EFFECTS OF RICE BRAN AND PHYTASE SUPPLEMENTATION ON EGG LAYING PERFORMANCE AND EGG QUALITY OF LAYING HENS

By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

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Chairman: Professor Abdul Razak Alimon, PhD

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Rice bran (RB) is a major by-product from the rice milling process. It contains high amounts of phytate and non-starch polysaccharides, which are considered to be two major antinutritional factors that limit the use of RB in poultry diets. This laying trial was conducted in which 150 Lohmann Brown laying hens were fed diets based on maize or rice bran to evaluate the effects of RB inclusion level and commercial microbial phytase Natuphos®5000 on their performance and egg quality. Five replicate groups of 18 week-old hens were assigned to six dietary treatments comprised of a control maize-soybean meal (MS) diet (Diet 1) and three diets, Diets 2, 3 and 4 with three levels of RB, 15, 25 and 35% respectively. In addition, Diet 4 was also supplemented with 1050 (Diet 5) and 1400 I.U/kg diet (Diet 6) of phytase enzyme (Natuphos®5000, BASF). Body weight (BW), feed consumption, feed conversion ratio (FCR), egg production and egg characteristics which includes egg weight, shell thickness, albumen height and yolk colour were measured in this study. At the end of the experiment (week 53), all five birds in

each replicate were slaughtered and ileal content was harvested for amino acid (AA) and mineral analysis. In this study, using up to 35% RB in the diets was not detrimental to laying hens. Although feed consumption of the hens was similar among the treatments, supplemental of phytase significantly increased overall egg production. Phytase also improved shell thickness, body weight, feed conversion ratio and egg weight, but statistically it is non significant.

Results of this study showed that percentage phosphorus in the excreta decreased and phosphorus (P) retention increased in birds fed 35% RB diets supplemented with phytase. From the improvement of P availability for birds, supplemental phytase enzyme reduces the need for conventional phosphate supplements and alleviates P pollution of the environment.

In conclusion the utilization of RB at a dietary level up to 35% is beneficial to the laying hens industry in Malaysia. In addition, since the price of RB was cheaper than other major ingredients, dietary inclusion of 35% full-fatted rice bran (FFRB) lowered feed cost per egg produce by laying hens.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KESAN DEDAK PADI DAN PENAMBAHAN PHYTASE TERHADAP PENGELUARAN DAN KUALITI TELUR AYAM PENELUR.

Oleh

RADIM ANAK DADANG

Februari 2006

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Dedak padi merupakan hasil sampingan utama daripada industri perkilangan beras. Ia mempunyai kandungan phytat dan polisakarid bukan kanji yang dianggap sebagai anti-nutrisi dan menjadi penghalang utama penggunaanya sebagai rangsum poultry. Kajian telah dibuat menggunakan 150 ekor ayam penelur Lohmann Brown dan diberi makan rangsum dedak padi-jagung untuk melihat kesan penggantian jagung menggunakan dedak padi serta penambahan phytase (Natuphos®5000) terhadap pengeluaran dan kualiti telur. Enam kumpulan replikat yang mengandungi 25 ekor ayam penelur berumur 18 minggu telah diberi enam rangsum dengan rangsum kawalan menggunakan jagung-hampas kacang soya (61% jagung). Tiga rangsum lagi (Diet 1, Diet 2, Diet 3) mengandungi peratusan dedak padi masing-masing 15%, 25% dan 35%. Rangsun keempat, Diet 4 telah ditambah dengan enzim phytase pada kadar berbeza iaitu 1050 I.U dan 1400 I.U dan menjadikannya rangsum kelima dan keenam (Diet 5 dan Diet 6).

Dalam kajian ini, berat badan, kadar pengambilan makanan, kadar tukaran makanan, kadar pengeluaran telur dan ciri-ciri telur seperti berat telur, tebal cengkerang telur,

ketinggian albumen, warna kuning telur dan Haugh unit telah diukur. Di akhir kajian, beberapa ekor ayam daripada Diet 1, Diet 4, Diet 5 dan Diet 6 telah dikorbankan dan kandungan usus telah diambil untuk kajian asid amino dan mineral.

Kajian ini menunjukkan penggunaan 35% dedak padi dalam rangsum tidak menyebabkan kemudaratan kepada ayam penelur. Walaupun kadar pengambilan makanan hampir sama bagi semua kumpulan, penambahan enzim phytase mempengaruhi kadar pengeluaran telur dengan tidak bererti.

Keputusan kajian juga menunjukkan peratusan fosforus dalam tinja telah berkurangan daripada ayam penelur yang diberi rangsum 35% dedak padi bila ditambah dengan enzim phytase. Didapati penambahan phytase dapat mengekalkan fosforus dalam ayam menjadi lebih baik dan dengan itu dapat mengurangkan penambahan fosforus secara konvensional dalam rangsum seterusnya kadar pencemaran fosforus kepada alam sekitar.

Daripada kajian ini dapat disimpulkan bahawa penggunaan dedak padi hingga 35% dapat memberi faedah kepada industri ayam penelur di Malaysia. Tambahan pula harga dedak padi yang lebih murah berbanding bahan makanan utama akan dapat mengurangkan kos pengeluaran telur ayam.

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I certify that an Examination Committee has met on 22nd February 2006 to conduct the final examination of Radim Anak Dadang on her Master of Science thesis entitled "Effects of Rice Bran and Phytase Supplementation on Egg Laying Performance and Egg Quality of Laying Hens" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or currently submitted for any other degree at UPM or other institutions.

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ABBREVIATIONS

AA	Amino Acid
AAS	Atomic Acid Spectrometer
ADF	Acid Detergent Fibre
AID	Apparent Ileal Digestibility
AH	Albumin Height
ME	Metabolisable Energy
BDG	Brewer's Dried Grain
BW	Body Weight
Ca	Calcium
CF	Crude Fibre
CP	Crude Protein
Cu	Copper
DM	Dry Matter
d	Day
EDTA	Ethylene di-amine tetra acetate
EE	Ether Extract
FCR	Feed Conversion ratio
Fe	Iron
FFRB	Full Fatted Rice Bran
DFRB	Defatted Rice Bran
FTU	Unit of Phytase
g	gram
GIT	Gastrointestinal Tract
HPLC	High Pressure Liquid Chromatography
I	Iodine
iP	Inorganic Phosphorus
I.U	International Unit
kCal	Kilo Calorie
kg	kilogram
Mg	Magnesium
MS	Maize-soya meal

ml	mililitre
NDF	Neutral Detergent Fibre
NRC	National Research Council
P	Phosphorus
pP	Phytate Phosphorus
NPP	Non-phytate Phosphorus
NSPs	Non-starch Polysaccharides
PKM	Palm Kernel Meal
pP	Phytate Phosphorus
RB	Rice Bran
Sd	Standard Deviation
SDW	Sample Dry Weight
SE	Standard Error
SEM	Standard Error of Mean
Se	Selenium
wt	Weight
Zn	Zinc
ul	Micro Litre
K	Kalium
µm	Micro mol
mm	Millimeter
Mn	Manganese

CHAPTER I

INTRODUCTION

The poultry industry is singularly the most important livestock industry in Malaysia. In the past two decades, production of poultry meat and eggs has increased substantially and over a short period, has reached a stage of self-sufficiency with the capacity for further expansion. Increase in human population and better family income lead to increase demand of quality food particularly protein source. Efficient use of resources has been attributed to better farm productivity, profitable and assured viability of the investment. The feed and animal production industry is faced with a number of challenges, not the least of which are the pressures to produce high quality products to satisfy customer needs in a cost effective manner.

The major constraint to the development of the poultry industry is the increasing cost of the feed ingredients. There is a heavy reliance on imported raw materials for feed production. Development of feed based on other alternatives excluding maize is still experimental. Imported and local ingredients are used. The imported ingredients range from cereal grains and animal proteins such as soybean meal, corn gluten meal, fish meal and various micro-ingredients such as vitamins, mineral and other additives used to improve feed efficiency and growth. Maize is the major ingredient imported.

Locally available raw materials form about 30-40% of the feed ingredients. The major local materials used are rice bran, wheat pollard, copra cake, palm kernel cake, sago, tapioca and broken rice.

Feed ingredients account for about 90% of the cost price of compound feed, and the industry's ability to produce reasonably priced quality feeds depend on the sources used. As practiced elsewhere, the composition of a compound feed is determined by four main criteria; the price and availability of ingredients, the nutritional value, specific requirements of livestock in question and specific national rules and regulations.

In Malaysia, compound feed is usually prepared by using least-cost formulation techniques commonly used elsewhere. The major concern today is the reduction of imports and to find alternative sources of feed ingredients which pose various constraints.

An important criterion for any feed ingredients is its availability and continuous supply, which are essential to maintain a constant formula. However, often the availability of locally available feed ingredients is not quite dependable. Another important criterion is quality i.e the nutrient quality as determined by analysis. Analysis value for locally available feed ingredients are often out of date or not available and feedmillers have to depend on approximate values in their usage. There are also problems with fluctuation in nutrient quality and adulteration.

When one feed ingredient is replaced with another, there is always a replacement cost to make it equivalent to the ingredients that is substituted. For example, when local rice bran replaces maize, supplementation of methionine, lysine and xanthophyll pigments are needed to maintain the quality or growth of chicken and egg yolk colour. Furthermore, anti-nutritional factors in rice bran and the availability of rice bran locally have to be considered. There is often considerable fluctuation in local feed ingredient prices as most suppliers follow international prices leading to high cost in formulation.

The potential of rice bran as an energy source cannot be exploited due to presence of certain anti-nutritional factors like trypsin inhibitors, crude fibre and heme agglutinin. These antinutritional factors, however, can be removed by different processes such as cooking or treating with weak acid or alkali.

Problems such as high moisture content, mould growth, and rancidity are often associated with the keeping quality of local feed ingredients. This resulted in these ingredients have to be purchased in smaller volumes for which the suppliers often quote high prices and additional anti-oxidants and preservatives have to be added incurring extra costs.

Statements of the Problem

From the above discussion it appears that the optimal inclusion rate and utilization of rice bran in laying hens need further investigation. To what extent the pP of corn-rice bran-soybean meal based could be utilized is still unknown. It is also

imperative to determine the level of substitution of costly imported corn by cheaper rice bran in layer chicken diets.

Objectives of the Study

Since there is limited information on the effects of rice bran on the egg production of laying hens as well as the effect of supplementation of phytase on egg production of laying hens, this experiment was carried out with the following objectives;

1. To determine the effects of different levels of rice bran on production performance and egg quality and composition of laying hens.
2. To evaluate the effects of phytase supplementation on minerals availability in the rice bran based diets.
3. To determine the effects of phytase on digestibility of calcium, phosphorus and other nutrients.

CHAPTER II

LITERATURE REVIEW

Introduction

Feed constituted the major cost of poultry meat and egg production, usually 65-70%, all over the world (Ehtesham and Chowdhury, 2002). Commercial layer feeds typically contain in excess of 60% corn, and the inclusion of RB in these diets may has great economic potential. The poultry producers are always interested for high production but with minimum expenditure of nutrients to economize their feeding practices. Feed ingredients for poultry, especially the energy sources are scarce in Malaysia and the poultry industry mainly depends on the imported feed ingredients, only a small amount is locally produced (Alimon, 1993).

The commercial poultry producers are using compounded feed mixture, in which corn is used as the main source of energy. Addition of any cheaper alternative source of energy to the compounded feed could reduce the price of the feed as well as the production cost (Ravindran and Sivakenesan, 1996). For this reason, nutritionists are looking for new high nutritive value, protein and energy and less costing feed resources such as rice bran. This new feeding alternatives can be successfully used for egg production, due to their high protein content, vitamin, minerals and energy (Popeescu and Ciurascu, 2003).

Approximately two-thirds of the phosphorus (P) in plants is present as phytic acid in the form of myo-inositol hexaphosphates (Cromwell, 1980). Phytate phosphorus (pP) has low P availability, which leads to the use of inorganic P source to meet the P requirement of most monogastric animals such as poultry and pigs (Singh and Krikorian, 1982).

There are many factors that influence the required non-phytate P (NPP) level and utilization by the bird such as dietary calcium (Ca), inorganic P (iP), vitamin D_3, age and type of birds, dietary ingredients and feed processing (Sebastian et al., 1998). Mohammed et al., (1991) demonstrated that both dietary Ca and P concentrations influenced the pP utilization by poultry.

The breakthrough in feed enzyme production has provided a quantum leap in animal nutrition. Feed enzymes enhance digestibility/availability of dietary nutrients and subsequent animal performance to sustain livestock production. Furthermore, feed enzymes also provide a very important tool for managing nutrient excretion to make the environment healthier.

Feed Industry in Malaysia

Feeds play a very important and critical role in the livestock industry. Adequate and proper nutrition is essential for increasing productivity thereby ensuring success of the industry. The Government has contributed greatly towards this by allowing free access to raw materials import as well as the introduction of incentive for import by lowering or abolishing duties. This has assured the

unimpeded growth of the feed industry which in turn benefits the livestock industry as a whole (Department of Veterinary Services, 1994).

Malaysia imports most of its feed ingredients and since feeds constitute about 70% of the total production costs for poultry meat and eggs production, there is thus little margin left for other costs such as labour, overheads and profits. The over-dependence on imported feed ingredients greatly affect the competitiveness of the local industry compares to other countries (especially Thailand) and other major poultry exporters like USA and Brazil (Rashid, 2003).

Feed industry in Malaysia with a total of 43 feedmills produced 42 million metric tones of feed (Raghavan, 2003/04). However, raw ingredients for animal feeds are not produced in the country. As such the intensive livestock industry, particularly pig and poultry are dependent on imported feedstuffs. The imported ingredients ranged from cereal grains, vegetable and animal proteins such as soybean meal, corn gluten meal, fish meal and meat and bone meal, mineral sources and various micro-ingredients: vitamins, minerals and other additives used to improve feed efficiency and growth. In contrast, the ruminant industry is not able to sustain on imported feedstuffs. It mainly depends on locally available feedstuffs. The major materials used are crop residue and other agro-industrial by-products such as rice bran, copra cake, palm kernel cake, oil palm frond, sago, tapioca and broken rice (Loh, 2001).

Most of compound feeds for non-ruminant (poultry and pigs) are based on maize mixed with other ingredients to provide the necessary amino acid, vitamins and

minerals including many other additives. Most of the ingredients used in the ration are imported although to some extent locally produced ingredients are also included. However, the usage of locally produced ingredient depends on supply, cost and also quality. The local produced ingredients are tapioca and fishmeal. However, the amount produced is not sufficient to meet the requirement of the local feed industry. The by-products of oil extraction and milling factories that produced rice bran, soybean meal, wheat bran and pollard always available and usually included in pigs and poultry feeds (Loh, 2001).

Rice Industry in Malaysia

In the global scenario, paddy production and rice consumption for the period 1996-2000 have been stable at 580 and 380 million tonnes per annum respectively. The area under this crop has also been maintained at about 151 million hectares. Because of its political, economic and social significance in the eight agricultural countries of the Association of Southeast Asian Nations (ASEAN), rice remains the most important crop grown in South East Asia (Mutert and Fairhurst, 2002). China and India are the major paddy producers, with their combined production accounting for about 50% of the total world paddy produced. Most of the rice produced is consumed in the home countries. However, some 20 to 27 million tonnes are exported annually. The major rice exporting countries are Thailand and Vietnam with their combined export accounting for about 45% of the rice traded in the world. Malaysia imports about 600,000 tonnes of rice every year and this account for about 2.7% of the world import trade (Malaysia Agricultural Directory and Index, 2003).

Rice is Malaysia's only major grown cereal crop. The rice industry is a major source of income in rural areas. The total area under paddy in Malaysia in 1999/2002 season is estimated to be 701,000 hectares. Total paddy production increased from 1.7 million tonnes in 1985 to 2.1 million tonnes in 1995. The demand for rice in both international and domestic markets is expected to increase. In the domestic market, the consumption of rice projected to increase from 1.8 million tonnes in 1995 to 2.3 million tonnes in 2010 due to population increase (Third National Agricultural Policy, 1998-2010).

According the Malaysia Agricultural Directory and Index, (2001/2002), to report of the soil and climate of the country are suitable, indeed, highly suitable, for food crop, very little rice is grown simply because it is more profitable to grow industrial crops like oil palm and rubber instead. However, rice is the staple food for the country, and the government has termed it a strategic crop with production to continue even though it is more profitable to grow oil palm. Thus, a guaranteed price, above the world market price, is offered for the crop and imports restricted. A self-sufficiency ratio of 65% has been set and to achieve this, ‘granary’ areas have been developed to concentrate on paddy growing.

Malaysia Agricultural Directory and Index, (2003) stated that the total area under paddy in Malaysia has not changed very much during the last five years. During the 1997/98 season, there were about 674,000 hectares, increasing to 699,000 hectares in the 1999/2001 seasons and then to 701,000 in the 2001/02 season. Most of the crop grown in Peninsular Malaysia is wetland paddy and it covers about 520,000 hectares. Kedah, Kelantan and Perak are the major paddy growing states

in Peninsular Malaysia and more than 50% of the crop is planted during the main season. Production of the dry land paddy is, however, carried out mainly in Sarawak and Sabah.

As mentioned in the Malaysian Third National Agricultural Policy (1998-2010), over the 1985-1995 period, the country recorded an increase in average yield and total paddy production. National yield recorded an increase from 2.7 tonnes per hectare to 3.2 tonnes per hectares during the period. Peninsular Malaysia recorded an average yield of 3.7 tonnes per hectares in 1995, while Sarawak and Sabah average 1.2 and 2.7 tonnes per hectares respectively.

Laying Hens Diet

In layer diets, various ranges of dietary energy levels can be used without affecting egg production. Numerous researches have been extensively studied a wide range of metabolizable energy (ME) levels ranged from 2400 to 3200 kcal (e.g Combs and Helbacka, 1960; Peterson et al., 1960; Touchburn and Naber, 1962; Morris, 1968; De Groote, 1972; Damron and Harms, 1976; Lillie et al., 1976; Jensen, 1977; Elwinger, 1981, 1982; Piliang et al., 1982; Mannion and McCloud, 1984). However, if the energy levels are too low, performance can be affected (Combs and Helbacka, 1960; Peterson et al., 1960; Kondra et al., 1974; Gleaves et al., 1977; Moran and Evans, 1977; Piliang et al., 1982; Cherry et al., 1983). Even though layers are able to adjust their energy intake over a large range of dietary energy levels, they tend to over consume calories in diets containing higher

concentrations of metabolizable energy (Morris, 1968) leading to increased body weight and greater maintenance requirement (Vohra et al., 1975).

Stilborn and Waldroup (1990) used rice bran (RB), alfalfa meal, wheat bran and maize gluten feed in layer diets containing 2500, 2600, 2700 and 2800 kcal metabolizable energy kg^{-1} (10.46, 10.88, 11.30, and 11.72 M.I kg^{-1}) and compared with diets based on maize and soybean meal. They found that the layer performance was influenced more by the source of low-energy feed than by dietary energy levels per se. They also found that the hen-day production, egg weight, feed intake, feed per dozen eggs and weight gains for alfalfa meal, RB or wheat bran, diets were similar to that for MS control with equivalent energy content. Egg production was similar for hens fed on a low ME level of 2601 kcal kg^{-1} with these feedstuffs and hens given higher energy diets. Increasing corn gluten feed levels significantly reduced layer performance. They concluded that the layer performance on the lower metabolizable energy, ME (kg^{-1}) diets is highly dependent on the type of feed used.

Cereal By-products

Choct, (1997) reported that cereal by-products, such as rice bran, are important feed ingredients in Asia, but their efficient use in monogastric diets is hindered by the presence of nonstarch polysaccharides (NSPs) and phytate. The general proximate analysis of some byproducts and non-traditional feedstuffs are shown in Table 2.1.

Table 2.1: Average crude protein (CP), lysine (Ly), digestible energy (DE), fat, NSC, starch (S), fiber (F), calcium (Ca) and phosphorus (P) content of some byproducts and non-traditional feedstuffs (As feed basis).

Feedstuff	CP %	Ly %	ME (kcal/kg)	Fat %	*NSC %	S %	F %	Ca %	P %
Traditional (for comparison)									
Corn	9	0.3	1.5	3.6	70	60	2	0.05	0.27
Oats	12	0.4	1.3	5.0	50	30	11	0.08	0.34
Alfalfa Hay	18	0.8	1.0	2.0	15	3	20	1.30	0.2
Soybean Meal	44	2.8	1.4	1.4	30	7	6	0.03	0.6
Almond Hulls	5	0.1	1.1	3.6	31	3	13	0.2	0.1
Beet Pulp Dry	9	0.5	1.1	0.6	31	4	15	0.2	0.1
Brewers Grain	-	0.8	1.3	9	14	10	15	0.2	0.6
Canola Meal	22	2.5	1.2	4.1	18	9	10	0.6	1.0
Corn Gluten Feed	36	0.6	1.3	3.2	27	23	8	0.2	0.7
Cottonseeds, Whole	21	1.4	1.4	18.0	5	-	23	0.1	0.6
Cottonseed, Hull	24	-	0.6	-	3	-	40	0.1	0.1
Distillers Grain	28	0.8	1.5	12.0	19	16	9	0.1	0.7
Hominy Feed	10	0.4	1.5	5.5	54	22	6	-	0.6
Rice Bran	13	-	1.4	8.0	34	22	12	-	1.6
Soybean Hulls	11	0.5	1.1	2.3	15	5	32	0.5	0.1
Sunflower Meal (with hulls)	25	0.9	0.8	1.0	18	-	22	0.3	0.9
Sunflower Hulls	5	-	0.8	3.9	3	-	44	0.3	0.1
Wheat Mids	15	0.8	1.4	3.6	41	34	7	0.1	0.8

*NSC is non-structure carbohydrate when primary contains starches, sugar and pectin
Source: David Freeman, Oklahoma Cooperative Extension Service.

According to Guenter, (1997) about two thirds of the P in plant ingredients for poultry and pigs is in the form of salts of phytic acid (myoinositol hexakisphosphates, phytates), which are not very soluble and of very limited digestibility.

The level of pP in feedstuffs generally depends on the part of the plant from which it is derived. Ravindran et al., (1995a) reported that oilseed meals and cereal by-products contain large amounts of pP, whereas cereals and grain legumes contain moderate amounts. Phytate phosphorus content of various feedstuffs are shown in Table 2.2.

Table 2.2: Phytate phosphorus content of various feed ingredients.

	Phytate-P (g/100 g DM)	Phytate-P (as % of total)
Cereal:		
Corn	0.24	72
Wheat	0.27	69
Barley	0.27	64
Sorghum	0.24	66
Rice(unpolished)	0.27	77
Grain legumes:		
Field peas	0.24	50
Oilseed meals:		
Soybean meal	0.39	60
Rape seed meal	0.70	59
Sunflower meal	0.89	77

Source: Adopted from Ravindran et al., (1995a).
Note: DM, dry matter.

Rice Bran

Bran is a byproduct resulting from the processing of rice by removing the outer layers from brown rice to whiten the kernel. It consists mostly of pericarp, tegument, aelurone, whole germ and crushed germ and starchy endosperm, in the form of dust and smalls fragments; it includes varying amounts of scarps of husk and impurities. When the term is used to distinguish “bran” from “white bran”, it refers to the byproducts obtained in the first stages of whitening. In practice, the demand for white bran is usually much lower than the actual production and in many countries it is not even marketed. As a result, white bran and bran are usually mixed in the proportion in which they were produced, being offered commercially as a single byproducts under the general term “bran”. Even though the process, which consists in removing certain outer layers of the caryopsis, is basically the same in all cases, it can be carried out in a number of ways, some of which have a vital bearing on the characteristics of the end products. The bran thus produced may have quite different properties; therefore their articular identity should be differentiated and maintained (United Nation Industrial Development Organisation, 1985).

In the processing of whole rice (Figure 2.1), the first step is removal of husks. Once the husks have been removed, the rice becomes ‘brown rice’. The second step is removal of bran which yields white rice. White rice is milled from brown rice as very little brown rice is consumed. Milling removed the outer layer of the rice caryopsis producing white rice, which is almost entirely endosperm. White

rice is usually further process or polished and the residue is named as rice polishings (Daghir, 1995).

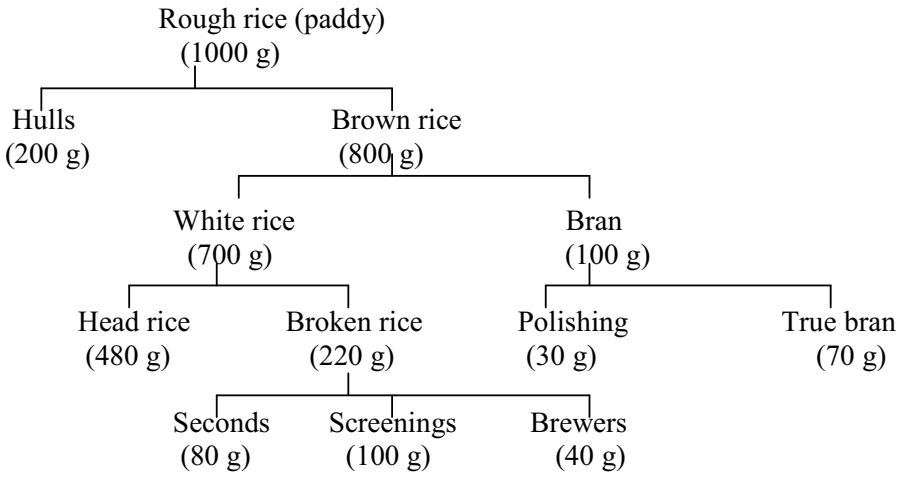

Figure 2.1: The different fraction of paddy as a result of milling (After Warren, 1985).

Basically there are two rice by-products used in feeding poultry. These are rice bran and polishings. Rice bran is recognized variable in its composition depending on its severity with which the rice is threshed and the extent to which the oil is extracted (Daghir, 1995).

Feltwell and Fox, (1978) reported that the feeding value of rice polishings depend upon the degree of polishing to which the grain has been subjected. It is unlikely to contain much of the rice flour. Panda, (1970) gave a figure of 11-12% crude protein and 12-13% oil for rice polishing produced in India, which is closed to

Scott et al., (1982), who list this products as having 12% crude protein and 12% oil.

In Australia, the by-products of white rice milling are referred to as rice pollard and include the true bran and polishing. Rice bran is about 10% of brown rice and may contain 20-25% of the total protein, 80% of the oil, more than 70% of the mineral and vitamins and up to 10% of the starch endosperm (Houston, 1972).

On the other hand, Arosemena et al., (1994) reported that RB is a high fat by-products feedstuff where the fat content ranged from 18.24 to 24.25%. The mean fat percentage of 20.48% was much higher than the 15.1% EE reported by NRC (1989), a difference which could be a consequence of the processing technique. The average ash value of 6.78% observed in his study was lower than the value reported by NRC, (1989). The same author also reported that the mean percentage of Ca in RB was much lower than the mean of P percentage (0.08% and 1.72% respectively). However, considerable variation was found in the content of some microminerals including zinc (Zn), manganese (Mn), copper (Cu), ferum (Fe), and selenium (Se) (86ppm, 276ppm, 10ppm, 115ppm and 174ppm respectively). Little information exists in the literature about the content of macrominerals and microminerals in RB. The treatment and handling of the rice such as defatted, parboiled, and nonparboiled rice after or during the milling process greatly altered the chemical composition of the RB produced (Saunders, 1990). The same author reported variability in ether extract (EE) content of RB ranging from 0.5 to 32.0%, and the variability in chemical composition reflected processing differences.

The RB also contains the lipase enzyme, which cause the RB to become rancid in a matter of hours and unfit for human consumption. As a result almost all of the 40-50 million tones of RB produced each year is discarded mainly in the Far East and South-East Asia, and use largely as animal feed. Variation in chemical composition of the rice bran may occur in older rice mills due to adulteration of the rice hulls, which have virtually no anti-nutritional value for poultry and to the nature of the milling process (Farrell and Warren, 1982).

Since the bran has little economic value, a high degree of milling is not practiced in many countries unless the white rice is used to meet special needs, e.g. export market. Frequently as little as 40% of the maximum yield of bran is recovered (Saunders, 1986).

Both rice bran and rice polishings can be used in poultry rations at fairly high levels if they are low in rice hulls and if the high oil level in them can be stabilized by an antioxidant so that much of the energy value will not be lost through oxidative degredation (Daghir, 1995). Cabel and Waldroup, (1989) tested the effects of adding an antioxidant or a metal chelator or both on the nutritive quality of RB stored at high temperature (35-38^{o}C) and high humidity (80-90%). They found that rancidity development in stored RB up to 4 weeks slowed by the addition of the antioxidant.

Since the production of the edible oil from RB can provide a much needed source of energy for people in rice-producing areas, Randall et al., (1985) developed a process to stabilize the bran and prevent enzymatic hydrolysis of its

oil. The stabilization process heats the freshly milled bran in an extrusion cooker 130^{o}C. The hot extruded bran is maintained at near 100^{o}C on an insulated holding belt for 3 minutes prior to cooling in an ambient air stream. The processed rice bran is in the physical form of small, free flouring flakes with a low microbiological load. Lipolytic enzymes are permanently inactivated and the extracted bran residue retains the flakes form thereby reducing dusts and fines. Sayre et al., (1987) conducted feeding trial with broilers to compare this stabilized rice bran with the raw bran. Free fatty acid content in oil from raw rice bran stored at elevated temperature (32^{o}C) reached 81% whereas free fatty acid in stabilized bran oil remained at about 3%. Chicken fed stabilized rice bran made significantly greater gains than those fed raw rice bran diets.

Extraction of the rice bran for its edible oil is practiced in few countries, notably Japan, Korea and India when it can occur shortly after milling (Saunders, 1986) giving a stable defatted bran. Saunders, (1986) concluded that the extrusion cooking process (Randall et al., 1985) was the only viable method of stabilizing the oil in RB, although microwave treatment is worth considering (Xian and Farrell, 1991) if costs can be reduced to economic levels. The extraction of the oil from RB has resulted in the availability of increasing amounts of defatted rice bran (DFRB), which has been used for animal feedstuff. Farrell, (1994) found that the feeding value of DFRB is equal to the value of FFRB in chicken diets when diets are adjusted for ME by adding oil.

Rancidity of Bran

Besides variation in the chemical composition, other major disadvantage associated with RB is that become rancid rapidly due to the breakdown of the lipid fraction that occurs during storage (Farrell, 1994). Oil stability is good in un-milled rice. Once milled there is an immediate and rapid release of free fatty acids, and a further breakdown by the action by the lipoxygenase shown to be present in the rice bran (Shaheen et al., 1975). Storage temperature and humidity of rice important in determinating the rate of hydrolysis of the oil (Farrell, 1994). Shaheen et al., (1975) reported that 60% of the oil was affected at four weeks after milling and another report showed 50% to be affected within 6 weeks (Warren and Farrell, 1990a). Further deterioration of these fatty acids can occur resulting in extreme hydrolytic and oxidative rancidity and poor livestock acceptability, although Hussein and Kratzer, (1982) reported no difference in the ME between fresh and rancid RB measured in adult cockerels. These workers showed considerable depression (>18%) in the growth rate of chicks when given diet containing 60% rancid RB compared to fresh RB.

Hussein and Kratzer, (1982) also found that the free fatty acid content of fat in RB was increased from 13.7% before storage to 42.8% during 3-month storage period. Gunawan and Tangendjaja, (1988) found that chick growth was depressed when diet containing 60% rice bran was stored at 25°C, for 12 days, then no further decline in growth rate was observed to 50 days of storage. However storage reduced ME of the RB by 148 kcal/kg per week.

Cabel and Waldroup, (1989) demonstrated that 250ppm of ethoxyquin was effective in reducing rancidity development in rice bran for up to 4 weeks even when the temperature and humidity were high. Tangendjaja et al., (1981) concluded that oxidation of RB oil was less important than hydrolysis as the cause of the oil instability. There may be due to the natural antioxidants in the bran.

The addition of 250ppm ethoxyquin proved effective in significantly reducing the initial peroxide value and 1000 ppm ethylenediamine tetra-acetic acid (EDTA) significantly lowered 20h active oxygen method value. They therefore concluded that rancidity development in stored rice bran can be slowed by the addition of ethoxyquin or EDTA (Daghir, 1995).

Chemical Composition of Rice Bran

Juliano, (1985) reported that rice bran has antinutritional factors like trypsin inhibitor, haemaglutinin and phytin/phytate phosphorus. Most of the antinutritional factors are protein in nature and heat liable except its pP.

A major of problem with RB for poultry is its variation in chemical composition which may be associated with depressed performance for poultry (Farrell, 1994). Rice bran varies in its composition due to the variation in the milling process and adulteration with hulls. The presence of small amount of large particle size in RB is an indication of good milling standard (Warren and Farrell, 1990a).

Variation in the composition of Malaysian rice bran was reported by Ukil, (1999) where the average dry matter (DM), crude protein (CP), EE and ash contents of the whole bran were 92.58, 12.11, 6.53 and 7.15%, respectively. The CF values were 4.38-8.59%. The average acid detergent fibre (ADF), non detergent fibre (NDF) and lignin values were 6.15, 16.4 and 5.13% respectively. The variation of the Australian RB was reported by Warren and Farrell, (1990a) (Table 2.3), Indian RB by Zombade et al., (1982) and Indonesian RB by Tagendjaja and Lowry, (1985). Houston, (1972) in a review of literature reported that CP values ranged from 98-154g/kg, lipid 98-154g/kg and ash 71-206g/kg. Zombade et al., (1977) reported that the EE and the available carbohydrate are the major nutrients in RB contributing to energy. These two nutrients account for 86.4% of the total ME.

Rice bran also contains a relatively high percentage of non-starch polysaccharides, with arabinose and xylose being predominant (Annison et al., 1995). This may have an adverse effect on the digestion of some dietary components. The main influence of non-starch polysaccharides in poultry diets is it increased viscosity of digesta and excretion of sticky droppings (Iji, 1999). High viscosity in intestinal content causes reduction in enzyme activities and nutrient absorption (Smits and Annison, 1996).

Although the RB may vary considerably in chemical composition and therefore in nutritive value (e.g Tandendjaja and Lowry, 1985; Farrell, 1994; Ukil, 1999) it is probably the most widely used agricultural by-product available.

According to Ukil, (1999) Malaysian RB is reasonably uniform in their chemical composition and considerable research has been conducted on this product to determine its nutritive value with poultry (Hamid and Jalaludin, 1987; Alimon, 1993; Radim, 1997; Ukil, 1999). Rice bran ash is high in phosphorus, potassium, and magnesium. However, the Ca: P ratio is very low. The amount of minerals in Malaysian rice bran is shown in Table 2.4.

Table 2.3: Chemical analysis of Australian rice cultivars, minerals and some indispensable amino acids (%) (Warren and Farrell, 1990d).

	Mean (range)	SEM	n
Dry matter	918 (900-933)	3.1	45
Crude protein	153 (182-141)	5.6	45
Ash	220 (204-223)	4.2	22
Fibre			
Neutral detergent	215 (201-222)	6.0	4
Acid detergent	107 (94-116)	4.5	4
Lignin	39 (29-52)		4
Minerals			
Calcium	0.39 (0.27-0.51)	0.01	21
Phosphorus	17.1 (16.2-18.1)	0.42	10
Magnesium	6.9 (6.1-7.7)	0.55	21
Zinc	49.0 (44.2-53.9)	3.1	21
Iron	42.8 (37.9-48.1)	2.4	12

	Australian			South East Asia		
Amino acid	Pelate	Starbonnet	Calrose	Average (n=10)	Good sample[a] (n=15)	Poor sample[a] (n=15)
Arginine	12.4	15.9	12.3	11.6	11.8	9.4
Isoleucine	5.6	6.6	5.1	5.1	4.3	4.0
Leucine	10.1	11.5	11.7	10.3	10.4	8.5
Lysine	8.5	9.1	8.2	8.2	7.0	5.9
Methionine	2.4	4.3	2.4	2.8	3.0	2.5
Phenylalanine	5.0	5.9	7.6	6.5	6.9	5.6
Threoonine	5.4	6.4	5.3	5.8	5.6	4.6
Tyrosine	5.1	6.0	5.9	4.6	4.9	3.9
Valine	7.6	8.8	11.4	7.7	7.7	6.4

[a] Creswell, (1987)

Table 2.4: Minerals content of Malaysian rice bran. (Adapted from Ukil, (1999).

Minerals	Amount
Calcium (%)	0.038-0.056
Phosphorus (%)	1.26-1.79
Magnesium (%)	-
Manganese (µg/g)	46.76-61.64
Zinc (µg/g)	29.3-36.3
Iron (µg/g)	-
Copper (µg/g)	1.5-4.39

Components Metabolizability of Rice Bran

Comparisons of the metabolizablity of chemical components in rice, defatted and full fat rice bran based on diet containing 40%, were determined in adult cockerels and young broiler chickens aged between 15 to 20 days (Warren and Farrell, 1990b). Only starch and neutral detergent fibre did not differ in their metabilizability due to aged of bird; DM and ME were similar for the sample of DFRB. Ether extract metabolizability was significantly lower for young chickens than the adult birds. Rice bran that came from different rice cultivars caused the wide variations in metabolizable energy values. As a consequence, ME was also lower by 35% and 28% for Carlose and Starbinnet cultivars respectively. These large differences may help to explain the wide variation in the values reported for ME in rice bran; the NRC (1994) gives a value of only 8.79kcal/kg. Normand and Ory, (1984) concluded from in vitro studies that water-soluble hemicellulose in RB reduced lipase activity. Previously Normand et al., (1981) had found hemicellulose

extracted from rice bran. The apparent DM metabolizability was low in adult and young bird because of high fibre content of DFRB.

Amino Acid Contents of Rice Bran

Although RB is considered as a source of energy, it also contains a considerable amount of protein .The profile of some essential amino acids of RB, as shown in Table 2.5 indicates a good balance and compares favorably with other cereal bran, but none is present in high amount. However, the profiles of some AA of RB are better than the other cereal grains (Warren and Farrell, 1990a). Houston et al., (1969) attributed the variable of amino acids contents in RB to the variability in the bran samples and analytical procedures itself.

Table 2.5: Amino acids content of rice bran[1]

Amino acid (%)	Australian rice bran	South East Asian rice bran
Arginine	1.23-1.59	1.16
Isoleucine	0.51-0.66	0.51
Leucine	1.01-1.17	1.03
Lysine	0.82-0.91	0.82
Methionine	0.24-0.43	0.28
Phenylalanine	0.50-0.76	0.65
Threonine	0.53-0.64	0.58
Tyrosine	0.51-0.59	0.46
Valine	0.76-1.14	0.77

[1]Adapted from Farrell, (1994).

Trace Minerals and Vitamins in Rice Bran

Rice bran is also an excellent source of B-vitamins and vitamin E but contains little or no vitamin A, C, and D. The amount of minerals and vitamins in RB is shown in Table 2.6.

Table 2.6: Minerals and vitamins content of rice bran.

Composition	Rice bran
Minerals[1]	
Calcium (%)	0.027-0.051
Phosphorus (%)	1.62-1.81
Magnesium (%)	0.61-0.77
Zinc (µg/g)	44.2-53.9
Iron (µg/g)	37.9-48.1
Vitamins[2]	
Thiamine (µg/g)	10.6
Riboflavin (µg/g)	5.7
Niacin (µg/g)	309.0
Pyridoxine (µg/g)	19.2

[1]Adapted from Warren and Farrell, (1990c)
[2]Adapted from Houston, (1972).

The trace minerals normally added to swine and poultry diets are Zn, Cu, Mn, Fe, Se, and iodine (I). They play very important roles in the growth and maintenance of tissues. However, research conducted concerning some trace minerals has been minimal. Minerals that are generally adequate or only slightly deficient in practical diets have traditionally been overlooked. Requirements for some of these minerals may vary because of the biological availability in practical diets. In the case of Mn,

it has been shown that some ingredients reduce its availability in the inorganic form.

As reported by Scott et al., (1982) rice bran is an excellent plant source of Mn but the availability of Mn is low. Halpin and Baker, (1987) reported the utilization of Mn might be impaired by some factors present in natural feed ingredients, such as phytic acid, fiber, and other mineral elements. In another study, Halpin and Baker, (1986) reported that RB was shown to reduce tissue Mn deposition when dietary Mn was in excess of requirement. Addition of RB to the diet also caused the reduction of Mn deposition in chick tibias, pancreas, and gallbladder (Thompson and Weber, 1981; Halpin and Baker, 1987). However, addition of RB to diets containing a level of Mn below the chick's requirement significantly increased tissue Mn concentrations (Halpin and Baker, 1986).

Availability of Nutrients in Rice Bran

There is disagreement as to the effect of rice bran on nutrient availability, especially of minerals. Kratzer et al., (1974) concluded that 'there is no interference of the RB with trace minerals in the diet'. This is contrary to the result of Warren and Farrell, (1990c) who found that osteoporosis occurred in laying hens on diets with 25% DFRB and adequate concentrations of minerals when shell grit, offered free choice, was omitted for 6 weeks. Further observation confirmed that rice bran increases the excretion of calcium significantly and of phosphorus and magnesium non-significantly when added in incremental amounts (0-50%) to the diets of growing chickens and adults cockerels (Warren and Farrell, 1991).

Deolankar and Singh, (1979) used radiolabel calcium and iron to demonstrate a lower distribution in various organs of both elements on RB compared with maize-based diets. The author concluded that these effects were due to reduced absorption of these elements. Subsequent studies by Singh et al., (1987) confirmed previous observations and concluded that the Ca content of the femur in the chickens was much lower when RB replaced corn in the diets containing 1% Ca.

There are very few studies on the digestibility AA in RB. Nitis, (1973) used excreta to determine their apparent digestibility and reported values that were often similar or slightly lower than in soybean meal. Raharjo and Farrell, (1984) found that the determination of AA digestibility using excreta analysis was unreliable compared with measurements made at the terminal ileum.

Warren and Farrell, (1991) used ileal cannulae in adult cockerels and ileal contents from slaughtered chickens (5 weeks of age) to determine the AA digestibility in RB. In chickens DFRB had lower AA digestibility than the FFRB. They may have associated with cultivar rather than with oil content. In adult cockerels there were differences due to harvest in overall amino acids digestibility between two DFRB tested.

Ravindran et al., (1999b) conducted a study to determine the microbial phytase on the ileal AA digestibilities in the rice polishings using 5 weeks old broilers. He found that the mean digestibility of 15 amino acids with and without phytase was 62.1 and 66.9% respectively. When individual amino acids were considered, the increment in digestibility was relatively higher in threonine and valine.

Rice Bran in Layer Diets

Rice bran, a by-product of rice milling is being used as a feed ingredient in poultry and pig production. Some variation in maximum inclusion of RB in poultry diets has been reported in published experiments. In experiments with chicks, cereal grains have been replaced with RB, and it was found promising in certain substitutions (Dafwag and Shwarmen, 1996; Khalil et al., 1997). Warren and Farrell, (1990b) carried out experiment with broiler chickens using various levels of DFRB. They found that substitution with DFRB at 7- 21% in a basal diet improved growth and FCR of broilers from 3-13 days of age. The authors also reported that feed intake did not decline significantly until the DFRB content of the diet exceeded 20% of diet. They noted when all diets were balanced to be equal in nutrients, growth rate and feed conversion ratio differed from the controls according to inclusion rate.

According to Farrell, (1994), RB can be used up to 20% in chicken diets without or with a small depression in performances. Farrell and Martin, (1998a) carried out experiments with broiler chickens and ducks by using rice bran. The authors reported that there was a significant decline in growth rate and food intake of chicks with increasing rice bran inclusion (0, 20, and 40%). However, these levels of RB did not alter growth rate, feed intake, or FCR ratio of duckling at 3-17 day of age. In diets of 19-35 day old ducks, 30% RB inclusion stimulated growth, while 60% RB inclusion depressed growth but not food intake.

Warren and Farrell, (1990b) carried out experiment with broiler chickens using various levels of DFRB. They found that substitution with DFRB at 7-21% in a basal diet improved growth and FCR of broilers from 3-13 days of age. The authors also reported that feed intake did not decline significantly until the DFRB content of the diet exceeded 20% of diet. They noted when all diets were balanced to be equal in nutrient, growth rate and FCR differed from the controls according to inclusion rate. Ukil et al., (1999) suggested that RB could be used up to 30% in diet for broilers.

In Argentina, Gallinger et al., (2004) conducted feeding trial using three hundred fifty, 1-d-old male broiler chicks to determine the responses of performance and bone mineralization of chicks grown on different concentrations of RB. They found that concentrations of RB in excess of 20% in the diet produced significant reductions of BW. Furthermore, feed conversion and tibia ash were impaired with diets containing more than 10% of rice bran. The authors also suggested that feed conversion and tibia ash were more sensitive than weight gain for detecting antinutritive factors in RB. In addition, they concluded that the adverse effects of RB on weight gain, feed conversion, and mineralization found here suggest that rice bran should be included in broiler diets at levels between 10 and 20% if strategies are not used to decrease the antinutritive activity.

Laying hens can tolerate higher dietary inclusions of FFRB or refer as rice pollard in Australia (Warren and Farrell, 1990a) than broiler chickens and often used in diets for layers in both developed and developing countries. Several investigators have described successful laying hens diets containing 20 to 45% of RB or polish

(Mahadevan et al., 1957; Panda and Gupte, 1965; Cuca and Avila, 1974; Apandi et al., 1974, Lodhi and Ichhponani, 1975. Din et al., (1979b) fed 74.7% RB and observed 43.5% egg production. A diet containing 74.7% autoclaved RB resulted in 64.6% egg production, which was not significantly different from the positive control diet.

An upper limit of 45% RB diets has been confirmed by Majun and Payne, (1977), Din et al., (1979a), Srichai and Balnave, (1981) and Balnave, (1982). However, egg production, shell thickness and yolk colour were adversely affected at 60% RB (Majun and Payne, 1977). On the other hand, Hamid and Jalaludin, (1987) found that egg production was reduced in a linear fashion when hens were given diets containing from 12.5% to 28.5% FFRB. Problems relating to the use of RB in poultry diets have been reported previously. Karunanjeewa and Tham, (1980) reported that the inclusion of 20% FFRB in layer diets in one experiment constantly reduced egg production and feed intake, and increased mortality. However, Srichai and Balnave, (1981) found no reduction in egg production and an increased in egg weight when giving 42% FFRB in one diet to pullets and hens, and did not report increased mortality.

In layers diets, Majun and Payne, (1977), Din et al., (1979a) and Balnave, (1982) found that FFRB bran can be used up to 45%. However, they found that a high level of inclusion (60% 74.7%, respectively) caused an adverse effect in egg production, shell thickness, and yolk colour and they reported that these deleterious effects were overcome by autoclaving the bran. On the other hand,

Lodhi and Ichhponani, (1975) found a reduction in egg size and egg mass when the laying hens fed DFRB 40% but there was no effect on laying performance.

Piliang et al., (1982) found a large improvement in egg production and egg quality when layer were fed on diets with a high proportion of RB and free choice calcium. The authors also observed that chicks hatched from eggs from hens on a diet containing 81.5% RB were zinc-deficient. Singh et al., (1987) showed that femur calcification was markedly reduced when chickens were fed on FFRB-based diets, particularly on diets containing the lowest levels of Ca (1%), relative to femur calcification on maize-based diets.

In a poultry nutrition study, Warren and Farrell, (1990b) found that osteoporosis occurred in laying hens on diets with 25% DFRB with adequate concentrations of minerals for 6 weeks. Piliang et al., (1982) fed pullets with diet contained 81.5% RB. Egg production was significantly lower than in pullets fed a standard breeder diet.

Effect of Rice Bran on Egg Quality

Farrell, (1994) reported that inclusion of RB above 60% in laying hens attributed to shell grit problem whilst Majun and Payne, (1977) reported Shell thickness and yolk colour adversely affected.

Balnave, (1982) stated that egg weight often increases in diet containing rice bran due to its high content of linoleic acid. On the other hand, Pilliang et al., (1982)

reported no significant differents in egg weight or shell deformation between hens fed the RB diets contained 73.8 to 91.0% with three different protein (14.3, 15.2 and 16.1%) and energy (2450, 2650 and 2850 kcal) levels.

Effect of Phytase on Rice Bran

Martin, (1995) demonstrated that supplementing duck diets with microbial phytase allow RB 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 Mn, Cu and Zn was also noted.

Phytase was shown to improve the nutritive value of RB, while tibia ash and growth rate was increased when phytase had been added to duck diet containing 20% RB (Farrell, 1994; Farrell and Martin, 1998b).

Leske and Coon, (1999) performed experiments with layers and broilers hens to determine effect of phytase on pP hydrolysis and total P retention of different feed ingredients, included as the sole source of P. In laying hens without phytase addition, pP hydrolysis of corn, soybean meal, and DFRB were 23.0, 25.7, and 36.1%, respectively. With addition of phytase (300 units/kg diet) each value significantly increased to 52.0, 62.4, and 50.9%, respectively. Total P retention increased from 28.6, 36.8, and 35.9% to 44.7, 53.4, and 43.0%, respectively, with the addition of phytase. In 22- day-old broilers without addition of phytase, pP hydrolysis of corn, soybean meal, wheat, barley and DFRB were 30.8, 34.9, 30.7, 32.2, and 33.2%, respectively. The addition of phytase (600 units/kg diet)

significantly increased each value to 59.0, 72.4, 46.8, 71.3, and 48.0%, respectively. The addition of phytase increased total P retention from 34.8, 27.0, 16.0, 40.3, and 15.5% to 40.9, 58.0, 33.8, 55.5, and 26.5%, respectively.

Tagendjaja et al., (2002) reported that no matter which phytase source was used in RB diets for laying hens, enzyme addition allowed to remove the iP source from the diet with no changes in performance or decrease in eggshell quality.

Phytic Acids

The currently accepted name of phytic acid is myo-inositol 1,2,3,4,5,6- hexakis dihydrogen phosphate or myo-inositol 1,2,3,4,5,6-hexakisphosphate. Phytic acid is an organic phosphate. It is an abundant component of plant seeds and is deposited in protein bodies as a mixed salt of mineral cations, such as K^{+}, Mg^{2+}, Ca^{2+}, Zn^{2+}, and Fe^{3+} (Shi et al., 2003). The phytic acid salts are generally referred to as phytate(s) (Sathe and Reddy, 2002). About 50% to 80% of the total phosphorus in seeds is found in this compound. Phytate in plants is usually chelated with cations, proteins and/or starches and this chelated form is called phytin (Ravindran et al., 1995a). The location of phytin within seeds differs among different plants. As stated by O'Dell and De Boland, (1976), for example, ninety percent of the phytin in corn is found in the germ portion of the kernel, while in wheat and rice most of the phytin is in the aleurone layers of the kernel and the outer bran.

When absorbed phytic acid serves as a major storage form for myo-inositol, P, and mineral cations for use during seedling growth. Myo-inositol phosphates, including phytic acid, play diverse roles in plants as signal transduction molecules, osmoprotectants, and cell wall constituents (Hegeman et al., 2001). They also reported phytic acid, a phosphorylated derivative of myo-inositol, functions as the major storage form of P in plant seeds. Morré et al., (1990), Loewus and Murthy, (2000) and Reddy et al., (1989), reported that Myo-inositol is a precursor to compounds in plants that function not only in P storage, but also in signal transduction, stress protection, hormonal homeostasis, and cell wall biosynthesis. The inositol of the phytic acid also serves as an importance precursor of polysaccharides and ascorbic acid, and another small molecular weight metabolites (Loewus and Kelly, 1963).

Gibbins and Norris, (1963) stated that phytic acid is distributed in several plant parts, however the concentration of phytic acids is primarily in the seeds and root. In maize kernels, nearly 90% of the phytic acid is accumulated in embryo and about 10% in aleurone layers. Maize endosperm contains only trace amount of phytic acid (O'Dell et al., 1972). In rice (Oryza sativa), barley (Hordeum vulgare), and wheat (Triticum aestivum), most of the phytic acid (approximately 90%) is found in the aleurone layers and only about 10% in embryo (Cromwell and Coffey, 1991).

Supplemented with iP to meet the P requirement for animal growth undigested phytic acid is eliminated and is a leading P pollution source (Cromwell and Coffey, 1991). Although phytic acid as an antioxidant is suggested to have potential

functions of reducing lipid peroxidation and some protective effects, phytic acid is considered to be an antinutritional substance in animal feed and human diets because it binds mineral cations and reduces their bioavailability (Zhou and Erdman, 1995).

According to Maddiah et al., (1964), and Vohra et al., (1965), formation of insoluble phytate makes both Ca and P unavailable. Zn, Cu, Co, Mn, Fe and Mg can also be complex, but Zn and Cu have the strongest binding affinity. As O'Dell and de Boland, (1976) and Knuckles et al., (1985) reported phytic acid may have a negative influence on dietary protein and amino acids. It also inhibits proteolytic enzymes such as pepsin and trypsin under gastrointestinal conditions (Singh and Krikorian, 1982).

Chemical Structure of Phytic Acids

The confirmation structures of phytic acid have been derived from X-ray analysis (Blank et al., 1971). This energetically most favourable conformation of phytic acid is shown in Figure 2.2. Costello et al., (1976) suggested that that phytic acid has a strong potential for complexing multivalent cations and positively charged proteins, since it exists as a strongly negatively charged molecule over a wide pH range.

Phytate or myoinositol 1,2,3,4,5,6-hakisphosphate is the major phosphorus (P) storage compound in plant seeds and can account for up to 80% of seeds total P

(Raboy, 1990). According to Raboy, (1997), phytin is degraded by the action of phytase during germination, which provides the growing seedlings with phosphate, mineral cations, and myoinositol. Apart from its storage function, phytate has also been assumed to play an important role in P homeostasis, buffering cellular P levels.

In most seeds phytic acid occurs as Mg phytate in one or all for three forms, either the insoluble Na_2-Mg-phytate and K_2-Mg_5-phytate or the soluble Ca_2-Mg-phytate or phytin O'Dell and De Boland, (1976). Phosphorus in seeds is stored primarily in the form of phytic acid (phytate, myo-inositol hexakisphosphate, $InsP_6$), which is a derivative of inositol. During seed development phytic acid is deposited in spherical inclusions known as globoids or as complexes with seed storage proteins in protein bodies (Prattley and Stanley, 1982; Lott et al., 1995). The stored phytate is hydrolyzed by the activity of phytase enzymes during germination to provide inorganic phosphate and myo-inositol to the growing seedling (see Figure 2.3). Biolgical breakdown of phytic acid is an enzymatic process involving phytase, with the end products being the inorganic phosphate and myoinositol (Gibbins and Norris, 1963).

Antinutritive Effects of Phytin

Phytin, which is present in many livestock feed ingredients (Table 2.7), is considered as both an antinutritional factor as well as a nutrient. Pallauf and Rimbach, (1997) mention that, because of its ability to chelate to cations in the diet, rendering these chelated cations partially or completely unavailable to the animal, it is often considered toxic, or anti-nutritive. According to Pallauf and

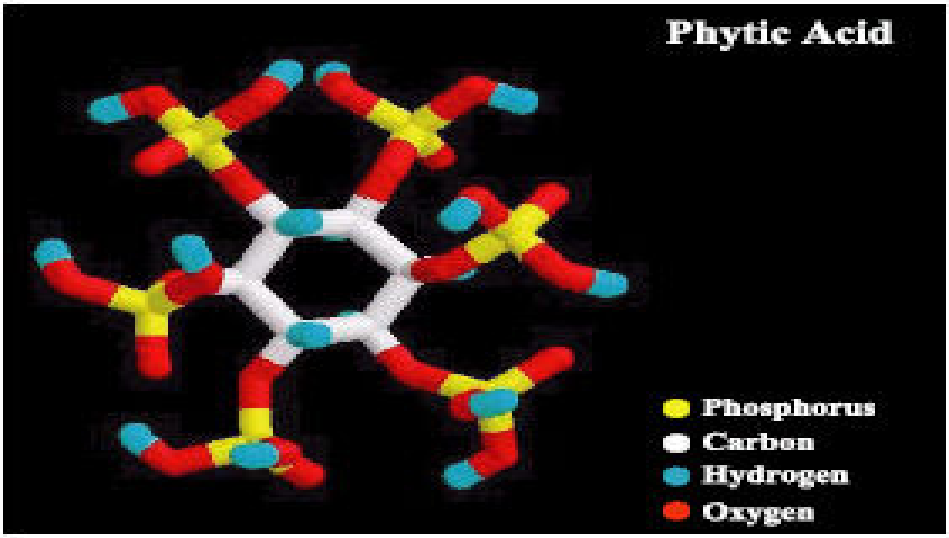

Figure 2.2: Phytic acid, the predominate storage form of phosphorus in mature seeds (figure courtesy of W. Schmidt - USDA/ARS).

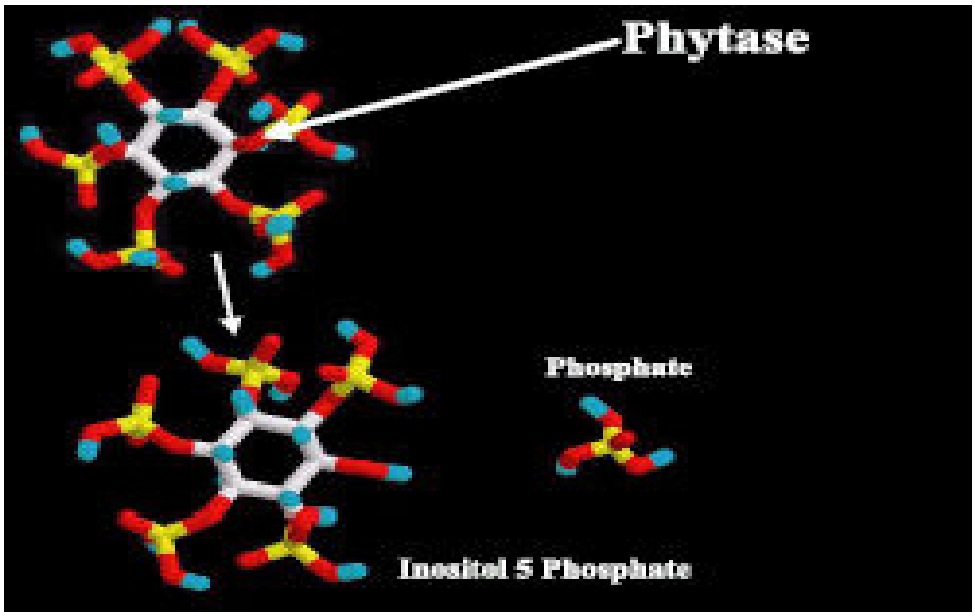

Figure 2.3: Diagram of release of phosphate from phytin by the enzyme, phytase. (Figure courtesy of W. Schmidt - USDA/ARS).

Rimbach, (1996) the strong antinutritive effect of the phytic acid is based on the unusual molecular structure of phytic acid.

Table 2.7: The concentration of phytic acid in common feed ingredients.

Feedstuff	Percentage
Corn	7.44
Sorghum	7.44
Wheat	5.67
Soybean meal	16.67
Canola meal	26.24
Cottonseed meal	32.98
Sunflower meal	27.30
Wheat middlings	27.66
Rice bran	54.96

Data obtained from Ravindran et al., (1999a).

At complete dissociation, the six phoshate groups of phytic acid carry a total of twelve negative charges. Therefore, phytic acid effectively binds different mono-, di-, and trivalent cations and their mixtures, forming insoluble complexes (Reddy et al., 1989). The formation of insoluble phytate-mineral complexes in the intestinal tract prevents mineral absorption. Furthermore, at all pH values normally encountered in feeds, phytin carries a strong negative charge and is capable of binding di- and trivalent cations such as Ca, cobalt, Cu, Fe, Mg, Mn, nickel and Zn in very stable complexes (Pallauf and Rimbach, 1997). This reduces the bioavailability of essential minerals to the animal (Davies, 1982).

On the other hand, phytin is recognized as a nutrient because it contains P, (Reddy et al., 1982) and is the main natural source of P in animal feeds of plant origin. According to Ravindran et al., (1995a) the P content of phytic acid is 28.2%, and pP accounts for approximately 50-80% of the total P in plant feedstuffs.

Despite the plant feedstuffs content adequate levels of total P to meet the dietary requirements of most animals, the pP in general, is poorly available to monogastric animals, including poultry, and this availability varies both within and between ingredients. Although most evidence indicates that monogastric species are capable of utilizing at least a portion of the dietary pP, however, there is wide variation in the reported ability of various monogastric animals, including rats, humans, pigs and chickens to utilize pP. Before pP can be absorbed it must be hydrolyzed from the phytin molecule. The hydrolysis of pP by monogastric animals is a complex process that is influenced by many factors including, but not limited to, age, presence of endogenous or exogenous phytases, level of dietary Ca, Ca to total P ratio (Ca: tP), level and type of vitamin D and others. The effect of high levels of Ca on intestinal pH may be partly responsible for some of the deleterious effects that high dietary Ca levels have on pP hydrolysis.

Studies on broiler hens by Nelson, (1976) indicated that the birds were found to inefficiently use pP and they hypothesized that this was due to a minimal quantity of intestinal phytase. More recent studies with chickens indicate that pP utilization is variable and that dietary factors including the level of Ca, non-phytin P, total P and vitamin D, as well as feed processing and feed or ingredient particle size, may influence pP hydrolysis in the gastrointestinal tract. The pP utilization by chickens is reported to be as low as 0% to 15% (Nelson, 1976; Scheideler and Sell, 1987; Carlos and Edwards, 1998) and as high as 70% to 75% (Mohammed et al., 1991; Mitchell and Edwards, 1996).

Studies with rats have shown that both age and dietary factors, such as high levels of Ca and P, influence pP hydrolysis in the intestinal tract. Wise and Gilburt, (1982) studied the influence of level of Ca in dietary on pP hydrolysis in rats. The authors confirmed the negative affect of Ca on pP release where rats were shown to utilize only 25% of the pP in a high Ca (1.3%) commercial diet but were capable of digesting 50% of the pP in a low Ca (0.7%) diet. The negative effect of pP and total P level on the release of pP in the gastrointestinal tract (GIT) was confirmed in another study where rats fed diets with marginal available P levels (0.34%) exhibited higher pP availability than rats fed a P-adequate (0.4%) diet (Moore and Veum, 1983). In pigs, Sandberg et al., (1993) found that increasing Ca in the diet led to decreased total GIT pP hydrolysis. In contrast, Ketaren et al., (1993) found only 33% pP utilization by pigs; however, the Ca level of the test diet was twice that of the diet used by Sandberg et al., (1993).

In feeding trial study, Shafey et al., (1991) measured the pH of GIT contents of 12 day old broilers fed diets containing either normal (1.07%) or high (2.53%) Ca, and an available P level of 0.46% for both Ca levels. They found the pH of the GIT contents of birds increased as the digesta moved distally, starting at the proventriculus. Digesta pH in birds fed 1.07% Ca level was 4.89 in the crop, 1.98 in the proventriculus, 3.14 in the gizzard, 5.53 in the duodenum, 6.06 in the jejunum, 6.62 in the ileum. The authors indicated that high dietary Ca concentration (2.53 vs 1.07%) increased the pH in the crop (5.32 vs 4.89) and ileum (7.39 vs 6.62). This increase in pH of the GIT contents causes the pP molecule to be ionized and thus, more readily form complexes with divalent metal cations like Zn, Ca, Mg and Fe (Wise, 1983).

As mention by Maenz et al., (1999), Zn, among mineral cations, has the highest affinity for chelating with pP and is capable of forming a strong complex with phytin. These researchers also reported that the affinity for phytin was different for different cations with Zn having the greatest affinity followed by the ferrous form of Fe, Mn, ferric form of Fe, Ca and Mg. Since the Ca is present in highest concentration relative to other metal ions in the diet, it has the highest practical implications. However, the inhibitory effect of Ca on pP hydrolysis can be prevented by a Ca chelator like ethylene di-amine tetra acetate (EDTA) (Maenz et al., 1999). They found that to prevent the inhibitory effect of Ca on pP hydrolysis a concentration of greater than 5ml of EDTA was required.

Calcium added to broiler and laying hen diets as well as Ca bound in dicalcium-monocalicum and monocalicum-dicalcium phosphates can reduce P utilization and allow passage through bird digestive systems undigested. High Ca levels in a layer diet can contribute to poorer phytate breakdown and P absorption by the hen. In a study by Van der Klis et al., (1996), phytate breakdown decreased from 34% on the basal diet with 30g Ca/kg to only 10% on a diet with 40g Ca/kg.

Sebastian et al., (1997) using male broilers, observed significant interaction of P and Ca level and phytase for apparent ileal digestibility (AID) and CP and most of AA and they highlighted the importance of maintaining an appropriate Ca:P ratio in diets. Zhang et al., (1999) found that addition of phytase at 600 FTU/kg increased AID, compared with no added phytase, when P and Ca levels were normal or subnormal, but decreased AID in low P, normal Ca diets. Also Biehl and Baker, (1997b) noted that supplementation of phytase at 600 and 1200 FTU/kg

improved AA utilization in Leghorn roosters fed diets based on soybean meal but not in those fed diets based on peanut meal. Thus, dietary Ca:P ratios and other factors such as protein sources affect efficacy of phytase on AID of AA.

Birds with a dietary deficiency of vitamin D do not use P well. Edwards, (1983) found that addition of 1,25-dihydroxy vitamin D_3 to the feed reduced broiler phytate P excretion by 35% and improved total P retention by more than 20%. Boling et al., (2000) demonstrated a 50% reduction in faecal P in laying hens consuming a low P (0.10% aP) diet plus phytase (300 U/kg) compared to a normal commercial P level diet (0.45% aP). The authors indicated that the corn-soybean meal diet with added phytase supported optimal egg production from 20 to 70 weeks of age.

In general, the variation in the availability of pP observed in different research trials could be due to difference in experimental design, research methodologies, ingredient compositions of the diets as well as processing of the diets, analytical methods, age, species and breed. According to Nelson et al., (1968), different ingredients have varying amounts of pP. Also plant feedstuffs have different location of pP within the plant (Ravindran et al., 1995a). Some feed ingredients like wheat and rye have endogenous phytase (Nelson, 1967), which might influence the extent of pP availability.

There are conflicting reports on the degree of utilization of pP in poultry (Ravindran et al., 1995a). In dietary ingredients of plant origin, the major portion of the total P is present as pP which is poorly available to the monogastric animals

especially for poultry (Cosgrove, 1980) and poultry can utilize only one third. Available literatures indicate that pP utilization by poultry ranges from 0 to over 50% (Nelson, 1976; Cromwell, 1989; Edwards, 1983; 1993; Mohamed et al., 1991). Phytate P is not absorbed by the bird due to the absence of the enzyme phytase in their intestines (Taylor, 1965, Nelson, 1967, Sohail and Roland, 1999). Inorganic or nonphytate P is therefore added in the feed to meet the demand. Unavailable phytate P is excreted in manure, and may cause manure to contain more P than plant can use. The inability of poultry to utilize pP, necessitates dietary addition of iP which increases feed cost and excretion of P and other minerals, and creates environment pollution (Nahashon et al., 1994, Aoyagi and Baker, 1995; Denbow et al., 1995). Environment consequences of high P level in ground water include destruction of the ecosystem (Aoyagi and Baker, 1995). Since phytic acid is an antinutritive component in plant derived food and feed, and therefore enzymatic hydrolysis of phytic acid is desirable.

Feed Enzyme

Feed enzymes include phytases; carbohydrases (including α-amylase, α-galactosidase, NSP-degrading enzymes); proteases; and lipases. They are largely effective in enhancing the digestibility of pP, carbohydrates (including starch), oligosaccharides and non-starch polysaccharides, proteins and lipids, respectively.

Enzymes are biological catalysts that are able to hydrolyze and thereby neutralize the negative effects produced by certain viscous compound in the cereals (Zhang et al., 2000). Enzymes are able to eliminate the effects of non-nutritive, non-starch,

non-water soluble polysaccharides when added to poultry and pig diet, which results in increase rates of growth and reduced environmental pollution due to the decrease output of manure and gases such as ammonia (Campbell and Bedford, 1992; Chesson, 1993; Bedford, 1995; Marquardt, 1997; Marquardt and Bedford, 1997; Zhang et al., 1997).

In recent years, much attention has been focused on improving nutrient utilization of low quality or inferior feed ingredients with the aim of reducing feed costs (Farrell and Martin, 1998a). Such improvement has been achieved by exogenous enzyme supplementation to the diets of poultry (Scott et al., 1998, Farrell et al., 1993; Yi et al., 1996a). Enzyme supplementation of fibre feedstuffs results in improved performance in a variety of ways. Various workers, (Günal and Yasar, 2004; Bedford, 1997; Taibipour and Kermanshahi, 2004) have reported the beneficial effect of reduced digesta viscosity with enzyme supplementation. Results of many experiments indicated that enzyme supplementation of poultry diets improved the nutritional value of cereal grains and their by-products. Improvements in phytate utilization (Simons et al., 1990; Kornegay et al., 1996) due to added enzymes have been reported.

Phytase

Phytase(s) (myo-inositol hexakisphosphate phosphohydrolase) are phosphates enzymes that catalyze the hydrolysis of myo-inositol hexakisphosphate (phytic acid) to inorganic monophosphate and lower myoinositol phosphates, and in some cases to free myo-inositol. The Enzyme Nomenclature Committee of the

International Union of Biochemistry, (1979) distinguishes two types of phytase: 3-phytase (EC 3.1.3.8) and 6-phytase (EC 3.1.3.26). 3-Phytase is typical for microorganisms and 6-phytase for plants. Phytase is widespread in nature. Phytase activity has been reported in plant and animal tissues and in a variety of microorganisms (Kerovuo, 2000).

The 3-phytase initiates dephosphorylation of phytin at 3 position, yielding 1,2,4,5,6-pentakisphosphate and inorganic phosphate; the 6-phytase initiates the dephosphorylation of phytin at the 6 position, yielding 1,2,3,4,5-pentakisphosphate and Pi. The 3-phytase does not always completely dephosphorylate phytic acid while the 6-phytase does (Wodzinki and Ullah, 1996). It has been stated that the microorganism normally produce the 3-phytase and that the 6-phytase is normally found in plants (Reddy et al., 1982; Nayini and Markakis, 1986).

According to Liu et al., (1998), fungal species are the most widely used microorganisms for the expression of phytases. The phytase produced by *Aspergillus niger* is the most extensively studied and possesses two separate pH optima, one at 2.5 and one at 5.5, with temperature optima of approximately 60°C. Eeckhout and De Paepe, (1994) reported that all of the agronomic species of cereals, legumes, and oilseeds possess some phytase activity, however, only cereals such as barley, wheat, rye, and triticale possess appreciable amounts of phytase activity.

Phytase enzymes have been shown to increase P availability and utilization in hens (Nelson et al., 1971), turkeys (Ravindran et al., 1995b; Qian et al., 1996), and

swine (Simons et al., 1990). Phytase enzymes have been a subject of research focus for a number of scientific groups with the researchers reporting increases P availability and utilization in chickens (Nelson et al., 1971) turkeys (Ravindran et al., 1995b and Qian et al., 1996) and in swine (Simons et al., 1990).

Phytase present in the feed ingredients themselves has been shown to improve phytate phosphorus utilization by monogastric animals (Han et al., 1998). High levels of plant phytase are found in wheat, rye, their hybrid triticale and barley (Viveros et al., 2000), yet there is a large variation in phytase acyivity within the different varieties of these plants (Nys et al., 1996). The pH and temperature that occur during feed processing (Wodzinski and Ullah, 1996) as well as the low pH in the upper portion of the GIT (Phillippy, 1999) may inactivate the enzyme. It has been shown that there is a decrease in unprotected phytase activity when pelleting temperatures exceed 75^{o}C (Pointellart, 1993). Phillippy, (1999) also demonstrated that wheat phytase lost substantial activity when incubated with pepsin, a proteolytic digestive enzyme. The variability and instability of these natural phytases decreases the viable use of plant ingredients as a reliable source of the enzyme in the animal feeds.

Nelson et al., (1971), Ballam et al., (1984) and Nahashon et al., (1994) carried out studies to determine the effect of phytase on increasing plant P availability and reducing P excretion of poultry. The authors have measured growth parameters and performance. Nelson, (1976) and Sooncharernying and Edwards, (1993) in their studies calculated the digestibility or retention of dietary pP with and without phytase addition by measuring pP in the diet and excreta. Other investigators (e.g

Denbow et al., 1995; Yi et al., 1996a) have determined P equivalency values for added phytase based on performance parameters and retention. They found live weight gain was significantly improved by phytase additions, and the retentions of P, Ca and ash in the total carcass were linearly related to the addition of phytase.

Zimmermann et al., (2003) conducted an experiment to determine whether phytases of different origin (cereal phytase originating from wheat and rye or in combination with supplemented microbial phytase, Natuphos) exhibit additivity in their response on coefficient of apparent P absorption, utilizing pigs as the experimental animal. The study concluded that different sources of phytase exhibit linear additivity in their response on apparent P absorption in growing pigs, provided that an existing differences in equivalency values between cereal and microbial phytase.

The addition of microbial phytase to diets for nonruminants has been shown to increase the availability of P (Nelson et al., 1968; Cromwell et al., 1991), as well as other minerals (Biehl et al., 1995; Radcliffe et al., 1995; Simpson and Wise, 1990), protein and amino acids (Johnston, 2000; Johnston et al., 2004), carbohydrates (Johnston et al., 2004), and energy (Rojas and Scott, 1969).

More research has been conducted on the effect of phytase on minerals. Most research has indicated that the addition of phytase increases the digestibility and (or) the availability of Zn in diets for pigs and chicks. Phytase increased Zn digestibility and availability in nursery pigs (Lei et al., 1993; Adeola, 1995; Adeola et al., 1995; Hargrave et al., 2000; Valencia and Chavez, 2002), growing-finishing

pigs (Nasi, 1990; Gebert et al., 1999b; Waltz and Pallauf, 2002; Brady et al., 2003), and chicks (Biehl et al., 1995; Zanini and Sazzad, 1999; Paik et al., 2000; Lim et al., 2001; Lan et al., 2002; Viveros et al., 2002). However, some research has indicated that phytase addition has no effect on Zn digestibility in pigs (Murry, 1994) or chicks (Roberson and Edwards, 1994; Sebastian et al., 1996a; Um et al., 2000) or that it decreases Zn digestibility in growing-finishing pigs (Gebert et al., 1999a).

Research has indicated that adding phytase increased the retention of Cu in nursery pigs (Adeola, 1995; Adeola et al., 1995; Valencia and Chavez, 2002), growing-finishing pigs (Nasi, 1990; Gebert et al., 1999b), and chicks (Sebastian et al., 1996b; Um et al., 2000; Lim et al., 2001; Lan et al., 2002). However, in other studies Cu retention or availability was not affected in growing-finishing pigs (Murry, 1994; Gebert et al., 1999a) or chicks (Sebastian et al., 1996a; Biehl et al., 1997a) or decreased in growing-finishing pigs (Brady et al., 2003) or chicks (Aoyagi and Baker, 1995).

The addition of phytase has been shown to increase the retention of Fe for pigs (Gebert et al., 1999b; Valencia and Chavez, 2002) and chicks (Paik et al., 2000, Um et al., 2000). However, other reports have indicated that phytase has no effect on Fe digestibility or availability in pigs (Nasi 1990; Murry, 1994; Gebert et al., 1999a) or chicks (Sebastian et al., 1996a; Biehl et al., 1997a).

There is less research on the effects of phytase addition on Mn retention in pigs and chicks. Windisch and Kirchgessner, (1996) and Lan et al., (2002) indicated

that phytase addition increased Mn retention in pigs and chicks, respectively. However, Adeola, (1995) and Adeola et al., (1995) reported no effect of phytase addition on the retention of Mn for nursery pigs. Also, Sebastian et al., (1996a) indicated that adding phytase to chick diets had no effect on retention of Mn, and at dietary levels of 1% Ca, phytase addition decreased Mn retention. Brady et al., (2003) reported that phytase addition decreased Mn retention in growing-finishing pigs.

Research has indicated that phytase may have positive effects on energy availability in diets for chicks (Namkung and Leeson, 1999; Ravindran et al., 1999b, 2000, 2001; Camden et al., 2001; Lan et al., 2002; Selle et al., 2003b; Shirley and Edwards, 2003). However, other reports have indicated that phytase has no effect on energy availability (Biehl and Baker, 1997; Ledoux et al., 2001; Murai et al., 2002).

Selle et al., (2003c) found that adding phytase to wheat-sorghum-SBM based diets increased chick AME in one experiment but had no effect in two others. Johnston and Southern (2000) reported that phytase (600 phytase units/kg) provided 45.7 kcal of ME/kg in corn-soybean meal diets for chicks. However, the ME matrix values for Natuphos indicates it provides 31 and 24 kcal/kg for starter and growing-finishing broilers, respectively.

Study in pigs, Johnston et al., (2004) found that phytase addition increased apparent ileal gross energy digestibility in pigs with no effect on total-tract energy digestibility. Similar finding was reported by O'Doherty et al., (1999) who

concluded that adding phytase to finishing pig diets increased energy digestibility. However, other researchers indicated that adding phytase had no effect on energy digestibility in pigs (Murry, 1994; O'Quinn et al., 1997; Gebert et al. 1999b; Waltz and Pallauf, 2002; Sauer et al. 2003).

Phytase in Poultry Diets

The poor digestive utilization of phytin-bound P by monogastric animals and its consequences on diet cost, environment, and digestibility of minerals and proteins have lead to extensive research efforts directed toward understanding the process of phytic acid digestion. Plethora of data available in the literature have been shown that microbial phytase supplementation of plant-based diets consistently improved the utilization of plant phytin-bound P (Nelson et al., 1968. Ravindran et al., 1995, 2000, 2001; Kies et al., 2001).

The use of microbial phytase as a feed additive in poultry diets is becoming a current routine for reducing the excretion of large quantities of P from intensive poultry production operations in manure. Phytase is a cost-effective method known to reduce P excretion without affecting bird health or performance.

Nelson et al., (1968) first utilized phytase in poultry diets by isolating it from a fungus and incubating soybean meal with the crude phytase extracts prior to feeding. They found that chicks utilized the hydrolyzed pP as efficiently as the phosphorus from inorganic source. Recent advances in recombinant DNA

technology have made it possible to synthesize commercial preparations of phytase enzymes to add to poultry diets.

A result of study undertaken by Sooncharenying and Edwards, (1993) showed that inositol 1,2,3,4,5,6-hexakisphosphate (IP6) retention of 3-wk-old broiler chicks on a corn-soybean diet with no phytase supplementation to be as high as 32%. This with the agreement with the values for corn (30.8%) and for soybean meal (34.9%) hydrolysis of IP6 found by Leske and Coon, (1999).

Simons et al., (1990) reported that the performance of broilers fed a diet containing corn, soybean, sorghum, and sunflower seed meal, but no feed grade phosphate, the total P retention found to be 49.8%. The authors also observed that total P retention increased (56.5 and 64.5%, respectively) with the addition of phytase (250 units and 1,500 units/kg of diet, respectively). These results were in line with finding of Qian et al., (1997) who reported ranges of total P retention (50.9 to 68%) of a corn-soybean diet, with linear increases in P retention observed with supplemental phytase and cholecalciferol.

Mohammed et al., (1991) reported that the pP retention by 3- to 4-wk-old broilers of a corn-soybean diet found to be 50.1%, with a total Pretention of 41.5%. Sebastian et al., (1996a) carried out an experiment to determine the effects of supplemental microbial phytase on the performance of broiler chickens. They determined total P retention by 3-wk-old male broilers of a corn-soybean diet to be 51% and retention of total P increased to 63.5% with the addition of phytase. Total P retention of the corn and soybean meal in a study conducted by Leske and Coon,

(1999) was 34.8% and 27.0%, respectively, with no addition of phytase and these values were somewhat lower than the values reported by other researchers for corn-soybean diets.

Broz et al., (1994) found that the total P retention values of a corn-soybean diet with no added inorganic phosphates reported to be 44% as determined with 3-week-old broilers, however, total P retention was increased to 52% with the addition of phytase.

Leske and Coon, (1999) indicated that the feed ingredients utilized in the diet influenced pP hydrolysis and total P retention for broilers, and that the effect of supplementation with phytase was also feed ingredient dependent. They reported that laying hen hydrolysis of IP6 without phytase supplementation for both corn and soybean meal were 23.0% and 25.7% respectively. They also observed that the total P retention values for a corn and soybean meal were 36.8% and 28.6%, respectively. On the other hand, Nelson, (1976), determined laying hen hydrolysis of pP of a diet containing corn and soybean meal to be 8% which was smaller than that reported by Leske and Coon, (1999).

Leske and Coon, (1999) reported no significant differences between laying hens and broilers in P retention of the corn diet. The authors, however observed that laying hen total P retention was found to be significantly greater for soybean meal with no phytase supplementation ($P = 0.0342$), defatted rice bran without phytase supplementation ($P = 0.0001$), and defatted rice bran with phytase supplementation ($P = 0.0001$).

Nelson, (1976) determined laying hen hydrolysis of pP of a diet containing corn and soybean meal to be 8% in laying hens. Edwards, (1983) found breed and strain differences in pP utilization of a corn-soybean diet. Leske and Coon, (1999) also found hydrolysis of IP6 of soybean meal with no phytase supplementation by broilers was significantly greater than that of laying hens ($P = 0.0259$).

Ahmad et al., (2000) reported that additional phytase to a low P diets resulted in improved growth rate, relative retention of calcium and phosphorus, and bone mineralization. The authors also concluded that phytase supplementation to normal P diets increased ($P<0.05$) BW and feed intake as compared to the control.

Study with 5-wk-old broilers fed a variety of cereals, oilseed meals, and cereal byproducts supplementing with 1,200 phytase units/kg of diet by Ravindran et al., (1999b) showed improvement in the ileal digestibility of all amino acids. From the results of this study the authors also revealed significant negative correlations between dietary phytin concentration and CP digestibility and mean AA digestibility of the ingredients evaluated, as well as a significant negative correlation between inherent AA digestibility and phytase response. Ravindran et al., (2000) performed experiment with broiler chicks. In this trial, the researchers observed that the addition of microbial phytase had alleviated the influence of negative effects of dietary phytin on AID of essential amino acids. Furthermore, the digestibility of amino acids in broilers was improved by microbial phytase supplementation of a lysine-deficient diet (Ravindran et al., 2001).

A number of studies have demonstrated a generally positive effects of supplemental phytase on AA digestibility (Yi et al., 1996a; Biehl and Baker, 1997b, Sebastian et al., 1997; Ravindran et al., 1999a, 2000) and AME (Farrell et al., 1993; Farrell and Martin, 1998; Namkung and Leeson, 1999; Selle et al., 1999; Ravindran et al., 2000) (Table 2.8).

In feeding trial, Applegate et al., (2003) fed broiler chickens different levels of total phosphorus with phytase (600 U/kg). They concluded that no differences were found in the water-soluble phosphorus concentrations in the litter collected at 49 day of age. Based on these results the authors therefore suggested that phytase activity should not affect the water-soluble P content in the manure of animals fed phytase-supplemented diets.

Zhang et al., (1999) noted that supplementation of 600 FTU/kg Natuphos phytase had no significant effect ($P>0.05$) on AID of CP and AA in 7 wk-old broiler chicks. They also reported feed conversion was not affected by phytase inclusion approaches. An improvement in utilization of ME was indicated in broilers when phytase was added to sorghum-soybean basal diets (Farrell et al., 1993). Phytase may have a small but significantly positive effect on improving utilization of the essential amino acids in chicks fed soybean meal basal diets (Biehl and Baker, 1997b).

Table 2.8: The effects of microbial phytase on apparent ileal digestibilities (%) amino acids in poultry (Adapted from Adeola and Sands, 2003)

Study/diet	Arginine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine	Tripthopan	Valine
Biehl and Baker (1997a)[a]										
1. Diet	93.4	95.7	96.4	95.1	93.3	78.2	81.2	90.8	-	95.1
2. Diet 1 + phytase	95.9	97.3	97.8	96.6	96.8	79.8	81.6	91.7	-	96.6
Sebastian et al. (1997)[b]										
1. Diet	91.6	85.2	82.5	89.5	90.5	91.5	86.9	79.0	-	85.6
2. Diet 1 + phytase	90.1	82.7	76.4	86.4	86.0	89.5	82.5	75.2	-	81.6
Namkung and Leeson (1999)[c]										
1. Diet	86.2	88.3	78.7	82.2	84.2	91.4	81.0	74.1	78.0	77.7
2. Diet 1 + phytase	82.6	84.3	82.8[e]	84.1	85.9	91.3	83.3	74.7	79.7	81.2[e]
Zhang et al. (1999)[d]										
1. Diet	91.8	89.6	87.4	90.0	88.2	90.4	89.6	81.4	-	86.7
2. Diet 1 + phytase	91.8	89.8	87.9	90.3	88.8	90.4	89.9	82.2	-	87.1

Diet 1 - soybean meal containing 46.5% CP

[a]True digestibility using cecectomized roosters fed 30 g of soybean meal containing 46.5% CP. Phytase was added to Diet 2 at 1,200 units/kg.

[b]Apparent ileal digestibility in chicks during the period from 1 to 21 day posthatching fed a 22.4% CP corn-soybean meal diet containing 0.58% Ca and 0.44% P. Phytase was added to Diet 2 at 600 units/kg.

[c]Apparent ileal digestibility in chicks during the period from 1 to 21 day posthatching fed a 23% CP corn-soybean meal diet containing 0.8% Ca and 0.6% P. Phytase was added to Diet 2 at 1,150 units/kg.

[d]Apparent ileal digestibility in chicks during the period from 1 to 21 day posthatching fed a corn-soybean meal diet containing 0.8% Ca and 0.6% P. Phytase was added to Diet 2 at 600 units/kg.

[e]Phytase effect, $P < 0.05$.

Pourreza and Classen, (2001) used phytase (500 and 1000 FTU) and xylanase (2700 and 5400 EXU) individually and in combination to investigate effects of supplemental phytase and xylanase in a corn-soybean meal based diet containing 25% wheat bran, on broiler (21 days of age) performance and P degradability and nitrogen digestibility. The authors found that added phytase significantly ($P<0.01$) improved FCR (1.59 vs 1.62g/g) and tibia ash (464.4 vs 444.3g/kg). They also found phytate P degradability was significantly ($P<0.02$) improved by added phytase (41.4 vs 27.8%) and the protein digestibility was also increased (81.7 vs 79.4%) significantly ($P<0.01$) by 500 FTU/kg added phytase.

Research has indicated that phytase may have positive effects on energy availability in diets for chicks (Namkung and Leeson, 1999; Ravindran et al., 1999b, 2000, 2001; Camden et al., 2001; Lan et al., 2002; Selle et al., 2003b; Shirley and Edwards, 2003). However, other reports have indicated that phytase has no effect on energy availability (Biehl and Baker, 1997; Ledoux et al., 2001; Murai et al., 2002). Response to enzyme supplementation is depends on the bird's age, which apparently related to both the type of gut microflora present and the physiology of the birds. Older birds, because of the enhanced fermentation capacity of the microflora in their intestine, have greater capacity to deal with negative viscosity effects (Choct et al., 1995).

Ahmed et al., (2004) conducted study with 144 unsexed Van Cobb broiler chicks from 21 days to 42 days of age to investigate effect of phytase supplementation on the birds performance. In this experiment, the chicks were fed on soybean meal (SM) based iso-nitrogenous and iso-energetic diet incorporating phytase with the

levels of 0.0, 0.50, 1.00 and 1.50 g/kg diet for better utilization of the basal diet. The authors found that the growth rate, feed intake, feed consumption, dressing yield and profitability increased as the level of phytase supplementation increased and the level of phytase had no effect on survivability. They also reported that the addition of phytase seemed to be effective to overcome the antinutritive effect of phytate phosphorus and non-starch polysaccharides (NSP) on broiler performance. From this study results, the researchers therefore, concluded that 1.50 g/kg phytase may be incorporated in MS based broiler diet for profitable production.

Iyayi and Davies, (2005) studied the effect of enzyme supplementation of palm kennel meal and brewer's dried grain on the performance of broiler chickens. They have found that phytase supplementation of diets based on brewer's dried grain and palm kernel meal had significantly ($p<0.05$) increased the weight gain and feed intake at the starter phase. At the finisher phase, while feed intake was significantly ($P<0.05$) increased with enzyme supplementation, the weight gain was not significantly affected ($P>0.05$). The authors also found that the FCR also did not significantly ($P>0.05$) change with enzyme supplementation at the starter phase, but at the finisher phase, feed conversion was significantly ($P<0.05$) poorer. Apparent digestibility of CP, crude fat and crude fibre was significantly ($P<0.05$) higher with enzyme supplementation.

Several studies have been carried out with broiler fed on low protein based diets with exogenous phytase. These studies indicated that phytase addition improve birds growth performance (Ahmad et al., 2000; Lan et al., 2002) and also improve

P digestibility for certain plant based feedstuffs and overall utilization of broiler diet (Rutherfurd et al., 2002).

Response of Laying Hens to Phytase Supplementation

The responses of laying hens to enzyme-supplemented feeds are well documented. Typically, enzymes added to layer feed appear to have little effects on egg mass but improve feed efficiency (Benabdeljelil and Arbaoui, 1994), energy utilization (Wyatt and Goodman, 1993), and laying rate (Poultry International, 1996).

Simon and Versteegh, (1992) conducted an experiment with layers by using 11 mash diets from 24 to 52 weeks of age. They found the deficiency symptom on layer performance, eggshell and skeletal quality which become increasingly pronounced during the trial with the basal diet (0.14% aP) were found to be completely compensated even by the lowest mono-calciumphosphate (MCP) dose (0.6g P/kg) or by the lowest phytase supplementation (200 FTU/kg).

In a study with laying hens Van der Klis et al., (1997) tested the efficacy of microbial phytase using a phytase preparation from a genetically modified *Aspergillus niger* fungi strain. The authors reported results of three experiments in which they measured ileal absorption of phosphorus as an indication of phytase efficacy and monitored supplementations impact on production parameters (egg production, laying rate, feed intake, feed conversion and egg weight). Their results indicated that phosphorus absorption increased considerably, from 22% (control diet) to 50% in laying hens by dietary supplementation of microbial phytase. They

also found production performance in phytase supplemented hens comparable to hens receiving iP supplementation from monocalcium phosphate.

Gordon and Roland, (1997) carried out an experiment to evaluate the performance of commercial laying hens fed various P levels, with and without supplemental phytase. They used five levels of NPP (0.1-0.5%) and two levels of phytase (0 and 300 U/kg feed). In their study, the authors observed significant differences in production parameters between supplemented and unsupplemented diets. They reported that when unsupplemented with phytase, the NPP diet at 0.1% decreased egg production by 8.1% over the trial period (21 to 38 weeks), relative to other unsupplemented diets of 0.2-0.5% NPP. They noted that without enzyme supplementation resulted in a 5.8% decrease in feed consumption, lighter body weights, weaker bone strength and bone quality, an elevation in mortality and decrease in egg weight and egg specific gravity in hens fed only 0.1% NPP. However, supplementation of 0.1% NPP with phytase completely corrected these adverse effects with increases in egg weight, egg weight, egg specific gravity, feed consumption and BW and a drop in mortality. Furthermore, the performance of hens consuming 0.1% NPP with phytase performed as well as hens fed diets containing higher levels of NPP without phytase. Gordon and Roland, (1997) also observed that hens fed 0.1% NPP with phytase supplementation excreted 25 % less P than those consuming the same diet without phytase (3.71 and 4.98mg/g, respectively).

Peter, (1992) reported that laying hens fed a low nonphytate P (NPP) diet with phytase has significantly higher ($P<0.05$) egg production, egg weight, and feed

consumption than hens consuming the low NPP diet without supplemental phytase. Phytase also improved nitrogen absorption in laying hens (Van der Klis et al., 1997), and improved nitrogen and amino acids digestibility in broilers (Ravindran et al., 2001). Lim et al., (2002) in his study found that high NPP level and phytase increased egg production in the second 10-week period (31-40 week), agree with those of Scott et al., (1999), who reported increased egg production in hens fed a phytase supplemented diet from 36 to 51 weeks.

A number of research studies with the layers have reported positive responses to enzyme-supplemented feeds. Typically, enzymes added to layer feed appeared to have little effect on egg mass but improved feed efficiency (Benabdeljelil and Arboui, 1994; Vukic Vranjes and Wenk, 1995), energy utilization (Wyatt and Goodman, 1993; Vukic Vranjes and Wenk, 1995), and laying rate (Poultry International, 1996). In laying hens, Wyatt and Goodman, (1993) concluded that corn-fed layers exhibited better feed efficiency than those fed enzyme-supplemented barley-based diets Nevertheless, enzyme supplementation improved the utilization of barley diets.

Jalal and Scheideler, (2001) reported results from experiment with laying hens fed corn soybean meal diets containing low level of NPP (0.10%), and supplemented with phytase. Based on the results of this experiment the authors concluded that supplementation of phytase improved feed intake, FCR, egg mass, and elicited a response in shell quality and egg components. Keshavarz, (2000) conducted a study with laying hens to evaluate the effect of addition of phytase (300 units/kg diet) on the pP retention and total P excretion. The researchers found that phytase

supplementation significantly increased pP retention by about 15% while daily total P excretion was 47% less for 66-week-old hens fed phytase-supplemented diets than for the unsupplemented phytase control group fed 0.30% NPP in the diet.

The result obtained from P digestibility trials and long term feeding studies conducted by BASF (2004) indicated that 300 phytase units is equivalent to an addition of 1.0 gram of P from monocalcium phosphate. They also reported that the efficacy of phytase in layers was higher than in pigs or broiler chickens, and an average of 500-600 phytase units is necessary to replace the same amount of P from monocalcium phosphate.

In a study with broiler chickens Simons et al., (1990) have shown that microbial phytase supplementation of a low P maize-soybean diet increased the availability of P to over 60% and decreased the amount of P in the droppings by 50%.

In broilers, Juanpere et al., (2005) indicated that addition of phytase (500unit/kg) to diets based on corn, wheat, or barely increased apparent ME in corn diets. They also found the phytase increased total P retention in all diets and decreased P excretion in corn and barely diets. Payne et al., (2005) used corn-soybean meal diet to compare phytase sources (Natuphos and Ronozyme) in commercial broilers. The researchers reported that an incremental addition of phytase linearly increased ($P<0.07$) daily gain and feed intake. In addition, they found that broilers fed Natuphos had higher ($P<0.07$) toe ash percentage in experiment 1 and 2, and higher ($P<0.02$) daily gain and feed intake in experiment 2 than those fed

Ronozyme. They also found overall daily gain and feed intake were linearly increased ($P<0.05$) by incremental phytase addition in experiment 3 and both Natuphos and Ronozyme produced similar growth and bone ash traits in commercial broilers.

Microbial phytase has been shown to increase the availability of pP for swine and poultry (Cromwell and Coffey, 1991; Qian et al., 1996). Phytase also has been shown to increase energy (Namkung and Leeson, 1999; Ravindran et al., 1999b) and AA (Sebastian et al., 1997; Ravindran et al., 1999b; Johnston, 2000) digestibility in diets for poultry. The amount of Ca and available P that phytase releases has been studied extensively (Denbow et al., 1995; Mitchell and Edwards, 1996; Gordon and Roland, 1998) and the values range from approximately 0.09 to 0.10%.

Several other studies have indicated that microbial phytase supplementation increased the availability of pP in broiler chickens (Nelson etal., 1998: Denbow et al., 1995: Kornegay et al., 1996, Sebastian et al., 1996a) and turkeys (Yi et al., 1995: Qian et al., 1996). Improvement in the P availability resulting from phytase supplementation are generally reported to in the range of 20-40%. The amount of pP released by microbial phytase depends on the concentration (Kornegay 1996) and source (Simons et al., 1990) of the added phytase and the dietary phytate (Ravindran et al., 199b: Yi et al., 1995), calcium (Schoner et al., 1993) and vitamin D3 contents (Edwards, 1993: Qian et al., 1995) and the Ca: P ratio (Schoner et al., 1993, Qian et al., 1995).

Effect of Phytase on Egg Quality

Simons and Versteegh, (1993) noted that when phytase was added to layer diets, has a positive effects on egg weight. On the other hand, Boling et al., (2000) reported that no significant differences (P>0.05) on egg size, egg mass and egg specific gravity when laying hen fed with corn-soybean meal based diet supplemented with 100, 200, 250 or 300 U of phytase/kg diet.

Other study reported that supplementation of 0.1% NPP with phytase on laying hens fed various phosphorus levels completely corrected the adverse effects with increases in egg weights and egg specific gravity (Gordon and Roland, 1997 ; Peter, 1992).

There are inconsistencies regarding the effects of phytase on eggshell quality. Several investigators reported a beneficial effects on eggshell quality from phytase supplementation (Punna and Roland, 1999), whereas others did not observed any beneficial effect (Van der Klis et al., 1997; Parsons, 1999). The experiment reported here was conducted to determine the effect of phytase on the productivity, egg quality and P excretion of laying hens (Lohman Brown) fed 2 different levels of phytase Natuphos® at the 35% RB based diet.

Puna and Roland, (1999), conducted a performance trial to evaluate the influence of 300 FTUphytase/kg on first cycle laying hens fed P deficient diet. The investigators used level of 0.1, 0.3 and 0.4% available phytase with or without phytase. Egg weight, egg specific gravity and eggshell quality were among the

criteria used by the authors in the trial. Others were feed consumption, egg production and mortality. They concluded that interactions between available P and phytase for feed consumption (P<0.05), egg production (P0.001) and egg weight (P<0.04) indicated that phytase correlated all deficiency symptoms in hens consumning 0.1% aP but showed no influence on hens fed aP levels >0.2%.

Um and Paik (1999) concluded that performance and eggshell thickness of laying hens fed with diets having 0.24% and 0.12% available P and phytase was higher or equal to the control group which was a diet having 0.37% available phosphorus and no phytase.

Casartelli et al., (2005) conducted an experiment to evaluate the effects of the enzyme phytase in diets formulated with different P sources on performance, eggshell quality and P excretion of commercial laying hens. In regard to phyatase addition, the investigators noted that birds fed diets with no enzyme and therefore with higher leves of the total P laid heavier eggs (P<0.05) in comparison to birds fed phytase-supplementd with low levels of available P. They also noted that the treatment with phytase improve eggshell quality (specific gravity and shell percentage).

Site of Phytase Activity

According to Simons et al., (1990), microbial phytase, unlike plant phytase, is active over a wide range pH and is thus active within the proventriculus and gizzard. Liebert et al., (1993) reported in chickens that 69-86% of added microbial

phytase activity was detected in the crop and that 31-38% of added phytase activity was detected in the proventriculus in broiler chicks. No phytase activity was detected in the small intestine. The disappearance of pP in the crop and proventriculus supports the observation that the crop and proventriculus are the main sites of phytase activity in poultry.

Results from several post-slaughter and cannulation experiments with pigs conducted by Jongbloed et al., (1992) and Kemme et al., (1997) have shown dietary phytase activity, whether from plants or fungal were predominantly active in the stomach. In pigs, phytase activity or breakdown of pP was generally very low or was not observed in the lower small intestine. Significant amounts of phytate P (40-50% of the total) would be passed into the large intestine, where most it would be hydrolysed, but little of the hydrolysed P would be absorbed. Jongbloed et al., (1992) also reported that over 85% of the hydrolysis of phytate by microbial phytase takes place in stomach.

CHAPTER III

MATERIAL AND METHODS

Animals, Treatments and Experimental Design.

One hundred and fifty 18-weeks-old Lohmann Brown laying hens were randomly and equally distributed to 6 treatment groups (25 laying hens in each group). Each treatment was replicated five times, consisting of 5 birds per replicate. The 5 replicates from each treatment group were randomly distributed into two-tier battery cages. The birds of five adjacent cages considered an experimental replicate. The experimental design was a Completely Randomized Design (Figure 3.1).

The birds were obtained from a commercial layer farm in Selangor. Birds were brought from it original farm to the Poultry Research Unit late in the evening to avoid the birds from stress during transportation. They were separated into different dietary treatments from the first day they reached the research unit. The study was conducted at the Poultry Research Unit, Universiti Putra Malaysia, Serdang for a period of 33 weeks.

Housing and Sanitation

Individual hens was house per cage with dimension 26 x 46 x 28cm arranged on two-tier, high-rise battery cages system in a stair step arrangement (Plate 3.1).

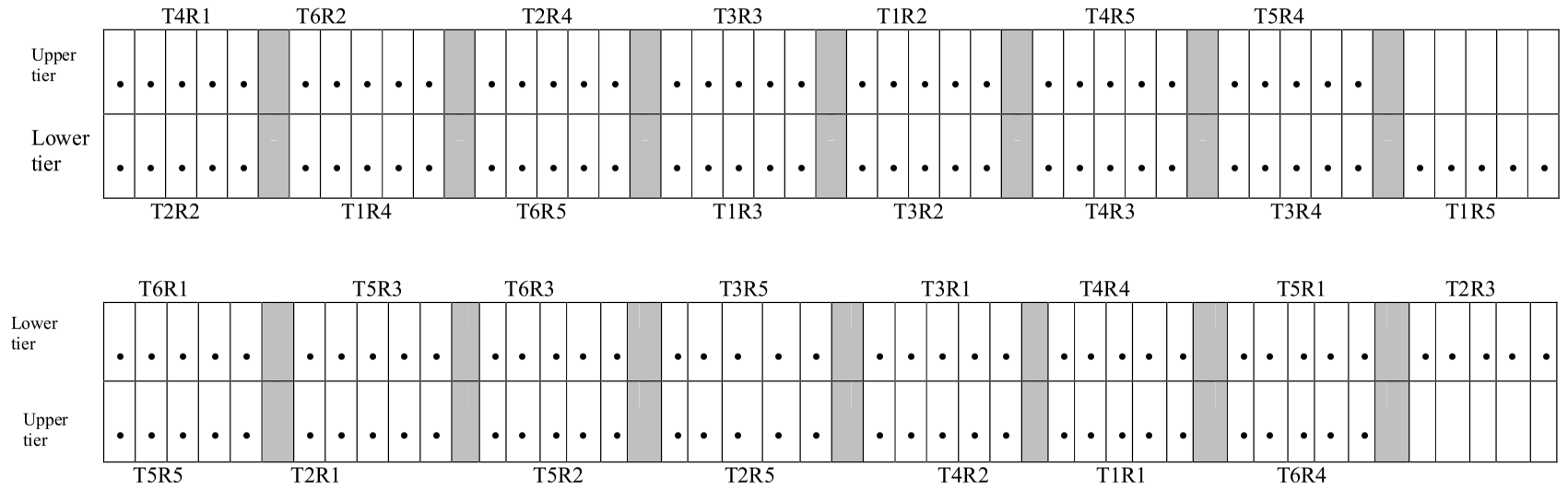

Figure 3.1: The distribution of laying hens in battery cages.

The experiment was conducted in an open-type laying house. The house was built at East-West orientation to avoid the chicken being exposed directly to the sunlight that may cause stress to the chicken. The shed was bird-proofed using $^{1}/_{4}$ inch diameter wire mesh covering the side walk and roof openings. The floor of the house was cemented.

Trough feeders which were hung in front of the battery cages and automatic nipple drinkers were used in this experiment. Each replicate in a group had their own feeder without sharing with other replicate group nearby. Each bird has own nipple drinker in cage.

Cleaning, washing and sanitizing of the house premises, feeders, drinkers and all other equipments was done two weeks prior to the arrival of the birds. Lime powder was strewed on the floor and drains as disinfectant.

Ambient Temperature

During experiment all period, the house temperature and relative humidity were recorded in the morning (between 9.30 to 10.00 a.m) and afternoon (between 2.30 to 3.00 p.m). The range of temperature recorded was from 24.8-27^{o}C in the morning and 29-37^{o}C in the afternoon. The relative humidity were 80-86.8% in the morning and 50.6- 68% in the afternoon.

Feeding, Watering and Birds Managements

Initially the birds were fed on commercial diet for one week after arrival at the research area for pre-adaptation at the new environment. Then the birds were fed with the formulated diets for another two weeks (19-20 weeks of age) for the adaptation to the experimental diets. The experimental diets were then fed to the hens until 53 weeks of age (33 weeks experimental period).

The lighting program was 12 hours light natural daylight. Ventilation was totally depended on the natural ventilation as no mechanical fans were used. Feed and water was supplied *ad-libitum*. No vaccination was done during the experimental period.

The droppings were removed twice a week. Birds in all treatments groups received identical care and management.

Feed Formulation of the Experimental Diets

The experimental diets were formulated to meet the nutrient requirements for laying hens at 90% production (NRC, Nutrients Requirements for Poultry 1984, 1994). Six isonitrogenous (16% crude protein) and isocaloric (3000kcal ME/kg) diets namely Diet 1, Diet 2, Diet 3, Diet 4, Diet 5 and Diet 6 were formulated using rice bran at 0, 15, 25% for Diet 1, Diet 2 and Diet 3, respectively. Diet 4, 5 and 6 were formulated to contain similar rice bran at 35%.

Plate 3.1: Birds kept on two tier battery cages in a stair step arrangement.

Diets 5 and 6 were supplemented with a commercial preparation of 1050 I.U and 1400 I.U phytase Natuphos®5000 (BASF Corporation) respectively. The composition and nutrient composition of the diets are shown in Table 3.1. In this experiment the diet formulated without RB was used as the control diet. The feed was in the mash form.

Mixing of Vitamin Mineral Premix, Mineral and Other Feed Additives

Calculated amount of vitamin mineral premix, amino acids and other feed additives were weighed separately by using an electronic balance and mixed properly with 5kg capacity mixer in the nearby feed mills located at the experimental area. Components required in higher quantity were taken in mixer one after another. On the other hand, components required in small quantities was first mixed with a portion of another component required in higher quantity and was added slowly to the components of the mixer, and finally the premix-additive mixer was prepared.

Preparation of the Diets

Predetermined amount of feed ingredients was weighed separately and placed in the feed mixer of 50kg capacity. The feed ingredient required in the higher amount was introduced in the machine first, whereby the other ingredients were added slowly. These major feed ingredients were allowed to mix for a few minutes and the mixture of premix-additive were added slowly and allowed to mixed for another 20 minutes. Finally, palm oil was added and allowed to mix for another 20 minutes. The diets were then put in the plastic bag and stored in the house

Table 3.1: Compositions of the formulated rice bran based diets for laying hens (21-53 weeks).

Ingredients	Dietary Treatment			
	Diet 1	Diet 2	Diet 3	Diet 4, 5[a], 6[b]
	----------%----------			
Maize	61.00	48.00	40.60	31.90
Rice Bran	0.00	15.00	25.00	35.00
Soybean Meal	21.40	20.00	17.40	16.08
Fish Meal	5.00	5.00	5.00	5.00
Palm Oil	3.00	3.00	3.00	3.00
Common Salt	0.50	0.50	0.50	0.50
VitaminMineral Premix*	0.50	0.50	0.50	0.50
Dicalcium Phosphate	0.50	0.50	0.50	0.50
Limestones	8.00	7.40	7.40	7.40
DL Methionine	0.05	0.05	0.05	0.06
Lysine	0.05	0.05	0.05	0.06
Calculated Composition				
Crude Protein	16.17	16.16	16.001	16.005
ME kcal/kg	3104	3043	3023	2983
Crude Fibre	2.57	3.89	4.76	5.63
Ether Extract	6.44	7.53	8.19	8.93
Total Phosphorus	0.48	0.51	0.51	0.52
Non Phytate Phosphorus	0.32	0.33	0.34	0.35
Calcium	3.48	3.48	3.25	3.25
Lysine	0.88	0.90	0.90	0.90
Methionine	0.33	0.32	0.32	0.31
Methionine + Cystine	0.59	0.56	0.54	0.53
Cystine	0.26	0.24	0.23	0.22
Analysed Composition				
ME kcal/kg	3109	3023	3021	3001
Crude Protein	16.21	16.09	16.07	16.05
Crude Fibre	2.230	2.871	3.730	4.075
Ether Extract	6.71	7.58	8.14	10.21
Calcium	3.483	3.489	3.315	3.350
Total Phosphorus	0.488	0.491	0.492	0.492

***** Vitamin mineral premix provided the following per kilogram of the premix. Vitamin A, 6,000,000 I.U; Vitamin D_3, 1,000,000 I.U; Vitamin B_1, 6g; Vitamin B_2, 2.5g; Vitamin B_6, 1.25g; Vitamin B_{12}, 5mg; Vitamin K, 1g; Pantothenic acid, 5g; Niacin, 12.5g; Folic acid, 0.5g; Manganese, 50g; Zinc, 30g; Iron, 40g; Copper, 5.0g; Cobalt, 0.5g; Selenium, 0.07g and Antioxidant.

[a]Diet 5 supplemented with phytase at 1050 I.U

[b]Diet 6 supplemented with phytase at 1400 I.U

before delivered to the birds. Mixing of the diets was conducted every two weeks.

Sampling of the Mixed Diet

At the final stage of mixing, approximately 100 gram of the diets was taken in a plastic bag. The plastic bag was sealed and the samples were preserved in the freezer for proximate and mineral analysis.

Data Collection

Data on various production parameters were collected from the age of 21 weeks to 53 weeks. The initial weights of the birds were taken at 18 weeks at placement and then at week-21, week-29, week-45 and at the end of the experiment (Week 53). Eggs were collected twice a day, the first collection before 8.00 a.m and the second collection just before 5.00 p.m.

Weekly egg production was determined by collecting eggs laid per replicate every day and pooling together for counting the total collection for seven days. Eggs from each replicate were marked and placed in separate egg trays. Egg production was calculated based on the hen-day egg production of every treatment. The percentage of hen-day egg production was computed as the percentage of the total number of eggs over total number of days by number of hens i.e.

$$\%\ \text{Hen - day production} = \frac{\text{Total number of eggs}}{\text{Total number of days x Number of hens}}\ \text{x}\ 100$$

For egg quality assessment, three eggs from each replicate (15 eggs per treatment per colllection) were collected three times a week in three consecutive days to get a total of 270 samples of egg per week. The eggs were marked according to the diet, replicate group and cage number of the hen. The eggs were weighed individually using a balance and was recorded in grams to the nearest hundredth of a gram. Egg weighs were taken by collecting the fresh eggs per replicate per day and then weighning. The various weights were recorded against each replicate. The average egg weight for each replicate was later determined at the end of the experimental period. The eggs were refrigerated at $4^{o}C$ immediately after collection for later analysis. The egg mass was calculated based on the weight of individual egg from each hen daily (gram of egg/hen/day).

The feed intake was measured weekly. The weekly feed intake was calculated by substracting the residual feed, collected at the end of the week, from the total feed offered. The feed conversion ratio was calculated based on the feed intake for every dozen of eggs for each group in every weekly interval. The feed intake was computed as follows:

$$\%\ \text{Feed intake} = \frac{(\text{g feed offered - g residue feed})}{\text{g feed offered}} \text{ x } 100$$

Mortality was recorded on daily basis. Dead birds were sent to the laboratory for post mortem to determine the cause of death.

Measurement of Egg Quality

Determination of internal egg quality was carried out after the determination of egg weight. The egg quality assessment was conducted three times a week. At least five eggs from each treatment were used for assessment of albumen height, yolk colour and shell quality (shell thickness). Since the eggs were stored, Haugh unit calculations were not performed due to an increased in variability attributed to the calculations.

The fresh eggs (30 eggs i.e 5 eggs per replicate) were broken out on a plate non-absorbent surface (smooth-surface transparent glass slab) for labumen height and yolk colour assessment.

Albumen Height

The height of the thick albumen was measured with a tripod micrometer (Egg Quality Scale, Ogawa Seiki Co., Tokyo, Japan) as described by Haugh (1973).

Egg Yolk Colour

In every assessment of yolk colour (three times a week), five eggs from each treatment were broken into glass plate. The yolk colour was scored with the aid of Roche Yolk Colour Fan (F. Hoffman-La Roche Ltd. Switzerland).

Eggshell Thickness

The two egg membranes were pulled off the shell immediately after being broken and the shells so peeled (90 shells/week) were air dried for a day. The eggshell thickness was measured at three different locations (both ends and the middle) with a micrometer gauge (12 Cms Vernier Calipers, India). The values so obtained were used to compute the average shell thickness per replicate.

Feed Digestibility Determination

At the age of 30 weeks, 3 hens from Diets 1, 4, 5 and 6 were assigned to carry out the digestibility trial. Since the literature shows the performance of laying hens was declined with increasing the rice bran levels in the diet, therefore, the diet containing the highest rice bran level (35%) was chosen to evaluate the feed digestibility with and without the addition of phytase. Excreta were collected for 3 consecutive days (72 hours) for every 3 weeks interval for 3 times of collections. The trial was performed following the method of Din et al., (1979). The digestibility was computed as follows:

$$\% \text{ Digestibility} = \frac{\left(\text{g Nutrient consumed - g Nutrient in excreta}\right)}{\text{g nutrient consumed}} \text{ x } 100$$

Collection of Faecal Samples

The excreta were collected on galvanized zinc trays lined with plastic sheets. Dropped feathers, feed particles or foreign materials were removed to prevent

contamination. Approximately 150-200g samples were collected daily. Each of the excreta samples was mixed and homogenized individually. The faecal samples were placed in plastic container and stored in the freezer. After completion of each collection and before the next sample collection done, the sample were taken on a pre dried aluminium tray, mixed and placed in the oven for drying set at 65^{o}C until the weight were constant. The dried samples were grounded by using 1-mm mash screen and kept in plastic container before analysed for proximate components.

Sampling for Ileal Digestibility of Amino Acids

At the final week of the trial, birds from three replicates of Diets 1, 4, 5 and 6 were given a measured amount of feed (120g/bird/day) twice a day, half in the morning and half in the evening. After three days of feeding, birds were slaughtered. The ileum was dissected as soon as possible after slaughtered. The ileum was defined as extending from Meckle's diverticulum to a point of 40mm proximal to the point of ileo-cecal junction (Payne et al., 1968). Ileal contents were removed and pooled according to dietary treatment. These composite samples were lyophilized and preserved in the freezer and freezed dried before analysis.

Sample Preparations for Chemical Analysis

Preparation of Feed Sample

The feed samples were ground with a grinder with 1-mm mesh screen before analysis. The feed sample was analysed for gross energy, Ca, P, Zn and Fe

following the methods of A.O.A.C (1984). The gross energy of the feed was used to calculate ME value g^{-1} of the diet on the dry matter basis.

Preparation of Faecal Samples

The faecal samples were ground with a grinder with 1-mm mesh screen before analysis. The faecal sample were analysed for gross energy, Ca, P, Zn and Fe following the methods of A.O.A.C (1984). The gross energy of excreta were used to calculate ME value g^{-1} of the diet on the dry matter basis. This was done by subtracting the excreta gross energy (energy output) from gross energy intake and then divided by the total dry matter intake (Din et al., 1979b).

Preparation of Yolk and Albumen Samples

In laboratory, five fresh eggs from each replicate (25 eggs from each treatment) were boiled weekly and subdivided into two portions; egg white (albumen) and egg yolk according to the replicate and treatment group. The yolk and albumen were then dried separately in the oven at $62^{o}C$ for 72 hours or until completely dry. Then dry sample were kept in plastic bottle and kept in the fridge for further analysis. Proximate analysis was carried out on the yolk and albumin samples.

Proximate Analysis

Determination of Dry Matter and Moisture

Approximately 3 grams samples were placed in a previously weighed empty crucible and the weight of samples and crucibles was recorded. The crucible were covered with the lids and kept in the oven set at 105^{o}C for 24 hours. After 24 hours of drying, the crucibles were taken out from the oven, cooled in the desiccators for approximately half hour and weighed. The dry matter is the weight of sample after drying minus the weight of sample before drying (wet sample). The dry matter content of sample is the weight of sample minus the moisture content.

Determination of Ash

Crucibles weight were taken and then filled with approximately 3 grams of samples. The crucibles with samples were covered with the lid and placed in the muffle furnace. The temperature was increased gradually to 550^{o}C or 600^{o}C and the samples were ignited for 5-6 hours or until white, light gray or reddish ash is obtained. The ash content of sample is the weight of samples after ignited.

Determination Crude Protein (CP)

About 0.3-0.5g of sample was put in the digestion tube. One tablet (Kjedahl catalyst tablets, Ajaxchemical Pvt. Limited) and 7.5ml of concentrated sulphuric acid were added to the tube. The tubes containing samples were vortex for mixing and placed on the digestion block located under the fume hood. The temperature of

the fume hood was set at 100^{o}C and allowed heating for 30 minutes then the temperature was increased to 150^{o}C and heated for another 30 minutes. After a ring like appearance was form on the surface of the tube, a few drop of hydrogen peroxide (H_2O_2) was added following the method of Thomas et al., (1967) and repeated for 3-4 times. The temperature of the heating block was gradually increased to 400^{o}C and allowed to heat until digestion complete where liquid samples in the tube become colourless. Two blank tubes were placed in each batch of forty tubes and each sample was replicated twice.

After cooling the digesta were transferred into 100ml volumetric flask. The tube were forcibly rinsed with de-ionised distill water which were transferred to the volumetric flask. Then volumetric flasks were allowed to cool before pouring de-ionised distilled water up to the mark to get the final volume. The crude protein was determined by using the Kjeltec Auto Analyser. Details of calculation of CP procedure were shown in the appendix.

Determination of Ether Extract (EE)

The ether extract or the lipid content of samples was determined by means of Soxtec System (Soxtec System HT 1043 Tecator). The extraction cup were cleaned, dried and cooled in desiccators and weighed. Approximately 2g of sample was weighed and then placed into an extraction thimble. About 50ml of solvent (petroleum benzene) was used for extraction. The samples were allowed to extract for the first 30 minutes in the boiling point by adjusting the indicator at “Boiling” position, rinsed for another 30 minutes in rinsing position and finally another 30

minutes for evaporation. After evaporating the solvent, the cups were released and dried in the oven for 2-3 hours at 105^{o}C. The cups were then cooled in the desiccators before taking the weight with the extract. The EE content is the weight of extraction cup with ether extract minus the empty extraction cup.

Determination of Crude Fiber (CF)

Approximately 1.5g of samples was placed in 600ml beaker and 150ml of 1.25% H_2SO_4 was added. The solution containing the samples was heated for 30 minutes. Then contents of the beaker were filtered through a California Buchner funnel with Whatman 541 filter paper. The beaker was thoroughly rinsed with hot water to clean up the entire sample particles. The residue was washed with hot water again to drain out all the acids and the filtrate was transferred into the beaker with the filter paper.

One hundred and fifty ml of 1.25% NaOH was added and allowed to boil for another 30 minutes. Upon completion of boiling, the samples were filtered through a pre cleaned sintered glass crucible. The filter paper was washed and removed. The filtrate in the crucible was washed with warm water to drain out the NaOH repeated at least three times. Then it was washed with ethanol followed by rinsing using diethyl ether.

The sintered glass with residue was dried in the oven at 105^{o}C overnight. Then the crucible were taken out from the oven and allowed to cool in the desiccators and then weighed. After taking the weight, the glass crucible was then placed in the

muffle furnace and ignited for 3 hours at 550^{o}C. The crucible was allowed to cool below 200^{o}C and then transferred from the furnace to the desiccators, allowed to cool to room temperature and weighed. The crude fibre content was the weight of the sintered glass with dry fibre minus the sintered glass with ash.

Determination of Minerals

The determination of inorganic constituents involved the activity of destroying the inorganic matter as the first step to prepare the samples for mineral analysis. The inorganic matter can be destroyed either by two methods; wet or dry ashing. Both methods were followed depending on the type and amount of samples during analysis. In this experiment, the method use was dry ashing.

Dry Ashing

The porcelain crucibles with the lids were cleaned and soaked in HCI (6N) rinsed with deionised distilled water. Then the crucibles were dried at 105^{o}C and cooled in desiccators. Approximately 1g of sample weighed and put into the crucible, covers with lids and placed in the muffle furnace and ignited at 200^{o}C for an hour. The temperature was then gradually increased to 550^{o}C and allowed to ignite for at least 5 hours. After 5 hours ignition, the crucibles were allowed to cool under the temperature below 200^{o}C. Then by using the metal tong, the crucibles with lids were taken out from the muffle furnace and transferred into the desiccators to cool it down. The crucibles were then placed on the hot plate and 6ml of 5N HCl were added. Then the samples in the crucibles were boiled for half hour and allowed to

cool. The contents of the crucibles were transferred into 100-ml volumetric flasks and the volume was made. The crucibles were rinsed with deionised distilled water for at least distill water. Finally the samples solution was filtered into plastic container with Whatman 541 filter paper and preserved for mineral determination.

Determination of Phosphorus

Phosphorus was determined following the method of Hardd et al., (1981) with slight modification. After the destruction of inorganic matter by dry ashing, the phosphorus (P) remains dissolved in sample solution (acid). The concentration of P in the solution was determined calorimetrically as a yellow phospho-vanadomolibdate complex.

Twenty gram of ammonium molybdate was dissolved in 400ml warm water ($50^{o}C$) and cool. One gram of ammonium metavanadate was dissolved in 300 ml boiled deionised distilled water, cooled and then 140ml of concentrated nitric acid was added gradually with stirring. Finally the molybdate solution was added. The solution was allowed to cool and volume was made up to 1 litre. A series of standards were prepared. In the spectrofotometer cells, (cuvette) 0.5ml of standard was pipetted using micropipette followed by the sample solution. Starting with the standard and following sequentially with the sample solution, 2.0ml of deionised distilled water and 0.5ml of ammonium molybdate-ammonI.Um metavanadate reagent was added. The standard and the samples were allowed to stand for 20 minutes and the absorbency was determined at 420nm. The formula used for calculation is shown in the Apendix A.

Determination of Calcium, Zinc, and Ferum

Samples were analyzed based on dry matter content of each samples. Each 1.000-2.000g samples were placed in a digesting tube before adding 10ml of 98% sulfuric acid. The mixture were shaken gently until it mixed well before heated on a heating block at 140^0C until it was completely digested. The next procedure would be cooling of the digested samples in room temperature. A reasonable amount of sample solution was transferred into the sample tubes and the concentration was determined with atomic absorption spectrophotometer (A.O.A.C., 1990).

Determination of Gross Energy

Gross energy values of the samples (feed, eggs and faeces) were determined using a bomb calorimeter (Parr Adiabatic, Parr Instrument Co. III, USA). The sample holder assembly in the holding ring on the front of the master cabinet so that the terminal on the sample holder mates with the terminal of the master cabinet. Approximately 0.5-1g of samples was made into a pellet by using a pellet press. The samples were placed in the crucible and press. The crucible was tarred before placing the samples. The samples weight was recorded and the crucible-containing sample was placed into the sample cup holder. Ten centimeters of fuse wire for oxygen bomb (NO.45C10 Parr Instrument Co., Moline, ILL U.S.A) was tied to the electrodes carefully on the bomb head and bent into a loop to touch the sample. The sample was then assembled on the combustion chamber by placing the bomb

cap in position and turning it until tight. The bomb was fitted with the oxygen cylinder and oxygen was passed to 30 atmospheric pressure.

Two litre of water was then filled into the oval bucket and placed into the Parr Adiabatic Calorimeter. The bomb was then placed in the oval bucket. The temperature probes were lowered and power sitched on. The bomb ignited and the temperature was recorded after it become stable.

After a stable temperature was recorded, the calorimeter lid was opened, the bomb removed and the pressure was released slowly. The bomb cap then unscrewed from the combustion chamber and removed. The entire inner bomb surface was rinsed with deionised distilled water and the solution was collected in conical flask. A few drops of methyl red indicator was added and titrated with Na_2CO_3 solution. The volume of Na_2CO_3 used for titration was recorded. All the unburned pieces of fuse were removed from the electrode, values were subtracted from the initial length and recorded. The amount of heat that is released when a substance is completely oxidized in a bomb calorimeter is called gross Energy (GE). The heat of combustion is measured in calories. The formula for GE calculation is shown in the appendix A.

Determination of Amino Acid

Amino acids in the diets and ileal digesta were analysed, following hydrolysis, using high pressure liquid chromatography (Water and Associates, Model Number, Water Pump 501 HPLC Pump, Waters 484, Tunable Absorbance Detector). This

method involved three steps: (i) hydrolysis of protein of peptide samples to yield free amino acids (ii) pre-column derivatisation of the samples and (iii) analysis by reverse phase HPLC.

In the pre-column steps, protein and peptide samples were first hydrolysed with HCl, then derivatised with phenylisothiocynate (PITC) to produce phynylthiocrabamil (PTC) amino acids. These amino acids derivatives may then be analysed by HPLC in amounts as low as 1 picomole. PTC amino acids have a broad ultra violet spectrum with maximum absorbancy near 269ηm. A fixed wavelength detector operating at 254ηm produces excellent result. The list of different eluents and reagent and its amount are presented in Appendix A.

Samples and Glassware Preparation for Determination of Amino Acid

Samples of digesta which were collected from the ileum and preserved at negative 20^{o}C were taken out, thawed for 5 hours and freeze dried for 96 hours. After freeze-dried, the samples were ground directly to pass through 1 mm mesh sieve. All glassware required were wash with 6N HCl, rinsed with milli-Q water and dried before conducting the analysis.

Acid Hydrolysis

An estimated of enough samples were taken to have 40mg crude protein (weight=4 g/% CP) in a hydrolysis tube, and 15ml 6N HCl was added. The tube was flushed with liquid nitrogen for 30 seconds and then sealed immediately with Teflon-lined

cap. The tubes were then placed in an electric oven, which was set at 110^{o}C for 24 hours. After 24 hours of acid hydrolysis, the tubes were taken out from the oven and allowed to cool in room temperature before 10ml of internal standard solution (AABA, 2.5µl/ml) was added. The contents of the tubes were then quantitatively transferred to a 50ml volumetric flush with washings of Milli-Q water. Nearly 10ml of sample dilution was filtered by using filter paper after mixing and the initial filtrate was discarded. The filtrated sample was preserved at -20^{o}C until further analysis.

Drying

The preserved samples was taken out from the freezer and keep in the room temperature until the samples is defrosted. A small volume was filtrated again through 0.2µm aqueous syringe filter, of 25mm diameter, the initial filtrate was discarded. Ten µl of samples filtrate was pipetted into Pierce Standard tube (6 x 50mm) by using micripipette. The tubes were placed in the reaction vial and vacuum (knob screw out) to 100 millitor.

Redrying Procedure

The redrying solution consistes of 2:2:1, mixture (by volume) of methanol (HPLC grade): Milli-Q quality water (or equivalent): triethyamine. Redrying (20 µl) solution was added to each sample tube. The tubes were vortex to mix and were placed n the reaction vial. The button on the reaction vial cap was slided to the

open position and the vial was installed into the workstation. The vacuum pump was turned on and the vacuum valve was opened slowly to prevent rapid evaporation of the sample. The samples were redried to 70 millitor. The vacuum valve was closed before removing the reaction vial from the workstation.

Derivatisation Procedure

Before each analysis conducted, fresh derivatisation reagent was prepared. The reagent consists of 7:1:1:1 solution (by volume) of methanol: trithylamine: water: phenylisothiocynate (PITC) and was prepared as follows: By using a Hamiltom syringe or an adjustable micropipette, 70µl of methanol was placed into a fresh sample tube. 10µl of each triethylamine, water and PITC was added and vortex for mixing. 20µl of the derivatisation reagent was added to each of the redried sample. It was mixed by vortexing for a few seconds. The tubes were placed in the reaction via and capped then allowed to stand for 20 minutes at room temperature and was installed into the workstation. The vacuum valve was opened and the vials were allowed to dry to 65 millitor. Upon completion of drying, the vials were removed from the workstation and tubes were removed. The samples were then ready for free amino acid analysis by using HPLC.

Start up and Operation

The column was installed in the column heater and all the fittings were checked for tightness. The column heater temperature was set up to 38^{o}C. Then the column was purged with 90% Eluent 1, and 10% Eluent 2, and allowed to equilibrate until the

based line stabilized. The high pressure limit of both the pump was set to 3500. The flow rate was leave at 0. The model 440 was turned on. It was make sure that the model 730 contained enough paper. The bottles containing Eluent A and Eluent B were connected to the ESS.

Standard Preparation

One ml of standard and 1 ml of internal standard (2.5µl AABA) was placed in a small tube and diluted with 3ml 0.1 (N) HCl and vortex for mixing. 20µl of standard solution was taken to the sample tube and dries in the workstation. The standard was redried and derivatised following the earlier procedure.

The derivatised standard was reconstituted with 200µl of sample diluent solution and vortex for mixing. Eight µl of standard was injected and a representative chromatogram was generated. The HPLC machine was calibrated with a second injection.

A hundred µl of sample diluent was added to the sample and vortex for mixing and finally transferred to a limited volume. Twenty µl of sample was injected into the HPLC. The calculation of amino acid content is shown in the appendix.

Statistical Analysis

Data obtained on various parameters were tabulated, and mean and standard deviation of means were analyzed. The data on each parameter were subjected to statistical analysis using analysis of variance (ANOVA) technique using SPSS software package, Version 12 (2003) in accordance with a completely randomized design. Duncan's multiple range test (DMT) was applied to compare the means. Statement of significance were based on $P<0.05$. To facilitate the statistical analysis of the data, all of the parameters were keyed in into Microsoft Excel and then transferred to the SPSS software package.

CHAPTER IV

RESULTS

Body Weight

The body weights (BW) of layers, fed different levels of rice bran and the 35% RB diet with two levels of microbial phytase are presented in Table 4.1. The mean BW weight of hens fed on Diet 1, 2, 3, 4, 5and 6 were 1618.81, 1626.96, 1618.01, 1625.05, 1615.49 and 1623.02 grams per bird, respectively. The highest BW was 1626.96 on Diet 2 (15% RB) and the lowest BW was 1615.49 grams on ration Diet 5 (35% RB with 1050 I.U phytase.

The overall BW changes of Lohmann Brown laying hens in various dietary treatments for 33-week period did not differ significantly (P>0.05). Regardless of treatments groups, all birds showed positive growth. The results again indicated that inclusion of RB in the diets at high levels of RB (25 or 35%) produced body weight values that were equal or superior to those produced by corn based diets.

The body weights of layers at different ages are shown in Table 4.1. No significant differences were observed between layers fed the different level of inclusion of rice bran at any phase. Birds fed the diets supplemented with 1050 or 1400 I.U/kg Natuphos phytase were similar (P>0.05) to the non-supplemented corn-based diet (Diet 1) at all phases.

Table 4.1: Body weight of laying hens fed different level of rice bran and phytase from week 21 to week 53.

	Body Weight (g/bird)				
Treatment	(Week 21)	(Week 29)	(Week 45)	(Week 53)	Mean
Control Diet 1(0)[1]	1554.04 ± 17.90	1656.00 ± 21.78	1622.04 ± 17.19	1644.29 ± 18.51	1618.80 ± 10.75
Diet 2 (15)	1593.48 ± 27.32	1642.40 ± 18.76	1621.75 ± 18.88	1650.96 ± 16.94	1626..96 ± 10.75
Diet 3 (25)	1583.08 ± 20.68	1627.96 ± 16.58	1594.48 ± 19.36	1666.52 ± 17.92	1618.01 ± 9.77
Diet 4 (35)	1592.44 ± 29.24	1635.04 ± 21.78	1597.40 ± 21.10	1677.42 ± 17.33	1625.05 ± 11.77
Diet 5 (35)[2]	1589.84 ± 31.42	1630.64 ± 23.05	1617.50 ± 20.24	1624.42 ± 22.73	1615.49 ± 12.34
Diet 6 (35)[3]	1566.12 ± 14.91	1658.92 ± 16.59	1635.24 ± 18.38	1631.80 ± 20.11	1623.02 ± 9.33
SEM	9.85	8.06	7.82	7.79	4.35
Level of significant	NS	NS	NS	NS	NS

[1]Figures within parenthesis indicate percentage of rice bran.

[a,]Mean (± SE) within a column having different superscripts are significantly (P ≤0.05) different.

NS-Not significant

[2]Supplemented Natuphos® phytase 1050 I.U

[3] Supplemented Natuphos® phytase 1400 I.U

The results of the present study indicated that consuming the 35% of RB meal with microbial phytase (1050 or 1400 I.U phytase/kg diet) did not improve the body weight gain of Lohmann Brown laying hens.

Mortality

Data on the mortality of Lohmann Brown laying hen on the various dietary levels of rice bran are given in Table 4.2. Hens consuming the 35% RB diet with phytase experienced 0% mortality during the study. Mortality percentage of hens consuming the six diets were 4, 4, 0, 0, 4, and 0% for Diet 1, Diet 2, Diet 3, Diet 4, Diet 5 and Diet 6, respectively. The mortality pattern during the study was not influenced by the inclusion of RB in the diet (Table 4.2).

Table 4.2: Mortality of laying hens fed different level of rice bran and phytase from week 21 to week 53.

Treatment	Phase 1 (wk 21-28)	Phase 2 (wk 29-36)	Phase 3 (wk 37-44)	Phase 4 (wk 45-53)	Total
Control Diet 1(0)[1]	0	1	0	0	1
Diet 2 (15)	0	0	1	0	1
Diet 3 (25)	0	0	0	0	0
Diet 4 (35)	0	0	0	0	0
Diet 5 (35)[2]	0	0	1	0	1
Diet 6 (35)[3]	0	0	0	0	0
Total	0	1	2	0	3

Feed Intake

A summary of feed intake (gram/bird/day) for the 33 weeks (wk 21-53) experimental period is presented in Table 1 (Appendix C) for all dietary

treatments. No significant (P>0.05) differences in daily feed intake were observed among dietary treatment groups. The average feed consumed by the hens fed on Diets 1, 2, 3, 4, 5 and 6 was 117.3, 120.13, 122.41, 121.39, 122.02, and 119.34 g per bird per day respectively. The highest feed consumption was 122.4 1g on Diet 3 (25% RB), while the lowest feed consumption was 117.3 g on Diet 1 (0% RB).

The overall feed intake of hens were not significantly different (P>0.05) between 0, 1050 and 1400 I.U phytase/kg dietary enzyme groups. Hens that received 1050 or 1400 I.U phytase/kg diet consumed similar amount of feed consumed by the hens fed on the 35% RB diet only, Diet 4, and to those consumed the control (fed no rice bran and no phytase) diet (Diet 1). It is observed that supplementation of the basal Diet 4 (35% RB) with phytase did not stimulate feed intake.

The average feed intake of Lohmann Brown laying hens during each phase for all dietary treatments are given in Table 4.3. As can be seen in Table 4.3 the average feed intake of all hens during Phase 1 (wk 21-28) of age for Diet 1, Diet 2, Diet 3, Diet 4, Diet 5 and Diet 6 were 124.2, 115.9, 127.97, 124.6, 119.7 and 114.6 g per hen per day, respectively. There was no significant effect of diet on feed consumption at this stage of age. Similar to that, at Phase 2 (29-36 wk of age) feed consumption was not significant (P>0.05) on Diet 2 and Diet 3 with rice bran 15 and 25% respectively, than on other diets.

During the Phase 1 (21-28 wk of age) no significant (P>0.05) differences was observed in the feed consumption of layers consuming the 35% RB diet, (Diet 4) with those fed Diet 5 or 6 with phytase (Table 4.3).

During Phase 2 (wk 29-36 of age), the birds fed the 35% RB (Diet 4) without phytase consumed 5.33g and 4.82 g feed per bird per day less than those fed the same diet with 1050 and 1400 I.U phytase/kg, respectively. The difference in consumption of those diets decreased to 2.28 and 4.96 g per bird per day as the study progressed so that over the last Phase (45-53 wk of age) of the study, hens receiving the 35% RB without phytase consumed similar or higher (P=0.05) than those on the same diet, Diet 4, with phytase.

While there was no significant differences (P>0.05) in feed intake of hens from week 21 up to week 44 of age (Phase 1 to Phase 3), differences were observed at Phase 4 (wk 45-53 of age) (Table 4.4). By Phase 4 (wk 45-53 of age), the hens on Diet 2, 4 and 5 had significantly higher feed intake than those fed the control diet. For the overall period (wk 21-53) the average feed consumption of the hens fed Diet 4 or Diet 5 were higher, but not significant, (P>0.05) than those fed Diet 1 (0% RB).

While there was no significant differences (P>0.05) in feed intake of hens from week 21 up to week 44 of age (Phase 1 to Phase 3), differences were observed at Phase 4 (wk 45-53 of age) (Table 4.4). By Phase 4 (wk 45-53 of age), the hens on Diet 2, 4 and 5 had significantly higher feed intake than those fed the control diet. For the overall period (wk 21-53) the average feed consumption of the hens fed Diet 4 or Diet 5 were higher, but not significant, (P>0.05) than those fed Diet 1 (0% RB).

Table 4.3: Average feed intake of laying hens fed different levels of rice bran and phytase from week 21to week 53.

Treatment	Feed Intake (g/hen/day)				
	Phase 1 (Week 21-28)	Phase 2 (Week 29-36)	Phase 3 (Week 37-44)	Phase 4 (Week 45-53)	Mean
Control Diet 1(0)[1]	124.15[a] ± 4.33	116.08[a] ± 2.54	115.72[a] ± 4.29	113.72[a] ± 4.06	117.30 ± 1.98
Diet 2 (15)	115.89[a] ± 4.15	125.23[a] ± 2.13	114.42[a] ± 2.42	124.45[b] ± 2.07	120.13 ± 1.58
Diet 3 (25)	127.97[a] ± 3.04	125.72[a] ± 3.44	117.80[a] ± 3.28	118.62[ab] ± 1.61	122.41 ± 1.57
Diet 4 (35)	124.64[a] ± 4.11	119.27[a] ± 4.38	116.08[a] ± 3.35	125.11[b] ± 2.19	121.39 ± 1.81
Diet 5 (35)[2]	119.67[a] ± 4.79	124.60[a] ± 3.64	118.36[a] ± 3.22	125.09[b] ± 2.00	122.02 ± 1.74
Diet 6 (35)[3]	114.63[a] ± 4.36	124.09[a] ± 3.22	121.04[a] ± 5.05	117.79[ab] ± 2.49	119.34 ± 1.93
SEM	1.72	1.38	1.46	1.15	0.73
Level of significant	NS	NS	NS	P<0.05	NS

[1]Figures within parenthesis indicate percentage of rice bran.

[a,b] Mean (± SE) within a column having different superscripts are significantly (P ≤0.05) different.

[2]Supplemented Natuphos® phytase 1050 I.U

[3] Supplemented Natuphos® phytase 1400 I.U

NS-Not significant

Feed Conversion Ratio

Also presented in Appendix C (Table 1) is the average feed conversion ratio (FCR), expressed as kilogram feed consumed per dozen egg, values for layer chickens fed on rations containing various portions of RB and microbial phytase. The average FCR values for birds fed on Diet 1, Diet 2, Diet 3, Diet 4, Diet 5 and Diet 6 were 1.57, 1.69, 1.74, 1.68, 1.70, and 1.64 respectively. Overall, the most efficient feed utilization (FCR) was 1.58 in hens fed the control diet while the poorer feed utilization was 1.74 by hens on ration Diet 3 (25% RB).

It is seen from Table 1 in Appendix C, that there was non-significant ($P>0.05$) difference among experimental groups as far as phytase supplementation was concerned. However, highly significant ($P<0.05$) differences were observed in FCR between the control diet (Diet 1) and the other experimental diets with exception of Diet 6 (35% RB plus 1400 I.U phytase/kg diet).

During the entire experimental period (33 weeks), feed conversion ratio of hen on all dietary treatments with rice bran inclusion (15, 25, and 35% RB) were not significantly ($P>0.05$) different. At the end of the experimental period, FCR was found best (1.64) in the 35% RB plus 1400 I.U phytase/kg (Diet 6) group. On other hand, the poorest FCR (1.74) was observed in Diet 3 (25% RB).

Supplementation of Diet 4 (35% RB) with 1400 I.U phytase/kg improved feed efficiency, to be comparable to the control diet (Diet 1) fed group.

Supplementation of this diet, however, with phytase at rate 1050 I.U phytase/kg diet did not influence ($P>0.05$) feed conversion efficiency.

No significant difference ($P>0.05$) in the FCR values of the hens from Phase 1 (21 to 28 wk of age) to Phase 3 (37 to 44 wk of age) regardless of dietary treatments. At the last phase of the study, Phase 4, wk 45-53 of age, however, the FCR values of birds fed Diet 1 (control diet) was significantly better ($P<0.05$) than those of birds fed on all other treatments dietary (Diet 2, Diet 3, Diet 4, Diet 5 and Diet 6) (Table 4.4).

The FCR values of birds at Phase 4 become poorer with increasing rice bran inclusion. However, addition 1400 I.U phytase/kg diet to Diet 4 (35% RB) significantly improved ($P<0.05$) the birds feed conversion ratio values. Similarly, there was significant difference ($P<0.05$) in overall FCR values of the birds (21-53 weeks) fed Diet 1, Diet 2, Diet 3, Diet 4 and Diet 5 compared to those birds receiving the control diet (Diet 1). Inclusion of phytase at the level of 1400 I.U/kg diet in Diet 4 (35% RB) significantly ($P<0.05$) improved the FCR of birds (Table 1, Appendix C).

Table 4.4: The feed conversion ratio of laying hens fed different level of rice bran and phytase from week 21-53.

Treatment	Feed Conversion Ratio (kg feed/dozen egg)				
	Phase 1 (Week 21-28)	Phase 2 (Week 29-36)	Phase 3 (Week 37-44)	Phase 4 (Week 45-53)	Mean
Control Diet 1(0)[1]	$1.71^{a} \pm 0.08$	$1.52^{a} \pm 0.04$	$1.55^{a} \pm 0.07$	$1.54^{a} \pm 0.07$	$1.57^{a} \pm 0.66$
Diet 2 (15)	$1.65^{a} \pm 0.08$	$1.61^{a} \pm 0.04$	$1.62^{a} \pm 0.04$	$1.74^{b} \pm 0.022$	$1.69^{b} \pm 0.02$
Diet 3 (25)	$1.91^{a} \pm 0.11$	$1.72^{a} \pm 0.07$	$1.63^{a} \pm 0.05$	$1.77^{c} \pm 0.018$	$1.74^{b} \pm 0.08$
Diet 4 (35)	$1.78^{a} \pm 0.13$	$1.61^{a} \pm 0.09$	$1.58^{a} \pm 0.04$	$1.81^{c} \pm 0.0274$	$1.68^{b} \pm 0.04$
Diet 5 (35)[2]	$1.67^{a} \pm 0.11$	$1.65^{a} \pm 0.08$	$1.66^{a} \pm 0.04$	$1.81^{c} \pm 0.024$	$1.70^{b} \pm 0.04$
Diet 6 (35)[3]	$1.60^{a} \pm 0.14$	$1.60^{a} \pm 0.05$	$1.69^{a} \pm 0.08$	$1.66^{b} \pm 0.041$	$1.64^{ab} \pm 0.04$
SEM	0.05	0.03	0.02	0.02	0.02
Level of significant	NS	NS	NS	$P<0.05$	$P<0.05$

[1]Figures within parenthesis indicate percentage of rice bran.

[a,b,c] Mean (± SE) within a column having different superscripts are significantly ($P \leq 0.05$) different.

[2]Supplemented Natuphos® phytase 1050 I.U

[3] Supplemented Natuphos® phytase 1400 I.U

NS-Not significant

Laying Performance

Hen-day Egg Production

The average daily egg production (%) for laying hens fed Diets 1, 2, 3, 4, 5 and 6 over the whole experimental period (21 to 53 wk of age) were 88.83%, 84.75%, 84.27%, 83.82%, 86.66%, and 88.16%, respectively (Figure 4.1 and Table 2, Appendix C).

Hens consuming Diet 2 (15% RB), Diet 3 (25% RB) and Diet 4 (35% RB without phytase) for the overall period (21-53 wk) exhibited significantly ($P<0.05$) difference hen-day egg production ffrom hens fed the control diet (fed no rice bran), (Table 2, Appendix C).

However for the entire 21 to 53 week period, Diet 4 (35% RB) supplemented with 1050 I.U phytase/kg diet slightly increased hen-day egg production (from 83.8 to 86.7%) to be comparable to that of the control (0% RB) diet fed group (88.8%). While addition of phytase at level of 1400 I.U phytase/kg of diet to Diet 4 (containing 35% RB) significantly ($P<0.05$) increased hen-day egg production (88.2%).

No significant differences ($P>0.05$) in hen-day egg production were observed among treatments from week 21 to 28 (Table 4.5). The hen-day egg production of layers fed Diet 6 at Phase 1 (21 to 28 wk of age) were slightly higher than those fed Diet 1, 2, 3, 4, or 5 but statistically not significant ($P>0.05$).

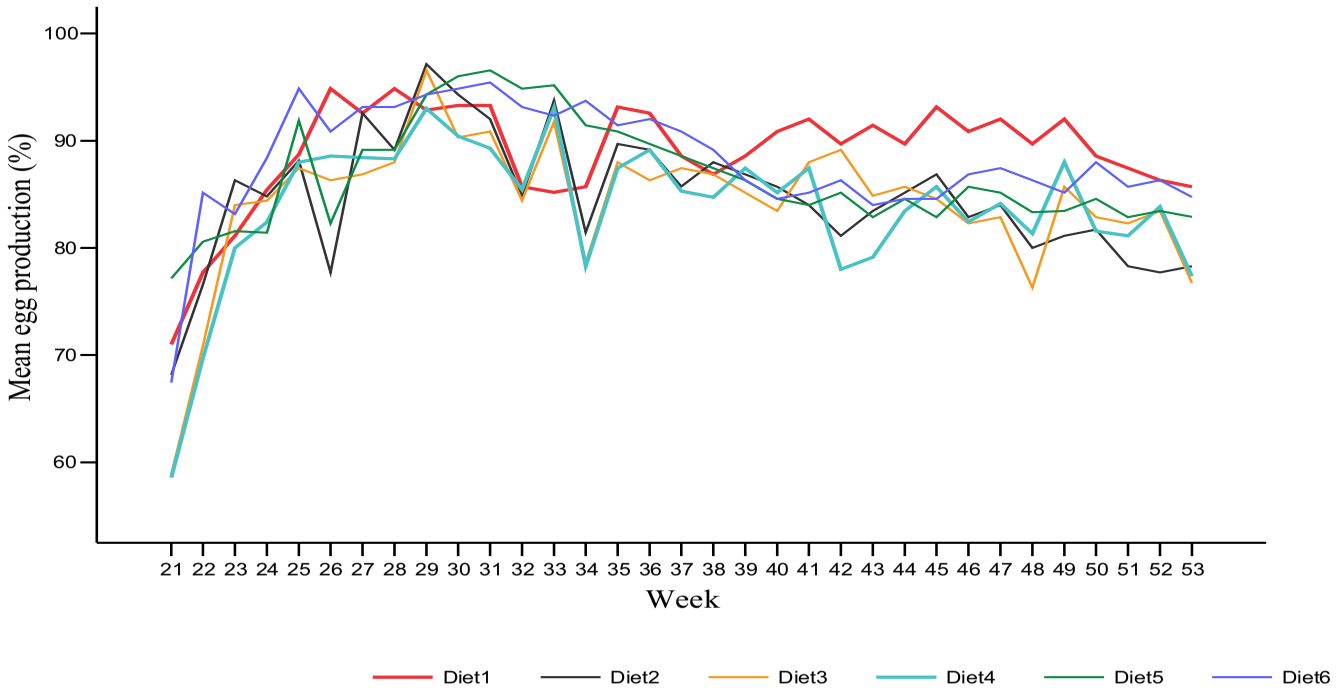

Figure 4.1: Egg production (%) of Lohmann Brown fed different level of rice bran in the diets.

By week 29, hen-day egg production was significantly lower for hens consuming 25% and 35% rice bran diets (Diet 3 and Diet 4, respectively). However at this phase, supplemented Diet 4 (35% RB) with 1050 or 1400 I.U phytase/kg of diet significantly ($P<0.05$) increased hen-day egg production compare to that of the control (0% RB) group.

By Phase 3 (37 to 44 wk of age), the birds fed Diet 1 (control diet, containing 0% RB) achieved significantly ($P<0.05$) higher egg production than those fed Diets 2, 3, 4, 5 and 6 respectively. On the other hand, at this age, no difference ($P>0.05$) was observed in the egg production of hens fed Diets 2, 3, 4, 5 and 6.

Hen-day egg production of hens fed the 35% RB diet without phytase during the Phase 3 (wk 37 to 44) of age, began to decline (Table 4.5). During the period from 29 to 36 wk (Phase 2) of age before this decline, the laying rate was 88.3%. Hen-day egg production of hens on the unsupplemented diet continue to decline and by 45 weeks of age, production was only 82.8% compared with an average of 89.5% for the control diet (Diet 1) during the same period. From 37 weeks of age (Phase 3) onwards, hen-day egg production of hens fed negative control diet (fed 0% rice bran, Diet 1) remained higher than that of other groups (Table 4.5).

During the Phase 3 (wk 37 to 44), the percentage of production of hens fed Diet 5 and Diet 6 dropped to 85.4 and 86.11% respectively. By Phase 4 (wk 45-53) of age, the egg production of phytase unsupplemented hens had fallen to 82.8% whereas phytase supplemented hens maintained production at 86.12%. However, addition 1400 I.U phytase to Diet 4 RB) significantly ($P<0.05$) improved the hen-

Table 4.5: Influence of dietary rice bran and supplemental phytase on egg production.

Treatment	Egg Production (%)				
	Phase 1 (Week 21-28)	Phase 2 (Week 29-36)	Phase 3 (Week 37-44)	Phase 4 (Week 45-53)	Mean
Control Diet 1(0)[1]	$85.78^{a} \pm 3.06$	$90.22^{ab} \pm 1.38$	$89.71^{a} \pm 0.60$	$89.52^{c} \pm 0.89$	$88.83^{b} \pm 0.88$
Diet 2 (15)	$82.92^{a} \pm 2.87$	$90.29^{ab} \pm 1.83$	$85.00^{b} \pm 0.76$	$81.21^{a} \pm 1.01$	$84.75^{a} \pm 0.10$
Diet 3 (25)	$80.84^{a} \pm 3.70$	$88.35^{a} \pm 1.91$	$86.32^{b} \pm 0.66$	$81.89^{a} \pm 1.08$	$84.27^{a} \pm 0.15$
Diet 4 (35)	$80.50^{a} \pm 3.89$	$88.26^{a} \pm 1.69$	$83.82^{b} \pm 1.24$	$82.84^{a} \pm 1.02$	$83.82^{a} \pm 0.16$
Diet 5 (35) + phytase[a]	$84.14^{a} \pm 1.84$	$93.61^{b} \pm 0.91$	$85.43^{b} \pm 0.67$	$83.80^{ab} \pm 0.36$	$86.66^{ab} \pm 0.87$
Diet 6 (35) + phytase[b]	$87.02^{a} \pm 3.15$	$93.40^{b} \pm 0.50$	$86.11^{b} \pm 0.86$	$86.12^{b} \pm 0.40$	$88.16^{b} \pm 0.93$
SEM	1.27	0.65	0.42	0.51	0.43
Level significant	NS	P<0.05	P<0.05	P<0.05	P<0.05

[1]Figures within parenthesis indicate percentage of rice bran.

[a,b,c] Mean (± SE) within a column having different superscripts are significantly (P ≤0.05) different.

Phytase[a] : Supplemented Natuphos® phytase 1050 I.U

Phytase[b] : Supplemented Natuphos® phytase 1400 I.U

NS-Not significant.

day egg production compared to those birds on Diet 4, 35% RB, without phytase. The percentage of egg production of birds at Phase 3 (37 to 44 wk) and Phase 4 (45 to 53 wk) of age showed a significant ($P<0.05$) depression ($P<0.05$) at higher level of rice bran.

As can be seen in the Figure 4.2, the pattern of hen-day egg production to the production phases is consistent within a given treatment but the egg production responses vary across the treatments.

Results of this study showed that the average daily egg production decreased significantly ($P<0.05$) when level of RB inclusion increase regardless of the phytase supplementation. The output (Figure 4.3) shows results of a linear model to describe the relationship between egg production (%) and RB (%) inclusion. Regression of egg production against level of RB in the diet yielded the equation:

$$\text{Egg production (\%)} = 88.1 - 0.14136\ (\text{RB}) \qquad (r = -0.289,\ P = 0.0008)$$

Since the correlation coefficient (r) equals to -0.289, this is an indication of a relatively negative and weak relationship between the variables. There is a statistically significant relationship between egg production and RB (%) at the 99% confidence level since the $P<0.01$.

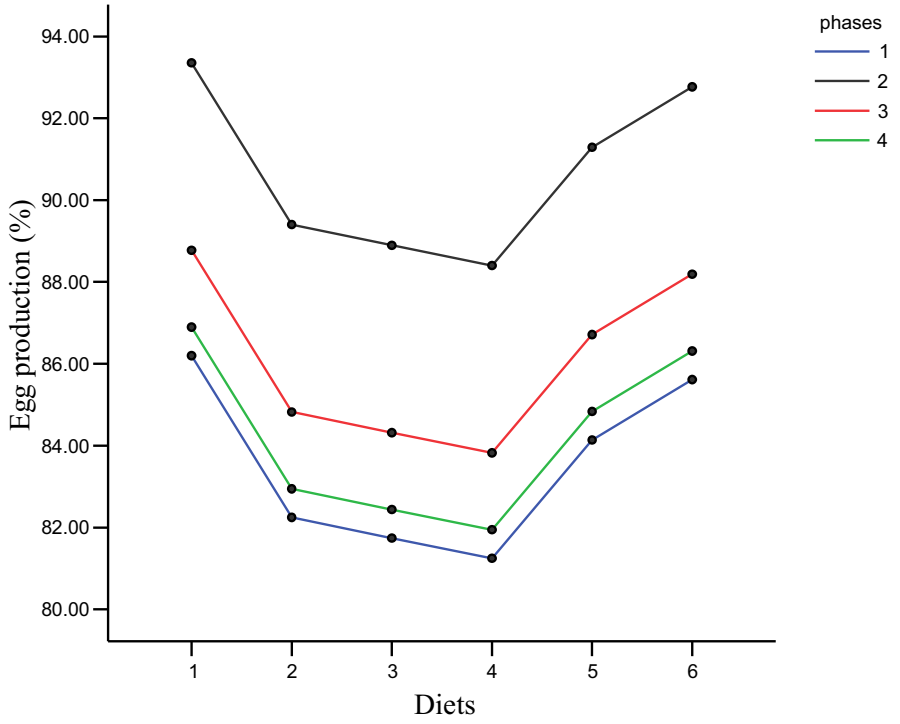

Figure 4.2: Egg production (%) of Lohmann Brown layer hens fed various levels of rice bran and two levels of phytase at different phases. Diet 1 (0% RB), Diet 2 (15% RB), Diet 3 (25% RB), Diet 4 (35% RB), Diet 5 (35% RB + 1050 I.U phytase) and Diet 6 (35% RB + 1400 I.U phytase)

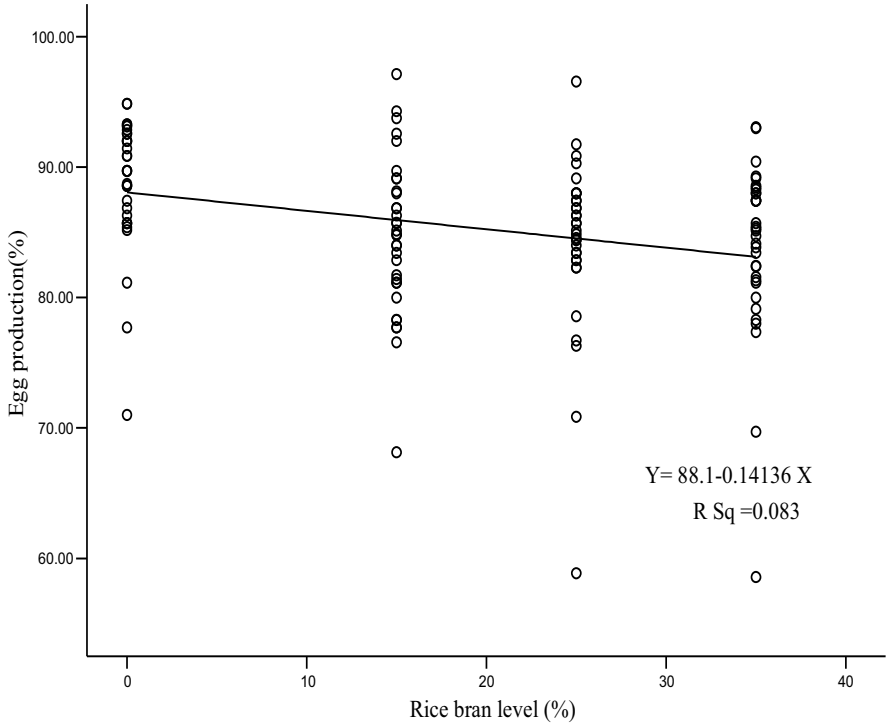

Figure 4.3: Correlation coefficient of egg production (%) of Lohmann Brown fed different levels of RB and two level of phytase. Diet 1 (0% RB), Diet 2 (15% RB), Diet 3 (25% RB), Diet 4 (35% RB), Diet 5 (35% RB + 1050 I.U phytase) and Diet 6 (35% RB + 1400 I.U phytase)

Egg Weight

As shown in Table 4.6, at Phase 1 (wk 21-28) of age, egg weights was significantly lower ($P<0.05$) for hens consuming 35% RB diet (Diet 4) compared to supplemented diet with 1400 I.U phytase kg^{-1} (Diet 6) and the control diet (Diet 1). By the 37 wk of age, no significant ($P>0.05$) differences were observed in egg weights among treatment groups regardless of RB level or microbial phytase inclusion. In addition, the results also demonstrated that increasing dietary RB from 0 to 35% resulted in significantly ($P<0.05$) decreased egg weights during the Phase 1 (wk 21-28) and Phase 2 (wk 29-36).

Table 4.6 showed that the egg weights of Lohmann Brown laying hens were increased from week 29 to 36 for all dietary treatment groups. During the Phase 2 (wk 29-36) to the beginning of Phase 3 (wk 37 to 44), egg weights from the birds consuming the 15, 25, and 35% rice bran without phytase diets increased 1.9, 5.2, 7.0g, respectively compared to 3.8g from birds consuming the maize meal based diet.

On the other hand, egg weights of birds consuming the 35% RB diet with 1050 or 1400 I.U phytase/kg diet increased 3 or 0.7g respectively during the same period. No change in egg weights were observed for all dietary treatment groups as the study progressed (Table 4.6).

The average egg weights throughout the trial period 33 weeks (Table 4.6) was highest in the control diet, Diet 1, (57.53g), intermediate in Diet 6 (57.30),

Table 4.6: Egg weight of laying hens fed different level of rice bran and phytase from week 21 to week 53.

Treatment	Egg Weight (g/egg)				
	Phase 1 (Week 21-28)	Phase 2 (Week 29-36)	Phase 3 (Week 37-44)	Phase 4 (Week 45-53)	Mean
Control Diet 1(0)[1]	$55.48^{bc} \pm 1.08$	$59.36^{c} \pm 0.71$	$58.17^{a} \pm 0.41$	$57.15^{a} \pm 2.02$	$57.53^{a} \pm .66$
Diet 2 (15)	$54.41^{abc} \pm 0.99$	$56.35^{a} \pm 0.80$	$58.90^{a} \pm 0.61$	$58.30^{a} \pm 0.64$	$57.03^{a} \pm .48$
Diet 3 (25)	$52.94^{ab} \pm 1.01$	$58.14^{abc} \pm 0.80$	$58.38^{a} \pm 0.37$	$58.72^{a} \pm 0.64$	$57.20^{a} \pm .54$
Diet 4 (35)	$51.83^{a} \pm 0.72$	$58.86^{bc} \pm 0.55$	$58.25^{a} \pm 0.85$	$58.65^{a} \pm 0.63$	$56.95^{a} \pm .61$
Diet 5 (35) + phytase[a]	$54.57^{abc} \pm 0.95$	$57.56^{abc} \pm 0.75$	$57.51^{a} \pm 0.32$	$58.23^{a} \pm 0.36$	$57.01^{a} \pm .408$
Diet 6 (35) + phytase[b]	$56.00^{c} \pm 0.51$	$56.73^{ab} \pm 0.74$	$58.31^{a} \pm 0.68$	$58.20^{a} \pm 0.41$	$57.32^{a} \pm .33$
SEM	0.40	0.33	0.23	0.38	0.22
Level of Significant	P<0.05	P<0.05	NS	NS	NS

[1]Figures within parenthesis indicate percentage of rice bran.

[a,b,c] Mean (± SE) within a column having different superscripts are significantly (P ≤0.05) different.

Phytase[a] : Supplemented Natuphos® phytase 1050 I.U

Phytase[b] : Supplemented Natuphos® phytase 1400 I.U

NS-Not significant

Diet 3 (57.1g) and Diet 2 (57.03g) and lowest in Diet 4 (56.95g) and Diet 5 (57.00g). The effect of RB inclusion and addition of phytase on egg weights was statistically no significant (P>0.05).

As shown in Table 4.6 average egg weights increased throughout the experimental period from 55.48, 54.41, 52.94, 51.83, 54.57 and 56.00g during the first 21 to 28 weeks of lay to 57.15, 58.30, 58.72, 58.65, 58.23, and 58.20g at last phase of study (wk 45-53) for Diets 1, 2, 3, 4, 5 and 6, respectively.

Egg Mass

The results in Table 4.7 indicted that the average egg mass (gram egg/hen/day) throughout the experiment period (wk 21 to 53) was highest in the control diet, Diet 1, (51.63g), intermediate in Diet 6 (50.62g), Diet 4 (50.12g) and Diet 5 (49.77g) and lowest in Diet 2 and Diet 3 (48.92g and 48.86g, respectively). The effect of RB inclusion and addition of phytase on egg mass was statistically not significant (P>0.05).

The result in Table 4.7 shows egg mass was not affected by dietary treatment at Phase 1 (wk 29-36), Phase 2 (wk 37-44) and Phase 3 (wk 45-53). However, at Phase 4 (45-53 wk), egg mass was significantly lower (P<0.05) for hens consuming 15% RB diet (Diet 2) compared to the control diet (Diet 1), Diet 4 (containing 35% RB) and Diet 6 (35% RB with 1400 I.U phytase/kg diet).

Table 4.7: The egg mass of laying hens fed different level of rice bran and phytase from week 21 to week 53.

Treatment	Egg mass (g/bird/day)				
	Phase 1 (Week 21-28)	Phase 2 (Week 29-36)	Phase 3 (Week 37-44)	Phase 4 (Week 45-53)	Mean
Control Diet 1(0)[1]	48.90 ± 2.49	54.45 ± 1.42	52.148^{c} ± 0.40	51.06 ^{b} ± 1.54	51.63 ± .85
Diet 2 (15)	45.98 ± 1.65	52.57 ± 0.93	50.06^{ab} ± 0.22	47.32^{a} ±0.57	48.92 ± .64
Diet 3 (25)	43.24 ± 2.61	52.56 ± 0.49	50.54^{abc} ± 0.47	49.08^{ab} ± 0.71	48.86 ± .89
Diet 4 (35)	44.51 ± 2.39	54.23 ± 1.02	51.30^{bc}± 0.87	50.40^{b} ± 0.59	50.12 ± .89
Diet 5 (35) + phytase[a]	47.38 ± 1.65	53.88 ± 0.90	49.13^{a} ± 0.45	48.80^{ab} ± 0.37	49.77 ± .62
Diet 6 (35) + phytase[b]	49.17 ± 1.87	52.96 ± 0.51	50.35$^{a\ b}$± 0.73	50.07^{b} ± 0.38	50.62 ± .55
SEM	0.89	0.38	0.26	0.35	0.31
Level of significant	NS	NS	P<0.05	P<0.05	NS

[1]Figures within parenthesis indicate percentage of rice bran.

^{a,b,c} Mean (± SE) within a column having different superscripts are significantly (P < 0.05) different.

Phytase[a] : Supplemented Natuphos® phytase 1050 I.U

Phytase[b] : Supplemented Natuphos® phytase 1400 I.U

NS-Not significant.

Although at Phase 4 inclusion of RB showed reduction in egg mass compared to the control diet (fed no RB), however, egg mass values of birds fed on diets containing 25% and 35% RB were not significantly different from those of birds on the control diet (0% RB).

Egg Quality

The results of egg quality characteristics (the eggshell thickness, yolk colour and albumin height) for the entire 33 weeks trial are presented in Table 3, (Appendix C).

Eggshell Thickness

The overall results on eggshell thickness in Table 3, (Appendix C) illustrated that the eggshell thickness was highest in Diet 6 and Diet 5 (0.415 and 0.413mm, respectively), intermediate in Diet 2 and Diet 3 (0.403 and 0.404mm, respectively) and lowest in Diet 4 (0.401mm) and Diet 1 (0.402mm).

The data of overall eggshell thickness (Table 3, Appendix C) shows that the eggshell thickness from hens consuming 35% RB diet without phytase (Diet 4) was similar to those consuming same diet supplemented with 1050 I.U phytase/kg diet. However, the phytase supplementation at level of 1400 I.U kg^{-1} to the 35% RB diet increased eggshell thickness to the level significantly ($P<0.05$) higher than the respective unsupplemented 35% RB diet and the control diet (Diet 1) fed groups. Effects of inclusion of RB was not statistically significant ($P>0.05$) on eggshell

thickness. On the other hand, supplemental phytase (1050 or 1400 I.U kg/kg diet) has a significant ($P<0.05$) effect on shell thickness of egg.

No differences ($P>0.05$) in eggshell thickness was observed during the Phase 2 (wk 29-36), Phase 3 (wk 37-44) and Phase 4 (week 45-53) on experimental diets at (Table 4.8). However, at Phase 1 (week 21-28), eggshell thickness was higher in birds fed Diet 4 (35% RB) even when 1050 or1400 I.U phytase/kg diet was added to the Diet 4 (positive control diet).

Table 4.8 also shows that the eggshell thickness was reduced over the course of the study from hens fed the 15, 25 and 35% RB without phytase.

Eggshell thickness from birds consuming negative control diet (Diet 1) and hens consuming the 35% diet with 1400 I.U phytase/kg diet (Diet 6) were increased over the course of the study.

Yolk Colour

The overall yolk colour was significantly ($P<0.05$) inferior in birds on rice bran-based diets, Diet 2 to Diet 6, (Table 3, Appendix C). The intensity of the egg yolk colouration decreased with increasing rice bran inclusion (Plate 4.1). Yolk colour index from hens consuming 35% RB without phytase was significantly ($P<0.05$) reduced (5.30) compared to those fed corn-soybean based diet (9.76). When the 35% RB diet (Diet 4) was supplemented with phytase, there was no improvement in

Table 4.8: The Effect of different levels of rice bran and phytase in diet on eggshell thickness from week 21 to week 53.

Treatment	Egg Shell Thickness (mm)				
	Phase 1 (Week 21-28)	Phase 2 (Week 29-36)	Phase 3 (Week 37-44)	Phase 4 (Week 45-53)	Mean
Control Diet 1(0)[1]	$0.40^{a} \pm .004$	$0.40 \pm .003$	$0.41 \pm .004$	$0.41 \pm .015$	$0.40^{a} \pm .004$
Diet 2 (15)	$0.41^{bc} \pm .004$	$0.40 \pm .003$	$0.41 \pm .006$	$0.40 \pm .004$	$0.40^{ab} \pm .002$
Diet 3 (25)	$0.40^{ab} \pm .003$	$0.41 \pm .006$	$0.41 \pm .004$	$0.40 \pm .004$	$0.40^{ab} \pm .002$
Diet 4 (35)	$0.42^{c} \pm .004$	$0.42 \pm .013$	$0.41 \pm .004$	$0.41 \pm .005$	$0.40^{a} \pm .005$
Diet 5 (35) + phytase[a]	$0.40^{ab} \pm .009$	$0.40 \pm .020$	$0.41 \pm .004$	$0.40 \pm .005$	$0.41^{ab} \pm .004$
Diet 6 (35) + phytase[b]	$0.40^{ab} \pm .003$	$0.41 \pm .004$	$0.42 \pm .004$	$0.43 \pm .016$	$0.42^{b} \pm .005$
SEM	.002	.004	.002	.004	.002
Level of significant	*	NS	NS	NS	*

[1]Figures within parenthesis indicate percentage of rice bran.

[a,b,c] Mean (± SE) within a column having different superscripts are significantly ($P \leq 0.05$) different.

Phytase[a] : Supplemented Natuphos® phytase 1050 I.U

Phytase[b] : Supplemented Natuphos® phytase 1400 I.U

NS-Not significant; *$P<0.05$

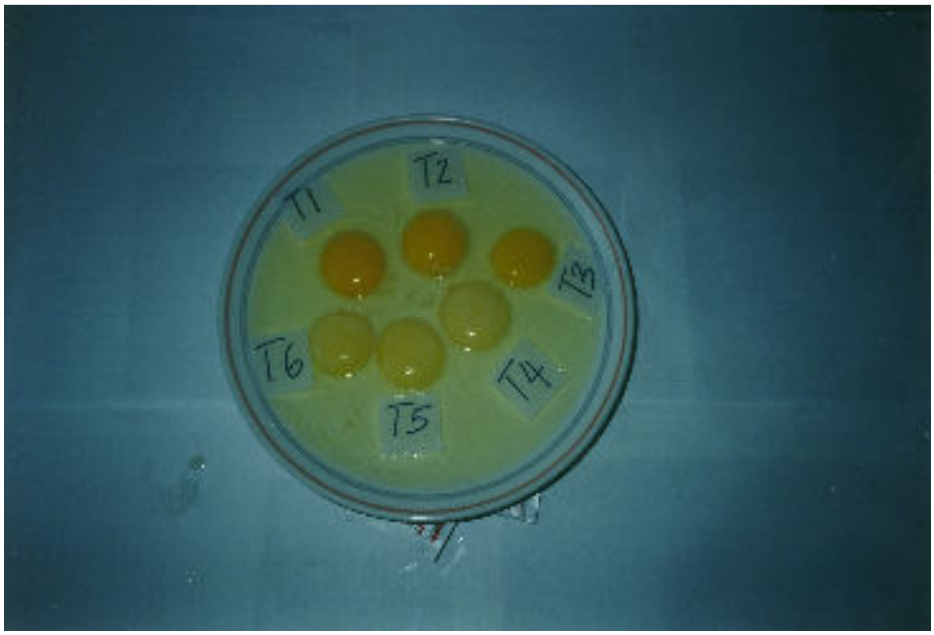

Plate 4.1: Yolk colour of birds fed on Diet 1 (0%RB, T1), Diet 2 (15% RB, T2), Diet 3 (25% RB, T3), Diet 4 (35% RB, T4), Diet 5 (35% RB+ 1050 I.U phytase, T5) and Diet 6 (355 RB+ 1400 I.U phytase, T6).

yolk colour. Increasing RB in hens diet from 15% to 35% depressed ($P<0.05$) yolk colour index by 1.94.

At Phase 1 (21-28 wk of age), yolk colour was significantly ($P<0.05$) higher for the corn-soybean based (control diet, 0% RB) diet fed group and was least for the birds fed on Diet 4, 35% RB without phytase, (Table 4.9).

Also during the Phase 1 (week 21 to 28), the yolk colour was significantly ($P<0.05$) depressed with the increased rice bran content. This trend of depression in yolk colour index with increasing RB content was maintained during the other Phases (2, 3, and 4). There was no phytase effect at the all phases (Table 4.9) except at Phase 1 where phytase addition at level of 1400 I.U/kg feed improved yolk colour index but not significant ($P>0.05$).

Albumen Height

Average albumin height of Diets 1, 2, 3, 4, 5 and 6 was 9.78, 9.65, 9.84, 9.78, 8.99, and 9.99 mm, respectively (Table 3, Appendix C). There was not significant difference ($P>0.05$) of dietary treatment on albumen height.

Albumen height was not influenced by differences in RB or phytase in diets during the Phase 1, Phase 3, and Phase 4 (Table 4.10). No differences ($P>0.05$) in albumen height also was observed during second phase (wk 29-36) of age, on experimental Diets 2, 3, and 4. However, at this phase albumen height of birds fed on the 35% RB diet supplemented with phytase (1050 and 1400 I.U kg^{-1} diet) were significantly

Table 4.9: The effect of different levels of rice bran and phytase in diet on yolk colour from week 21 to week 53.

Parameter	Yolk Colour (1-15)				
	Phase 1 (Week 21-28)	Phase 2 (Week 29-36)	Phase 3 (Week 37-44)	Phase 4 (Week 45-53)	Mean
Control Diet 1(0)[1]	$9.75^{d} \pm 0.25$	$9.63^{c} \pm 0.18$	$9.75^{d} \pm 0.16$	$9.89^{c} \pm 0.26$	$9.76^{d} \pm 0.11$
Diet 2 (15)	$6.75^{c} \pm 0.16$	$6.88^{b} \pm 0.23$	$7.75^{c} \pm 0.16$	$7.56^{b} \pm 0.24$	$7.24^{c} \pm 0.12$
Diet 3 (25)	$6.00^{b} \pm 0.27$	$6.38^{b} \pm 0.18$	$6.25^{b} \pm 0.16$	$5.67^{a} \pm 0.24$	$6.06^{b} \pm 0.12$
Diet 4 (35)	$5.13^{a} \pm 0.30$	$5.25^{a} \pm 0.25$	$5.75^{ab} \pm 0.25$	$5.11^{a} \pm 0.20$	$5.30^{a} \pm 0.13$
Diet 5 (35) + phytase[a]	$5.13^{a} \pm 0.23$	$5.63^{a} \pm 0.18$	$5.63^{a} \pm 0.18$	$5.22^{a} \pm 0.15$	$5.39^{a} \pm 0.01$
Diet 6 (35) + phytase[b]	$5.00^{a} \pm 0.27$	$5.38^{a} \pm 0.18$	$5.50^{a} \pm 0.20$	$5.56^{a} \pm 0.24$	$5.36^{a} \pm 0.11$
SEM	0.26	.23	0.24	.25	.12
Level of significant	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05

[1]Figures within parenthesis indicate percentage of rice bran.

[a,b,c,d]Mean (± SE) within a column having different superscripts are significantly ($P \leq 0.05$) different.

Phytase[a] : Supplemented Natuphos® phytase 1050 I.U

Phytase[b] : Supplemented Natuphos® phytase 1400 I.U.

Table 4.10: The effect of different levels of rice bran and phytase in diet on albumen height from week 21 to week 53.

Parameter	Albumen height (mm)				
	Phase 1 (Week 21-28)	Phase 2 (Week 29-36)	Phase 3 (Week 37-44)	Phase 4 (Week 45-53)	Mean
Control Diet 1(0)[1]	9.68 ± 0.44	10.22[b] ± 0.41	10.20 ± 0.36	9.10 ± 1.13	9.78 ± 0.43
Diet 2 (15)	9.35 ± 0.48	9.73[b] ± 0.37	9.44 ± 0.44	10.03 ± 0.21	9.65 ± 0.19
Diet 3 (25)	9.66 ± 0.41	10.15[b] ±0.40	9.72 ± 0.30	9.83± 0.29	9.84 ± 0.17
Diet 4 (35)	9.77 ± 0.35	10.16[b] ± 0.31	9.52 ± 0.46	9.70 ± 0.26	9.78 ± 0.17
Diet 5 (35) + phytase[a]	8.86 ± 0.39	8.61[a] ± 0.37	8.38 ± 1.21	9.98 ± 0.32	8.99 ± 0.34
Diet 6 (35) + phytase[b]	9.81 ± 0.51	9.64[a] ± 0.36	10.38 ± 0.32	10.13 ± 0.23	9.99 ± 0.18
SEM	0.18	0.18	0.31	0.21	0.10
Level of significant	NS	P<0.05	NS	NS	NS

[1]Figures within parenthesis indicate percentage of rice bran.
[a,b] Mean (± SE) within a column having different superscripts are significantly (P ≤0.05) different.
Phytase[a] : Supplemented Natuphos® phytase 1050 I.U
Phytase[b] : Supplemented Natuphos® phytase 1400 I.U
NS-Not significant.

lower ($P<0.05$) than those received other dietary treatments (Diets 2, 3 and 4) and those fed on the control diet (Diet 1).

Egg Chemical Composition

The effects of RB inclusion and phytase addition on chemical composition of egg from Lohmann Brown laying hens for the overall period of the study is summarized in Table 4.11. The calculation was based on dry matter basis of egg yolk and albumen.

Crude Protein

Crude protein (CP) content of egg yolk from birds fed on dietary treatment containing 15, 25, and 35% rice bran were significantly ($P<0.05$) lower compared to those hens consumed the control diet, Diet 1 (0% RB). Supplementation of the 35% RB diet with 1400 I.U phytase kg^{-1} improved the crude protein content to similar level of those fed on control diet (Diet 1). Dietary treatments however, had no significant effect ($P>0.05$) on crude protein content of egg albumen (Table 4.11).

Ether Extract

Ether extract content of egg yolk measured from hens consuming the control diet (0% RB) was lower (58.8%) than those fed on other dietary treatments containing various levels of rice bran (Diets 2, 3, 4, 5 and 6). When RB

included at levels of 25% or 35% with or without phytase supplementation, the ether extract of egg yolk was significantly ($P<0.05$) increased compared to those fed on the control diet (0% RB). However, phytase supplementation to the higher dietary rice bran (Diet 4, 35% RB) did not influence ether extract content of egg yolk (Table 4.11). There was no significant difference ($P>0.05$) of ether extract content of egg albumen regardless of the levels of rice bran and phytase inclusion.

Ash

Ash content of egg yolk measured from birds fed on Diet 4 (35% RB) was significantly ($P<0.05$) higher than the birds on Diet 2 (15% RB), Diet 3 (25% RB), Diet 5 (35% RB) and the control diet (Diet 1, 0% RB). No significant difference ($P>0.05$) was noted in the overall ash content of egg yolk of birds (21-53 weeks) offered Diet 4 (35% RB) and Diet 6 (supplemented with phytase, 1400 I.U/kg diet). It was also noted that neither inclusion level of rice bran nor level of phytase has significant ($P<0.05$) effect on ash content of egg albumen.

Table 4.11: Chemical composition of egg (based on dry matter basis) from laying hens fed diets with different levels of rice bran with or without phytase (week 21-53).

Parameter(%)	Dietary Treatment						
	Control Diet 1(0)[1]	Diet 2 (15)	Diet 3 (25)	Diet 4 (35)	Diet 5 (35) + phytase[a]	Diet 6 (35) + phytase[b]	SEM
				Yolk			
Crude protein	43.26[b] ± .25	42.51[a] ± .17	42.3[a] ± .21	42.55[a] ± .16	42.24[a] ± .29	42.70[ab] ± .15	.09
Ether Extract	58.79[a] ± .22	59.28[a] ± .29	60.03[b] ± .27	60.46[bc] ± .27	60.92[c] ± .19	61.06[c] ± .24	.15
Ash	3.05[a] ± .01	3.05[a] ± .01	3.08[a] ± .01	3.23[b] ± .02	3.08[a] ± .01	3.24[b] ± .02	.01
				Albumen			
Crude protein	86.42[a] ± .55	87.51[a] ± .35	86.66[a] ± .57	84.14[a] ± .74	87.46[a] ± .36	86.12[a] ± .67	.23
Ether Extract	.036[a] ± .006	0.06[a] ± .01	0.174[a] ± .06	0.168[a] ± .07	0.170[a] ± .07	0.142[a] ± .04	.02
Ash	4.96[a] ± .05	4.91[a] ± .08	4.94[a] ± .07	4.91[a] ± .17	5.07[a] ± .12	4.93[a] ± .14	.04

[1]Figures within parenthesis indicate percentage of rice bran.

[a,b,c] Mean (± SE) within a row having different superscripts are significantly (P ≤0.05) different.

Phytase[a] : Supplemented Natuphos® phytase 1050 I.U

Phytase[b] : Supplemented Natuphos® phytase 1400 I.U

Digestibility of Nutrients

Results of the effect of phytase supplementation to Diet 4 (35% RB) on feed digestibility, digestibility of dry matter (DM), crude protein (CP), Calcium (Ca), phosphorus (P), zinc (Zn), ether extract (EE), crude fiber (CF) and apparent metabolizable energy (AME) are presented in Table 4.12.

The digestibility of CP, CF, and EE in hens fed Diet 1, Diet 4, Diet 5 and Diet 6 were 70.6, 70.8, 72.3 and 71.1%; 28.1, 26.8, 26.6, and 24.9%; and 85.9, 84.9, 84.6, and 84.6% respectively (Table 4.12).

Table 4.12: Digestibility (%) of nutrients and apparent metabolisable energy (AME) contents of diets.

Parameters	Dietary Treatment			
	Control Diet (0)[1]	Diet 4 (35)	Diet 5 (35 + phytase[a])	Diet 6 (35 + Phytase[b]) SEM
Dry Matter	66.80[a] ± 2.80	64.43 [a] ± 0.88	64.90[a] ± 0.68	64.61[a] ± 3.87
Crude Protein	70.57[a] ± 0.40	70.77 [a] ± 0.78	72.27[a] ± 0.53	71.08[a] ± 0.96
Calcium	59.22[a] ± 1.28	55.92 [a] ± 0.86	56.41[a] ± 0.48	60.87[a] ± 1.55
Phosphorus	63.98[a] ± 1.32	46.05[b] ± 1.12	46.23[b] ± .1.33	59.43[a] ± .736
Zinc	38.11[a] ±0.77	36.85 [a] ± 0.97	36.70[a] ± 0.46	37.70[a] ± 1.07
Ether Extract	85.88[a] ±0 .82	84.90 [a] ± 0.24	84.64[a] ± 0.24	84.62[a] ± .54
Crude Fiber	28.06[a] ± 0.43	26.76[a] ± 0.17	26.60[ab] ± 0.15	24.88[b] ± .27
AME (kcal/kg)	2996.01[a] ± 11.10	2989.60[a] ± 0.89	2990.64[a] ± .83	2994[a] ± 6.54
Feed Digestibility	83.03[b] ± 0.63	76.95[a] ± 2.62	77.62 [a] ± 0.70	78.92[a] ± 0.60

[1]Figures within parenthesis indicate percentage of rice bran.
[a,b,c] Mean (± SE) within a row having different superscripts are significantly (P ≤0.05) different.
Phytase[a] : Supplemented Natuphos® phytase 1050 I.U
Phytase[b] : Supplemented Natuphos® phytase 1400 I.U

The results of digestibility of nutrients in Table 4.12 shows that no significant (P>0.05) differences were observed in the digestibility of DM, CP, Ca, Zn, EE, and AME between the dietary treatments. However, although there was no significant difference (P>0.05) but feeding basal Diet 4 (35% RB) resulted in the digestibility tended to reduce by 2.4, 0.20, 3.3, 1.26, 0.98% for DM, CP, Ca, Zn, and EE respectively at 35% of RB inclusion as compared to control diet (0% RB). The apparent metabolisable energy of the diets were 2996.01, 2989.6, 2990.64 and 2994 Kcal /kg for Diets 1, 4, 5 and 6 respectively. The digestibility of DM, CP, Ca, Zn, EE, and AME were not affect significantly (P>0.05) with levels of phytase.

The digestibility of CF of birds fed the negative control diet (0% RB) and Diet 4 (35% RB) were significantly (P<0.05) greater than those fed Diet 6, 35% RB supplemented with 1400 I.U phytase/kg diet. In addition, the digestibility of digestibility of CF of birds fed positive control diet was similar to those hens fed Diet 4 and Diet 5 (35% RB plus 1050 I.U phytase/kg diet).

Table 4.12 also shows the digestibility of phosphorus. Total phosphorus digestibility using 35% RB or/and phytase was 17.93, 17.75, and 4.55% lower for Diets 4, 5 and 6, respectively, than the control group (Diet 1). In addition, there was no significant (P>0.05) reduction in the phosphorus digestibility for the group fed on Diet 6 but not for other groups as compared to the control group (Diet 1).

The results obtained herein showed that the digestibility of P was significantly (P<0.05) reduced by inclusion of 35% RB. The addition of phytase at level 1050 to the 35% RB diet did not influence the digestibility of P. Increasing the dose of

phytase to 1400 I.U/kg diet, however, resulted in a significantly ($P<0.05$) greater P absorption (in percentage units) compared to the unsupplemented positive control diet (Diet 4).

The percentage of digestibility of feed in the control diet (Diet 1) was higher than in other dietary treatments but not significant ($P>0.05$). As evidence in Table 4.12 there is no effect of supplemental phytase on digestibility with rice bran based diets.

Mineral Excretion

Table 4.13 shows the amount of mineral excreted base on dry matter basis in the feces. No significant difference ($P>0.05$) was observed in excretion of Ca, Zn, Fe regardless RB inclusion or phytase supplementation levels. However, despite there was no significant difference ($P>0.05$) but the excretion of Ca and Fe were reduced at the 1400 I.U of phytase supplementation. The significant differences ($P<0.05$) between dietary treatments were observed in amount of excreta %P and P retention.

Excretion of Ca was the highest in the control diet and lowest in the diet that contained 35% RB and 1400 I.U/kg diet ($P>0.05$). From the calculation of Ca retention, the birds fed Diet 6 (35% RB plus 1400 I.U phytase/kg diet) had a higher percentage of retention ($P>0.05$). Addition of phytase at high level changed the percentage of Ca retention but not significant ($P>0.05$).

Table 4.13: Means ± SE of mineral excreted (%) based on dry matter basis in feces by layers fed 35% RB based diet supplemented with two levels of phytase Natuphos®.

Rice Bran (%)	0 %	35 %			SEM
Phytase (I.U)	0	0	1050 I.U	1400 I.U	
Phosphorus	0.487[a] ± .01	0.714[b] ± .02	0.676[b] ± .02	0.492[a] ±.01	.03
Calcium	0.781[a] ± .04	0.713[a] ± .03	0.749[a] ± .01	0.683[a] ± .03	.02
Zinc	.019[a] ± .003	.019[a] ± .003	.020[a] ± .001	.019[a] ± .001	.0003
Iron	.007[a] ± .001	.008[a] ± .001	.008[a] ± .001	.008[a] ± .0003	.0003
Copper	.0020[a] ± .00013	.0023[b] ± .00003	.0021[a] ± .00326	.0019[b] ± .00011	.00005

[a,b] Means (± SE) within a row having different superscripts are significantly ($P \leq 0.05$) different.

Phosphorus in excreta was highest in layer fed 35% RB without phytase supplementation ($P<0.05$). This could be due to the higher phytate phosphorus content in rice bran as compared to corn. Phytase addition at level 1400 I.U significantly lowered ($P<0.05$) the percent of P in excreta of birds fed 35% RB diet by 13%, but this amount was still higher than the control group.

The excretion of P and Cu was significantly higher ($P<0.05$) for birds offered the 35% RB basal diet (Diet 4) and Diet 5 (35% RB supplemented with 1050 I.U of phytase) as compared to negative control diet (0% RB) and Diet 6 (35% RB supplemented with 1400 I.U of phytase). However, the supplementation of the 35% RB based diet with 1400 I.U phytase significantly ($P<0.05$) reduced the amount of P and Cu excretion to a level similar to the negative control diet (Diet 1). There were no significant difference ($P>0.05$) of Zn and Fe excretion observed in this study in birds offered Diet with 0% RB or 35% RB with or without phytase supplementation in the diets.

Intake and Excretion of Phosphorus

Available phosphorus intake values and excretion were of particular interest and were calculated for each dietary treatment (Table 4.14). The mean daily P intake value for the 21 to 53 week period for hens consuming the 35% RB (diet 4) diet was 1.323 g while the mean P intake values for hens fed on the 35% RB based diet supplemented with phytase (1050 or 1400 I.U/kg) for the overall period (21-53 wk) were 1.26 and 1.21 respectively. Birds fed on maize-soybean based diet (Diet

1) had the highest (P<0.05) P intake (1.354g). Markedly higher (P<0.05) amount of P was excreted in the faeces of birds fed Diet 4.

Table 4.14: Digestibility of phosphorus of hens fed 35% RB based diet supplemented with phytase.

Parameters	Dietary Treatment				
	Control Diet (0)	Diet 4 (35)	Diet 5 (35 + Phytase[a])	Diet 6 (35 + phytase[b])	SEM
Phosphorus intake (g)/bird/day	1.35[d] ±0.015	1.32[c] ±0.005	1.26[b] ±0.002	1.21[a] ±0.004	0.172
Phosphorus excreted (g)/bird/day DM	0.49[a] ±0.013	0.71[b] ±0.017	0.68[b] ±0.018	0.49[a] ±0.010	0.032
Phosphorus excretion (%)	36.02[a]±1.32	53.93[c] ±0.012	53.77[c] ±1.33	40.56[b] ±0.735	2.240

[1]Figures within parenthesis indicate percentage of rice bran.
[a,b,c] Mean (± SE) within a row having different superscripts are significantly (P ≤0.05) different.
Phytase[a] : Supplemented Natuphos® phytase 1050 I.U
Phytase[b] : Supplemented Natuphos® phytase 1400 I.U

As evidence in Table 4.14, the rate of P excretion was reduced with increasing level of supplemented phytase (from 0.676 to 0.49g). It was calculated that hens fed the 35% RB diet with 1400 I.U of phytase excreted 31% less phytate P than those consuming the same diet without phytase (0.49 and 0.714 g/bird/day, respectively).

Ileal Digestibility of Amino Acids

Results concerning ileal digestibility of amino acids in Lohmann Brown hens fed 35% RB based diet supplemented with phytase are presented in Table 4.15. The effect of phytase addition to the 35% RB diets on ileal digestibility (%)

Table 4.15: Ileal digestibility of the amino acids (%) of layer chicken fed 35% RB based diet supplemented with two levels of phytase Natuphos®.

Rice Bran (%)	0 %	35 %			
Phytase (I.U)	0	0	1050 I.U	1400 I.U	Level Significant
Apartic acid	63.80[a]	62.18 [a]	63.80 [a]	67.76 [a]	NS
Glutamic acid	55.57 [a]	55.27 [a]	53.79 [a]	56.86 [a]	NS
Serine	58.45 [a]	55.30 [a]	56.15 [a]	61.42 [a]	NS
Glycine	94.70 [a]	91.21[b]	91.96 [b]	95.45 [a]	P<0.05
Histidine	72.74 [a]	69.97 [a]	70.64 [a]	73.65 [a]	NS
Arginine	69.99 [b]	65.76 [a]	68.85 [ab]	70.83 [b]	P<0.05
Threonine	69.16 [a]	67.66 [a]	66.10 [a]	68.73 [a]	NS
Alanine	80.42 [a]	78.95 [a]	79.26 [a]	81.74 [a]	NS
Proline	73.24 [a]	72.04 [a]	70.29 [a]	73.50 [a]	NS
Tyrosine	56.82 [a]	53.07 [a]	54.28 [a]	57.89 [a]	NS
Valine	66.14 [a]	61.46 [a]	63.47 [a]	64.07 [a]	NS
Isoleucine	71.99 [a]	69.54 [a]	71.31 [a]	71.55 [a]	NS
Leucine	62.04 [a]	58.52 [a]	63.60 [a]	63.13 [a]	NS
Phenylalanine	59.17 [a]	60.38 [a]	60.11 [a]	62.33 [a]	NS
Lysine	66.37 [a]	60.87 [a]	61.94 [a]	63.19 [a]	NS
Cystine	60.05 [a]	55.88 [a]	55.45 [a]	55.66 [a]	NS

Means within a row with different superscripts (a,b) are significantly different ($P \leq 0.05$).
NS-Not significant

of amino acids is shown in Table 4.15. Only glycine and arginine were significantly ($P<0.05$) improved by phytase (1400 I.U/kg diet) supplementation (Figure 4.4).

Inclusion of 35% RB in layer diet significantly decreased ($P<0.05$) the digestibility of glycine and arginine. Phytase addition at 1050 I.U I.U/kg diet did not improve ($P>0.05$) the glycine digestibility. However, it improved the digestibility of arginine but not significant ($P>0.05$). On other hand, supplementation of phytase at 1400 I.U/kg diet significantly ($P<0.05$) improved the digestibility of glycine and arginine.

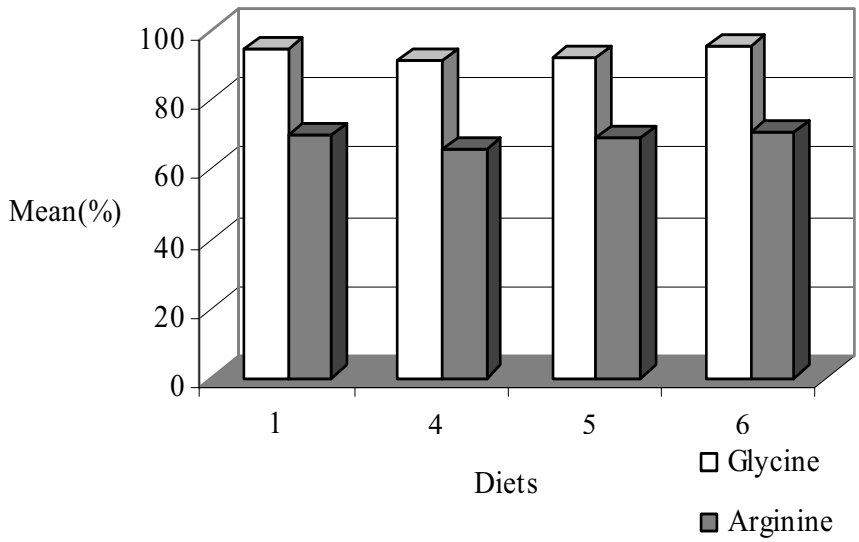

Figure 4.4: Digestibility of glycine and arginine in Diets 1, 4, 5 and 6.

CHAPTER V

DISCUSSION

In many countries, different types of feeds are used depending on availability and local condition and there exists a largely untapped potential for utilizing feedstuffs for poultry. In Malaysia, the traditional feed is generally made of maize and soybean, which are expensive. Increasing cost of feed ingredients (grains, fishmeal, oil-cakes, etc.) has accelerated the search for cheaper substitutes. One such byproduct is rice bran, a by-product obtained after milling brown or paddy rice and perhaps the most widely used agricultural byproduct in the diet of ruminant and non ruminant animals as a source of energy. Feed constituted the major cost of poultry meat and egg production, usually 65-70%, all over the world. Commercial layers feeds typically contain corn in excess of 60%, and the inclusion of RB in poultry diet may have great economic potential. RB is also rich in crude fibre (CF) and phytate phosphorus (pP) contents. The chemical composition of locally available RB was found to be uniform (Ukil, 1999).

Body Weight and Mortality

The body weight gain data the absence of phytase suggest that a dietary RB inclusion at 35% from 21-53 weeks was sufficient to satisfactorily maintain the body weights. It was clear that RB could be used up to 35% level as there was no significant difference in body weights of the hens in all the six treatment groups and all were in a state of positive weight gain. These results are in agreement with

previous findings in broilers (Sayre et al., 1988; Masood et al., 1995 and Jeshwani et. al., 1996) who concluded non-significant difference in body weight by feeding RB. In contradiction to these results; Gallinger et al., (2004) concluded that concentrations of RB in excess of 20% in broiler diets produced significant reductions of body weight. However, laying hens can tolerate higher dietary inclusions of RB than broiler chickens (Warren and Farrell, 1990a). Mortality is not correlated to high inclusion of RB which was in agreement with work of Wiranda et al., (1982) and Din et al., (1979a). In addition, the survivability results of birds during experimental period on Diet 5 and Diet 6 indicated that enzyme supplementation had no effect on mortality. This result coincides with the finding of Alam et al., (2003); Lan et al., (2002) and Pillai et al., (1995). They also reported no effect of phytase supplementation on survivability results.

Feed Intake and Feed Conversion Ratio

The overall feed intake of Lohmann Brown laying hens in different groups for the 33-wk period did not differ significantly ($P>0.05$) due to dietary treatments. The results of feed intake obtained in this study were in disagreement with Karunanjeewa and Tham (1980) who reported that feed intake was constantly reduced in layers fed at 20% RB. The results of this study during each phase and for the overall experimental period 33 weeks, revealed that microbial Natuphos phytase supplementation at both levels (1050 or 1400 I.U/kg) did not stimulate intake on the Diet 4 with 35% RB.

The result of this study also showed that the overall efficient of feed utilization (FCR) was 1.57 in chickens fed on Diet 1 (0% RB) while the least efficient feed utilization was 1.74 by the chickens fed on Diet 3 (25%RB). There was significant ($P<0.05$) difference among the different experimental groups as far as rice bran inclusion concerned. Inclusion of rice bran at level 35% in diet without phytase addition produced feed conversion ratio in Lohmann Brown laying hens poorer than those obtained with the reference (corn-soybean) diet. Data from the literature indicated that the FCR rates are usually increased (i.e become poorer) as the content of RB in that diet increase. These results support the conclusions of Warren and Farrell, (1990b), who found that inclusion 20% RB diet yields a poorer FCR. The reason of poor FCR values of birds fed on RB based meal could be attributed to rancidity of the lipid fraction and other constituents in RB may be responsible for this effect. However, in another experiment using hens by the same authors, inclusion of RB up to 40% in diet produced no change in FCR. Gallinger et al., (2004) also reported that FCR values were impaired with diets containing more than 10% RB and suggested that the FCR and tibia ash were more sensitive than weight gain for detecting antinutritive factors in RB.

No significant ($P>0.05$) differences were observed in FCR among different groups as far as phytase supplementation was concern. However, the results of the study shows an uptrend of FCR value. These results are in accordance with the findings of Zhang et al., (1997) who reported that FCR values in broiler were improved ($P>0.05$) when 0.5% crude enzyme was added to a diet containing 40% RB. The author however, found the beneficial effects of enzymes (0.2 and 0.5%) especially for the chicks, 7-14 day-old that were fed 25% RB. This results were also in

agreement with Farrell and Martin, (1993) who found addition of 1000 units/kg phytase to duck finishing diets improved the FCR at three levels of rice bran (0, 30 and 60%). In broilers, Jalal and Scheideler, (2001) found that addition of phytase improved feed conversion ratio. Contrary to this finding, Farrell, (1994) reported that the addition of phytase gave a significant increased in growth rate and feed intake but not FCR when the data was analysed from those diets containing 40% RB.

Egg Production

The data of production performance parameters for the entire trial period show that the laying rate of hens fed rice bran based diets resulted in a significant ($P<0.05$) reduction in egg production compared to the control diet (Diet 1). This indicated that the plant phosphorus from different portions of rice bran diet is not sufficient for laying hens. This result is in agreement with Karunanjeewa and Tham, (1980) and Hamid and Jalaludin, (1987) who reported constantly reduced egg production when hens were given diets containing from 12.5 to 28.5% RB. Contrary to these findings, Majun and Payne, (1977) and Din et al., (1979) reported no effect of RB on laying performance when it formed <40% of the diet. The results of this study indicated that the amount of phytase supplementation in the 35% RB diet (Diet 4) had a marked effect on the performance of Lohamnn Brown laying hens at different ages. Furthermore, supplementation of the Diet 4 (35% RB) with microbial phytase (1050 or 1400 I.U phytase/kg diet) improved the hen-day egg production of layers (86.7% and 88.2%, respectively) to be comparable to that the production (88.9%) of birds on the control (0% RB) diet. These observations are in

line with those of Simon and Versteegh, (1993) who noted increase in egg production when phytase was added to layer diets. This possibly highlight an additional role of phytase in growth promotion. Hydrolysis and subsequent utilization of phytate-associated nutrients including proteins, lipids, carbohydrates, and minerals (including divalent cations such as Cu, Zn, and Ca) may be involved in these growth improvements (Ravindran et al., 1995a).

Despite inclusion of RB in laying hens up to level of 35% significantly reduced ($P<0.05$) the egg production, the effect, however was not detrimental. The overall laying pattern of hens fed on the RB up to 35% was comparable to the Lohmann Brown standard performance (Figure 5.1).

Egg Quality

The results of the overall period indicated that egg weight, egg mass, albumen height and yolk colour are superior in birds on the control (0% RB) diet over other dietary treatments. Based on the overall egg weights, egg mass and albumen height results obtained herein, it showed that there was no significant effect of RB or phytase level on these parameters. Inclusion of RB effect was not statistically significant on eggshell thickness. On the other hand, among the egg quality characteristics, the eggshell thickness showed a significant increase ($P<0.05$) as the level of phytase increased. Phytase supplementation at a level of 1400 I.U kg^{-1} to the 35% RB diet increased eggshell thickness to the level significantly ($P<0.05$) higher than the respective unsupplemented 35% RB diet or the control diet (Diet 1) fed groups. This may be due to liberation of inorganic P

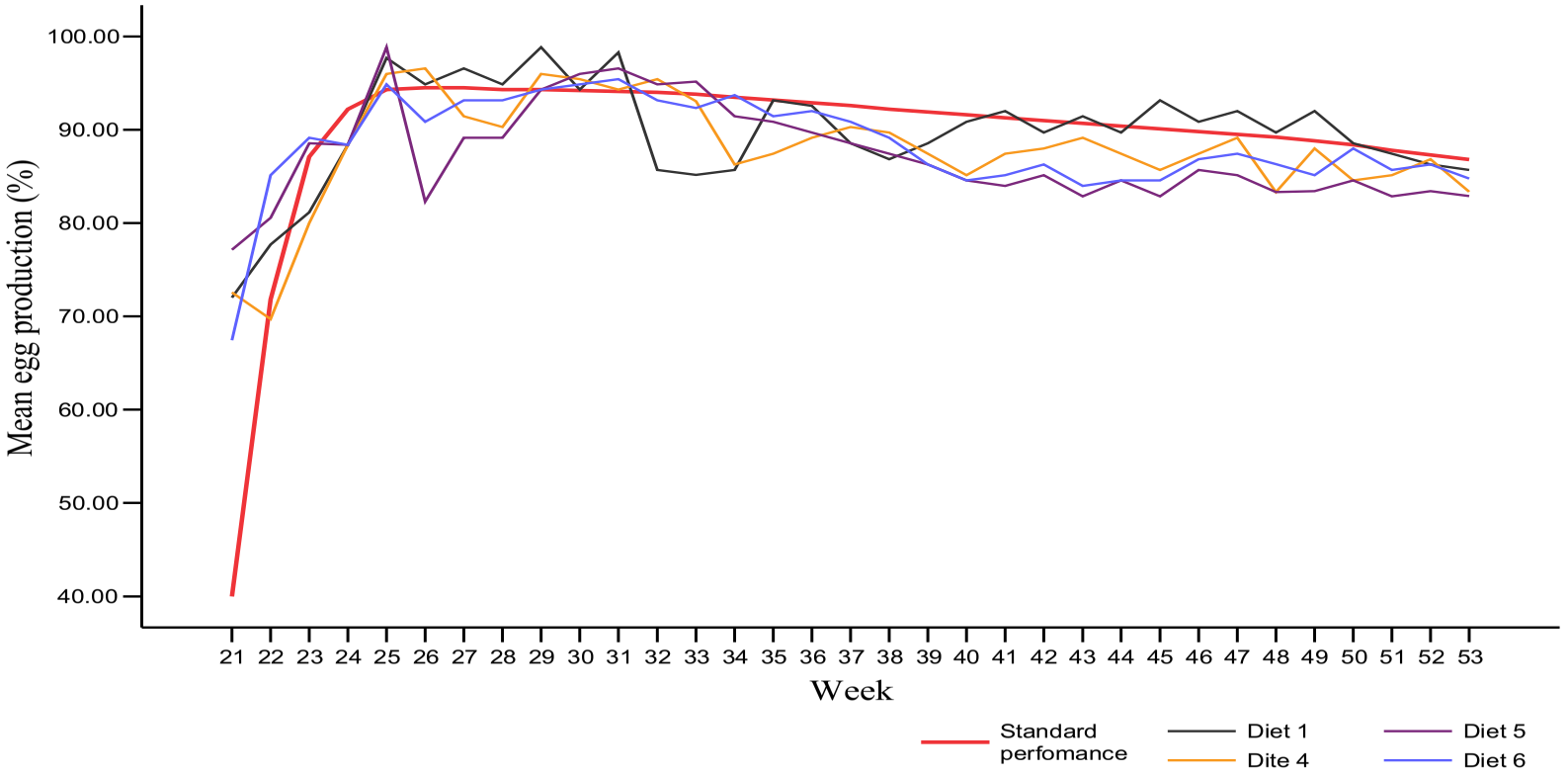

Figure 5.1: Egg production (%) of Lohmann Brown fed 35% of RB with or without phytase supplementation as compared to standard performance (The standard performance adapted from the Lohmann Brown Management Guide, 2005).

and Ca from the phytate molecular by supplemental enzyme. Among the egg quality characteristics, the yolk colour showed a significant decreased ($P<0.05$) as the level of rice bran increased. Based on the yolk colour index results presented here are in agreement with Majun and Payne, (1977) who reported that yolk colour was adversely affected on diet containing RB. The higher yolk colour score was observed in eggs from layers fed in the maize diet (control diet) compared to the other rice bran diets owing to the higher colour content of xanthophylls (a pigment that imparts a golden yellow colour to egg yolk) in maize than in rice bran. Supplementation of basal Diet 4 (35% RB) with phytase either at 1050 or 1400 I.U kg^{-1} was found ineffective in influencing yolk colour. There were no significant differences in the crude protein content, the ether extract, and ash content of egg albumen regardless of the RB inclusion levels or phytase addition. The results of external and internal quality characteristics demonstrated that in spite of some variation in the amount of RB and phytase in the diets, it did not, in any way, affects qualities of the egg albumen and yolk chemical content.

Digestability of Nutrients

No significant difference was observed in excretion of Ca, Zn and Fe regardless of RB inclusion or phytase supplementation levels. While the excretion of P and Cu was significantly higher ($P<0.05$) for birds offered the 35% RB basal diet. The same diet but supplemented with 1050 I.U of phytase, the Cu excretion was not significant ($P>0.05$) as compared to negative control diet (0% RB). However, supplementing the 35% RB based diet with 1400 I.U of phytase significantly ($P<0.05$) reduced the amount of P and Cu excretion to level similar to the negative

control diet. The feed nutrient digestibility experiment using the 35% RB based diet showed that in comparison to control group, in the experimental groups the digestibility of calcium decreased ($P>0.05$) by 4.7, and 2.8% for Diet 4 and Diet 5, respectively while the calcium digestibility of the bird group fed on Diet 6 was 1.7% larger ($P>0.05$) than those of the control group.

As shown in Table 5.1, the percentage P digestibility (46.1) and analyzed P (4.92g/kg) in the positive control diet gives digested P at 2.27g/kg; the difference-undigested P at 2.65g/kg-would be available for hydrolysis by the added microbial phytase. Given that the addition of 1050 or 1400 units of microbial phytase to the positive control diet reduced digested P from 2.65 to between 2.64 and 1.99g/kg, it follows that the added microbial phytase hydrolyzed approximately 12.6% of the undigested P. As mentioned by Adeola and Sands (2003) the efficiency of the hydrolytic process is dependent on several factors such as phytase activity, form and location of phytate in feedstuffs, and conditions in the gastrointestinal tract. This dependency raises more research questions in the improvement of the efficiency of hydrolysis of the phytate molecule.

In conclusion, the diet consisted of 35% RB must be supplemented with phytase (1400 I.U/kg) in order to maintain hen-day egg production as found in those control hens. In comparison with the unsupplemented positive control diet, this microbial phytase was able to liberate approximately 12.6% more P from the undigested phytate-P as calculated from both nutrient digestibility and retention and thereby significantly reduce the excretion of P by the laying hens.

Table 5.1: Efficacy of phosphorus digestibility in hen fed 35% RB diet supplemented with phytase.

Parameters	Dietary treatment		
	Diet 4	Diet 5	Diet 6
Rice bran (%)	35	35	35
Phosphorous analysis content (g/kg)	4.92	4.92	4.92
Phytase I.U /kg	0	1050	1400
Digestibility of P (%)	46.1	46.23	59.43
Digested P g/kg	2.27	2.28	2.92
Undigested P g/kg	2.65	2.64	1.99

The results of this study also showed that no significant (P>0.05) differences in the digestibility of DM, CP, Ca, Zn, EE, and AME were observed between the dietary treatments regarding of RB inclusion and microbial phytase addition. Similar to the result of the present study, no change in DM digestibility was reported when low P rice bran basal (35% RB) diets were supplemented with phytase in 42-49 days old broiler chicks (Ukil 1999).

Addition of phytase (1050 or 1400 I.U/kg) to Diet 4 (35% RB) did not improve the digestibility of Ca. This finding was in agreement with Ukil (1999) who did not find any significant improvement in the digestibility of Ca of 42-49 day of age chicks by supplementing diet with phytase (350–1500 FTU of phytase). Phytase added at level of 1050 I.U kg^{-1} did not significantly (P>0.05) decrease P and Ca excretion. However, by increasing the level of phytase supplementation to 1400 I.U kg^{-1}, this able to decrease the P excretion significantly (P<0.05) but not Ca. The results of the current study also showed that P release from Diet 4 (without phytase) was 1.5 times greater than P release from Diet 6 (containing 35% RB with 1400 I.U of phytase) suggesting that phytin phosphorus has been hydrolysed by phytase. Such conclusion was reached by Farrell and Martin, (1998b) with rice bran. This conclusion in line with the finding of Leske and Coon, (1999) who reported that that addition of phytase significantly (P<0.05) increased the hydrolysis of phytate phosphorus and total P retention of DFRB in laying hens. Based in phosphorus excretion results presented here are in agreement with the previous studies (Van der Klis et al., 1997) who indicated that phosphorus absorption increased considerably from 22% (control) to 50% in laying hens by supplementing microbial phytase to diet. Furthermore, improvement in phosphorus

absorption by dietary supplementation with microbial phytase was previously shown by others in broilers (e.g Simons et al., 1982: Ukil, 1999).

Phosphorus digestibility was significant ($P<0.05$) different between the negative control diet and Diet 4 and Diet 5 (Diet 1 vs Diet 4 and Diet 5). The digestibility of phosphorus of hens fed Diet 6 was not significantly different ($P<0.05$) from control diet (Diet 1). The AME values were not different between the two control diets (Diet 1 and Diet 4), and the diets containing enzymes were similar to Diet 1 (Table 4.16). The digestibility of CP was not different between control diets, and both levels of Natuphos phytase produced a CP digestibility that was numericaly higher ($P>0.05$) than either of these controls. Dietary treatment had little effect on DM, Zn, and EE digestibiltities (Table 4.16) . Chickens consuming the negative control diet (containing no RB) had higher, but nonsignificant ($P>0.05$), values of digestibilities of DM, Zn, and EE than any of the chickens raised on the other dietary treatment diets.

The main effects of microbial phytase indicated that exogenous phytase had no influence on the ileal digestibility of overall amino acids except glycine and arginine. The efficacy of microbial phytase depends on both the amino acids sufficiency and protein source(s). Biehl and Baker, (1997) observed that phytase was not effective in liberating amino acids bound to phytate complex in corn peanut meal diet. On other hand utilization of amino acid in soybean meal diets were improved by exogenous phytase. Martin et al., (1998), in experiments with RB based diets in duckling noted that phytase had no significant effect on the digestibility of amino acids. They observed that RB inclusion consistently reduced

the digestibility of all the amino acids. The diets of the present study were adequate in amino acid especially lysine and methionine content (NRC, 1994). A part from the adequacy of amino acids and protein in the diet, the location of phytate in cereal grains and oil seeds vary among sources, which may explain the result found herein. Due to different location of phytate within feed ingredients, substances that increase the availability of nutrients bound to phytate may vary in their efficacy depending on the particular ingredients fed to the monogastric animals.

Although a 3.11% increase in AA digestability values from 16 essential AA was observed in the present study, we were unable to detect significant differences in AA digestibilities due to the phytase supplementation except for glycine and arginine. Bielh and Baker, (1997b) observed a 2% increase in true AA digestibility values for nine amino acids and cystine when 1200 U/kg phytase was included with corn soybean meal. Addition of 750 U/kg phytase to a corn - SBM diet for turkey poults yielded 1 to 29% improvement in AA digestibility (Yi et al., 1996). Similarly, nonsignificant effects of phytase on AID of most AA were noted in broiler chicks (Sebastian et al., 1997 and Zhang, et al., 1999) and pigs (Jongbloed et al., 1993). Only 2 indispensable amino acids, glycine and arginine, were shown to be affected by phytase addition and improvements in the digestibility. Dilger et. al., (2004) reported only improvements in apparent ileal digestibility of tryptophan and valine while Sebastian et al., (1997) noted improvements in apparent ileal digestibility only of methionine and phenylalanine . By contrast, Kornegay, (1996) using broiler chicks, observed a linear increase in AID of all AA except methionine and proline as phytase was increased from 0 to 750 FTU/kg. Dilger et

al., (2004) also observed increasing phytase levels from 0 to 1000 FTU/kg resulted in significant (linear $P<0.05$) improved in digestibility of nitrogen, phosphorus and most of amino acids.

CHAPTER VI

CONCLUSION

The literature concerning the effect of various levels of RB on the performance of laying hens is conflicting (Panda and Gupte, 1965; Mahadevan et al., 1975; Srichai and Balnave, 1981; Hamid and Jalaludin 1987; Stilborn and Waldroup 1990; Warren and Farrell, 1990a; and Din et al., 1979b). The finding of this study showed that RB could be included up to 35% in the diet of laying hens (21-53 wk of age) without any deleterious effects on body weights, mortality, feed intake, egg weight and the digestibility of the nutrients except Cu and P. However, the feed conversion ratio became poorer (increased) with the 35% RB inclusion in diet. This may be due to increased dietary fibre content. The results of this study also revealed the adverse effect of feeding RB to laying hens which were reflected in overall egg production and yolk colour.

Phytate phosphorus of RB has been identified as the major growth-limiting factor (Juliano, 1985). Phytate may render poor availability of minerals (Warren and Farrel, 1990a) and excessive excretion of phosphorus in faeces which can cause environmental pollution. In the present study, the egg production, eggshell thickness, feed conversion ratio, the digestibility of P and ileal digestibility of glycine and arginine were improved with higher level of supplemental phytase. The digestibility of DM, CP, Ca, P and AME were not significantly affected either by levels of phytase or inclusion of RB. However, the digestibility tended to increase with increasing dose of supplemental phytase. The main effects of phytase indicated that exogenous phytase had no influence on the ileal digestibility of

amino acids except glycine and arginine. Apart from the adequacy of protein and amino acids in the diet, location of phytate in cereal grains and oil seeds various among sources, which may explain the result obtained herein.

Any added enzyme activity should increase phosphorus digestibility substantially (Nelson et al., 1971). This was true only for phytase at the high level and confirmed the results of performance variables. The lower dose (1050 I.U) of phytase enzyme had no apparent effect, but 1400 I.U/kg of diet caused greater phosphorus digestibility. The 35% RB diet resulted in lower calcium retention than that of the 35% RB with phytase diet, likely because of the interactions that have been observed in digestibility of these two nutrients (Shafey et al., 1990; Huyghebaert et al., 1992). Calcium digestibility with the high dose of Natuphos phytase approached that of the negative control diet. We were unable to find reports of magnesium digestibility in the literature. In this trial, magnesium digestibility was probably too low and variable to give a useful information.

The positive effects of supplementing phytase to 35% RB diets at level 1400 I.U phytase activity/kg of feed significantly improved egg production (from 83.8% to 88.2%), decreased phosphorus excretion (from 53.9% to 40.6%) and calcium excretion (from 44.1 to 39.1%). Current results support a reduction in faecal P with the addition of phytase to the diet. The results obtained herein have substantial impact on environmental pollution. It is well known that large amount of undigested dietary P, a substantial amount of P is excreted via faces. The poultry manure is usually used in the land, ponds, and lakes as fertilizer, which is a common method of managing poultry waste. Excessive P levels in lakes, ponds,

and slow moving waterways can cause water pollution. These excessive P levels could be reduced from poultry manure by addition of phytase in the diet.

The most affected nutrient was calcium, which has been shown by others to be improved by phytase addition (Sebastian et al., 1996; Zanini and Sazzad, 1999). As the pH conditions within the intestinal tract are favorable for phytate-divalent cation complex formation, microbial phytase hydrolysis of phytate likely ameliorated these complexes and therefore allowed greater calcum utilization. Also, this observation is likely a response to the increased retention of P as an attempt by the bird to maintain a favorable ratio of these two nutrients for physiological normality.

Efficiency of the hydrolytic process is dependent on several factors such as phytase activity, form and location of phytate in feedstuffs, and conditions in the gastrointestinal tract (Adeola and Sands, 2003). This dependency raises more research questions in the improvement of the efficiency of hydrolysis of the phytate molecule. This, taken with the fact that pH is likely a contributing factor in the formation of phytate-protein complexes within the gizzard-proventriculus of the chicken (Adeola and Sands, 2003), suggests that this microbial phytase may not have liberated sufficient quantities of non-P nutrients to affect digestibility.

Based on the present findings the following conclusions can be made

- Using RB up to 35% in laying hens diet significantly ($P<0.05$) decreased the egg production and yolk colour.

- Supplementation with phytase at levels 1050 and 1400 I.U/kg^{-1} feed to the feed with 35% RB significantly improved ($P<0.05$) egg production but not yolk colour.
- Addition of phytase improved P utilization which is shown in the result of shell thickness and also improved the laying rate.
- Supplementation phytase phytase at levels 1050 and 1400 I.U/kg^{-1} feed also had significantly ($P<0.05$) reduced P excretion via faces which has a substantial impact on environmental pollution.
- Supplementation with 1400 I.U phytase generally increases the ileal digestibility of amino acids although in many cases are not significant ($P>0.05$). In glycine and arginine, it is significantly different ($P<0.05$) and addition of phytase brings the digestibility to normal.
- Economically when adding a high level of rice bran will reduce the cost of feed as the price of RB is cheaper than corn. Therefore, by adding a very little amount of phytase (1050 and 1400 I.U/kg^{-1} feed) in the feed to overcome the shortening due to the phytic acid, the cost is offset by reduction of feed cost.

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APPENDIX A

PROXIMATE ANALYSIS OF PROCEDURE

Determination of Dry Matter and Moisture

Apparatus:
1. Porcelain crucible with lids
2. Oven
3. Desiccators
4. Weighing balance with 0.0001 g accuracy

Procedure:

1. Crucibles were soaked and cleaned with detergent and rinsed with distilled water before dried over night at 105^0C.
2. Crucibles weight were taken and cooled in the desiccators and weight were taken (W1).
3. Approximately 3 g of samples were put in the crucibles and the weight were taken again (W2).
4. Crucibles with samples were covered with lids and kept in the oven set at 105^0C.
5. After 24 hours drying, the crucibles and samples weight were taken after cooled in desiccators for half and hour (W3).
6. Moisture and dry matter content were calculated by using the following formula:

$$\% \ Moisture = \frac{W_3 - W_1}{W_2 - W_1} x \ 100$$

Where,

W_1 = Dry weight of crucible

W_2 = Weight of fresh sample + crucible

W_3 = Weight of crucible + dry sample

% DM = 100 - % Moisture

Determination of Crude Protein (CP)

Apparatus:
1. Digestion tube with holding rack
2. Weighing balance with 0.0001 g accuracy
3. Kjedahl catalyst tablet
4. Concentrated sulphuric acid
5. Vortexer
6. Fume hood
7. Hydrogen peroxide
8. Volumetric flask
9. Deionised distilled water
10. Kjeltec Auto Analyser

Procedure:

1. Cleaned, dried and marked digestion tubes were placed on the holding rack.
2. About 0.3-0.5 g samples were put in the digestion tubes.
3. One Kjedhal catalyst tablet and 7.7 ml of concentrated sulphuric acid were added to the tubes and vortexed before putting under the fume hood.
4. The temperature of the fume hood was set at 100^0C and allowed heating for 30 minutes before increased to 150^0C and heat again for another 30 minutes.
5. When the ring like appearance was form, a few drop of hydrogen (H_2O_2) was added. The procedure repeated 3-4 times.
6. The heating block temperature was gradually increased to 400^0C and allowed heat until the digestion completed.
7. The digestion tubes with digested samples were cooled and the digesta transferred into 100ml volumetric flask.
8. The tubes were forcibly rinsed with deionised distilled water and transferred to the volumetric flask.
9. The volumetric flasks were cooled before pouring deionised distilled water up to the mark to get the final volume.

10. 20 ml of the diluted samples were placed in the tube of auto analyzer and the readings were recorded.
11. The CP content was calculated using the following formula:

$$\%\ Crude\ Protein = \frac{1.401\ x\ N\ x\ ML\ (Tit. - Blank)\ x\ Dil.\ x\ Factor}{S.W.D.}$$

Where,

N = Normality of the acid

Tit. = Titration Reading

Blank = Blank Reading

Dil. = Dilution Factor

Factor = 6.25

Determination of Ether Extract (EE)

Apparatus:
1. Extraction cups
2. Weighing balance with 0.0001g accuracy
3. Extraction thimbles
4. Petroleum benzene
5. Desiccators
6. Soxtec System

Procedure:

1. The extraction cups were cleaned, dried and cooled in desiccators and weighed (W_1).
2. Approximately 2 g of sample was weighed and then placed into an extraction thimble.
3. The thimbles were placed into the extraction unit together with the previously weighed extraction cups and 50ml of solvent (petroleum benzene) was added.

4. The samples were allowed to extract for the first 30 minutes in the boiling point by adjusting the indicator at "Boiling" position, rinsed for another 30 minutes in rinsing position and finally another 30 minutes for evaporation.
5. The cups were released and dried in the oven for 2-3 hours at 105^{o}C.
6. The cups were then cooled in the desiccators before taking the weight with the extract (W_2).
7. The following equation was used to determine the percentage of lipids.

$$\% \ Ether \quad Extract \quad = \frac{W_2 - W_1}{S.W.D.} x \ \ 100$$

Where,

W_1 = Weight of empty dry cup

W_2 = Weight of empty dry cup + Ether Extract

S.D.W = Sample Dry Weight

Determination of Crude Fibre (CF)

Apparatues:
1. 600 ml beaker
2. H_2SO_4
3. California Buchner funnel
4. Whatman 541 filter paper
5. 1.25% NaOH
6. Sintered glass
7. Ethanol
8. Diethyl ether
9. Desiccators
10. Weighing balance with 0.0001 g accuracy

Procedure:

1. Approximately 1.5 g of samples was placed in 600ml beaker and 150ml of 1.25% H_2SO_4 was added and heated for 30 minutes.
2. The contents of the beaker were filtered through a California Buchner funnel with Whatman 541 filter paper.
3. The residue was washed with hot water again to drain out all the acids and the filtrate was transferred into the beaker with the filter paper.
4. One hundred and fifty ml of 1.25% NaOH was added and allowed to boil for another 30 minutes and then filtered through a pre cleaned sintered glass crucible.
5. The filtrate was washed with warm water to drain out the NaOH and repeated at least three times, then washed with ethanol followed by rinsing using diethyl ether.
6. The sintered glass with residue was dried in the oven at 105^{o}C overnight and weighed (W_1) after cooling**.**
7. The crucible were then placed in the muffle furnace and ignited for 3 hours at 550^{o}C.
8. Upon ignition, the rucible were allowed to cool below 200^{o}C and then transferred from the furnace to the desiccators, allowed to cool and weighed (W_2).
9. The percentage of the crude fiber was calculated by using the formula below:

$$\% \ Fibre \ = \frac{W_1 - W_2}{Original \quad S.D.W.} \ x \ 100$$

Where,

W1 = Weight of sintered glass crucible + Dry fibre

W2 = Weight of sintered glass crucible + Ash

S.D.W = Sample Dry Weight

Determination of Gross Energy

The energy was determined by using the following formula:

$$Wstd = \frac{(HWstd + C_1 + C_2)}{\Delta\ tstd} \qquad [3.12]$$

Where,

$H = 6318\ cal / g$

$Wstd$ = Weight of benzoic acid

C_1 = ml of Na_2CO_3

C_2 = fuse used

$\Delta\ tstd$ = Change of temperatur e for benzoic acid

$\Delta\ sample$ = Change of temperature for sample

$$H = \frac{Wst\ x\ \Delta\ t(sample) - (C_1 - C_2)}{Weight\ of\ sample}$$

H = Energy value of sample

APPENDIX B

ELUENTS AND REAGENTS REQUIRED FOR DETERMINATION OF AMINO ACIDS

PICO TAG REAGENTS

Sodium acetate trihydrate
Triethylamine (TEA-Waters Part Number 88121 or equivalent)
Glacial acetic acid
Acetonitrile
Water (Milli-Q quality or equivalent)
Disodium hydrogen phosphate (Na_2HPO_4)
Phosphoric acid

Eluent A

1) Sodium acetate trihydrate 19g
2) Water 1 litre
3) 0.5 ml of TEA
4) Titration to pH6.4 with glacial acetic acid
5) Filteration of the solution
6) 60 ml of acetonitrile and 940ml of prepared solution
7) Final volume 1 litre

Eluent B

1) 600 ml of acetonitrile in one cylinder
2) 400 ml of Milli-Q quality water in another cylinder
3) Mixing in two reagents
4) Degasing by sonicating under vacuum for 20 seconds.

Sample Diluent

1) 710 mg of sodium hydrogen phosphate
2) Water 1 litre
3) Titration to pH7.40 with 10% phosphoric acid
4) Mixing up the resulting solution with acetonitrile so that equals 5% by volume.

Required Eluents and Reagents

Amino Acid	Mol. Wt.	Amino Acid	Mol. Wt
Lysine	156.19	Glutamic Acid	147.13
Proline	115.13	Glycine	75.07
Alanine	80.09	Cystine	240.30
Valine	117.15	Methionine	149.21
Isoleucine	131.18	Leucine	131.18
Tyrosine	181.19	Phenylalanine	165.19
Histidine	155.18	Ammonium Chloride	53.18
Arginine	174.42	Aspartic Acid	133.11
Threonine	119.12	Serine	105.0

Calculation of Amino Acids

$$RRF = \frac{Peak\ area\ of\ \ AABA\ in\ standard}{Peak\ area\ of\ individual\ amino\ acid}$$

$$RRF\ of\ amoni\ acid = \frac{Peak\ area\ of\ individual\ amino\ acid\ in\ sample}{Peak\ area\ of\ \ AABA}$$

$$M\ mole\ of\ individual\ a\min o\ acid = Solution\ concentration\ x \frac{}{Dilution\ \ factor}$$

$$Mg\ amino\ acid\ /\ g\ sample = \frac{m\ mole\ amino\ acid}{Weight\ of\ \ sample}\ x\ Molecular\ wt.\ of\ \ AA$$

APPENDIX C

Table 1: Effects of diets containing various portions of rice bran (RB) with or without microbial phytase on body weights (BW), feed intake, and feed conversion ratio (FCR) measured in laying hens between 21 and 53 weeks.

Dietary Treatment	Rice Bran Content (%)	Phytase I.U/kg	Initial BW (21 weeks) g	Final BW (53 weeks) g	Feed intake g/bird/day	Hen-day Egg Production (%)	FCR g/dozen egg
Diet 1 (control diet)	0	0	1554.04 ± 17.90	1644.29 ± 18.51	117.30 ± 1.98	88.83^{b} ± 0.88	1.565^{a} ± 0.66
Diet 2	15	0	1593.48 ± 27.32	1650.96 ± 16.94	120.13 ± 1.58	84.75^{a} ± 0.10	1.687^{b} ± 0.02
Diet 3	25	0	1583.08 ± 20.68	1666.52 ± 17.92	122.41 ± 1.57	84.27^{a} ± 0.15	1.740^{b} ± 0.08
Diet 4	35	0	1592.44 ± 29.24	1677.42 ± 17.33	121.39 ± 1.81	83.82^{a} ± 0.16	1.684^{b} ± 0.04
Diet 5	35	1050	1589.84 ± 31.42	1624.42 ± 22.73	122.02 ± 1.74	86.66^{ab} ± 0.87	1.699^{b} ± 0.04
Diet 6	35	1400	1566.12 ± 14.91	1631.80 ± 20.11	119.34 ± 1.93	88.16^{b} ± 0.93	1.636^{ab} ± 0.04
SEM	-	-	9.85	7.79	0.727	0.430	0.020
Level significant			NS	NS	NS	P<0.05	

[ab]Mean (±SE) within a column having different superscripts are significantly (P< 0.05) different.
NS-Not significant.

Table 2: Effects of diets containing various levels of rice bran (RB) with or without microbial phytase in diets on egg production egg weight, and egg mass measured between (week 21-53)

Dietary Treatment	Parameter				
	Rice bran %	Phytase I.U/kg	Hen-day Egg Production (%)	Egg Weight (g)	Egg Mass (g/hen/day)
Diet 1	0	0	$88.83^{b} \pm 0.88$	$57.528^{a} \pm .66$	$51.63^{a} \pm .85$
Diet 2	15	0	$84.75^{a} \pm 0.10$	$57.027^{a} \pm .48$	$48.92^{a} \pm .64$
Diet 3	25	0	$84.27^{a} \pm 0.15$	$57.095^{a} \pm .54$	$48.86^{a} \pm .89$
Diet 4	35	0	$83.82^{a} \pm 0.16$	$56.949^{a} \pm .61$	$50.12^{a} \pm .89$
Diet 5	35	1050	$86.66^{ab} \pm 0.87$	$57.008^{a} \pm .40$	$49.77^{a} \pm .62$
Diet 6	35	1400	$88.16^{b} \pm 0.93$	$57.321^{a} \pm .33$	$50.62^{a} \pm .55$
SEM	-	-	0.430	0.208	0.311
Level significant			P<0.05	NS	NS

[1]Figures within parenthesis indicate percentage of rice bran.
[a,b]Mean (± SE) within a column having different superscripts are significantly ($P \leq 0.05$) different.
NS-Not significant.

Table 3: The effects of inclusion of different levels of rice bran with or without phytase supplementation in diets on egg quality (week 21 to 53).

	Diet 1(0)[1]	Diet 2 (15)	Diet 3 (25)	Diet 4 (35)	Diet 5 (35)[a]	Diet 6 (35)[b]	SEM	Level of significant
Egg shell thickness	$0.40^{a}\pm .00$	$0.40^{ab}\pm .00$	$0.40^{ab}\pm .00$	$0.40^{a}\pm .01$	$0.41^{ab}\pm .00$	$0.42^{b}\pm .01$	0.00	P<0.05
Albumen(mm)	9.78 ±0.43	9.65 ±0.19	9.84 ±0.17	9.78 ±0.17	8.99 ± 0.34	9.99 ±0.18	0.10	NS
Yolk colour (1-15)	9.76^{a} ±0.10	7.24^{b} ±0.12	6.06^{c} ±0.12	5.30^{d} ±0.13	5.39^{d} ±0.10	5.36^{d} ±0.11	0.12	P<0.05

[1]Figures within parenthesis indicate percentage of rice bran.

[a,b,c,d] Mean (± SE) within a column having different superscripts are significantly (P ≤0.05) different.

[a]Supplemented Natuphos® phytase at level of 1050 I.U

[b]Supplemented Natuphos® phytase at level of 1400 I.U

NS-Not significant.

Table 4: Mean ± SE of feed intake (g/day), and fecal excretion (g/day) and feed digestibility (%) of layers fed 35% RB based diet supplemented with two levels of phytase Natuphos®.

Rice Bran (%)	0 %	35 %			SEM
Phytase (I.U)	0	0	1050 I.U	1400 I.U	
Feed Intake	115.96[a] ± 4.07	120.60[a] ± 3.06	121.41[a] ± 2.85	118.76[a] ±3.72	1.70
Fecal Excretion	19.47[a] ± 0.43	27.62[c] ± 0.63	27.05[c] ± 0.51	24.84[b] ± 0.29	0.56

[a,b] Means (± SE) within a row having different superscripts are significantly ($P \leq 0.05$) different.

[a,b,c] Mean (± SE) within a raw having different superscripts are significantly ($P \leq 0.05$) different

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