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Organ Histopathology of Laying Chickens to Bio-Control Methods of Aflatoxin Contamination

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Aflatoxin is toxic and carcinogenic to both crops and livestock. Use of different methods of aflatoxin mitigation has not been very effective. Information on the biological methods in aflatoxin mitigation is scanty. Therefore, effect of aflatoxin bio-control method on organ weight and histopathology of layers were investigated. 700 point-of-lay Bovan Nera layers (LC) were randomly allotted to four dietary treatments (Aflasafe maize-based diet AMBD, FF+ toxin binder, Aflatoxin-contaminated diet with toxin binder (ACDTB) and Aflatoxin-contaminated diet without toxin binder (ACDWTB). There were 5 replicates per treatment and experiment lasted for 14 weeks. Histopathology of liver, kidney, spleen, bursa of fabricius and ileum were assessed using standard procedures. Data were analysed using descriptive statistics and ANOVA at $\alpha_{0.05}$. Bursa histopathology of layers fed ACDTWB showed lymphoid depletion and hepatocellular necrosis, while those fed AMBD showed lymphoid proliferation and hepatocellular aggregates. Layers fed ACDTB and ACDWTB showed severe periportal hepatic degeneration and necrosis, with severe periportal cellular infiltration by mononuclear cells. This was classified as ranging from moderate to severe congestion of the parenchyma as observed in the lungs. The submucosal lymphoid population was expanded in the ileum of layers fed AMBD and those fed FF+toxin binder showed severe villi atrophy. Aflasafe maize-based diet enhanced integrity of the organ weights and histopathology of layers. The use of bio control method of aflatoxin mitigation (aflasafe) in poultry diet is recommended.

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1. INTRODUCTION

Mycotoxins are naturally-occurring secondary fungal metabolites produced by moulds that depletes the quality of various agricultural products [1]. Aflatoxins are a subgroup of mycotoxin [2], produced by the strains of *Aspergillus flavus* and *Aspergillus parasiticus* which can also be present as contaminants in a variety of food and feedstuffs [3,4,5]. Fungi infestation have adverse implications for poultry production [6,7], including, increased mortality, anemia and liver condemnation, [8,7] observed enlarged, pale and friable liver as well as haemorrhagic patches on the surface of the kidney of broiler chickens fed aflatoxin contaminated diets. The reactive intermediates of aflatoxins metabolize by binding to macromolecules with consequent interruption of transcriptional and translational processes [9], Aflatoxin B1 is the most prevalent form and has been known for its hepatotoxicity, carcinogenicity, genotoxicity and immunotoxicity in livestock. Since definitive ways for complete detoxification of mycotoxin-contaminated food and feed do not subsist, new ways of mitigating mycotoxicosis are being investigated [10,11]. This involves the use of Aflasafe, a bio-control method produced through the transfer of toxigenic strains of *Aspergillus flavus* on agricultural fields with atoxigenic strains. The mechanism of action is by physical exclusion of the toxigenic strain during infection and by competing for nutrients required for aflatoxin biosynthesis by the toxigenic strains, thereby reducing the overall toxigenicity of *A. flavus* population. The authors of this research was therefore prompted to investigate the effect of this biocontrol method on the organ weights and pathology of vital organs in layers.

2. MATERIALS AND METHODS

2.1 Site of Study

This study was carried out at the God's Grace Farm, a commercial layers farm known for egg production, located in Lagun Town, along Ibadan- Iwo road, Oyo State in the South-Western part of Nigeria.

2.2 Experimental Materials

Aflatoxin-contaminated maize grain was obtained from the Plant Pathology Unit, International Institute of Tropical Agriculture, (IITA), Ibadan, Nigeria as well as the aflasafe maize grains. Other ingredients used for the feed formulation were purchased from God's Grace commercial layers farm, located in Lagun Town, along Ibadan- Iwo road.

2.3 Aflatoxin Contaminated Maize Grain

The culturing and inoculation of the carrier ingredient was done at the plant pathology unit, international institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Spore of the fungus producing aflatoxin (*Aspergillus flavus*) was prepared for growth on 5'2 medium 5/2 agar medium (5% V8 juice and 2% agar, PH 5.2). Prepared aflatoxin extracts and aflatoxin standards were separated using thin-layer chromatography (TLC) plates. (Silica gel 60, 250 µm) by development with diethyl ether-methanol water (96:3:1), visualized under ultraviolet light and scored visually for presence or absence of aflatoxin, having a 2 mg limit of detection. Aflatoxins were quantified using scanning densitometer, CAMAG TLC scanner 3 with – CATS 1,4,2 software (Camag AG, Muttenz, Switzerland) [12].

2.4 Experimental Birds and Management

This study was carried out using a total of 700, 30-week old Bovan Nera black hens with a mean body weight of 2.0 kg. There were four (4) treatments with five replicates per treatment. Birds were randomly allotted into groups with 175 birds per treatment and 35 birds/ replicate. Data collection lasted for 14 weeks. The layers were housed in battery cages having linear feed troughs and nipple drinkers for running water. All the birds were fed basal diets for 2 weeks after which they were fed the experimental diets and fresh water was provided *ad libitum*.

2.5 Experimental Diets

There were four experimental diets compounded to meet the nutrient requirement of layers. Birds in Treatment 1 were fed diet 1, which contained feed formulation comprised of maize grains from Aflasafe treated grains (AMBD). Birds in Treatment 2 were fed diet 2, which contained normal feed formulation used in the farm industry to feed broilers including the toxin binders (FF). Birds in Treatment 3 were fed diet 3, which contained aflatoxin-contaminated maize grains

Table 1. Composition of the experimental diet for layers

Ingredients (kg)	AMBD	FF+Toxin binder	ACDTB	ACDWTB
Aflasafe Maize	50.22	-	-	-
Farm Maize	-	50.22	-	-
Contaminated Maize	-	-	50.22	50.22
Soyabean Meal	23.10	23.10	23.10	23.10
Wheat offal	13.46	13.46	13.46	13.46
Salt	0.35	0.35	0.35	0.35
Bone Meal	3.01	3.01	3.01	3.01
Oyster Shell	9.04	9.04	9.04	9.04
Lysine	0.20	0.20	0.20	0.20
Methionine	0.20	0.20	0.20	0.20
Vitamin C	0.01	0.01	0.01	0.01
Layer Premix	0.30	0.30	0.30	0.30
Toxin Binder	-	0.10	0.10	-
Oxytetracycline	0.10	0.10	0.10	0.10
Total (kg)	100	100	100	100
Calculated nutrients				
Crude Protein (%)	17.30	17.30	17.30	17.30
Crude Fibre (%)	4.56	4.56	4.56	4.56
Metabolizable Energy (Kcal/kg)	2598.25	2598.25	2598.25	2598.25
Avail. Phoshorus	0.68	0.68	0.68	0.68
Lysine	1.05	1.05	1.05	1.05
Methionine	0.46	0.46	0.46	0.46
Avail. Calcium	4.34	4.34	4.34	4.34

Premix supplied per kg diet; Vitamine A (10,000 IU), Vitamine D3 (2,000 IU), Vitamine E (20 IU), Vitamine K (2.0 mg), Thiamine (2.0 mg), Riboflavin (3.0 mg), Pyridoxine (4.0 mg), Niacin (2.0 mg), Cobalamin (0.05 mg), Panthotenic acid (200 mg), Folic acid (0.5 mg), Biotin (0.08 mg) Choline Chloride (0.2 g), Manganese (0.006 g), Zinc (0.003 g), Iron (0.005 g), copper (0.006 g), Iodine (0.0014 g), Selenium 90.24 mg), Cobalt (0.25 mg), Antioxidant (0.125 g). AMBD= Aflasafe maize-based diet, FF =Farm feed + Toxin binder, ACDTB =Aflatoxin-contaminated diet with toxin binder and ACDWTB = Aflatoxin-contaminated diet without toxin binder

and binders compounded with other feed ingredients (ACDTB). While birds in Treatment 4 were fed diet 4 which contained, aflatoxin-contaminated grains without toxin binders (ACDTWB).

2.6 Evaluation of Organs

2.6.1 Histopathological investigation

At the end of the feeding trial in this study, 20 birds were randomly selected per treatment, weighed and sacrificed. Dissection was done through the lower abdominal incision. Samples of the kidney, liver, thymus, gizzard, spleen, heart, GIT, lungs, bile, adrenal gland, bursa of fabricus, pancreas, ileum, ovary and reproductive organ were harvested for histopathological investigation. Eviscerated weight was also determined by weighing the carcasses after removal of the internal organs. The tissues were observed and cut into small pieces of not more than 4mm thick into pre-labelled cassettes. These were further immersed in 10% formal saline for 24 hours to fix. Tissue Processing involves Embedding, Microtomy, Floating, Drying and staining. The staining technique used is haematoxylin and eosin technique [13].

3. RESULTS

3.1 Effect of the Experimental Diets on Relative Organ Weights of Layers

The results of the relative organ weights of layers fed the experimental diets are shown in Table 2. Among the parameters evaluated, the relative organ weights (%) of the spleen, ovary, thymus, liver, pancreas, gizzard and gastro intestinal tract were significantly ($P<0.05$) influenced by the experimental diet. The relative spleen weight of layers fed AMBD (0.08±0.03%), ACDTB (0.14±0.06%) and that of ACDWTB (0.12±0.03%) were not significantly ($P<0.05$) different compared to the control value (0.10±0.03%), the least spleen weight was

recorded in birds fed the AMBD. The same trend was observed in the relative gizzard weight of the layers. The relative weights of ovaries (0.93±0.3%) of birds fed the AMBD were not significantly (P<0.05) influenced compared to that of the control (FF+toxin binder), however, the relative weights of ovaries of hens fed the ACDTB (0.34±0.2%) and ACDWTB (0.44±0.4%) were significantly (P<0.05) lower than the FF+toxin binder (0.99±0.5%). This same trend was observed in the relative thymus weight values. The relative bursa weight of the layers was observed to be lowest in birds fed FF+toxin binder (0.03±0.01%) and ACDTWB (0.03±0.02%) and highest in birds fed AMBD (0.05±0.02%). The relative liver weight was observed to be significantly affected by the varying dietary treatment. The lowest weight was recorded in birds fed AMBD (1.87±0.2%), although not statistically different from the control (FF+toxin binder) (2.11±0.4%) and the highest weight was observed in hens fed ACDWTB (2.42±0.6%). The relative lung weight of birds fed the experimental diets ranged within 0.51±0.1% to 0.55±0.1%, with the least weight observed in hens fed the AMBD (0.51±0.1%) and the highest observed in birds fed the ACDTB (0.55±0.1%). The relative abdominal fat was not significantly altered by the dietary treatment. The highest weight was recorded in birds fed AMBD (2.24±1.4%) and the lowest relative abdominal fat weight was in laying chickens fed the ACDWTB (1.33±0.7%). Compared to the control (FF+toxin binder), birds fed the ACDWTB (0.20±0.01%), showed a significantly (p<0.05) higher relative pancreas weight and birds fed the AMBD (0.16±0.02%), FF (0.16±0.02%) and ACDTB (0.18±0.01%) were not significantly affected. The relative gastro-Intestinal tract weight of birds fed the AMBD (9.53±1.5%) and those obtained in birds fed the ACDTB (9.64±1.5%) were significantly lower than the FF+toxin binder (11.52±1.58%), while the relative weight of birds fed the ACDWTB (10.08±2.1%) was not significantly different from the weight obtained in birds fed the FF+toxin binder.

3.2 Experimental Influence of Aflatoxin on Organ Pathology of Layers

In this study, the toxic effects of aflatoxin on liver, kidney, bursa of Fabricus, lungs, spleen, heart, ileum and ovary were clearly observed after feeding 200 ppb aflatoxin to layers for 14 weeks.

3.3 Effect of Experimental Diet on Histology of the Lungs

The histopathological evaluation of the lungs of layers in the groups that received AMBD, FF+Toxin binder, ACDTB and ACDTWB showed no visible lesion, however, moderate to severe congestion of the parenchyma was observed in the lung as (Plates 1-4).

Table 2. Relative organ weights of laying hens fed the experimental diets

Parameters	AMBD	FF+Toxin binder	ACDTB	ACDWTB
Kidney (%)	0.63±0.12	0.68±0.14	0.67±0.13	0.73±0.14
Spleen (%)	0.08±0.03^{b}	0.10±0.03^{ab}	0.14±0.06^{a}	0.12±0.03^{ab}
Ovary (%)	0.93±0.3^{a}	0.99±0.5^{a}	0.34±0.2^{b}	0.44±0.4^{b}
Thymus (%)	0.04±0.01^{b}	0.03±0.01^{b}	0.07±0.04^{a}	0.05±0.02^{ab}
Bursa (%)	0.05±0.02	0.03±0.01	0.04±0.01	0.03±0.02
Liver (%)	1.87±0.2^{b}	2.11±0.4^{ab}	2.37±0.4^{a}	2.42±0.6^{a}
Lungs (%)	0.51±0.1	0.52±0.1	0.55±0.1	0.52±0.1
Abd. Fat (%)	2.24±1.4	1.25±1.0	2.01±1.3	1.33±0.7
Pancreas (%)	0.16±0.02^{b}	0.16±0.02^{b}	0.18±0.01^{ab}	0.20±0.01^{a}
Heart (%)	0.43±0.07	0.55±0.10	0.56±0.21	0.49±0.08
Repr.Organ (%)	2.47±0.4	2.44±0.7	1.48±1.2	2.25±1.9
Adr. Gland (%)	0.006±0.002	0.009±0.006	0.007±0.002	0.006±0.002
Bile (%)	0.10±0.05	0.11±0.03	0.11±0.03	0.12±0.04
Gizzard (%)	2.69±0.36^{b}	2.98±0.33^{ab}	3.18±0.6^{ab}	3.32±0.6^{a}
GIT (%)	9.53±1.5^{b}	11.52±1.58^{a}	9.64±1.5^{b}	10.08±2.1^{ab}

ab: Means along the same row with different superscripts are significantly (P<0.05) different. AMBD =Aflasafe maize-based diet, FF+Toxin binder = Farm feed + toxin binder, ACDTB= Aflatoxin-contaminated diet + toxin binder, ACDWTB= Aflatoxin-contaminated diet without toxin binder.GIT= Gastro intestinal tract. Repr. Organ – Reproductive organ, Eviscer Weight (g) – Eviscerated weight, Adr. Gland – Adrenal Gland

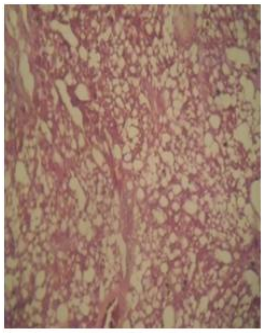

Plate 1. AMBD

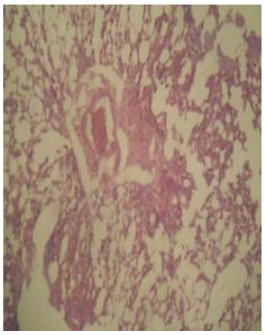

Plate 2. FF+Toxin binder

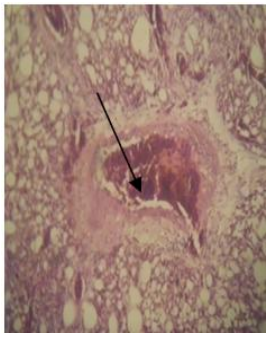

Plate 3. ACDTB

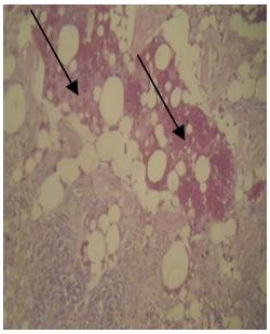

Plate 4. ACDWTB

3.4 Effect of Experimental Diet on Liver Histology

Although the sections from the liver of laying chickens given the AMBD showed no histopathological alterations and no visible lesion was observed. Observation of the ilea of layers fed FF diet+toxin binder showed a moderate portal congestion. The sinusoids were very prominent, although, no visible lesion was noticed (Plates 5 and 6). Histopathological findings of the liver of layers fed ACDTB and ACDWTB showed severe periportal hepatic degeneration and necrosis, with severe periportal cellular infiltration by mononuclear cells. Severe diffuse hepatic vacuolar degeneration and necrosis were also observed as shown in Plates 7 and 8.

3.5 Effect of Experimental Diet on the Histology of the Ileum

Histopathological findings of the ileum of layers fed AMBD and FF+toxin binder showed no observable visible lesion and the mucosal glands are intact. However, the submucosal lymphoid population was expanded in the ileum of laying hens fed AMBD and those fed FF+toxin binder showed severe villi atrophy as shown in Plates 9 to 10. Sections of ileum histology of layers fed ACDTB and those of ACDWTB showed no visible lesion and the mucosal glands are intact. Also severe villi atrophy was noticed. But, the mucosal glands are intact and severe villi atrophy was observed (Plates 11 to 12).

3.6 Effect of Experimental Diet on Histopathology of the Kidney

The findings recorded in the histopathological evaluation of layers fed AMBD and FF+Toxin Binder showed massive congestion of the kidney at the renal interstitium and haemorrhage of the renal parenchyma (Plates 13-14). No visible lesion was observed. Layers fed ACDTB and ACDWTB showed a moderate interstitial cellular infiltration by mononuclear cells, although no visible lesion was noticed, there were zones of interstitial haemorrhage in the kidney of birds fed ACDTWB (Plate 15 to 16).

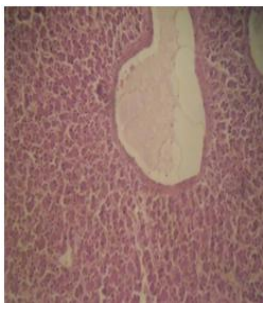

Plate 5. AMBD

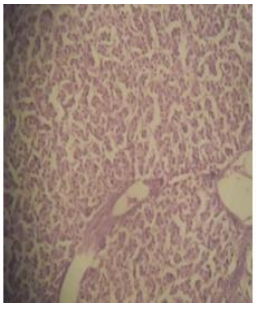

Plate 6. FF+Toxin binder

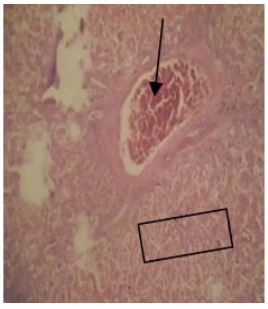

Plate 7. ACDTB

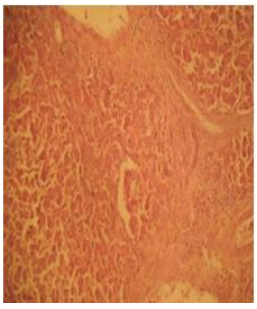

Plate 8. ACDWTB

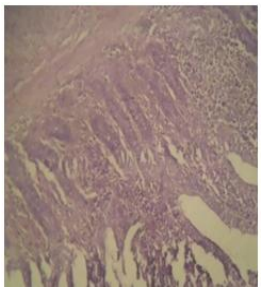

Plate 9. AMBD

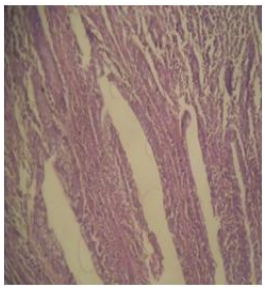

Plate 10. FF+Toxin binder

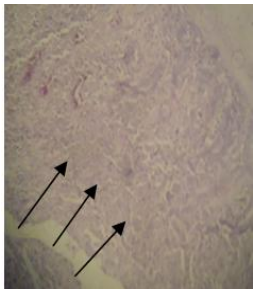

Plate 11. ACDTB

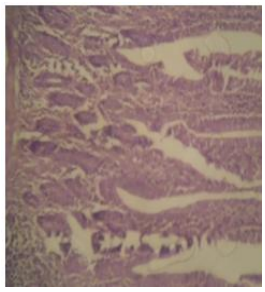

Plate 12. ACDWTB

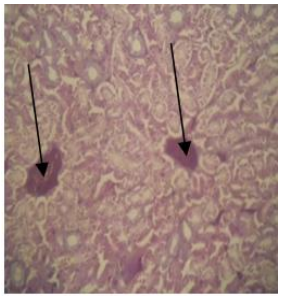

Plate 13. AMBD

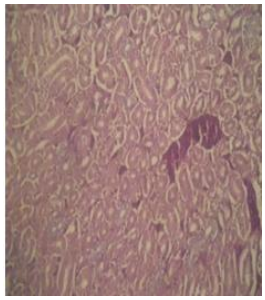

Plate 14. FF+Toxin binder

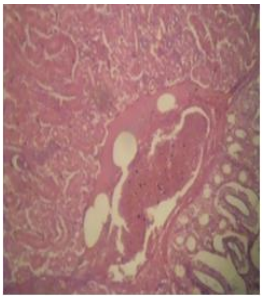

Plate 15. ACDTB

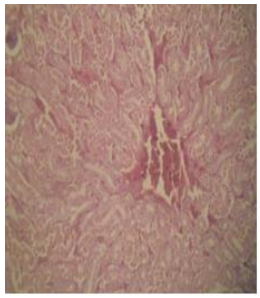

Plate 16. ACDWTB

3.7 Effect of Experimental Diets on the Ilea Measurements of Layers

Result of the ilea measurement of layers fed varying experimental diets is shown in Table 3. It was observed that the villus width (VW) and the crypt depth (CD) were not significantly affected by the dietary treatment. However, the mean values recorded for the villus height (VH) were significantly influenced. The VH of birds fed AMBD (254.17±43.61 µm) and ACDTWB (217.50±67.18 µm) were not significantly affected by the dietary treatment, although, birds fed ACDTB (298.50±30.41 µm) were significantly higher compared to the control value (FF+toxin binder) (161.63±22.45 µm).

4. DISCUSSION

4.1 Relative Organ Weights of Layers Fed Experimental Diets

Among the organ weights evaluated, the relative weights of spleen, ovary, thymus, liver, pancreas and Gastro intestinal tract of the experimental birds were influenced by the dietary treatments while those of the kidney, Bursa, Lungs, Heart, reproductive organ, adrenal gland, Bile, Abdominal fat and Eviscerated weights were not affected.

Spleen is a reticuloenthothelial system. Fragile, worn-out red blood cells are recycled in the spleen, also platelets and white blood cells are stored in the spleen. The spleen weight of birds fed ACDTB (treatment 3) and ACDWTB (treatment 4) which were higher compared to the control could be as a result of an alteration in its structure especially an increase in size due to AF effect. The enlarged spleen has been documented to store excessive number of the body's platelets causing thrombocytopenia (low platelet count). This is as reflected in their reduced platelet count of birds in this study. The result of this work corroborate with the reports of [14], who observed an increase in relative spleen weight of birds fed 1.0mg AF/kg compared to the control diet.

The relative weight of the ovary of birds fed ACDTB and ACDWTB diets, which were lower is in agreement with the observation of [15,16], who noticed ovary atresia i.e degeneration of immature ovarian follicles or subsequently re-absorbed during follicular phase in layers fed a diet containing 8000 ppb AFB for 7 days. According to [17], aflatoxicosis causes

Table 3. Ilea measurement of layers fed experimental diets

Parameters (µm)	AMBD	FF+Toxin binder	ACDTB	ACDWTB
Villus height	254.17±43.61[ab]	161.63.±22.45[b]	298.50±30.41[a]	217.50±67.18[ab]
Villus width	30.50±10.61	25.40±1.09	25.35±12.37	42.15±2.33
Crypt depth	34.50±6.36	46.67±10.13	35.40±1.98	33.62±3.98

ab: means on the same rows with different superscripts are significant (P<0.05).
AMBD =Aflasafe maize-based diet, FF+Toxin binder = Farm feed + toxin binder, ACDTB= Aflatoxin-contaminated diet + toxin binder, ACDWTB= Aflatoxin-contaminated diet without toxin binder

pathological changes in the chicken ovaries, which has detrimental effect on egg production. However, the relative weight of the ovary of birds fed AMBD and FF+toxin binder were not significantly different. This showed that the ovary weight of birds fed the AMBD without toxin binder still had a physiologically normal size. The relative thymus weight of birds which received ACDTB is significantly higher compared to those under AMBD. Thymus is a lymphoid organ which plays a vital role in production and maturation of T-lymphocytes/T-cells which helps to defend the body from potentially deadly pathogens such as bacteria, viruses and fungi. It produces a hormone, thyroxin which stimulates the development of T-cells. The observed relative thymus weight of birds on ACDTB diets, which was significantly higher compared to the control is in contrast with the observation of [18], who administered 0.5 ppm AFB1 to cob broiler chicks for 5 weeks and noticed a significant decrease in the thymus weight, but observed an increase in the relative thymus weight when AFB, and T2 – toxin combination was used.

Liver is a complex organ with many functions, including lipid metabolism. AFB1, is a hepatotoxin which means that the liver is the target organ. Aflatoxin causes accumulation of fat in the liver resulting in enlarged, pale and fatty liver. Perhaps, this is the reason for an increase in the liver weight of birds in ACDTB and ACDWTB respectively, compared to the control. The result of this study corroborate the findings of [19], but disagrees with the observation of [20] who noticed a decrease in liver weight of birds when given 100 mgAF/kg.

The pancreas of birds fed ACDTB and ACDWTB with numerically higher weight compared to the control, had a similar result with [21]. He observed a significant increase in the relative pancreas weight of birds as a result of AF contamination of the diet. This also agrees with the observation of [22] with ducks fed 200 ppb AF diet. The relative gizzard weight of birds in AMBD, ACDTB and ACDWTB were not significantly different compared to the control. The gizzard of birds is a unique organ with strong muscles whose grinding muscular activity act as the “teeth” of the bird. The recorded weight of gizzards is similar to the result of [21] who observed an increase in the gizzard weight of Isa brown laying birds at the end of 52 weeks of experiment. It was in contrast with [18] who did not observe any statistical difference in the gizzard weight of Broiler Breeder hens fed 300, 400 and 500 ppb AF compared to the control.

The relative weight of GIT of birds in treatments 1 and 4 were not statistically different compared to the control. However, the weight of GIT of birds fed ACDTB was significantly lower compared to the control. Studies indicate that AF could stimulate the fore part of gastro intestinal tract directly, causing pathologic changes, which will therefore affect their nutrient absorption abilities [18]. Perhaps, this is the cause of a significant reduction in the GIT weight.

4.2 Organ Histopathology of Layers Fed Experimental Diets

In this study, the detrimental effects of aflatoxin were investigated from the point of pathological changes. The microscopic investigation of various organs in these studies showed that aflatoxin adversely affected the organs attributed with the hematopoietic, immune and the reticulo endothelial system [23,24,25,8]. Changes in the vital organs of birds affected by aflatoxicosis induce negative effects on the overall performance of the chickens. The reported lymphoid proliferation of spleen and bursa of fabricus fed AMBD was also observed in birds fed FF+toxin binder. This could be as a result of the absence to moderate level of aflatoxin in the diets, which enhanced normal pathology of the organs. This is obvious in the various performance indices of birds given AMBD and FF+toxin binder, which showed positive result. The spleen examined showed numerous distinct lymphoid follicles with mild to moderate distinct lymphoid depletion. This is similar to the results

of [25,26] who observed spleens showing lymphoid depletion, an increase in the number of germinal centres and reticulum cell hyperplasia in aflatoxin treated birds.

The mild to moderate lymphoid depletion of the spleen and bursa of fabricus of birds fed ACDTB and ACDTWB, is similar to the findings of [25] who did not observe any considerable lesion in the spleen of broiler chickens fed 100 ppb dietary aflatoxin for 42 days except that he observed very light lymphocytic depletion in few cases. Also, the moderate histopathological changes observed in this study are in agreement with the previous studies using various lower levels of AF (100-500ppb) in broilers [27,28] and in wild turkeys [29]. [30] have reported cellular depletion in the follicle medulla of the bursa of Fabricius which appeared first and persisted during the recovery phase in experimental aflatoxicosis. Also, in the study conducted by [26], it was discovered that the bursa of the Fabricius of the groups of birds fed 100 and 150 ppb aflatoxin revealed a lack of cortico-medullary differentiation, generalized lymphoid depletion and heterophilic infiltration. These findings support the results in this study, indicating the efficacy of aflasafe maize-based diet as the biological control of aflatoxin in poultry diet.

The histopathological examination of the liver in this study showed hepatocellular necrosis, abundant diffuse inflammatory cellular aggregates and blood vessel congestion. This is in agreement with the findings of [26] who noticed that the livers of the groups of birds fed 100 and 150 aflatoxin revealed vacuolar degeneration, fatty degeneration, lymphoid aggregation and hepatocytes degeneration which showed fatty changes coalesced to form fatty cysts. Previous studies have stated that the periportal fibrosis and bile-duct hyperplasia findings, in particular, may constitute chronic aflatoxicosis cases and indicate the regenerative changes in the liver [31]. The observed marked widespread of hepatocellular necrosis and abundant diffuse inflammation of the cells aggregates with blood vessels and moderate congestion is in agreement with [23,32] and [33] who observed histological alterations such as hepatocyte necrosis, Steaotosis and blood vessel congestion in the liver of chickens affected with aflatoxicosis. This suggests that high concentrations of aflatoxin in the diet caused dead cells, which have been documented to increase with increasing aflatoxin concentrations. This agrees with the findings of [34], who reported extensive necrosis and filtration in *Clarias gariepinus* fed with mouldy maize contaminated with aflatoxin. The study conducted by [35] agrees with the present study, who, from their findings, discovered the regenerative reversible lesion in growing hen fed commercial poultry feed. AFB1 is principally a hepatotoxin and hepatocarcinogen and the liver is considered to be target organs for AF and is primarily affected in aflatoxicosis cases. [24] graded hepatocellular degeneration in livers into three degrees; Degree 1(Slight); Mild hepatocellular swelling in both centrilobular and mid zonal areas. Degree 2 (Moderate); Clear hepatocellular swelling in both centrilobular and mid zonal areas. Degree 3 (Severe); Diffuse and severe hepatocellular swelling, cytoplasmic paleness and rupture. The birds fed ACDTWB fell into degree 3 which was a severe case. The use of aflasafe maize-based diet completely prevented the severity of aflatoxin contamination in the liver and enhanced normal tissue pathology through presence of numerous multifocal dense cellular aggregates. Birds fed the FF and ACDTB showed histopathological results categorized as degree 1 (slight). This shows that although both feeds were not totally free of aflatoxin, both contained aflatoxin binder. It suggested that the binder used was not effective in binding completely the aflatoxin concentration in the diet. The observed widespread sloughing off of the tubular epithelium (acute tubular necrosis) in group of birds fed ACDTWB corroborate the result obtained by [18] who observed that all experimental aflatoxin groups (300, 400 and 500 ppb) showed significantly more kidney lesions than the control group. The marked congestion of the renal blood vessels noticed in group of birds fed ACDTB also is similar to the result obtained by [18] who observed a significant reduction in kidney lesion compared to their respective control group. Use of aflasafe in the diet showed no visible lesion in the kidney. No pathological alteration was observed. This shows that the use of aflasafe enhanced a normal histopathology, depicting better performance of the birds. The kidneys which showed congestion at the renal blood vessels, marked widespread with sloughing off of the epithelium revealed the adverse effect of aflatoxin contamination in the chickens diet. This observation corroborate the findings of [26], who observed congestion, focal haemorrhages, increased glomerular cellularity and vacuolar degeneration of tubular epithelium in all toxin fed groups (100 and 150 ppb aflatoxin) and in addition, occasional thickening

of basement membrane in the 150 ppb group aflatoxin fed birds. This adverse effect were absent in those birds fed the AMBD and those fed the FF diet containing toxin binder. This shows the efficacy of aflasafe without a necessary addition of toxin binder. The observation recorded in the kidney in group of birds fed FF was similar to that of AMBD. The lungs of groups of birds fed AMBD and FF+toxin binder showed no visible lesion. No histopathological alteration of the lungs was noticed, this is a reflector of the fact that use of aflasafe maize-based diet showed a normal pathological appearance of the lungs which was similar compared to that observed in birds fed the FF+toxin binder diet. Groups of birds fed ACDTB and ACDTWB showed moderate to severe congestion of the parenchyma which is of the moderate (degree 2) category. This shows the adverse effect of aflatoxin on animal tissues.

4.3 Ilea Measurement of Layers Fed Experimental Diets

The villi of layers fed AMBD that showed normal muscularis externa, submucosa crypts could be as a result of the use of afaltoxin-free diet (Aflasafe maize-based diet) which resulted into normal villi. This is similar to the group of birds fed FF which also showed similar histopathological result. Those fed ACDTB and ACDTWB showed severe necrosis of the upper half of the villi and peri-cryptal accumulation of inflammatory cells respectively. This could be as a result of the adverse effect of aflatoxin on the group of birds fed ACDTB and ACDTWB on the group of birds. Binders, documented to be a non-nutritive entity was added to the livestock feed for the purpose of binding with the aflatoxin in the diet. An expression of severe necrosis in the birds fed ACDTB is a reflection of the ineffectiveness of the binder to bind the aflatoxin, this is shown by the histopathology result of ileum. According to [36] the intestinal crypt depth usually increased with increasing AF concentrations and not the villus length, thus influencing the villus: crypt ratio. It is known that enterocytes must differentiate during their time along the crypt-villus axis to fully express their digestive functions. What is not known, therefore, is whether AF changed enterocyte differentiation or migration rates along the length of the villus or not. Changes in intestinal morphology such as shorter villi and deeper crypts have been associated with the presence of toxins in chickens and turkeys [22,37]. Published data on the effects of AF- contaminated corn on gut morphology are scanty. The height of intestinal villi and depth of crypts reflects the surface area for nutrient absorption [16]. The ileal, villi height crypt depth in ducklings consuming the contaminated diets in an AF-contaminated corn dose-dependent manner were observed to be compromised. It is likely that the changes observed in the VH, VW and CD are due to the toxicity and damage of AF- contaminated corn on the intestinal mucosa.

5. CONCLUSION

The observation recorded in the organ weights and histopathology showed that the adverse effects of aflatoxin causes an alteration in the tissue integrity. This can be prevented by the use of feed ingredients that contains clinically low or no aflatoxin contamination. The use of biological means of mitigating aflatoxin (aflasafe-maize grain) in layers diet is recommended.

ETHICAL APPROVAL

As per international standard or university standard written ethical permission has been collected and preserved by the author.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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