Effects of Supplementing Different Levels of Vitamin A to Aflatoxin B_1 Contaminated Diets on the Performance of Broiler Chickens

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ABSTRACT

This study was conducted to evaluate the effects of supplementing different levels of vitamin A on the performance of broiler chickens fed on diets contaminated with aflatoxin B_1 (AFB_1) for 42 days. Conducted as a Completely Randomised Design, the study had 5 treatments, 3 replications and 10 chicks per experimental unit. Control/Treatment A had no AFB_1 and vitamin A added. Other dietary treatments were contaminated with AFB_1 at 35µg/kg. Except for Treatment B, the rest were supplemented with vitamin A at 3000, 6000 and 11000 IU/kg, respectively. In the Starter Phase, AFB_1 significantly ($P \leq 0.05$) reduced feed intake, bodyweight gains and feed conversion ratios. However, in the Grower and Finisher Phases, only bodyweight gains and feed conversion ratios were reduced. This affected final bodyweights and dressing out percentages, where those fed on contaminated diets performed poorly compared to those on Control diets. It was noted that performance of chickens improved significantly with increasing levels of vitamin A supplementation. The toxic effects of AFB_1 on feed intake were ameliorated by dietary supplementation of vitamin A at 6000 IU/kg and above. However, amelioration of the deleterious effects of AFB_1 on bodyweight gains and feed conversion ratios was achieved when vitamin A was supplemented in the diets at 3000 IU/kg. Levels of AFB_1 contamination used in the current study did not cause any death. Contamination of broiler diets with AFB_1 also significantly ($P \leq 0.05$) decreased serum concentrations of total protein, albumin, triglyceride and cholesterol. Feeding AFB_1-contaminated diets also increased the serum concentrations of alkaline phosphatase, aspartate aminotransferase and alanine aminotransaminase. However, the serum concentrations of alanine aminotransaminase normalized when feed diets were supplemented with vitamin A at 11000 IU/kg. It was thus, concluded that supplementation of vitamin A to AFB_1-contaminated rations has capacity to reduce toxic effects of AFB_1 in broiler chickens.

Keywords: Broiler, Aflatoxin B_1, contamination, vitamin A, supplementation.

INTRODUCTION

Feed safety is an essential prerequisite to ensuring human health and there is a worldwide concern about the dangers of food poisoning (WHO, 2006). Feeds may be contaminated with physical, chemical, biological and other toxic agents that affect both animal health and productivity. Among the major biological feed contaminants, mycotoxins are probably of greatest concern to tropical countries including Zambia. Mycotoxins are secondary metabolites produced by different species of fungi with the most common ones being aflatoxins, ochratoxin and fumosin. Aflatoxins are of greatest concern for tropical countries due to predisposing hot and humid conditions that prevail in these areas (FAO, 2004). They are derived from fungi of genus *Aspergillus;* with *A. flavus, A. parasiticus* and *A. nomius* being the most common species. The aflatoxins produced by these species are differentiated

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according to the colour of light they emit under the ultraviolet light that is either blue or green and hence the B or G varieties. A metabolite of B_1 has been identified in milk to give the M varieties. Based on the ring structure in the Molecule various varieties are categorized as either 1 or 2 to give the terms B_1, B_2, G_1, G_2, M1 and M2 varieties (Yunnus et al., 2013). Of all varieties, Aflatoxin B_1 is the most abundant and toxic of all naturally occurring mycotoxins. They are generally storage mycotoxins that proliferate under hot and humid conditions and mostly infect grain legumes and cereals.

Aflatoxin B_1 contaminations are usually a consequence of interactions among the fungi, host animal and the environment. High levels of aflatoxins contamination in feed results in acute necrosis of the liver and hyperplasia of the bile duct resulting in reduced digestibility of fatty acids and proteins (Brasel and Hussein, 2001). Aflatoxin B_1 also exerts inhibitory effects on biological processes including protein and DNA synthesis (Denli et al., 2005). Lower levels of aflatoxin contamination may not exhibit any clinical symptoms but are usually associated with considerable decrease in productivity and suppressed immunity (Tedesco et al., 2004; Bailey et al., 2006; Shi et al., 2006;). This is usually manifested in reduced feed intake, growth and feed efficiency. For chickens consuming contaminated feeds, there are also residues of mycotoxins in poultry products that are known to cause adverse effects on human health (Micco et al., 1988; Oliveira et al., 2000; Binthivok, et al., 2002; Rizzi et al., 2003). Aflatoxin B_1 has also been associated with cancer in humans (Kellerman et al., 1988).

Control of aflatoxins poisoning in domestic animals should be aimed at minimizing feed contamination through proper storage, processing or decontamination of feed ingredients (FAO, 2004). The objective of this study was to evaluate the effects of supplementing different levels of vitamin A, a potential anti-oxidant on the performance of broiler chickens fed on diets contaminated with Aflatoxin B_1.

MATERIALS AND METHODS

Experimental Site and Materials

This study was conducted at the University of Zambia in the School of Agricultural Sciences for a period of 42 days. The materials used for the study included 150 unsexed one day-old broiler chicks (weighing on average 46.3 g) from a local hatchery (Ross Breeders Zambia Ltd). Feed ingredients for the formulation of experimental diets were purchased from a local supplier (Livestock Services Cooperative Society, Lusaka, Zambia). These included maize meal No.3, Soybean meal, Limestone flour, Di-calcium phosphate, methionine, lysine, broiler premix and salt. Retinol (vitamin A) acetate and Aflatoxine (aflatoxin B_1) were sourced from HiMedia Laboratories (Pvt) Ltd, 23 Vadhani Industrial Estate, Ghatkopav, Mumbai, India.

Basal Diets and Dietary Treatments

Throughout the study, standard broiler starter, grower and finisher diets were formulated (Table 1) for feeding experimental chickens from days 0 -14, 15 -28 and 29-42, respectively.

Initially, all the diets were found to contain 9000 IU of vitamin A per kg before being supplemented with treatment levels. Except for the control, treatment diets were contaminated with AFB_1 by dissolving 1mg in 10ml of chloroform. Thereafter, the resulting solution was mixed with 2kg of basal diets. This resulted in the production of a toxin-premix that was allowed to dry overnight in a fume hood for the solvent to evaporate. The dried toxin-premix was finally mixed with the rest of basal diets to give a concentration of 35µg/kg. This was slightly higher than the 20 µg AFB_1 per kg regulatory upper limit recommended for human foods and animal feeds (Muzaffer *et al*., 2003 and WHO, 2006).

Table 1. Composition and nutrient concentration of the basal diets (% as fed-basis)

Item	Diet		
	Starter	Grower	Finisher
Ingredient (%)			
Maize meal	53.50	63.77	68.36
Soyabean meal (solvent extract)	40.50	30.73	26.64
Tallow	2.88	2.44	2.11
Dicalcium phosphate	0.23	0.26	0.28
Calcium carbonate	2.04	1.99	1.93
Lysine	0.01	0.02	0.01
Methionine	0.12	0.09	0.07
Vitamin and mineral premix	0.42	0.4	0.3
Sodium chloride	0.30	0.30	0.30
Total	100	100	100
Chemical composition			
ME (kcal/kg)	2870	3000	3100
Crude protein (%)	22	19	18
Calcium (%)	0.9	0.9	0.9
Phosphorus (%)	0.42	0.42	0.42
Moisture (%)	12	12	12
Crude Fat (%)	5	7	9
Crude Fiber (%)	5	5	5

The contaminated diets were then mixed with assigned levels of vitamin A (powder) as shown in Table 2.

Table 2. Levels of AFB_1 contamination and vitamin A supplementation in the dietary treatments.

Treatment	AFB_1 (µg/kg)	Level of Vitamin A Supplementation (mg/kg)	(IU/kg)
A (Control)	0	0	0
B	35	0	0
C	35	0.9	3,000
D	35	1.8	6,000
E	35	3.3	11,000

1 IU = 0.0003 mg

For vitamin A supplementation, treatment diets were separately and sequentially mixed with 20 g (initially with 5 g and finally 15 g), 80 g and 900 g of broiler premix, di-calcium

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phosphate meal and maize meal. Lastly, the final premix was the mixed with rest of basal dietary treatments.

Management of Broiler Chickens

The birds were housed under a deep-litter system where 1m x 1m wire mesh frames were placed on the floor to demarcate treatment groups. Feed and water were offered *ad libtum* throughout the trial. Each treatment was allocated to 3 replicates of 10 birds each. Feed intake was recorded daily by weighing the feed offered and leftover from the previous day. At the beginning of the trial, initial body weights of birds were recorded and evened out before distributing chickens into treatment groups. The birds were also vaccinated by ocular route against Newcastle disease at 14 and 24 days of age using the freeze-dried live lentogenic ND virus, Lasota strain. While at days 10 and 28 of age, the birds were vaccinated by ocular route against Infectious Bursal Disease using the freeze-dried live IBD virus, Virgo 7 intermediate-hot strain. The ND and IBD vaccines were sourced from Biovac Ltd, Or-Akiva 30600, Israel. For lighting, the chickens were subjected to 23 hours of light every day and during the starter phase, brooding was provided by using of 250W infrared bulbs as the main source of heat.

Data Collection and study Parameters

Feed consumption and mortalities were recorded everyday while data on body weights were collected once every week. Weekly body weight gains were determined by subtracting the weight of chickens between two weeks while feed conversion ratios were calculated by dividing weekly feed intakes with body weight gains. At the end of the experiment (day 43), 5 chickens from each pen were randomly selected for collection of 2 ml per bird of blood for determination of serum biochemical parameters. Within an hour of blood collection, the serum was separated from the rest of the blood by centrifuging at 2,500 *g* for 15 minutes and stored at − 80°C until required for analysis. The serum was analyzed for the concentrations of liver function enzymes (alkaline phosphatase, aspartate aminotransferase and alanine aminotransaminase), and content of total triglycerides, cholesterol, albumin and proteins. These serum biochemical parameters were measured by using the UV-Spectrophotometer (Ultrospec 2000, Pharmacia Biotech, Germany) and commercial kits (Human Gesellschaft für Biochemica und Diagnostica mbH, Wiesbaden, Germany), following the manufacturer's instructions. After collecting of blood samples, the chickens were slaughtered and processed to determine dressed weights and dressing out percentages.

Experimental Design and Statistical Analysis

The study was designed as a Completely Randomized Design and all collected data were compiled and summarized using Microsoft Excel computer software and analyzed for statistics using the Analysis of Variance (ANOVA) to determine presence of significant differences among treatment means at probability $P \leq 0.05$. A Tukey's comparison test was used to separate significantly different treatment means. All analyses were done on a GenStat statistical software package (GenStat, 2012).

RESULTS

Growth Performance of Broiler chickens

Starter Phase: 1 - 14 days

The performance of broiler chickens fed different treatment diets during the Starter phase were as presented in Table 3. Contamination of starter diets with AFB_1 reduced feed intake in broiler chickens. This was demonstrated by having significant differences ($P \leq 0.05$) in feed

intake between the chickens that were fed on the control diet (uncontaminated) and those fed on AFB_1 contaminated diet but without vitamin A supplementation. Supplementing contaminated diets with vitamin A at 6000 and 11000IU/kg improved feed intake in chickens to a level that was similar to that of those that were fed the control diet. The results on bodyweight gains were similar to that of feed intake in that chickens fed on the Control diet had significantly ($P \leq 0.05$) higher bodyweight gains than those that were fed on AFB_1-contaminated diets but without vitamin A supplementation. It was also observed that there were no significant differences in feed conversion ratios of broilers on the Control diet and those fed on AFB_1 contaminated diets that were supplemented with vitamin A at 6000 and 11000 IU per kg. Supplementing AFB_1 contaminated diets with 3000IU of vitamin A was not effective in improving feed intake and body weight gains. There were no cases of mortality during the starter phase, which may be an indication that the contamination levels used in this experiment may not have been toxic enough to cause deaths in growing chickens.

Table 3. Effect of supplementing different levels of vitamin A to AFB_1-contaminated diets on growth performance of broiler chickens in the Starter Phase (0 – 14 days).

Treatment	*Vitamin A (IU/kg)*	*AFB_1 (µg/kg)*	*Parameter*		
			FI (g)	*BWG (g)*	*FCR*
A (Control)	0	0	622.00^{a}	294.5^{a}	2.11^{c}
B	0	35	604.33^{b}	252.5^{c}	2.39^{a}
C	3,000	35	612.67^{b}	264.4^{bc}	2.32^{ab}
D	6,000	35	622.00^{a}	268.2^{bc}	2.32^{ab}
E	11,000	35	622.00^{a}	282.0^{ab}	2.21^{bc}
Lsd			4.20	10.3	0.08

Means within a column with different superscript letters differ significantly ($p<0.05$) according to Tukey's method. AFB_1 = Aflatoxin B_1, FI = Feed Intake, BWG = Body weight gain and FCR = Feed Conversion Ratio.

Grower Phase: 15 – 28 days

The performance of broiler chickens during the grower phase in terms of feed intake, body weight gains and feed conversion ratios were as tabulated in Table 4. Unlike in the starter phase, there were no significant differences in feed intake among birds fed different treatment diets. The reason for lack of significant differences among treatments during the grower phase could have been due to the fact that the birds were given limited amounts of feed which resulted in all treatments having same feed intake levels. There were, however significant differences among treatments on bodyweight gains that also affected feed conversion ratios. Significant differences ($P \leq 0.05$) were recorded between chickens that were fed on control diets and those whose diets were contaminated with AFB_1 but without vitamin A supplementation. Supplementing AFB_1 contaminated diets with increasing levels of vitamin A in the grower phase improved body weight gains only slightly as improvements were still significantly lower than that of birds fed uncontaminated or control diets. It must however be appreciated that increasing levels of vitamin A supplementation beyond 3000IU/kg improved the response of broiler chickens to AFB_1 contamination. The results on body weight gains were also reflected in feed conversion ratios where chickens feed uncontaminated diets had significantly better performance while those fed contaminated diets with increasing levels of

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vitamin A supplementation had significantly better feed conversions than those fed un-supplemented diets.

Table 4.Effect of supplementing different levels of vitamin A to AFB_1-contaminated diets on growth performance of broiler chickens in the Grower Phase (15 – 28 days).

Treatment	*Vitamin A (IU/kg)*	*AFB_1 (µg/kg)*	*Parameter FI (g)*	*BWG (g)*	*FCR*
A (Control)	0	0	1378	864.20^{a}	1.60^{d}
B	0	35	1378	650.87^{d}	2.12^{a}
C	3,000	35	1378	653.43^{cd}	2.11^{ab}
D	6,000	35	1378	663.07^{c}	2.08^{b}
E	11,000	35	1378	837.70^{b}	1.65^{c}
Lsd			0.0	5.88	0.02

Means within a column with different superscript letters differ significantly ($p<0.05$) according to Tukey's method. AFB_1 = Aflatoxin B_1, FI = Feed Intake, BWG = Body weight gain and FCR = Feed Conversion Ratio.

Finisher Phase: 29 – 42 days

Like in the Grower phase, there were no significant differences among treatment means on feed intake among chickens fed on various treatment diets (Table 5). The results on body weight gains and feed conversion ratios were significantly higher in broiler chickens whose diets were contaminated with AFB_1 and supplemented with vitamin A at 11000 IU per kg. This was followed by chickens fed the control diet and those fed contaminated diets that also had vitamin A supplementation at 3000 and 6000IU/kg. Thus, body weight gains and feed conversion ratios of broiler chickens fed diets exposed to AFB_1 contamination and supplemented with 3000IU and 6000IU per kg had a similar response to those fed the Control diets. Only chickens fed on contaminated diets without vitamin A supplementation had significantly inferior body weight gains and feed conversion ratios.

Table 5. Effect of supplementing different levels of vitamin A to AFB_1-contaminated diets on growth performance of broiler chickens in the Finisher Phase (29 – 42 days).

Treatment	*Vitamin A (IU/kg)*	*AFB_1 (µg/kg)*	*Parameter FI (g)*	*BWG (g)*	*FCR*
A (Control)	0	0	1500	938.0^{ab}	1.60^{bc}
B	0	35	1500	885.2^{c}	1.70^{a}
C	3,000	35	1500	906.8^{bc}	1.65^{ab}
D	6,000	35	1500	916.1^{bc}	1.64^{ab}
E	11,000	35	1500	967.8^{a}	1.55^{c}
Lsd			0.0	20.4	0.04

Means within a column with different superscript letters differ significantly ($p<0.05$) according to Tukey's method. AFB_1 = Aflatoxin B_1, FI = Feed Intake, BWG = Body weight gain and FCR = Feed Conversion Ratio.

Body Weights, Dressed Weights and Dressing Percentages

Results on chicken dressed weights and dressing out percentages are shown in Table 6. There were significant differences ($P \leq 0.05$) among all treatments with the control having highest dressed figures that were followed by chickens fed contaminated diets that were supplemented with 11000IU/kg of Vitamin A. There were no significant differences in dressed weights between chickens fed contaminated diets that were supplemented with either 3000IU or 6000IU per kg of vitamin A. However, the results on dressing out percentages between the two treatments were significantly different from each other. The lowest dressed weights and dressing out percentages were recorded in birds that were fed AFB_1 contaminated diets that were also not supplemented with any vitamin A. It was thus; noted that dressed weights and dressing percentages increased with increasing levels of vitamin A supplementation to AFB_1 contaminated diets although the response failed to reach that of chickens fed on control diets.

Table 6. Effect of supplementing different levels of vitamin A to AFB_1-contaminated diets on body weights, dressed weights and dressing percentages in broiler chickens.

Treatment	*Vitamin A (IU/kg)*	*AFB_1 (µg/kg)*	*Study Parameters*	
			Dressed Weight (g)	*Dressing Percentage (%)*
A (Control)	0	0	1520.7^{a}	70.99^{a}
B	0	35	1118.1^{d}	60.92^{e}
C	3,000	35	1194.3^{c}	63.83^{d}
D	6,000	35	1229.6^{c}	64.96^{c}
E	11,000	35	1473.0^{b}	69.00^{b}
Lsd			17.4	0.26

Means within a column with different superscript letters differ significantly ($p<0.05$) according to Tukey's method. AFB_1 = Aflatoxin B_1.

Serum Biochemical Parameters

Concentrations of Liver Function Enzymes

The results on the concentrations of liver functional enzymes in blood serum including alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransaminase (ALT) were as presented in Table 7. Levels of ALP, AST and ALT were significantly different ($P \leq 0.05$) among all chickens fed different treatment diets. As for ALT, no significant differences were observed between broilers fed the Control diets and those that were given AFB_1-contaminated diets that were also supplemented with 11000 IU/kg levels of vitamin A. The reduction in blood enzyme concentrations of liver functional metabolites with increasing levels of vitamin A supplementation demonstrated the reducing effect of the vitamin A supplementation on AFB_1 toxicity.

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Table 7. Effect of supplementing different levels of vitamin A to AFB_1-contaminated diets on the concentration of liver function enzymes in broiler chickens.

Treatment	*Vitamin A (IU/kg)*	*AFB_1 (µg/kg)*	*Parameter*		
			ALP (u/L)	*AST (u/L)*	*ALT (u/L)*
A (Control)	0	0	984.37^{e}	10.00^{e}	1.75^{d}
B	0	35	1668.63^{a}	26.83^{a}	7.00^{a}
C	3,000	35	1459.57^{b}	22.67^{b}	5.25^{b}
D	6,000	35	1337.83^{c}	15.65^{c}	3.50^{c}
E	11,000	35	1053.43^{d}	13.42^{d}	1.75^{d}
Lsd			4.13	1.98	0.00

Means within a column with different superscript letters differ significantly ($p<0.05$) according to Tukey's method. AFB_1 = Aflatoxin B_1, ALP = Alkaline phosphatase, AST = Aspartate aminotransferase, ALT = Alanine aminotransaminase.

Concentration of serum total Proteins, Triglycerides, Cholesterol and Albumin

The results on serum concentration of triglycerides, cholesterol, total proteins and albumin were as presented in Table 8 and clearly indicate that the effect of AFB_1 contamination on the concentration of these metabolites in chickens supplemented with different levels vitamin A..

Table 8: Effect of supplementing different levels of vitamin A to AFB1-contaminated diets on the concentration of total Triglycerides (TG), Cholesterol (C), Proteins (TP) and Albumin (ALB) in broiler chickens.

Treatment	*Vitamin A (IU/kg)*	*AFB_1 (µg/kg)*	*Parameter*			
			TG (mg/dL)	*C (mg/dL)*	*TP (g/dL)*	*ALB (g/dL)*
A (Control)	0	0	216.00^{a}	282.77^{a}	6.210^{a}	3.813^{a}
B	0	35	97.93^{e}	121.13^{e}	2.833^{e}	1.507^{e}
C	3000	35	106.07^{d}	157.00^{d}	3.203^{d}	1.723^{d}
D	6000	35	122.87^{c}	171.90^{c}	3.680^{c}	2.107^{c}
E	11000	35	144.20^{b}	182.97^{b}	4.537^{b}	2.660^{b}
Lsd			1.87	2.30	0.150	0.055

Means within a column with different superscript letters differ significantly ($p<0.05$) according to Tukey's method. FB_1 = Aflatoxin B_1, ALB = Albumin, TP = Total Protein, TG = Triglycerides and C = Cholesterol.

Contamination of broiler chicken diets with AFB_1 reduced the concentration of total proteins, triglycerides, cholesterol and albumin as demonstrated by the significant differences ($P \leq 0.05$) in the content of these metabolites between chickens fed control diets and those fed AFB_1 contaminated diets without vitamin A supplementation

There were also significant differences ($P \leq 0.05$) in the concentration of these parameters among chickens fed AFB_1 contaminated diets that were supplemented with different levels of vitamin A. In all cases, the concentration of all metabolites increased with increasing levels of vitamin A supplementation. This was a clear demonstration of the ameliorating effect of vitamin A on AFB_1 toxicity. It must be appreciated though that even at the highest level of vitamin A supplementation, the serum metabolites failed to reach concentration levels recorded in the control diets. The increase in serum levels of triglycerides, cholesterol, total proteins and albumin with increasing levels of vitamin A supplementation was an indication of the reducing effect of vitamin A on AFB_1 toxicity.

DISCUSION

Mycotoxins are a major concern in poultry production as they do not only contaminate feeds but are also a potential danger to human health (Resanovic et al., 2009). The severity of the problem may be demonstrated by the fact that up to 25% of world grain is said to be contaminated with different types of mycotoxins (Johnson and Mannon, 1985).

Contamination of feed ingredients with mycotoxins often result in deterioration of quality that is manifested through changes in nutritional profiles, organoleptic characteristics and general hygienic properties (Markovic et al., 2005). There are also mycotoxin residues in poultry meat, eggs and other derived products as a result of chickens consuming a contaminated diet, which is a threat to human health (Binthivok et al., 2002). This calls for the need to find means of decontaminating or reducing the effects mycotoxins in poultry in order to avoid economic losses and protect human health. Results from this study have demonstrated that contamination of broiler chicken diets with AFB_1 results in reduced feed intake, body weight gains and feed conversion ratios. This is especially critical during the starter phase. These findings are in agreement with several other studies that have also shown that aflatoxin B_1 contamination in feeds impairs broiler chickens performance through reduced feed intake, body weight gains and feed utilization (Agag, 2004; Tedesco et al., 2004; Bailey et al., 2006; Saif, 2003; Shi et al., 2006; Yunus et al. 2013. In this study, no mortalities were observed and this was in agreement with those of Denli et al., (2005) who also found out that low exposure of chicks to AFB_1 (< 50 µg/kg of diets) contamination may not lead to their deaths but merely lower their productivity. It has thus; been confirmed that it takes relatively higher concentrations of mycotoxins in the diet to cause mortality in chickens (Binder, 2007). However, because of negative effects on production even under chronic poisoning, current recommendations are that growing chickens should not receive more than 20ug/kg in the diet (Denli et al., 2005).

Toxic effects of aflatoxins in poultry are usually as a result of liver damage and inhibition of protein synthesis (Miazzo et al., 2005; Resanovic et al., 2009; Abidin et al., 2011;). As demonstrated in this study, this is often manifested by a reduction in the concentration of blood metabolites including total proteins, triglycerides, cholesterol and albumin. These findings agree very well with those of Oguz et al. (2000) who also found that AFB_1 contamination decreases serum concentration of total proteins and albumins. Fernandez *et al*., (1995) also reported that the synthesis of albumin and most of the globulins takes place in the liver and in chronic aflatoxicosis, hypoalbuminaenia occurs. The damage is usually manifested through hyperplasia, necrosis, cirrhosis and fibrosis (Lafi et al., 2010). Even

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though not considered in this study, aflatoxin toxicosis is also known to reduce blood glucose concentration and also tends to interfere with calcium and vitamin D metabolism that result in weakened legs in broiler chickens (Hamilton, 1984). Reductions in serum total protein and albumin concentrations in chickens fed AFB_1 contaminated diets is said to be a result of inhibition of amino acid transport and mRNA transcription that leads to inhibition of DNA and protein synthesis (Thaxton et al., 1974).

As observed in this study, changes in the concentration of blood metabolites are often associated with corresponding increases in the activity of liver function indicator enzymes including alkaline phosphatase, alanine transaminase and aspartate aminotransferase (Amer et al., 1998). Other studies have also shown that AFB_1 levels of 0.1 to 5mg/kg significantly increases activity of liver functional enzymes including ALT and AST accompanied with a decrease total proteins and albumin contents in blood serum (McKenzie et al., 1998). The increased concentration of liver function enzymes in blood serum was associated with damage to hepatocytes. This was also observed by Oguz et al., (2000) who reported that AFB_1 contamination increases serum levels of ALP, ALT and AST. The increase in the concentration of liver function enzymes in the serum has been associated with AFB_1 on cellular damage in the liver and kidneys.

This study also demonstrated that increasing levels of vitamin A supplementation in chickens fed AFB_1 contaminated diets improves the productive performance of broiler chickens. It can therefore be stated that increasing levels of vitamin A supplementation promotes protein synthesis as was demonstrated by increasing total protein content in chickens fed contaminated diets that were supplemented with levels of vitamin A above 6000IU/kg. These results agree with findings of Brasel and Hussein (2001) who reported that lipid metabolism is affected by AFB_1 due to the reduction on enzymes synthesis and activity, which mainly occurs under chronic exposure.

Vitamin A supplementation significantly ameliorated the toxic effects of AFB_1 on the broilers which counteracted the serum biochemical changes and resulted in improved productive performance of broiler chickens. These results are also comparable to those of Thys et al., (2012) in chickens and that of Muzaffer et al., (2003) whose trial on Japanese quails revealed reduction in feed consumption in those exposed to AFB_1 and the efficacy of vitamin A in reducing its toxicity. It can thus be stated that vitamin A is a biological antioxidant that has capacity to significantly prevent aflatoxin induced damage in tissues such as the liver, kidney and gizzards of poultry (Denli et al., 2005).

CONCLUSION

In this study, it was demonstrated that feeding of AFB_1-contaminated diets to broiler chickens negatively affects performance by reducing feed intake, body weight gains and feed conversion ratios. This was mostly done through damage to the liver and kidney that result in elevated serum concentrations of alkaline phosphatase, aspartate aminotransferase and alanine aminotransaminase. There was also a corresponding reduction in the concentration of serum metabolites including triglycerides, cholesterol, total proteins and albumin. It was demonstrated that the toxic effects of AFB_1 could be ameliorated by supplementing diets with levels of vitamin A above 6000IU. The upper limit for vitamin A supplementation to completely minimize toxic effects of AFB_1 in broiler chickens needs further evaluations.

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REFERENCES

[1]. Abidin et al. (2011). Mycotoxins in broilers: pathological alterations induced by aflatoxins and ochratoxins, diagnosis and determination, treatment and control of mycotoxicosis. *World's Poultry Science Journal*, *67,* 485-496.

[2]. Agag, B. I. (2004). Mycotoxins in foods and feeds 1-Aflatoxin: Review Articles., *Ass. Univ. Bull. Environ. Res*., *7*(1), 173-206.

[3]. Amer et al. (1998). Effect of aflatoxicosis on kinetic behavior of ceftiofur in chickens. *Research Veterinary Science*, *65,* 115-118.

[4]. Bailey et al. (2006). Efficacy of montmorillonite clay (NovaSil PLUS) for protecting full-term broilers from aflatoxicosis. *Journal of Applied Poultry Research*, *15*, 198–206.

[5]. Binder, E. M. (2007). Managing the risk of mycotoxins in modern feed production. *Animal Feed Science Technology*, *133*, 149-166.

[6]. Bintvihok et al. (2002). Residues of aflatoxin in liver muscles and eggs of domestic fowls. *Journal of Veterinary Science*, *64,* 1037–1039.

[7]. Denli et al. (2005). Effect of dietary conjugated linoleic acid on carcass quality, serum lipid variables and histopathological changes of broiler chickens infected with aflatoxin B_1. *South African Journal of Animal Science*, 35, 109–116.

[8]. Fernandez et al. (1995). Changes in the prothrombin time, haematology and serum proteins experimental aflatoxicosis in hens and broiler chickens. *Research in Veterinary Science*, *58,* 119-122.

[9]. Food and Agricultural Organisation (FAO). 2004. *Worldwide regulations for mycotoxins in food and feeds in 2003*. FAO Food and Nutrition Paper 81, Rome, Italy.

[10]. GenStat. (2012). *GenStat Procedure Library Release PL22.2*. (14th ed.) VSN International Ltd.

[11]. Hamilton, P. B. (1984). Determining safe levels of mycotoxins. *Journal Food Protection*, *47,* 67-73.

[12]. Hussein, H. S., & Brasel, J. M. 2001. Rewiev: Toxicity, metabolism and impact of mycotoxins on humans and animals. *Toxicology*, *167*, 101-134.

[13]. Kellerman et al. (1988). *Plant poisonings and mycotoxicoses of livestock in Southern Africa*. (2nd ed.). Oxiford University Press.

[14]. Lafi et al. (2010). Histopathology of the liver affected with aflatoxins in broiler chicks. *Al-Anbar Journal Veterinary Science*, *3*, 1.

[15]. Mannon, J., & Johnson, E. (1985). Fungi down on the farm. *New Scientist*, *105*, 12-16.

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www.ajsc. leena-luna.co.jp

[16]. McKenzie et al. (1988). Aflatoxicosis in turkey poults is prevented by treatment of naturally contaminated corn with ozone generated by electrolysis. *Poultry Science*, *77*(8), 1094-102.

[17]. Miazzo et al. (2005). Efficacy of sodium bentonite as a detoxifier of broiler feed contaminated with aflatoxin and fumonisin. *Poultry Science*, *84*, 1-8.

[18]. Micco et al. (1988). Long-term administration of low doses of mycotoxins to poultry. Residues of aflatoxin B_1 and its metabolites in broilers and laying hens. *Food Additive Contamination*, *5,* 303-308.

[19]. Markovic et al. (2005). Mould and mycotoxin contamination of pig and poultry feed. *Proc. Nat. Sci.*, *109*, 89-95.

[20]. Muzaffer et al. (2003). Effects of vitamin a supplementary in the feed to reduce toxic effects of aflatoxin B_1 on japanese quails (*Coturnix contunix japonica*). *International Journal for Poultry Science*, *2*(2), 174-177.

[21]. Oguz et al. (2000). Effect of clinoptilolite on serum biochemical and haematological characters of broiler chickens during aflatoxicosis." *Research Veterinary Science*, *69*, 89-93.

[22]. Oliveira et al. (2000). Aflatoxin B_1 residues in eggs of laying hens fed a diet containing different levels of the mycotoxin. *Food Additive Contamination*, *17*, 459–462.

[23]. Resanovic et al. (2009). Mycotoxins in poultry production. *Proc. Nat. Sci*., *116*: 7-14.

[24]. Rizzi et al. (2003). Aflatoxin B_1 and clinoptilolite in feed for laying hens: effects on egg quality, mycotoxin residues in livers, and hepatic mixed-function oxygenase activities. *Journal of Food Protection*, *66*, 860-865.

[25]. Saif, Y. M. (2003). *Diseases of poultry. (11th ed.).* Blackwell publishing co ISU press. U.S.A., Pp.1109-1113.

[26]. Shi et al. (2006). Efficacy of modified montmorillonitenanocomposite to reduce the toxicity of aflatoxin in broiler chicks. *Animal Feed Science Technology*, *129*, 138-148.

[27]. Tedesco et al. (2004). Efficacy of silymarin-phospholipid complex in reducing the toxicity of aflatoxin B_1 in broiler chicks. *Poultry Science*, *83,* 1839-1843.

[28]. Thys et al. (2012). *Evaluation of a commercial product in broilers fed aflatoxin B_1 contaminated feed*. Nutrex nv, Lille, Belgium and Zootechnical Centre, Catholic University of Leuven, Leuven, Belgium.

[29]. Thaxton et al. (1974). Immunosuppression in chickens by aflatoxin. *Poultry Science*, *53* (2), 721-5.

[30]. World Health Organisation. (2006). *Impacts of aflatoxins on health and nutrition*. Report of an Expert Group Meeting.

[31]. Yunus et al. (2013). Aflatoxin B_1 in affecting broiler's performance, immunity, and gastrointestinal tract: A review of history and contemporary issues toxins. *Basel*. *3*(6), 566–590.