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Thanabal C
Senior BDM, Alltech Biotechnology Private Limited, Palladam, Tamil Nadu, India

Ramamurthy N
Professor and Head (Retd.), Dept. of Poultry Science, MVC, Chennai, Tamil Nadu, India

Richard Churchil R
Professor and Head, Poultry Research Station, TANUVAS, Chennai, Tamil Nadu, India

Tensingh Gnanaraj P
Registrar, TANUVAS, Chennai, Tamil Nadu, India

Manju G Preedaa
Veterinary Assistant Surgeon, Animal Husbandry, Tamil Nadu, India

Corresponding Author:
Thanabal C
Senior BDM, Alltech Biotechnology Private Limited, Palladam, Tamil Nadu, India

Ameliorative effects of *Phyllanthus niruri* on production performance of Guinea fowls raised with aflatoxin contaminated feed

Thanabal C, Ramamurthy N, Richard Churchil R, Tensingh Gnanaraj P and Manju G Preedaa

Abstract

This study was conducted to investigate the effect of aflatoxin and protective role of *Phyllanthus niruri* herb against the effect of aflatoxin on production performances (Viz. Body weight, Body weight gain, Feed intake, Feed efficiency and Livability) of Guinea fowls. A total of 270 day old keets were divided into six groups (45 chicks each) as following: The T1 Control (without Aflatoxin), T2 (diet with 1 PPM aflatoxin B1), T3 (diet with 2 PPM aflatoxin B1), T4 (diet with 1% herb powder alone), T5 (1 PPM aflatoxin B1 and 1% herb powder) and T6 (2 PPM aflatoxin B1 and 1% herb powder). The results revealed that the inclusion of Aflatoxin B1 (2 ppm) level adversely affected the bodyweight, weight gain and feed efficiency at fortnight during experimental period. Inclusion of 1 ppm aflatoxin B1 in diet does not influence the production parameters. Supplementation of 1 percent *Phyllanthus niruri* herb powder to control diet caused significant depression in the weekly body weight, Weight gain, feed consumption and feed efficiency. Inclusion of 1 percent *Phyllanthus niruri* herb powder in the aflatoxin B1 (2 ppm) supplemented diet significantly counteracted the toxin effects of aflatoxin B1 on final body weight, weight gain and feed efficiency at 12th week of age. In conclusion, Guinea fowl production performance was not affected up to 1 ppm level aflatoxin B1 contamination in diet and the negative effects of aflatoxin B1 at 2 ppm level in production performance of Guinea fowls was improved by supplementation of 1 percent *Phyllanthus niruri* herbal powder on diet.

Keywords: Guinea fowl, aflatoxin, *Phyllanthus niruri* and Production performance

Introduction

Traditional backyard poultry keeping has been practiced since time immemorial in different parts of the world. Worldwide, this backyard poultry sector consists of Chicken (63%), Ducks (11%), Geese (9%), Turkeys (5%), Pigeons (3%) and Guinea fowls (3%) [1]. Even though Guinea fowl rearing constitute only 3 percent at present, it is gaining significance as alternate poultry farming owing to some important characters of this species. The contamination of poultry feeds with mycotoxin is a major problem causing great economic loss to the poultry farmers. Mycotoxins are the toxic metabolites synthesized by some naturally growing fungi on animal feed, feed ingredients and other agricultural crops when the temperature, moisture and aeration are favorable. The aflatoxin contamination in feed is due to the use of toxin contaminated feed ingredients like maize and oil cakes or long storage of compounded feed. Mycotoxins have adverse effects on both the health and productivity in almost all species of domestic animals including poultry. In general, mycotoxicosis results in reduced feed intake, diminished feed conversion, decrease in production and susceptibility to various infections depending upon the types of toxins ingested.

Aflatoxin is the most potent and commonly occurring toxicant in the feedstuffs [2]. Although Guinea fowl is the most resistant of all the poultry species to dietary aflatoxin, scientific evidence based on research is scanty. Barring a single earlier study [3], there is complete paucity of research in India and abroad on the subject. On the other hand, intensive rearing of Guinea fowls for producing day-old and grown up keets for backyard farming is on the rise in many parts of the country. Therefore, evaluating the impact of aflatoxin on intensively reared Guinea fowl and it is necessary to develop appropriate programme for the prevention and control of mycotoxins. The use of herbs has recently emerged as a promising way to counter mycotoxicosis. Certain herbal extracts targeted at inactivation of the toxins and inhibition of toxin production by the fungi has shown to be effective in control of mycotoxicosis [4].

Therefore, the use of a known herbal hepato-protectant, *Phyllanthus niruri* for counteracting aflatoxicosis was tested in the study.

Materials and Methods

Production of Aflatoxin

Aspergillus parasiticus NRRL 2999 strain procured from National Centre for Agricultural Utilization Research, Microbial Genomics and Bioprocessing Unit, 1815N University Street, Peiria, Illinois 61604, USA was used to produce aflatoxin. The fungus was maintained by sub culturing it on potato dextrose agar at 10 days interval and aflatoxin for experimental induction was produced on rice [5]. The aflatoxin content of cultured rice powder was quantified by using Thin Layer Chromatography [6] at Pharmacovigilance Laboratory for Animal Feed and Food Safety (PLAFFS), Madhavaram Milk Colony, Chennai.

Preparation of experimental diet

The feed ingredients free of any toxins were only used for the preparation of experimental diets. Guinea fowl mash feed were procured from Central Feed Technology Unit (CFTU), Kattupakkam and analyzed to ensure the absence of aflatoxin. Weighed amount of powdered culture material containing known amount of aflatoxin was added to the herb free control diet (T1) to prepare two more experimental diets containing aflatoxin B1 at 1 (T2) and 2 ppm (T3) levels. One percent of Deoiled rice bran was replaced by *Phyllanthus niruri* herb powder to prepare T4 diet. Weighed amount of powdered culture material containing known amount of aflatoxin was added to T4 diet to prepare two more experimental diets containing both herb powder (1%) and aflatoxin B1 at either 1 (T5) or 2 ppm (T6) level.

Biological Experiment

A biological trial was conducted in the University Research Farm, Madhavaram Milk colony, Chennai with 270-day old keets purchased from SRS Hatcheries Ltd., from Palladam, Tamil Nadu. The experiment was conducted for a period of twelve weeks duration. The keets were reared under cage system and good management practices were followed during the experiment and were provided with respective treatment feed and water Ad libidum throughout the experimental period. No vaccination or antibiotic administration was done during the experimental period.

Experimental design

Two hundred and seventy keets were used in this experiment. The keets were wing banded, weighed individually and randomly distributed into six treatment groups with three replicates each consisting of 15 keets.

Experimental groups can be classified as follows:

T1 – Control diet

T2 - 1 ppm aflatoxin B1 added to control diet

T3 - 2 ppm aflatoxin B1 added to control diet

T4 – 1 percent *Phyllanthus niruri* herb powder added to control diet

T5 - 1 ppm Aflatoxin B1 plus 1 percent *Phyllanthus niruri* herb powder added to control diet

T6 - 2 ppm Aflatoxin B1 plus 1 percent *Phyllanthus niruri* herb powder added to control diet

Body weight

The body weight of individual bird was recorded at biweekly interval and average biweekly body weight and weight gain per bird were calculated for all the treatment groups.

Feed intake

Feed intake of birds was recorded for each replicate at fortnightly.

Feed efficiency

Feed efficiency (kg of feed per kg weight gain) at bi-weekly interval was calculated for each replicate based on the data on body weight gain and feed intake.

Livability

Mortality was recorded on its occurrence during the experimental period and biweekly livability was calculated. All the dead birds were subjected to detailed autopsy examination.

Statistical analysis

All the statistical analysis was performed by using SPSS software (version 20.0) as per [7]. The means were compared by One-way ANOVA for significant differences among treatments.

Results and Discussion

Bodyweight

The mean body weight of Guinea fowls recorded at biweekly interval and overall mean bodyweight have been depicted in table 1. The statistical analysis of the data on day-old body weight of keets did not reveal any significant difference while data on biweekly body weight from second to twelfth week of age revealed significant ($P\leq0.01$) differences among the treatment groups. The insignificant difference of day-old body weight of keets among treatments indicated proper randomization of experimental units.

In this study, Guinea fowl consuming 1 ppm aflatoxin B1 containing diet had statistically similar body weight to that of Control group from 2nd week to 12th week of age except significant reduction ($P<0.05$) at 10th week of age. This agreed with the findings of Johri *et al*., 1988 [3], who also observed no effect of aflatoxin at same dietary level in Guinea fowl between 4 and 12 weeks of age. This relative resistance of Guinea fowl to dietary aflatoxin compared to other poultry species might be due to differences in aflatoxin B1 biotransformation pathways [8]. Epoxide production in chicken is less compared to that of ducks conferring more resistance to chicken against aflatoxicosis. As there is no published literature to prove the biological pathway offering resistance against aflatoxin in Guinea fowl, the mechanism described earlier for chicken may holds good in Guinea fowls also. This may be attributed to the minimal growth depressing effect of aflatoxin observed in the study at 1 ppm level.

In the present study, the depression in body weight at 2 ppm aflatoxin B1 level, became significant from 2nd to 12th week of age compared to numerical difference at 1 ppm aflatoxin B1 level. The growth depressing effect of different dietary concentration of aflatoxin has been confirmed by several earlier authors at different dose levels in chicken [9] and Japanese quails [10]. The depression in body weight during aflatoxicosis might be due to inhibition of protein synthesis, lipogenesis and decreased activities of several digestive enzymes and absorption of essential nutrients [11]. The comparison of current results with literature provides sufficient evidence to suggest that Guinea fowl is resistant to aflatoxin at lower level of 1 ppm; whereas, noticeable growth depression has been reported at a level of 1 ppm or less in other species of poultry [12].

In this study, supplementation of 1 percent *Phyllanthus niruri* herb powder in aflatoxin free diet had significantly lower body weight than control during all fortnights. On the contrast, Jagadeeswaran, 2011 [13] had claimed significant improvement in the growth of *Phyllanthus niruri* supplemented broilers. Similarly, the supplementation of herbal growth promoter containing *Phyllanthus niruri* to broilers significantly improved body weight [14]. The depression in body weight in the present study may be due to decrease in feed consumption of treatment group during initial stages of experiment.

The depression in body weight caused by 2 ppm aflatoxin B1 was significantly counteracted at tenth and twelfth week of age by supplementation of 1 percent *Phyllanthus niruri* herb powder. This was in congruence with the findings of Sundaresan *et. al.* [15], who found significant alleviation of growth depressive effect of aflatoxin (100 ppb) at 28 days of age in broiler chicken by dietary inclusion of 1 percent *Phyllanthus niruri* herb powder.

Feed intake

The biweekly feed intake of Guinea fowl receiving 1 ppm aflatoxin B1 treated feed had similar feed intake compared to Control throughout the experiment; however, at 2 ppm level significant (P<0.05) reduction was evident only during second fortnight only. This was in partial resemblance with the findings of Johri [3], who claimed that feed intake was not influenced in Guinea fowls by 0.2 to 1 ppm of aflatoxin in diet. However, many researchers stated decreased feed intake at various doses of aflatoxin in feed of broilers [16], Japanese quails [9] and ducks [16].

The feed consumption of birds receiving *Phyllanthus niruri* herb powder in aflatoxin free diet had significantly reduced compared to control in first and second fortnights of experiment; thereafter, the feed intake became like control. Similarly, Sundaresan *et al.* [15] observed reduced feed intake in broilers up to six weeks of age supplemented with *Phyllanthus niruri* herb powder. This initial reduction in feed intake might be due to its bitter taste attributable to the presence of tannins, saponins, alkaloids and terpenoids in the herb powder and the birds became habituated after some time to the taste and feed intake became normal.

The birds supplemented with *Phyllanthus niruri* herb powder in 2 ppm aflatoxin containing diet showed significantly lower feed intake in earlier part of experiment (first fortnight) only compared to 2 ppm aflatoxin alone fed group followed by numerical reduction in second and third fortnights. There after, numerically higher feed intake was recorded after third fortnight. Similarly feed intake was not significantly improved, even though aflatoxin (100 ppb) contaminated diet supplemented with *Phyllanthus niruri* herb powder [15].

Feed efficiency

Aflatoxin at 1 ppm level was found to cause no deleterious effect in Guinea fowls. This was supported by the findings of Johri [3] in Guinea fowls at same level of aflatoxin. Aflatoxin at 1 ppm level [16, 18] or even less than that has been reported to cause deleterious effect on feed efficiency of other species of poultry [19, 20]. At 2 ppm level, the biweekly feed efficiency in Guinea fowls was inferior to control group throughout the experiment.

Table 1: Influence of Aflatoxin and *Phyllanthus niruri* herb powder supplemented diet on body weight (g) of Guinea fowls

Treatment	Age (Weeks)						
	0	2	4	6	8	10	12
Control diet	24.24±0.39 (45)	80.00^{a}±1.84 (42)	221.88^{a}±5.38 (42)	424.99^{a}±9.55 (42)	619.54^{a}±12.06 (39)	813.51^{a}±12.61 (39)	993.24^{a}±13.90 (39)
Aflatoxin B1 (1 ppm)	24.49±0.37 (45)	77.83^{a}±1.43 (41)	214.42^{ab}±4.01 (41)	408.82^{ab}±5.75 (40)	594.60^{a}±9.09 (37)	773.73^{b}±10.55 (37)	956.54^{ab}±11.10 (37)
Aflatoxin B1 (2 ppm)	24.36±0.34 (45)	69.96^{b}±1.55 (42)	190.80^{d}±4.01 (41)	358.11^{d}±8.13 (40)	507.00^{d}±8.90 (37)	653.51^{e}±11.35 (37)	807.44^{e}±12.06 (37)
Herb powder (1%)	24.28±0.40 (45)	69.75^{b}±1.50 (45)	205.18^{bc}±4.17 (45)	389.42^{bc}±8.02 (45)	554.58^{b}±10.02 (42)	745.84^{bc}±11.86 (42)	936.98^{bc}±12.86 (42)
Herb powder (1%) plus Aflatoxin B1 (1 ppm)	24.55±0.37 (45)	70.03^{b}±1.51 (40)	201.75^{bcd}±4.87 (40)	388.72^{bc}±10.03 (40)	550.19^{bc}±12.83 (37)	729.68^{cd}±14.91 (37)	908.71^{c}±15.40 (37)
Herb powder (1%) plus Aflatoxin B1 (2 ppm)	24.18±0.36 (45)	69.36^{b}±1.26 (42)	197.91^{cd}±4.66 (41)	371.66^{cd}±8.69 (41)	520.85^{cd}±11.07 (38)	695.43^{d}±12.86 (38)	865.01^{d}±14.81 (37)
Overall mean	24.35±0.15	72.79±0.68	205.40±1.95	390.76±3.70	558.10±5.05	736.02±6.06	912.60±6.75
F Value	0.15^{NS}	9.77**	6.13**	8.07**	15.69**	20.50**	24.48**

^{NS} - Non significant, **- Significant (*P*≤0.01)

Means bearing different superscripts within the column differ significantly (P≤0.05)

Figures in parenthesis indicate the number of observations.

Table 2: Influence of Aflatoxin and *Phyllanthus niruri* herb powder supplemented diet on feed intake of Guinea fowls

Treatment	Duration (Weeks)						
	0 – 2	3 – 4	5 – 6	7 - 8	9 - 10	11 - 12	0 – 12 (Cumulative)
Control diet	115.53^{ab}±2.23 (42)	397.63^{a}±4.73 (42)	599.96±7.13 (42)	666.67^{ab}±2.37 (39)	761.50±4.45 (39)	812.83±9.25 (39)	3354.20±21.37 (39)
Aflatoxin (1 ppm)	124.43^{a}±4.43 (41)	371.03^{ab}±7.94 (41)	588.67±17.71 (41)	705.70^{a}±15.10 (37)	752.17±20.48 (37)	841.87±25.25 (37)	3383.85±64.45 (37)
Aflatoxin (2 ppm)	117.76^{a}±2.23 (42)	363.10^{b}±15.38 (41)	581.30±17.54 (40)	635.20^{b}±5.54 (37)	749.17±10.37 (37)	816.87±12.52 (37)	3263.41±52.67 (37)
Herb powder (1%)	104.46^{c}±2.23 (45)	351.10^{b}±9.70 (45)	575.56±4.43 (45)	622.20^{b}±5.88 (42)	755.57±4.43 (42)	822.23±4.47 (42)	3231.11±14.64 (42)
Herb powder (1%) plus Aflatoxin B1(1 ppm)	106.67^{bc}±3.84 (40)	352.90^{b}±8.60 (40)	585.70±16.84 (40)	617.77^{b}±6.94 (37)	765.60±13.76 (37)	843.80±10.62 (37)	3272.48±55.38 (37)

Herb powder (1%) plus Aflatoxin B1 (2 ppm)	106.67^{bc}±3.84 (42)	357.66^{b}±6.50 (41)	571.40±14.30 (41)	671.10^{b}±34.08 (38)	778.63±6.74 (38)	845.33±10.91 (38)	3330.77±59.59 (38)
Overall mean	112.59±2.08	365.57±5.01	583.77±5.31	653.11±9.30	760.44±4.62	830.49±5.74	3305.94±21.23
F Value	5.84**	3.37**	0.53^{NS}	4.61**	0.87^{NS}	1.16^{NS}	1.48^{NS}

^{NS} – Non significant, **- Significant (P≤0.01).
Means bearing different superscripts within the column differ significantly (P≤0.05).
Figures in parenthesis indicate the number of observations.

Table 3: Influence of Aflatoxin and *Phyllanthus niruri* herb powder supplemented diet on feed efficiency of Guinea fowls

Treatment	Duration (Weeks)						
	0-2	**3-4**	**5-6**	**7-8**	**9-10**	**11-12**	**0 - 12 (Cumulative)**
Control diet	2.10^{b}±0.10	2.80^{ab}±0.15	2.97^{b}±0.09	3.57^{c}±0.13	4.03^{bc}±0.13	4.47^{c}±0.03	3.50^{c}±0.07
Aflatoxin (1 ppm)	2.33^{ab}±0.09	2.73^{ab}±0.09	3.03^{b}±0.09	3.80^{c}±0.21	4.20^{bc}±0.15	4.60^{bc}±0.21	3.64^{c}±0.07
Aflatoxin (2 ppm)	2.60^{a}±0.10	3.00^{a}±0.15	3.47^{a}±0.18	4.50^{a}±0.09	5.10^{a}±0.10	5.30^{a}±0.06	4.20^{a}±0.07
Herb powder (1%)	2.33^{ab}±0.09	2.57^{b}±0.03	3.13^{ab}±0.09	3.87^{bc}±0.15	3.97^{c}±0.19	4.33^{c}±0.18	3.56^{c}±0.06
Herb powder (1%) plus Aflatoxin B1 (1 ppm)	2.37^{ab}±0.13	2.67^{ab}±0.09	3.13^{ab}±0.12	3.93^{abc}±0.09	4.27^{bc}±0.15	4.70^{bc}±0.20	3.73^{bc}±0.11
Herb powder (1%) plus Aflatoxin B1 (2 ppm)	2.33^{ab}±0.07	2.77^{ab}±0.03	3.30^{ab}±0.10	4.43^{ab}±0.30	4.43^{b}±0.09	5.00^{ab}±0.15	3.96^{ab}±0.09
Overall mean	2.34±0.05	2.76±0.05	3.17±0.06	4.02±0.10	4.33±0.10	4.73±0.10	3.76±0.07
F Value	3.23*	3.15*	3.55*	4.39**	8.85**	5.42**	11.421**

* - Significant (P≤0.05), **- Significant (P≤0.01).
Means bearing different superscripts within the column differ significantly (P≤0.05)

Aflatoxin at this level was also found to cause poor feed efficiency in broilers [21]. However, no report is available on the effect of 2 ppm aflatoxin on feed efficiency of Guinea fowls. The poor feed efficiency in aflatoxin feeding could be attributed to decreased nutrient utilization due to hepatic cell damage and decreased pancreatic enzymes that affects synthesis of protein and amino acid utilization.

The birds received 1 percent *Phyllanthus niruri herb* powder supplementation on aflatoxin free diet in this study had statistically similar biweekly feed efficiencies to that of control throughout the experimental period. Similarly, no significant change in weekly feed conversion ratio of broilers supplemented with 1 percent crude powder of *Phyllanthus niruri* compared to control [14].

The toxic effects of aflatoxin on feed efficiency observed during first, second, third and fifth fortnights was partially restored towards control diet by the supplementation of *Phyllanthus niruri* herb powder (10 g/ kg) in 2 ppm aflatoxin B1 containing feed. The improvement of feed efficiency in fifth fortnight was statistically significant which was like the findings of Sundaresan *et al*. 2007 [15], who observed superior feed efficiency in aflatoxin plus herb powder supplemented broilers compared to control. The possible mechanism of *Phyllanthus niruri* herb powder in bringing about this beneficial effect could be attributed to the presence of various phytochemicals like phyllanthin, hypophyllanthin etc., which may increase protein anabolism by protecting the liver from deleterious effect of aflatoxin by inhibiting many drug metabolizing P450 enzymes [22].

Livability

Table 4: Influence of Aflatoxin and *Phyllanthus niruri* herb powder supplemented diet on livability (%) of Guinea fowls

Treatment	Duration (weeks)						
	0-2	**3-4**	**5-6**	**7-8**	**9-10**	**11-12**	**Total (0-12)**
Control diet	6.67	0.00	0.00	0.00	0.00	0.00	6.67
Aflatoxin (1 ppm)	8.89	0.00	2.22	0.00	0.00	0.00	11.11
Aflatoxin (2 ppm)	6.67	2.22	2.22	0.00	0.00	0.00	11.11
Herb powder (1%)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Herb powder (1%) plus AFB1 (1 ppm)	11.11	0.00	0.00	0.00	0.00	0.00	11.11
Herb powder (1%) plus AFB1 (2 ppm)	6.67	2.22	0.00	0.00	0.00	2.22	11.11
Overall mean	6.67	0.74	0.74	0.00	0.00	0.37	8.52

In the present study, the aflatoxin B1 treated birds irrespective of levels (1 and 2 ppm) with the addition of herb powder showed higher percentage of mortality than control group in twelve weeks of age. Zhao *et al.* 2010 [23] reported dose related mortality in broilers fed 0 to 2 ppm aflatoxin. However, Johri *et al*. 1988 [3] observed no significant difference in mortality of aflatoxin (1 ppm) fed Guinea fowls and control group. Impaired liver functions and protein/ lipid utilization mechanisms might have affected the performance and general health of the birds. In addition, aflatoxin, a potent immunosuppressant could have affected the cell mediated immunity response due to impaired lymphoblastogenesis and impairment of lymphokine production [24].

There was no mortality recorded in 1 percent *Phyllanthus niruri* herb powder supplemented group and thereby the overall livability in *Phyllanthus niruri* herb powder alone supplemented group was 100 percent throughout the experiment. Similarly reported decrease in mortality percentage in broilers fed with liver tonic containing *Phyllanthus niruri* as one of the components [25].

Conclusion

In the present study, it could be concluded that Guinea fowl also susceptible to aflatoxin B1 at a level of 2 ppm. Supplementation of *Phyllanthus niruri* herb powder alone does not cause any improvement in growth. The dietary inclusion of *Phyllanthus niruri* herb powder in Guinea fowls intoxicated with 2 ppm aflatoxin B1 has resulted in significant improvement of final body weight and feed efficiency. Therefore, the use of *Phyllanthus niruri* in ameliorating toxicity of higher level of aflatoxin B1 contaminated feed in Guinea fowl can be considered after considering margin of economic returns.

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