Chapter 5

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

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5.1. Introduction

NSP are not digested by poultry and are mostly excreted. The NSP, primarily arabinoxylans, correlate closely with the AME values of a range of cereals (Choct and Annison, 1992b). If the NSP could be degraded to their monomeric constituents, the nutritive value of rice bran would theoretically be improved by 15-20%. Several treatments have been shown to be effective in improving the nutritive value of rice bran, including soaking the cereals, adding NSP-degrading enzymes and heat treatment. Microwave cooking results in the simultaneous production of heat throughout the food product; it gives a uniform temperature profile throughout the sample (Rizk *et al.*, 1994).

The experiment examined the relative improvement in the metabolisable energy content of rice bran for poultry after treatment by water soaking, enzyme supplementation and microwave heating.

5.2. Materials and Methods

Experimental Diets

The experiment consisted of six dietary treatments in a **2** x **3** factorial design. The **2** treatments were: soaking and no soaking; and the **3** diets were: control, enzyme, and enzyme + microwave. Each diet contained a fixed level of 30% full fat rice bran. The six experimental diets were as follows :

- Diet 1 : 70% of basal diet (Table 5.1) + 30% full fat rice bran. This product was designated as Control.
- Diet 2 : Control + 7 L of water, continuous stirring for 48h at 25°C. It was then dried at 45°C and hammer-milled to pass a 1 mm screen. This product was designated RB + S.
- Diet 3 : Control + enzyme (11.2 mL) which had been diluted in 300 mL water. This product was designated RB + E.
- Diet 4 : Control + enzyme (11.2 mL) + 7 L of water, continuous stirring for 48h at 25°C. It was then dried and hammer milled to pass a 1 mm screen. This product was designated RB + E + S.
- Diet 5 : Control + enzyme (11.2 mL) + microwave. This product was designated RB+E+MV.
- Diet 6 : Control + enzyme (11.2 mL) + 7 L of water, continuous stirring for 48h at 25°C, and was then microwaved. It was then dried at 45°C and hammer-milled to pass a 1 mm screen. This product was designated RB+E+MV+S.

Diet Mixing

All ingredients used for the control diet (Table 5.1) were accurately weighed, and thoroughly mixed in a rotary mixer (described in Chapter 3). A recommend amount of enzyme A was diluted in 300 mL water and sprayed into the ingredients using a pressure-spray while feed mixing continued.

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

Ingredients	Amount (g/kg)
DL Methionine	2.0
Maize (8.5% CP)	423.5
Rice Pollard (13% CP)	300.0
Tallow (Stabilised)	10.0
Limestone	18.0
Salt	1.5
Vitamins and minerals	5.0
Meat meal 52	30.0
Soybean 48% (expeller)	210.0

The AME Trial

One hundred and forty four mixed sex broiler chicks (21 days of age) were used in the experiment. There were six treatments, replicated six times with four birds per treatment. The birds were allocated to treatments on an approximate equal liveweight basis. The AME procedure was as described in Chapter 3.

5.3. Results

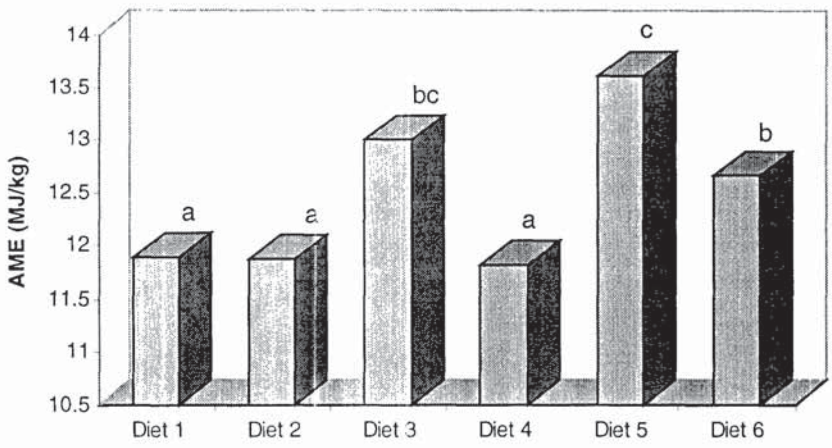
Data from the experiment are shown in Table 5.2. There was evidence of an increase (P<0.05) in AME (Diets 3,5 and 6). However, water soaking (Diet 4) of rice bran decreased AME (P<0.05) (Figure 5.1). Feed intake and weight gain of the birds were significantly increased (P<05) by water soaking. Addition of an enzyme (xylanase) to diets 3, 5 and 6 markedly increased the AME (P<0.05), but feed intake, weight gain and FCR were not significantly improved. Moreover, birds fed diets 5 and 6 which contained enzyme and were microwave treated had significantly better AME and growth compared to those fed the other diets. The AME values for control, enzyme, and enzyme + microwave diets were : 11.89; 13.01 and 13.61 MJ/kg, respectively. The three diets (diet 1, 3 and 5) differed significantly (P<0.05) (Figure 5.1). The moisture content of excreta of the birds was not affected by treatments.

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

Diet	AME (MJ/kg)	FCR (feed : gain)	WG (g/d/b)	FI (g/d/b)	Excreta Moisture (%)
1. Control	11.89	2.39	32.08	79.46	79.62
2. RB + S	11.87	2.14	39.71	83.48	80.01
3. RB + E	13.01	2.32	33.87	78.28	80.09
4. RB + E + S	11.82	2.29	37.45	83.99	80.20
5. RB + E + MV	13.61	2.33	38.97	89.47	80.33
6. RB + E + MV+S	12.66	2.18	43.39	92.40	77.82
Pooled SEM	0.22	0.11	2.92	4.77	1.00
F test and level of significance :					
Treatment	16.09**	0.15	2.28	2.72	0.31
Diet	15.88*	2.74	4.77*	1.17	0.82
Treat X diet	3.86	0.55	0.27	0.04	1.24

* P<0.05; ** P<0.01.

Figure 5.1. Effects of enzyme supplementation and microwave treatment on the AME of a high rice bran diet in broilers.



abc Means with unlike letters differ significantly at P<0.05

5.4. Discussion

Water soaking of rice bran depressed AME, but increased feed intake and weight gain of the birds. The negative effect on the AME was possibly due to an increased oxidation of lipids during soaking and subsequent drying at 45°C. The positive effect of soaking, on the other hand, was probably due to the activation of endogenous phytase present in rice bran, which increased the availability of organic phosphorus to the birds.

Diment (1984) reported that oxidation of lipids might have contributed to the rather excessive loss in nutritive value he observed in soaked rough rice. Roberts and Yudkin (1960) reported that when ground rough rice was water-soaked and dried, then an adverse effect was noted on both poult growth and feed efficiency. However, Barber (1978) found favourable nutritional responses from water soaking of cereal grains, especially from barley, when they were used in broiler rations.

Addition of xylanase to the diets markedly increased AME, but had little effect on bird performance. This suggests that phosphorus was limiting in the current diets to a greater extent than energy. The current diets contained 30% rice bran, which was much higher than commercial inclusion levels. This rice bran contained a high level of phytate, which presumably increased the availability of organic phosphorus to the birds.

Bedford and Morgan (1996) confirmed that starch digestibility may account for up to 35% of the improvement in AME as a result of xylanase supplementation. Under Australian conditions, Choct *et al.* (1996) demonstrated that supplementation of a low-ME wheat diet with a commercial glycanase preparation increased the AME by 24% and the FCR by 25% in 3-4 week old broiler chickens. Taken together, these reports show that enzyme addition to a high NSP basal diet may result in an improvement in feed conversion ratio, and a reduction in feed intake. In contrast, Campbell *et al.* (1984) did not observe any significant effect of enzyme addition from three to four weeks of age, but during the finisher period observed that while enzyme addition did not affect weight gain, it did reduce feed intake by 2.9% and thus improved feed conversion efficiency.

In the current work, birds fed the diets which contained enzyme and were microwave-treated metabolised significantly more energy than birds in the other groups. This suggests that the microwave treatment enhanced the activity of the enzyme and released more absorbable nutrients from the NSP of rice bran. Again, the lack of response on bird performance was probably due to phosphorus limitation in the current diets.

Chapter 6

The Effect of Xylanase, Phytase and Lipase Supplementation on the Performance of Broiler Chickens Fed a Diet with a High Level of Rice Bran

6.1. Introduction

Utilisation of many feedstuffs by poultry is limited by the lack of endogenous enzymes necessary for hydrolysis of their fibre components. Dietary enzymes offer a mechanism whereby nutrient utilisation can be enhanced and/or the negative effects of anti-nutritive activity may be reduced or eliminated. Realisation of the potential of this technology to improve feeding value of feed ingredients requires the establishment of specific substrates and enzyme requirements (Classen and Campbell, 1990).

Rice bran contains 20-25% of NSP, which consist predominantly of arabinoxylans and cellulose (Choct, 1997). It also contains a substantial amount of phytate as well as oil. Supplementation of various enzymes including xylanases (Rotter *et al.*, 1989; Petterson and Aman, 1989; Adrizal *et al.*, 1996) and phytase (Martin, 1995) has yielded positive results in birds fed high rice bran diets.

The current experiment was conducted to examine the effects of xylanase, phytase and lipase as well as their interactions on energy metabolism and performance of broilers fed a diet containing 30% defatted rice bran.

6.2. Materials and Methods

The Enzyme Cocktails

The xylanase and lipase were kindly supplied by Novo Nordisk, Australia and the phytase by BASF, Australia. The recommended dosages were: phytase 130 mg/kg, lipase 200 mg/kg and xylanase 800 mL/kg.

Chicks and Diets

One hundred and ninety two mixed sex broiler chickens (21 days of age) were used in a 2 x 2 x 2 factorial design. There were 8 treatment groups, replicated six times with four chicks per group. The diet allocations were as follows:

Diet 1 :	Control - Practical type diet containing 30% defatted rice bran
Diet 2 :	Control + Xylanase
Diet 3 :	Control + Lipase
Diet 4 :	Control + Phytase
Diet 5 :	Control + Xylanase + Lipase
Diet 6 :	Control + Xylanase + Phytase
Diet 7 :	Control + Lipase + Phytase
Diet 8 :	Control + Lipase + Xylanase + Phytase

The diets were isocaloric and isonitrogenous and were cold pelleted (Table 6.1).

Table 6.1. Ingredient composition of the basal diet

Ingredients	Amount (%)
DL-Methionine	0.30
Lysine Mono HCL	0.10
L-Threonine	0.03
Rice pollard 13%	30.00
Triticale	10.00
Wheat 12%	29.12
Soybean oil	1.00
Tallow (stabilised)	2.00
Dicalcium phosphate	2.00
Limestone	1.70
salt	0.25
Vitamin and minerals	0.50
Soybean "48% (expeller)"	23.00
Total	100.00

AME Trial

A classic AME trial was conducted following the procedure outlined in Chapter 3-Material and Methods. Feed intake, weight gain, and excreta moisture were measured for days 21-27 and feed conversion ratio calculated.

6.3. Results

All the data are presented in Table 6.2.

Feed intake and weight gain. None of the enzyme products (xylanase, phytase and lipase) had a significant effect on weekly feed intake and weight gain of the birds. However, feed intake tended to drop and weight gain tended to increase in response to supplementation of all three types of enzymes. Thus, weight gain of the birds was increased by 0.6% with lipase, 3.7% with phytase and 2.4% with xylanase. There were no interaction of the enzymes on these two parameters.

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

Diet		FI	WG	FCR	AME	EM
Lipase						
0		700	473	1.496	12.51	75.3
+		691	470	1.475	12.38	75.5
Phytase						
0		693	463	1.499	12.40	75.4
+		698	480	1.461	12.48	75.4
Xylanase						
0		707	466	1.519	12.29	74.9
+		684	477	1.441	12.60	76.0
Lipase by phytase						
0	+	695	479	1.455	12.52	74.9
0	0	705	467	1.516	12.50	75.6
+	+	702	480	1.467	12.45	75.9
+	0	680	459	1.482	12.31	75.2
Lipase by xylanase						
0	+	696	487	1.433	12.59	76.2
0	0	704	459	1.539	12.43	74.4
+	+	673	466	1.450	12.61	75.8
+	0	710	473	1.500	12.14	75.3
Phytase by xylanase						
0	+	682	469	1.459	12.59	75.5
0	0	703	457	1.540	12.22	75.3
+	+	686	484	1.424	12.61	76.4
+	0	711	475	1.499	12.36	74.4
F values and level of significance :						
Main effects :						
- Lipase (A)		0.37	0.09	0.20	1.90	0.13
- Phytase (B)		0.16	2.02	2.40	0.69	0.00
- Xylanase (C)		2.35	0.79	10.12*	10.67*	1.99
Interactions :						
AB		1.28	0.86	0.17	0.42	0.77
AC		1.01	1.31	2.38	2.71	0.77
BC		0.02	0.02	0.01	0.45	1.32
ABC		0.25	1.17	1.53	15.61*	0.43

* Significant at P<0.01.

Feed conversion ratio (FCR) and apparent metabolisable energy (AME) value. Xylanase had a marked effect (P<0.01) on both FCR and AME, decreasing FCR from 1.52 to 1.44 and increasing AME from 12.29 to 12.60 MJ/kg. There was also a highly significant (P<0.01) interaction between the three enzymes on AME. The lipase and phytase both improved FCR but had not effect on AME. The improvement in FCR by lipase, phytase and xylanase was 2.1%, 2.6% and 5.4%, respectively.

Excreta moisture content. The moisture content of the excreta was not affected by any of the enzyme. There was also no interaction between any combination of enzymes on excreta moisture.

6.4. Discussion

In the current experiment, supplementation of the diet with xylanase significantly (P<0.01) improved the AME and feed conversion. Considering the excellent performance of the control birds, the improvement is of great practical significance.

The use of glycanases in cereal-based diets is now a common practice in the poultry industry throughout the world. The enzymes break down non-starch polysaccharides, thereby removing their anti-nutritive effect on nutrient digestion and absorption (Choct, 1997). Rice bran contains 20-25% NSP, which consist of equal amounts of arabinoxylan and cellulose (Choct, 1997). The NSP in rice bran are mainly insoluble and do not increase gut viscosity in poultry (Annison *et al.*, 1995). However, the insoluble NSP are not inert as they can absorb large amounts of water to influence gut motility and may also complex with biologically active molecules such as bile acids (Kritchevsky, 1976). It is therefore envisaged that partial degradation of the rice bran NSP may disrupt these interactions as well as release small polymers, which may in turn be effectively fermented by the hindgut microflora. In addition to the marked effect of the xylanase on AME and FCR, there were also significant interactions between lipase, phytase and xylanase on

AME and FCR, showing an additive effect on energy metabolism and feed conversion. The effect of the xylanase on AME and FCR was large and difficult to account for by an increase in energy contribution by fermentation alone. The enzyme was also devoid of phytase ac ivity and released free sugars could have been low (see Chapter 4). It is therefore possible that the improvement in AME and FCR was due partly to effect of the enzyme on the wheat and triticale components of the diet.

Rice bran contains a considerable amo int of phytate, which is poorly utilised by monogastric animals (Warren and Farrell, 1990b). Martin (1995) has demonstrated that supplementation of duck diets with microbial phytase allows rice bran to be used at high levels (up to 60%) without detrimental effects. Phosphorus excretion was reduced by 9.6% and significant decreases in excretion of the mineral elements manganese, copper and zinc were also noted. The effectiveness of phytase in improving phytate phosphorus digestion and its subsequent reduction in organic phosphorus output to the environment has attracted a great deal of scientific and commercial interest. Addition of a phytase at 500 U/kg of feed to pigs increased the digestibility of phosphorus from 44.2% to 52.4% (18.6% increase) and calcium from 44.2 to 51.7% (17.0 % increase). Supplementation of piglet diets with 1500 U phytase/kg also significantly improved the weight gain (from 424 to 529 g/d) and feed conversion ratio (from 1.65 to 1.52) (Jongbloed *et al.*, 1993). In poultry, up to a 40% reduction in phosphorus excretion with phytase use has been reported for broilers (Simons *et al.*, 1990). Increased egg production and positive effects on egg weight and tibia ash were also noted when phytase was added to layer diets (Schutte, 1991). Yi *et al.* (1996) found that graded levels of phytase progressively increased body weight gain and feed intake and feed conversion efficiency of broilers. The improvement of body weight gain resulting from the addition of phytase was primarily a result of increased feed intake and utilisation of dietary phosphorus (Simons *et al.*, 1990; Broz., 1991). In the current study phytase supplementation tended ($P<0.10$) improve weight gains and feed efficiency of broilers. Weight gains were improved by 3.7% and FCR by 2.6% compared to the controls. Since the basal diet contained adequate levels of P (1.16% total P or 0.49% non-phytate P), the observed phytase responses appear to be independent of its effect of P availability. These 'extra-phosphoric' effect of phytase may be attributed to the release of protein and amino acids from phytase-protein complexes.

Lipase addition alone was not effective in improving performance of the chickens. It is well known that raw rice bran contains an extremely active lipolytic enzyme, lipase, which hydrolyses the triglycerides and releases free fatty acids. Therefore, supplementation of lipase may have been redundant.

The current study demonstrates that combination of xylanase and phytase can allow high levels (up to 30%) of rice bran to be included in broiler diets without any detrimental effect.

Chapter 7

General Discussion and Conclusion

The studies reported in this thesis were directed to the general theme of improving the feeding value of rice bran-based diets for broiler chickens, using feed enzymes. The studies also examined the possibility of releasing sugars from rice bran by enzyme supplementation and microwave treatment.

The digestibilities of NSP are very low in poultry and large amounts of NSP are excreted. Most cereal grains and their by-products contain large amounts of arabinoxylan and cellulose as the main NSP. The structure of both polymers is well characterised and the enzyme technology is widely available for a complete breakdown of these substrates. Large bulks of the NSP are polymers of 6-carbon sugars (glucose, galactose, mannose) which can be used efficiently as energy sources. Cellulose is a straight chain 1-4 ß-glucan and requires a combination of cellobiohydrolase, endoglucanase and ß-glucosidase for complete breakdown to glucose. The (1-3), (1-4) - ß-glucan of barley and oats can be rapidly broken down to glucose with the combination of endoglucanase and β-glucosidases. Rice bran, for instance, contains approximately 20-25% NSP, half of which is cellulose (Saunders, 1986). With development of highly sophisticated enzymes, all these NSP represent a large source of potential energy for monogastrics. It is known that a long period of incubation of NSP-rich materials with appropriate enzymes may lead to increased release of free sugars from the NSP, which may in turn be used by poultry as energy sources. In the current study, both a multi-activity glycanase (Enzyme A) and a xylanase-based product (Enzyme B) were unable to release substantial amounts of sugars from rice bran *in vitro*. This confirms the view that the positive effect of enzyme supplementation on monogastric performance is not due to the complete breakdown of the NSP and a subsequent absorption of the released sugars, rather it is due to the ability of the enzymes to cleave large molecules of NSP, thereby removing their anti-nutritive effects on nutrient digestion and absorption (Choct, 1997).

All enzymes require certain amounts of energy in order to act on their substrates. Appropriate frequencies of microwave energy appear to energies the enzymes. Thus a five-minute microwave treatment of enzyme/rice bran mixture resulted in release of higher amounts of sugars from rice bran than 48h incubation with the enzyme at 40°C. The effect of microwave treatment and enzyme supplementation on AME in broilers fed a diet containing 30% rice bran was also demonstrated in Experiment 2. A significant increase ($P<0.05$) in AME and weight gain was observed following enzyme and microwave treatments. However, these treatments had little or no effect on bird performance. A simple water soaking of rice bran significantly ($P<0.05$) increased bird performance although it decreased AME of the diet. In general, bird performance was poorer than expected Experiment 2 indicating possible nutrient imbalance or deficiency. The current diet contained 30% rice bran, which was much higher than commercial inclusion levels. Rice bran contains a high level of phytate and it was possible that the diet was marginally deficient in phosphorus. This is supported by the fact that an increase in the moisture level and temperature can activate endogenous enzymes including phytase present in rice bran which would increase the availability of organic phosphorus to the birds.

The limiting factors in rice bran as a feedstuff for poultry are its high levels of NSP, phytate and lipids. The NSP and the phytate are poorly utilised by poultry and the lipids are prone to rancidity. Various enzymes appear to offer promise in overcoming some of these limitations of high rice bran diets in poultry. Experiment 3 was conducted to examine this possibility. Three different enzymes, namely xylanase, phytase and lipase, were used in all possible combinations to investigate their individual effects as well as interactions on AME and bird performance. Once again, the xylanase significantly enhanced

AME of the diet and improved feed conversion of the birds. Rice bran contains substantial amounts of NSP that are predominantly insoluble. Also these NSP do not appear to possess any anti-nutritional activity as inclusion of isolated rice bran NSP in broiler diets does not depress AME or bird performance (Annison *et al.*, 1995). Although supplementation of rice bran containing diets with xylanase possibly degraded the NSP to an extent, the large improvements in AME and FCR observed in the current experiment were unexpected. Even though partial depolymerisation of the NSP can lead to increased fermentation in the hindgut of the chicken (Choct *et al.*, 1996), the energy contribution from fermentation is estimated to be only 6-8% of the total energy (Annison *et al.*, 1968). It is therefore possible that the significant improvements in AME and FCR obtained in the current experiment may partially be due to the effect of the xylanase on the wheat and triticale components of the diet. The beneficial effect of xylanase supplementation of wheat or triticale diets on AME and bird performance is well documented in the literature.

Phytase addition increased ($P<0.1$) weight gain by 3.7% and FCR by 2.6% compared to the control. An enormous amount of literature is available on the positive effect of phytase supplementation on bird performance (Ravindran *et al.*, 1996; van der Klis *et al.*, 1996). The positive effect of phytase on monogastric performance is due to its ability to degrade phytic acid complex in feedstuffs, thereby eliminating its adverse effects on nutrient utilisation, and, in particular, making phytate phosphorus available to the animal. The potential benefits of using phytase in rice bran diets are not limited to increasing nutrient availability. It can also reduce P output via excreta into the environment.

Supplementation of the diet with lipase elicited little or no effect. Martin (1995) failed to obtain a positive response when he added lipase to a diet containing 40% rice bran. Pluske *et al.* (1997) reported that the AME of rice bran was increased by 0.3 MJ from 12.2 to 12.5 MJ/kg in adult cockerels force-fed 40 g rice bran with or without a lipase. Although the data on use of lipase in monogastric diets are not extensive, it appears that effect of lipase on nutrient digestibilities in poultry is minimal.

It may be concluded that : (a) the release of free sugars from rice bran *in vitro* by the current glycanases on the market is negligible; (b) supplementation of diets containing high levels of rice bran with xylanase can increase the AME of the diet. Especially, application of microwave technology in combination with highly efficacious enzymes may hold promise in utilising NSP as energy sources for monogastric animals in the future; (c) phytate is a factor limiting the nutritive value of rice bran and use of phytase is an effective solution to this problem; and (d) lipase supplementation does not appear to be a cost-effective measure in increasing the nutritive value of rice bran.

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