REDUCING INDICES OF OXIDATIVE STRESS CAUSED BY DIETARY AFLATOXINS WITH ANTIOXIDANTS AND VITAMIN K SUPPLEMENTATIONS

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ABSTRACT
Aflatoxins are unavoidable toxic contaminants in animals' feed. Nutrition-derived vitamins and mineral with antioxidant properties such as vitamins E, C and selenium can be used to counteract the Oxidative stress (OS) effects of aflatoxins on broiler chickens. Limited information exists on the combination and dosage of these antioxidants and vitamin K, that will be effective in counteracting OS effects of aflatoxins. Response of broiler chickens fed aflatoxin-contaminated diets to different combinations of Supplemental Dietary Antioxidants (SDA) and VK was investigated. One-day-old (n = 180) Arbor Acres broiler chick (unsexed) were randomly allotted into six treatment diets comprising Negative Control (NC), Basal Diet (BD), diet containing 270ppb aflatoxins and other BD's containing vitamins: E (VE), C (VC), K (VK) and selenium (Se) at 200mg, 250mg, 3mg and 0.3mg/kg diet, respectively in four combinations: D1- (BD+VE+VC), D2- (BD+VE+VC+Se), D3 (BD+VE+VC+VK) and D4- (BD+VE+VC+VK+Se) Serum biochemical enzymes, reduced/oxidised glutathione (GSH/GSSG) and lipid peroxidation (LP) index (Malondialdehyde-MDA nmol/mL) were assayed. Experimental design was completely randomised and study lasted for 42 days. Data were analysed with ANOVA at α0.05. The SDA and VK significantly reduced LP activity by 30% reduction in MDA level from 128.29±31.16 (BD) to 42.00±10.40 (D4), compared to 10-fold increase in BD (128.29±31.16), against NC (12.41±10.56). Combinations of SDA and VK improved oxidative-stress index (GSH:GSSG) by 60% rise in D4 (2.19±0.92:1), as against 22.8% in BD (0.83±0.58:1), when compared to NC (3.64±1.44:1). Addition of supplemental dietary antioxidants and vitamin K reduced lipid peroxidation and oxidative stress effects of aflatoxins in broiler chickens.
Keywords: Aflatoxins, lipid peroxidation, oxidative stress, dietary antioxidants, broiler chickens

INTRODUCTION

Aflatoxins contamination of animal feeds and/or feed materials is a severe risk to the animal's health and productivity and also to humans (Nazhand *et al*., 2020) consuming products derivable from animals that were fed aflatoxins-contaminated diet. Following the ingestion of dietary aflatoxins, they are taken up into the blood streams and ferried into the liver via the hepatic portal vein. The liver is endowed with cytochrome P450 enzymes, which are a large group of monooxygenase enzymes that are involved in the oxidative metabolism of a wide range of xenobiotics (Ewuola and Bolarinwa, 2016) such as mycotoxins. This biotransformation process in the liver converts fat-soluble aflatoxins into hydrophilic substances, to facilitate their disposal and clearance out of the animal's body by the kidney into urine or the liver into bile. However, the biotransformation process results in the production of varying degree of free-radicals (FR) like superoxide anion and reactive oxygen species (ROS) such as hydrogen peroxide (Reed *et al*., 2011).

Uncontrolled FR/ROS production within a biological system has been implicated for oxidative stress or oxidative tissue damage (Omar, 2013). Reactive oxygen species can attack vital cell components such as polyunsaturated fatty acids within cell membranes, resulting in lipid peroxidation and the depletion of the antioxidant defence system, leading to oxidative stress. Oxidative damage is essentially the expression of the tissue damage that the disease-causing organism will also produce, making the animal more vulnerable to succumb to the disease (Romero *et al*., 2013).

Aflatoxin also exhibits anticoagulant property, in view of the structural similarity between aflatoxin and coumarin. Bababunmi (1989), reported that the addition of vitamin K was effective in reversing the prolonged blood clotting ability of aflatoxin in the liver slices of rat.

Dietary supplementation of antioxidant vitamins is a promising method of counteracting the adverse effects of aflatoxins. However, there are little documented information on *in vivo* trials on the combination and dose of antioxidants that will be effective in mitigating the adverse effects of aflatoxins in animals. This study was conducted therefore, to investigate the effect of different combinations of vitamins C (VC) and E (VE), selenium (Se) and vitamin K (VK) in broiler chickens fed dietary aflatoxins to mitigate its oxidative stress effects.

MATERIALS AND METHODS

Experimental Animals, Management and Diets

One hundred and eighty (180) one-day-old Arbor Acres broiler chicks (mixed sexes) were randomly allotted into six dietary treatments. Maize grains inoculated with *Aspergillus flavus*, isolate 3228, obtained from the Plant Pathology Unit of International Institute of Tropical Agriculture (IITA), Ibadan, were cultured by adapting the method of Athenkeng *et al*. (2008), to formulate the basal diet. The grain culturing lasted for 10 days and the contaminated grains were oven dried at about 60 – 70 ºC. The level of aflatoxin contamination in the basal diet was quantified to be 270 ± 16.0 ppb total aflatoxins, (aflatoxins B_1 - AFB_1 and B_2 - AFB_2), per kg of feed, using standard TLC procedure of (AOAC, 1990).

Each dietary treatment was replicated thrice with 10 chicks in each replicate. NC was the negative control (aflatoxins-free diet) while BD was Basal diet (the diet with 270ppb aflatoxins but without SDA and VK) or positive control and BD's containing. vitamins: E (VE), C (VC), K (VK) and selenium at 200mg, 250mg, 3.0mg and 0.3mg/kg of feed respectively. Aflatoxins-free diet was produced by using maize grains harvested from aflasafe® treated maize plots. The test diets were formulated to be isonitrogenous and isocaloric, to ensure that observed treatment differences were not adduced to differences in the nutrients' levels of the test diets. The starter diet contained 22% CP and 3,000kcal/kg ME, while the finisher diet contained 19% CP and 3,100kcal/kg ME (approximately). Feed was offered *ad libitum* and the experiment lasted for 42 days.

Dietary Treatments Layout

NC = Negative Control; D2 = BD + [(VE + VC) + Se]
BD = Basal Diet; D3 = BD + [(VE + VC) + VK]
D1 = BD + (VE + VC); D4 = BD + [(VE + VC) + Se + VK].

Where: VE- Vitamin E, VC- Vitamin C, VK- Vitamin K, Se- Selenium

Parameters Measured

At day 42, two birds were randomly selected from each replicate for blood sample collection via jugular venipuncture and 3ml was collected for some selected serum biochemical indices analysis. Serum was separated by centrifugation × 4,000rpm for about 15 minutes and kept frozen at about -20ºC until it was analysed. Alkaline phosphatase (ALP) was done by the method of Rosalki *et al*. (1993). Serum malondialdehyde (MDA) was assayed using Elabscience® Malondialdehyde Colorimetric Assay kit (through the TBA method). Lipid peroxidation activity was determined indirectly by reacting breakdown products from lipid peroxidation with thiobarbituric acid (TBA) to estimate level of MDA in the serum samples, adapting the method used by Ohkawa *et al*. (1979). Total Glutathione (T-GSH) and oxidised glutathione (GSSG) were assayed using the method of Rahman *et al*. (2006). Using Elabscience® Total Glutathione/Oxidised Glutathione assay test kits. The reduced glutathione (GSH) in the sample was determined from the total and oxidised glutathione in the equation that follows as earlier stated by Rahman *et al*. (2006). ([GSH]total = [GSH] + 2 × [GSSG]).

Experimental Design and Statistical Analysis

Experimental design was complete randomization. Analysis of variance was used to analyse all data collected for the variables measured, using SAS (2012) software package. Differences between treatment means were separated with Duncan' Multiple Range Test (DMRT). Means were declared significant based on 0.05% level of probability.

RESULTS

Table 1. shows the effect of different combinations of SDA and VK on serum biochemical indices of broilers chickens fed aflatoxins-contaminated poultry feed. The four supplemented treatment diets (D1 to D4) had significantly lower ($P<0.05$) ALP values (144.21±74.33; 120.03±61.48; 116.36±49.88

and 77.56±22.10 U/L) respectively, compared to birds fed BD (215.59±25.01 U/L), while birds on diets D2, D3 and D4 had reduced ALP values similar to NC (76.88±26.08 U/L). The least MDA value was recorded in birds fed NC diet (12.41±10.56 nmol/mL) while birds fed D3 to D6 had significantly reduced (P<0.05) MDA values below that of birds fed BD, which had significantly higher (P<0.05

)**Table 1: Serum biochemical indices of broilers offered aflatoxins-contaminated poultry feed supplemented with different combinations of supplemental dietary antioxidants and vitamin K**

	Dietary treatments							
Parameters	NC	BD	D1	D2	D3	D4	SEM	P-value
ALP (U/L)	76.88±26.08^{c}	215.59±25.01^{a}	144.21±74.33^{b}	120.03±61.48^{bc}	116.36±49.88^{bc}	77.56±22.10^{c}	16.82	<0.0001
MDA (nmol/mL)	12.41±10.56^{d}	128.29±31.16^{a}	79.22±38.65^{b}	80.18±12.58^{b}	72.54±24.99^{b}	42.00±10.40^{c}	8.49	<0.0001
T-GSH (µmol/mL)	10.06±6.83	4.51±1.45	8.97±6.00	8.85±6.63	10.07±6.63	10.95±8.91	2.29	0.43
GSH (µmol/mL)	5.03±0.97^{a}	2.19±1.16^{b}	5.38±1.36^{a}	5.13±1.59^{a}	4.75±1.22^{a}	5.24±1.59^{a}	0.47	0.0001
GSSG (µmol/mL)	1.53±0.51^{b}	3.08±1.05^{a}	2.97±0.99^{a}	2.74±0.68^{a}	2.81±0.97^{a}	2.59±0.73^{a}	0.30	0.01
GSH:GSSG	3.64±1.44^{a}	0.83±0.58^{c}	1.98±0.89^{b}	1.94±0.78^{b}	1.88±0.72^{b}	2.19±0.92^{b}	0.33	<0.0001

^{abcd} Means of treatments along a column with different superscripts differed significantly (P<0.05). ALP- Alkaline Phosphatase, TP- Total protein, ALB- Albumin, GLB- Globulin, MDA- Malondialdehyde, SOD- Superoxide dismutase, T-GSH- Total glutathione, GSH- Reduced glutathione, GSSG- Oxidised glutathione, GSH: GSSH- Oxidative stress index, SEM- standard error of means, P-value-probability level, NC- Negative control, BD- Basal Diet, D1- [BD + (VE+VC)], D2- [BD + (VE+VC)+Se], D3- [BD + (VE+VC)+K], D4- BD + (VE+VC)+VK+Se], Se – Selenium.

level of 128.29±31.16 nmol/mL. The GSH values in all treatments contaminated with aflatoxins but having SDA with or without Se and/or VK did not differ significantly (P>0.05) from that of birds fed NC diet (5.03±0.97 µmol/mL), while there was significant reduction (P<0.05) in the GSH value of birds on BD (2.19±1.16 µmol/mL). The GSSG value of birds fed NC diet was significantly reduced (P<0.05) when compared to the rest of the treatments containing aflatoxins (having SDA with or without Se and/or VK), which showed no significant differences to one another. Oxidative stress (OS) index denoted by GSH:GSSG, was significantly lower (P<0.05) in birds fed BD (0.83±0.58) while birds fed all four treatment diets having SDA with or without Se and/or VK had similar GSH:GSSG ratio that ranged between 1.88±0.72 and 2.19±0.92, but were significantly higher (P<0.05) than that of birds fed BD. Birds fed NC diet had significantly higher (P<0.05) GSH:GSSG ratio (3.64±1.44) compared to other treatment diets.

Discussion

The SDA and VK combination in diet D4 may probably be effective in reducing hepatic injury arising from FR/ROS production, resulting in reduction in hepatic damage as shown by ALP values in birds fed D4 which had similar ALP value with birds fed NC, which were least expected with liver damage due to the non-presence of aflatoxins in it. The presence of vitamin K (anti-haemorrhagic vitamin) in the diet of birds on D4 may had been effective in reducing high level of hepatic and biliary tracts haemorrhages, as reported by Peles *et al*. (2019). Results for lipid peroxidation activity indicated by the level of serum MDA showed that birds fed D1 – D4 had reduced MDA levels due to the addition of SDA with or without Se and/or VK addition, while the treatment without mitigation (BD) had very high MDA. This may probably be due to the synergistic role of vitamins C and E in minimising oxidative attack on hepatic microsomal PUFA, by donating hydrogen ions to the expected FR/ROS from hepatic cytochrome P450 activity. The MDA level of birds fed D4 was not reduced as low as seen in birds fed NC, probably because even under normal physiological condition, some degree of lipid peroxidation takes place (Ayala *et al*., 2014). Oxidative stress status is the ratio of GSH:GSSG (Pastore *et al*., 2001), shows that the addition of SDA with or without Se and/or VK, increased GSH of birds fed all four contaminated but treated diets due to the sparing effect of VE and VC on glutathione peroxidase activity (the endogenous antioxidant that uses GSH), in reducing or neutralising FR/ROS. However, the result of high levels of GSSG in the presence of aflatoxins in all contaminated treatment diets was indicative of the ability of aflatoxins in depleting the antioxidants principles in animals, leading to immune suppression by reducing the animals' resistance to diseases (Corrier, 1991).

CONCLUSION AND RECOMMENDATION

Nutrition-derived antioxidants and vitamin K supplementations in contaminated broiler chicken's feed resulted in reduced lipid peroxidation activity and improved oxidative stress index, marked by higher GSH:GSSG ratio. However, additional mitigation strategy that will perhaps reduce the absorption of

the toxin from the GIT is required to increase oxidative stability and minimize peroxidative damage in broiler chickens fed on aflatoxins-contaminated diets out of inevitable circumstances.

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