Reduction of phytic acid and aflatoxin content to rice bran through fermentation by *Rhizopusspp*. combined with deproteinated chitin waste addition

Ahmad Sofyan,[1] Ema Damayanti, and Hardi Julendra

*Division of Feed and Animal Nutrition,Research Unit for Development of Chemical Engineering Processes (BPPTK),Indonesian Institute of Sciences (LIPI), Indonesia, Jl. Jogja-Wonosari Km. 31, Gading, Playen, Gunungkidul, Yogyakarta, 55861, Indonesia

ABSTRACT : The research was conducted to evaluate the effect of deproteinated-chitin waste (DCW) addition on rice bran fermented by *Rhizopus*spp.asan animal feed. Waste from deproteinated-chitin contain essential mineral as supported nutrient sources for inoculum growth. The experiment was designated into two treatments consisted of rice bran without fermentation/control (T0) and fermentation by *Rhizopus*spp.combined with addition of DCW (T1). Parameters measured were dry matter, ash, crude protein, crude fiber, ether extract and nitrogen free extract. Aflatoxin and phytic acid were also analyzed to evaluate antinutrient factors. Data were analyzed using least significant difference (LSD) to compare between treatment means. Results indicated that treatment affectedthe quality of rice bran. Phytic acid and crude fiber contents on rice bran treated by T1 decreased 35.1% and 38.4%,respectively, comparedwith control (T0). Aflatoxin also tended to decrease by treatment (T1) i.e. 89.4% less than control (T0). Ether extract of fermented rice bran increased from 10.5% to 16.9%, conversely nitrogen free extract reduced from 50.4% to 49.1%. Mineral content increased from 1.08% to 1.31% and 0.63% to 0.76%, respectively, for Ca and P contents, in which positively correlatedwith increasing ash. However, treatment did not affect crude protein content (17.6 vs.16.9%).It was concluded that combination treatment of DCW addition and fermentation using inoculum *Rhizopus*spp. improved rice bran quality.

Key words: aflatoxin, animal feed, phytic acid, Rhizopus spp., rice bran

INTRODUCTION

Quality of raw material of animal feed has a significant influence to support animal productivity. Feedstuff quality can be related with balance of nutrient composition and presence of anti nutrient compound. Anti nutrient factor contained animal feed could be ascribed from plant toxic compound and contamination during feed processing. Feed quality and safety are raised as global issue due to some of animal disease could be affected by anti nutrient factor. Phytic acid is plant toxic compound which containes rice bran and sorghum, whereas aflatoxin is not only naturally contained in feed grain but also affected by moulds contamination.

Aflatoxin is one of the most potent carcinogens and hepatotoxin (Lesson and Summer, 1997). Aflatoxins have also a high impact in both human and animalhealth, causing significant economic losses in the poultry industry,especially by diminution of avian growth, feed efficiency, and product quality. Aflatoxin affects the whole organism, particularlyliver and kidney(Martínez-de-Anda et. al., 2010). There are the numbers of effective preventive aflatoxin with the ammonia, hexane, hydrogen, hydrogen peroxide (Lesson and Summer, 1997) and with ozone generated by electrolysis (McKenzie et al. 1998).

Beside the presence of aflatoxin in feed, there are constrain of phosphorous availability. Phytic acid, myo-inositol hexakis (C6H18O24P6) are found in many plant tissues and related food products. Phytate constitutes 1–4% by weight of cereals and oil seeds and it is the primary phosphorus and myoinositol reserve in most seeds and usually accounts for 60–90% of the total phosphorus (El-Batal and Karem. 2001). Almost plant phosphorous in phytic-phosphorous complex which consequence in reducing bioavailability and increasing phosphor in manure (Lesson and Summer, 1997).

[1*] Corresponding author: ahmad.sofyan@lipi.go.id

However, almost those treatments are not economically. Feed processing technology using fermentation is the popular methods to increase quality of food or feed materials. Many research reported that fermentation has not only improve nutrient composition but also could prevent contamination. Feed contamination could be prevented by the bio-detoxification treatment using *N. corynebacteroides*fermentation (Tejada-Castaneda et al. 2008). Kusumaningtyas et al. (2006) had also reported that *Saccharomyces cerevisiae*, *Rhizopusoligosporus*or their combination was able to reduce aflatoxin content in feed.

In order to reduce aflatoxin contamination was also conducted by the combination of physical, chemical and biological treatments. However, it implies the additional cost or possibly the inefficiency fermentation processes. Due to some of reagent or chemical substances could inhibit inoculants or microbial growth whereas the high cost them. Liquid waste from deproteinated processing in the chitosan production is chemical substance containing essential mineral which is able to use as additive in biomass fermentation. The aims of this experiment to evaluate effect of deproteinated-chitin waste (DCW) addition on rice bran fermented by *Rhizopus*spp. as an animal feed.Waste from deproteinated-chitin contain essential mineral as supported nutrient sources for inoculum growth.

MATERIALS AND METHODS

Materials and Equipments

Materials used in this experiment were rice bran, inoculum (*Rhizopus* sp.) isolated from tempeh, waste from the deproteinated process of chitosan which contained essential minerals (Table 1), potato dextrose broth (PDB), distilled water and chemicals for protein and amino acids analyses. The equipments included thermometer, hygrometer, pH-meter, incubator, oven vacuum, spectrophotometer and high performance liquid chromatography (HPLC).

Table 1. Protein and mineral composition of liquid chitosan waste[1]

Nutrients	%
Protein	0.9065
K	0.0080
Na	2.4637
Cu	0.0002
Fe	0.0005
Mg	0.0008
Ca	0.0800
Zn	0.0006

[1]Source: Angwar et al. (2004)

Treatment and Fermentation

The experiment was conducted in3 stages, 1) preparation of inoculum (*Rhizopus* spp.) and deproteinatedchitin waste (DCW), 2) inoculation and incubation, 3) harvesting and drying. Inoculum was isolated from tempeh then cultured into PDB medium and incubated for 3-5 d. DCW was inserted into glass bottles and sterilized using an autoclave set in the temperature of 121^0 C for 15 minunder 2 atm of pressure.

Rice bran was prepared by weighing around 10 kg each fermentor as an experimental unit. Before inoculation, DCW was added intorice bran mixed with distilled water to make the mixture reached 40%. *Rhizopus* spp.was added at the rate of 5% into rice bran mixture when the temperature approached at 30^0C. Incubation was carried out at room temperature with a temperature range from 22to 32^0C and relative humidity of 75-90% during 7 d. After incubation, rice bran was fermented and

dried using oven at 70^0C for 8h. Samples were taken each treatment for proximate, phytic acid and aflatoxin analyses.

Variables

Variables measured were dry matter (DM), crude protein (CP), ether extract (EE), nitrogen free extract (NFE), crude fiber (CF), phytic acid and aflatoxin content of rice bran (RB) and fermented rice bran (FRB). DM, CP, EE, NFE, and CF were determined by proximate analysis (AOAC, 1990). Phytic acid content was measured by spectrometry methods (Davies and Reid, 1979).In order to analyze aflatoxin, sample was prepared adopting the method of Stubblefield and Shotwell (1981) and measured using HPLC.

Data Analysis

Data were arranged in a completely randomized design and analyzed statistically using Least Significant Difference method (Gomez and Gomez, 1984)

RESULTS AND DISCUSSION

Rice bran is one of the local feedstuff is commonly used for monograstric and ruminant diets. It is high content crude protein however has limiting factor as anti nutrient. The abundance of phytic acid contained in rice bran became to P-phytic complexesis unavailable formonogastric animals. Some of researcher concerned it to make the anti nutritional factor could be reduced. In this experiment, rice bran treated by combination of chemical treatment and fermentation. Chemical composition of rice bran before and after treatment showed on Table 2.

Table 2. Nutrient content of rice bran before and after fermentation

Variable	Control	Treated	Reduction (%)
DM, %	89.76± 0.00	93.03± 0.08	-3.71
Ash, %, DM basis	4.90± 0.10^b	6.83± 0.01^a	-39.35
EE, %, DM basis	10.46± 0.01^b	16.87± 0.19^a	-61.30
CP, %, DM basis	17.46± 0.05^a	16.89± 0.43^a	3.27
CF, %, DM basis	16.76± 0.02^a	10.33± 0.05^b	38.36
NFE, %, DM basis	50.41± 0.02^a	49.07± 0.30^b	2.66
Calcium, %, DM basis	1.08± 0.02^b	1.31± 0.00^a	-21.92
Phosphorus, %, DM basis	0.63± 0.00^b	0.76± 0.03^a	-20.65

^{ab}Within a row, means without a common superscript differ (P<0.05).

Based on Table 2, nutrient composition of RB fermented by *Rhizopus*spp. combined with DCW showed increasing EE, ash, Ca and P and decreasing CF and NFE. Moreover, CP content tended to be constant. CF could be reduced up to 38% by the chemical and fermentation treatment. In addition, mineral content of treated RB showed higher than that of control which was indicated by increasing value up to 20%. It implies the nutritional improvement for poultry feed.

Nutritional values improvement of RB was affected by chemical treatment and fermentation process. In addition, DCW containing alkali and mineral residue from chitin processing might affectstructural carbohydrate content in RB. Therefore, this condition and mineral residue of DCW became favorable to the fermentation processes by *Rhizopus*spp. Vadiveloo et al. (2009) reported that alkali (NaOH) treatment reduced structural carbohydrate of rice husk which was indicated by acid detergent fiber (ADF) values less than control.

Mineral avaibility from DCW also influenced the growth of *Rhizopus*spp. DCW contained nitrogen or protein, macro and micro-mineral, which affected microbial growth. Žnidaršìč et al. (1999) studied that mineral supplementation and chitin addition to medium supported fungal

growth such as *Rhizopusnigricans* and enzyme production. Enhanced activity of microbial implied on fermentation of RB. Some of fungal species were reported having the ability to reduce phytic acid (Nair and Duvnjak, 1990; Mukesh et al. 2004; Kim et al. 2006) and prevent contamination of fungus producing toxin(Kusumaningtyas et al. 2006;Tejada-Castaneda et al. 2008).

Referring to the data from Table 3, aflatoxin and phytic acid contents of RB could be reduced by the treatment. In addition, DCW and fermentation using *Rhizopus*spp. tended to decrease aflatoxin up to 89% and phytic acid up to 35%. It means that presence of *Rhizopus*spp. inoculum on RB could increase degradation of phytic acid and reduced activity of fungal producing toxin through competition mechanism.

Table 3. Aflatoxin and phytic acids content before and after fermentation

Variable	Control	Treated	Reduction (%)
Aflatoxin[nt]	11.55	1.22	89.44
Phytic acid	2.18 ± 0.05 [a]	1.41 ± 0.02 [b]	35.11

[abc]Within a row, means without a common superscript differ (P<0.05).
[nt]Not statistically tested.

Aflatoxin, the secondary metabolite, whichis produced by Aspergillusflavus (Yu et al. 2005) could be inhibited by *Rhizopus*spp. Occurrence of those microbes might compete *A. flavus*growth as aflatoxin producers. Those was caused by *Rhizopus*spp. excrete substances or secondary metabolites which was imply growth inhibition of *A. flavus*. Kusumaningtyas et al. (2006) also reported that *Saccharomyces cerevisiae*, *Rhizopusoligosporus* or their combination were able to reduce aflatoxin content in feed. The ability of*Rhizopus*to reduce aflatoxinwas related to the fast grow and compete with *A. flavus*.

Beside reduction of aflatoxin, phytic acid content in treated RB had also lower than that in control (1.41 vs 2.18%). El-Batal and Karem (2001) stated that phytic acid was reduced in rape seed meal by *Aspergillusniger*during solid state fermentation.Nairand Duvnjak (1990) reported that *Rhizopusoligosporus*had capability to reduce phytic acid content on canola meal. Reduction of phytic acid contained in RB was related to the phytase enzyme activity which was produced by *Rhizopus*spp.Pal Vig, and Walia (2001) had also reported that *Rhizopusoligosporus*not only reduced the phytic acid content up to 42.4%but also declined glucosinolate-sthiooxazolidonesand CFup to 43.1%, 34% and 25.5%, respectively in rapeseed.

Based on the result, it seems that this treatment is potentially to be applied in industrial scale to improve the nutritional quality of agricultural by-product, e.g. RB. The combination of processes have multiply impact on environmental contamination from the liquid waste from chitosan production and mineral losses to the soil water.

CONCLUSIONS

The use of waste from DCW was able to optimize the fermentation processes through the mineral supply, destruct the phytic acid and reduce the aflatoxin contamination. The combination treatment of DCW addition and fermentation using inoculum *Rhizopus*spp. improved RB quality.

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