



# Supplemental zinc and *Vernonia amygdalina* leaf meal comparatively mitigated induced performance and blood related aflatoxicity in cocks

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## Abstract

Contamination of poultry feed with mycotoxins such as *Aflatoxin flavus* has been a major challenge to the poultry industry due to the resultant toxic effects it poses. Hence, any nutritional strategy to mitigate the effects of this contamination will be a novel approach to enhancing productivity of the birds. The aim of this experiment was to evaluate the influence of dietary zinc (Zn) and *Vernonia amygdalina* leaf meal (VALM) on haematobiochemical indices and growth indicators in cocks raised on diets containing aflatoxin B<sub>1</sub> (AFB<sub>1</sub>). A total of 150 Isa White cocks of 24 weeks of age were allotted into 5 experimental diets: A (control), B (AFB<sub>1</sub> diet), C (AFB<sub>1</sub> diet + Zn), D (AFB<sub>1</sub> diet + 2.50 g/kg VALM) and 5 (AFB<sub>1</sub> diet + 5.0 g/kg VALM). A total of thirty birds were allotted to each treatment which was replicated five times with each replicate containing six birds in a completely randomized design. The growth performance parameters, haematological indices and serum biochemical indicators in cocks fed diet B were observed to be adversely affected significantly (P<0.05). However, significant (P<0.05) enhancements of the parameters were noticed among the cocks fed Zn and varied additions of VALM. The results highlight the capacity of Zn and VALM as veritable agents to mitigate the possible effects of AFB<sub>1</sub> poisoning domestic chickens.

## INTRODUCTION

Feed ingredients have been shown to be prone to contamination by mycotoxins, leading to contamination of poultry feed (Akinmusire *et al.*, 2018). Mycotoxicosis has become one of the most serious fungal diseases, due to climatic change and modern agricultural practices, causing loss

to farmers and diseases to consumers (Osborne and Stein, 2007). Mycotoxins are secondary metabolites of fungi mainly belonging to the genera *Aspergillus*, *Alternaria*, *Fusarium*, *Cladosporium*, *Claviceps* and *Penicillium* (Peng *et al.*, 2018). The most frequently detected globally are aflatoxin B<sub>1</sub> (AFB<sub>1</sub>), aflatoxin

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B<sub>2</sub> (AFB<sub>2</sub>), aflatoxin G<sub>1</sub> (AFG<sub>1</sub>), and aflatoxin G<sub>2</sub> (AFG<sub>2</sub>); fumonisins (FBs); trichothecenes (for example, deoxynivalenol (DON) and T-2 toxin (T-2); and ochratoxin A (OTA) (Kemboi *et al.*, 2020).

Aflatoxin B<sub>1</sub> is the most common and also the most toxic in both acute and chronic terms (Kemboi *et al.*, 2020). The carcinogenicity of AFB<sub>1</sub> is common in several animals and the liver is the main target organ (Kensler *et al.*, 2016). The biochemical and haematological parameters of poultry birds have been reported to be changed due to the effects of AFB<sub>1</sub> contamination of the feed (Basmacioglu *et al.*, 2005). In chronic and subclinical aflatoxicoses, changes in biochemical and hematological parameters occur before clinical symptoms develop (Aravind *et al.*, 2003). Significant changes in serum biochemical and haematological parameters are seen in aflatoxicoses, and these can assist in the diagnosis of toxicity (Basmacioglu *et al.*, 2005).

Blood is useful for assessing the health status, clinical evaluation for survey of physiological or pathological conditions and diagnostic and prognostic evaluation of various types of diseases in animals (Alade *et al.*, 2005). The commonly used haematological parameters are erythrocyte (Red Blood Cells - RBC) leucocytes (White Blood Cells - WBC), haemoglobin concentration (HBC), packed cell volume (PCV) and values like mean corpuscular volume or cell (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) (Chineke *et al.*, 2006). It has also been reported that AFB<sub>1</sub> can adversely affect growth performance of many species (Deng

*et al.*, 2010). The level of aflatoxin in the diet affects the weight gain rate. For every 1 mg/kg increase in aflatoxin content in the diet, the growth rates of pigs and broilers decrease by 16 and 5%, respectively (Dersjant-Li *et al.*, 2003).

Because AFB<sub>1</sub> contamination of feed leads to generation of reactive oxygen species (ROS) and the formation of free radicals, oxidative stress is the resultant effect of ingestion of AFB<sub>1</sub> contaminated feeds by animals (Hamid *et al.*, 2013). The increased number of free radicals is due to the malfunctioning of the antioxidant system and it can lead to damage of the structure of DNA, proteins, and lipids (Assi, *et al.*, 2017). Antioxidants, on the other hand, are used in feed to prevent the deleterious effects of oxidation and thereby neutralize the adverse effects of oxidative stress on animals.

Many of the natural antioxidants of interest are of plant origin and belong to the phenolic and polyphenolic class of compounds as well as carotenoids and antioxidant vitamins, among others. For instance, fish that were fed with medicinal plants (i.e., whole plants, leaves, roots, or their extracts) have been shown to exhibit better levels of haematological parameters (Anjusha *et al.*, 2019). In particular, *Vernonia amygdalina* (VA) has some beneficial effects in disease management in poultry, such as anti-coccidiosis, anti-bacterial and anti-parasitic, as an antioxidant and as a growth promoter by enhancing the gastro intestinal enzymes thus increasing feed conversion efficiency (Gbolade, 2009; Olobatoke and Oloniruha, 2009). The biologically-active compounds of VA are saponins and alkaloids (Muraina *et al.*, 2010), terpenes, steroids, coumarins,

flavonoids, phenolic acids, lignans, xanthenes and anthraquinone (Cimanga *et al.*, 2004). For instance, Tokofai *et al.* (2021) reported that diet containing VA leaf meal (VALM) increased the feed intake and weight gain of poultry birds. Serum biochemical parameters such as serum proteins, enzymes and metabolites can serve as indicators for hepatic function (Chen *et al.*, 2016). Also, Olarotimi *et al.* (2023) documented the hepatic phytoprotective potentials of VALM. In the same way, VALM was reported to have effects in reducing toxicity and regulating lipid metabolism in the liver (Onasanwo *et al.*, 2017).

Zinc (Zn), on the other hand, is a nutritionally essential micro mineral playing a significant role in many biochemical processes such as amino acid metabolism and gene expression. Zinc is a structural component of the enzyme superoxide dismutase present in the cytoplasm of cells. For instance, dietary supplementation of Zn was found to adequately mitigate aflatoxin induced toxicity in broilers (Sharma and Singh, 2019). Therefore, the objective of this study is to compare the ameliorative effects of organic and inorganic antioxidants (VA and Zinc, respectively) on the growth performance and blood profiles of cocks fed AFB1- contaminated diets.

## **MATERIALS AND METHODS**

### **Experimental Site and Animals**

The study was carried out at the Teaching and Research Farm, Adekunle Ajasin University, Akungba-Akoko, Ondo State. A total of 150 sexually mature Isa White cocks of 24 weeks of age were used for the study. They were raised in battery cages and managed under strict bio-security system.

The birds were evenly allotted to five experimental treatments. Each treatment received a total of 30 cocks replicated five times with six cocks per replicate. The experimental diets and clean water were given *ad libitum* for 12 weeks' period of the study.

### **Preparation of Vernonia amygdalina leaf meal (VALM)**

Fresh leaves of *Vernonia amygdalina* were harvested within the premises of the Adekunle Ajasin University, Akungba-Akoko, Ondo State, and Southwestern Nigeria. The leaves were adequately validated at the university's herbarium. They were, thereafter, air-dried for seven days after which the dried leaves were reduced to powdery form using an electric blender [Bajaj, Model: Bravo Dlx Mixer Grinder (410175)]. The resultant powder *Vernonia amygdalina* leaf meal (VALM) obtained was kept in an air-tight container and kept in a dry place till it was used in the preparation of the experimental diets.

### **Aflatoxin B<sub>1</sub> production**

Autoclaved maize grains were cultured with a toxigenic strain of *Aspergillus flavus* (NRRL 6513) inoculum to produce aflatoxin B<sub>1</sub>. The method involved the addition of 500 g of yellow corn kernels and 500 mL of distilled water in a 30.5×61cm autoclavable polyethylene bag. The maize was inoculated by drawing an aqueous suspension from a lyophilized culture of the inoculum 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 14 days. Seven to eight days after inoculation, holes were punched near the tops of the bags to promote aeration. After a two-week incubation

period, the culture material was air dried for 48 hours. The *A. flavus* -contaminated maize grains were pulverized and quantified for AFB<sub>1</sub> concentration. Briefly, 10g of the contaminated maize was mixed with 1kg of the basal diet and quantified for dietary contents of AFB<sub>1</sub>. The result was 0.25 mg/kg AFB<sub>1</sub>. Thus, 40g/kg of the 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/kg AFB<sub>1</sub>. The experimental diets included the basal diet, and four other diets containing 1mg AFB/kg (Table 1).

### Formulation of Experimental Diets

A basal diet (Table 1) was formulated for the birds and analyzed for proximate composition (AOAC, 1995) after which the basal diet was divided equally into 5 portions coded A, B, C, D and E. Diet A (control/basal), Diet B (Diet A + 1.0mg AFB<sub>1</sub>/ kg of diet), Diet C (Diet B + 50g Zinc/kg), Diet D (Diet B + 2.5g VALM/ kg) and Diet E (Diet B + 5.0g VALM/kg).

**Table 1. Ingredient composition of the experimental diet**

| Ingredients                 | Quantity (kg) |
|-----------------------------|---------------|
| Maize                       | 40            |
| Soybean meal                | 6             |
| Wheat Offal                 | 22            |
| Palm Kernel Meal            | 28            |
| Bone Meal                   | 1.4           |
| Limestone                   | 2             |
| Salt                        | 0.35          |
| Premix                      | 0.25          |
| <b>Total</b>                | <b>100</b>    |
| <b>Calculated Nutrients</b> |               |
| ME (Kcal/Kg)                | 2536.9        |
| Crude Protein (%)           | 14.89         |
| Calcium (%)                 | 1.31          |
| Phosphorus (%)              | 0.46          |
| Crude Fibre (%)             | 5.06          |

### Performance of birds

The feed intake and weight gain were recorded weekly throughout the experiment. 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:

$$PI = (TFI \times FCP)/100$$

$$EI = (TFI \times FME)/1000$$

Both protein and energy utilization indices were determined as the ratio of PI and EI to TWG respectively:

$$PU = PI/TWG$$

$$EU = EI/TWG$$

### Blood sampling

At the end of the 12-week experiment, 15 birds were randomly selected from each treatment for blood sampling. The birds were fasted overnight and blood samples were collected from the jugular veins into EDTA bottles for haematological analyses. Also, blood samples were collected in dry, clean centrifuge glass tubes without any coagulant to separate the serum for the determination of serum oxidative status and anti-oxidative enzymes indicators. Blood samples were left for 15 minutes at room temperature. The tubes were centrifuged for 10 minutes at 3000 rpm to obtain clean supernatant serum. The harvested serum samples were kept frozen at -20°C until the determination of serum biochemical components.

### 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

### Growth performance

From the results of the growth performance of this study (Table 2), it was observed that the average final weights (AFW) of the birds were significantly higher among the birds on diets D and E when compared with the weights of birds on other treatments. The total weight gained by the birds during the study ranged from 0.45 to 0.91 kg. Birds on treatments D and E had the highest weight gains, which were significantly ( $P < 0.01$ ) different from the other

The protein intake (PI) by the birds ranged from 0.94 to 1.01 kg, with treatment B having the highest protein intake (1.01 kg), which was significantly ( $P < 0.05$ ) different from the other treatments. For the protein utilization (PU), birds in treatments A and C had the lowest PU ratios, while those in treatments B and D had the highest ( $P < 0.05$ ). Furthermore, the energy intake (EI) by the birds ranged from 15.67 to 16.90 kcal, with birds in treatment B having the highest ( $P < 0.05$ ) energy intake (16.90 kcal), which was significantly different from the other treatments ( $P < 0.01$ ). For the energy utilization, cocks in treatments A and C had the lowest energy utilization, while those in treatments D and E had the highest.

**Table 2. Growth Performance of Cocks Fed Diets with Aflatoxin B<sub>1</sub>, Zinc and *Vernonia amygdalina* Leaf Meal**

| Parameters                        | A                   | B                  | C                  | D                  | E                  | SEM   | P-Value |
|-----------------------------------|---------------------|--------------------|--------------------|--------------------|--------------------|-------|---------|
| Average initial Weights (kg/bird) | 1.57                | 1.56               | 1.59               | 1.60               | 1.58               | 0.09  | 0.08    |
| Average Final Weights (kg/bird)   | 2.30 <sup>b</sup>   | 2.01 <sup>c</sup>  | 2.32 <sup>b</sup>  | 2.41 <sup>a</sup>  | 2.49 <sup>a</sup>  | 0.13  | 0.01    |
| Total Weight Gain (kg/bird)       | 0.73 <sup>b</sup>   | 0.45 <sup>c</sup>  | 0.73 <sup>b</sup>  | 0.81 <sup>a</sup>  | 0.91 <sup>a</sup>  | 0.15  | 0.01    |
| Total Feed Intake (kg/bird)       | 6.42 <sup>b</sup>   | 6.85 <sup>a</sup>  | 6.38 <sup>b</sup>  | 6.37 <sup>b</sup>  | 6.35 <sup>b</sup>  | 0.23  | 0.04    |
| Feed Conversion Ratio             | 8.79 <sup>b</sup>   | 15.22 <sup>a</sup> | 8.74 <sup>b</sup>  | 7.86 <sup>bc</sup> | 6.98 <sup>c</sup>  | 1.68  | 0.02    |
| Protein Intake (kg/bird)          | 0.95 <sup>b</sup>   | 1.01 <sup>a</sup>  | 0.94 <sup>b</sup>  | 0.94 <sup>b</sup>  | 0.94 <sup>b</sup>  | 0.11  | 0.01    |
| Protein Utilization               | 1.30 <sup>b</sup>   | 2.25 <sup>a</sup>  | 1.29 <sup>b</sup>  | 1.16 <sup>c</sup>  | 1.03 <sup>c</sup>  | 2.67  | 0.03    |
| Energy Intake (kcal/bird)         | 15.84 <sup>ab</sup> | 16.90 <sup>a</sup> | 15.74 <sup>b</sup> | 15.72 <sup>b</sup> | 15.67 <sup>b</sup> | 12.37 | 0.01    |
| Energy Utilization                | 21.70 <sup>b</sup>  | 37.56 <sup>a</sup> | 21.57 <sup>b</sup> | 19.41 <sup>c</sup> | 17.22 <sup>d</sup> | 27.05 | 0.02    |

Means in a row without common superscripts are significantly ( $P < 0.05$ ) different. SEM = Standard Error of Means, Level of significance =  $P < 0.05$ . Diets A = Control/Basal, B = Basal + 1.00 mg/kg AFB<sub>1</sub>, C = Diet B + 50 mg/kg Zn, D = Diet B + 2.50 g/kg VALM, E = Diet B + 5.0 g/kg VALM.

treatments. The total feed intake by the birds ranged from 6.35 to 6.85 kg, with treatment B having the highest feed intake (6.85 kg), which was significantly ( $P < 0.05$ ) different from the other treatments. For the feed conversion ratio (FCR), which is the efficiency of the birds in converting feed into body mass, Birds in treatments C, D, and E had the best FCR, while treatment A had the least.

### Haematological effects

Table 3 displays the haematological findings of the roosters that were administered diets containing AFB<sub>1</sub>, *Vernonia amygdalina*, and zinc. The observed PCV values exhibited a range spanning from 31.04 to 41.03%. Notably, roosters in treatment E had the highest ( $P < 0.05$ ) PCV value, whereas those in treatment C had the lowest PCV value. The treatments A and D birds exhibited elevated

**Table 3. Haematology of Cocks Fed Diets with Aflatoxin B<sub>1</sub>, Zinc and *Vernonia amygdalina* Leaf Meal**

| Parameters                              | A                   | B                  | C                  | D                   | E                   | SEM  | P-Value | **Ref         |
|---|---------------------|--------------------|--------------------|---------------------|---------------------|------|---------|---------------|
| <b>Haemogram</b>                        |                     |                    |                    |                     |                     |      |         |               |
| PCV (%)                                 | 40.08 <sup>ab</sup> | 31.04 <sup>c</sup> | 39.80 <sup>b</sup> | 40.37 <sup>ab</sup> | 41.03 <sup>a</sup>  | 2.53 | 0.02    | 35.90 – 41.00 |
| RBC (x10 <sup>6</sup> mm <sup>3</sup> ) | 4.71 <sup>a</sup>   | 3.31 <sup>c</sup>  | 4.10 <sup>ab</sup> | 3.98 <sup>b</sup>   | 4.75 <sup>a</sup>   | 0.32 | 0.01    | 4.21 – 4.84   |
| MCHC (g/dl)                             | 33.03               | 32.44              | 32.56              | 33.03               | 33.00               | 0.28 | 0.42    | 32.41 – 33.37 |
| MCV (fl)                                | 80.12 <sup>c</sup>  | 110 <sup>a</sup>   | 80.00 <sup>c</sup> | 84.11 <sup>b</sup>  | 83.01 <sup>b</sup>  | 8.41 | 0.01    | 81.60 – 89.10 |
| MCH (pg)                                | 29.02 <sup>b</sup>  | 46.28 <sup>a</sup> | 26.00 <sup>c</sup> | 28.02 <sup>bc</sup> | 28.14 <sup>bc</sup> | 2.83 | 0.04    | 27.20 – 28.90 |
| Hb (g/dl)                               | 13.09 <sup>b</sup>  | 9.17 <sup>c</sup>  | 12.09 <sup>b</sup> | 13.52 <sup>a</sup>  | 13.65 <sup>a</sup>  | 0.67 | 0.03    | 11.60 – 13.68 |
| <b>Differentials WBC (%)</b>            |                     |                    |                    |                     |                     |      |         |               |
| Heterophils                             | 33.50 <sup>b</sup>  | 21.72 <sup>c</sup> | 33.50 <sup>b</sup> | 34.00 <sup>a</sup>  | 35.07 <sup>a</sup>  | 0.35 | 0.02    | 23.70 - 35.10 |
| Eosinophils                             | 2.00 <sup>a</sup>   | 1.01 <sup>c</sup>  | 1.54 <sup>b</sup>  | 1.51 <sup>b</sup>   | 1.59 <sup>b</sup>   | 0.20 | 0.01    | 1.20 - 3.10   |
| Basophils                               | 2.50                | 2.00               | 2.05               | 2.00                | 2.50                | 0.20 | 0.12    | 2.10 - 3.10   |
| Lymphocytes                             | 64.09 <sup>a</sup>  | 41.02 <sup>b</sup> | 64.08 <sup>a</sup> | 64.11 <sup>a</sup>  | 65.52 <sup>a</sup>  | 2.20 | 0.03    | 45.01-70.05   |
| Monocytes                               | 2.35 <sup>a</sup>   | 0.82 <sup>ab</sup> | 2.10 <sup>b</sup>  | 2.29 <sup>ab</sup>  | 2.41 <sup>a</sup>   | 0.57 | 0.04    | 1.10 - 2.50   |

Means in a row without common superscripts are significantly (P<0.05) different.

SEM = Standard Error of Means, Level of significance = P<0.05; PCV = Packed Cell Volume,

RBC = Red Blood Cells, MCHC = Mean Corpuscular Haemoglobin Concentration,

MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular Haemoglobin, Hb = Haemoglobin,

WBC = White Blood Counts. Diets A = Control/Basal, B = Basal + 1.00 mg/kg AFB<sub>1</sub>, C = Diet B + 50 mg/kg Zn,

D = Diet B + 2.50 g/kg VALM, E = Diet B + 5.0 g/kg VALM.

\*\*Ref: Chicken haematology reference range ([https://en.wikivet.net/Chicken\\_Haematology](https://en.wikivet.net/Chicken_Haematology)).

red blood cell count, while those in treatment B had the lowest (P<0.05) count. The mean corpuscular haemoglobin concentration (MCHC) values exhibited a high level of consistency among all treatments, as evidenced by the absence of statistically significant differences (P>0.05). The mean corpuscular volume (MCV) values span a range of 80.00 to 110.00 fl, with treatment B cocks exhibiting the greatest (P<0.05) MCV mean and treatment C cocks displaying the lowest. In terms of mean corpuscular haemoglobin (MCH) values, it is seen that treatment B birds exhibit the greatest MCH, while treatment C birds display the lowest MCH. The detected differences exhibit statistical significance (P<0.05). The haemoglobin (Hb) levels vary between 9.17 and 13.65 g/dl, with treatment E exhibiting the greatest (P<0.05) hemoglobin concentration, while treatment B demonstrates the lowest.

For the differentials WBC, the heterophils

percentage ranges from 21.72 to 35.07%, with treatment E having the greatest value and treatment B having the lowest. The detected differences exhibit statistical significance (P<0.05). The percentage of eosinophils varies between 1.01 and 2.00%, with treatment A exhibiting the greatest value and treatment C displaying the lowest value. The detected differences exhibit statistical significance at a level of P<0.05. The percentages of basophils exhibit a rather uniform distribution among all treatments, and no statistically significant disparities were identified (P>0.05). The percentages of lymphocytes exhibit a range spanning from 41.02 to 65.52%. Notably, birds on treatment E demonstrate the highest lymphocyte count, while treatment B birds exhibit the lowest count. The detected differences exhibit statistical significance (P<0.05). The percentages of monocytes vary between 0.82 and 2.41%, with group E exhibiting the greatest (P<0.05) monocyte count and treatment B cocks displaying the lowest level.

## Serum proteins, enzymes and metabolites

The results of the serum proteins, enzymes and metabolites are presented in Table 4. The serum albumin varied significantly ( $P < 0.05$ ) among the groups. Treatment A has the lowest albumin levels (12.70 g/l), while treatment E has the highest (25.01

differ among the treatments, with treatment A having the highest (30.10  $\mu\text{mol/l}$ ) and treatment C having the lowest (16.51  $\mu\text{mol/l}$ ). The serum glucose levels are significantly ( $P < 0.05$ ) variable across the groups, with treatment A having the highest (9.80 mmol/l) and treatment B having the lowest (6.71 mmol/l).

**Table 4. Serum Biochemistry of Cocks Fed Diets with Aflatoxin B<sub>1</sub>, Zinc and *Vernonia amygdalina* Leaf Meal**

| Parameters                       | A                   | B                   | C                   | D                    | E                   | SEM  | P-Value | Ref          |
|----------------------------------|---------------------|---------------------|---------------------|----------------------|---------------------|------|---------|--------------|
| <b>Serum Proteins (g/l)</b>      |                     |                     |                     |                      |                     |      |         |              |
| Albumin                          | 19.21 <sup>c</sup>  | 12.70 <sup>d</sup>  | 18.92 <sup>c</sup>  | 22.01 <sup>b</sup>   | 25.01 <sup>a</sup>  | 0.62 | 0.01    | 15 – 33*     |
| Globulin                         | 20.03 <sup>c</sup>  | 12.30 <sup>d</sup>  | 20.08 <sup>c</sup>  | 21.99 <sup>b</sup>   | 25.20 <sup>a</sup>  | 0.55 | 0.01    | 16 - 43*     |
| Total Protein                    | 39.24 <sup>c</sup>  | 25.00 <sup>d</sup>  | 39.00 <sup>c</sup>  | 44.00 <sup>b</sup>   | 50.21 <sup>a</sup>  | 0.36 | 0.04    | 39 - 70*     |
| <b>Serum Enzymes (U/L)</b>       |                     |                     |                     |                      |                     |      |         |              |
| Aspartate aminotransferase       | 110.15 <sup>c</sup> | 160.01 <sup>a</sup> | 110.11 <sup>c</sup> | 150.04 <sup>ab</sup> | 140.17 <sup>b</sup> | 0.78 | 0.02    | 118 – 298*   |
| Alanine aminotransferase         | 18.91 <sup>b</sup>  | 48.12 <sup>a</sup>  | 16.51 <sup>c</sup>  | 16.10 <sup>c</sup>   | 18.01 <sup>b</sup>  | 0.99 | 0.04    | 21.7- 46.5*  |
| <b>Serum Metabolites</b>         |                     |                     |                     |                      |                     |      |         |              |
| Creatinine ( $\mu\text{mol/l}$ ) | 15.01 <sup>b</sup>  | 30.10 <sup>a</sup>  | 16.25 <sup>b</sup>  | 14.25 <sup>b</sup>   | 15.27 <sup>b</sup>  | 3.92 | 0.01    | 5.6 – 22.2** |
| Glucose (mmol/l)                 | 9.80 <sup>a</sup>   | 6.71 <sup>c</sup>   | 9.12 <sup>a</sup>   | 9.71 <sup>a</sup>    | 9.82 <sup>a</sup>   | 0.58 | 0.01    | 9.7 – 13.3*  |

Means in a row without common superscripts are significantly ( $P < 0.05$ ) different. SEM = Standard Error of Means, Level of significance =  $P < 0.05$ . Diets A = Control/Basal, B = Basal + 1.00 mg/kg AFB<sub>1</sub>, C = Diet B + 50 mg/kg Zn, D = Diet B + 2.50 g/kg VALM, E = Diet B + 5.0 g/kg VALM. \*Ref = Board *et al.* (2018), \*\*Ref = Kaneko *et al.* (2008)

g/l). Treatments B, C, and D fall in between. For globulin and total protein, similar trend to albumin were observed. There are significant differences ( $P < 0.05$ ) among the groups. Treatment A has the lowest globulin levels while treatment E has the highest.

For the serum enzymes, treatment A shows the lowest AST levels (110.15 U/L), while treatment B has the highest (160.01 U/L). Treatments C, D, and E have intermediate values. Alanine Aminotransferase levels exhibit significant variation as well. Treatment B has the highest ALT levels (150.04 U/L), while treatment C shows the lowest (110.11 U/L). Treatments D and E have intermediate values.

For the serum metabolites, creatinine levels

## DISCUSSION

### Growth performance

The significant reductions in average final weights (AFW) and the total weight gain (TWG) observed among the birds on diet B is suggestive of the adverse effects AFB<sub>1</sub> on growth performance of domestic birds. These results were not in variance with previous findings on the debilitating effects of AFB<sub>1</sub> on weight increase (Yunus *et al.*, 2011, Fouad *et al.*, 2019). The substantial increase in AFW and TWG observed among the birds fed supplemental Zn (diet C) is testimonial of the restorative potentials of dietary Zn on the adverse effects of AFB<sub>1</sub>. This supported the report of Kumar *et al.* (2021) outlining the effects of Zn supplementation on growth performance of domestic chickens. In the

same vein, the higher significant AFW and TWG of the birds in treatments D and E suggested that inclusions of varied levels of VALM in diets D and E were more effective in promoting weight gain in the birds. These findings were consistent with previous research that has demonstrated the positive impact of VALM on the weights of birds (Tokofai *et al.*, 2021).

Despite the reduced weight gain among the cocks in diet B, the observed higher TFI, FCR, PU, EI and EU is suggestive that AFB1 might have negative effects on the energy and protein contents and metabolism of the feed, hence, making the birds to consume higher feeds than necessary to meet up with their energy and protein requirements. This increase TFI as observed in this report was in line with the finding of Al-shawabkeh *et al.* (2009). However, the ameliorative effects of supplemental Zn and VALM on AFB1 were glaring among the birds fed diets C, D and E respectively. This further strengthened the positions of Kumar *et al.* (2021) and Tokofai *et al.* (2021) on the protective effects of dietary Zn and VALM on the effects of AFB1.

### **Haematological effects**

From the present study, it was evident that AFB1 had affected the roosters' overall health status and physiological response to the administered diet B as indicated by the significant reductions in packed cell volume (PCV), red blood cell count (RBC), haemoglobin (HB), heterophils, eosinophils, lymphocytes, and monocytes of the cocks. These findings suggest potential variations in the immune response and inflammatory processes among the different dietary groups, indicating the influence of dietary components on the

roosters' immune systems. These hematological findings collectively underscore the significance of dietary composition in impacting various blood parameters and immune responses in the roosters, thereby emphasizing the critical role of nutrition in avian health and well-being.

The results of this study was not different from the findings of Dönmez *et al.* (2012) who documented similar effects on the haematological responses of rams fed diets containing AFB1. However, the ameliorative effects of Zn and VALM were observed in diets C, D and E as attested to by the improved haematological indices among the cocks on diets C, D and E respectively. Our results on the ameliorative effects of Zn on the dietary contamination of feed by AFB1 was in sync with the report of Zakaria *et al.* (2017) while the findings of Tokofai *et al.* (2021) also highlighted the restorative role of VALM on the haematological responses of chickens.

### **Serum proteins, enzymes and metabolites**

The significant increases in the blood concentration of creatinine, and the two serum enzymes studied in this research pointed to the incidence of liver dysfunction or complete damage in the cocks as occasioned by AFB1 inclusion. Aflatoxin B1 had been fingered to predispose domestic chickens to hepatic cell damage due to the generation of reactive oxygen species causing oxidative damage (Wang *et al.*, 2023). However, hepatoprotective effects of Zn in diet C were evident in this study. Zinc has been explained to be effective in forestalling hepatic oxidative damage in livestock (Zhang *et al.*, 2022). The hepatic phytoprotective potentials of



VALM have been documented (Olarotimi et al., 2023). Farombi and Owoeye (2011) highlighted the chemopreventive properties of *V. amygdalina* in scavenging free radicals, inducing detoxification and inhibiting stress response proteins in animals.

Furthermore, the results observed for the serum glucose and proteins concentrations in diet B are suggestive that the inclusion of AFB<sub>1</sub> had offset the normal serum protein and glucose metabolisms among the cocks in this group. Previously, AFB<sub>1</sub> was reported to have direct implications on serum protein levels (Chen et al., 2016) and glucose (Amiridumari et al., 2013). Nonetheless, the notable enhancements in serum proteins and glucose levels observed with the inclusion of Zn and VALM in diets C, D, and E suggest the potential of these dietary additives to counteract the detrimental effects of AFB<sub>1</sub> and improve serum protein and glucose profiles. This finding resonates with Attia et al. (2022), who highlighted the beneficial effects of supplemental Zn on serum protein and glucose levels in humans. Similarly, Abedini et al. (2018) expounded on the enhancement effects of dietary Zn supplementation on serum proteins and glucose of laying hens.

## CONCLUSION

The observed adverse effects of AFB<sub>1</sub> on weight gain, immune response, and hepatic function underscore the deleterious consequences of aflatoxin contamination in poultry feed. The notable improvements in growth performance, haematological indices, and serum metabolites among the roosters fed diets supplemented with Zn and VALM provide compelling evidence of the potential protective and restorative effects

of these dietary additives. Notably, the demonstrated hepatoprotective properties of Zn and VALM underscore their efficacy in mitigating hepatic damage induced by AFB<sub>1</sub>, as indicated by the enhanced liver function parameters and serum profiles. Overall, this study contributed to the growing body of research on AFB<sub>1</sub> management in poultry nutrition, emphasizing the significance of dietary strategies in safeguarding avian health and performance in the face of prevalent AFB<sub>1</sub> challenges.

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