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## Efficacy of Glucomannan-Containing Yeast and Glucomannan Extracted from *Amorphophallus oncophyllus* Against Aflatoxins in Broiler Chicken

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**Abstract:** The objective of the study was to evaluate and compare the efficiency of glucomannan extracted from *Amorphophallus oncophyllus* (GRE) with commercial aflatoxin binder glucomannan yeast product (GYP) in broiler chicken fed aflatoxin contaminated feed. A total of 63 one day old chicks were assigned to 9 equal groups according to dietary treatments: A (basal feed), B (basal feed+aflatoxin 50 µg/kg), C (basal feed+aflatoxin 2 mg/kg), D (basal feed+aflatoxin 50 µg/kg+GYP 1 g/kg), E (basal feed+aflatoxin 50 µg/kg+GRE 1 g/kg), F (basal feed+aflatoxin 50 µg/kg+GRE 2 g/kg), G (basal feed+aflatoxin 2 mg/kg+GYP 1 g/kg), H (basal feed+aflatoxin 2 mg/kg+GRE 1 g/kg) and I (basal feed+aflatoxin 2 mg/kg+GRE 2 g/kg). The body weight decreased significantly ( $p<0.05$ ) with the inclusion of aflatoxins in the diet at 2 mg/kg diet. Both GYP and GRE were found almost equally effective in combating the negative impact by improving the body weights. Feed intake and FCR of birds fed basal diet with aflatoxin at 2 mg/kg decreased significantly ( $p<0.05$ ) when compared to the control. Supplementation of both GYP and GRE improved the feed intake and FCR of broiler chicken. Aflatoxins at 2 mg/kg (group C) significantly ( $p<0.05$ ) decreased PCV but not at 50 µg/kg level. GYP and GRE supplementation helped to overcome the negative effect of aflatoxin by increasing PCV compared to group C. There was no significant ( $p>0.05$ ) effect on Hb, MCHC and glucose levels among various treatment groups. The weight of liver varied significantly ( $p<0.05$ ) among the treatment groups but no change in heart, spleen and kidney weights were observed. The highest increase in the relative weight of liver was seen in the group fed aflatoxins at 2 mg/kg level. Supplementation GYP and GRE helped in reducing the liver enlargement effect of aflatoxins. In conclusion, GRE proved to be an effective alternative to GYP particularly at the rate of 2 mg/kg in providing protection against aflatoxin contaminated feed in broiler chicken.

**Key words:** Aflatoxin, *Amorphophallus oncophyllus*, anemia, glucomannan, performance

### INTRODUCTION

Aflatoxin is the most prevalent mycotoxin produced by *Aspergillus flavus* and *A. parasiticus* (Deiner *et al.*, 1987; Kurtzman *et al.*, 1987). Aflatoxins consist of around 20 types with B1, B2, G1 and G2 commonly found in foods. Among these, B1 is an extremely hepato-toxic and carcinogenic compound (Girish and Devegowda, 2006). Aflatoxins occur as natural contaminants of poultry feeds (Edds and Bortell, 1983) and common signs of aflatoxicosis in poultry include apathy, anorexia with lowered growth rate, increased mortality, reduced weight gain, egg wt. and production (Oguz, 2012). They produce devastating effects on the general well-being and productivity of a poultry enterprise (Devegowda *et al.*, 1998a).

Extensive research has been conducted to prevent mycotoxicosis that mainly includes physical, chemical, nutritional and biological approaches. At present, to

control mycotoxicosis in poultry, adsorbents or binders are used (Surai and Dvorska, 2005). Commercial aflatoxin binders include glucomannan containing yeast products (GYP), hydrated sodium calcium aluminosilicate (HSCAS), zeolite, bentonite, kaolin and activated charcoal (Scheideler, 1993; Jindal *et al.*, 1993, 1994; Edrington *et al.*, 1997; Santurio *et al.*, 1999; Miazzo *et al.*, 2000; Rosa *et al.*, 2001). Glucomannan is a cell wall derivative of *Saccharomyces cerevisiae* which has been reported to have considerable binding ability against various mycotoxins (Aravind *et al.*, 2003; Devegowda and Murthy, 2005; Yildirim *et al.*, 2011). Source of glucomannan is not only the cell wall of *S. cerevisiae* but also tuber of *Amorphophallus oncophyllus* plant (Zhang *et al.*, 2001). Application of GRE and its derivatives have been extended greatly from food and food additives to various fields such as pharmaceutical, biotechnical and chemical industries (Vuksan *et al.*, 1999; Zhang *et al.*, 2005).

The *in vivo* efficiency of GYP against aflatoxins were reported extensively but there is lack of literature regarding effect of glucomannan extracted from *A. oncophyllus* (GRE) which has only been studied *in vitro* (Susanto *et al.*, 2014). Therefore, the present study was conducted with the objective to evaluate and compare the efficiency of GRE with commercial aflatoxin binder glucomannan yeast product (GYP) in broiler chicken.

## MATERIALS AND METHODS

**Materials used:** GYP used in the experiment was commercially available product Mycosorb® and glucomannan extracted with ethanol from the tuber of *A. oncophyllus* (GRE).

**Extraction of glucomannan:** Extraction of Glucomannan from *A. oncophyllus* Tubers of *A. oncophyllus* were collected from Sambit, Ponorogo, East Java, Indonesia. Skin of tuber was peeled and slices of pulp measuring 0.5cm thickness were obtained and oven dried at 80°C for 8 h. Dried slices were grinded and sieved through 100 mm mesh. Flour of *A. oncophyllus* obtained was boiled in glass with water at 30 ml/g flour at a temperature of 45°C with stirring for 1 h. Flour became gelly and was set aside in at a room temperature; filled with 96% ethanol (1:2), stirred and sieved again. The resultant extract was poured on aluminium foil and oven dried at 60°C for 48 h. Dried flour was grinded to obtain Glucomannan Extraction of *A. oncophyllus* (GRE).

**Aflatoxin production:** Aflatoxins were produced by Indonesian Research Centre for Veterinary Science on Bogor, West java, Indonesia. First of all Inoculum *Aspergillus flavus* was used in potato dextrose broth media. *A. flavus* grew and produced aflatoxins. Multi-step extraction methods with acetonitrile were used to extract aflatoxin from potato dextrose broth media. Resultant extract was separated for aflatoxins by column fractionation. Fraction that contained aflatoxin was purified and estimated by thin layer chromatography (TLC) as per the method of AOAC (Association of Official Analytical Chemists, 2005). TLC had aflatoxin detection limit up to 0.65 µg/kg. Aflatoxins in solid adsorbent of TLC were removed and diluted by Chloroform, centrifuged and supernatant containing aflatoxins was collected and dried in order to obtain pure aflatoxins.

**Broiler chicken and diets:** Sixty three one day old commercial broiler chicken were randomly divided into nine groups (A to I), each having 7 birds. The details of treatments include: A (basal feed), B (basal feed+aflatoxin 50 µg/kg), C (basal feed + aflatoxin 2 mg/kg), D (basal feed + aflatoxin 50 µg/kg + GYP 1 g/kg), E (basal feed + aflatoxin 50 µg/kg + GRE 1 g/kg), F (basal feed + aflatoxin 50 µg/kg + GRE 2 g/kg), G (basal feed + aflatoxin 2 mg/kg + GYP 1 g/kg), H (basal feed +

aflatoxin 2 mg/kg + GRE 1 g/kg) and I (basal feed + aflatoxin 2 mg/kg + GRE 2 g/kg). Feed and water were provided *ad libitum*.

## Data collection

**Performance:** The birds were weighed individually and feed consumption was calculated per replicate every day from first to fifth week. and feed conversion ratio was accordingly worked out.

**Haematological parameters:** Five birds per treatment were utilized and blood was collected by brachial pectoralis venipuncture. Blood was taken in EDTA tubes and was analyzed for Packed cell volume (PCV), Haemoglobin (Hb), Mean corpuscular haemoglobin concentration (MCHC) and Glucose at Laboratory of Pathology, Indonesian Research Center Veterinary Science, Bogor, Indonesia.

**Relative weight of visceral organs:** At 35 days of age, 5 birds in each treatment were humanely euthanized. Weights of liver, kidney, heart and spleen were recorded. The relative weights were adjusted to 1 kg live weight and means were calculated.

**Statistical analysis:** Data obtained were subjected to Analysis of Variance (ANOVA) using SPSS version 19. The means were compared using Tukey's test by considering the differences significant at  $p < 0.05$ .

## RESULTS AND DISCUSSION

Results of proximate analysis of basal diet revealed moisture 11.02%, crude protein 19.2%, crude fat 4.94%, calcium 0.99%, phosphorus 0.7% and gross energy 4.052 Kcal/kg. Feed ingredient used for formulating basal feed did not contain any aflatoxin.

The performance of broiler chicken fed aflatoxins, GYP and GRE is presented in Table 1. A significant ( $p < 0.05$ ) effect on the body weight was observed among various treatment groups with highest weight of  $1508.57 \pm 60.27$  g in the control group fed basal diet without the inclusion of aflatoxin, GYP or GRE. The body weight decreased significantly ( $p < 0.05$ ) with the inclusion of aflatoxins in the diet particularly in the group C wherein aflatoxin was used at 2 mg/kg diet. The results are in agreement with the other workers who also reported reduction in the body weight of chicken fed aflatoxin contaminated feed (Basmacioglu *et al.*, 2005; Girish and Devegowda, 2006; Wang *et al.*, 2006; Khaki *et al.*, 2012). With regard to GYP and GRE, both were found almost equally effective in combating the negative impact of aflatoxins as could be seen from the improved body weights in the birds fed GYP and GRE in the diet, thus proving that GRE also has the efficacy to trap aflatoxins in the gastrointestinal tract. Feed intake of birds fed basal diet with aflatoxin at 2 mg/kg decreased significantly ( $p < 0.05$ ) when compared

Table 1: Effect of GYP and GRE on performance of broiler chicken fed aflatoxin containing feed

Treatment						
Group	Aflatoxin	GYP (g/kg)	GRE (g/kg)	Body weight (g)	Feed intake (g/chicken)	FCR
A	-	-	-	1508.57±60.27 <sup>b</sup>	2587.74±111.77 <sup>b</sup>	1.77±0.05 <sup>a</sup>
B	50 µg/kg	-	-	1244.83±271.51 <sup>ab</sup>	2217.58±502.26 <sup>ab</sup>	1.86±0.06 <sup>ab</sup>
C	2 mg/kg	-	-	981.40±49.24 <sup>a</sup>	1959.03±170.32 <sup>a</sup>	2.09±0.18 <sup>c</sup>
D	50 µg/kg	1	-	1318.86±178.19 <sup>b</sup>	2316.47±350.55 <sup>ab</sup>	1.83±0.02 <sup>ab</sup>
E	50 µg/kg	-	1	1308.14±136.14 <sup>b</sup>	2303.56±253.85 <sup>ab</sup>	1.83±0.02 <sup>ab</sup>
F	50 µg/kg	-	2	1318.57±120.54 <sup>b</sup>	2300.35±235.91 <sup>ab</sup>	1.81±0.03 <sup>ab</sup>
G	2 mg/kg	1	-	1333.29±198.22 <sup>b</sup>	2494.19±352.507 <sup>ab</sup>	1.94±0.08 <sup>b</sup>
H	2 mg/kg	-	1	1310.71±139.50 <sup>b</sup>	2471.43±286.18 <sup>ab</sup>	1.96±0.16 <sup>b</sup>
I	2 mg/kg	-	2	1326.29±183.44 <sup>b</sup>	2460.88±358.81 <sup>ab</sup>	1.92±0.05 <sup>ab</sup>

Values within columns with different superscripts are significantly different (p<0.05)

Table 2: Effect of GYP and GRE on hematological parameters of broiler chicken fed aflatoxin containing feed

Treatment							
Group	Aflatoxin	GYP (g/kg)	GRE (g/kg)	PCV (%)	Hb (mg %)	MCHC	Glucose (mg/dl)
A	-	-	-	27.75±1.7 <sup>a</sup>	7.5±0.8	25.23±2.57	192.0±53.33
B	50 µg/kg	-	-	27.75±1.7 <sup>a</sup>	7.5±0.6	27.09±2.55	166.0±18.92
C	2 mg/kg	-	-	20.5±2.5 <sup>b</sup>	6.0±0.8	26.04±2.08	164.7±86.7
D	50 µg/kg	1	-	28.5±4.1 <sup>a</sup>	6.75±1.0	26.08±6.45	189.2±7.14
E	50 µg/kg	-	1	25.5±1.5 <sup>ab</sup>	6.67±1.2	26.04±3.73	238.0±11.75
F	50 µg/kg	-	2	25.25±4.6 <sup>ab</sup>	6.75±1.0	27.79±5.57	243.0±52.4
G	2 mg/kg	1	-	20.96±11.0 <sup>ab</sup>	5.18±2.8	24.04±1.36	184.0±42.52
H	2 mg/kg	-	1	28.5±6.6 <sup>a</sup>	6.75±2.6	23.17±7.03	198.5±53.6
I	2 mg/kg	-	2	27.25±1.0 <sup>a</sup>	7.25±0.5	26.63±3.06	185.7±42.27

Values within columns with different superscripts are significantly different (p<0.05)

Table 3: Effect of GYP and GRE on relative visceral organ weights (g/kg LBW\*) of broiler chicken fed aflatoxin containing feed

Treatment							
Group	Aflatoxin	GYP (g/kg)	GRE (g/kg)	Liver	Heart	Spleen	Kidney
A	-	-	-	27.37±4.47 <sup>a</sup>	5.03±0.98	3.16±1.33	9.39±0.73
B	50 µg/kg	-	-	26.15±3.58 <sup>a</sup>	6.05±0.99	2.23±0.44	8.48±1.13
C	2 mg/kg	-	-	39.09±5.48 <sup>b</sup>	4.82±0.77	3.31±0.88	7.90±1.63
D	50 µg/kg	1	-	31.28±8.63 <sup>a</sup>	5.49±1.54	3.26±2.00	8.36±1.49
E	50 µg/kg	-	1	27.59±3.41 <sup>a</sup>	5.09±0.85	2.64±1.09	7.98±1.06
F	50 µg/kg	-	2	29.96±7.11 <sup>a</sup>	5.00±0.83	3.32±1.19	8.91±1.14
G	2 mg/kg	1	-	32.91±5.70 <sup>ab</sup>	4.81±0.45	2.48±1.30	8.17±1.22
H	2 mg/kg	-	1	33.78±7.39 <sup>ab</sup>	6.35±1.46	7.01±10.32	8.41±1.35
I	2 mg/kg	-	2	22.14±14.15 <sup>a</sup>	5.82±1.25	7.82±9.24	7.50±0.94

Values within columns with different superscripts are significantly different (p<0.05). \*LBW: Live body weight

to the control. The reduction in the feed intake as a result of aflatoxins has also been reported by earlier worker (Kubena *et al.*, 1998; Afzal and Zahid, 2004; Girish and Devewgoda, 2006; Khaki *et al.*, 2012; Celik *et al.*, 2000; Kermanshahi *et al.*, 2009; Shabani *et al.*, 2010). Supplementation of both GYP and GRE improved the feed intake in broiler chicken. Aflatoxins also resulted in deterioration of feed conversion ratio (FCR) while as supplementation of both GYP and GRE resulted in improvement of FCR. Improvement in the FCR with the use of GYP has also been reported by Girish and Devewgoda (2006). Thus, the supplementation of GYP and GRE resulted in improvement in the overall performance of broiler chicken fed aflatoxin containing feed, thus confirming the reports of Kubena *et al.* (1993); Aravind *et al.* (2003); Basmacioglu *et al.* (2005) and Yildirim *et al.* (2011) who found similar results with the inclusion of GYP in the diet of broiler chicken.

The haematological parameters of broiler chicken fed aflatoxin, GYP and GRE based diets are presented in Table 2. Aflatoxins at 2 mg/kg significantly (p<0.05) decreased PCV but not at 50 µg/kg level. The reduction in PCV by aflatoxins has also been reported by other researchers (Tung *et al.*, 1975; Donmez *et al.*, 2012; Khaki *et al.*, 2012). Further, 50 µg/kg aflatoxin did not decrease PCV, thus confirming the reports of Khaki *et al.* (2012) that reduction in PCV of chicken would occur if fed contains minimal levels of aflatoxin as 0.5 mg/kg. Aflatoxins caused blood clotting disorders (coagulopathy) in birds as was seen by the hemorrhagic diarrhea in case of birds fed aflatoxin at 2 mg/kg diet. Bilgic and Yepyldere (1998) and Celik *et al.* (2000) also reported pethelial hemorrhages in liver and kidneys of broiler chicks fed aflatoxins. Moreover, GYP and GRE supplementation helped to overcome the negative effect of aflatoxin by increasing PCV. Low PCV is indicative of



Fig. 1: There are hemorrhagic diarrhea (a) and face of chicken doldrums (b) in group C (contaminated aflatoxin 2 mg/kg)

anemia and high PCV shows dehydration or polycythaemia (Lanza *et al.*, 1983; Campbell and Ellis, 2007). As GYP and GRE bind aflatoxins in the alimentary canal and reduce their entrance in the bloodstream, so there was no coagulopathy in the groups fed GYP and GRE supplemented diets. Further, there was no significant ( $p>0.05$ ) effect on Hb and MCHC among various treatment groups indicating that the anemia caused by aflatoxins was normochromic-normocytic type. Also there was no change ( $p>0.05$ ) in the blood glucose levels among various treatment groups including control.

The relative weight of vital organs of broiler chicken is presented in Table 3. The results revealed that only the weight of liver varied significantly ( $p<0.05$ ) among the treatment groups but no change in heart, spleen and kidney weights were observed. The highest increase in the relative weight of liver was seen in the group fed aflatoxins at 2 mg/kg level. Wang *et al.* (2006) also reported increased relative weight of liver in chicken fed mold contaminated corn ( $p<0.05$ ). Enlargement of liver is an indication of increased metabolic activity in detoxification of aflatoxins (Miazzo *et al.*, 2000; Anandkumar *et al.*, 2005; Girish and Devewgoda, 2006). Supplementation GYP and GRE helped in reducing the liver enlargement effect of aflatoxins. GRE at 2 g/kg was more protective than 1 g/kg against adverse effects of aflatoxin. Similar results of protection against liver enlargement with GYP against aflatoxins has also been reported by Girish and Devegowda (2006).

**Conclusion:** In conclusion, aflatoxins resulted in various adverse effects like decreased performance, normochromic-normocytic anaemia and enlargement of liver weight in broiler chicken. Supplementation with GYP and GRE helped to alleviate such adverse effects of aflatoxins. GRE proved to be an effective alternative to

GYP particularly at the rate of 2 mg/kg in providing protection against aflatoxin contaminated feed in broiler chicken.

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