# AMELIORATIVE POTENTIALS OF *Psidium guajava* LEAF MEAL ON ELECTROLYTE BALANCE, SERUM PROTEIN, ENZYMES AND GLUCOSE IN HENS FED AFLATOXIN $B_1$-CONTAMINATED DIETS

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**ABSTRACT**
*The poultry industry faces significant challenges due to mycotoxin contamination, particularly aflatoxin $B_1$ ($AFB_1$) in feed. Therefore, developing nutritional interventions to mitigate the adverse effects of mycotoxins is crucial for optimizing bird productivity. This study assessed the ameliorative potentials of Psidium guajava leaf meal (PGLM) on electrolyte balance, serum protein, enzymes and glucose in hens fed aflatoxin $B_1$-contaminated diets. A total number of 96 point-of-lay Isa Brown pullets were randomly assigned to four dietary treatments for 16 weeks. Diet A was the control/basal diet, Diet B was basal diet with 1mg of $AFB_1$/kg, Diet C was diet B with 5g of PGLM/kg and Diet D was diet B with 10g of PGLM/kg. Each treatment with 24 birds was divided into three replicates in a completely randomized design. The results showed that the electrolyte balance i.e. sodium (150.00), chloride (80.00), phosphate (1.81) and calcium ions (2.40) of hens fed diet B were adversely ($P<0.05$) affected. However, supplementation with PGLM at 5g/kg (Diet C) showed a protective effect restoring the phosphate ion (2.10), and the inclusion of PGLM at 10g/kg increased the calcium (2.63), sodium (190.00), and chloride ions (85.13) in the birds. Diet B significantly ($P<0.05$) decreased the serum protein, globulin (11.02), albumin (17.10) and total protein (28.12) and statistically ($P>0.05$) increased the serum enzymes, alanine aminotransferase (44.10), aspartate aminotransferase (51.00) and glucose (5.28) in the layers compared with birds fed diets A, C, and D. The potential of the leaf meal to ameliorate the impact of the toxin, however, increased with increase in the PGLM concentrations. These findings highlighted the potential of PGLM to mitigate the possible effects of $AFB_1$-induced mycotoxicity in hens.*
**Keywords: Leaf meal, Mycotoxin, Aflatoxicosis, Poultry, Layers.**

## INTRODUCTION

Layers production serves as an important aspect of the global food sector, because it produces eggs (Saki *et al*., 2010). However, layers production is subject to various health and disease challenges, such as bacterial, viral and parasitic infections. One of the biggest health and disease challenges faced by layers is caused by mycotoxin (Nakavuma *et al*., 2020).

Mycotoxin are secondary metabolites produced by micro fungi that are capable of causing diseases (mycotoxicoses) and death in humans and animals. They are of different classes which include aflatoxins. Aflatoxins are mycotoxins that come in various forms; the primary forms of aflatoxin include $B_1$, $G_1$, $B_2$ and $G_2$ (Navakuma *et al*., 2020). When chickens consume feed contaminated with aflatoxin $B_1$, it can result in several negative consequences. One major primary effects of aflatoxin on layers is oxidative stress in the kidney through the formation of free radicals which alter the antioxidant reactions mediated by reactive oxygen species (ROS) scavenging enzymes (Olarotimi *et al.*, 2023). Aflatoxin $B_1$-induced electrolyte imbalance increases the risk of dehydration, respiratory distress, and mortality in layers. Jayasri *et al.* (2023) reported decrease in the glucose, total proteins, albumin, globulin, calcium and phosphorus of chicken fed aflatoxin-contaminated diets.

Recently, plant materials such as *Vernonia amygdalina* has shown exceptional substitute as bio fungicide and to mitigate the effect of aflatoxin $B_1$ ($AFB_1$) in cock (Gbore *et al.*, 2023). However, the potentials of *Psidium guajava* leaf in mitigating the adverse effects of $AFB_1$ in layers has not been fully explored. *Psidium guajava* is rich in vitamins, fatty acids, tannins, phenols, flavonoids, and essential oils, and the antimicrobial properties of *P. guajava* leaf extracts against fungi and bacteria has been reported (Richard *et al.*, 2013). The use of *P. guajava* leaf meal may offer an environmentally friendly, cost-effective, and safer solution to mitigate the adverse impacts of fungal and aflatoxin contamination in poultry. Hence, this study investigated the ameliorative potentials of *P. guajava* on electrolyte balance, serum protein, enzymes and glucose of hens fed $AFB_1$-contaminated diets.

## MATERIALS AND METHODS

### Experimental site and animals

The research was conducted at the Teaching and Research Farm of Adekunle Ajasin University, Akungba-Akoko, Ondo State with 96 Isa Brown point-of-lay. The birds were divided randomly to four treatments with each

containing 24 hens with three replicates. The experiment lasted 16 weeks and the hens had access to clean drinking water and the experimental diet.

**Preparation of *P. guajava* leaf meal (PGLM), aflatoxin $B_1$ production and experiment diets**.
Fresh leaves of *P. guajava* were collected, air-dried for seven days and converted to powder. Aflatoxin $B_1$ was produced by inoculating autoclaved maize grains with a toxigenic *Aspergillus flavus*, yielding $AFB_1$-contaminated grains as described by Gbore *et al.* (2023). The contaminated maize was incorporated into the basal diet containing 2529Kcal/kg metabolizable energy and 16% crude protein, as determined by the methods of AOAC (2005) to produce 1.0mg of $AFB_1$/kg. Four experimental diets were formulated as outlined: Diet A (control/basal diet), Diet B (basal diet containing 1.0mg of $AFB_1$/kg), Diet C (basal diet containing 1.0mg of $AFB_1$ plus 5g of PGLM/kg), Diet D (basal diet containing 1.0mg of $AFB_1$ plus 10g of PGLM/kg).

**Blood Sampling**
At the end of 16 weeks, 12 hens/treatment were selected for blood sampling. Blood sample from each hen was collected into plain bottles for serum biochemical studies. The blood samples were centrifuged for 10 minutes at 3,000 rpm to obtain clean supernatant serum. The harvested serum samples were used to determine the serum proteins (albumin, globulin and total protein) as described by Adu *et al*. (2018), and serum enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined using the Randox Laboratories Ltd, UK test kits. Serum electrolytes ($Na^+$, $Cl^-$, $PO_4^{3-}$ and $Ca^{2+}$) were analyzed by auto analyzer (Kodak Ektachem; Eastman Kodak Company, Rochester, New York).

**Data Analysis**
All data generated were subjected to one-way analysis of variance using Statistical Analysis System (SAS, 2008). The significant treatments were compared using Duncan's multiple range tests at 5% level of significance.

**RESULTS AND DISCUSSION**
**Electrolyte balance**
The electrolyte balance of layers fed $AFB_1$-contaminated diets with or without *Psidium guajava* leaf meal are presented in Table 1. The results showed that hens on diet B had the lowest ($P<0.05$) sodium (150.00), chloride (80.00), phosphate (1.81) and calcium ions (2.40) compared with layers fed diets C sodium (170.10), chloride (80.22), phosphate (2.10), calcium ions (2.52), and diets D sodium (190.00), chloride (85.13), phosphate (2.00), calcium ions (2.63).
Electrolyte balance is crucial for the physiological functioning and health of poultry. The highest sodium, chloride, phosphate and calcium ion concentration exhibited in layers fed diet D which was significantly higher than those of layers fed diet B indicated that PGLM at 10 g/kg helped enhanced sodium, chloride, phosphate and calcium ion levels despite the presence of $AFB_1$. The lowest sodium, chloride, phosphate and calcium ion observed in layers fed diet B could be due to the impact of $AFB_1$ on renal function, as aflatoxicosis is known to disrupt normal kidney function and fluid balance (Surai, 2002). Layers fed diet C showed some protective effect of PGLM $AFB_1$-induced electrolyte imbalance. The significantly increased levels of calcium ion observed in layers on Diets C and D indicated that PGLM supplementation could enhance calcium absorption and metabolism, which is essential for eggshell formation and overall bone health (Leeson and Summers, 2001).

**Table 1: Electrolyte balance of layers fed aflatoxin $B_1$-contaminated diets with or without *Psidium guajava* leaf meal**

| | Diets | | | | | P-Value |
|---|---|---|---|---|---|---|
| **Parameters** | **A** | **B** | **C** | **D** | **SEM** | |
| $Na^+$ (mmol/l) | $160.00^b$ | $150.00^c$ | $170.10^{ab}$ | $190.00^a$ | 11.01 | 0.01 |
| $Cl^-$ (mmol/l) | $83.10^b$ | $80.00^c$ | $80.22^{ab}$ | $85.13^a$ | 2.00 | 0.03 |
| $PO_4^{3-}$ (mmol/l) | $2.00^a$ | $1.81^b$ | $2.10^a$ | $2.00^a$ | 0.16 | 0.04 |
| $Ca^{2+}$ (mmol/l) | $2.41^c$ | $2.40^c$ | $2.52^b$ | $2.63^a$ | 0.03 | 0.03 |

$^{abc}$ 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/kg $AFB_1$, C = Diet B + 5 g/kg PGLM, D = Diet B + 10 g/kg PGLM. Sodium ion ($Na^+$), Chloride ion ($Cl^-$), Phosphate ion ($PO_4^{3-}$), Calcium ion ($Ca^{2+}$)

**Serum Proteins, Enzymes and Glucose**
Table 2 presents the serum proteins, enzyme activities and glucose concentrations of hens fed $AFB_1$-contaminated diets with or without *Psidium guajava* leaf meal. Hens fed diet B had the lowest serum protein concentration,

globulin (11.02), albumin (17.10) and total protein (28.12) when compared with hens on diet A, globulin (12.00), albumin (22.31) and total protein (34.31) , diet C, globulin (12.00), albumin (22.49) and total protein (34.49) and diet D, globulin (19.04), albumin (23.11) and total protein (42.15). The lowest globulin levels found in layers fed diet B, indicated the negative impact of $AFB_1$ on protein synthesis and immune function (Surai, 2002). The lowest albumin level observed in diet B reflected the detrimental impact of $AFB_1$, which could lead to hypoalbuminemia and impaired liver function (Zain, 2011). However, PGLM supplementation at level of 10g/kg mitigated the negative effects of $AFB_1$ on albumin levels.

The highest ALT level in birds fed diet B indicated liver damage, as elevated ALT levels are a marker of hepatic injury (Surai, 2002). The lowest ALT level recorded in layers fed diet D, which was similar to that of the control, suggested that PGLM at 10 g/kg effectively protected the liver from $AFB_1$-induced damage. The highest AST level observed in layers fed diet B indicated severe hepatic stress and damage due to $AFB_1$ as reported by Zain (2011). The lowest AST level recorded in layers fed diet D, which was similar to those on the control diet, indicated hepatoprotective effects of PGLM at a higher supplementation level.

**Table 2: Serum proteins, enzyme activities and glucose concentrations of hens fed aflatoxin $B_1$-contaminated diets with or without *Psidium guajava* leaf meal**

| Parameters | A | B | C | D | SEM | P-Value |
|---|---|---|---|---|---|---|
| **Serum Protein (g/l)** | | | | | | |
| Globulin | 12.00$^{b}$ | 11.02$^{b}$ | 12.00$^{b}$ | 19.04$^{a}$ | 1.40 | 0.02 |
| Albumin | 22.31$^{a}$ | 17.10$^{b}$ | 22.49$^{a}$ | 23.11$^{a}$ | 1.52 | 0.01 |
| Total Protein | 34.31$^{b}$ | 28.12$^{c}$ | 34.49$^{b}$ | 42.15$^{a}$ | 0.71 | 0.01 |
| **Serum Enzymes (U/L)** | | | | | | |
| Alanine aminotransferase | 30.00$^{bc}$ | 44.10$^{a}$ | 35.20$^{b}$ | 23.11$^{c}$ | 4.34 | 0.03 |
| Aspartate aminotransferase | 38.03$^{c}$ | 51.00$^{a}$ | 48.16$^{b}$ | 38.00$^{c}$ | 5.61 | 0.02 |
| **Serum Glucose (mmol/l)** | 4.95$^{b}$ | 5.28$^{a}$ | 4.80$^{b}$ | 4.51$^{c}$ | 0.21 | 0.01 |

$^{abc}$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/kg $AFB_1$, C = Diet B + 5 g/kg PGLM, D = Diet B + 10 g/kg PGLM.

Glucose levels are influenced by stress and metabolic function. The highest level of serum glucose in layers fed diet B might be a stress response to $AFB_1$ toxicity, leading to hyperglycemia (Jayasri *et al*., 2023). While the lowest serum glucose level observed in diet D suggested the potential role of PGLM at 10 g/kg diet in enhancing glucose homeostasis, possibly through its anti-oxidative and oxidative stress-relieving properties (Jayasri *et al*., 2023).

## CONCLUSION

The study demonstrated that $AFB_1$ contamination significantly disrupted electrolyte balance in layers, particularly affecting sodium, chloride, phosphate, and calcium ions. The inclusion of *P. guajava* leaf meal, especially at 10g/kg, in the diet mitigated these effects. This demonstrated the potential of PGLM as a natural dietary supplement to counteract the adverse impacts of $AFB_1$-contamination in poultry feed. Also, these findings suggested that supplementation with PGLM, especially at 10g/kg, had beneficial effects on the serum glucose, enzyme activities and protein metabolism of layers exposed to $AFB_1$-contaminated diets, potentially mitigating the adverse effects of the mycotoxin on these parameters. The findings in this study suggested that since PGLM is abundant and affordable, it could be, recommended to be utilized at 10g/kg as an antioxidant promoter. This may reduce the risk of side effects occasioned by the exposure of layers to $AFB_1$-contaminated diets. It may also help to maintain electrolyte balance and reduce oxidative stress and inflammation in birds exposed to mycotoxin-contaminated diets.

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