



© 2010 Copyright by Islamic Azad University, Rasht Branch, Rasht, Iran Online version is available on: www.ijas.ir

### ABSTRACT

Rice bran (RB) is a major by-product, produced from the rice milling process. This study was conducted to evaluate the performance, egg quality, and blood biochemical parameters of 288 Hy-line laying hens fed diets with rice bran and commercial microbial phytase Natuphos<sub>®</sub> 5000. The experiment was a  $4\times2$  factorial one with four RB levels (0, 5, 10, and 15%) by two phytase levels (0 and 5000 FTU/kg diet). These diets were fed to experimental groups from 30 up to 42 week old. The results indicated that there were no significant phytase effects or rice bran levels, on hen-day egg production, feed intake, egg mass, yolk index, egg shell weight, egg shell thickness, haugh unit, specific gravity, and shell weight/surface area compared with control group. Inclusion of the RB or phytase in laying hens diets did not significantly influence biochemical parameters (cholesterol, triglyceride, uric acid, glucose, total protein and albumin), and cholesterol in yolk. The results suggested that supplementing 5000 FTU/kg diet of phytase enzyme for layers resulted in no further improvement; it was also indicated that use of RB at a dietary level is beneficial to the laying hens industry, up to 15% in Iran. Moreover, since the price of RB was cheaper than other major ingredients, dietary inclusion of rice bran (RB) would be beneficial.

KEY WORDS biochemical, layer hens, performance, phytase, rice bran.

## INTRODUCTION

Rice bran (RB), a by-product of rice milling is being used as a food ingredient in poultry and pig production. Choct (1997) reported that cereal by-products such as RB, are important feed ingredients in Asia, but their efficient use in monogastric diets is hindered by the presence of non starch polysaccharides (NSPs) and phytate. About 90% of the phosphorus in rice bran is in the form of phytic acid or phytate combined with several other minerals and only 18% of this is available.

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 In experiments with chicks, cereal grains have been replaced by RB, and it was found promising in certain substitutions (Dafwag and Shwarmen, 1996; Khalil and Hohler Henkel, 1997). Warren and Farrell (1990a) carried out an experiment with broiler chickens using various levels of defatted rice bran (DFRB). They found that substitution with DFRB at 7-21% in a basal diet improved growth and feed conversion ratio of broilers of 3-13 days old. The authors also reported that feed intake did not decline significantly until the DFRB content of the diet exceeded 20% of diet. They noted that according to inclusion rate, when all diets were balanced to be equal in nutrients, growth rate and feed conversion ratio were different from that of the control group.

Several experiments have been published in recent years demonstrating the efficacy of microbial phytase in laying hens fed diets based on corn (Van Der Klis *et al.* 1997; Gordon and Roland, 1998; Scott *et al.* 1999) or based on a

combination of wheat and corn (Kaminska, 1997).

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% full fat rice bran (FFRB).

Problems relating to the use of RB in poultry diets have been previously reported. In the case of broilers, Juanpere *et al.* (2005) indicated that addition of phytase (500 FTU/kg) to diets based on corn, wheat and barley increased apparent ME in corn diets.

Phytate not only reduces phosphorus availability, but also impairs the utilization of other minerals such as Ca, Fe, Zn, Cu and Co (Ravindran et al. 1995); it also has a negative effect on protein digestibility and energy utilization; probably due to inhibition of digestive enzymes including pepsin, trypsin and  $\alpha$ -amylase (Farrell, 1994; Ravindran et al. 1995). It has been shown that microbial phytase increase the availability of Phytate Phosphorus (PP) for swine and poultry (Qian et al. 1996). It also increases energy (Ravindran et al. 1999b) and amino acid digestibility (Ravindran et al. 1999b; Johnston, 2000) in diets for poultry. Gordon and Roland (1997) found an improvement in egg production, feed consumption, egg weight and eggspecific gravity. Francesch et al. (2005) found an improvement in egg production, weight gain, and feed consumption for hens fed a diet low in NPP with supplementary phytase when compared to hens fed a low NPP diet without supplemental phytase.

A major problem of RB, used for poultry, is its variation in chemical composition which may be associated with impaired performance for poultry (Farrell, 1994). Feeding laying hens with diets containing a high level of rice bran is common in rice-producing countries such as Iran. As a byproduct of the rice milling industry, rice bran is an attractive alternative ingredient for the feed industry due to its moderate nutritional quality and economics. With high phytate P content, rice bran is an excellent candidate for phytase application in feeds.

From the above discussion it appears that the issues regarding optimal inclusion rate and utilization of rice bran in laying hens needs further investigation. Since there is a limited information on the effect 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 to evaluate the effects of microbial phytase on production performance, egg quality and composition and biochemical parameters of layers feed diets containing different levels of rice bran.

## MATERIALS AND METHODS

### Experimental design and diets

Rice bran was obtained from a commercial processor

(North of Iran), and then it was ground in a hammer mill (Table 1). Four corn-soybean meal diets were formulated to produce various levels of Rice bran (0, 5, 10 and 15%). Each diet was supplemented with or without phytase enzyme (Natuphos<sub>®</sub>5000, BASF) and fed to the hens (*Hy*-Line W-36) in a factorial arrangement (Rice bran and enzyme supplement) resulting in a total of 8 experimental treatments. The feed rations used for poultry feeding are summarized in Table 2. Each treatment was assigned to 9 replicate cages with 4 hens per cage (n=36) for a total of cages. The composition of experimental diets and calculated analysis are shown in Table 1.

The control diet was formulated to meet nutrient requirements recommended by the Hy-Line W-36 management guide (Anonymous, 1998). Diets were formulated to be iso-nitrogenous and equal in essential amino acids, calcium, and phosphorus. Ingredients used in this experiment were from the same batch as in the first experiment. Hens were fed to the experimental diet for a 12-wk period, beginning at the age of 30-wk and continuing to 42-wk old. This experiment was conducted in an environmentally controlled room that was continuously maintained at 26 °C. The supplement used in this experiment, 5000 FTU/kg, is a microbial enzyme produced by *Aspergillus niger* and contains a minimum of 5,000 units of phytase per g (Natuphos<sub>®</sub>5000, BASF, activity determined by the manufacturer).

The supplement was incorporated into the feed formulations at a rate of 0.05%. Hens were exposed to a daily lighting schedule of 16L:8D.

All the birds were kept under uniform environmental conditions throughout the experimental period. Diets were presented in mash form and provided daily according to the expected intake and the hens had free access to water.

#### Measurements

Egg production was recorded daily and was incorporated into the feed three different locations (middle, broad and narrow end) using a micrometer gauge (Mitutoyo code 7027), and the mean value was calculated. Dried shells were ashed at 600 °C for 4 hours, and shell ash was analyzed for calcium and phosphorus content (AOAC, 1990). During each 28-day period, three eggs were randomly selected from each replicate for two consecutive days to determine the Haugh Unit using the method of Haugh (1937).

#### Hen parameters

Egg production (EP) was determined each day and was calculated on hen per day basis. Hens were given *ad libitum* access to 105 g of feed for every hen; unconsumed food was measured each morning. Feed conversion (FC) was calculated as the ratio of grams of feed to grams of egg produced per hen-day.

#### Table 1 Composition of laying hen diets (as fed basis<sup>1</sup>)

	Rice bran%					
	0 (R1, R1+E)	5 (R2, R2+E)	10 (R3, R3+E)	15 (R4, R4+E)		
Ingredient composition (%) <sup>2</sup>						
Corn grain	63.030	58.430	53.730	49.090		
Rice bran	0.000	5.000	10.000	15.000		
Soybean meal	23.760	23.210	22.690	22.150		
Vegetable oil	1.800	2.000	2.230	2.140		
Oyster shell	8.240	8.280	8.450	8.460		
Di calcium phosphate	2.040	1.980	2.010	1.980		
Salt	0.380	0.370	0.360	0.360		
Vitamin premix <sup>3</sup>	0.250	0.250	0.250	0.250		
Mineral premix <sup>4</sup>	0.250	0.250	0.250	0.250		
DL-Methionine	0.180	0.180	0.170	0.170		
Lysine-HCl	0.010	0.010	0.010	0.010		
Chemical composition (g/kg)						
Metabolizable energy (kcal/kg)	2800	2800	2800	2800		
Crude protein (%)	16.000	16.000	16.000	16.000		
Crude fiber (%)	3.050	3.480	3.910	4.430		
Calcium (%)	3.650	3.650	3.650	3.650		
Available phosphorus (%)	0.500	0.500	0.500	0.500		
Sodium (%)	0.180	0.180	0.180	0.180		
Lysine (%)	0.800	0.800	0.800	0.800		
Methionine (%)	0.450	0.450	0.450	0.450		
Methionine+systeine(%)	0.700	0.700	0.700	0.700		
Tryptophan (%)	0.230	0.230	0.230	0.230		

<sup>1</sup>Experimental diets contained different levels of rice bran (R1, R2, R3 and R4) were supplemented (R1-R4 and R1-R4+E) or not supplemented (R1) with a mixture of commercial.

<sup>2</sup>Dry matter content 900 g/kg. <sup>3</sup>Premix supplied per kg of diet: 9000 IU vitamin A, 1.78 mg vitamin B<sub>1</sub>, 6.6 mg vitamin B<sub>2</sub>, 30 mg niacin, 10 mg pantothenic acid, 3 mg vitamin B<sub>6</sub>, 0.15 mg biotin, <sup>4</sup>Premix supplied per kg of diet: 10 mg Cu, 0.99 mg I, 50 mg Fe, 100 mg Mn. 0.08 mg Se, 100 mg Zn.

Diet 1, 2, 3 and supplemented with phytase at 5000 IU/g (0.01% of Diet).

Table 2 Performance of laying hens fed Rice bran with or without enzyme supplementation

Variable	Egg weight (g/egg)	Egg production (%)	Egg mass (g/hen/day)	Feed intake (g/hen/day)	FCR (g feed/g egg)
Rice Bran (%)					
0	56.520	89.530	50.920	101.520	2.030
5	56.280	88.350	53.020	103.600	2.050
10	56.330	88.480	50.170	101.200	2.030
15	56.170	87.170	48.920	103.830	2.140
SEM	0.340	1.620	1.500	1.700	0.036
Enzyme (E)					
Without E	56.540	87.760	51.340	101.20	2.040
Phytase	56.110	89.000	50.170	103.850	2.080
SEM	0.255	0.990	1.060	1.200	0.025
Interaction (E×RB)	$NS^{a}$	NS	NS	NS	NS
SEM	0.510	1.980	2.130	2.410	0.050

<sup>a</sup> NS: not significant (P>0.05).

### Egg parameters

Eggs were saved 1 d each week to measure egg weight and egg specific gravity. Egg mass was calculated as a factor of egg weight and hen-day EP. Bi-weekly, 2 eggs per hen were saved to determine percentages of albumen, and yolk index. Yolk color was measured bi-weekly on one egg per cage (20 eggs/treatment) using a Roche color fan (Vuilleumier, 1969).

Every 28-d, 4 eggs per replicate (16 eggs per treatment) were individually weighed and the egg specific gravity (g/

mL) was also measured. During the 28-day periods, eggs from each dietary group (16 eggs) were taken to determine egg shell quality parameters.

Egg shell weight and shell thickness were determined by randomly collecting 4 eggs from each replicate. After the eggs were broken, the shells were washed and dried at the room temperature for determining the shell weight. Shell thickness was measured by a micrometer gauge (Measure, 24 21/1type) on three parts of shell from the equator of each egg. These measurements were pooled. Shell weigh/area

was also recorded, and shell ash was determined after being dried at room temperature for 3 days.

Blood samples were drained from the wing vein of all birds at the end of the experiment; serum was separated and used for biochemical assays. Serum cholesterol, triglyceride, total protein, albumin, uric acid and glucose were measured using the commercial kits on an auto-analyzer (Technicon RA-1000). Yolk cholesterol was extracted using the method of Folch *et al.* (1956) as modified by Washburn and Nix (1974), from 2 eggs of each replicate. Yolk cholesterol was determined by the colorimetric Libermann-Burchard method (Kenny, 1952).

#### Statistical analysis

All data were subjected to an ANOVA using the GLM program of SAS (Littell *et al.* 1991; SAS, 2002). The model used for all data included the use of the main effects of diet 4, phytase 2, along with interactions among these effects. Means for treatments showing significant differences in the analysis of variance were compared using Duncan's multiple range tests. All statements of significance are based on the probability level of 0.05.

## **RESULTS AND DISCUSSION**

Results of feeding the diets containing different levels of rice bran and phytase to layers are presented in Tables 2, 3 and 4. The results indicated that there were no significant differences between all the dietary groups regarding henday production, egg weight, egg mass, feed intake, and feed (P<0.05): The production performance of layers fed with phytase-supplemented diets was not significantly different from that of layers fed to the control diet without phytase supplementation (P>0.05).

No significant differences were observed among dietary treatments of 31 to 42 weeks in yolk index, egg shell weight, egg shell thickness, Haugh unit, egg specific gravity and shell weight/surface area (P>0.05) (Table 3).

The results of blood plasma and egg yolk cholesterol contents are presented in Table 4. Inclusion of rice bran in laying hens diets did not significantly affected egg yolk and blood plasma cholesterol (P>0.05). Despite phytase supplementation, inclusion of different levels of rice bran in diets significantly decreased Roche color compared to the control diet (P<0.05).

In many countries, different types of feeds are used depending on availability and local condition and there is a largely untapped potential for utilizing feed stuffs for poultry. In Iran, the most common feed is generally made of corn and soybean meal, which are expensive. Increasing cost of feed ingredients (grains, fish meal, oil seeds meals, etc.) has accelerated the search for cheaper substitutes. One such by-product is rice bran, a by-product obtained after milling brown or paddy rice and perhaps the most widely used agricultural by-product in the diet of animals used 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 fiber (CF) and phytate phosphorus (pP) contents. The experiment reported here was conducted to determine the effect of phytase on the productivity, egg quality and biochemical parameters of laying hens (Hy-Line) fed to the four different levels of RB and phytase.

The overall feed intake of Hy-Line laying hens in different groups for 12-wk period was not significantly different (P>0.05) due to the dietary treatments. The results of feed intake obtained in this study were consistent with the results of Karunanjeewa and Tham (1980) who reported that feed intake was constantly reduced in layers fed to the 20% RB diet. The results of this study, during overall experimental 12-wk period, revealed that phytase supplementation did not stimulate feed intake on the diets containing 5, 10 and 15% RB. The result of this study also showed that the overall efficiency of feed utilization (FCR) was 2.03 in layers fed on diet 1 (0% RB) while the least efficient feed utilization was 2.14 by the layers fed on diet 4 (15% RB). There was no significant difference among different experimental groups as far as rice bran inclusion concerned (P>0.05). Inclusion of rice bran the level of 15% in diet without phytase addition produced feed conversion ratio in laying hens poorer than those obtained with the basal (corn-soybean) diet. Data reported in the literature indicated that the FCR rates are usually increased (i.e. become poorer) as the content of RB in that diet increases. These results support the conclusions achieved by Warren and Farrell (1990b). They found that inclusion of 20% RB increased the FCR. The reason of poor FCR values of birds fed on RB in level 20% could be attributed to rancidity of the lipid fraction and other constituents in RB such as high fiber. However, in another experiment done by the same authors in which hens were the subject of the study, inclusion of RB up to 40% in diet, produced no change in FCR. Gallinger et al. (2004) also reported that FCR values were impaired in broiler chicks with diets containing more than 10% RB and suggested that the FCR and tibia ash were more sensitive than weight gain for detecting anti-nutritive factors in RB.

As far as phytase supplementation was concerned no significant differences were observed in FCR among different groups. However, the results of the study showed an uptrend of FCR value. These results concord with findings of Zhang *et al.* (1997) who reported that FCR values in broiler were improved (P>0.05) when 0.5% 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. Such results were not in agreement with those of Farrell *et al.* (1993), who found addition of 1000 FTU/kg phytase to duck finishing diets improved the FCR at three levels of rice bran (0, 30 and 60%). In the case of 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 make a significant increase in growth rate and feed intake (P<0.05) but it was not the case for FCR when the data were analyzed from those diets containing 40% RB.

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; Din et al. 1979b). This result might be related to higher biological value of rice bran and fat as reported by Khan (2004) as well. The data gathered from production performance parameters for the entire experimental period showed that the laying rate of hens fed to rice bran based diets led to a non-significant reduction in egg production compared to the control diet (P>0.05) (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 contrast with that obtained by Karunanjeewa and Tham (1980) and Hamid and Jalaludin (1987) who reported constantly reduced egg production when hens were given diets containing 12.5 to 28.5% RB. Similar to these findings were that of Majun and Payne (1977) and Din et al. (1979a) who reported that RB had no effect on laying performance when it formed <40% of the diet. The results of this study indicated that the amount of phytase supplementation in different levels of RB diet did not have a noticeable effect on the performance of laying hens. Furthermore, the improvement of RB levels to 15% in experimental diets did not change the hen's daily egg production of layers too much to be comparable with that of the production of birds on the control (0% RB) diet. These observations are in contrast with those of Simon and Versteegh (1993) who noted an increase in egg production when phytase was added to layer diets.

Simons and Versteegh (1993) noted that when phytase was added to layer diets, it had positive effects on egg weight. On the other hand, Boling *et al.* (2000) reported that no significant differences were observed on egg size, egg mass and egg specific gravity when laying hens fed to corn-soybean meal based diet supplemented with 100, 200, 250 or 300 FTU/kg of phytase/kg diet. Another study reported that supplementation of 0.1% NPP with phytase on laying hens fed to 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 egg shell quality. Several investigators reported beneficial effects of phytase supplementation on egg shell quality from (Punna and Roland, 1999), whereas, others did not observe any beneficial effect (Van Der Klis et al. 1997; Parsons, 1999). Casartelli et al. (2005) conducted an experiment to evaluate the effects of the phytase enzyme in diets formulated with different P sources on performance, egg shell quality and P excretion of commercial laying hens. Regarding phytase addition, the investigators noted that birds fed diets with no enzyme, and therefore with higher levels of the total P laid heavier eggs (P<0.05) in comparison with birds fed to the phytase supplementd with low levels of available P. They also noted that treatment accompanying with phytase improves egg shell quality (i.e. egg specific gravity and shell percentage). In this experiment Phytase-supplemented treatment groups were able to perform similarly to the control treatment group, suggesting that phytase source was effective in releasing phytate P to partially meet the P needs of the laying hen.

Based on the overall egg weights, egg shell weight (g), egg shell thickness (mm), haugh unit, specific gravity and shell weight/surface area results obtained herein, it was apparent that there was no significant effect of RB or phytase level on these parameters (P>0.05). Inclusion of RB was not significantly effective on Yolk index (P>0.05). Haghnazar and Rezaei (2004) reported that egg weight increased as the level of rice bran increased probably due to the elevated level of linoleic acid in the diet. In the present study this discrepancy was associated to provision of linoleic acid in all experimental diets.

The nutritional content of rice bran varies widely because of differences in hull content, degree of polishing during milling, and varietal differences in the grain. In some instances the oil is extracted during the milling process, while in others it remains in the bran where it contributes significantly to the energy content but also leaves the bran susceptible to potential development of rancidity. Reported energy values for rice bran vary widely, mostly because of wide variations of nutrient composition (IIussein and Kratzer, 1982; Zombade *et al.* 1982).

Phytase supplementation, at a level of 5000 I.U kg-1 to 5, 10 and 15% RB diet, had no effect on egg shell thickness. Among the egg quality characteristics, the yolk color showed a significant decrease (P<0.05) as the level of rice bran increased. The yolk color index results presented here agree with those of Majun and Payne (1977) who reported that yolk color was adversely affected by diets containing RB. Higher yolk color score was observed in eggs from layers fed in corn diet (control diet) compared to the other rice bran diets owing to the higher color content of xantho-

Dietary treatment	Yolk index	Eggshell weight(g)	Eggshell thickness (mm)	Haugh unit	Specific gravity	Shell weight/surface area
Rice Bran (TP)						
0	44.720	5.450	0.421	91.107	1.079	0.750
5	45.980	5.330	0.419	92.145	1.083	0.753
10	44.290	5.530	0.425	91.435	1.092	0.777
15	44.410	5.670	0.437	91.560	1.088	0.760
SEM	0.636	0.110	0.007	1.860	0.004	0.009
Enzyme (E)						
Without E	45.160	5.530	0.422	89.820	1.091	0.772
Phytase	44.550	5.520	0.429	93.300	1.079	0.750
SEM	0.450	0.078	0.005	1.3150	0.003	0.007
Interactiona <sup>a</sup> (E×TP)	NS	NS	NS	NS	NS	NS
SEM	0.900	0.156	0.011	2.630	0.005	0.001

### Table 3 Eggshell quality of laying hens fed Rice Bran with or without enzyme supplementation

<sup>a</sup> NS: not significant (P>0.05).

Table 4 Biochemical parameters (mg/dL) of laying hens fed Rice bran with or without enzyme supplementation

Variable	Cholesterol	Triglyceride	Uric acid	Glucose	Total Protein	Albumin
Rice Bran (RB)						
0	159.600	3231.200	4.800	220.000	6.370	2.970
5	184.370	3385.500	5.720	199.600	6.060	3.040
10	170.420	3080.200	6.070	181.300	5.040	3.015
15	172.750	3443.800	5.400	183.300	5.850	2.270
SEM	16.400	245.000	0.780	15.800	0.680	0.180
Enzyme (E)						
Without E	139.690	3154.7	5.570	195.760	5.120	2.700
Phytase	148.870	3050.7	5.430	196.350	6.540	2.940
SEM	9.500	180.8	0.550	11.200	0.415	0.124
Interaction effects (E×TP)	NS	NS	NS	NS	NS	NS
SEM	11.500	280.5	1.100	11.200	0.830	0.248

NS: not significant (P>0.05).

Values are means of eight observations per treatment and their pooled SEM.

	¢ 11	
Variable	Roche color fan (%)	Cholesterol in yolk
Rice Bran (RB)		
0	$4.200^{a}$	231.330
5	$3.850^{b}$	242.660
10	3.800 <sup>b</sup>	230.660
15	$3.740^{b}$	230.000
SEM	0.120	11.400
Enzyme (E)		
Without E	3.550	241.830
Phytase	3.850	234.660
SEM	0.150	7.400
Interaction (E×TP)	NS	NS
SEM	0.150	11.400

\*The means within the same column that have at least one common letter, do not have significant difference (P>0.05).

NS: not significant (P>0.05).

phylls (a pigment that imparts a golden yellow color to egg yolk) in corn than in rice bran. Supplementation of diets with phytase at 5000 FTU/kg was found ineffective in influencing yolk color. There were no significant differences in cholesterol in yolk regardless of the RB inclusion levels or phytase addition (P>0.05).

Cholesterol, triglyceride, uric acid, glucose, total protein and albumin were not significantly affected neither by levels of phytase nor inclusion of RB. Although the extent of phosphate with drawal practiced in this study is not at this point recommended commercially, hens consuming the rice bran with phytase performed as well as hens fed to the diets containing corn-soybean based without phytase.

# CONCLUSION

The findings of this study showed that RB could be included up to 15%, in the diet of laying hens (30-42 wk old) without any deleterious effects on egg weights, egg mass, egg production, feed intake and egg weight. Supplementation with phytase at level of 5000 I.U/kg feed to the feed with 5, 10, and 15% RB did not significantly improve egg production (P>0.05).

# ACKNOWLEDGEMENT

The authors acknowledge the assistance given by the farm at the Animal Research Institute, IAU, Maragheh Branch, with special thanks to Dr. Ahadi.

# REFERENCES

- Anonymous. (1998). Hy-Line variety W36 management guide. Hy-Line international, West Des Moines, IA.
- AOAC. (1990). Official Methods of Analysis. Association of Official Analytical Chemists, Washington, DC.
- Boling S.D., Douglas M.W., Shirley R.B., Parsons C.M. and Koelkebeck K.W. (2000). The effects of various dietary levels of phytase and available phosphorus on performance of laying hens. *Poult. Sci.* **79**, 535-538.
- Casartelli E.M., Jungqueira O.M., Laurentiz A.C., Filardi R.S., Lucas J.R.J. and Araujo L.F. (2005). Effect of phytase in laying hen diets with different phosphorus sources. *Brazilian J. Poult. Sci.* **7**, 93-98.
- Choct M. (1997). Enzymes in animal nutrition: the unseen benefit. Department of Animal Science, University of New England, Armindale, NSW2351, Australia. http://www.idrc.ca//books-/focus/821/chap5/html.
- Dafwang I.I. and Shwarmen E.B.N. (1996). Utilization of rice offal in practical rations for broiler chicks. *Niger. J. Anim. Prod.* **23**, 21-23.
- Din M.G., Sunde, M.L. and Bird H.R. (1979a). Effects of feeding plant by-products diets on growth and egg production. *Poult. Sci.* 58, 1274-1283
- Din M.G., Sunde M.L. and Bird H.R. (1979b). Measuring metabolizable energy with mature hens. *Poult. Sci.* 58, 441-445.
- Farrell D.J. (1994). Utilization of rice bran in diets for domestic fowl and ducklings. J. World's Poult. Sci. 50, 115-131.
- Farrell D.J., Martin E.A., du Preez J.J., Bongarts M., Betts M., Sudaman A. and Thomson E. (1993). The beneficial effects of a microbial phytase in diets of broiler chickens and ducklings. *Physiol. Anim. Nutr.* 69, 278-283.
- Folch J., Less M. and Solane Stanley G.H. (1956). A simple method for the isolation and purification of total lipid from animal tissues. *J. Biochem. Chemist.* **226**, 497-509.
- Francesch M., Broz J. and Brufau J. (2005). Effects of an experimental phytase on performance, egg quality, tibia ash con-

tent, and phosphorus bioavailability in laying hens fed on maize- or barley-based diets. *Br. Poult. Sci.* **46**, 340-348.

- Gallinger C.I., Suarez D.M. and Irazusta A. (2004). Effects of rice bran inclusion on performance and bone mineralization in broiler chicks. *J. Appl. Poult. Res.* [Abstr.].
- Gordon R.W. and Roland D. (1997). Performance of commercial laying hens fed various phosphorus levels, with and without supplemental phytase. *Poult. Sci.* **76**, 1172-1177.
- Gordon R.W. and Roland D.A. (1998). Influence of supplemental phytase on calcium and phosphorus utilization in laying hens. *Poult. Sci.* **77**, 290-294.
- Haghnazar A. and Rezaei M. (2004). To determine the metabolizable energy of rice bran and the use of it in layer ration. XII. World's Poultry Congress. 8-13 June, Istanbul.
- Hamid H. and Jalaludin S. (1987). Effect of rice bran on production performance of laying hens offered diets with two levels of energy and protein. In: Advances in Ani Feeds and Feeding in the Tropics (Eds. R).
- Haugh R.R. (1937). The Haugh for measuring egg quality. United State Egg Poultry Magazine, No. 43, p. 552-555 and 572-573.
- IIussein A.S. and Kratzer F.H. (1982). Effect of rancidity on the feeding value of rice bran for chickens. *Poult. Sci.* 61, 2450-2455.
- Jalal M.A. and Scheideler S.E. (2001). Effect of supplementation of two different sources of phytase on egg production parameters in laying hens and nutrient digestibility. *Poult. Sci.* **80**, 1463-1471.
- Johnston S.L. (2000). The effect of phytase on nutrient availability in diets for swine and poultry. Ph.D. thesis., Louisiana State Univ., Baton Rouge.
- Juanper J., Perez A.M., Venderll E. and Angulo-Brufau J. (2005). Assessement of potential interactions between phytase and glycosidase enzyme supplementation on nutrient digestibility in Broiler. *Poult. Sci.* 84, 571-580.
- Kaminska B.Z. (1997). Effect of supplemental phytase to laying hen diets of different phosphorus content. J. Anim. Feed. Sci. 6, 369-378.
- Karunajeewa H. and Tham S.H. (1980). The influence of triticale, rice pollard and dietary fatty acid on growth, egg size and productivity of white leghorn layers. Proceedings of 1980 South Pacific Poultry Science Convention, WPSA, Auckland. New Zealand, 36-43.
- Kenny P.C. (1952). The determination of cholesterol by the Liebermann-Burchard reaction. *J. Biol. Chem.* **52**, 612-619.
- Khalil D. and Hohler Henkel H. (1997). Utilization of rice bran and peanut meal in broilers. 1. Characterization of the feed efficiency of rice bran/peanut meal diet. *Arch. Gefluegelkd*. 61, 88-94.
- Khan A.D. (2004). Making rice bran a cereals alternative. *Feed Int. June.* Pp. 18-19.
- Littell R.C., Freund R.J. and Spector P.C. (1991). SAS\_System for Linear Models. 3<sup>rd</sup> Ed. SAS\_Series in Statistical Applications. SAS Inst., Inc., Cary, NC.
- Mahadevan P., Pandittesekera D.G., White J.S.L. and Arumugam V. (1975). The effects of tropical feeding stuffs on growth and first year egg production. *Poult. Sci.* 36, 286-295.

Majun G.K. and Payne C.G. (1977). Autoclave rice bran in layers'

diets. Br. Poult. Sci. 18, 201-203.

- Qian H., Kornegay E.T. and Denbow D.M. (1996). Phosphorus equivalence of microbial phytase in turkey diets as influenced by calcium and phosphorus ratios and phosphorus levels. *Poult. Sci.* 75, 69-81.
- Panda B. and Gupte S.M. (1965). Utilization of rice by-products in animal industry. *J. Food Sci. Technol.* **2**, 120-123.
- Parsons C.M. (1999). The effects of dietary available phosphorus and phytase level on long-term performance of laying hens. In: Layer Nutrition and Managemant. BSF Corporation, Mt. Olive, N.J. Pp. 24-33.
- Peter W. (1992). Investigations on the use of phytase in the feeding of laying hens, In: Proceeding XIX World's Poultry Congress, Amsterdam, Netherlands. Pp. 62.
- Punna S. and Roland D.A. (1999). Influence of supplemental microbial phytase on first cycle laying hens fed phosphorus deficient diets from day one of age. *Poult. Sci.* 78, 1407-1411.
- Ravindran V., Bryden W.L. and Kornegay E.T. (1995). Phytates: occurrence, bioavailability and implications in poultry nutrition. *Poult. Avian Biol. Rev.* 6, 125-143.
- Ravindran V., Cabahug S., Ravindran G. and Bryden W.L. (1999b). Influence of microbial phytase on apparent ileal amino acid digestibility of feedstuffs for broilers. *Poult. Sci.* 78, 699-706.
- SAS Institute. (2002). SAS User's Guide: Statistics. Version 9.1 Ed. SAS Institute Inc., Cary, NC.
- Scott T.A., Kampen R. and Silversides F.G. (1999). The effect of phosphorus, phytase enzyme, and calcium on the performance of layers fed corn-based diets. *Poult. Sci.* 78, 1742-1749.
- Simon P.C.M. and Versteegh H.A.J. (1993). The effect of the

addition of low doses of microbial phytase to layer feed on the technical results and skeleton and egg shell quality. Spelderholt publication No. 589 (NL).

- Srichai Y. and Balnave D. (1981). Egg weight response to dietary supplementation with sun flower oil and rice pollard. *Aust. J. Agric. Res.* 32, 183-188.
- Stilborn H.I. and Waldroup P.W. (1990). An evaluation of lowenergy feedstuffs in diets for laying hens. Anim. Feed Sci. Technol, Ainz. Feed Sci. Technd. 27, 327-339.
- Van Der Klis J.D., Versteegh H.A.J., Simons P.C.M. and Kies A.K. (1997). The efficacy of phytase in corn-soybean meal based diets for laying hens. *Poult. Sci.* 76, 1535-1542.
- Vuilleumier J.P. (1969). "Roche Color Fan" an instrument for measuring yolk color. *Poult. Sci.* 48, 767-779.
- Warren B.E. and Farrell D.J. (1990b). The nutritive value of full fat and defatted Australian rice bran II. Growth studies with chickens, rats and pigs. *Anim. Feed Sci. Technol.* 27, 229-246.
- Warren B.E. and Farrell D.J. (1990a). The nutritive value of fullfat and defatted Australian rice bran. 1. Chemical composition. Anim. Feed Sci. Technol. 27, 219-228.
- Washburn K.W. and Nix D.F. (1974). A rapid technique for extraction of yolk cholesterol. *Poult. Sci.* 53, 1118-1122.
- Zhang Z., Marquardt R.R., Guenter W. and Han Z. (1997). Effect of different enzyme preparations supplemented in a rye-based diet on the performance young broilers and viscosity of digesta and cloacal excreta. *Chinese J. Anim. Sci.* 34, 3-6.
- Zombade S.S., Lodhi G.N. and Lchhponani J.S. (1982). Evaluation of raw and parboiled rice bran. 11. Feeding value for broilers. *Indian J. Anim. Sci.* 52, 325-329.