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RESEARCH ARTICLE

Amelioration of Aflatoxicosis through a Bio-Technologically Derived Aflatoxin Degrading Commercial Product in Broilers

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Aflatoxins (AF) are secondary fungal metabolites which severely depress performance in poultry. A trial was undertaken on broiler chicken to study the effects of AF (500 µg/kg) and to assess a biotechnologically derived product (BTP) comprised of culture extracts of toxin degrading microbes, antifungal agents, MOS and bio-antioxidants (Destrox^{R}) over hydrated sodium calcium aluminosilicate (HSCAS) to combat aflatoxicosis. The experimental design consisted of T_1-basal diet; T_2-AF (500 µg/kg); T_3-AF (500 µg/kg) + HSCAS (1 kg/ton); T_4 and T_5-AF (500 µg/kg) with BTP 200 and 400 g/ton, respectively. The 42 day study revealed that, addition of neither HSCAS nor BTP did significantly improve body weight gain and feed intake over T_2. However, significantly ($P<0.05$) improved feed conversion ratio was observed in T_4 (2.017) against T_2 (2.150). All serum biochemical parameters were significantly altered in T_2 in comparison to T_1. A significant improvement was observed in total protein, albumin and ALT in T_3 and T_4 groups as compared to T_2. An improvement in dressing percentage (69.63% v/s 66.76%) and relative weight of liver, heart and spleen were observed in T_4 in comparison to T_2, while in T_3 the dressing percentage and relative weight of liver and spleen were increased. Higher mortality was observed in T_2 and it was significantly ($P<0.05$) reduced in T_3 and T_4. In conclusion, AF (500 µg/kg) found to significantly depress bird performance and addition of BTP @ 200 g/ton was found to moderately alleviate toxicity, while HSCAS and higher BTP level did not show any ameliorative effects.

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INTRODUCTION

Aflatoxins (AF) are the most toxic and commonly encountered mycotoxins produced by *Aspergillus flavus* and *A. parasiticus*. Aflatoxins are known to cause severe growth depression, immunosuppression and mortality at higher levels in poultry (El Miniawy *et al*., 2014; Bilal *et al*., 2014). Aflatoxins are also concerned from consumer health point of view since they are responsible for toxin residues in poultry meat (Khan *et al.*, 2013; Ishfaq *et al*., 2014; Nazir *et al*., 2014), which may pose a carcinogenic threat to humans. Hence, prevention, decontamination and detoxification of AF are worldwide issues of great importance. Among various detoxification methods, addition of inert sorbents like hydrated sodium calcium aluminosilicate (HSCAS), zeolites, bentonites and activated carbons is one of the approaches to reduce aflatoxicosis in animals. These compounds act to reduce the bioavailability of mycotoxins and prevent absorption from gut. However, some of them decrease the bioavailability of amino acids and/or minerals (Dawson, 1999). Nowadays research activities are increasingly focused upon the use of biological products (esterified-glucomannan, MOS) to alleviate aflatoxicosis and to overcome the inherent drawbacks of inorganic sorbents (Raju and Devegowda, 2002).

Among the biological methods, microbial degradation of AF is a new approach and appears more appropriate choice, since it is more specific, efficient and eco-friendly. Among microbes, *Flavobacterium*

aurantiacum NRRL B-184 (Smiley and Draughon, 2000), *Mycobacterium fluoranthenivorans sp.* Nov. DSM44556T (Teniola *et al*., 2005), *Stenotrophomonas maltophila* (Guan *et al*., 2008) and *Mycobacterium segmentis* (Laplikar *et al*., 2012) are found to bio-degrade AF at *in vitro* conditions. A biotechnologically derived product (BTP) commercially available as Destrox^R (M/s Animal Biotech Pvt. Ltd., Bangalore, India) and containing culture extracts of toxin degrading microbes, antifungal agents, mannanoligo-saccharides (MOS), and bio-antioxidants, was tested *in vitro* and found to have a considerable toxin degrading/ binding ability (50.00 to 84.38%) and further *in vivo* broiler study with 250 µg/kg AF revealed numerical improvement in broiler performance with 200 g/ton of BTP (Kumar *et al*., 2015). In continuation, this study was undertaken to study the effects of moderately high levels (500 µg/kg) AF and to comparatively evaluate BTP and HSCAS to overcome the toxicity in broiler chicken.

MATERIALS AND METHODS

Production and quantification of toxin: Aflatoxin was produced using *Aspergillus parasiticus var globosus* MTCC-411 as per the method of Shotwell *et al.* (1966). The fermented cultures were harvested (6th day), autoclaved, oven dried, pulverized and AF was extracted (Romer, 1975) and quantified by TLC (Anonymous, 1995). The culture material had a total aflatoxin content of 277.31 mg/kg (B_1 63.12; B_2 4.73; G_1 31.56 and G_2 0.59 per cent), which was used to derive experimental diets to contain total AF of 500 µg/kg of feed.

Mycotoxin binding agents: The BTP used was a commercial product (Destrox^R) from M/s Animal Biotech Pvt. Ltd., Bangalore, India and contained culture extracts of toxin degrading microbes, antifungal agents, MOS and bio-antioxidants. HSCAS was procured from a commercial supplier from Hyderabad, India.

Experimental design: One hundred fifty day-old straight run commercial broiler chicks were wing banded and allotted randomly to fifteen homogenous groups of ten chicks each. Each treatment was randomly assigned to three groups and all the chicks were reared on battery cages under uniform management practices throughout the experiment and vaccinated for New Castle Disease and Infectious Bursal Disease. A practical type broiler diet (Table 1) was prepared to serve as control (T_1). Four test diets were prepared by incorporating AF and toxin binders to the control diet *viz*., T_2-AF (500 µg/kg; toxin control), T_3-AF (500 µg/kg) + HSCAS 1 kg/ton, T_4-AF (500 µg/kg) + BTP 200 g/ton and T_5-AF (500 µg/kg) + BTP 400 g/ton. Each of such diets prepared for starter (0-21 days) and finisher phase (22-42 days) were offered in mash form.

Parameters studied: The feed intake of chicks in each replicate was recorded daily and the BW of individual bird was recorded at weekly interval. The mortality of the bird was recorded as and when occurred. Mortality corrected feed conversion ratio was calculated as unit feed intake to the unit body weight gain.

At the end of the trial (42nd day), two birds from each replicate were randomly selected, starved for 12 hours with the provision of water *ad lib* and sacrificed by cervical dislocation. The dressing percentage was calculated as the per cent of carcass weight obtained after removing the feathers, neck, legs and internal viscera to its live body weight. The weight of the giblet organs *viz.*, heart without pericardium, liver without gall bladder, gizzard without food contents and spleen were recorded and expressed as the percentage of pre-slaughter BW (g/ 100 g).

Blood was collected from randomly selected birds (2 birds per replicate) on 42nd day of age. Serum was collected after 8 to 10 hours as per the standard procedures and was stored at -20^0 C for subsequent analysis. The individual serum samples were analyzed for total proteins, albumin, total cholesterol, ALT and AST using an automatic analyzer. The data generated were analyzed statistically using GLM procedure of SAS and the Tukeys studentized range test was used to detect differences ($P<0.05$) among different treatment means.

RESULTS

The body weight gains (BWG; Table 2) of birds under different treatments were significantly ($P<0.05$) different during both the phases. At pre-starter phase, there was a significant ($P<0.05$) reduction in BWG in toxin control (T_2) by 27.99 and 19.53% at pre-starter and finisher phases, respectively. Addition of either HSCAS or BTP failed to significantly ($P<0.05$) improve BWG. However, the performance of T_4 although failed to significantly separate from T_2, was numerically higher (7.60 & 3.32%) during both the phases. However, during finisher phase there was a numerical reduction in BWG with higher level of BTP. The phase wise and cumulative feed intake under different treatments (Table 2) found to be significantly ($P<0.05$) affected by the treatments. In both phases the feed intake was significantly ($P<0.05$) reduced by 500 µg/kg of AF. However, in the treatments with added HSCAS or BTP, there was no significant improvement in the feed intake. In the feed conversion ratio (FCR), during starter phase as one could expect, the FCR was significantly ($P<0.05$) better in T_1 over rest of the treatments. In contrast, during the finisher phase a significantly ($P<0.05$) better FCR was observed in T_4 and T_1.

In serum biochemical profiles (Table 3), the total protein (TP) significantly ($P<0.05$) decreased by 54.3% in T_2 as compared to control and with addition of BTP @ 400 g/ton a significant ($P<0.05$) improvement equivalent to control was observed. Addition of BTP @ 200 g/ton significantly ($P<0.05$) improved TP over T_2 by 48.68% and addition of HSCAS numerically improved by 23.75% but both treatments still incomparable to control. The serum albumin was significantly ($P<0.05$) reduced in T_2 in comparison to T_1 and in the treatments T_3 and T_5 significant ($P<0.05$) improvement over T_2 was evident. The ALT and AST, were significantly ($P<0.05$) increased in T_2 and further a significant ($P<0.05$) reduction of ALT and a numerical reduction of AST was evident in the T_4 when compared to T_2, however was not comparable to control. On contrary, AST and ALT levels in T_3 and T_5

Table 1: Ingredient and chemical composition of the control diet

Ingredient (Kg/ton)	Starter diet	Finisher diet
Maize	525.00	600.00
Rice polish	58.80	32.80
Soya extractions	269.00	240.00
Ground nut extractions	80.00	60.00
Sun flower extractions	30.00	30.00
Dicalcium phosphate	20.50	20.50
Calcite powder	12.25	12.25
Salt	3.50	3.50
Trace mineral premix[1]	0.95	0.95
Total	1000.00	1000.00
Additives (Kg/ton)		
Breevit[2]	0.75	0.75
Digistim[3]	0.75	0.75
DL-Methionine	1.65	1.65
Lysine	0.80	0.80
Aaviax[4]	0.50	0.50
Lipocare[5]	1.00	1.00
Doxymix[6]	0.03	0.03
Tylomix[7]	0.40	0.40
Analyzed composition of the basal diet (%)		
Dry matter	90.79	90.50
Crude protein	22.01	20.03
Ether extract	3.32	2.98
Crude fiber	6.27	5.84
Total ash	8.86	7.70
Nitrogen free extractives	59.54	63.45

[1]Fe-90000 ppm, I-2000 ppm, Cu-15000 ppm, Mn–90000 ppm, Zn-80000 ppm, Se-300 ppm; [2]Contained per Kg: Vit A-20 mIU, D_3-4.0, E-60.0 g, C-100 g, B_1-4.0 g, B_2-20.0 g, B_6-6.0 g, B_{12}-0.03 g, Niacin-60.0 g, Calcium-D-Pantothenate 30.0 g, Biotin-0.20 g, Folic Acid- 4.0 g and Vit-K-8.0 g; [3]Herbal Liver stimulant; [4]Semduramicin 5%; [5]Lecithin derived choline along with specific enzymes; [6]Doxycycline 99%; [7]Tylosin phosphate 10%.

were non-significant from T_2. The serum cholesterol levels were significantly ($P<0.05$) reduced in T_2 and nevertheless addition of neither HSCAS nor BTP was effective in correcting the hypocholestremia.

In dressing percentage (Table 4), with the addition of AF in T_2, a significant ($P<0.05$) reduction in was evident. Addition of either HSCAS or BTP @ 200 g/ton significantly ($P<0.05$) improved dressing percentage in comparison to T_2 however, the improvement was not comparable to the best observed in T_1. The relative weight of liver, kidney, heart and spleen were significantly ($P<0.05$) different whereas, gizzard weight was similar among the treatments. The relative liver weight significantly ($P<0.05$) increased in T_2 by 96.41%, and in T_3 and T_4 there was a significant ($P<0.05$) improvement in comparison to T_2 respectively, by 15.38 and 11.83%, yet the improvement was not equivalent to control. A similar trend was observed in relative weight of heart among the treatments. Whereas, in relative weight of kidney, the trend was slightly different in that, among toxin fed groups it was only T_3 which significantly ($P<0.05$) equated to control in spite of failing to separate from rest of the AF fed treatments. The relative size of the spleen was significantly ($P<0.05$) reduced with the addition of AF (T_2) by 33.33% as an indication of the immune-suppressive effect of AF. Addition of either HSCAS or BTP significantly ($P<0.05$) improved the relative weight of spleen by 42.31, 64.62 and 65.38% in T_3, T_4 and T_5, respectively over the least observed in T_2.

The mortality percentage was significantly ($P<0.05$) increased in T_2 (33.3%) in comparison to control (6.7%), and was significantly ($P<0.05$) reduced in treatments T_3 (20.0%) and T_4 (20.0%). Although, the mortality in T_5 (26.7%) was comparable to T_3 and T_4, yet failed to separate from the T_2.

DISCUSSION

The significant ($P<0.05$) growth depressing effect of 500 µg/kg of AF observed in this study is supported by studies of Girish and Devegowda (2006), Manegar *et al.* (2010) and Muhammed *et al.* (2012). The growth depression observed is obvious and is due to reduced protein synthesis (Yang *et al*., 2012), reduced pancreatic enzyme secretion resulting in reduced nutrient assimilation (Marchioro *et al*., 2013) and as a consequence of reduced feed intake as observed in this study. Very similar to our previous study (Kumar *et al*., 2015), the relative ineffectiveness of HSCAS could be due to insufficient dosage as compared to other studies, where more than 5.0 kg was used (Girish and Devegowda, 2006; Zhao *et al*., 2012) and/or because of undesirable adsorptive property of HSCAS to minerals and amino acids (Dawson, 1999) which might have aggravated the existing toxicity by impairing the nutrient availability. The improved performance with BTP @ 200 g/ton of feed is supportive to our previous findings (Kumar *et al*., 2015) with low levels of AF (250 µg/kg). The trend observed in reduction of BWG with increased BTP dosage is similar to our previous findings with 250 µg/kg AF (Kumar *et al*., 2015). The reduction in feed intake due to AF is suggestive of appetite reduction due to impaired nutrient metabolism caused due to damaged liver (Yang *et al.*, 2012) and is well documented in other studies (Girish and Devegowda, 2006; and Muhammad *et al.*, 2012). The 500 µg/kg of AF, as observed in this study is also well known to impair the FCR in other previous studies as well (Manegar *et al.*, 2010; Abd El-Ghany and Hatem, 2013). The improved FCR with low levels of BTP appears to be due to low feed intake and numerically better BWG observed in this treatment (T_4). In contrary, the poorest FCR observed in T_5 is quite obvious in view of its effect on BWG and feed intake. The negative impact of 500 µg/kg AF on FCR could be due to sparing much of the nutrients towards detoxification process which otherwise supposed to be utilized for BWG and also due to damaged liver which impairs nutrient metabolism.

The findings of this study that 500 µg/kg of AF significantly ($P<0.05$) decreases serum proteins is in accordance with the earlier reports of Manegar *et al.* (2010) and Muhammed *et al.* (2012) and can be attributable to impaired amino acid transport, mRNA transcription and hence the inhibited protein synthesis and due to impaired hepatic tissue and anti oxidant system leading to increased liver apoptosis which obviously ends up with reduced liver protein synthesis (Yang *et al.*, 2012). The increase in serum levels of ALT and AST is justifiable due to the damage of hepatocytes caused by the AF which enhance the leakage of these enzymes from the hepatocytes to the blood stream. Muhammed *et al.* (2012) reported a significant increase in ALT and AST levels in AF fed birds very similar to findings of the present study. In contrast, Manegar *et al.* (2010) and Zhao *et al.* (2010) reported significant ($P<0.05$) reduction in AST and non-significant alteration in the ALT of birds fed 200-600 µg/kg and 1 mg/kg AF, respectively. Neither the HSCAS nor the BTP could ameliorate the altered AST and ALT, which is quite obvious since the AST and ALT are known to alter even with a very low level of AF (Kumar *et al.*,

Table 2: Phase wise and cumulative growth performance of broilers under different treatments

Tr. No.	Body weight gain (g/bird)*		Feed intake (g/bird)*		Feed efficiency (unit feed/unit gain)*	
	Starter	Finisher	Starter	Finisher	Starter	Finisher
T_1	690.4[a]	1311[a]	1262[a]	2821[a]	1.825[a]	2.171[a]
T_2	497.1[b]	1055[b]	1008[b]	2356[b]	1.989[b]	2.255[b]
T_3	500.3[b]	949.2[b]	969.0[b]	2213[b]	1.942[b]	2.330[b]
T_4	534.9[b]	1090[b]	1013[b]	2302[b]	1.905[b]	2.166[a]
T_5	495.6[b]	974.6[b]	1012[b]	2276[b]	2.050[b]	2.337[b]
SEM	16.62	44.6	36.48	113.2	0.046	0.030

T_1-Basal diet; T_2-AF (500 µg/kg); T_3-AF (500 µg/kg) + HSCAS (1 kg/ton); T_4 and T_5-AF (500 µg/kg) with BTP 200 and 400 g/ton, respectively. *Within a column, means bearing different superscript significantly (P<0.05) differ.

Table 3: Serum biochemical parameters of birds under different treatments at the end of 42-day trial

Treatment	Total Protein* (g/100ml)	Albumin* (g/100ml)	ALT* (IU/L)	AST* (IU/L)	Cholesterol* (mg/dl)
T_1	4.615[a]	1.915[a]	16.47[c]	147.45[b]	135.1[b]
T_2	2.105[c]	1.150[c]	38.55[a]	222.26[a]	118.3[a]
T_3	2.605[bc]	1.665[b]	44.45[a]	212.54[a]	117.4[a]
T_4	3.130[b]	1.135[c]	33.71[b]	238.73[a]	117.4[a]
T_5	5.080[a]	1.605[b]	44.55[a]	230.72[a]	119.3[a]
SEM	0.240	0.193	2.10	5.77	8.58

T_1-Basal diet; T_2-AF (500 µg/kg); T_3-AF (500 µg/kg) + HSCAS (1 kg/ton); T_4 and T_5-AF (500 µg/kg) with BTP 200 and 400 g/ton, respectively. *Within a column, means bearing different superscript significantly (P<0.05) differ.

Table 4: Dressing percentage and relative weight (g/ 100 g live weight) of organs under different treatments

Treatment	Dressing percentage*	Liver*	Kidney*	Heart*	Gizzard[NS]	Spleen*
T_1	73.47[a]	2.039[c]	0.738[b]	0.583[b]	2.732	0.195[a]
T_2	66.76[c]	4.005[a]	1.154[a]	0.746[a]	2.787	0.130[b]
T_3	69.84[b]	3.381[b]	0.958[ab]	0.649[ab]	3.111	0.185[b]
T_4	69.63[b]	3.531[b]	1.054[a]	0.546[b]	2.794	0.214[b]
T_5	67.22[c]	4.152[a]	1.216[a]	0.566[b]	2.835	0.215[b]
SEM	0.74	0.124	0.088	0.045	0.078	0.019

T_1-Basal diet; T_2-AF (500 µg/kg); T_3-AF (500 µg/kg) + HSCAS (1 kg/ton); T_4 and T_5-AF (500 µg/kg) with BTP 200 and 400 g/ton, respectively. *Within a column, means bearing different superscript significantly (P<0.05) differ; NS- non-significant (P≥0.05).

2015), and probably a low amount of toxin may still prevail in the gut after the action of HSCAS/BTP, resulting in alteration in serum enzyme profile. The reduction in serum cholesterol observed herein is similar to other studies (Al-Jubory *et al*., 2001; Manegar *et al.*, 2010) where a reduction in cholesterol due to AF was reported as a result of limited biosynthesis due to damaged liver. The non-significant effect of HSCAS on the cholesterol is quite obvious since the used dosage was low as described previously.

The reduction in dressing percentage due to AF is perceptibly associated to the effect of aflatoxin on impairment of protein synthesis (Yang *et al.,* 2012) leading to decreased muscle mass production. The increased relative weight of liver observed is due to increased lipid deposits due to impaired fat metabolism and is well documented in other studies (Girish and Devegowda, 2006; Manegar *et al*., 2010; Abd El-Ghany and Hatem, 2013). Addition of HSCAS or BTP @ 200 g/ton resulted in significant (P<0.05) reduction in liver weight in comparison to T_2 yet could not equate to the control. However, high dosage of BTP was found to be of no use similar to our findings in previous study (Kumar *et al*., 2015). As regards relative weight of heart, a trend similar to relative weight of liver was observed. The relative weight of kidney was also significantly (P<0.05) increased due to nephrotoxic effect of AF. The significantly (P<0.05) reduced spleen relative weight with AF is indicative of its immunosuppressive role (Girish and Devegowda, 2006; Manegar *et al.,* 2010; Indresh *et al.*, 2013). Addition of either HSCAS or BTP significantly (P<0.05) increased the relative spleen size which shows immunomodulatory role of both these agents. A similar trend with 1 mg/kg AF on the relative weights of kidney, spleen and gizzard was reported by Girish and Devegowda (2006) and addition of HSCAS and modified glucomannan was found to moderately improve these parameters.

The high mortality observed is attributed to combined effect of hepatotoxicity and necrosis leading to liver failure (Yang *et al.,* 2012) and immunosuppression resulting in increased susceptibility to diseases (Yunus *et al.,* 2009). The magnitude of mortality observed in this study is very close to the observation of Manegar *et al.* (2010) and Bhaskar *et al.* (2003) who reported 21.66 and 23.33% mortality, respectively with 600 and 200 µg/kg AF. The mortality was moderately reduced with addition of either HSCAS or BTP which is a clear reflection of moderate ameliorating effect of both these agents on serum protein profile, relative weights of liver, heart, kidney and spleen ensuing in improved general health and immune status of birds. This shows a positive role of toxin binders HSCAS and BTP in prevention of mortality. The BTP at higher levels did not ameliorate the toxic effects of AF, for which the reason is unknown at this moment and needs further research, with graded levels of BTP (50 to 500 g/ton) with narrow increments of 50 grams.

Conclusion: The study revealed significant (P<0.05) effect of 500 µg/kg AF on various growth performance parameters in broiler chicken. Addition of neither HSCAS nor BTP did significantly improve body weight gain and feed intake. In contrast, addition of BTP @ 200 g/ton significantly (P<0.05) improved the FCR on par with that of control. Considering all the parameters, it can be concluded that BTP @ 200 g/ton is moderately effective

in ameliorating effects of 500 µg/kg of AF and in contrast HSCAS @ 1 Kg/ton and higher level of BTP could not ameliorate the toxic effects of aflatoxin, which needs further investigations.

Author's contribution: BKC carried out the experiment, BSVR designed the experiment and guided during the experiment. RGG, TMP, BNS and SNK helped in laboratory work, analysis of data and drafting and revision of manuscript. All authors interpreted the data, critically revised the manuscript for important intellectual contents and approved the final version.

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