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DEGRADATION OF PHYTATE IN THE RICE BRAN BY *Aspergillus ficuum* AND ITS EFFECT ON THE BIOLOGICAL VALUE OF CALCIUM AND PHOSPHOROUS IN CHICKEN

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Abstract

The research was conducted to evaluate the effect of fermentation by *Aspergillus ficuum* on the biological value of Calcium (Ca) and Phosphorous (P) of rice bran in the chicken. In experiment 1, the phytase-producing Ability of *Aspergillus ficuum* was tested; the mold produced 2.529 Activity Unit (AU) of phytase when grown for 88 hours in rice bran medium. In Experiment 2, *in vitro* and *in vivo* studies using cock and broilers were carried out on rice bran before and after fermented utilizing *Aspergillus ficuum*. Fermentation of rice bran reduced phytate by about 83.25 %. *In vivo study* showed that the Ca and P retentions of fermented rice bran (55.65 % and 79.07 %) were higher than those of unfermented rice bran (42.02 % and 47.02 %); furthermore biological availability of P also increased from 0.157 % in unfermented rice bran to 0.527 % in the fermented one.

Key words: Phytate, *Aspergillus ficuum*, Phytase, Rice bran, Calcium, Phosphorous

Introduction

Rice bran, as other carbohydrate resource, is often used to compose chicken ration, instead of corn, due to the high energy, protein, vitamins, and several minerals contents. Several researchers indicated that the use of rice bran in the chicken ration was limited. In the commercial ration, rice bran is only used from 10 to 20 %, as it is able to inhibit growth rate and decrease biological utilization of certain minerals, especially on broiler and growing chicken. The reason is that it contains high crude fibre and phytate. It was reported that rice bran was composed of 1.44 % Phosphorous, in which 80 % in form of phytate (Halloran, 1980).

Phytic acid (myo-inositol hexa dihydrogen phosphate) and phytate minerals as $CaMg_5$-phytate is a phosphorous deposit in centre of rice; it can be digested by poultry if the phosphate are hydrolysed by phytate enzyme (phytase); whereas phytase activity in poultry especially chicken is not able to utilize this material in the body. Phytase hydrolysed phytate to be inositol and phosphorousic acid, therefore, it is able to increase phosphorous deposit in the body.

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Isolated phytase from other resources such as *Aspergillus ficuum* can be used for hydrolisation of phytate in the feed (Shieh and Ware, 1968). A research reported that *A. ficuum* produced phytase, and alpha-amylase (Hayashida and Teramoto, 1986); celobio-hydrolase (Hayashida *et al.*, 1988), beta-fructofuranosidase (Ettalibi *et al.*,1990), and inulinase (Carnniti *et al.*, 1991).

An alternative for degradation of phytate in this research is fermentation technology or metabolism process, in which phytase produced by *Aspergillus ficuum* will hydrolyse substrate (rice bran) to produce other chemical substances.

Materials and Method

Materials

The research used: IR 64 cultivar rice bran, cultivated *Aspergillus ficuum*, *Extract Toge Agar*, broilers and cocks.

Research methodology

The research was divided into two steps:

Preliminary research. This research addressed to study the ability of *A. ficuum* to produce phytase, when it was grown on rice bran medium (solid state fermentation). The process to obtain the enzyme is outlined in Figure 1. Enzyme activity was determined using a modified method recommended by Alltech Biotechnology Centre.

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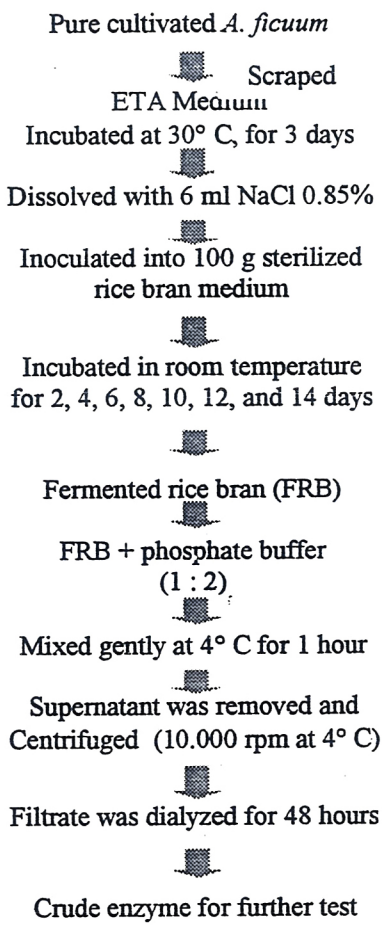


Figure 1. Fermentation process of rice bran by *A. ficuum* to obtain crude enzyme

Having been known that *A. ficuum* produced phytase enzyme in the rice bran substrate, the research was then continued to the main research.

Main research procedure. Steamed rice bran was inoculated utilizing 0.50 % *A. ficuum* inoculants, then fermented for 3 days in room temperature (ca. 28 ^{0}C). The fermented rice bran was dried at 50 ^{0}C for 24 hours, thereafter was ready for biological tests. Retentions of calcium and phosphorous were determined with 10 cockerels in each of fermented and unfermented rice bran using method conducted by Nwokolo *et al.* (1976).

Table 1. Composition and nutrient content of test diets

Ingredients	FRBD	uFRBD	D1	D2	D3	D4
	%					
Yellow corn	23.80	23.80	55.10	55.10	55.10	55.10
Soybean meal	35.60	35.60	38.00	38.00	38.00	38.00
Unfermented Rice bran	30.00	-	-	-	-	-
Fermented Rice bran	-	30.00	-	-	-	-
Palm oil	7.60	7.60	3.80	3.80	3.80	3.80
$CaCO_3$	2.32	2.32	1.92	1.19	0.46	-
Methionine	0.23	0.23	0.23	0.23	0.23	0.23
Lysine	-	-	0.04	0.04	0.04	0.04
NaCl	0.25	0.25	0.36	0.34	0.32	0.30
Premix	0.20	0.20	0.20	0.20	0.20	0.20
Tri-Ca phosphate	-	-	0.35	1.10	1.85	2.60
Nutrients						
Protein	21.24	21.24	21.30	21.30	21.30	21.30
F a t	12.11	12.11	6.20	6.20	6.20	6.20
Fiber	4.83	4.83	3.43	3.43	3.43	3.43
A s h	7.07	7.07	5.51	5.51	5.51	5.51
Calcium	0.90	0.90	0.90	0.90	0.90	0.90
Total Phosphorous	0.82	0.82	0.47	0.62	0.77	0.92
Available Phosphorous	tested	tested	0.15	0.30	0.45	0.60
Methionine	0.56	0.56	0.56	0.56	0.56	0.56
Lysine	1.19	1.19	1.19	1.19	1.19	1.19
T S A A	0.91	0.91	0.91	0.91	0.91	0.91
Triptophane	0.26	0.26	0.27	0.27	0.27	0.27
ME (kcal/kg)	3000	3000	3000	3000	3000	3000

The availability of phosphorous was determined using method recommended by International standards (Nwokolo et al, 1976). Minerals and chemicals composition were in the form of feeding trial. Six test diets were fed on 6 groups day old broilers that each consists of 32 birds for 3 weeks.

The phosphorous availability of fermented and unfermented rice bran was calculated referred to regression model based on the relationship between tibia ash percentage of broilers at 3 weeks of age and the logarithmic value of available phosphorous in the test diet D1, D2, D3, and D4.

Result and Discussion

Phytase producing ability of *Aspergillus ficuum*

Based on the preliminary research the phytase produced by solid state fermentation in rice bran medium referred to the following equation:

$$Y = 1.73\ X^{1.28} Exp.^{-0.35\ x} \quad \text{with } R^2 = 0.9724$$

The highest activity level of phytase was obtained at 2.529 Activity Unit with fermentation duration of 88 hours.

Compared to the research conducted by Shieh and Ware (1968), who cultivated Aspergillus *ficuum* in cornstarch, the activity was lower (2.529 vs 10.500 AU). The difference is clearly due to the medium. This reason is on the line with the research conducted by Upton and Fogarty (1977), who observed amylase and protease produced by *Trichoderma viridae*. There was a difference in enzyme activity produced by the same fungi if they were cultivated in the different medium. The other research was conducted by Refnita (1990); the result indicated that *Aspergillus niger* cultivated in Rice bran produced phytase with an activity between 1.66 – 2.88 AU, and fermentation duration between 72 – 120 hours.

However, the result shows that rice bran can be used as a medium for cultivation of *Aspergillus ficuum* to produce phytase.

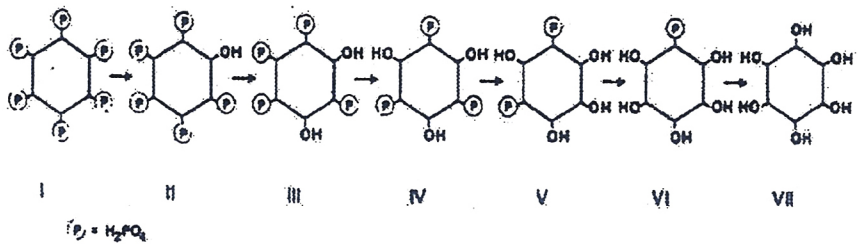
The effect of fermentation on biological changes of calcium and phosphorous

Table 2. Biological Value of Ca and P before and after fermentation

Biological Value	Before fermentation	After fermentation
	.....%	
Phosphorous Retention	47.020	79.070
Phosphorous Availability	0.157	0.527
Calcium Retention	42.020	55.650

Retention and availability of phosphorous

According to Cosgrove (1980) dephosphorousilation of phytic acid (myo-inositol hexa dihydrogen phosphate), chemically and enzymatically runs several steps and produces 5 levels intermediate products as illustrated below:



The illustration above shows that each step of dephosphorousilation process produces one molecule of free phosphate acid. Table 2 indicated that retention and availability of phosphorous of fermented rice bran was significantly higher than those produced from unfermented rice bran. Phytase was produced during

fermentation and then dephosphorousilized phytate. This process occurred in several steps with the final product of inositol and free phosphate, in which they can be utilized by monogastric animal. It will be understood that both retention value and bioavailability of phosphorous from fermented rice bran were higher than those produced by unfermented rice bran, while phytate in unfermented rice bran has not been dephosphorousilized by the enzyme.

Calcium retention

According to McCall et.al. (1953) and Houston (1972), phosphorous deposite in rice bran is in the form of phytin ($CaMg_5$-phytate). The minerals in phytin are insolvable and cannot be utilized by animals. Hydrolisation of phytin by phytase occured during fermentation, the final product of this process were free phosphorous, and also free calcium in which can be better utilized by the animal.

Conclusion

Based on the results, it can be concluded that rice bran can be utilized as a medium for cultivation of *Aspergillus ficuum* to obtain phytate enzyme (phytase). Enzyme activity obtained was 2.529 AU with fermentation duration of 88 hours. Fermentation rice bran by *Aspergillus ficuum* is able to increase retentions value of Calcium and Phosphorous; and the Phosphorous bioavailability as well.

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