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Behavioral Changes and Clinical Signs in Aflatoxicosis Induced Broilers and Amelioration with Probiotics and Silymarin

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Abstract

The global poultry industry faces a persistent challenge from aflatoxins. Instead of chemical treatments, use of probiotics and phyto-constituent like silymarin was thought of to combat aflatoxins. This study investigated the behavioral aspects of aflatoxicosis in broiler chickens as a cost-effective diagnostic tool for poultry farmers and assessed the effectiveness of silymarin and multi-strain probiotics in mitigating aflatoxin-induced behavioral changes. Aflatoxicosis was induced in broiler with 0.5, 1 and 3 ppm aflatoxin and clinical signs were observed in broiler chicken exposed to three levels of aflatoxin. The study found that aflatoxin-exposed birds exhibited reduced appetite, increased water intake, altered faecal consistency, poor feather condition and diminished comb growth. However, treatment with probiotics and silymarin, alone or combination, significantly alleviated these effects and restored normal behaviour in broiler. To conclude, this study suggested that poultry farmers can suspect aflatoxicosis based on observable behavioral changes and highlighted the efficacy of probiotics and silymarin in countering aflatoxin's harmful effects in commercial broilers chicken, enhancing their overall health.

The poultry industry is a thriving sector of animal husbandry worldwide. Despite its significant growth, the poultry farming continues to grapple with a persistent challenge: Aflatoxins. Aflatoxins are pervasive mycotoxins that pose a substantial threat to the health of both animals and humans, especially in tropical and subtropical regions. These mycotoxins have the potential for severe consequences due to their carcinogenic, neurotoxic, immunotoxic, mutagenic, teratogenic, estrogenic, and hepatotoxic properties (Casteel and Rottinghouse, 2000). Among the primary aflatoxins, AFB1 stands out as the most potent toxin and is classified as a human carcinogen (Talebi *et al*., 2011).

Traditionally, the detoxification of aflatoxins has involved physical and chemical treatments of feed. However, one promising strategy for detoxification is the use of probiotics. Probiotics consist of live microorganisms that provide health benefits to the host. In the poultry industry, they are used as feed additives, typically in small quantities, and are considered non-nutrient feed additives. Commercial probiotic preparations are commonly employed to enhance various performance parameters in poultry, including mean egg weight, market-aged body weight, and feed conversion ratio (Plaza-Diaz *et al*., 2019).

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In the pursuit of solutions for various health issues in both humans and animals, plant-based compounds have been relied upon for centuries. More recently, the poultry industry has turned its attention to plant-derived substances to improve broiler performance (Oso *et al*., 2019). One such natural product of interest is milk thistle, which has a history spanning over two millennia as a therapeutic herb. It is considered one of the most effective herbal remedies for liver-related ailments, including toxin-induced liver diseases, viral hepatitis, liver cirrhosis, and hepatocellular carcinoma (Pickova *et al*., 2020).

Aflatoxicosis apart from producing production loss through changes in the molecular structures of organs involved, it also produces a variety of behavioural changes to mimic the levels of toxicity. Certain behavioural changes are highly unique to the level of toxicities and such clinical and behavioural changes in the broiler birds are attempted for evaluation in this study so as to have a knowledge on the behavioural changes at farm level.

In light of these considerations, this study was designed to explore the potential of natural products such as silymarin and multi-strain probiotics as alternative strategies to the commonly used chemical binders in combating the behavioural changes induced by aflatoxins in-order to detect the toxicity by poultry farmers.

Materials and Methods

A. parasiticus fungal culture was regularly sub-cultured in potato dextrose agar every 10 days to maintain its viability. Broken maize was used as the substrate to produce aflatoxin as per the method of Shotwell *et al*. (1966). Newly hatched day-old straight run broiler chicks were obtained, wing-banded, weighed and housed in battery brooders as per CCSEA recommendations with IAEC approval. The birds were randomly distributed into 15 different groups of 12 birds in each group. They were fed with 0.5, 1 and 3 ppm AF containing feed from day 0 to 6 weeks of age. Commercial multi-strain probiotic containing total viable count of NLT $2*10^9$CFU of *Bacillus coagulans, Bacillus licheniformis*, *Lactobacillus acidophilus, Lactobacillus plantarum, Saccharomyces cerevisiae, Lactobacillus rhamnosus, Enterococcus faecium* and *Bifidobacterium bifidum* (0.15% in feed) and silymarin (10 g/kg) were given individually and in combination from first day to 6 weeks of the study period.

Group	Treatment	No. of birds
I	Control	12
II	Multi-strain Probiotic (0.15% in feed)	12
III	Silymarin (10 g/kg feed)	12
IV	AF (0.5 ppm)	12
V	AF (1.0 ppm)	12
VI	AF (3.0 ppm)	12
VII	AF (0.5 ppm) + Multi-strain Probiotic (0.15% in feed)	12
VIII	AF (1.0 ppm) + Multi-strain Probiotic (0.15% in feed)	12
IX	AF (3.0 ppm) + Multi-strain Probiotic (0.15% in feed)	12
X	AF (0.5 ppm) + silymarin (10g/kg)	12
XI	AF (1.0 ppm) + silymarin (10g/kg)	12
XII	AF (3.0 ppm) + silymarin (10g/kg)	12
XIII	AF (0.5 ppm) + Multi-strain Probiotic + silymarin (10g/kg)	12
XIV	AF (1.0 ppm) + Multi-strain Probiotic + silymarin (10g/kg)	12
XV	AF (3.0 ppm) + Multi-strain Probiotic + silymarin (10g/kg)	12

Behavioural changes and clinical signs like desirability towards feed and water, alertness, consistency of faeces, feather condition, comb growth and appearance of nervous signs were recorded twice daily. Each sign was subjectively scored from 0 (absent) to 3 (based on intensity) and number of birds in each group showing the sign. Total score for each clinical sign was obtained at the end of the trial period by summing up the average of daily observation. Mortality, if any was also recorded (Hussain *et al*., 2016).

Results and Discussion

Scoring of clinical signs in broilers fed with different levels of aflatoxin and treated with probiotics and silymarin for 42 days is given in table. Visual assessment of broiler chicken from control, probiotic and silymarin treated birds were alert and active, showed normal behaviours like towards feed and water consumption, consis-

Table : Scoring of clinical signs in broilers fed with different levels of aflatoxin and treated with probiotics and silymarin for 42 days

Groups	Alertness (Normal – depressed)	Desirability towards feed (Normal to less interest)	Inclination to water (Normal to more inclination)	Feather condition (normal to ruffled broken)	Comb growth and colour (Normal to small pale)	Faeces consistency (Normal to watery greenish brown)	Nervous signs (Absent/ Present)
I – Control	0	0	0	0	0	0	0
II – Probiotic (Pro) (0.15% in feed)	0	0	0	0	0	0	0
III – Silymarin (Sil) (10 g/kg)	0	0	1	0	0	1	0
IV – AF 0.5 ppm	24	21	20	27	18	19	0
V – AF 1 ppm	76	90	82	84	70	84	2
VI – AF 3 ppm	157	207	189	225	142	192	0
VII – AF 0.5 ppm + Pro	3	2	3	4	1	5	0
VIII – AF 1 ppm + Pro	39	45	38	43	35	44	2
IX – AF 3 ppm + Pro	151	195	189	201	124	189	0
X – AF 0.5 ppm + Sil	4	2	4	4	2	5	0
XI – AF 1 ppm + Sil	39	36	47	34	34	39	0
XII – AF 3 ppm + Sil	150	180	174	174	127	183	0
XIII – AF 0.5 ppm + Pro + Sil	3	3	4	4	2	5	0
XIV – AF 1ppm + Pro + Sil	38	38	44	39	36	39	0
XV – AF 3ppm + Pro + Sil	153	168	171	171	130	180	0

Scoring details: Absence of sign – 0; Severity intensity of sign: 1-3

tency of faeces was firm and normal in colour, feather condition was shiny and uniform, comb growth was good with bright red colour and no evidence of nervous signs. Dullness, decreased desirability towards feed and increased inclination towards water in AF treated groups with the severity of toxin. The consistency of faeces had changed from firm, green colour droppings to loose and watery reddish brown colour in AF treated birds (Fig. e). AF treatment revealed lustreless, ruffled, broken and scarce feather condition in broiler chicken. Comb growth was reduced and was pale in AF fed birds (Fig. a-c). Nervous signs like lameness and ataxia were observed in only two birds fed with 1 ppm AF (Fig. d). No mortality was recorded in all three doses (0.5, 1 and 3 ppm) of AF treated birds. Treatment with probiotic and silymarin alone or in combination exhibited significant improvement in the appearance of clinical signs when compared to the intoxicated broilers. Birds were alert and active and revealed more desirability towards feed, less inclination to water, improved lustre and condition of feather, improved comb growth and colour (Fig. f), improved consistency and colour of faeces when compared to the toxin fed group of birds.

Decreased desirability towards feed, increased inclination towards water intake, loose

Fig (a). 0.5 ppm AF group- Broiler – 6 week – Small comb and scarce feathers

Fig (b). 1 ppm AF group- Broiler – 6 week – Small pale comb and scarce feathers

Fig (c). 3 ppm AF group- Broiler – 6 week – Very small comb, ruffled feathers

Fig (d). 1 ppm AF group- Broiler – 6 week – Neurological sign – ataxia

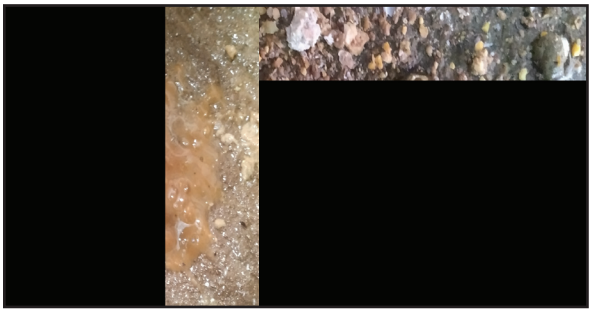

Fig (e). 3 ppm AF group – Faeces – 6 week – greenish brown watery droppings

Fig (f). 0.5 ppm AF +P group- Broiler – 6 week – Improved comb and feather condition

and watery reddish brown colour droppings and lustreless, ruffled, broken and scarce feather condition with small pale combs were observed in AF treated birds. Similarly, Hussain *et al.* (2016) explained the dose related increase in the expression of clinical signs in broilers intoxicated with AFB1 at the level of 50, 100, 200, 400 and 800 ppb in diet for 28 days from 14 days of age. The difference in findings with the previous study would be attributed to the source of toxin, strain and age of bird used in the study. Throughout the study only two birds from 1 ppm AF group showed nervous signs like lameness and ataxia and no mortality was recorded throughout the study period. Similarly, Ashry *et al.* (2022) reported that no mortality and clinical signs like retarded growth, paralysis, lameness, wing paralysis and whitish diarrhoea were observed in broilers treated with 2.5 ppm aflatoxin in feed for 35 days. Clinical signs caused by intoxication of AF diet was counteracted by probiotic and silymarin alone or in combination at 0.5 ppm level. When the toxicity level increased to 1 ppm and 3 ppm, treatments with probiotic and silymarin were able to reduce the effect of toxicity but could not completely ameliorate the toxicity of AF. Desirability towards feed and water, feather condition, comb growth and faeces consistency were improved by probiotic and silymarin supplementation in broilers. This indicated the AF detoxifying ability of both probiotic and silymarin.

Summary

In summary, the study observed distinct behavioral changes in broiler chicken exposed to aflatoxin, including reduced appetite, increased water consumption, watery faeces, poor feather condition, and diminished comb growth with pale appearance. However, treatment with probiotics and silymarin, either individually or in combination, significantly alleviated these adverse effects. Treated birds exhibited improved appetite, healthier feathers, enhanced comb growth, and normal faecal consistency. This study suggests that poultry farmers can potentially suspect aflatoxicosis in their birds based on these observable behavioral changes. Furthermore, it underscores the effectiveness of probiotics and silymarin in mitigating the harmful effects of aflatoxin exposure in broiler chicken, ultimately enhancing their overall health and well-being.

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