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Original Article

Dietary inclusion of *Psidium guajava* leaf meal improves quality parameters of eggs from hens fed aflatoxin B_1-contaminated diets

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Abstract
The study investigated the impact of varied levels of *Psidium guajava* leaf meal (PGLM) on internal, external, and biochemical egg qualities of hens exposed to diets contaminated by aflatoxin B_1 (AFB_1). Ninety-six point-of-lay Isa Brown pullets were randomly assigned to four dietary groups; each replicated six times with four birds per replicate in a completely randomized design (CRD). The experimental diets included: A (control), B (basal with 1 mg AFB_1/kg diet), C (Diet B with 5 g PGLM /kg diet), and D (Diet B with 10 g PGLM /kg diet). Among the internal egg qualities, albumen weight, yolk length, and yolk width were significantly reduced ($P<0.05$) in hens fed Diet B, containing 1 mg AFB_1/kg, whereas other parameters showed no significant difference ($P>0.05$). However, supplementation with 5 and 10 g PGLM/kg in Diets C and D, respectively, significantly improved these parameters. External egg qualities such as shell weight, thickness, ratio, and index were significantly affected ($P<0.05$), with reductions observed in birds fed Diet B. Biochemical analysis of yolk components indicated significant reductions ($P<0.05$) in SOD, CAT, GPx, TAC, and HDL-C levels, and increases ($P<0.05$) in MDA, TG, TC, and LDL-C levels in birds fed Diet B compared to Diet A. Diets C and D mitigated these adverse effects, showing significant improvement ($P<0.05$) across these parameters. Contamination of layer diets with 1 mg AFB_1/kg negatively impacts egg quality, while supplementation with 5 to 10 g PGLM /kg effectively mitigates these effects. Thus, incorporating PGLM into AFB_1-contaminated diets shows promise for maintaining egg quality in layers.

INTRODUCTION

Layers are hens raised for egg production (Kamboh *et al*., 2015). Poultry eggs are cheap and valuable food for human nutrition. The demand for this food is likely to increase as the global population increases (Kamboh *et al*., 2015). The Nigerian poultry industry comprises approximately 180 million birds and produces the largest annual egg production of 650 metric tons (NIAS, 2021). Meat and egg production are the primary sectors

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within poultry farming, alongside other areas including chick production, point-of-lay production, feed manufacturing, poultry equipment production, as well as poultry processing and marketing (Alders *et al*., 2018). Layers production plays an all-important part in food security of the world's population by providing eggs (Saki *et al*., 2010). Laying hen can be exotic and indigenous and have been reportedly bred for increased performance, increased productivity and impacting the environment as a whole (Ijaiya *et al*., 2010).

The egg is adjudged a complete protein food because it has both all the essential and non-essential amino acids. Eggs provide a very high-quality protein that contains all essential amino acids in the correct proportions needed by the body for optimal growth and maintenance (Adu *et al.,* 2017). However, the quality of eggs and the health status of the laying hens are influenced by environmental factors, such as ambient temperature and nutrition (Adu *et al*., 2017). Climatic conditions in most regions of Africa are characterized by high relative humidity, high temperatures, and little aeration (Sirma *et al*., 2018). These conditions make crops more liable to mycotoxin contaminations as they accelerate growth and mycotoxin biosynthesis by toxigenic fungi (Sarma *et al*., 2017). Mycotoxins lower the profitability due to high economic losses in poultry industry by decreasing the growth rate, feed conversion efficacy, carcass yield and egg yield quality (Morrison *et al*., 2017).

Mycotoxin contamination is a major problem to agricultural products in Nigeria, and over 40% of agricultural products have been reported to be prone to mycotoxins (Darwish *et al.,* 2014). Mycotoxicosis is a chronic condition resulting from the prolonged ingestion of low levels of fungal metabolites, leading to measurable declines in performance and the manifestation of nonspecific physiological changes (Nakavuma *et al.,* 2020). Mycotoxins produce a variety of diseases, collectively called "mycotoxicoses," directly or in combination with other primary stressors such as pathogens (Akinmusire *et al*., 2018). Among the commonly known mycotoxins, ochratoxin A (OTA), deoxynivalenol, T-2, zearalenone (ZEN) and aflatoxins (AFs), are major contaminants of feed and feed ingredients (Njobeh *et al*., 2012).

Aflatoxins are a group of mycotoxins mainly produced by several fungal species in the genus *Aspergillus*, it includes *A. flavus, A. parasiticus, A. pseudotamarii,* and *A.nomis*. Among these species, *A. flavus* and *A. parasiticus* are well known (Mamo *et al.,* 2021). *Aspergillus parasiticus* is less widely distributed and *A. flavus* is the most widely reported fungus in foodstuffs (Mamo *et al.,* 2021). Aflatoxin-contaminated diets could cause economic loss to the farmer because it is present in variety of foods including peanuts, cottonseed meal, corn, and other grains, as well as animal feeds (Kanyi, 2018; Negash, 2018). Negash (2018) deduced that aflatoxins B_1, B_2, G_1 and G_2 are the most prevalent toxins that interfere with the metabolism of carbohydrates, fats and proteins in livestock. In maize, the most vigorous aflatoxin (aflatoxin B_1) is produced from an abundant amount of *A. flavus* and *A. parasiticus* (Zaki *et al.,* 2012). Aflatoxins disrupt cellular redox balance by increasing the production of reactive oxygen species (ROS), thereby overwhelming the body's natural antioxidant defense systems. Antioxidants, therefore, play a crucial role in preventing and mitigating the harmful effects of mycotoxins by neutralizing reactive oxygen species (ROS), enhancing endogenous defense systems, improving immune function, supporting liver and kidney function as well as enhancing growth performance and productivity.

Antioxidants are substances which can significantly delayed or prevent oxidation of a substance when present in low concentration (Olayemi *et al.,* 2019). It protects susceptible substrates by the removal of free radical initiators and propagators by transferring a hydrogen atom to stabilize the free radicals to stop the lipoperoxidation chain (Olayemi *et al*., 2019). In the search for a solution to these negative consequences, extensive research has been performed on the use of phytochemicals such as bitter leaf meal and moringa leaf meal as alternatives to inorganic antioxidants in poultry diets (Daramola, 2019). Phytochemical screening of these aforementioned leaves showed the presence of phenolic acids, flavonoids, tannins, cardiac glycosides, saponin and glucosinolates, which are of great value in preventing the onset or progression of many human and animal diseases (Daramola, 2019). The health promoting effect of antioxidants from plants is thought to arise from their protective effects by counteracting reactive oxygen species (Saleh *et al*., 2018). Another of such plant is *Psidium guajava.*

The guava tree (*Psidium guajava*) is native of Tropical America. The archaeological remains place it in Brazil or somewhere between Mexico and Peru (Singh, 2018). Its leaves and bark are used as phytopreparations in animals and humans, due to their antibacterial, antiemetic, anti-inflammatory, anthelmintic, antiseptic, antitoxic, astringent, carminative, spasmodic and tonic properties (Naseer *et al*. 2018). Salazar *et al*. (2017) found improvements in egg production and mass conversion, when *P. guajava* powder was included as part of a mixture of medicinal plants. Zargar *et al*. (2020) reported that the use of *P. guajava* leaves reduced histopathological lesions and cholesterol in broilers. Geidam *et al*. (2015) showed that with the use of the leaf of this medicinal plant, the diarrheal syndrome in broilers with *E. coli* decreased. Also, the crude extract of *P. guajava* leaf reduced the serum concentration of malondialdehyde (MDA) as an indicator of oxidative stress in laying hens (Martínez *et al*., 2020). According to Wang *et al*. (2021), the addition of *P. guajava* leaf could organize and redistribute energy resources through similar or dissimilar metabolic pathways and finally enhance antioxidant ability of livestock. Therefore, the aim of the present study is to examine the effects of dietary inclusion of *Psidium guajava* leaf meal on quality parameters of eggs from hens fed aflatoxin B_1-contaminated diets

MATERIALS AND METHODS

Preparation of *Psidium guava* Leaf Meal (PGLM)

Guava leaf was collected from different areas in Akungba-Akoko, Ondo State, Nigeria. It was sliced into small sizes for effective drying and to maintain green colour even when dried. The guava leaf was air dried for about 4 weeks in an open ventilated space away from sunlight. The air-dried guava leaf was milled into fine particles using a local milling machine. The ground sample was stored in a clean air tight container to prevent contamination. The test ingredient was added at different levels to make different dietary treatments.

Aflatoxin B_1 production

Maize grains were milled into smaller pieces using a local milling machine. The milled maize was autoclaved and cultured with a toxigenic strain of *Aspergillus flavus* (NRRL 6513) inoculum to produce aflatoxin B_1 (Singh and Shrivastava, 2012). The method involved a mixture of 500g of yellow corn kernels and 500 mL of distilled water in a 30.5×61cm autoclavable polyethylene bag, autoclaved for 30 minutes and allowed to cool down. The autoclaved maize was inoculated by drawing an aqueous suspension from a lyophilized culture into a sterile 5 mL syringe fitted with a 19-gauge needle and injecting 1 mL through the side of each bag. Bags of inoculated maize were incubated in the dark at 20 to 22°C for two weeks. Seven to eight days after inoculation, holes were punched near the tops of the bags to promote aeration. After the two-week incubation period, the cultured material was air dried for 48 hours. The *Aspergillus flavus*-contaminated maize grains were pulverized and quantified for AFB_1 concentration.

Formulation of Experimental Diets

A basal diet (Table 1) was formulated for the birds and analyzed for proximate composition. For quantification of AFB_1, 10g of the contaminated maize was mixed with 1kg of the basal diet and quantified for dietary contents of AFB_1. The result was 0.25 mg AFB_1/kg. Thus, 40g/kg of the contaminated maize was replaced for 40g/kg of pure maize to produce the experimental diet. The resultant feed was quantified; the result was 1.005 mg AFB_1/kg after which the basal diet was divided equally into four portions coded (Diets A, B, C and D). Diet A (control/basal), Diet B (Diet A + 1mg AFB_1/kg), Diet C (Diet B + 5g PGLM /kg), Diet D (Diet B + 10g PGLM /kg).

Table 1.Ingredients Composition of the Experimental Basal Diet

Ingredient	Quantity (kg)
Maize	45
Soybean meal	15
Wheat Offal	31
Limestone	6
Bone Meal	1.9
Lysine	0.3
Methionine	0.3
Salt	0.25
Layer premix	0.25
TOTAL	**100**
Calculated Nutrients	
Crude protein (%)	16.21
ME (Kcal/ME)	2,529.10
Calcium (%)	2.87
Phosphorous (%)	0.46
Crude fibre (%)	4.83
Fat (%)	3.41
Lysine (%)	1.11
Methionine (%)	0.54

Experimental Site

The research was carried out at the Poultry Unit of the Teaching and Research Farm, Adekunle Ajasin University, Akungba-Akoko, Nigeria. It is situated at 326m above sea level and lies at a latitude of 07° 28' 11 North and longitude of 5° 44'10 East in the humid rain forest zone of western Nigeria (NGIA, 2020). It is in the tropical climate of broadly two seasons; rainy season (April -

October) and dry season (November - March) characterized by a mean annual minimum and maximum temperatures of 21 and 32 °C, respectively and precipitation of 1500 - 2000 mm per annum. The annual relative humidity range is 41 - 91% (Olabode, 2014). The study was carried out for eight weeks following the research ethics and guidelines of the Animal Science Department of the institution. This study was conducted from the month of September to November.

Research Birds and Management

A total of 100 point-of-lay Isa Brown pullets were purchased from a reputable farm. Four birds were housed in each cell in a battery cage with floor covered with wood shavings for a period of two weeks for physiological adjustment before the commencement of the feeding experiment. Thereafter, the birds were weighed for their initial weight and 96 birds were randomly assigned to four treatments. Each treatment was replicated 6 times with 4 birds per replicate in a completely randomized design making it 24 birds per treatment. Feed and water were provided *ad libitum* throughout the 8 weeks of the experiment and the recommended medications were administered when necessary. Throughout the period of the study, the birds were subjected to the same management practices such as sanitary conditions, feed and water on daily basis. Data on feed consumption was taken weekly while egg production was taken daily and stored in a cool dry place. The percentage of daily egg production was calculated as total number of eggs produced/ total number of hens present) x 100. The weekly feed intake was determined as the total feed given for the week minus the left over. The feed conversion ratio (FCR) was determined as the ratio of total feed intake (TFI) to total weight gain (TWG):

FCR = TFI/TWG.

Protein intake (PI) and energy intake (EI) were also determined as described by Olarotimi and Adu (2022) from the total feed intake, feed crude protein (FCP) and feed metabolizable energy (FME) contents. i.e.

PI= (TFI × FCP)/100

EI = (TFI × FME)/1000

Both protein and energy utilization indices were determined as the ratio of PI and EI to TWG respectively: PU = PI/TWG and EU = EI/TWG respectively.

Egg Quality Assessment

At about 50% laying performance, eggs collected were analysed weekly. For egg qualities determination, a total of 20 eggs per treatment, i.e. 5 eggs per replicate were randomly selected on weekly basis. Internal and external qualities such as egg weight, length, width, index, surface area; yolk weight, height, diameter, ratio, index; albumen weight length, height, diameter, index, ratio; shell weight, thickness, ratio and Haugh Unit (HU) were determined (Olumide *et al*., 2016).

The albumen weight was calculated by subtracting the sum of the weights of the shell and the yolk from the total egg weight. Shell thickness was measured with micrometer screw gauge. The eggs were carefully broken, the contents removed and the shells air-dried for two days before weighing. Percentage shell weight was measured by finding the ratio of the shell weight to the egg weight, expressed in percentage. Shell surface area was determined as reported by (Crosara *et al*., 2019). The shell surface area was calculated by the formula:

$SSA = W^{0.667} \times 4.67$

Where

SSA = Shell Surface Area

W = Average Egg Weight

0.667 and 4.67 are constants.

Hen day production (HDP) in percentage was calculated by adding all the eggs per replicate on daily basis using the formula below:

$$\frac{Ne}{Nb} \times 100$$

Where

Ne = Number of eggs laid per replicate per day

Nb = Number of birds per replicate

Yolk index was calculated as the ratio of the yolk height to the yolk width. Yolk height and width was measured with a ruler calibrated in centimetre with the aid of optical pins and mathematical compass. Yolk index and Haugh Unit (HU) was determined according to the methods of Oluyemi and Robert (2000).

$$\text{Yolk Index} = \frac{\text{yolk height}}{\text{yolk width}}$$

$HU = 100 \log (H + 7.57 - 1.7W^{0.37})$

Where H = observed albumen height in mm

W = observed weight of eggs in grammes.

Yolk weight percentage is the ratio of the yolk weight to the egg weight and was calculated by the formula:

$$\%\text{ Yolk Weight} = \frac{\text{yolk weight}}{\text{egg weight}} \times 100$$

The width and the length of the eggs was measured with the aid of Vernier calliper calibrated in centimetres. Egg width (maximum) was divided by the maximum egg length to get the egg shape index. Albumen heights in millimetres were taken with the aid of optical pin which was used for the calculation of Haugh Units. The use of egg mass rather than egg number is to ensure better comparisons of flocks. It is estimated as:

EM = % HDP × EW. Where HDP is Hen Day production, EW is Egg Weight in grams (Fikru *et al*., 2015).

Egg surface area (ESA) was determined as reported by Adu and Olarotimi (2020) using the formula:

ESA/SSA=W0.667×4.67; W=Average Egg Weight, 0.667 and 4.67 are constants. SSA; Shell surface area.

Egg Specific Gravity (ESG) was determined using the formula reported by Adu and Olarotimi (2020).

$ESG = 1.9754EW/1.9140EW - ESW$

EW; egg weight, ESW: egg shell weight; 1.9754 and 1.9140 are constants. Shell weight per unit egg surface area:

$(SWUSA) = (3.9782EW)^{0.666}$

Other egg qualities parameters were estimated using Paganelli's equations as reported by (Hegab and Hanafy, 2019). SA; Surface area of egg

- Area vs. egg volume (V, cm^3):

$SA = 4.951\ EV^{0.666}$

- Egg density (ED, g $cm^{-3)}$ vs. egg weight:

$ED = 1.038\ EW^{0.006}$

- Shell density (ShDgcm^{-3}) vs. egg weight:

$SD = 1.945\ EW^{0.014}$

- Shell Volume (SV, cm^3) vs. egg weight:

$SV = 2.48 \times 10^{-2}\ W^{1.118}$

Statistical Analysis

The design used for this experiment was Completely Randomized Design (CRD). Data collected were subjected to statistical analyses using one-way analysis of variance (ANOVA) procedure of SAS (2008). The significant treatments were compared using Duncan's multiple range tests at 5% level of significance.

RESULTS

Internal egg qualities of layers fed aflatoxin B1-contaminated diets with *Psidium guajava* leaf meal

The results of the internal egg (albumen and yolk) qualities of layers fed aflatoxin B_1-contaminated diets with *Psidium guajava* leaf meal are presented in Table 2. The parameters significantly (P<0.05) influenced by the inclusions of 1mg AFB1/kg and PGLM at 5 and 10g/kg were albumen weight, yolk length and width. There were significant (P<0.05) reductions in the means of these parameters among the hens fed diet B when compared with hens on diet A. However, varied inclusions of PGLM significantly (P<0.05), as observed in diets C and D, enhanced the values of these parameters. While inclusion of 5 g PGLM /kg did not significantly (P>0.05) improve yolk length and width in hens fed diet C when compared with hens fed diet B, 10 g PGLM /kg performed significantly better. There were no significant (P>0.05) differences in the means of other parameters across all the experimental diets.

Table 2: Internal Egg Qualities of Layers Fed Aflatoxin B_1-Contaminated Diets with *Psidium guajava* Leaf Meal

Parameters	A	B	C	D	SEM	P-value
Albumen						
Length (cm)	4.80	4.61	4.54	4.60	0.12	0.71
Width (cm)	3.71	3.70	3.63	3.66	0.06	0.19
Weight (g)	37.12[a]	34.10[b]	37.34[a]	37.00[a]	1.70	0.04
Height (mm)	0.71	0.69	0.71	0.73	0.02	0.49
Haugh Unit	70	69	69	69	0.83	0.36
Yolk						
Length (cm)	2.81[a]	2.61[b]	2.62[b]	2.80[a]	0.08	0.01
Width (cm)	2.60[a]	2.21[b]	2.42[ab]	2.53[a]	0.07	0.01
Weight (g)	15.00	15.01	14.99	13.75	1.09	0.14
Height (cm)	1.41	1.35	1.40	1.42	0.07	0.09
Index	0.54	0.61	0.59	0.56	0.04	0.63

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.

Egg shell quality of layers fed aflatoxin B1-contaminated diets with *Psidium guajava* leaf meal

The results of the external egg quality of layers fed aflatoxin B1-contaminated diets with or without *Psidium guajava* leaf meal are presented in Table 3. Of all the parameters studied, only the shell weight, shell thickness, shell ratio and shell index were significantly (P<0.05) influenced. There were significant (P<0.05) reductions in the means of these parameters among the

Table 3: External egg quality of layers fed aflatoxin B1 with *Psidium guajava* leaf meal

Parameters	A	B	C	D	SEM	P-Value
Egg Shell						
Shell Weight (g)	6.40^{a}	5.62^{b}	6.41^{a}	6.50^{a}	0.23	0.01
Shell Thickness (mm)	0.51^{a}	0.48^{b}	0.52^{a}	0.50^{a}	0.04	0.01
Shell Ratio (%)	11.72^{a}	9.31^{b}	12.09^{a}	11.69^{a}	0.62	0.04
Shell Surface Area (cm^2)	71.12	72.00	70.01	69.89	2.52	0.34
Shell Index	9.00^{a}	7.80^{b}	9.02^{a}	9.14^{a}	0.38	0.03
Shell Density(gcm^{-3})	2.14	2.11	2.10	2.12	0.00	0.37
SWUSA (gcm^{-2})	38.02	38.00	36.99	37.01	1.34	0.20
Shell Volume (cm3)	2.43	2.41	2.28	2.30	0.14	0.63
Egg						
Weight (g)	59.00	60.01	56.00	57.00	3.11	1.12
Volume (cm3)	48.98	40.00	38.00	39.00	4.70	0.81
Density (gcm-3)	1.20	1.50	1.47	1.46	0.13	0.51
Length (cm)	5.30	5.00	4.92	5.06	0.22	2.35
Width (cm)	4.51	4.07	3.90	4.10	0.33	1.11
Shape Index	84.00	80.00	80.00	79.00	3.41	4.25
Surface Area (cm2)	65.00	58.00	56.00	57.00	4.40	0.53

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.

hens fed diet B when compared with birds on diet A. However, inclusions of 5 and 10 g PGLM /kg improved these parameters when compared with the values recorded among the hens on diet B.

Egg biochemical qualities of layers fed aflatoxin B1-contaminated diets with *Psidium guajava* leaf meal

The antioxidant status and lipid profiles of eggs from hens fed AFB_1-contamiated diets with or without PGLM are shown in Table 4. For the antioxidant status, there was significant ($P<0.05$) increase in the MDA concentrations of the eggs produced by hens on diet B as compared to those on the control diet. Furthermore, there were significant ($P<0.05$) decreases in all the antioxidant enzyme levels of the yolk collected from hens on diet B when compared with those on the control. The yolk SOD, CAT, TAC and GPx contents were significantly lowered by the inclusion of 1mg AFB_1/kg. However, dietary fortification of diet B with 5 g and 10 g PGLM as observed in eggs collected from hens on diets C and D respectively significantly ($P<0.05$) reduced the egg yolk concentration of MDA when compared with the eggs from hens on diet B. The

Table 4: Egg Biochemical Qualities of Layers Fed Aflatoxin B1-Contaminated Diets with *Psidium guajava* Leaf Meal

Parameters	A	B	C	D	SEM	P-Value
Antioxidant status						
MDA (mg/g)	0.89^{b}	3.10^{a}	0.55^{c}	0.50^{d}	0.02	0.01
SOD (U/ml)	0.72^{a}	0.54^{b}	0.66^{ab}	0.81^{a}	0.07	0.01
TAC (mM Trolox Eq.)	7.74^{a}	6.50^{b}	6.76^{b}	7.96^{a}	0.16	0.04
GPx (U/L)	116.00^{a}	84.40^{b}	116.00^{a}	132.00^{a}	7.07	0.03
CAT (U/mg prot.)	2.79^{a}	1.86^{b}	3.32^{a}	3.05^{a}	0.36	0.02
Lipid Profiles (mmol/l)						
TC	6.00^{b}	7.39^{a}	5.02^{b}	3.41^{c}	0.59	0.02
TG	1.65^{c}	2.39^{a}	1.58^{c}	1.95^{b}	0.08	0.01
HDL-C	1.03^{b}	0.65^{c}	1.08^{b}	1.33^{a}	0.07	0.01
LDL-C	4.34^{bc}	6.77^{a}	5.59^{ab}	3.63^{c}	0.61	0.04

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. Total Cholesterol (TC); High Density Lipoprotein Cholesterol (HDL-C); Triglyceride (TG); Low Density Lipoprotein Cholesterol (LDL-C); Total Antioxidant Activity (TAC); Malondialdehyde (MDA); Superoxide Dismutase (SOD); Catalase (CAT); Glutathione Peroxidase (GPx).

inclusions of PGLM in diets C and D brought about significant (P<0.05) enhancement of the antioxidant profiles of the yolk when compared with eggs collected from the hens on diet B. Better results were recorded among the hens fed diet D for all of the parameters when compared with the results from the hens on diet C.

DISCUSSION

Internal and external egg qualities

Egg qualities play a significant role in determining the marketability, consumer acceptance, and nutritional value of eggs. External egg qualities such as good eggshell quality is essential for preservation of egg contents and embryonic life. Eggs with poor shell quality are rejected both as table eggs and as hatching eggs, leading to heavy economic losses (Devegowda and Ravikiran, 2008). The significant reduction observed in egg shell weight of hen fed diet B was indicative of the adverse effects of AFB_1 on egg quality. The present results had validated the opinion of Devegowda and Ravikiran (2008) that dietary mycotoxins in laying hens can predispose them to or precipitate eggshell defects. Other study had also established the fact that dietary AFB_1 is capable of reducing eggshell weight of laying hens (Zaghini *et al*. 2005). The observed reduction in shell thickness and non-significant effects in egg weight were in agreement with the reports of Aly Salwa and Anwer (2009) and Verma *et al*. (2003).

The significant reduction in the albumen weight of hens on diet B could be due to inclusion of AFB_1 in the diet. Ochieng *et al*. (2021) and Seifi *et al*. (2021) had previously reported the negative effect of mycotoxins on egg albumen weight. Since diets contaminated with aflatoxins are reported to cause liver malfunctioning, thus negatively affecting liver synthesis and transport of yolk precursors (Ochieng *et al.*, 2021), the significant reduction observed in the egg yolk length and width among the hens on diet B may indicate a potential physiological response or alteration in egg formation in hens exposed to AFB_1. This is similar to the changes in egg quality parameters, including egg weight and shell thickness, in response to AFB1 contamination in poultry diets reported by Khatoon *et al.* (2015) that.

However, inclusions of PGLM in diets C and D were observed to significantly mitigate the adverse effects of AFB_1 on the internal and external egg qualities studied. The improvement observed in albumen weight; yolk width and length were similar to the findings of Kedir *et al*. (2023) that illustrated the potency of leaf supplements such as rosemary (*Rosmarinus officinalis*) leaf meal in the enhancement of egg production and quality parameters. The observed enrichment of the egg albumen and yolk with the inclusions of PGLM in the present study may be attributed to the high bioactive contents, such as carotenoids and flavonoids, present in the leaf of this plant (Ahmad *et al*., 2018).

External egg qualities

Aflatoxin contamination in poultry feed can lead to reduced shell quality in hens. A study by Ghosh *et al*. (2016) found that aflatoxin exposure negatively impacts eggshell thickness and strength. The reduction in the egg shell weight, egg shell ratio and egg shell index from hens on diet B in the present study was in line with the report of Fouad *et al*. (2019) that highlighted that the presence of aflatoxin AFB_1 in poultry diets decreases egg quality. These results were also in agreement with the findings of Verma *et al*. (2004) that reported a reduction in egg weight and shell-thickness for groups of hens fed a diet containing 1 - 2mg AFB_1/kg. The significant increases observed in these qualities among the birds on diets C and D were also in accordance with the study that found increase in egg weight by use of dietary sodium bentonite aflatoxin binder and its effects on production performance of laying hens (Gul *et al*., 2017). The enhancement egg weight could be attributed to the presence of PGLM in Diets C and D. Also, the significant increase in the egg shell weight, egg shell ratio and egg shell index of birds on diets C and D of this study could be a proof that PGLM may be effective in mitigating the harmful impact of aflatoxin exposure.

In another development, Khatoon *et al.* (2018) demonstrated that dietary supplementation with certain herbal extracts reduced the adverse effects of aflatoxin exposure on egg production and quality parameters in laying hens. Egg shell quality parameters such as shell thickness, shell surface area, shell density, SWUSA, and shell volume that were not significantly affected in this study by both AFB_1 and PGLM could be due to the fact that AFB_1 primarily targets the liver, leading to hepatotoxicity, immunosuppression, and impaired protein synthesis (Hua *et al*., 2021). However, eggshell formation occurs in the shell gland (uterus) of the hen's reproductive tract and relies more on calcium metabolism and carbonic anhydrase activity than liver function directly (Sinclair-Black *et al.,* 2023).

Unless the aflatoxin dosage is high enough to severely disrupt calcium metabolism, its effect on shell quality parameters may be minimal.

Although shell thickness, shell surface area, shell density, SWUSA, and shell volume were not also affected by the inclusions of PGLM, this could be because the primary benefits of PGLM are more aligned with mitigating oxidative stress and liver damage caused by AFB_1 than enhancing shell mineralization. Unlike additives such as vitamin D3, calcium supplements, or phytase, which directly influence mineral availability and shell strength, PGLM may lack sufficient levels of nutrients or hormones that impact shell surface area, thickness, or density.

Egg biochemical qualities

Aflatoxin contamination in poultry feed has a profound impact on the biochemical composition of hens' eggs (Fouad *et al*., 2019). It affects protein and lipid content, vitamin and mineral levels, antioxidant capacity, and functional properties, ultimately reducing the nutritional quality and safety of the eggs (Ofori-Attah *et al*., 2024). The biochemical alterations caused by AFB_1 are primarily due to their toxic effects on the liver, which is a crucial organ for metabolism and detoxification in hens (Popescu *et al*., 2022). For instance, the MDA concentration which is a measure of lipid peroxidation which increased as well as the significant reductions in the antioxidant enzymes of the eggs in the present study indicated that AFB_1 contamination enhanced the promotion of oxidative stress in chickens. This position buttressed the assertion of Yilmaz *et al*. (2015) that AFB_1 increases lipid peroxidation and decreases both enzymatic and non-enzymatic antioxidants, leading to oxidative stress. In the same vein, the significant increase in the egg lipid profiles such as TC, TG and LDL-C and the elevation of the HDL-C concentrations in the present study further strengthened the position that AFB_1 adversely affected the quality of eggs produced by the hens fed the contaminated diets. Aflatoxins disrupt various metabolic pathways, leading to imbalances in nutrient assimilation and deposition in eggs (Mavrommatis *et al*., 2021). Studies had shown that elevated TC, TG and LDL-C can raise the risk of heart disease (Spence *et al*., 2010; Li *et al*., 2020). Therefore, the eggs produced by the hens on diet B might be risky to the absolute well-being of the consumers.

Ensuring feed quality and regular monitoring for aflatoxin contamination are essential to mitigate these adverse effects and maintain the health of the hens and the quality of the eggs they produce. The potentials of PGLM to reduce oxidative stress through multiple mechanisms, including direct scavenging of free radicals, enhancing endogenous antioxidant defences, inhibiting oxidative enzymes, and preventing lipid peroxidation have been well established (Jayachandran *et al*., 2018; Kumar *et al*., 2021).

The significant reduction in the MDA concentration and the elevations of the antioxidant enzymes of the eggs from hens fed diets 3 and 4 were testimonial of the free radical scavenging power of PGLM bioactive components. These actions collectively contribute to various health benefits, highlighting the potential of guava leaf as a natural therapeutic agent for managing oxidative stress-related conditions. Also, PGLM has proved to be effective in reducing bad cholesterol (LDL-C) and TG levels in this study. The mechanisms through which this happens include inhibiting cholesterol absorption, enhancing cholesterol excretion, providing antioxidant protection, reducing inflammation, and improving lipid metabolism (Díaz-de-Cerio *et al*., 2017; Kumar *et al*., 2021). These effects collectively contribute to improved cardiovascular health. Therefore, regular consumption of eggs from hens fed diets 3 and 4, alongside a balanced diet and healthy lifestyle, could be a beneficial strategy for managing cholesterol levels and promoting overall heart health.

CONCLUSION

It could, therefore, be concluded that feeding laying hens with diets containing up to 1 mg AFB_1/kg confers adverse effects on the internal, external and egg biochemical qualities of the layers. Supplementing such diets with 5 – 10 g PGLM /kg, however, could be a strategy to mitigate the adverse effects of AFB_1 contamination on the egg qualities. Consumption of eggs from hens fed diet B could be potentially dangerous to consumers due to the elevated levels of TC, TG, LDL-C and MDA levels of the eggs while supplementation with PGLM could make the eggs safer for consumers.

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