72[c]	14.38[d]	0.067
AME_{FFRB} d 4-7	-	-	-	20.10	17.93	1.468
AME_{FFRB} d 18-21	-	-	-	17.93	16.90	0.762
AME_{FFRB} d 32-35	-	-	-	18.59	16.01	0.656

[a,b,c,d] within each analysis, values not having the same superscript are significantly different (P < 0.05)

3.3.2 Effects of Enzyme Supplementation on Lipid Digestibility

(i) Analysis of basal diet

Addition of lipase preparation did not increase lipid digestibility in the basal diets (Table 3.3). There was a significant increase (P < 0.05) in lipid digestibility as birds aged, however, this only occurred in the basal diet supplemented with enzyme.

(ii) Analysis of FFRB-containing diet

Addition of lipase preparation did not increase lipid digestibility in the diets containing FFRB (Table 3.3). There was a significant increase ($P < 0.05$) in lipid digestibility as birds aged, however this only occurred in the FFRB diet supplemented with enzyme.

Table 3.3 The effects of enzyme supplementation on lipid digestibility (%)

	Basal		SE	9% FFRB + Basal		SE
	+ Enz	-Enz		+ Enz	-Enz	
Day 4-7	92.2	93.0	0.93	90.9	90.5	0.66
Day 18-21	94.4	93.3	0.43	92.6	93.5	0.38
Day 32-35	95.3	94.9	0.37	94.7	93.2	0.49

3.3.3 Effects of Enzyme Supplementation on GR, FI and FCR

The daily gain, feed intake and FCR over specific periods of time over the 35-day rearing period of birds fed different diets are presented in Table 3.4.

Table 3.4 The effects of Australian FFRB diets supplemented with enzyme on daily gain, FI, and FCR

	Basal Diet		SE	Basal + 9 % FFRB		SE
	+ Enz	-Enz		+Enz	-Enz	
Day 0-7						
Gain, g/bird/d	23.7	24.4	0.31	23.1	22.5	0.33
Food intake, g/bird/d	25.4	25.3	0.32	26.4	25.3	0.42
FCR, g feed/g gain	1.07	1.05	0.006	1.14	1.12	0.013
Day 8-13						
Gain, g/bird/d	45.0	42.9	0.62	43.8	42.8	0.56
Food intake, g/bird/d	57.4	55.9	0.83	57.3	56.2	0.68
FCR, g feed/g gain	1.31	1.32	0.018	1.31	1.32	0.014
Day 14-21						
Gain, g/bird/d	66.9	63.7	0.87	65.8	65.1	0.61
Food intake, g/bird/d	97.1	93.4	1.49	97.9	94.4	0.88
FCR, g feed/g gain	1.45	1.49	0.014	1.49	1.45	0.010
Day 22-31						
Gain, g/bird/d	79.0	78.3	1.61	84.5	83.4	1.25
Food intake, g/bird/d	129.6	131.4	2.71	139.8	139.6	1.90
FCR, g feed/g gain	1.68	1.68	0.022	1.69	1.68	0.015
Day 32-35						
Gain, g/bird/d	82.8	79.2	2.19	89.7	90.3	1.79
Food intake, g/bird/d	156.4	157.4	3.19	164.1	164.1	2.72
FCR, g feed/g gain	1.91	2.00	0.028	1.86	1.86	0.037
Day 0-35						
Gain, g/bird/d	57.8	56.4	0.69	59.4	59.0	0.48
Food intake, g/bird/d	90.2	89.5	1.23	94.2	92.1	0.77
FCR, g feed/g gain	1.56	1.57	0.010	1.59	1.56	0.008

Day 0-7

(i) Analysis of basal diet

No differences were found in daily gain, FI and FCR on birds fed basal diets supplemented with or without enzyme preparation.

(ii) Analysis of FFRB-containing diet

No differences were found in daily gain, FI and FCR on birds fed 9% FFRB diets supplemented with or without enzyme preparation.

Day 8-13

(i) Analysis of basal diet

A trend for an improvement (P = 0.089) in daily gain was found in the basal diet plus enzyme compared with basal diet without enzyme. No differences were found in FCR.

(ii) Analysis of FFRB-containing diet

No significant differences in daily gain, FI and FCR were found when the two 9% FFRB diets were compared.

Day 14-21

(i) Analysis of basal diet

Enzyme inclusion increased daily gain numerically in the basal diet (P = 0.061), but no significant differences in FI and FCR were found.

(ii) Analysis of FFRB-containing diet

Enzyme inclusion increased FI in the 9% FFRB diet (P = 0.047) with no improvement in daily gain, thus FCR deteriorated (P = 0.087).

Day 22-31

(i) Analysis of basal diet

Enzyme addition showed no significant improvement in the basal diet on daily gain, FI and FCR.

(ii) Analysis of FFRB-containing diet

Enzyme addition showed no significant improvement in the 9% FFRB diet on daily gain, FI and FCR.

Day 32-35

(i) Analysis of basal diet

Although addition of enzyme increased daily gain on the basal diet over this period, the effect was not statistically significant (P = 0.326).

(ii) Analysis of FFRB-containing diet

Enzyme supplementation had no effect in daily gain, FI and FCR on birds fed 9% FFRB diet.

Day 0-35

(i) Analysis of basal diet

No improvements in daily gain and FCR were seen in the basal plus enzyme diet.

(ii) Analysis of FFRB-containing diet

No significant differences were found in daily gain in birds fed 9% FFRB diet with and without enzyme preparation. However, the FCR deteriorated numerically (P = 0.066).

3.3.4 Effects of Enzyme Supplementation on Digestive Tract Characteristics

The weight of the small intestine, caeca, liver plus gall bladder and pancreas per unit body weight (g/kg body weight) are presented in Table 3.5.

Table 3.5 Weight of small intestine, caeca, liver plus gall bladder, and pancreas per unit body weight (g/kg body weight)

	Basal		SE	9% FFRB + Basal		SE
Organ	**+ Enz**	**- Enz**		**+ Enz**	**- Enz**	
Small intestine	24.0	24.7	0.30	23.5[c]	26.1[d]	0.23
Caeca	3.7[a]	4.4[b]	0.12	4.0	3.7	0.05
Liver + gall bladder	25.2	24.2	0.42	24.9	25.0	0.07
Pancreas	1.8	2.0	0.08	2.1	2.1	0.03

[a,b,c,d] within each analysis, values not having the same superscript are significantly different (P < 0.05)

(i) Analysis of basal diet

Inclusion of enzyme decreased caecal weight per unit body weight (P = 0.007) in birds fed basal diet.

(ii) Analysis of FFRB-containing diet

In the 9% FFRB diet, enzyme addition numeraically lowered the small intestine weight per unit body weight (P = 0.072).

3.4 Discussion and Conclusion

In this study, the AME content of both the basal and FFRB diets was higher in diets supplemented with the enzyme preparation, which agrees with the findings of Pluske et al. (1997) who also found higher AME content when a lipase preparation was added to diets substituted with 20 and 40% Australian FFRB. In contrast to the low AME content for FFRB of 9.6 to 10.8 MJ/ kg in 15 to 20-day-old chickens reported by Warren and Farrell (1990c), a higher AME content of FFRB was found in this study. This might be due to the fact that a lower level of FFRB was used, and the unexpectedly higher AME content of the FFRB diets achieved than that of the basal diets. Even though the average value of AME content of FFRB in diets with enzyme was much higher than the AME content of FFRB-containing diets without enzyme during days 4-7 and 18-21, the differences between them were not statistically significant as the results were very variable. This may be due to the fact that a small error (variation from the sample mean) in the AME content of either the basal or rice bran-containing diets had a large influence on the AME value of FFRB. This may partly be explained by the use of a low level of FFRB (9%) in this study. Hence, and in the equation used for calculation of AME content of an ingredient (see 2.8.3), the small denominator of 0.09 (proportion of FFRB used in the diet) makes a big difference to the AME content of FFRB. Therefore, higher inclusion levels (> 40%) should be used before a more reliable AME content of FFRB can be obtained. However, an inclusion rate of 40% FFRB may not be practical under commercial conditions, as under this inclusion rate the growth rate of broilers will be depressed.

This study showed that lipid digestibility increased as the birds aged ($P < 0.05$), which agrees with the data of Martin and Farrell (1998). However, this increase was only significant in diets supplemented with enzyme (Table 3.3). This suggests that low lipid digestibility at a younger age is more likely to associated with underdeveloped intestinal absorptive surface and/or low bile acids secretion rather than insufficient lipase activity. Therefore, lipid digestibility improved to a greater extent in enzyme-supplemented birds than in those without enzyme supplementation when the absorptive surface become more developed and secretion of bile acids increased as the birds aged. This suggests that there is a possibility of the enzyme preparation improving lipid digestibility. However, the results were not obvious due to the high lipid digestibility of the mostly unsaturated fatty acids in the diets.

The FFRB oil constituted only about 24% of the total lipid content in the 9% FFRB diets, and only 18-20% of this were saturated fatty acids (≈ 5% of total lipid). Therefore the percentage of saturated lipid (plus small amount from MBM) in the FFRB diets was relatively low compared with the amount of unsaturated lipids present in the diet. Thus it was not surprising to observe no significant differences in lipid digestibility in diets with and without enzyme supplementation. This is because the unsaturated and medium fatty acids within the duodenum would have acted synergistically to increase the digestibility of the more poorly digestible saturated fatty acids (Young et al., 1963; Zollitsch et al., 1997).

The lipid digestibility of both basal and FFRB diets found in this study was higher than that of Martin and Farrell (1998) who did not use soybean oil, and which was about 70%. This may be because soybean oil is made up of mainly unsaturated fatty acids (≈ 96%) (Scott et al., 1982), thus causing a higher lipid digestibility. An attempt was made in this experiment to quantify the lipid digestibility of FFRB. However, the results obtained were very variable. A small error in the lipid digestibility of either the basal or FFRB-containing diets may have a large influence on the lipid digestibility of FFRB. This may be partly explained by the use of low level of FFRB (9%) in this study. Therefore, attempts to improve lipid digestibility of FFRB diets and hence bird performance using lipase preparation were not successful. However, other

components of the lipase-based enzyme (xylanase, cellulase and protease) must have worked in one way or another on basal diet between days 8 to 21 to improve growth.

Annison et al. (1995) concluded that rice bran NSP (predominantly arabinose and xylose) contained no anti-nutritive activity and was not detrimental to broiler chickens when the isolated fraction was included in their diets. In addition, Farrell and Martin (1998a) found that gut viscosity declined with increasing rice bran inclusion (20 and 40%) in broiler chickens. Aboosadi et al. (1996) found no increase in AME content or improvement in performance when an array of cell-wall degrading enzymes were added to the diets of broiler chickens. Therefore the higher AME content of FFRB-containing diets supplemented with the enzyme preparation observed in the present study, though only significant between days 32-35, most likely occurred as a result of the exogenous protease, which was present in the enzyme preparation, acting against the protein component of the diet and hance increasing its availability. However, the possibility of xylanase and cellulase in the enzyme preparation contributing towards the higher AME content of enzyme supplemented FFRB diets cannot be eliminated. This is because the high lipid levels (> 5%) diets in this study may have depressed the digestibility of some nutrients. Nitsan, Dvorin, Zoref and Mokady (1997), who conducted a study on effect of addition of soybean oil on metabolisable energy and broiler performance, found that high lipid level (> 5%) suppressed starch digestibity. Therefore, there is a possibility that the digestibility of other carbohydrate compounds (e.g. cellulase and arabinoxylan) were reduced too. Thus, the cellulase and xylanase activities in the enzyme preparation might have acted against the targeted nutrients.

In this study, a low level of FFRB inclusion (9%) substituted in the basal diet resulted in a higher average GR value of birds, when compared to basal diet (Table 3.4), which was in contrast to the finding of Warren and Farrell (1990c) and Martin and Farrell (1998) who used higher levels of FFRB inclusion (20-40%). Enzyme inclusion in this study had no positive effect on the performance of birds fed FFRB diets. However, some positive effects were found in the basal diet from days 8 to 21 (Table 3.4; Figure 3.1). No improvement in GR on chickens fed 9% FFRB diets supplemented with enzyme preparation was found in this study, even though the AME content was improved. The reason behind this is unknown.

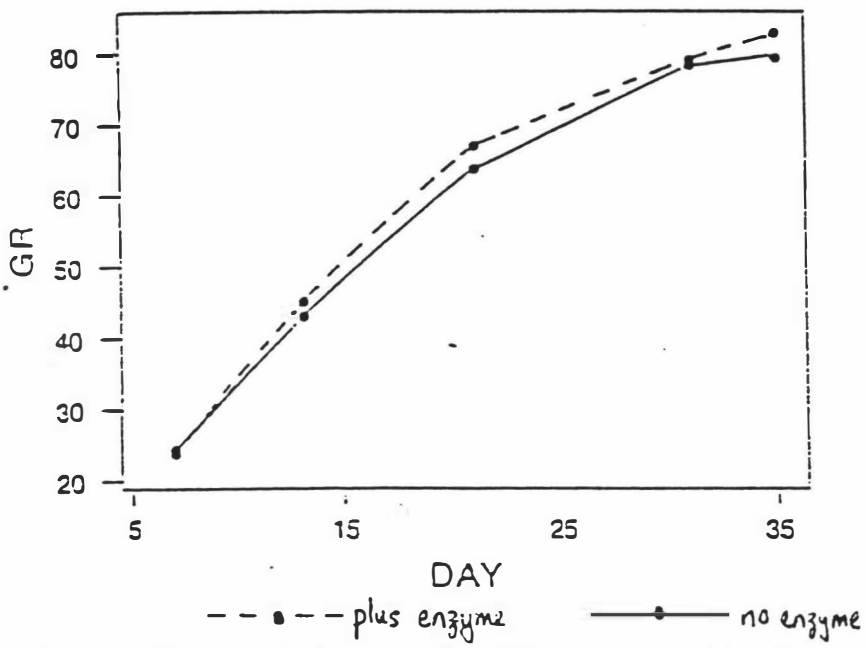


Figure 3.1 Daily Gain of Birds Fed Basal Diets with and without Enzyme

The lack of improvement in performance of chickens fed enzyme-supplemented FFRB diets relative to FFRB diets not supplemented with enzyme is in contrast to the findings of Pluske et al. (1997), who used higher levels of Australian FFRB (20 and 40%). This may due to the fact that, at higher inclusion levels of FFRB (20 and 40%), the high concentration of NSP (predominantly from FFRB) binds some minerals (Vohra et al., 1965) and thus inhibits some intestinal enzyme activities. Lewis, Fields, Craft, Yang and Reiser (1987), working with rats, and Luo and Dove (1996), working with pigs, stated that lipase and phospholipase A need Cu for intestinal activities, and Caldwell and Kung (1953) suggested that ∝-amylase need Ca for their intestinal activities. However, at a lower level of inclusion of FFRB (9%), such as that used in this study, the negative effect of NSP of FFRB on mineral binding might be negligible. Therefore, in the study by Pluske et al. (1997), the xylanase and cellulase component of the enzyme preparation in diets with higher levels of FFRB inclusion might have played more significant roles in liberating the minerals and enhancing the endogenous enzyme activities, which subsequently improved the performance of the chickens. In addition, at 20 and 40% FFRB inclusion level, more substrates (e.g., lipid) are available for the lipase in the enzyme preparation to act against, as FFRB contents about 20% lipid. From the approximate calculation based on the ingredients used in Pluske et al. (1997), the 20% FFRB diets contained about 9% lipid content and the 40% FFRB diets contained a lipid content of approximately 11%, which is much higher than the lipid content of the diets used in this study.

Addition of the lipase preparation affected the growth of some parts of the intestinal tract. However, the effects the enzyme had on the intestinal tract were different for different diets. For the basal diet, enzyme inclusion decreased caecal weight per unit body weight ($P = 0.007$), which may due to reduced fibre fermentation in the caecum. This is probably because of the xylanase and cellulase components of the enzyme preparation increasing the digestibility of dietary fibre in the intestine. Therefore, this reduced the amount of dietary fibre available for fermentation in the caecum. In the 9% FFRB diet, enzyme addition lowered the small intestine weight per unit body weight ($P = 0.072$). This may be due to the presence of lesser amounts of viscous indigestible polysaccharide in the small intestine of chickens fed FFRB diet supplemented with enzyme preparation. The exogenous xylanase and cellulase in the enzyme preparation might have acted against the targeted polysaccharides, and thus reduced the amount of indigestible cell wall polysaccharide in small intestine. In an experiment using rats, Ikegami, Tsuchihashi, Harada, Tsuchihashi, Nishide and Innami (1990) demonstrated that viscous indigestible polysaccharide caused an enlargement of intestinal tract. This diet*enzyme interaction agrees with the finding of Brenes, Smith, Guenter and Marquardt (1993) who found that a crude enzyme mixture increased organ size of birds fed barley-based diets, but had no effect on wheat-based diets.

From the discussion above, it is concluded that attempts to significantly increase lipid digestibility of FFRB diet to improve AME content of the diet were not successful. However, and given that significantly higher AME contents were found in diets supplemented with the enzyme preparation (between days 18-21 for basal diet, and between 32-35 for 9% FFRB diet), it is likely that the exogenous protease and possibly cellulase and xylanase activities acted to increase the AME content of the FFRB diets. These preparations may be more effective in diets containing mainly saturated fat, which has a lower digestibility, e.g., 40-79% in beef tallow (Carew, Machemer, Sharp and Foss, 1972), rather than the predominantly unsaturated lipids in this study with a digestibility of 84-95%, as reported by Carew et al. (1972) with corn oil in broilers up to 15 days of age.

CHAPTER 4: Experiment 2

Increasing the Nutritive Value of Thai Full-fat Rice Bran (FFRB) for Broiler Chickens for the First Two Weeks of Life

4.1 Introduction

Full-fat rice bran is produced and available abundantly in Asian region. It is rich in gross energy (≈21 MJ/kg DM) and crude fat (≈200g/kg DM) content. However, there are limitations to its use in the broiler industry. A lower ME value of FFRB for chickens compared to cockerels was reported by Warren and Farrell (1990c), an effect attributable in part to low pancreatic lipase secretion. Use of a lipase preparation was shown to increase the AME of FFRB for chickens by Pluske et al. (1997). Furthermore, data presented in Experiment 1 of this thesis showed a positive response of a lipase preparation on AME content. However, Wang et al.(1997) reported that a crude enzyme preparation added to diets containing rice bran produced equivocal results in chickens as it improved performance with Malaysian rice bran, but not that of Chinese rice bran. Part of this difference may be caused by differences in processing method or varietal or environmental differences during growth. It was also reported by Wang et al. (1997) that different concentrations of FFRB inclusion in diets caused different performance responses in chicken.

The objective of this experiment was to investigate the digestibility of energy and lipid, and monitor the production response of broiler chickens fed different concentrations of Thai FFRB (9 and 18%) with and without addition of a lipase preparation. The response of the broiler chickens fed 9% Thai FFRB in this experiment was compared to that of broilers fed Australian FFRB (Experiment 1). Warren and Farrell (1990b) and Farrell (1994) suggested that inclusion levels of 20% FFRB depressed bird performance. Therefore 18% FFRB was used in this current study to investigate if supplementation with a lipase preparation can overcome the problems described by the authors above. The hypothesis tested in this experiment was that the addition of a lipase-based enzyme preparation would improve lipid digestibility and AME content in all diets, especially 18% FFRB diet as it had the highest lipid content. The AME

content and lipid digestibility of both basal and treatment diets were determined to confirm mode(s) of action of the lipase preparation in the first and second weeks of life. Weekly production indices [Growth Rate (GR), Feed Intake (FI) and Feed Conversion Ratio (FCR)] of birds fed different diets throughout the two weeks growing period were also determined.

4.2 Materials and Methods

4.2.1 Animals

A total of 360 day-old male broiler chickens of a commercial Ross Strain were used. On the day of arrival, each bird was weighed and placed into a narrow weight class. Birds of relatively low or high body weight were discarded. Five birds were assigned to each of 54 pens such that all pens had a nearly similar average weight. The 54 pens were randomly assigned to six different treatment group, B+, B-, 9+, 9-, 18+ and 18- (see 4.2.3 below), of which each treatment had 9 replicates.

4.2.2 Experimental Procedures

A limited quantity of FFRB obtained from Thailand (CP group, Bangkok) meant that the experiment only lasted for two weeks. All birds were given *ad libitum* access to feed and water. The birds from each cage and the feed were weighed at the beginning and the end of each excreta collection period (day 4 to day 7 and day 11 to day 14). Therefore growth rate and feed intake of day 0 to day 3, day 4 to day 7, day 8 to day 11 and day 11 to day 14 were measured. However, these measurements were resolved on a weekly basis.

Total daily excreta collection procedures, taking and processing of subsamples, and laboratory analysis of GE, DM and crude fat are similar to those described in section 2.6.2 and 2.7. The equations shown in section 2.7.2 and 2.7.3 were used in the determination of AME content of the diets and test ingredient (FFRB).

On the completion of the experiment, six birds from each treatment group were randomly selected and euthanased by lethal intracardial injection with sodium pentobarbitione. The weight of birds, pancreas, and liver plus gall bladder, full and

empty small intestine, and full and empty caecum were weighed (section 2.6.3). All the weights measured were then resolved on a per body weight unit basis.

4.2.3 Diets

Two diets (B+ and B-) were formulated according to the Standard Ross Poultry Grower Formulation recommendation. Four treatment diets (9+, 9-, 18+ and 18-) substituted with 9 and 18% of Thai FFRB to the basal diet, respectively, were also prepared. Diet B- was the basal diet, whereas Diet B+ was the basal diet plus the lipase preparation. Diet 9+ and 9- each contained 9% Thai FFRB and 91% basal diet. Diet 18+ and 18- each contained 18% Thai FFRB and 82% basal diet. Diet 9+ and 18+ were supplemented with 0.1% of lipase preparation. The lipase preparation was added at the expense of maize. Fish meal was used as part of the main protein source that was supplied solely by meat and bone meal in Experiment 1.

Table 4.1 Composition of experimental diets (air-dry basis).

Ingredients (%)	B+	B-	9+	9-	18+	18-
Maize	58.7	58.8	53.39	53.49	48.11	48.21
Soyabean	23	23	20.93	20.93	18.86	18.86
Soyabean Oil	1.5	1.5	1.37	1.37	1.23	1.23
Meat and Bone Meal	9.5	9.5	8.65	8.65	7.79	7.79
Fish Meal	6	6	5.46	5.46	4.92	4.92
Rice Bran	-	-	9	9	18	18
L-lysine	0.25	0.25	0.23	0.23	0.21	0.21
DL-Methionine	0.35	0.35	0.32	0.32	0.29	0.29
Vitamin	0.05	0.05	0.046	0.046	0.041	0.041
Mineral	0.15	0.15	0.14	0.14	0.12	0.12
NaCl	0.4	0.4	0.36	0.36	0.33	0.33
Lipozyme	0.1	-	0.1	-	0.1	-
Analysis Value (as is basis)						
GE (MJ)	17.45	17.26	17.17	17.21	17.5	17.18
Crude Fat (%)	4.9	4.71	6.00	6.00	7.21	7.21
Dry Matter (%)	88.66	88.53	87.80	88.44	88.46	88.06
Calculated Value (as is basis)						
AME (Kcal/kg)	3105	3105	3094	3094	3082	3082
Crude Protein (%)	24	24	23	23	22	22
Fat (%)	6.1	6.1	6.9	6.9	7.7	7.7
Lysine (%)	1.49	1.49	1.4	1.4	1.3	1.3
Meth + Cyst (%)	1.02	1.02	0.97	0.97	0.92	0.92
Total Phosphorus (%)	1.05	1.05	1.11	1.11	1.18	1.18
Calcium	1.55	1.55	1.4	1.4	1.3	1.3

4.2.4 Statistical Analyses

Data were subjected to two analysis. The first analysis examined the differences between the basal diet with and without enzyme supplementation (one-way ANOVA). The second analysis examined the effect of the enzyme preparation on different levels of FFRB inclusion (2*2 factorial ANOVA with Bonferroni pairwise comparison, with respective factors being 9 and 18% FFRB diets with and without enzyme supplementation).

4.3 Results

4.3.1 Effects of Enzyme Supplementation on AME Content

The AME content of the diets and FFRB estimated between days 4-7 and 11-14 are presented in Table 4.2. Significance of main effects of level of FFRB and enzyme, and interactions on AME content, are presented in Table 4.3.

(i) Analysis of basal diets

Inclusion of enzyme in the basal diet increased the AME content numerically between days 4-7 ($P = 0.110$) and significantly between days 11-14 ($P = 0.002$).

(ii) Analysis of FFRB*enzyme interaction

Between days 4-7 and 11-14, AME content was significantly higher in diets with 9% FFRB than 18% FFRB (14.78 vs 14.21, $P < 0.001$; 14.69 vs 14.22, $P < 0.001$, respectively) and numerically in diets containing enzyme ($P = 0.080$). Inclusion of enzyme increased the AME content of FFRB numerically between days 4-7 ($P = 0.107$) and significantly between days 11-14 (11.94 vs 9.93, $P = 0.041$). However, no effect of level of FFRB or interactions were noted.

Table 4.2 The effects of Thai FFRB diets supplemented with a lipase preparation on AME content

	Basal Diet		SE	Basal + 9% RB		SE	Basal + 18% RB		SE
	+ Enz	-Enz		+Enz	-Enz		+Enz	-Enz	
AME (MJ/ kg DM)									
AME_{Diet} d 4-7	15.41	15.31	0.04	14.91	14.65	0.15	14.36	14.06	0.15
AME_{Diet} d 11-14	15.27[a]	15.02[b]	0.05	14.82	14.56	0.12	14.31	14.15	0.12
AME_{FFRB} d 4-7	-	-	-	10.82	9.74	0.40	10.03	8.37	0.38
AME_{FFRB} d 11-14	-	-	-	12.82	9.83	0.44	11.06	10.03	0.44

[a,b] within analyses, values not having the same supercript are significantly different ($P < 0.05$)

Table 4.3 Significance of main effects of FFRB level and enzyme, and their interactions, on AME content

	AME_{Diet}	AME_{FFRB}
Day 4-7		
Level of FFRB	***	NS
Enzyme	NS	NS
Level*Enzyme	NS	NS
Day 11-14		
Level of FFRB	***	NS
Enzyme	NS	*
Level*Enzyme	NS	NS

NS = P > 0.05; * = P < 0.05; ** = P < 0.01; *** = P < 0.001

4.3.2 Effects of Enzyme Supplementation on Lipid Digestibility

Addition of the lipase-based enzyme preparation did not enhance lipid digestibility of any diets (Table 4.4 and 4.5). Inclusion of 18% FFRB significantly depressed lipid digestibility in comparison to 9% FFRB from days 4-7. However, no significant depression in lipid digestibility was found between 9% FFRB diets and 18% FFRB diets during days 11-14. Lipid digestibility was significantly lower (P < 0.001) for basal and 9% FFRB diets during days 11-14, when compared with days 4-7.

Table 4.4 The effects of enzyme supplementation on lipid digestibility (%)

	Basal Diet		SE	Basal + 9% RB		SE	Basal + 18% RB		SE
	+ Enz	-Enz		+Enz	-Enz		+Enz	-Enz	
Day 4-7	94.5	94.9	0.38	91.7	91.0	0.57	79.0	79.1	1.85
Day 11-14	91.2	91.0	0.64	86.5	83.4	1.43	81.5	80.2	1.68

Table 4.5 Significance of main effects of FFRB level and enzyme, and their interactions, on lipid digestbility

	Lipid Digestibility (%)
Day 4-7	
Level of FFRB	***
Enzyme	NS
Level*Enzyme	NS
Day 11-14	
Level of FFRB	NS
Enzyme	NS
Level*Enzyme	NS

NS = P > 0.05; * = P < 0.05; ** = P < 0.01; *** = P < 0.001

4.3.3 Effects of Enzyme Supplementation on Gain, FI and FCR

The daily gain, FI and FCR of birds fed different diets are presented in Table 4.6. Table 4.7 presents the significance of main effects of level of FFRB and enzyme, and interactions on daily gain, FI and FCR.

Table 4.6 The effects of Thai FFRB diets supplemented with a lipase preparation on daily gain, FI and FCR.

	Basal Diet		SE	Basal + 9% RB		SE	Basal + 18% RB		SE
	+ Enz	-Enz		+Enz	-Enz		+Enz	-Enz	
Day 0-7									
Gain, g/bird/d	24.2[c]	23.4[d]	0.27	23.1	22.1	0.49	21.1	21.9	0.49
Feed intake, g/bird/d	24.7	24.1	0.25	24.2	23.7	0.45	23.0	24.1	0.45
FCR, g feed/g gain	1.02	1.03	0.006	1.05	1.08	0.008	1.09	1.10	0.008
Day 8-14									
Gain, g/bird/d	49.8[c]	47.7[d]	0.59	49.5[a]	46.1[b]	0.75	44.4[b]	44.7[b]	0.73
Feed intake, g/bird/d	59.3	57.8	0.59	59.9	57.9	0.76	58.3	59.4	0.74
FCR, g feed/g gain	1.19	1.21	0.007	1.21	1.26	0.010	1.32	1.33	0.015
Day 0-14									
Gain, g/bird/d	36.2[c]	34.7[d]	0.37	35.9[a]	33.3[b]	0.56	32.0[b]	32.6[b]	0.56
Feed intake, g/bird/d	40.7	39.8	0.32	41.6[a]	39.7[b]	0.66	39.5[b]	40.6[a]	0.66
FCR, g feed/g gain	1.13	1.15	0.006	1.16	1.19	0.009	1.24	1.25	0.011

[a,b,c,d] within analyses, values not having the same supercript are significantly different ($P < 0.05$)

Day 0-7

(i) Analysis of basal diets

Enzyme inclusion in the basal diet increased daily gain ($P = 0.039$), but had no significant effect on FI or FCR.

(ii) Analysis of FFRB*enzyme interaction

No significant interactions were observed between level of FFRB and enzyme inclusion on daily gain, FI and FCR. Daily gain (21.5 vs 22.6) and FCR (1.10 vs 1.06) were inversely related to the levels of FFRB ($P < 0.001$).

Day 8-14

(i) Analysis of basal diets

Growth rate was higher when enzyme was supplemented in the basal diet (49.8 vs 47.7, $P < 0.05$), however, no significant improvement was found in FCR.

(ii) Analysis of FFRB*enzyme interaction

Significant interactions between level of FFRB and enzyme were seen on daily gain (P = 0.019). The data showed that daily gain and FCR deteriorated with increasing FFRB substitution level. Inclusion of the enzyme preparation in FFRB diets improved FCR numerically (1.26 vs 1.30, P = 0.077).

Day 0-14

(i) Analysis of basal diets

Improvements in daily gain (36.2 vs 34.7, P = 0.011) and FCR (1.13 vs 1.15, P = 0.056) were seen when the enzyme preparation was added to the basal diet.

(ii) Analysis of FFRB*enzyme interaction

Significant interactions occured between level of FFRB and enzyme for daily gain (P = 0.016) and FI (P = 0.043). Bird performance (growth rate and FCR) deteriorated with increasing level of FFRB in the diets (P < 0.001), but FI was not affected. A significant main effect of enzyme was observed on FCR.

Table 4.7 Significance of main effects of FFRB level and enzyme, and their interactions, on daily gain, FI and FCR

	Gain	Feed intake	FCR
Day 0-7			
Level of FFRB	NS	***	***
Enzyme	NS	NS	NS
Level*Enzyme	NS	NS	NS
Day 8-14			
Level of FFRB	***	NS	***
Enzyme	*	NS	NS
Level*Enzyme	*	NS	NS
Day 0-14			
Level of FFRB	***	NS	***
Enzyme	NS	NS	*
Level*Enzyme	*	*	NS

NS = P > 0.05; * = P < 0.05; ** = P < 0.01; *** = P < 0.001

4.3.4 Effects of Enzyme Supplementation on Digestive Tract Characteristics

The weights of small intestine, caeca, liver plus gall bladder and pancreas per unit body weight (g/kg body weight) are presented in Table 4.8. Table 4.9 presents the significance of effects of level of FFRB and enzyme, and their interactions, on intestinal tract, liver plus gall bladder, and pancreas weights.

Table 4.8 Weight of small intestine, caeca, liver plus gall bladder and pancreas, per unit body weight (g/kg body weight)

	Basal		SE	9% RB+Basal		SE	18% RB+Basal		SE
	+	-		+	-		+	-	
Small intestine	34.3	35.2	1.15	37.7	39.6	1.70	41.8	41.5	1.08
Caeca	5.6	5.8	0.37	5.7[a]	4.4[b]	0.30	5.5[a]	6.1[a]	0.31
Liver + gall bladder	28.5	28.2	0.51	29.7	26.2	1.45	28.6	30.0	1.00
Pancreas	3.6	2.9	0.25	3.1	2.8	0.18	2.9	2.9	0.13

[a,b] within analyses, values not having the same supercript are significantly different ($P < 0.05$)

(i) Analysis of basal diets

No significance difference was found on all the organs measured between the birds fed basal diet with or without enzyme supplementation (Table 4.8).

(ii) Analysis of FFRB*enzyme interaction

Significant interaction was observed between level of FFRB and enzyme inclusion on caeca ($P = 0.035$). However, there was no interaction effect on small intestine, liver plus gall baldder and pancreas weight.

Table 4.9 Significance of main effects of FFRB level and enzyme, and their interactions, on digestive tract characteristics

	Small intestine	Caeca	Liver + gall bladder	Pancreas
Level of FFRB	NS	NS	NS	NS
Enzyme	NS	NS	NS	NS
FFRB*Enzyme	NS	*	NS	NS

NS = $P > 0.05$; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

4.4 Discussion and Conclusion

Inclusion of the enzyme preparation based on lipase improved AME content of all diets, though only numerically in FFRB diets, which agrees with the data of Pluske et al. (1997) who found a higher AME content in FFRB diets supplemented with the same lipase preparation. Data in the current study showed that a higher level of FFRB substitution (18%) in the basal diet significantly ($P < 0.001$) depressed AME content of the diets. The AME content of FFRB found in this study was in agreement with the AME content of 9.6 to 10.8 MJ/ kg reported by Warren and Farrell (1990c). However, the AME content of Thai FFRB found in this experiment was much lower than that of Australian FFRB in Experiment 1, although a similar AME content of diets with 9% FFRB was found in both experiments. The large difference was partly

due to lower AME content of Thai FFRB diets than basal diets in this experiment, and the higher AME content of Australian FFRB diets than basal diets in Experiment 1. Therefore, a large difference in AME of FFRB occurred when the formula (section 2.7.3) was used to correct for the AME content of basal diets.

No improvement in lipid digestibility was observed in this experiment with use of the enzyme preparation, which is similar to findings in Experiment 1. Therefore the higher AME content of diets supplemented with the enzyme preparation is more than likely attributable to the activity of xylanase, cellulase and protease in the enzyme preparation, either singularly or in combination. However, the higher AME content of FFRB in diets supplemented with the enzyme is unlikely to be explained by xylanase and cellulase activity. Annison et al. (1995) found that rice bran NSP (made up of predominantly arabinose and xylose) did not have detrimental effects on broiler chicken performance. Aboosadi et al. (1996) found no increase in AME content or improvement in performance when cell-wall degrading enzymes (cellulase, xylanase and arabinosidase) were added to the diets of broiler chickens. Therefore, the higher AME content of enzyme-supplemented diets may be due to the activity of protease components in the enzyme preparation acting against protein and amino acids in the small intestine, contributing to an increased AME content of the diets.

Despite the findings of Annison et al. (1995) and Aboosadi et al. (1996), there is a possibility that the high oil level (> 5%) might have depressed some digestibility of the nutrients in both basal and FFRB diets. Nitsan et al. (1997) conducted a study on effect of addition of soyabean oil on metabolisable energy and broiler performance, and found that a high lipid level (> 5%) suppressed starch digestibility. Therefore there is the possibility that the availability of other carbohydrates such as cellulose and arabinoxylan in the diets (from FFRB and other ingredients) were depressed. Therefore, xylanase and cellulase activities in the enzyme preparation might have acted against the targeted nutrients and increased the AME content of the diets supplemented with the enzyme preparation.

Although lipid digestibility in diets with 9% FFRB from days 4-7 in this experiment was similar to that found in Experiment 1, the lipid digestibility in this experiment fell as birds aged. This might be due to decreased pancreatic lipase secretion in the second week of life when the measurement was taken. Nitsan et al. (1990) showed that the activity of lipase in the pancreas of male broiler chicks decreased a few days after hatch and did not fully recover to the level at hatching until around 18 days of age. This is because the reserves that were produced during embryonic growth decreased rapidly, since synthesis during this period is less than that required for secretion in the intestine for maintaining the initial concentrations. Nir et al. (1993) showed that the activity of lipase in the contents of the small intestine of broiler chicks decreased at eight days of age and did not recover to the level at hatch until 15 days of age when the experiment ended.

The lower lipid digestibility of 18% FFRB diets (between days 4-7) may have arisen from the anti-nutritive factors of FFRB such as phytate and NSP. Even though Farrell and Martin (1998a) showed that Australian FFRB did not increase digesta viscosity, the NSP of Thai FFRB may have had some negative effects on nutrient digestibility. This is because FFRB from different regions can give different responses due to different processing methods and/or to varietal or environmental differences during growth (Wang et al., 1997). In addition, Normand et al. (1979) showed that NSP, which is higher in 18% FFRB diets, bound bile acids and thus reduced the digestibility of lipid in the intestine. A higher phytate concentration in 18% FFRB diets might bind with lipases or minerals needed for lipase activities, and thus reduced lipase activities in intestine which subsequently caused lower lipid digestibility. Vohra et al. (1965) indicated that phytate has a good affinity to Cu, which is essential for the lipase and phospholipase A activities in the intestine of rats (Lewis et al., 1987) and pigs (Luo and Dove, 1996). It is also possible that the impaired apparent digestibility of lipid in broilers was the result of endogenous lipid losses caused by binding of NSP to the bile of broiler chickens. This is because, contrary to that of mammals, the bile of the broiler chicken contains large amount of triacylglycerols (≈ 19%) and cholesterol esters (≈ 18%), which are present in only trace amounts (≈ 0.05%) in rats (Noble and Connor, 1984). Therefore, a higher NSP concentration in 18% FFRB diets that have high affinity to bile might have increased the amount of unabsorbed endogenous lipids in the chyme.

In contrast to the basal and 9% FFRB diets, no significant decrease in lipid digestibility was found in 18% FFRB diets as birds aged. The mechanism behind this is not understood. It may partly due to higher lipid content of the diets compared to basal and 9% FFRB diets. Krogdahl and Sell (1989), working on poult, indicated that development of lipase activity in intestinal content is dependent on fat content of the diet.

Full-fat rice bran oil comprised only about 27% and 45% of the total lipid present in the 9 and 18% FFRB diets, respectively. However, only 18-20% of the RB oil is saturated. Therefore, levels of saturated fatty acids (≈ 5 and 9% of total for 9 and 18% FFRB diets, respectively) were relatively low in the diets compared to unsaturated fatty acids presented in the diets. This low level of saturated fatty acids would have been digested by the synergistic action of the majority of unsaturated fatty acid within the duodenum. Lipid digestibility of more than 90% obtained in this study was higher than the study of Martin and Farrell (1998) of less than 75%. This may be because, in this experiment, the use of 1.5% soybean oil, which is highly digestible (≈ 96%) (Scott et al., 1982), increased the overall oil digestibility. No additional oil was used in the study of Martin and Farrell (1998). An attempt was made to obtain the lipid digestibility of FFRB. However, the results obtained were very variable. A small error in the fat digestibility of either the basal or FFRB containing diets may have a large influence on the lipid digestibility of FFRB. This may partly because of the use of low level of FFRB (9 and 18%) in this study.

In this study, inclusion of 18% FFRB in diets with and without enzyme preparation supplementation significantly depressed GR and deteriorated FCR ($P < 0.001$). However, at 9% FFRB inclusion, supplementation with the enzyme preparation significantly improved GR and FCR over the two-week growing period.

In conclusion, attempts to improve lipid digestibility using an exogenous lipase preparation were not successful. However, the AME content and bird performance were improved. This might be due to other components of the lipase-based enzyme that must have worked either singularly or in combination on the basal and 9% FFRB diets between days 0 to 14 to enhance growth. These preparations may be more effective in diets containing mainly saturated fat which has low digestibility of 40-79% in beef tallow (Carew et al., 1972), rather than the predominantly unsaturated lipids in this study which has digestibility of 84-95% reported by Carew et al. (1972) on corn oil up to 15 days of age.

CHAPTER 5: Experiment 3

Increasing the Nutritive Value of Thai Full-fat Rice Bran (FFRB) for Broiler Chickens Throughout the Entire Growing Period

5.1 Introduction

Full-fat rice bran (FFRB) is a by-product available abundantly and cheaply for use in the broiler industry. It has a high gross energy (≈ 21 MJ/kg DM) and crude fat (≈ 200g/kg DM) content. A lower ME value of FFRB for chickens compared to cockerels was reported by Warren and Farrell (1990c), an effect attributable in part to low secretion of pancreatic lipase. The high phytate level (1.3%) in FFRB may also contribute to this problem as it forms complexes with dietary protein, and some minerals (Ca, Zn, Cu, Fe and Mg), and make them biologically unavailable (Ravindran et al., 1995; Sebastian et al., 1998). This is because broiler chickens lack the endogenous phytase that hydrolyses phytate. Binding of phytate to Ca ions that is necessary for α-amylase activity reduces starch digestibility. The binding of phytate to protein makes the protein more resistant to proteolytic digestion. Use of exogenous lipase and phytase preparations targeting the two less digestible chemical component of FFRB fed to broiler chickens may therefore improve the nutritive value of FFRB and enhance broiler performance.

The objectives of this experiment were to investigate the effect of enzyme preparations (lipase and/or phytase) on broiler chickens fed 9 and 18% Thai FFRB diet throughout the entire growing period. Warren and Farrell (1990b) and Farrell (1994) suggested that FFRB inclusion of 20% depressed bird performance. Thus, a level of 18% (≈ 20%) was used in this experiment to investigate if the enzyme preparations could overcome the problems described by the authors above. The hypothesis tested was that addition of a lipase and/or phytase preparation would improve the bird performance. Weekly production indices [Growth Rate (GR), Food Intake (FI) and Feed Conversion Ratio (FCR)] of bird fed different diets throughout the entire rearing period of 35-day were determined to indicate the effects of the enzyme preparations, either singularly or in combination, on bird performance.

5.2 Materials and Methods

5.2.1 Animals

A total of 520 day-old male broiler chickens of a commercial Ross strain was used. On the day of arrival, each bird was weighed and placed into a narrow weight class. Birds of relatively low or high bodyweight were discarded. Six birds were then assigned to each of 72 pens such that all pens had a nearly similar average weight. The 72 pens were randomly assigned to nine different treatment groups: control, 9-, 18-, 9L, 18L, 9P, 18P, 9LP and 18LP (see 5.2.3 below), of which each treatment had eight replicates.

5.2.2 Experimental Procedures

All birds were given *ad libitum* access to feed and water. The birds and feed from each cage were weighed every seven days starting from 0 day-old to 35 days of age. Therefore, weekly GR, FI and FCR was determined. No collection of faecal samples was made in this experiment.

5.2.3 Diets

Nine diets (control, 9-, 18-, 9L, 18L, 9P, 18P, 9LP and 18LP) were formulated as shown below. The control diet contained no enzymes or FFRB inclusion. Diets 9-, 9L, 9P and 9LP were diets with 9% Thai FFRB inclusion, and 18-, 18L, 18P and 18LP were diets with 18% Thai FFRB inclusion. Diets 9- and 18- were negative controls with no enzyme added, 9L and 18L were supplemented with 0.1% of lipase preparation, 9P and 18P were supplemented with 0.05% of phytase preparation, and 9LP and 18LP were supplemented with both 0.1% of lipase and 0.05% of phytase preparations. All diets were formulated to have similar energy and crude protein levels.

5.2.4 Statistical Analyses

Data analyses were conducted using the GLM procedure of Minitab. The first analysis examined the effect of level of FFRB irrespective of enzyme treatment. FFRB diets of similar level of inclusion were pooled (e.g. pooled all 9% FFRB diets together, and

18% FFRB diets together) to examine the differences between the two level of FFRB inclusion. No significant effect of level of FFRB inclusion were found in the first analysis (above). Therefore, the second analysis examined the effect of enzymes (one-way ANOVA, with respective factors being no enzyme, plus lipase, plus phytase, and plus lipase and phytase). The third analysis compared all the FFRB diets to maize-soybean control diet (one-way ANOVA with pairwise comparison).

Table 5.1 Composition of experimental diets (air-dry basis)

Ingredients (%)	Control	9-	9L	9P	9LP	18-	18L	18P	18LP
Maize	65.3	58.6	58.5	58.55	58.45	50.7	50.6	50.65	50.55
Soyabean	23.7	20.4	20.4	20.4	20.4	19.4	19.4	19.4	19.4
Meat and Bone Meal	5.5	6.0	6.0	6	6	5.9	5.9	5.9	5.9
Fish Meal	4.5	5	5	5	5	5	5	5	5
Rice Bran	-	9	9	9	9	18	18	18	18
L-lysine	0.14	0.15	0.15	0.15	0.15	0.16	0.16	0.16	0.16
DL-Methionine	0.26	0.25	0.25	0.25	0.25	0.24	0.24	0.24	0.24
Vitamin	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Mineral	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
NaCl	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Lipozyme	-	-	0.1	-	0.1	-	0.1	-	0.1
Phytase	-	-	-	0.05	0.05	-	-	0.05	0.05
Analysis Value (as is basis)									
GE (MJ/kg)	16.93	17.14	17.01	17.13	17.09	17.39	17.31	17.25	17.41
Crude Fat (%)	3.48	4.56	4.49	4.34	4.54	5.50	5.33	5.35	5.40
Nitrogen (%)	3.77	3.83	3.72	3.62	3.72	3.69	3.55	3.69	3.78
Dry Matter (%)	88.63	89.57	89.34	89.10	88.94	89.30	89.31	89.48	89.36
Calculated Value (as is basis)									
AME (Kcal/kg)	3066	3048	3048	3048	3048	3028	3028	3028	3028
Crude Protein (%)	21	21	21	21	21	21	21	21	21
Fat (%)	4.2	5.38	5.38	5.38	5.38	6.4	6.4	6.4	6.4
Lysine (%)	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Meth + Cyst (%)	0.89	0.87	0.87	0.87	0.87	0.87	0.87	0.87	0.87
Total Phosphorus (%)	0.8	0.96	0.96	0.96	0.96	1.08	1.08	1.08	1.08
Calcium	1.01	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1

5.3 Results

The average daily gains, FI and FCR during the 35-day rearing period of birds fed different diets are presented in Table 5.2. Table 5.3 presents the significance of effects of level of FFRB on daily gain, FI and FCR. Table 5.4 presents the significance of enzymes and their interactions on daily gain, FI and FCR.

Table 5.2 The effects of diets based on Thailand FFRB and supplemented with enzymes on daily gain, FI and FCR.

	Control	SE	9% RB				SE	18% RB				SE
			-Enz	+Lip	+Phy	++		-Enz	+Lip	+Phy	++	
Day 0-6												
Gain, g/bird/d	21.0	0.37	20.3	19.8	19.3*	19.4*	0.19	19.3*	19.4*	19.1*	20.0	0.69
Feed intake, g/bird/d	22.3	0.86	22.0	21.7	21.4	21.0	0.16	21.3	21.7	21.0	21.8	0.61
FCR, g feed/g gain	1.07	0.04	1.09	1.10	1.11	1.08	0.01	1.10	1.12*	1.10	1.07	0.01
Day 7-13												
Gain, g/bird/d	45.1	0.68	44.7	44.9	44.4	43.9	0.32	44.0	42.9	44.3	45.2	0.24
Feed intake, g/bird/d	58.2	0.92	56.1	55.6	56.1	55.4	0.34	55.1	54.9	55.7	56.0	0.38
FCR, g feed/g gain	1.29	0.02	1.26	1.24*	1.27	1.26	0.01	1.25	1.28	1.26	1.24*	0.01
Day 14-20												
Gain, g/bird/d	68.1	0.67	66.0	66.9	66.7	66.2	0.42	67.2	67.1	66.3	67.7	0.42
Feed intake, g/bird/d	99.8	1.16	94.0*	95.0*	95.2*	94.8*	0.38	95.4*	95.0*	93.8*	98.3	0.61
FCR, g feed/g gain	1.46	0.05	1.43	1.42	1.43	1.43	0.01	1.42	1.42	1.42	1.45	0.01
Day 21-27												
Gain, g/bird/d	90.3	1.81	86.8	91.7	87.6	86.0	0.52	90.0	85.5	86.5	90.0	0.73
Feed intake, g/bird/d	148.2	3.27	135.5**	142.0	138.8*	138.2*	0.89	138.2*	132.7***	132.4***	145.4	1.01
FCR, g feed/g gain	1.64	0.02	1.56	1.55	1.59	1.61	0.01	1.54	1.55*	1.53*	1.62	0.01
Day 28-35												
Gain, g/bird/d	92.9	1.81	90.8	91.6	93.0	93.3	0.68	90.0	93.7	91.5	91.3	0.87
Feed intake, g/bird/d	173.1	2.90	162.4	163.3	166.0	162.0	1.34	162.3	169.0	162.1	160.2	2.42
FCR, g feed/g gain	1.86	0.03	1.79	1.79	1.79	1.74	0.01	1.81	1.81	1.78	1.76	0.02
Day 0-35												
Gain, g/bird/d	63.4	0.78	61.6	62.9	61.9	61.1	0.29	62.4	60.5	59.1	62.9	0.55
Feed intake, g/bird/d	100.3	1.23	93.9**	93.7**	95.3*	93.1***	0.4	94.1**	91.3***	90.2***	96.9	0.76
FCR, g feed/g gain	1.58	0.01	1.52	1.49**	1.54	1.53	0.01	1.51*	1.51*	1.53	1.54	0.01

Note: -Enz = no enzyme, +Lip = plus Lipase, +Phy = plus Phytase and ++ = plus Lipase and Phytase
Cells with asterisk(s) are significantly different with control, * = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$

Table 5.3 The effect of 9 and 18% FFRB on daily gain, FI and FCR, irrespective of enzyme treatment

	Level of FFRB		SE
	9%	18%	
Day 0-6			
Gain, g/bird/d	19.7	19.5	0.18
Feed intake, g/bird/d	21.5	21.4	0.16
FCR, g feed/g gain	1.10	1.10	0.006
Day 7-13			
Gain, g/bird/d	44.6	44.1	0.30
Feed intake, g/bird/d	55.8	55.4	0.37
FCR, g feed/g gain	1.26	1.26	0.006
Day 14-20			
Gain, g/bird/d	66.5	67.1	0.43
Feed intake, g/bird/d	94.7	95.7	0.53
FCR, g feed/g gain	1.43	1.43	0.007
Day 21-27			
Gain, g/bird/d	88.0	88.1	0.72
Feed intake, g/bird/d	138.5	137.5	1.21
FCR, g feed/g gain	1.58	1.56	0.012
Day 28-35			
Gain, g/bird/d	92.1	91.5	0.78
Feed intake, g/bird/d	163.5	163.3	1.86
FCR, g feed/g gain	1.78	1.79	0.018
Day 0-35			
Gain, g/bird/d	61.9	61.2	0.37
Feed intake, g/bird/d	94.0	93.0	0.59
FCR, g feed/g gain	1.52	1.52	0.001

Table 5.4 Significance of effects of lipase, phytase, and lipase plus phytase on daily gain, FI and FCR

	Gain	Feed intake	FCR
Day 0-6			
Lipase	NS	NS	NS
Phytase	NS	NS	NS
Lipase+Phytase	NS	NS	*
Day 7-13			
Lipase	NS	NS	NS
Phytase	NS	NS	NS
Lipase+Phytase	NS	NS	NS
Day 14-20			
Lipase	NS	NS	NS
Phytase	NS	NS	NS
Lipase+Phytase	NS	NS	NS
Day 21-27			
Lipase	NS	NS	NS
Phytase	NS	NS	NS
Lipase+Phytase	NS	NS	NS
Day 28-35			
Lipase	NS	NS	NS
Phytase	NS	NS	NS
Lipase+Phytase	NS	NS	NS
Day 0-35			
Lipase	NS	NS	NS
Phytase	**	NS	NS
Lipase+Phytase	NS	**	NS

NS = P > 0.1; * = P < 0.1; ** = P < 0.05; *** = P < 0.01; **** = P < 0.001

Day 0-6

An effect of lipase plus phytase was observed on FCR (P = 0.054) when enzymes in FFRB diets were examined. When all the FFRB-based diet were compared with control, GR of birds fed Diet 18- or 18L or 18P, or 9P or 9LP, were significantly depressed (P = 0.036, P = 0.050, P = 0.017, P = 0.029, and P = 0.050, respectively). Only FI of birds on Diet 18P and 9LP were numerically depressed (P = 0.075 and P = 0.075). Only FCR of birds fed 18L was significantly poorer than control (P = 0.050).

Day 7-13

The data showed no significant effects was found when enzymes were added to FFRB diets. When comparing all the diets with the control, no significant differences in GR and FI were found. In terms of feed efficiency, only birds fed Diet 9L and 18LP performed significantly better than the control (P = 0.036 and 0.023, respectively).

Day 14-20

The data showed no significant effects of enzymes when added to FFRB diets. When all the diets were compared with control, only birds fed Diet 18LP had similar GR and FI with control (P > 0.980). All the birds fed FFRB diets (except 18LP) had lower FI than control (P < 0.05). No significant differences were found in FCR between control and FFRB diets, although birds on 18% FFRB diets showed a trend of improving FCR (P = 0.101).

Day 21-27

The data showed that birds fed Diet 9L, 18- and 18LP grew as well as the controls. All birds fed FFRB diets, except on Diet 9L and18LP, had significant lower FI than control. Only birds fed Diet 18L and 18P utilized feed more efficiently than control (P = 0.046 and P = 0.050, respectively). No significant effect was found when enzymes were added to FFRB diets.

Day 28-35

The data showed no significant improvement when the enzyme-supplemented FFRB diets were compared to enzyme-free FFRB diets. When all FFRB diets were compared with control, no significant differences in performance were found.

Day 0-35

A phytase effect on GR ($P = 0.048$), and lipase plus phytase effect on FI ($P = 0.039$), were observed when effects of enzymes on FFRB diets were examined. Comparing all FFRB diets with the control diet showed that only birds fed Diet 9L and 18LP grew as well as the controls ($P = 0.9996$ and 0.9999, respectively). However, birds fed Diet 18L and 9P performed the worst among all the FFRB diets when compared with control ($P = 0.050$ and 0.001, respectively). All birds except those on 18LP ate significantly less feed than controls ($P < 0.05$). All birds on FFRB-based diets had better feed efficiency value than control. However, only Diet 18-, 9L and 18L were significantly better than control ($P = 0.028$, 0.003 and 0.033, respectively). Effects of phytase on gain ($P = 0.048$), and lipase plus phytase on FI ($P = 0.039$), were seen with FFRB-based diets.

5.4 Discussion and Conclusion

It was hypothesised in this experiment that the two enzymes or their combination should have targeted successfully at least one of the less digestible chemical component of FFRB, i.e., lipids and phytate, and resulted in improved bird performance. However, the results showed that supplementation of FFRB-based diets with lipase or phytase or their combination had no significant improvement on bird performance.

It was evident that supplementation of both lipase and phytase preparation increased FI of the birds fed 18% FFRB diet, but not that of the 9% FFRB diet. This might be because at this level of FFRB inclusion and at this diet specification, this enzyme mixture balanced the nutrients in the diet. The higher FI of birds fed 18% FFRB diets supplemented with both lipase and phytate preparations resulted in higher GR compared to birds fed other 18% FFRB diets.

Feed intake was significantly depressed in FFRB-based diets starting from the second week of life. The lower FI of most of the birds fed FFRB diets (except 18LP) would result in increased food retention time in digestive tract. Thus, an increase in the time the nutrients in FFRB diets were subjected to the action of the digestive enzymes in the tract would have occurred. This may have contributed to the better FCR value of birds fed FFRB diets, which contradicts the data of Martin and Farrell (1998) and Experiment 1 and 2 in this thesis. However, the lower FI had limited the growth of birds compared to those fed the control diet. The notion of decreasing ingestion of feed resulting in improved digestibility (above) is supported by the findings of Ledin (1984), who found that lower FI in rabbits resulted in higher mean retention time. In addition, Ambuhl, Williams and Senior (1979) and Williams and Senior (1978), working with rats, observed better organic matter and protein digestibility, respectively, with lower FI. Therefore, this might be partly the reason why the enzyme preparations did not have any effects on the FFRB diets.

The lower calculated AME content (3050 Kcal/kg) and crude protein content (21%) of diets in this experiment might have contributed to the lower FI of birds fed FFRB diets, and subsequently led to the lack of response to enzyme supplementation and growth. McDonald, Edwards, Greenhalgh and Morgan (1995) stated that, in poultry, severe deficiencies of amino acids reduce food intake whereas moderate deficiencies, insufficient to affect growth markedly, increase intake. Therefore, the crude protein content of 21% in the diets of this study might be too low for young broilers up to 27-days of age. Data in the current study showed that this specification might be more suitable for older broilers, as FI recovered from day 28-35. This argument is further supported by the data in Experiment 1 and 2, where FI was not depressed in both 9 and 18% FFRB diets that had a calculated AME value of about 3100 Kcal/kg and crude protein of 23% and 22% for 9 and 18% FFRB diets, respectively.

In addition, the phytate contents of FFRB might be responsible for the lower FI in FFRB diets than the control diet. The phytate content of FFRB might have bound with some minerals that are required for digestive enzymes activities. Lewis et al. (1987), working with rats, and Luo and Dove (1996), working with pigs, stated that lipase and phospholipase A need Cu for the intestinal activities, and Caldwell and Kung (1953) suggested that ∝-amylase needs Ca for their activities in intestine. Camovale et al. (1988) reported that a phytate-protein interactions reduced the availability of protein. Therefore, phytate content made the FFRB diets nutritionally less balanced than the control diet, and subsequently resulted in lower FI than the control diets.

In contrast to the findings of this study that performance of birds fed FFRB diets was not improved by supplementation of phytase-based enzyme preparation, Farrell and Martin (1998b), working with ducks, found that phytase inclusion in FFRB diets improved the performance of ducks from two-day-old to 40-day-old. However, the diets fed to ducks contained higher phytase activities (1000U/kg food), whereas in this study, only 125U/kg food of phytase activity was included in the diets. Therefore, besides the low nutrient specification issue discussed previously, the phytase activity in the FFRB diets supplemented with phytase preparation might be too low to release sufficient nutrients from phytate to balance the nutrition in the diets and improve the performance of the birds.

It is concluded, therefore, that use of lipase and phytase preparations to improve the performance of birds fed 9 and 18% FFRB diets was not successful. This may partly be due to FFRB and the effects of low nutrient specification interactions on FI subsequently affecting the normal food retention time in birds fed FFRB diets (Ledin, 1984). A sub-optimal phytase activity in the phytase preparation may partly be the reason why the phytase preparation did not improve the performance of the birds.

CHAPTER 6: General Discussion and Conclusion

In this thesis, two enzyme preparations were used in an attempt to improve the nutritive value of full-fat rice bran (FFRB) from Australia and Thailand. It was hypothesised that a lipase-based enzyme preparation would improve the AME content of FFRB by improving the digestibility of lipid (Experiment 1 and 2) and enhance performance of birds, and that the use of lipase and(or) phytase-based enzyme preparations (Experiment 3) would improve the performance of birds fed FFRB-containing diets. The results of Experiment 1 and 2 showed that supplementation of FFRB-based diets with the lipase-based preparation did not improve the lipid digestibility of the diets. The improved AME content of the enzyme-supplemented FFRB-containing diets and performance of birds fed a maize-soyabean meal basal diet plus enzyme (Experiment 1 and 2), and the 9% FFRB diet plus lipase preparation (Experiment 2), was most likely due to the side action of xylanase, cellulase and/or protease in the enzyme preparation. The data of the three experiments in this thesis showed that FFRB of different sources (e.g. Australia and Thailand) and nutrient specification level have substantial effects on the response of birds to the enzyme preparations.

Lipid digestibility in basal diets substituted with 9% FFRB was similar from day 4-7 in Experiment 1 and 2, however, the lipid digestibility in Experiment 2 fell as birds aged, which was in contrast to Experiment 1. This might be due to the different ages of birds when the measurements were taken in the two experiments. In Experiment 2, the second measurement was taken in the second week of life when pancreatic lipase secretion is decreased. In Experiment 1, however, the second and third measurements were taken in the third and fourth week of life, respectively, when pancreatic lipase secretion had recovered. Nitsan et al. (1990) showed that the activity of lipase in the pancreas of male broiler chicks decreased a few days after hatch and did not fully recover to the level at hatch until around 18-days-of-age. Nir et al. (1993) showed that the activity of lipase in the contents of the small intestine of broiler chicks decreased at eight days of age and did not recover to the level at hatch until 15 days of age, when their experiment ended. This is because the reserves that were produced

during embryonic growth decreased rapidly, since synthesis during this period is less than that required for secretion in the intestine for maintaining the initial concentrations.

When comparing performance indices of Experiment 2 with Experiment 1, the addition of the lipase preparation in the 9% Thai FFRB diet improved weight gain by 7.8% and FCR by 2.6% for the first two week of age. Significant improvements in GR and FCR were seen in the second week of life. In contrast, no improvement in GR and FCR was observed in birds fed the 9% Australian FFRB diet with enzyme supplementation during the first two weeks of life. These contradictory results agree with those of Wang et al. (1997), who showed that when enzyme was added, it improved performance with Malaysian rice bran but not the Chinese rice bran.

In the study using Australian FFRB (Experiment 1), lipase preparation reduced caecal weight/unit body weight in birds fed the basal diet, and reduced small intestinal weight/unit body weight of birds fed the 9% FFRB diet. In contrast, when Thai FFRB was used (Experiment 2), no significant difference in small intestinal weight was found in birds fed the 9% FFRB diets with and without lipase-preparation supplementation. The different results found in Experiments 1 and 2 may partly be due to differences in FFRB processing methods and varietal or environmental differences during growth. The proximate analysis carried out on the two FFRB indicated that the different response in caecal and small intestinal weight of birds fed two different FFRB might caused by a difference in hemicellulose content of the two FFRB. The two FFRB had similar neutral-detergent fibre/DM value (24.9 and 24.7, repectively, in Thai and Australian FFRB), but a lower acid-detergent fibre/DM value was found in Australian FFRB than Thai FFRB. Therefore, Australian FFRB was higher in hemicellulose content (neutral-detergent fibre/DM - acid-detergent fibre/DM), being 15.5 vs 14.1%. Other than the FFRB processing methods and environmental differences discussed above, part of the differences may be attributed to the age difference of the birds in different treatments when the measurements were taken. In the study with Australian FFRB, measurement was taken at 36 days of age, whereas in the study with Thai FFRB, the measurement was taken at 14 days of age. Therefore the non-significantly

lower small intestinal weight/body weight in birds fed 9% Thai FFRB diets with lipase preparation supplementation, which is significantly lower in Australian FFRB study, might be due to less obvious response of birds to the enzyme treatment as the digestive tract was still less-developed. The relative higher intestinal weight/unit body weight of younger bird in Experiment 2 (14-day-old) than older bird in Experiment 1 (36-day-old) agrees with the findings of Wang et al. (1997) that younger birds tend to have larger relative sizes of intestines than more mature birds.

In Experiment 3, lipase and(or) phytase-based preparations were used for the purposes of improving the digestibility of lipid and phytate in FFRB, which may then improve the performance of the birds. However, the results showed that supplementation of FFRB diets with lipase and(or) phytase-based preparations did not improve the performance of birds. The reasons of lack of responses on supplementation of enzymes preparation might be due to the lower AME and protein specification in the FFRB diets used in this experiment compared to Experiments 1 and 2. The lower nutrient specification might have lowered the FI of the birds, thus depressed their growth. McDonald et al. (1995) stated that severe deficiencies of amino acids reduce food intake in poultry. However, the improved feed efficiency may be attributable to higher retention time caused by lower FI (Ledin, 1984). The lower FI in FFRB diets compared to the control diet might be attributable to the NSP and phytate content of FFRB. The NSP content of FFRB might bind bile acids (Normand et al., 1979), thus reducing lipid digestibility. The phytate content of FFRB might have bound with some minerals (Vohra, 1965) that are needed for the digestive activities of enzymes in digestive tract (Caldwell and Kung, 1953; Lewis et al., 1987; Luo and Dove, 1996). Therefore, the NSP and phytate content of FFRB might have made the FFRB diets nutritionally unbalanced compared to the control diet. This might have caused the lower FI in FFRB diets. The lack of responses of phytase-based enzyme preparation added to FFRB diets might be due to the phytase activity in the diets being too low (125U/kg food). In the studies by Farrell and Martin (1998b) with ducks, higher phytase activities (e.g. 1000U/kg food) improved the performance of ducks fed more than 40% FFRB diets from two-days-old to 40-days-old.

The data in Experiments 1, 2 and 3 of this thesis showed that responses of birds to enzyme preparation were not only influenced by the factors of levels of FFRB inclusion, processing methods and varietal or environmental differences on the FFRB used. Nutrient specification levels also played an important role in the effectiveness of the enzyme preparations. A nutrient specification of diets that is insufficient in crude protein or some essential amino acids may influence their FI (McDonald et al., 1995), thus food retention time (Ledin, 1984) and nutrient digestibility in intestinal tract (Williams and Senior, 1978; Ambulhl et al., 1979), which can subsequently affect their performance.

It is therefore concluded that the use of a lipase enzyme preparation to improve the lipid digestibility of Australian and Thai FFRB diets (Experiment 1 and 2), and the use of lipase and (or) phytase preparations to improve performance of bird fed Thai FFRB (Experiment 3), were not successful. However, other components of the lipase-based enzyme prepration must have worked either singularly or in combination to improve the AME content of enzyme supplemented diets (Experiment 1 and 2). Response of birds to enzyme preparation varies according to the varietal and (or) environmental differences of FFRB used, and nutrient specification of the diets fed to the birds.

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