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Research Article

Effects of *Saccharomyces cerevisiae* Yeast Supplementation on the Performance and Egg Quality of Laying Hens Feed Low-Dose Aflatoxin B1-Contaminated Diets

Dadik Pantaya, Niati Ningsih and Suci Wulandari*

Department of Animal Science, Politeknik Negeri Jember, East Java-Indonesia
Corresponding author: suci@polije.ac.id

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Abstract

Background: Aflatoxin contamination in poultry feed remains a major challenge due to its adverse effects on productivity and economic performance. Poultry feed with aflatoxin contamination can have harmful effects on chicken production. **Purpose:** The study was conducted to evaluate the effect of bioactive yeast *Saccharomyces cerevisiae* (**Sc**) on reducing the negative effect of diets contaminated under low doses of aflatoxin B1 (AFB1) exposure (30 ppb) on the performance of laying hens. **Methods:** A total of one hundred, 24-week-old commercial line Hisex brown laying hens were randomly divided into four treatments with five replicates, with five hens in each replicate. The treatments comprised control P0 = basal diet (BD) without Sc and AFB1; P1 = BD + 30 ppb AFB1 without Sc; P2 = BD + 30 ppb AFB1 + 0.5 g Sc/kg diet; and P3 = BD + 30 ppb AFB1 + 1 g Sc/kg diet. The observed variables were 1) Layer performance, 2) the physical quality of eggs, and 3) liver histomorphology, and 4) Relative liver weight (%) and Serum glutamic oxaloacetic transaminase (SGOT)(U/L) value. **Results:** The results revealed that exposure to AFB1 at 30 ppb had no significant effect on egg production, feed consumption, the feed egg ratio (FER), liver histomorphology, relative liver weight (%), and SGOT liver enzyme activity in laying hens. However, feed contaminated with low doses of AFB1 significantly reduced eggshell thickness, while the supplementation of Sc to the contaminated feed significantly prevented such thinning ($P<0.02$). **Conclusion:** The conclusion drawn is that the addition of Sc to feed can counter the negative effect of AFB1 on the egg quality particularly eggshell thickness.

Keywords: Aflatoxin; laying hen; performance; *Saccharomyces cerevisiae*; yeast

INTRODUCTION

The production of poultry in Indonesia has been growing rapidly in recent decades alongside the growing innovation in science and technology, mainly related to poultry management and nutrition. One challenge to the poultry industry is economic losses as a result of aflatoxin contamination in feed. Aflatoxins are mycotoxins produced as secondary metabolites by fungi, particularly *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius*, which have carcinogenic, mutagenic, tetratogenic and immunosuppressive effects that harm livestock and humans (Petrović et al. 2022). Poultry fed with diets containing aflatoxin can suffer

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growth disorders, decreased productivity and health problems, leading to economic losses (Bansal et al. 2023).

Aflatoxin results in acute toxicity and chronic disease. The toxicity is caused by feed contaminated with high concentrations of aflatoxin, whereas chronic conditions are caused by low levels of contamination over a long period of time (Pantaya et al. 2016). Poultry feed with aflatoxin contamination can have harmful effects on chicken production. Long term low-dose contamination is difficult to avoid in tropical climates, and therefore tends to be ignored due to the limited information about its effect on poultry performance. In Asia-Oceanian countries, including Malaysia, the Philippines, Thailand, Indonesia and India (SEA), levels of 30.3% aflatoxin contamination in feeds and feed ingredients have been reported (Sun et al. 2023; Kępka-Borkowska et al. 2025).

Such contamination can be detected in animal feed ingredients when its concentration is between 50 ppb and 620 ppb (Kotinagu et al. 2015) (Anastasia et al. 2023). Different levels of aflatoxin contamination lead to variations in the productivity performance and quality of poultry products (Phan et al. 2021). The negative effect of aflatoxin residue can be eliminated by physical and chemical methods; however, these methods are less effective and not applied in this case. Therefore, this research focuses on preventing the absorption of AFB into the digestive tracts of poultry. One way of doing this is by using yeast. *Saccharomyces cerevisiae* yeast can reduce the absorption of toxins into the poultry digestive tracts, thus reducing toxicity in livestock (El-Sayed et al. 2022).

The benefits of the supplementation of *Saccharomyces cerevisiae* in the decontamination of aflatoxins that contain glucomannan and the overcoming of high-dose contamination of up to 200 ppb have been reported by several researchers (Kebede et al. 2020). However, limited information is available on the effect of adding *Saccharomyces cerevisiae* to feed contaminated with low-dose aflatoxin on the production of laying hens. The purpose of this study is therefore to evaluate the supplementation of *Saccharomyces cerevisiae* in terms of performance, the physical quality of eggs, blood profile, and the liver histomorphology of laying hens fed low doses of AFB1 contamination.

MATERIALS AND METHODS

Materials

One hundred pullets of twenty-four-week old Hisex Brown strain were obtained from commercial pullet breeders. The chickens were reared in thermoregulated pens, with feed and water provided *ad libitum*. The basal feed consisted of corn, commercial concentrate, rice bran and mix minerals, with its nutrient composition formulated according the NRC 1994 recommendations shown in Table 1.

Methods

A complete randomized design containing four treatments and five replicates was employed, with each replication consisting of five pullet birds placed in individual galvalume cages. The treatments were as follows P0 = basal feed (without AFB1 and Sc); P1 = basal diet + 30 ppb AFB1; P2 = basal diet + 30 ppb AFB1 + Sc 0.5 g/kg; and P3 = basal diet + 30 ppb AFB1 + Sc 1 g/kg. The variables observed were 1) Layer performance, 2) the physical quality of eggs, and 3) liver histomorphology, and

4) Relative liver weight (%) and Serum glutamic oxaloacetic transaminase (SGOT) activity.

Experimental Design

Preparation Contaminated Corn

Fifty grams of yellow corn kernels were mixed in a 250 mL flask with 20 mL of sterile distilled water. The flask was then covered with cotton and aluminium foil, autoclaved for 30 minutes at 121°C, cooled, and inoculated with the *A. flavus* previously grown on a 9 mm PDA plate. The flask was stored in an operated incubator at 29°C for 21 days, with the medium mechanically stirred daily from the third day. After 21 days, the flasks were taken from each chamber and from the incubator, dried in an oven at 60 °C overnight, and ground into a fine powder using a blender (Ochieng et al. 2022).

Quantification of Aflatoxin B1 by HPLC

Consider change to: AFB1 concentration was determined according to Pantaya et al. (2016). Briefly, 5 g of sample was extracted with 0.5 g NaCl and 30 mL methanol–water solution (20:80, v/v) in a 50 mL polypropylene tube, followed by filtration through Whatman No. 4 filter paper. Subsequently, 5 mL of filtrate was diluted with 95 mL phosphate-buffered saline (PBS, pH 7.4). An immunoaffinity column conditioned with 10 mL PBS was used for sample cleanup, and 50 mL of diluted filtrate was passed through the column at a flow rate of 3 mL/min. The column was then washed with 20 mL distilled water, and the eluate was collected, diluted to a final volume of 4.5 mL, and injected (100 µL) into a high-performance liquid chromatography (HPLC) system at a flow rate of 1 mL/min. AFB1 was detected using a fluorescence detector at excitation and emission wavelengths of 365 and 435 nm, respectively.

Layer performance

Feed consumption was calculated by subtracting the difference between the amount of feed given and the remaining feed. Hen day production (HDP) was measured by dividing the number of eggs by the number of chickens, while the feed egg ration was determined from the amount of feed consumed and the weight of the eggs.

Determination of liver histomorphology

Liver tissue samples were preserved in a 10% neutral-buffered formalin solution, then infiltered with liquid paraffin, and embedded in pure paraffin. Subsequently, the samples were stained using the hematoxylin-eosin method (Ma et al., 2012) and observed under a microscope.

Determination of blood hematology

The blood samples (around 1.5 ml) were drawn from the brachial vein in the superficial pectoralis wing area using a syringe, then put into a blood tube containing ethylene diamine tetra-acetic acid (EDTA) anticoagulant and stored in a cooler box. The samples were used for blood serum profile analysis. Blood profiles in the form of hemoglobin, erythrocytes, hematocrit, heterophile cells, leukocytes, basophils, lymphocytes, monocytes and platelets were calculated using a hemocytometer. That of hemoglobin was assessed using a complete reagent kit (Merckotest), and of R. Hematocrit using a microcapillary reader (USA). Concentrations of WBC and RBC were measured using an automatic hematology analyzer, XS series XS-1000i/XS-800i (Sysmex Corporation).

Determination of SGOT Activity

Blood samples were collected from the jugular vein and placed into tubes for serum separation. It was then homogenized with 0.1 M of cold phosphate buffer, pH 7.4. Assay chemistry control biorad levels 1 and 2 using Cobas C311 were made for the serum and liver. The concentrations of glutamate oxaloacetate transaminase (SGOT) were measured using commercial kits (Asan Pharmaceuticals Co., Ltd., Seoul, Korea) following the manufacturer's specifications (Subramaniyan et al. 2020). Samples were incubated at room temperature for one minute and then read with a Microlab 300 spectrophotometer (Thermo Fisher Genesys 10s UV-Vis, USA) at a wavelength of λ340 nm (Prahara et al. 2023).

Analysis of the Physical Quality of the Eggs

The egg quality (white and yolk) index was observed by measuring their height, length and width using a cutimeter. The value of the index was obtained by calculating the height divided by the average diameter, multiplied by 100%. Measurement of albumen height was made using a deep micrometer $HU = 100 \log (H + 2.75 - 7.57 W 0.37)$, where H = albumen height (mm) and W = egg weight (g). The thickness of the shell was measured from shell fragments using a caliper. Yolk colour measurement was made using a Yolk Colour Fan.

Table 1. Feed ingredient (%) and calculated nutrient composition (%) of the basal diets

Feed Ingredient	Proportion (%)
Yellow corn	51
Soybean meal	20
Rice brand meal	10
Fish meal	16
Palm oil	2
Mineral mix [1]	1
Proximate composition analysis	
Crude protein	19.0
Crude fat	5.0
Crude fibre	3.0
Ca	3.5
P	0.7
ME (Kcal/Kg)	3.100

[1] Supplied per kg of diet: 32 mg Mn ($MnSO_4 \cdot H_2O$), 16 mg Fe ($FeSO_4 \cdot 7H_2O$), 24 mg Zn (ZnO), 2 mg Cu ($CuSO_4 \cdot 5H_2O$), 800 µg I (KI), 200 µg Co ($CoSO_4$), and 60 µg Se.

Analysis Data

The data were analyzed using a completely randomized design with ANOVA, with the Minitab software package w used for statistical analysis (Minitab Inc, USA). The statistical model was $Yij=\mu+\alpha i+ \varepsilon ij$, where Yij is the observed value, μ is the overall mean, αi is the treatment effect, and εij is the experimental error. The post hoc Tukey test was applied when P<0.05 and the significant level was declared at (P<0.05).

RESULT AND DISCUSSIONS

Layer Performance

The effects of AFB1 and the dietary supplementation of Sc yeast on growth performance are presented in Table 2. The results indicate that the treatments did not make a significant difference to the performance of the laying hens, and those given a diet contaminated with low doses of AFB1 did not show signs of illness or health problems. Table 2 summarizes the experimental treatments, showing no significant differences between the groups in terms of egg production (Abd Al-Emier Almremdhy et al. 2024), feed consumption or the feed egg ratio (FER). The results indicate that the layer chickens have tolerance to low doses of AFBI, therefore low doses of 30 ppb/kg did not negatively affect their performance.

Table 2. Layer performance of layer chicken fed diets containing *Saccharomyces cerevisiae* yeast for 4 weeks

Variable	Treatment				*P-value*
	P0	P1	P2	P3	
Feed consumption (g/ekor)	119.64±0.71	119.01±0.39	119.81±0.34	119.53±0.45	0.465
Feed Egg Ratio (FER)	2.32±0.04	2.39±0.03	2.47±0.03	2.42±0.04	0.545
Egg production	95.72±0.41	92.42±0.32	94.48±0.42	92.73± 0.37	0.673

The means of a row with no common superscript are significantly different (P < 0.05).
P0= Basal diet (BD), P_1= BD+ AFB1 30 ppb/kg without Sc, P2= BD + AFB1 30 ppb/kg + 0.5 g Sc/kg diet, P3= BD + AFB1 30 ppb/kg + 1 g Sc /kg diet

The findings of this study are similar to research conducted by (Nalle et al. 2021), who reported no significant difference in the performance of laying hens with low-dose aflatoxin contamination in diets. However, AFB1 with contaminant doses of 100, 200, 500, and 1000 ppb in poultry diets has been shown to significantly reduce production performance and feed consumption of laying hens (Prakoso 2019). Similarly, (Herzallah 2013) found that contamination of aflatoxin in feed levels of 1mg/kg or 600 ppb had a significant negative effect on the performance and productivity of laying hens.

The physical quality of eggs,

The physical quality of eggs is presented in Table 3. No significant difference (P>0.05) was found in the white and yolk index, albumen height, egg weight or Haugh unit index (HU) in either control, treatments contaminated with AFB1 and supplementation with Sc yeast. However, consumption of low-dose (30 ppb AFB1) contaminated feed had a significant effect on eggshell thickness (P<0.002), indicating that supplementation with Sc partially alleviated the reduction in eggshell thickness induced by AFB1 exposure.

Supplementation of yeast in the diet may therefore prevent the reduction in eggshell thickness caused by AFB1 contamination. The thickness of the eggshell is one parameter in determining egg quality (Manafi 2018). Thickness is presumably due to the supply of Ca and P minerals, which are disrupted during eggshell formation, and is caused by metabolic disorders of carbonic anhydrase and by impaired metabolism of zinc as a co-enzyme (Ketta and Tůamová 2016). Carbonic anhydrase plays a role in regulating the absorption of Ca minerals, which are the main components of shell formation and can inhibit C and P deposition in them (Nasiri Poroj et al. 2023). Yeast walls are able to bind aflatoxins, thus reducing the impact of aflatoxins on mineral metabolism (Wei et al. 2024).

Table 3. The physical quality of eggs with the use of *Saccharomyces cerevisiae* for 4 weeks

Variable	Treatment				P-value
	P0	P1	P2	P3	
Egg weight (g)	54.75±2.67	53.31±1.99	54.81±0.66	53.69±2.54	0.68
Egg white index (mm)	1.20 ±0.04	1.23±0.02	1.22±0.03	1.24±0.05	0.55
Egg yellow index (mm)	1.05±0.02	1.03±0.02	1.02±0.05	1.07±0.03	0.18
Haugh unit (mm)	90.47±1.84	92.53±2.16	90.92±0.88	91.98±0.66	0.26
Shell thickness (mm)	0.50±0.00^a	0.43±0.05^b	0.50±0.00^a	0.50±0.00^a	0.00

The means of a row with no common superscript are significantly different ($P < 0.05$).

P0= Basal diet (BD), P$_1$= BD+ AFB1 30 ppb/kg without Sc, P2= BD + AFB1 30 ppb/kg + 0.5 g Sc/kg diet, P3= BD + AFB1 30 ppb/kg + 1 g Sc /kg diet

Liver histomorphology

The results related to the addition of *Saccharomyces cerevisiae* as an anti-aflatoxin agent in feed in terms liver function of the laying hens and the liver histomorphology are shown in Figure 1 To investigate the toxicological effects of AFB1, using a microscope, liver histological changes were observed, with a comparison of tissues between control groups, liver tissue from chickens fed with AFB1 contamination, on feed contaminated with AFB1 without yeast the liver appears to be slightly darker in colour (Figure 1). The results show that the AFB1 toxins yeast strains, particularly *S. cerevisiae*, tend to mitigate the adverse effects of AFB1-contaminated feed. Yeast is able to absorb the toxin AFB1, thereby reducing its bioavailability and toxicity (Hamad et al. 2017). Yeast strains have been shown to be able to reduce AFB1 by up to 54-66% (Tahir et al. 2018). They also help in mitigating oxidative stress by enhancing antioxidant defences (Khatoon et al. 2024).AFB1 contamination in feed leads to increased ROS production, causing oxidative stress and subsequent cellular damage (Wang et al. 2022). Such stress can cause cell damage due to the formation of superoxide anions, hydroxyl radicals and hydrogen.

Relative liver weight (%) and Serum glutamic oxaloacetic transaminase (SGOT)(U/L) value.

The liver is the main target organ of aflatoxin exposure; therefore, the study parameters include SGOT analysis and liver relative weight. The supplementation of Sc yeast to feed did not significantly affect the SGOT values, histology or relative liver weights of the layer hens exposed to AFB1 (Table 4). Singh (2019) made the same finding, that was no significant difference in the relative liver weight of broiler chickens given low doses of aflatoxin. A significant effect on increasing the activity value of SGOT gets with contamination of 250 ppb of AFB1 on feed. The liver SGOT value in this study was average, within the range of 175-224 AST. This value indicates that the protein metabolism of the laying hens is functioning normally. Disorders of protein metabolism in the liver will cause an increase in the value of aspartate aminotransferase found in the cytoplasm and can affect cell integrity. Our study shows that indicators related to protein metabolism in the liver were not affected by AFB1 contamination at the low dose of 30 ppb. This finding was reinforced by the liver size, which showed no significant difference (P>0.05) (Albadrani et al. 2024).

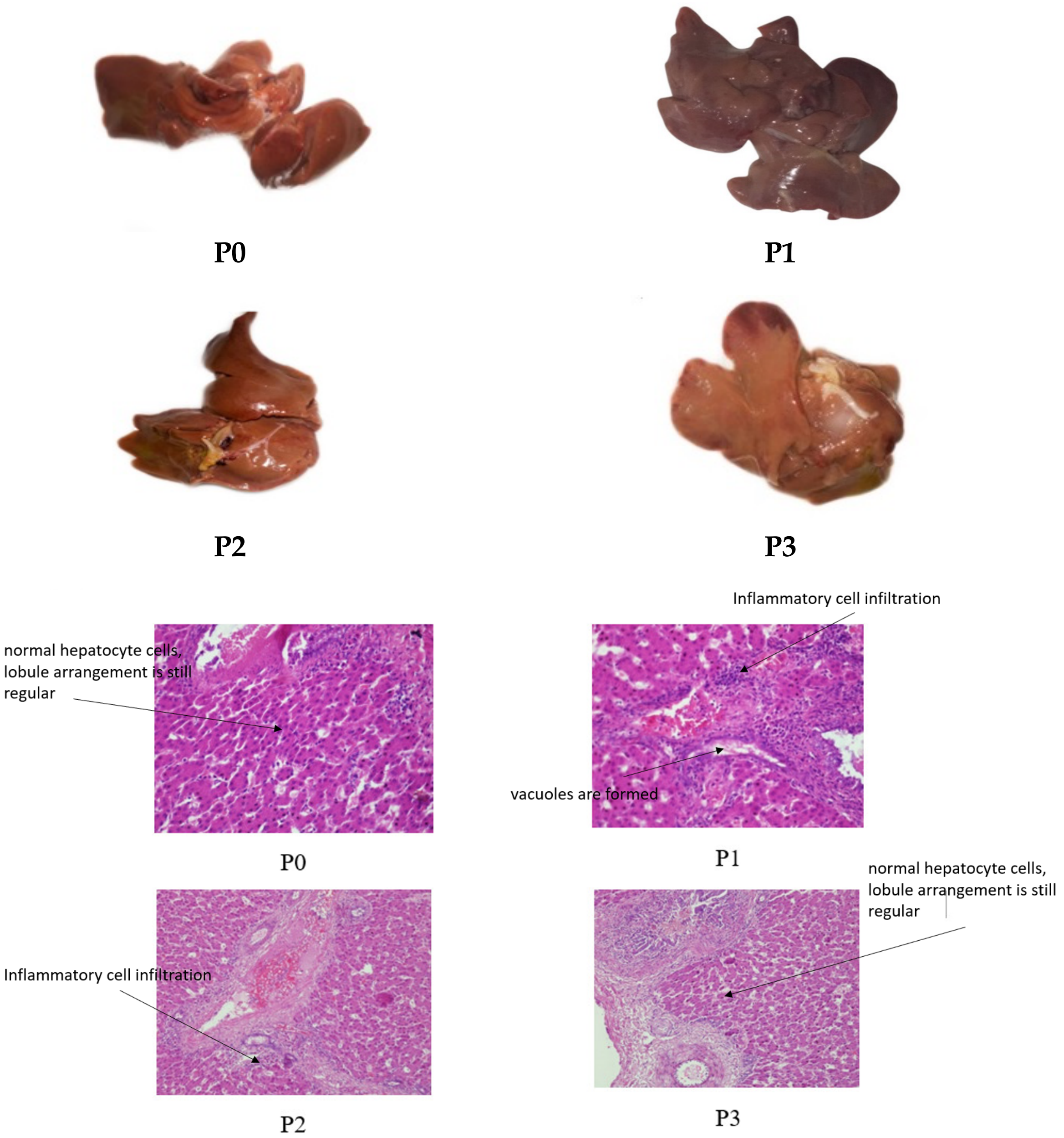

Figure 1. Liver histomorphology of laying hens with the use of *Saccharomyces cerevisiae* as a toxin agent. P0 = basal diet without AFB1 or *Saccharomyces cerevisiae* (Sc); P1 = basal diet + 30 ppb AFB1; P2 = basal diet + 30 ppb AFB1 + 0.5 g Sc/kg diet; and P3 = basal diet + 30 ppb AFB1 + 1 g Sc/kg diet. Relative liver weight (%) and serum glutamic oxaloacetic transaminase (SGOT) activity (U/L) were also evaluated to assess liver health and hepatic function

Table 4. Percentage Liver weight (%) and SGOT (U/L) value of the laying hens with *Saccharomyces cerevisiae* as toxins binder for 4 weeks

Variable	Treatment				P-value
	P0	P1	P2	P3	
Liver weight (%)	2.5±0.07	2.6±0.15	2.4±0.20	2.7±0.77	0.701
SGOT (U/L)	175.88±15.56	192.25±17.50	177.00±17.82	224.6±66.77	0.253

The means of a row with no common superscript are significantly different ($P < 0.05$).
SGOT : Serum Glutamic Oxaloacetic Transaminase
P0= Basal diet (BD), P_1= BD+ AFB1 30 ppb/kg without Sc, P2= BD + AFB1 30 ppb/kg + 0.5 g Sc/kg diet, P3= BD + AFB1 30 ppb/kg + 1 g Sc /kg diet

CONCLUSION

Dietary supplementation with *Saccharomyces cerevisiae* alleviated the reduction in eggshell thickness caused by low-level AFB1 contamination in laying hens, although it did not significantly affect laying performance, liver histomorphology, relative liver weight, or SGOT activity.

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AUTHORS' CONTRIBUTIONS

DP: contributed to the study conception and design. NN: performed the data analysis and interpretation. SW: was responsible for drafting and critically revising the manuscript.

CONFLICT OF INTEREST

The authors declare that there no conflicts of interest related to the financial, personal, or other relationships that could have influenced the work reported in this manuscript.

ETHICAL APPROVAL

The research was conducted at the Feed Technology Laboratory, State Polytechnic Jember, East Java, Indonesia. All the experimental activities complied with standard operating procedures and were approved by the Polytechnic ethics committee, with registration no. 052/PL17.4/PG/2025.

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