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Comparative analysis of various naturally occurring substrates for high Phytase activity for use in Pig and Poultry feed

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Abstract
Research indicates potential alternative feed obtained from plant products and agricultural wastes for pig and poultry. A comparison between commercial feed and phytase rich ecofriendly natural products has been conducted. Phosphorus (P) is an important element in the feeding of pigs and poultry. It is necessary for bone development, high production level and overall development of animals. But of the total phosphorus taken in the form of diet one third of the P is present in the forage as inorganic P which is easily digestible and the other two thirds is present as organic P especially in the form of phytic acid and phytate. The phosphate stored in this form has twin fallouts first it is unavailable to the poultry and pigs and secondly it poses serious environmental threats.

Keywords: phytase, phytic acid, animal feed, inorganic phosphorus

1. Introduction
The poultry industry and other livestock operations are facing growing concerns about the land application of manure contaminating surface waters (Sharpley, 1999) [8]. Manure fertilizer for crops provides necessary, but often more than required nutrients for crop growth. The surplus nutrients, phosphorus being of greatest concern, may leach into watersheds and contribute to eutrophication (Kuhar *et al*., 2009) [5]. Phosphorus is an essential mineral for broiler chicken metabolism and skeletal development. Phytate phosphorus is either unavailable or poorly utilized by monogastric animals due to insufficient quantities of endogenous phytase enzyme that aids in digestion of the phytic acid complex (Angel *et al*., 2002) [1]. Phytic acid can also act as an anti-nutrientdue to the ability of the complex to bind starch, proteins and trace minerals, such as phosphorus, zinc, iron, calcium and magnesium (Radcliffe, 2002) [7]. The addition of phytate-degrading enzymes can improve the nutritional value of plant-based foods by enhancing nutrient digestibility through phytate hydrolysis during digestion in the gut (Konietzny and Greiner, 2002) [4]. Research has shown that the supplementation of exogenous phytase to broiler diets is an effective means for increasing the availability of phosphorus to the bird and reducing phosphorus excretion by liberating phytate bound phosphorus (Angel *et al*., 2002) [1].
The organically bound P can be excreted almost totally. The enzyme phytase is necessary to hydrolyze the organic bound phosphor. This enzyme is not or practically not produced in the alimentary digestive tract of pigs and poultry but does occur in some grains like wheat and barley. Various natural sources also contain high amount of phytase and could be used as feed for pigs and poultry.

2. Materials and Methods
Various naturally occuring substrates along with commercial available pig and poultry feed were tested for their phytic acid content in the laboratory. Amount of phytase enzyme produced using each substrate individually was determined and a comparison was made between these substrates and commercial used substrates.

Extraction and Determination of phytic acid from different substrates
Finely ground sample (0.5 gm) was extracted with 25 ml of 0.2 N HCL for 3 hr with continuous shaking in a shaker and filtered through Whatman No.1 filter Paper. An aliquot (0.5 ml) of above extract was mixed with 1 ml of ferric ammonium sulphate solution in a test tube followed by 30 minutes heating in a preheated boiling water bath. The contents of the test tube were mixed and centrifuged at 3000 rpm for 30 minutes. 1 ml of supernatant was transferred to another test tube & bipyridine solution was added. Absorbance was taken at 519 nm in spectrophotometer against distilled water.

Standard Graph
Standard curve was prepared using different concentration (0.2 to 1.0 ml) of standard sodium phytate solution containing 40-200 µg phytic acid.

Effect of different substrates on the growth of bacteria
1 gm of different substrates was taken in different conical flasks & 30 ml of distilled water was added in each flask. Flasks were autoclaved and 1% inoculums was added to each flask and incubated at 37°C at 200 rpm. After 3 days of incubation cultures were harvested and checked for total protein content and phytase activity. Protein concentration was determined by Lowry's method using bovine serum albumin as standard. Phytase activity was assayed by Heinonen and Lahti method.

Phytase Estimation
The extra cellular crude phytase was centrifuged at 10,000g

for 15 min at 4 °C in order to remove any particulate matter. The supernatant was used as crude phytase enzyme and stored at 4 °C for further studies. Quantitative assay for estimating released phosphate was done by using an assay mixture containing 2 ml of crude enzyme mixed with 2ml solution of sodium phytate (0.5 w/v) prepared in 0.15 M Tris-HCl buffer pH 6.5. The reaction was stopped by adding an equal volume 4 ml of 10% trichloroacetic acid after 30 min of incubation at 37 °C. The liberated phosphate ions were quantified by mixing 0.5 ml of above assay mixture with a colour developing reagent containing acetone-5 N H_2SO_4—2.5% ammonium molybdate (2:1:1). 0.1 M citric acid was added and mixture was incubated for 15-20 minutes. Absorbance was measured at 355 nm (Heinonen and Lahti, 1981) [3]. One unit of phytase activity was defined as the amount of enzyme required to liberate 1 µmole of phosphate per min under the assay conditions. The specific phytase activity was defined as U/mg of protein.

3. Results and Discussion

Observation Table for amount of phytic acid

Table 1: Amount of phytic acid in various substrates

S. No.	Substrate	Amount of phytic acid (in µg/gm)
1.	Buck wheat	133.66
2.	Coriander	150.50
3.	Green gram dal	187.75
4.	Maize	154.90
5.	Oat	128.90
6.	Pig feed	165.30
7.	Poultry feed	145.80
8.	Ragi	142.73
9.	Sesame oil cake	165.32
10.	Sorghum	147.15
11.	Sugarcane bagasses	150.50
12.	Sugarcane leaves	127.52
13.	Wheat bran	139.70
14.	Wheat whole	109.56

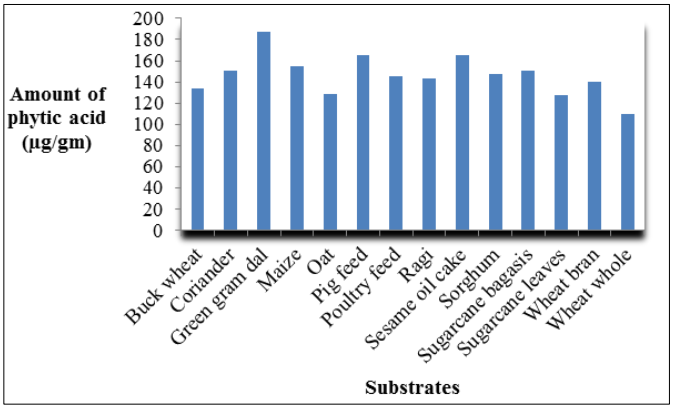

Fig 1: Amount of phytic acid in various substrates

Among various substrates tested for phytic acid content green gram dal accounts for maximum amount of phytic acid (187.75 µg/gm) followed by sesame oil cake (165.32 µg/gm) and commercial available pig feed contains 165.40 µg/gm of phytic acid.

Observation

Table 2: Effect of different Substrates used in culture media

S. No.	Substrate	Phytase activity (unit/ml)
1.	Calcium phytate	1.3223
2.	Buck wheat	0.9553
3.	Coriander	1.6723
4.	Green gram dal	1.8482
5.	Maize	0.7323
6.	Oat	1.009
7.	Pig feed	0.9228
8.	Poultry feed	0.7238
9.	Ragi	1.2438
10.	Sesame oil cake	0.8438
11.	Sorghum	0.8464
12.	Sugarcane baggases	0.9108
13.	Sugarcane leaves	0.9328
14.	Wheat bran	1.5667
15.	Wheat whole	1.7169

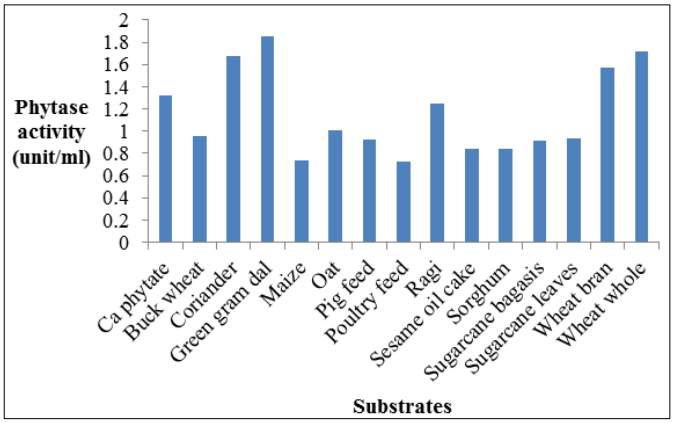

Fig 2: Effect of different substrates on phytase activity

From the above experiment it is clear that green gram dal has maximum phytase activity (1.8482 U/ml) followed by wheat whole (1.7169 U/ml) and coriander (1.6723 U/ml). These experiments show a high potential of these substrates as an alternative feed for animal livestock. Studies also reports use of different agricultural residues/wastes for bulk production of enzyme. As reported by Mandviwala & Khire (2000) different agricultural wastes like wheat bran, mustard cake, cowpea meal, groundnut cake, coconut cake, cotton cake and black bean were used for production of extracellular flour in solid state fermentation (SSF). *Aspergillus niger* NCIM 563 with maximum enzyme activity of (108 U g^{-1} dry mouldy bran, DMB) in cowpea meal. Studies on Environmental benefits of using plant products and agricultural solid waste also adds an extra advantage to their use as feed supplement.

4. Conclusion

Phytase enzymes have been studied both extensively and intensively and lot of sources of this enzyme have also been explored and discovered. But the physico-chemical and biochemical properties of some of the described phytases seem to be well known; especially the phytase produced by *Aspergillus sp*. Investigation in the field of molecular genetics yielded the possibility to overproduce this enzyme. Program aiming at improving the industrial production of phytase on a

cost-effective level should be emphasized because the efforts to use phytase in feed and food industries have been successful.

5. References

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