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Effect of dietary intervention on the performance and biochemical indices of broilers challenged with *Aspergillus flavus*

S. A. Bolu, O. A. Olatunde and V. Ojo

Department of Animal Production, University of Ilorin

Abstract

A study was conducted to determine the effects of dietary interventions of vitamins A, C, methionine and lysine singly and their combination on broilers challenged with *Aspergillus flavus*. The interventions were Vitamins A and C (A+C), methionine and lysine (METH+LYS) and their combination (A+C+METH+LYS). The experiment which was conducted for 8 weeks employed a completely randomized design. Feed intake, weight gain, nutrient retention and feed conversion efficiency were significantly influenced (P<0.05) by dietary supplementation of the Aspergillus challenged birds. Highest feed intake (42.81 g/bird/day) was observe for Aspergillus challenged birds supplemented with A+C+METH+LYS which compared favourably with the positive control birds (42.48 g/bird/day). The lowest feed intake was observed for the negative control birds (Aspergillus challenged without dietary intervention). Weight gain was highest for the positive control bird (20.14 g/bird/day). This value was similar to the value obtained for Aspergillus challenged birds supplemented with A+C+METH+LYS. Lowest weight gain was observed in the negative control birds (12.44 g/bird/day). These birds also recorded significantly (P<0.05) lowest feed conversion efficiency (3.09). Haematological and serum indices showed no significant differences, however, higher lymphocytes values were observed in challenged birds with dietary intervention. As a general immune modulator, vitamins A and C with lysine and methionine may be an attractive alternative to the on-farm use of vaccines in poultry in the management of aspergillosis.

Keywords: Vitamins A, C, Methionine, Lysine, Aspergillus Challenged Birds

Introduction

Aspergillus flavus is a major food-borne pathogen that produces aflatoxin, a toxic and carcinogenic compound (Anath and Farid, 2000). It is a leading cause of aflatoxicosis in poultry which results from ingestion of aflatoxin in contaminated feed. Its effect is toxic and immunosuppressive (Msucares.com). In broilers, a dose of 1.5 ppm has been shown to impair bile salt availability which causes a decrease in the absorption of fat soluble vitamins. Providing a diet containing high fat and high protein level and augmenting the ration with vitamin supplements may be of value in mitigating the effects of Aspergillus infection (Msucares.com). In pigs, treatments with vitamins and protein supplementation have shown to have some proactive effects (Reed and Kasali, 1987).

Vitamins are essential component of a well balanced diet whose supplementation is aimed at optimizing the immune response in chicken (Mohammad, 2009). Vitamin A is essential for the integrity of epithelial tissues which represent a major defense against the entry of pathogens (Mohammad, 2009).Vitamin C (ascorbic acid) has been reported as a non essential nutrient for poultry since birds are capable of synthesizing enough of the vitamin endogenously. However, stress condition may affect the effectiveness of synthesis giving rise to the need for supplementation (Bolu and Olatunde, 2005). Studies in poultry have shown that exogenous ascorbic acid given in feeds or drinking water or by injection improved performance of chicken during heat stress (Pardue and Thaxton, 1986; Pardue et al., 1985; Tuluen and Njoku, 2000; Bolu and Olatunde, 2005).

The levels and balance of amino acids in the diets are all important nutritional variables that affect the performance of chickens (Al-Saffar and Rose, 2002). In an *in vitro* test, liquid methionine hydroxyl analogue has been observed to have an inhibiting effect on *Aspergillus flavus* (Mercier et al., 2008). Mills et al. (1989) also reported an approximate inclusion of 1.33 and 0.52% lysine and methionine respectively in diets of broilers subjected to aflatoxin contaminated feed for maximum performance.

There is dearth of information on the use of vitamins, lysine and methionine in combination on the

expression of aflatoxicosis in broilers especially in the humid tropics which favours the growth of the fungus. This study was therefore aimed at investigating the effect of supplemental vitamins A and C, lysine and methionine and their combination on broilers challenged with *Aspergillus flavus*.

Materials and Methods

One hundred and twenty day old commercial broilers were used in this study. The chicks were weighed and randomly allotted to 5 treatment groups with 3 replicates of 24 chicks each. Birds were housed in an electrically heated metabolic battery cage.

Broilers fed the various treatments were challenged with *Aspergillus flavus* via the drinking water two weeks into the experiment. Yellowish caseous deposits in the lung were established as confirmatory lesion of aspergillosis in the challenged birds (MVM, 1986). The experiment also had positive (non-challenged birds) and negative (birds challenged without dietary supplementations) control groups. Dietary interventions of Aspergillus challenged birds include: vitamins A and C (A+C), methionine and lysine (METH+LYS) and vitamins A, C, lysine and methionine (A+C+METH+YS) which were incorporated into the basal diet formulated to meet the nutrient requirement (NRC, 1994) for broilers (Table 1). Routine management and vaccinations were followed. Feed and water were given *ad libitum* for the 56 days feeding trial.

Feed intake and weight gain were recorded weekly and used to determine the feed to gain ratio. Nutrient retention was determined at 4 weeks of age. Proximate analysis of the diet and faecal samples were determined according to the method of AOAC (1990). At the end of the experiment, 9 birds were selected/treatment fasted overnight and slaughtered by severing the jugular vein. Blood samples were collected and used for haematological and serological indices according to Maxwell et al. (1990) using Wintrobes microhaemotorits improved neubauer. Data obtained from the experimental trial were analyzed using the completely randomized design (Steel and Torrie, 1980). Significant differences were subjected to Duncan Multiple Range Test Duncan (1955).

Table 1: Composition of the experimental diet (kg/ton)

Ingredients	1*	2**	3	4	5
Maize	564.7	564.7	564.7	564.7	564.7
Groundnut	150	150	150	150	150
Soybean meal	216.8	216.8	216.8	216.8	216.8
Fish meal	25	25	25	25	25
Bone meal	25	25	25	25	25
Oyster shell	11	11	11	11	11
Methionine	1.0	1.0	1.0	2.0	2.0
Lysine	1.0	1.0	1.0	2.0	2.0
Salt	3.0	3.0	3.0	3.0	3.0
Vit. Premix	2.5	2.5	2.5	2.5	2.5
Vit A+C	-	-	+	-	-
METH+LYS	-	-	-	+	-
A+C+METH+LYS	-	-	-	-	+
Total	1000	1000	1000	1000	1000

Analyzed nutrient in percent content: crude protein 23.75; crude fat 64; crude fibre 5.8; total ash 6.6.
Premix supplied/ kg diets, vitamin A, 8x10 ^{6} iu, vitamin D3, I, 200iu vitamin E – 3iu; vitamin k3 – kastab (2mg), riboflavin (3mg), nicotinic acid (10mg), panthothenic acid (150 mg) manganese – 80mg, zinc 50mg, copper 2 mg, iodine 1.2mg, cobalt – 0.2mg, selenium – 0.1mg; *positive control (without A.flavus and dietary intervention); **negative control (without A.flavus and without dietary intervention)

Results

Daily feed intake and feed conversion efficiency showed significant differences ($P<0.05$) across the treatment means (Table 2). Highest feed intake (42.81g/bird/day) was observed for Aspergillus-challenged birds supplemented with A+C+METH+LYS which compared favourably with the positive control birds (42.48 g/bird/day). The lowest feed intake (38.67 g/bird/day) was observed for the negative control birds (Aspergillus-challenged without dietary intervention). Daily weight gain varied in response to dietary interventions of the challenged birds. Challenged birds with fed dietary supplementation of A+C+METH+LYS had a weight gain of 18.37 g/bird/day which was similar to the value observed for the birds fed the positive control (20.14 g/bird/day). Birds fed the negative control treatment had the least weight gain (12.48 g/bird/day). Feed conversion efficiency (Table 2) was highest (3.09) in the negative control birds and

was significantly different (P<0.05) from all the other treatments. The positive control birds had the least value (2.11). However this was not significantly different (P<0.05) from the values obtained for the challenged birds with dietary intervention of A+C, LYS+ METH, and the combination of A+C+LYS+METH.

The result of nutrient retention showed significant differences among treatment means. The positive control diet had the highest protein and fat retention (63.8 and 73.5%) respectively. This was however not significantly different (P>0.05) from the values observed for A+C+LYS+METH supplemented birds (61.1 and 72.0%) for protein and fat retention respectively. Birds on the negative control however had the least protein and fat retention (51.4 and 63.9%) respectively. The results of haematological values and serum biochemistry of the birds fed the different treatments (Tables 3 and 4) were not significantly influenced by the dietary treatments.

Discussion

Aflatoxin has been known to be toxic and reported to cause immunosuppression in birds (Pardue et al., 1985). These immunosuppressive effects of aflatoxins predispose the animals to many secondary infections due to other fungi, bacteria and viruses (Robens et al., 1992, Mclean, 1995). Aflatoxicosis results from the ingestion of aflatoxins that have been reported to cause impaired growth in poultry. It is also known to impair the availability of bile salts which decreases vitamins D3 production and also decreases the production of vitamins A in the liver (Anath and Farid, 2000). Feed refusal has also been reported to be a rapid and direct response to the presence of aflatoxin (Jewers et al., 1986). Mills et al. (1989) reported that contamination of broiler ration with aflatoxin resulted in a drastic reduction in performance both from growth and feed efficiency standpoint. These observations corroborate feed refusal and reduced weight gain observed in the negative control birds. High protein diet has been reported to prevent the inhibitory growth caused by aflatoxin, though at an increased cost of production (Jewers et al., 1986). Mohammad (2009) described vitamins supplementation as an essential component of a well balanced diet aimed at optimizing immune response in chicken. Dietary interventions were observed to have positively reduced the deleterious effects of aspergillosis on the performance of broilers

Table 2: Effect of dietary intervention on the performance of Aspergillus-challenged broiler chicks

Diets	Feed intake (g/bird/day)	Weight gain (g/bird/day)	Feed conversion efficiency	Protein	Fat retention (%)
Control	2.48^{a}	20.14^{a}	2.11^{b}	63.30^{a}	73.5^{a}
Control*	38.67^{b}	12.48^{c}	3.09^{a}	51.4^{b}	63.9^{c}
Vit. A+C	40.97^{b}	15.88^{b}	2.58^{b}	60.6^{a}	68.9^{b}
LYS +METH	39.08^{b}	15.14^{b}	2.58^{b}	53.9^{b}	66.6^{b}
Vit. A+C+ LYS + METH	18.37^{a}	2.33^{b}	61.1^{a}	72.0^{a}	42.81^{a}

$^{a-c}$values in a column with the same superscript are similar (P>0.05); *challenged broilers without dietary intervention

Table 3: Effect of dietary intervention on haematology of Aspergillus-challenged broiler chicks

Diets	Haemoglobin (g/dl)	Packed Cell Volume (%)	RBC (x 10^{12}k)	WBC (x 10^{9}k)	Neutro-phils (%)	Lympho-cytes (%)
Control	9.7	29	6.7	8.8	11	89
Control*	10.2	32	7.0	9.7	24	76
Vitamins A +C	9.7	16	5.6	13.4	8	92
LYS + METH	9.1	19	6.3	10.4	14	86
Vit. A + C + LYS + METH	19	6.7	10.4	16	84	9.9

*challenged broilers without dietary intervention

Table 4: Effect of dietary intervention on specific biochemical indices of aspergillus challenged broilers chicks

Diets	Serum protein (g/dl)	Aspartate amino transferase (U/l)	Alanine amino transferase (U/l)
Control	31	50	20
Control*	45	49	26
Vitamins A + C	33	31	40
LYS + METH	57	31	16
Vit. A + C + LYS + METH	43	42	18

*challenged broilers without dietary intervention

This effect was particularly observed in the aspergillus-challenged broilers fed A+C+METH+LYS. This effect also explained the improved performance observed with birds fed dietary interventions and the negative control bird. It is also noteworthy that the performance of birds fed supplemental combination of vitamins A+C+METH+LYS compared favourably with the positive control. This diet may have stimulated the immune response of the broilers and thus enhance their performance. This observation supports the reports of Reed and Kasali (1987) that vitamins and protein supplementation in pigs has some proactive effect on the incidence of aflatoxicosis. In the same vein, Msucares (2009) reported that providing a diet containing high protein level and augmenting the ration with vitamin supplementation may be of value in mitigating the effects of Aspergillus infection. El-Barkouky et al. (2010) also reported the inability of vitamin supplements alone in the diets of poultry may not totally prevent the negative effect of mycotoxin in broiler chicks. The result of haematological and biochemical values of the birds did not show significant differences ($P<0.05$) among treatment means. Though these values fall within the normal range reported for poultry, higher values of lymphocytes observed supports the findings of Jewers et al. (1989), that aflatoxin appears to have a dose related effect on the lymphatic tissues of poultry and that impaired function of lymphocytes and phagocytes appears to be the major aflatoxin–induced deficiency in immunogenesis. Haematological indices have been reported as diagnostic tools for various illnesses in domestic animals (Kecceci et al., 1998). The result on serum biochemical parameters showed that the effect of aspergillosis on these parameters may be transient and may not have changed drastically to suggest an immunorepressive effect.

Conclusion

The result of this study suggest that dietary vitamins A and C with increase in inclusion level of lysine and methionine can enhance the feed intake weight gain and feed conversion efficiency of the broiler chickens infected with *Aspergillus flavus*. Thus combination of vitamins and proteins supplementation may be an attractive alternative to the on-farm use of vaccine. It may also serve as a contribution to the effective prevention of aspergillosis in poultry since curative drugs have not been found effective in the control of the disease.

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