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Efficacy of Ascorbic Acid and Butylated Hydroxylanisole in Amelioration of Aflatoxicosis in Broiler Chickens

Research Article

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ABSTRACT

The effect of ascorbic acid (AA) and butylated hydroxyanisole (BHA) supplementation on aflatoxicosis in broiler chickens (1 to 42 days of age) was investigated (250, 500, 1000 and 2000 ppm for AA and BHA) in diet containing 1 ppm total aflatoxin (AF: 76.45% AFB1, 10.52% AFB2, 9.89% AFG1 and 3.14% AFG2). A total of 300 day-old broiler chicks were divided into ten treatment groups (T1=control; T2= T1+1 ppm AF; T3= T1+250 ppm AA; T4= T1+500 ppm AA; T5= T2+250 ppm AA; T6= T2+5000 ppm BHA; T7= T1+1000 ppm BHA; T8= T1+2000 ppm BHA; T9= T2+1000 ppm BHA; T10= T2+2000 ppm BHA). The results showed that inclusion of 1 ppm of total aflatoxin in the diet resulted in a significant decrease in body weight gain (BWG). Supplementation of AA or BHA to the AF contaminated diets increased BWG of broilers ($P<0.05$). Additing AA at both levels to the aflatoxin contaminated diet did not ameliorate the adverse effects of aflatoxicosis on FC. Incorporation of AA and BHA each at both levels, in aflatoxin contaminated diet could not ameliorate the ill effects of aflatoxicosis on feed efficiency in broiler chickens. The serum protein content of group T5 did not differ significantly ($P<0.05$) from that of T2, however, serum protein content in T6 was significantly ($P<0.05$) higher than that of T2. Indicating that inclusion of 250 ppm AA in the aflatoxin contaminated diet did not improve serum cholesterol content significantly. Inclusion of 500 ppm AA in the aflatoxin contaminated diet improved ($P<0.05$) the serum cholesterol content, however, the value was lower ($P<0.05$) than that of control. Serum uric acid content in T6 was higher ($P<0.05$) than that of T2, however. The aspartate animotransferase (ASAT) values in groups T5, T6 and T9 did not differ significantly ($P<0.05$) from that of T2. The alanine aminotransferase (ALAT) values in groups T5, T6, T9 and T10 did not differ significantly ($P>0.05$) from to that of control, indicating that both AA and BHA each at both levels significantly ($P<0.05$) ameliorated the adverse effects of aflatoxicosis on ALAT activities. It is thus concluded that dietary supplementation of AA at 250 and 500 ppm; and BHA at 1000 and 2000 ppm levels provided partial protection from adverse effects of aflatoxicosis caused by 1 ppm total AF in terms of BWG and blood biochemical parameters. However, inclusion of AA and BHA, each at both levels, in aflatoxin contaminated diet could not ameliorate the adverse effects of aflatoxicosis on feed efficiency. The present study further showed that BHA was more efficacious than AA in ameliorating the adverse effects of aflatoxicosis on BWG in broiler chickens.

KEY WORDS aflatoxicosis, ascorbic acid broiler, butylated hydroxyanisole, feed.

INTRODUCTION

Many mycotoxins can cause serious health problems in poultry and their presence in feedstuffs may result in a serious economic losses. Aflatoxins belong to a group of mycotoxins produced as secondary metabolites by fungi of the *Aspe-*

rgillus genus, especially *A. flavus*, *A. parasiticus* and *A. nomius* (Kurtzman *et al*. 1987). Crops contaminated with aflatoxins are a worldwide problem and approximately 25% of world's food supply is contaminated with mycotoxins annually. Contamination by aflatoxins can take place at any point along the food chain from the field, harvest, handling, transportation and storage (Giray *et al*. 2007). These compounds can enter the food chain mainly by ingestion through the dietary channel of humans and animals (Aycicek *et al*. 2005). Poultry are extremely sensitive to the toxic effects of AFB_1 (Arafa *et al*. 1981; Giambrone *et al*. 1985; Huff *et al*. 1986; Kubena *et al*. 1995; Klein *et al*. 2000). Aflatoxicosis in poultry causes listlessness, anorexia with lowered performance and increased mortality (Miazzo *et al*. 2000), anemia (Oguz *et al*. 2000), reduction of immune function (Oguz *et al*. 2003), hepatotoxicosis and haemorrhage (Ortatatli and Oguz, 2001). Thus, aflatoxins are deleterious to poultry and their contamination in feed is practically unavoidable (Coulombe *et al*. 2005). When contamination cannot be prevented, detoxification of aflatoxins is required while using contaminated feed. Several types of aflatoxin adsorbing agents effectively bind the aflatoxin in the gut of the animal and eliminate it via faeces without absorption from the gut into the systemic blood circulation (Doll and Danicke, 2004). It was first demonstrated that a hydrated sodium calcium aluminosilicate (HSCAS) was effective in ameliorating the toxic effects of aflatoxin *in vivo* (Phillips *et al*. 1988).

Later, several aflatoxicosis amelioration studies using clay-based adsorbents were performed in poultry (Huwig *et al*. 2001; Peraica *et al*. 2002). However, there are several possible disadvantages of these clay-based adsorbents such as their high inclusion rate which reduces nutrient density in the feed and their negative interactions with feed nutrients (Parlat *et al*. 1999; Miazzo *et al*. 2000; Rosa *et al*. 2001). The present study was conducted with the objective of amelioration of adverse effects of aflatoxin by the use of antioxidants (ascorbic acid and butylated hydroxylanisole) in broiler chickens.

MATERIALS AND METHODS

Aflatoxin production

Lyophilized preparation of *Aspergillus parasiticus* NRRL 2999 was obtained from U.S. department of agriculture, Peoria, Illinois (USA). The lyophilized preparation was revived on potato dextrose agar (PDA) medium and used for AF production.

AF was produced from *Aspergillus parasiticus* NRRL 2999 by fermentation of cracked maize as per the method described by Shotwell *et al*. (1966). The fermented maize was then steamed to kill the fungus spores, dried and then ground to a fine powder. The aflatoxin concentration from maize powder was extracted as per the method and measured using thin layer chromatography (TLC).

The total AF concentration in maize powder consisted of 76.45% AFB1, 10.52% AFB2, 9.89% AFG1 and 3.14% AFG2. The maize powder containing known concentration of AF was incorporated into the basal diets of certain dietary treatments to get the desired amount of 1ppm total AF.

Birds and diets

Three hundred day-old broiler (IR-3) chicks (obtained from CARI hatchery) were used in this study. These chicks were wing tagged, weighed individually and divided into ten (T1 to T10) treatment groups, each replicated three times and each replication consisted of 10 birds (each treatment group containing 30 chicks).

The chicks were housed in electrically heated compartments with continuous lighting and were given starter diet up to 21 days and finisher feed from 22 to 42 days of age. The composition of the basal diets is given in Table 1. There were ten experimental diets given to ten different treatment groups as below:

T1: Basal diet + 0 ppb aflatoxin (control)
T2: Basal diet + 1 ppm aflatoxin
T3: Basal diet + 250 ppm ascorbic acid
T4: Basal diet + 500 ppm ascorbic acid
T5: Basal diet + 250 ppm ascorbic acid + 1 ppm aflatoxin
T6: Basal diet + 500 ppm ascorbic acid + 1 ppm aflatoxin
T7: Basal diet + 1000 ppm BHA
T8: Basal diet + 2000 ppm BHA
T9: Basal diet + 1000 ppm BHA + 1 ppm aflatoxin
T10: Basal diet + 2000 ppm BHA + 1 ppm aflatoxin

Birds were inspected daily and their body weight and feedconsumption was recorded weekly. Feed intake, body weight gain and feed conversion ratio (feed intake/weight gain) were calculated.

At the end of feeding trial, at the age of 42 days, three from each replicate were selected randomly for taking organ weights and blood sampling. Serum was then separated and used for biochemical assays. Serum concentrations of total protein, total cholesterol, uric acid and the activities of aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) were determined by spectrophotometer using commercial kits (M/s. span diagnostics Ltd., Surat, India).

The data obtained were analysed statistically and differences in means were tested using Duncan's multiple range test.

Table 1 Ingredient and chemical composition of the basal diet

Feed ingredients	Starter (%)	Finisher (%)
Maize	58	60
Soybean meal	31	28.475
Sunflower cake	1.015	2
Rapeseed meal	4	4
Fish meal	3	3
Limestone	0.8	0/6
Di-calcium phosphate	1.4	1.2
Common salt	0.2	0.2
DL-methionine	0.08	0.04
L-lysine HCl	0.07	0.02
Trace mineral premix	0.11	0.1
Vitamin premix	0.15	0.15
B complex	0.015	0.015
Choline chloride	0.05	0.05
Sodium Bi-carbonate	0	0.05
Vitamin C	0.01	0
Coccidiostat	0.05	0.05
Total	100	100
Calculated composition		
Crude protein %	21.27	19.39
Metabolisable energy kcal/kg	2844.67	2866.79
Ca %	1.02	0.89
Available P %	0.44	0.41
Lysine	1.20	1.11
Methionine	0.50	0.46

The statistical model for the experiment was completely randomize design (CRD). All the statistical observations were recorded at 5% level of significance.

RESULTS AND DISCUSSION

The effect of dietary treatments on weekly and overall body weight gain (BWG) is presented in Table 2. During first and second week of age, there was no significant difference in BWG among different treatments. From 14 to 21 day of age, BWG of chicks fed control diet was higher than those fed 1 ppm aflatoxin (128.1 *vs.* 94.7 g; $P<0.05$). During fourth week of age, the BWG in control group was 218.96 g which significantly decreased to 110.68 g in aflatoxin alone fed group.

The BWG in T3, T4, T7 and T8 did not differ significantly from that of control (T1), indicating that AA and / or BHA supplementation alone neither had positive nor negative effects on BWG of broiler chickens.

The BWG in T5 did not differ significantly from that of T2, whereas, the BWG in T6 was significantly higher than that of T2.

This indicated that AA supplementation at 250 ppm could not improve BWG significantly, whereas, 500 ppm AA significantly improved the BWG, however, the gain was significantly lower than that of control. On the other hand, BHA inclusion to the aflatoxin contaminated diet at both levels (1000 and 2000 ppm), significantly improved the BWG, however the gain in T9 and T10 was significantly lower than that of control.

The BWG in T9 and T10 was significantly higher than that of T5 and T6, suggesting that BHA inclusion at both levels in the aflatoxin contaminated diet was more efficacious in ameliorating aflatoxicosis than that of AA inclusion at both levels.

During fifth week of age, the BWG in control was 246.17 g which significantly reduced to 150.57 g in T2. The BWG in T3, T4, T7 and T8 did not differ significantly from that of control.

The BWG in T5, T6, T9 and T10 was significantly higher than that of aflatoxin alone fed group, indicating that inclusion of AA and BHA at both levels each in the contaminated diet significantly improved the BWG, whereas, the gains were significantly lower than that of control.

Also, the BWG in T9 and T10 was significantly higher compared to T5 and T6, thus, BHA inclusion was more efficacious than AA in ameliorating the adverse effects of aflatoxicosis in broiler chickens.

During sixth week of age, the BWG in groups T3, T4, T7, T8 and T10 did not differ significantly from that of control, whereas, significantly lower BWG was recorded in T2 as compared to control.

The BWG in T5 and T6 did not differ significantly from that of aflatoxin alone fed group, showing that addition of AA (250 and 500 mg/kg) could not reduce the adverse effects of aflatoxicosis, whereas the values T5 and T6 were numerically higher than that of T2.

The BWG in T9 and T10 was significantly higher than that of T2, indicating that addition of BHA to the aflatoxin contaminated diets significantly increased the BWG. The BWG in T10 was statistically similar to that of control, indicating that BHA inclusion at 2000 mg/kg, in the aflatoxin contaminated diet significantly reduced the negative weight changes due to aflatoxin in broilers.

In case of overall BWG (1 to 6 weeks), BWG in control group was 1020.68 g which significantly reduced to 707.96 g due to aflatoxin feeding.

The BWG in groups T3, T4, T7 and T8 did not differ significantly from that of control, which suggested that neither AA nor BHA inclusion alone at any level had positive or negative effects on overall BWG of broiler chickens. The BWG in T5, T6, T9 and T10 was significantly higher than that of T2 and significantly lower an of T1.

Thus, addition of AA and BHA to the aflatoxin contaminated diets each at both levels significantly but partially ameliorated the adverse effects of aflatoxicosis on BWG in broiler chickens.

The BWG in T9 and T10 was significantly higher than those of T5 and T6. This indicated that addition of BHA was more efficacious than addition of AA in the aflatoxin contaminated diet.

No significant difference in BWG was recorded between T5 and T6; and between T9 and T10, indicating that both levels of AA and BHA were equally efficacious in ameliorating the adverse effects of aflatoxicosis on BWG in broiler chickens.

The results showed that inclusion of 1 ppm of total aflatoxin in the diet resulted in a significant decrease in the BWG. These results are in agreement with earlier workers who reported significant reduction in BWG at 0.3 ppm level of dietary aflatoxin (Raju and Devegowda, 2000; Sapocota *et al*. 2007).

Azzam and Gopal (1997) reported significant reduction in BWG at 0.2 ppm of aflatoxin in diet. Several other researchers (Johri *et al.* 1989; Miazzo *et al*. 2000; Ledoux *et al*. 1999; Kubena *et al*. 1998; Santurio *et al*. 1999; Verma, 1994; Rosa *et al*. 2001) also reported that dietary aflatoxin at 0.5 ppm levels or beyond in broiler diet adversely affected growth in a dose related fashion.

Weekly and overall feed consumption influenced by different dietary treatments (Table 2). During first and second week of age, no significant difference was recorded in FC among various treatments.

However, thereafter, significant variations in the FC were recorded. During third week of age, the FC in control group (T1) was 289.23 g which significantly reduced to 248.79 g due to aflatoxin feeding (T2).

The FC in T3, T4, T7, T8, T9 and T10 did not differ significantly from that of control. Feed conversion T5 and T6 did not differ significantly from that of T2, indicating that addition of both levels of AA in the aflatoxin contaminated diet could not improve the FC in broilers. During fourth week of age, the FC in groups T3, T4, T7, T8 and T10 did not differ significantly from that of control, however, the FC in groups T2, T5 and T6 was significantly lower than that of control.

Addition of both levels of AA to the aflatoxin contaminated diet did not ameliorate the adverse effects of aflatoxicosis on FC. However, addition of both levels of BHA to the contaminated diet significantly improved the FC, where, the FC in T10 did not differ significantly from that of control.

During fifth week of age, the FC in T1 was 537.95 g which significantly reduced to 388.95 g in T2. The FC in groups T3, T4, T7, T8, T9 and T10 did not differ significantly from that of control; whereas, the FC in groups T5 and T6 was statistically lower than that of control bird. Thus, addition of AA (250 and 500 mg/kg) to the aflatoxin contaminated diet did not ameliorate the adverse effects of aflatoxicosis.

However, addition of BHA (1000 and 2000 mg/kg) to the contaminated diets ameliorated the adverse effects of aflatoxicosis on FC in broiler chickens. During sixth week of age, the FC in control group was 656.98 g which significantly ($P<0.05$) reduced to 498.82 g in aflatoxin alone fed group.

The FC was in groups T3, T4, T7, and T8 did not differ significantly from that of control, indicating that addition of AA and BHA each at both levels did not alter the FC. The FC in groups T5 and T6 did not differ significantly from that of T2 which shows that addition of each level of AA to the aflatoxin contaminated diet did not ameliorate the toxic effects of aflatoxicosis on FC, on the other hand, addition of each level of BHA significantly ameliorated the adverse effects of aflatoxicosis, however, the FC at 1000 ppm level of BHA was significantly lower than that of control.

In case of overall FC (1-6 weeks), the FC in T1 was 2199.66 g which significantly reduced to 1699.27 g in T2. The FC in groups T3, T4, T7, and T8 did not differ significantly from that of control. Thus, addition of either AA or BHA alone at each level did not affect FC.

Feed conversion in T5 and T6 did not differ significantly from that of T2, indicating that addition of AA at both levels to the aflatoxin contaminated diet did not ameliorate the adverse effects of aflatoxicosis on FC. The FC in T9 and T10 was significantly higher than that of T2, indicating that both levels of BHA supplementation ameliorated the adverse effects of aflatoxicosis on FC in broiler chickens. FC in T9 was significantly lower than that of T1 indicating that 1000 ppm BHA may not be sufficient to ameliorate the aflatoxicosis caused by 1 ppm total aflatoxin, whereas, the FC in T10 did not differ significantly from that of control. The results showed that inclusion of 1 ppm of total aflatoxin in the diet resulted in a significant decrease in the FC. Decrease in FC may be due to anorexia and listlessness caused by aflatoxicosis (Oguz and Kurtoglu, 2000). These results are in conformity with earlier reports where aflatoxin contamination (0.3 ppm or higher) resulted in significant decrease in FC of broiler chickens (Kubena *et al*. 1990; Kubena *et al*. 1998; Ledoux *et al*. 1999; Santurio *et al*. 1999; Raju and Devegowda, 2000). Weekly and overall FCR was influenced by different treatments is given in Table 3.

During first, second and third week of age, no significant difference was recorded in FCR among various dietary treatments. During fourth week of age, the FCR in T1 was 1.716 which significantly increased to 2.653 in T2. The FCR in T3, T4, T7 and T8 did not differ significantly from that of T1. The FCR in groups T5, T6, T9 and T10 did not

Table 2 Effect of AA and BHA on body weight gain and feed consumption (g/bird) of broiler chicks on a diet containing total aflatoxin between 1 to 42 days of age

Treatments / Weeks	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}
	Body weight gain (BWG)									
1	54.04± 5.21	57.52± 1.58	54.50± 4.94	54.73± 4.34	57.73± 2.67	55.35± 3.56	58.01± 4.99	51.21± 2.58	59.80± 5.49	56.51± 4.75
2	94.98± 8.51	88.40± 4.01	94.84± 7.30	92.28± 8.41	79.50± 4.13	84.89± 1.45	94.22± 9.28	92.58± 5.98	91.60± 0.74	92.87± 4.29
3	128.11± 8.72^{bc}	94.77± 3.50^{a}	131.01± 8.95^{c}	134.74± 12.88^{c}	100.17± 5.50^{ab}	100.25± 0.83^{ab}	129.56± 9.35^{c}	136.57± 12.33^{c}	110.56± 4.69^{abc}	123.66± 10.94^{bc}
4	218.96± 6.57^{d}	110.68± 5.26^{a}	220.31± 6.97^{d}	211.88± 5.07^{d}	124.87± 2.15^{ab}	130.37± 4.36^{b}	208.53± 5.10^{d}	222.99± 4.88^{d}	163.11± 7.50^{c}	172.36± 4.40^{c}
5	246.17± 2.89^{d}	150.57± 2.51^{a}	247.86± 2.84^{d}	249.13± 3.13^{d}	174.35± 11.30^{b}	175.69± 5.05^{b}	249.45± 2.02^{d}	246.88± 3.32^{d}	211.08± 10.14^{c}	226.23± 8.01^{c}
6	278.40± 9.87^{d}	205.97± 14.67^{a}	279.07± 9.21^{d}	272.76± 10.41^{cd}	212.47± 5.66^{a}	223.63± 8.20^{ab}	281.76± 2.21^{d}	267.18± 11.99^{cd}	246.40± 9.37^{bc}	247.79± 9.21^{cd}
1-6	1020.68± 14.54^{d}	707.96± 16.90^{a}	1027.63± 12.63^{d}	1015.54± 16.03^{d}	749.03± 5.01^{b}	770.07± 4.79^{b}	1021.55± 18.39^{d}	1017.43± 8.31^{d}	882.57± 7.78^{c}	939.42± 10.48^{c}
Weeks	Feed consumption (FC)									
1	90.66± 6.01	86.37± 1.43	90.62± 5.10	90.41± 3.70	90.99± 4.42	92.95± 2.85	93.12± 4.06	87.91± 4.06	89.91± 9.67	88.37± 5.59
2	194.16± 17.44	187.99± 1.88	192.95± 17.78	196.95± 12.25	184.67± 6.90	185.54± 6.46	195.45± 11.48	194.29± 18.56	196.12± 2.69	206.41± 8.61
3	289.23± 4.65^{cd}	248.79± 16.78^{ab}	289.88± 3.44^{cd}	286.04± 6.36^{cd}	241.89± 17.56^{ab}	235.73± 6.95^{a}	292.90± 4.14^{d}	283.01± 3.80^{cd}	259.17± 6.23^{abc}	273.37± 14.19^{bcd}
4	430.66± 11.65^{c}	292.87± 6.45^{a}	429.84± 12.28c	422.94± 15.42^{c}	313.55± 17.50^{a}	311.33± 12.44^{a}	438.96± 3.45^{c}	414.05± 15.41^{bc}	373.74± 16.73^{b}	420.97± 21.94^{c}
5	537.95± 13.75^{b}	388.95± 35.77^{a}	536.42± 13.78^{b}	529.12± 9.44^{b}	374.04± 24.33^{a}	408.34± 12.77^{a}	519.17± 10.51^{b}	535.69± 7.25^{b}	482.31± 17.93^{b}	537.02± 30.46^{b}
6	656.98± 7.93^{c}	498.82± 31.22^{a}	656.19± 8.59^{c}	648.27± 12.26^{c}	509.61± 16.65^{a}	509.95± 10.34^{a}	654.72± 8.12^{c}	642.80± 7.99^{c}	560.21± 22.58^{b}	618.01± 15.23^{c}
1-6	2199.66± 12.44^{c}	1699.27± 27.37^{a}	2195.92± 11.61^{c}	2173.74± 28.16^{c}	1714.77± 40.10^{a}	1743.85± 21.28^{a}	2194.35± 18.60^{c}	2157.78± 15.68^{c}	1976.48± 26.97^{b}	2144.16± 27.73^{c}

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Table 3 Effect of AA and BHA on feed conversion ratio of broiler chicks on a diet containing total aflatoxin between 1 to 42 days of age

Treatments	Weeks						
	1	2	3	4	5	6	1-6
T_1	1.436±0.22	1.653±0.32^{a}	1.946±0.48	1.716±0.35^{a}	1.770±0.38^{a}	1.870±0.43^{a}	1.900±0.38^{a}
T_2	1.503±0.01	2.133±0.07^{b}	2.620±0.09	2.653±0.09^{f}	2.580±0.19^{b}	2.433±0.14^{b}	2.406±0.07^{b}
T_3	1.673±0.07	2.033±0.06^{b}	2.233±0.16	1.956±0.11^{abc}	2.163±0.04^{ab}	2.353±0.05^{ab}	2.136±0.01^{ab}
T_4	1.660±0.06	2.146±0.07^{b}	2.166±0.22	2.000±0.11^{abcd}	2.126±0.05^{ab}	2.380±0.06^{ab}	2.143±0.02^{ab}
T_5	1.583±0.07	2.326±0.07^{b}	2.420±0.16	2.513±0.13^{ef}	2.146±0.02^{ab}	2.403±0.10^{ab}	2.290±0.05^{b}
T_6	1.690±0.05	2.186±0.05^{b}	2.350±0.05	2.386±0.01^{cdef}	2.330±0.10^{b}	2.283±0.08^{ab}	2.263±0.04^{b}
T_7	1.616±0.07	2.093±0.09^{b}	2.280±0.14	2.106±0.06^{abcde}	2.083±0.02^{ab}	2.323±0.01^{ab}	2.150±0.02^{ab}
T_8	1.716±0.02	2.090±0.07^{b}	2.113±0.22	1.856±0.08^{ab}	2.170±0.05^{ab}	2.413±0.07^{ab}	2.120±0.01^{ab}
T_9	1.506±0.08	2.143±0.02^{b}	2.346±0.05	2.293±0.07^{bcdef}	2.296±0.14^{b}	2.336±0.05^{ab}	2.240±0.04^{b}
T_{10}	1.573±0.07	2.223±0.01^{b}	2.223±0.08	2.436±0.06^{def}	2.373±0.09^{b}	2.316±0.13^{ab}	2.283±0.05^{b}

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

differ significantly from that of T2, indicating that addition of both levels of AA and BHA could not ameliorate the ill effects of aflatoxicosis on FCR. During fifth week of age, the FCR in groups T3, T4, T5, T7 and T8 did not differ significantly from that of T1. However, the FCR in groups T6, T9 and T10 was significantly higher than that of T1. During sixth week of age, the FCR in T1 was 1.870 which significantly increased to 2.433 in T2, whereas, the FCR in rest of the treatments did not differ significantly from that of control. With regard to overall FCR (1-6 weeks), the FCR in control group was 1.9 which significantly increased to 2.406 in aflatoxin alone fed group, indicating that 1 ppm of total aflatoxin inclusion in the diet significantly deteriorated the feed efficiency. The FCR in groups T3, T4, T7 and T8 did not differ significantly from that of control, however, the FCR in T5, T6, T9 and T10 did not differ sig-

nificantly from that of T2. These detrimental effects of aflatoxin on FC BWG and FCR are due to anorexia, listlessness, inhibition of protein synthesis and lipogenesis (Oguz and Kurtoglu, 2000). In the present study, incorporation of AA and BHA each at both levels could not ameliorate the ill effects of aflatoxicosis on feed efficiency in broiler chickens. In the present study, aflatoxin contamination in diet at 1 ppm level showed poor feed efficiency. Raju and Devegowda (2000) also reported poor feed efficiency in broilers fed diets containing 0.3 ppm level of aflatoxin. Several other researchers have also reported a decrease in feed efficiency due to presence of aflatoxin (0.3 to 5 ppm) in diet (Scheideler, 1993; Kubena *et al.* 1998; Rosa *et al.* 2001; Verma, 1994; Reddy *et al.* 1982).

With regard to biochemical parameters (Table 4), the serum total protein in T1 was 4.95, which significantly reduced to 3.03 g/100 ml in T2. The total protein content in groups T3, T4, T7 and T8 did not differ significantly from that of T1. Protein content of group T5 did not differ significantly from of T2, indicating that 250 ppm level of AA in the aflatoxin contaminated diet could not improve the protein content significantly. However, protein content in T6 was significantly higher than that of T2, showing that 500 ppm level of AA in the contaminated diet significantly improved the total protein content, whereas, the value was significantly lower than that of control. The total protein content in T9 and T10 was significantly higher than that of T2. However, the values were significantly lower than that of control. Thus, addition of 500 ppm AA; and 1000 and 2000 ppm BHA in the aflatoxin contaminated diet partially and significantly ameliorated the adverse effects of aflatoxicosis on protein synthesis. Decrease in total serum protein due to aflatoxin contamination in the diet was also reported by several researchers (Harvey, *et al.* 1993; Kubena *et al.* 1998; Okotie-Eboh *et al.* 1997; Ledoux *et al.* 1999; Raju and Devegowda, 2000; Ahamad, 2000). The serum cholesterol content in T1 was 152.18, which significantly decreased to 110.46 mg/100 ml in T2. The cholesterol content in groups T3, T4, T7 and T8 did not differ significantly from that of T1. The cholesterol content in group T5 did not differ significantly from that of T2, indicating that inclusion of 250 ppm AA in the aflatoxin contaminated diet did not improve cholesterol content significantly. Inclusion of 500 ppm AA in the aflatoxin contaminated diet significantly improved the cholesterol content. However, the value was significantly lower than that of control. Cholesterol content in T2 was significantly lower than those of T9 and T10. Tuse inclusion of 1000 and 2000 ppm BHA in the aflatoxin contaminated diet, significantly increased in the cholesterol content; however, the values were significantly lower than that of control.

These results are in agreement with the other reports where decrease in cholesterol level was observed due to aflatoxin contamination in the diet (Okotie-Eboh *et al.* 1997; Bailey *et al.* 1998; Kececi *et al.* 1998; Raju and Devegowda, 2000; Ahamad, 2000).

With regard to uric acid content, it was 5.09 mg/100 mL in T1 which significantly reduced to 4.54 in T2. The uric acid content in T5 did not differ significantly from that of T2, indicating that 250 ppm level of AA in the aflatoxin contaminated diet did not alter uric acid content when compared to aflatoxin alone fed group. Uric acid content in T6 was significantly higher than that of T2. However, the value was significantly lower than that of control (T1). The uric acid content in T3, T4, T7, T8, T9 and T10 did not differ significantly from that of control. Thus, addition of 1000 and 2000 ppm BHA in the aflatoxin contaminated feed reversed the effects of aflatoxicosis on uric acid. Decrease in uric acid due to aflatoxin contamination in diet was earlier reported by several researchers (Bailey *et al.* 1998; Kececi *et al.* 1998; Ahmad, 2000). The serum ASAT activities value in T1 was 199.19 that significantly increased to 241.41 IU/L in T2. The ASAT values in groups T5, T6 and T9 did not differ significantly from that of T2, indicating that inclusion of 250 and 500 ppm AA; and 1000 ppm BHA to the aflatoxin contaminated diet did not change the ASAT values. The ASAT values in groups T10 was significantly lower than that of T2. Thus, addition of 2000 ppm BHA to the aflatoxin contaminated diet significantly lowered the ASAT activities; however, the activities were significantly to the aflatoxin contaminated diet significantly lowered the ASAT activities; however, the activities were significantly higher than that of control. Significant increase in the activities of ASAT was also reported by Basmacioglu *et al.* (2005) where 2 ppm aflatoxin was fed to broilers, whereas, Kubena *et al.* (1993) reported a decrease in ASAT activities at 3.5 ppm of aflatoxin in the diet of broilers. The serum ALAT activities value in T1 was 7.60 which significantly increased to 9.41 IU/L in T2. These results are agreement with Santurio *et al.* (1999) where increase ALAT activities 3 ppm level of aflatoxin. The ALAT values in groups T5, T6, T9 and T10 did not differ significantly from that of control, indicating that both AA and BHA each at both levels significantly ameliorated the adverse effects of aflatoxicosis on ALAT activities. In our study both AA and BHA partially reduced the harmful effects of aflatoxicosis in broiler chickens.

Aflatoxin could cause cell damage due to release of free radicals and lipid peroxidation involving cell membrane and fatty acids (Surai, 2001). Oxidative stress could either be a cause or consequence of cell damage (Halliwell and Gutteridge, 1999).

Table 4 Effect of AA and BHA on serum biochemical parameters of broiler chicks on a diet containing total aflatoxin between 1 to 42 days of age

Treatment	Total protein (g/100 mL)	Cholesterol (mg/100 mL)	Uric acid (mg/100 mL)	ASAT (IU/L)	ALAT (IU/L)
T1	4.95±0.04^{d}	152.18±2.52^{d}	5.09±0.06^{d}	199.19±1.52^{a}	7.60±0.09^{a}
T2	3.03±0.20^{a}	110.46±2.48^{a}	4.54±0.03^{a}	241.41±3.92^{c}	9.41±0.70b
T3	4.91±0.03^{d}	154.09±1.95^{d}	4.96±0.09^{cd}	189.16±5.75^{a}	7.73±0.19^{a}
T4	4.92±0.07^{d}	148.96±1.20^{d}	4.91±0.06^{bcd}	190.45±1.57^{a}	8.03±0.09^{a}
T5	3.29±0.18^{ab}	116.77±3.17^{ab}	4.69±0.12^{ab}	238.09±8.50^{bc}	8.82±0.61^{ab}
T6	3.63±0.17^{b}	121.25±0.87^{b}	4.83±0.06^{bc}	230.30±7.49^{bc}	8.63±0.64^{ab}
T7	4.96±0.04^{d}	150.42±3.12^{d}	4.97±0.03^{cd}	187.46±2.64^{a}	8.15±0.09^{ab}
T8	4.91±0.06^{d}	152.56±2.00^{d}	4.91±0.03^{bcd}	189.00±4.22^{a}	7.83±0.19^{a}
T9	4.10±0.12^{c}	125.26±3.56^{bc}	4.94±0.13^{bcd}	223.06±9.22^{bc}	8.20±0.39^{ab}
T10	4.15±0.12^{c}	130.51±4.99^{c}	4.93±0.04^{bcd}	218.70±10.14^{b}	7.85±0.24^{a}

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

Antioxidant (AA and BHA) ameliorate oxidative stress during aflatoxicosis by reducing the level of free radicals resulting in reduction in aflatoxin effects on birds. Shehata *et al.* (2009) also reported that adding 500 mg vitamin C/kg diet contaminated with 3 mg aflatoxin B1 may provide a safe and practical method for alleviate of aflatoxin B1 toxicity in fish diet and improve the economical efficiency. These results of vitamin C agree with those obtained by Salem *et al*. (2001) on rabbits.

These results of vitamin C may be due to increasing feed intake, digestibility of nutrients which had biological role in digestive enzyme biosynthesis and activation (Earp *et al.* 1970). Also, vitamin C improved the immunity of fish by enhancing the phagocytes ratio and serum lysozyme activity (Sahoo and Mukherjee, 2003). However Pardue (1987) did not find any significant effect of AA on aflatoxicosis in broilers.

Bilgrami *et al.* (1989) observed that feeding of aflatoxin without AA markedly reduced the growth rate in guinea pigs.

Vitamin C plays important roles in animal health as antioxidants by inactivating damaging free radicals produced through normal cellular activity and from various stresses (Chew, 1995).

Supplementation of vitamin C enhanced antibody production against *Edwarsiella ictaluri* in channel cat fish (Li and lovell, 1985). Aflatoxin B1 is detoxified primarily by glutathione S-transferases (GSTs). GSTs are inducible by many compounds including some antioxidants (AA and BHA) (Pickett and Liu, 1989). Further studies on ameliorating effect of antioxidants on aflatoxicosis in poultry are required.

CONCLUSION

DSietary supplementation of AA at 250 and 500 ppm; and BHA at 1000 and 2000 ppm levels provided partial protection from ill effects of aflatoxicosis caused by 1 ppm total AF in terms of BWG and certain blood biochemical paramters. However, inclusion of AA and BHA, each at both levels, in aflatoxin contaminated diet could not ameliorate the ill effects of aflatoxicosis on feed efficiency. The present study further showed that BHA was more efficacious than AA in ameliorating adverse effects of aflatoxicosis on BWG in broiler chickens.

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