PERFORMANCE OF LAYERS FED AFLATOXINS B_1-CONTAMINATED DIETS WITH OR WITHOUT *Psidium guajava* LEAF MAEAL

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ABSTRACT
This study evaluated the effects of P. guajava leaf meal (PGLM) performance of laying hens fed diets contaminated with aflatoxin B_1 (AFB_1). A total of 300 point of lay Isa Brown hens were assigned to 4 treatments designated diets A -positive control (basal diet), B (basal diet + 1 mg AFB_1/kg), C (diet B + 5g PGLM/kg, and D (diet B + 10g PGLM/kg diet). Each treatment was replicated 5 times with15 pullets/replicate in a completely randomized design. At the end of the 16 weeks feeding trials; total weight gain (TWG), egg mass (EG), hen day production percentage (%HDP) and net feed efficiency index (NFEI) were significantly ($P<0.05$) lower in hens fed diet B while feed conversion ratio (FCR) was high significantly ($P<0.05$) in hens fed diet B (AFB_1-contaminated diet) than all other treatments. Hens fed diet C and D showed better performance in most of the parameters. The potential of the P. gaujava leaf meal to ameliorate the impact of the toxin, however, increased with increase in the PGLM concentrations. The findings of the study indicated the potential of PGLM to ameliorate aflatoxin B_1-inducedoxidative stress with increase in the concentration of PGLM supplementation.
Keywords: Aflatoxicosis, Mycotoxin, Oxidative stress, Poultry

INTRODUCTION

Despite the potentials of animal agriculture, especially the egg producing sub-sector, to optimally support the fast-growing population in developing countries of the world, there are myriads of challenges facing this industry. Chief among these is the incidence of mycotoxin contamination. Mycotoxins are toxic secondary metabolites produced by various species of fungi such as *Aspergillus*, *Fusarium*, and *Penicillium* (Bennett and Klich, 2003). Mycotoxin contamination of food and feed is a global challenge leading to serious health issues in both humans and animals (Gbore *et al.,* 2023; Olarotimi *et al.*, 2023a). In poultry production, mycotoxin contamination is capable of causing adverse effects on bird's health, productivity, and overall food safety, thereby, posing a severe economic loss on the farmers' profitability and the productivity of the chickens.

Aflatoxins are a group of mycotoxins produced by certain species of *Aspergillus* fungi, particularly *A. flavus* and *A. parasiticus*. There are several types of aflatoxins, with the most common and significant ones being aflatoxins B_1, B_2, G_1, and G_2 (Kumar *et al.*, 2017). Aflatoxin B_1 (AFB_1) is the most toxic and prevalent aflatoxin. It is also the most carcinogenic, known to cause liver cancer (hepatocellular carcinoma), immune-suppression, and growth retardation in humans and animals (Hamid *et al.*, 2013).To prevent the adverse effects of poultry feed with AFB_1, the use of mycotoxin binders, in recent times, is the rule of thumb for commercial feed millers. A classic example of this is bentonite clay which is effective in binding AFB_1 when added to poultry feed usually at a 2% inclusion rate. However, clay-based toxin binders have their negative effects such as binding nutrients even at lower inclusions, thus, reducing essential nutrient availability needed for growth and production. Likewise, many of the clay-based toxin binders are costly and increase the cost of feed production, hence, reducing farmers' profitability margin (Olarotimi et al., 2023b).

It has been established that phyto-additives have the potential of improving performance and maintaining the physiological status in terms of enhancing the immunity and antioxidant status in birds (Oloruntola*et al.*, 2018). Leaf meals and phyto-additives such as *Vernonia amygdalina* (Gbore*et al.,* 2023; Olarotimi *et al.*, 2023a) and *Sidaacuta* (Olarotimi *et al.*, 2023b) have been previously used and reported to be effective in mitigating aflatoxicoses in cocks. There are abundant plants of different species in the tropics that are easily available and accessible all year round that could be potential candidates in ameliorating the possible effects of AFB_1 contamination in laying birds.

Psidium guajava (Guava) is a common phyto-therapeutic plant in sub-tropics which are rich in bioactive compounds. The leaves of *P. guajava* are reservoirs of antioxidants possessing anti-inflammatory, anti-cholinergic, anticancer and anti-cytotoxic properties (Lok *et al.*, 2023). They are equally sources of antibacterial, antifungal (de Freitas *et al.*, 2022). Hence, the objective of this study was to evaluate the effects of *P. guajava* leaf meal on performance of laying hens fed AFB_1-contaminated diets.

MATERIALS AND METHODS

Experimental Site, Hens and Management

The research took place at the Teaching and Research Farm, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria. A total of 300 point-of-lay (POL) Isa Brown pullet of sixteen (16) weeks old were purchased from Animal Care Farms Ogere, Ogun State for the study.They were placed on a commercial grower mash until they have reached 20% laying performance (24 weeks of age) before they were subjected to the experimental diets for a period of sixteen weeks. The pullets were weighed before the commencement of the study and the 300 pullets were randomly allotted to the four experimental treatments. Throughout the experimental period, the birds were fed twice daily (morning and afternoon) and water was also given *ad-libitum.* Leftovers were collected weekly to determine the weekly feed intake. Recommended vaccinations and other medications were administered as and when due.

Production of PGLM

The *P. guajava*leaf used for the study was sourced within the immediate environment of the institution and identified by an ethnobotanist at the University'sherbarium. The leaves were properly washed in salted clean water, drained and left dried in a shady screen which allowed adequate air flow and away from direct sunlight for a period of 7 days. Thereafter, the leaf was pulverized, using a laboratory table top milling machine, to make *P. guajava* leaf meal (PGLM), and securely stored in an air-tight container till when needed for experimental diets composition.

Production of AFB_1

For the production of AFB_1, maize grits were cultured with *Aspergillus flavus* and analysed for AFB_1 as described by Olarotimi *et al.,* (2023a). *A. flavus*-contaminated maize grains were pulverized and stored in an air-tight container to prevent moisture absorption from the atmosphere.

Formulation of Experimental Diets

A basal diet was formulated for the hens and analysed for proximate composition after which the basal diet was divided equally into 4 portions: A, B, C, and D. Portion A was the control /basal diet while portions B, C and D were used to constitute Diets B, C and D respectively. Diet A, positive control (basal diet), B (basal diet + 1 mg AFB_1/kg), C (diet B + 5g PGLM/kg, and D (diet B + 10g PGLM/kg diet). Each treatment was replicated 5 times with 15 pullets per replicate in a completely randomized design. A sample of this mixture was analysed to confirm the AFB_1 concentration and the result was approximately 1.01 mg/kg. The Feed Intake, Weight Gain, Feed Conversion Ratio (FCR), Net Feed Efficiency Index (NFEI), and Hen Day Production (% HDP) were evaluated.

Data Analysis

All data obtained were subjected to One-Way Analysis of Variance (ANOVA) of the Graph Pad Prism, software version 6.01 (2012). Significant differences among the treatment means were separated using the Tukey's Honestly Significant Difference ($\alpha_{0.05}$) option of the same software.

RESULTS AND DISCUSSION

Table 1 shows the results of performance of hens fed AFB_1-contaminated diets with or without *Psidium guajava* leaf meal.

Table 1: Performance of layers fed AFB_1-contaminated diets with or without PGLM

	Diets					
Parameters	**A**	**B**	**C**	**D**	**SEM**	**P-Value**
Initial Weight (g)	1170	1178	1183	1180	14.90	0.50
Final Weight (g)	1569	1544	1611	1659	12.73	1.48
Total Weight Gain (g)	399^{b}	366^{c}	428^{ab}	479^{a}	18.10	0.04
Feed Intake (g/bird/day)	112^{c}	128^{a}	119^{b}	122^{b}	0.76	0.02
Egg Mass (g)	41.89^{b}	26.68^{c}	40.37^{b}	47.66^{a}	2.05	0.01
% Hen Day Production	70.98^{b}	51.22^{c}	68.65^{b}	79.42^{a}	1.19	0.01
Feed Conversion Ratio	2.67^{bc}	4.80^{a}	2.95^{b}	2.56^{c}	1.26	0.03
Net Feed Efficiency Index	393.65^{b}	306.78^{c}	393.59^{b}	431.69^{a}	13.07	0.02

Values are means and SEM. Means in a row without common superscripts are significantly ($P<0.05$) different. SEM = Standard Error of Means, Diets: A = Control/Basal, B = Basal + 1.00 mg AFB_1/kg, C = Diet B + 5 g PGLM/kg, D = Diet B + 10 g PGLM/kg.

The hens fed diet B recorded significantly (P<0.05) lowest total weight gain (TWG), egg mass (EG), percent hen day production (%HDP), and net feed efficiency index (NFEI) when compared to others. This was an indication that AFB_1 has the potential to cause nutrient absorption and metabolism impairment, leading to reduced growth rates (Gowda and Ledoux, 2008). The lower total weight gain of hens fed diet B showed the detrimental impact of AFB_1 on livestock growth. Layers on diet C had similar weight gains to those on diets A and D. This showed that PGLM supplementation mitigated the negative effects of AFB_1 on weight gain.

The superior performances of birds fed Diets C and D over those fed Diet B in weight gain, egg mass, % Hen Day Production (%HDP) and Net Feed Efficiency Index (NFEI) aligned with studies indicating that antioxidants and phytochemicals in PGLM could mitigate the oxidative stress induced by AFB_1 (Kumar *et al.,* 2021). Likewise, the feed intake (FI) and Feed Conversion Ratio (FCR) of hens on diet B statistically increased (P<0.05) when compared with hens on diets A, C, and D. The highest feed intake exhibited by layers on diet B is likely due to the compensatory increase in feed consumption to offset the reduced nutrient utilization caused by AFB_1.

The similar feed intake observed in hens fed diets C and D suggested that PGLM supplementation helped normalized feed intake even in the presence of AFB_1, potentially by enhancing gut health and nutrient absorption (Helal, 2019). The low %HDP exhibited by layers on diet B highlighted the adverse effect of AFB_1 on reproductive performance especially egg production. AFB_1 disrupts hormonal balance and liver function, which are critical for egg production (Surai, 2002). On the other hand, the high %HDP recorded in on diet D demonstrated the potential of PGLM in protecting reproductive functions and enhancing productivity under AFB_1 challenge. This supported the report of antioxidant and hepato-protective properties of PGLM (Kumar *et al.*, 2021).

However, hens on diet D had the lowest FCR while the highest FCR was observed in hens on diet B. This showed that layers fed diet B utilized more feed to convert to body mass (egg and meat) which was the impacts of oxidative stress induced by AFB_1 on the birds and that PGLM at both supplementation levels potentially mitigated and enhanced nutrient bioavailability (Pauletto *et al.*, 2023). The low NFEI exhibited by layers on diet B were less efficient in converting feed into body mass and eggs, likely due to AFB_1-induced metabolic disruptions. The improved feed efficiency in birds on diets C and D confirmed the protective role of PGLM against AFB_1 toxicity (Zain, 2011). The potential of the leaf meal to ameliorate the impact of the toxin, however, increased with increase in the PGLM concentrations.

CONCLUSION

- This study demonstrated that AFB_1 exposure showed negative impacts in the performance, health and productivity of laying hens,
- Supplementation with PGLM, particularly at 10 g/Kg effectively mitigated the toxic effects of AFB_1 by improving growth and overall health of layers.

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