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## Phytase Activity in the Grain of 6 Varieties of Wheat Cultivated in Kurdistan Province

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**Abstract:** Phytase improves the bioavailability of phytate phosphorous in plant foods and reduces phosphorous pollution of animal waste. Purpose of this study was to determine phytase activity in the grains of different varieties of wheat as a part of poultry diet. Grain samples were ground with mill and passed through 0.5 mm sieve, then mixed thoroughly and dissolved in food buffer. The top layer was filtered through PVDF filters. Definite volumes of filtrate dissolved in Dilution buffer and incubated with substrate for 60 minutes at 37°C. After 10 minutes centrifugation of all samples at 5000 RPM, absorbance of supernatants was read at 415 nm. Finally phytase activity of samples was determined using the calibration curve which has been made from enzyme samples with known phytase activity. This work showed the highest Phytase activity for the Sardary variety.

**Key words:** Enzyme, phytase, wheat, phytic acid, phosphorous, poultry diet

### Introduction

Phosphorus is required for bone mineralization, immunity, fertility and growth. Swine and poultry digest only about 40-30% of the P found in feed stuffs of vegetable origin, with the remainder being tied up in the form of phytic acid, which is inaccessible to the non ruminant animals (Ravindran *et al.*, 1995). On the other hand phosphorous from farm animal's waste has become an environmental issue (Sharpley *et al.*, 1993, 1994; Parry, 1998). Dietary addition of microbial phytase or the inclusion of high phytase ingredients in pig, poultry and fish diets is now well documented to release a large portion of the naturally occurring phytate P (Keshavarz, 2003; Dvorakova, 1998) and thus greatly reduce the amount of inorganic P that must be added to meet animal's P requirement. Main objective of this study was to evaluate phytase activity of some different varieties of wheat to find if any variety has higher activity.

### Materials and Methods

With some changes, the standard method for determination of phytase activity was used (Engelen *et al.*, 2001) in this study.

#### Solutions

**Diluted Nitric acid:** While stirring, slowly added 70 mL Nitric acid to 130 mL Water.

**Tween 20 solution:** Quantitatively transferred 10 g Tween 20 into 100 mL volumetric flask, dissolved in 80 mL H<sub>2</sub>O, diluted to volume with H<sub>2</sub>O.

**Dilution buffer:** 1.76 g 4 M acetic acid (24 mL diluted to 100 mL), 30 g Na acetate.3H<sub>2</sub>O and 0.147 g Ca chloride.2H<sub>2</sub>O transferred into 900 mL H<sub>2</sub>O in 1 L

volumetric flask. Adjusted to pH 5.5 by drop wise addition of 4M acetic acid. 1 mL Tween 20 solution (2), added and diluted to volume with H<sub>2</sub>O.

**Feed buffer:** 30 g Na acetate.3H<sub>2</sub>O and 0.147 g Ca chloride.2H<sub>2</sub>O transferred into 1 L volumetric flask, dissolved in 900 mL H<sub>2</sub>O and 0.1 g Tween 20 added. Adjusted to pH 5.5 by drop wise addition of 4M acetic acid and diluted to volume with H<sub>2</sub>O.

**Phytic acid substrate:** 8.40 g phytic acid quantitatively transferred into 1 L volumetric flask and dissolved in 900 ml dilution buffer. Adjusted to pH 5.5 by drop wise addition of 4M acetic acid and diluted to volume with H<sub>2</sub>O.

**Ammonium heptamolybdate stock solution:** 100 g ammonium heptamolybdate transferred into 1L volumetric flask and dissolved in 900 mL H<sub>2</sub>O. 10 mL NH<sub>4</sub>OH added and diluted to volume with H<sub>2</sub>O.

**Ammonium vanadate stock solution:** 2.35 g ammonium vanadate transferred into 1 L volumetric flask and dissolved in 400 mL H<sub>2</sub>O at 60°C. While swirling 20 ml HNO<sub>3</sub> (7+13) was added slowly, cooled to room temperature and diluted to volume with water.

**Color stop mixture:** 250 mL heptamolybdate solution and 250 mL vanadate solution mixed and added slowly, while swirling, 165 mL HNO<sub>3</sub> (7+13). Cooled to room temperature and diluted to volume with water.

#### Determination

**Preparation of calibration curve solutions:** 1 mg amount of Phytase containing standard sample

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Table 1: Serial dilutions for calibration curve

	Solution 1		Solution 2		Solution 3		Dilution factor	FTU/ml
	Stock sol./ml	Buffer/ml*	Solution 1/ml	Buffer/ml	Solution 2/ml	Buffer/ml		
A	0.05	4.95	0.1	3.4	0.25	1.75	1/14000	2.98
B	0.1	4.9	0.1	3.4	0.25	1.75	1/28000	5.95
C	A-1	2	0.1	3.4	0.25	1.75	1/42000	11/90
D	C-2	2	0.1	3.4	0.25	1.75	1/82000	17.86
E	D-2	2	0.1	3.4	0.25	1.75	1/168000	35/71

\*Diluting buffer

Table 2: Some measured data for studied different varieties

Parameter/Variety	Sar	Azr	Alv	Bcr	Shr	Zar
1000 Grain Weight (g)	38.50	36.50	46.50	42.40	38.50	42.20
Crude Protein (%)	11.81	11.98	11.90	11.46	12.60	12.42
Phosphorous (%)	0.23	0.22	0.28	0.28	0.26	0.31
Ether Extracted fat (%)	1.20	1.30	0.90	0.95	1.20	0.78
Crude Fiber (%)	2.40	3.00	3.20	2.60	3.30	2.80
Phytase activity (FTU/kg)	1232.00	1230.50	1217.00	1215.00	1091.00	1133.00

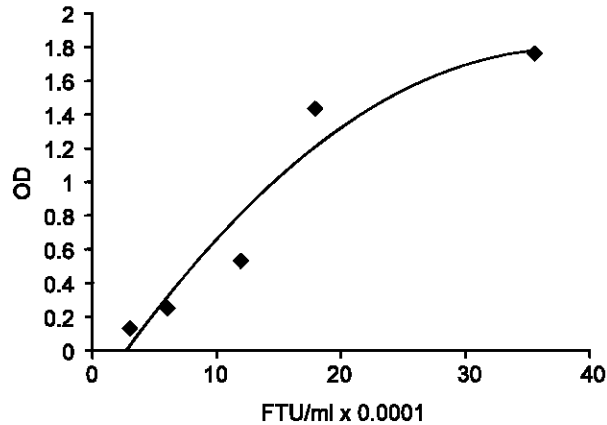


Fig. 1: Calibration curve for Phytase activity

weighted. Dissolved in dilution buffer and brought to volume by dilution buffer. Serial dilutions performed according to (Table 1).

**Test samples pretreatment:** 100 g of each sample ground with mill, until all material has passed through 1 mm sieve. Mixed thoroughly and 5 g weighted. 50 mL feed buffer added and stirred for 60 min. The top layer filtered through PVDF (pore size 0.45  $\mu$ m) filter over ice.

**Incubation: a) Standard/Samples:** Starting at time = 0 minutes, 4 mL phytic acid solution added to the first test tube. The tube put in a vortex mixer and thoroughly swirled contents. Next that tube placed into a water bath maintained at 37°C. They were incubated for 60 minutes. At regular time intervals, that procedure was repeated for each additional sample tube. After 60 minutes, 4 mL color/stop mix added and vortexed to terminate the incubation. After 15 minutes color developed.

**Standard/sample Blanks:** 4 mL color/stop mix added to the sample blanks, vortexed and placed those tubes on the bench. 4 mL phytic acid solution added to all sample blank tubes and vortexed. After 15 minutes color developed. All sample and sample/blank tubes centrifuged for 5 minutes at 5000 rpm. The absorbance measured at 415 nm with spectrophotometer, the instrument zeroed with water. In addition to phytase activity, some other parameters like raw protein and ... (Table 2) also have measured for the studied varieties.

### Results and Discussion

The presence of plant Phytase in some dormant seeds was demonstrated more than 50 years ago. Wheat, ray and barely were considered as being high in Phytase activity in contrast to oats and maize (McCance and Widdowson, 1944; Courtois and Perez, 1948). Recently there has been a renewed interest in levels of Phytase activity in various plant species and varieties in order to optimize dietary phosphorous supplementation and to reduce phosphorous excretion in areas of intensive chick production (Barrier-Guillot *et al.*, 1996). Using a standard sample with known Phytase activity from vetac company and serial dilution (Table 1) the calibration curve was drawn (Fig. 1). After processing of unknown samples their maximal absorption at 415 nm was determined and using the calibration curve, their enzymatic activity was revealed (Fig. 2). As that figure shows maximal activity was related to Sardaari (SAR) variant (1232 FTU/kg).

Table 3 shows some important ratios came from data's of Table 2. It can be seen FTU/Protein for Sardaari and Back cross (Bcr) variants samples is maximal. So Sardaari variety has a higher activity in equal amount of protein. This suggests a higher specific activity for those variants. Table 3 also shows Phytase

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Table 3: Some important ratios from comparison of studied varieties

Ratio/Variety	Sar	Azr	Alv	Bcr	Shr	Zar
FTU/kg/Protein	104.32	102.70	102.27	106.0	86.59	91.22
FTU/kg/P	5356.50	5593.20	4346.40	4339.3	4196.20	3654.80
FTU/kg/1000 GW*	32.00	33.40	26.00	28.7	28.30	26.80

\*1000 GW = 1000 Grain Weight

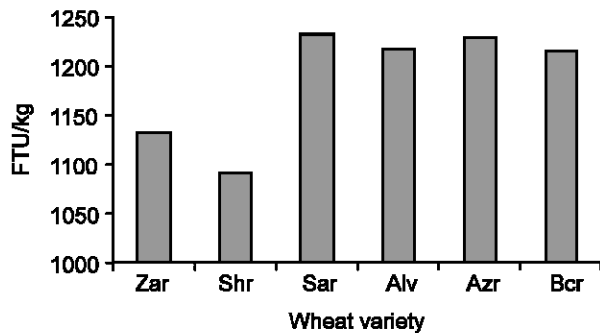


Fig. 2: Histogram of Phytase activity from different wheat varieties

activity/Phosphorous content for all varieties, with highest ratio for Sardaari and azar. This shows nutritional value for those varieties. Finally Table 3 shows Phytase activity/weight of 1000 grain and this ratio was also high for Sardaari.

Feedstuffs with high Phytase activity show large variations. Many factors could be responsible for the variation in Phytase activity, including the variety, the location of cultivation, fertilization, year, the duration and conditions of storage of the feedstuffs. The main source of variation in phytase activity is the variety effect. That was suggested in two early studies showing that hard wheat tended to express higher activities than soft wheat (Courtois et Pérez, 1948). We had in mind this fact in our studies and by fixing other effective parameters, just variety effect on phytase activity have been chosen as main parameter. These variations in e activity are of nutritional significance since large differences in available phosphorous for chickens and pigs have been previously demonstrated between various cereal and legume seed samples (Nelson, 1980; Cromwell, 1992). Results of this study suggests that Sardaary variety in equal situations has a significant higher activity for phytase and this could be a start point for further studies on this variety and its phytase both biochemical and genetically.

## References

- Barrier-Guillot, G.C., P. Casado, P. Maupetit, C. Jandreville and F. Gatel, 1996. Wheat phosphorous availability. 2. *In vivo* study in broilers and pigs; relationship with endogenous phytasic activity and phytic phosphorous content in wheat. *J. Sci. Food Agri.*, 70: 69-74.
- Cromwell, G.L., 1992. The biological availability of phosphorous in feedstuffs for pigs. *Pig News and Information*, 13: 75N.
- Courtois, J. and C. Perez, 1948. Teneurs en inositolphosphates et activitié phytasique de diverses graines. *Ann. Nutr. Alim.*, 2: 397-402.
- Dvorakova, J., 1998. Phytase : Sources, preparation and exploitation. *Folia Microbiol.*, 43: 323-338.
- Engelen, A.J., F.C. Vander Heft, P.H.G. Randsdorp and W.A.C. Somers, 2001. Determination of phytase activity in feed by colorimetric enzymatic methods: Collaborative Interlaboratory Study. *J. AOAC Int.*, 84: 629-633.
- Keshavarz, K., 2003. The effect of different levels of non phytate phosphorous with and without phytase on the performance of four strains of laying hens. *Poult. Sci.*, 82: 71-91.
- McCance, R.A. and E.M. Widdowson, 1944. Activity of the Phytase in different cereals and its resistance to dry heat. *Nature*, 153: 650.
- Nelson, T.S., 1980. Phosphorous availability in plant origin feedstuffs for poultry and swine. *Proc. Florida Nutr. Conf.*, pp: 59-84.
- Parry, R., 1998. Agricultural phosphorous and water quality. *J. Environ. Qual.*, 27: 258-261.
- Ravindran, V., W.L. Bryden and E.T. Kornegay, 1995. Phytates: occurrence, bioavailability and implications in poultry nutrition. *Poult. Avian Biol. Rev.*, 6: 125-143.
- Sharpley, A.N.T.C. Daniel and D.R. Edwards, 1993. Phosphorous movement in the landscape. *J. Prod. Agri.*, 6: 492-500.
- Sharpley, A.N.T.C. Chapra, R. Wedepohl, J.T. Sims, T.C. Daniel and K.R. Reddy, 1994. Managing agricultural phosphorous for protection of surface waters: Issues and options. *J. Environ. Qual.*, 23: 437-451.