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Impact of Dietary Aflatoxin-B1 on Juvenile Growth in White Pekin Ducks

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ABSTRACT

Background: Aflatoxicosis in ducks is reckoned as a challenge for nutritionists worldwide, for its adverse impacts on growth. A study was conducted to ascertain the precise level of Aflatoxin-tolerance in White Pekin ducklings during juvenile ages.

Methods: Day-old-ducklings (240) were randomly distributed into 3 experimental-groups *viz*., Control (<0.5 ppb), T_1 (200 ppb), T_2 (400 ppb) and reared for measuring weekly growth, feed efficiency and blood biochemical changes, with treatments terminated at 6 weeks age.

Result: There was significant depression in live weights for both dietary-toxin groups from 2nd week onwards till completion. Mortality (0-6 weeks) was 85% in T_2, while T_1 was in between control and T_2, both for mortality (45%) and morbidity. Feed-consumption and conversion for either toxin groups remained significantly ($p \leq 0.05$) poor, with T_2 resulting in erratic FCRs, ranging from 1.23 to 4.95, across weeks. A typical AFB1-induced lameness in ducklings beyond 3 weeks of AFB1-exposure, emerged as a hallmarked morbidity, in varied proportions, in T_1 and T_2, but not in control. The study confirms that acute-depression of juvenile growth and high-morbidity are distinct outcomes from AFB1's presence in Pekin diets @ 200 ppb level or higher, with such leg deformities emerging as hallmarked features of Pekins reared on AFB1-spiked diets. It can be concluded that, while the AFB1 content of juvenile White Pekin ducks should be kept limited to the recommended safe levels (<10 ppb); exceeding a threshold of 200 ppb is sure to cause poor growth and FCR, with adverse blood biochemical changes, high mortality, morbidity and lameness.

Key words: Aflatoxin, Duck, Feed, Juvenile, White pekin.

INTRODUCTION

Duck farming (*Anas platyrhynchos*) in India, is mostly concentrated in eastern-coastal states (Rath *et al.,* 2015), which pose big challenges in the storage of poultry feed ingredients due to high relative- humidity (Madgwick *et al*., 2011) leading to build-up of mycotoxins (Pachauri and Reisinger, 2007), specifically, Aflatoxin-B_1 (AFB1) with productivity-drops in growing and laying ducks (Pandey and Chauhan, 2007). There are conflicting reports regarding toxic threshold for naturally- occurring AFB1 in feed, beyond which morbidity could surface in the host (Fouad *et al.,* 2019). Further to European Commission (2006 and 2011) recommendations, Mishra *et al.* (2016) have recommended the acceptable level of aflatoxins (AFB1) in grains as 10 ppb, for safe-usages in Poultry feeds. While AFB1 levels ranging up to 500 ppb in feed of chickens were viewed insignificant; AFB1 upto 250 ppb in diets of Turkey Poults and AFB1 levels upto 100 ppb in diets of ducklings were also viewed insignificant, to impact productivity of the respective subjects (Shabani *et al.,* 2010; Diaz *et al.,* 2009 and Fin *et al*., 2012).

Keeping the above in view, a study was conducted to ascertain the precise level of Aflatoxin-tolerance in White Pekin ducklings during juvenile ages.

MATERIALS AND METHODS

The experiment was carried out at ICAR-CARI Regional Center, Bhubaneswar. White Pekin day-old ducklings (240, avg. hatch weight: 55 gms) were randomly divided into 3groups and each group having 4 replicates of 20 day old ducklings (DOD). A standard duck starter-feed (2950 Kcal/kg, 21.80% CP), with negligible (<0.5 ppb, mostly from background content in best of the sourced grains) AFB1 was treated as control diet. Two treatment diets (T_1 and T_2) were prepared from the control diet by spiking with lab-cultured and purified AFB1, to measure 200 and 400 ppb respectively. Randomly, the above three feeds were offered to the three groups, *ad libitum,* for a period of six weeks.

The culture and purification of AFB1 was carried out as per Verma *et al.* (2004) using *Aspergillus parasiticus* (strain: NRRL-299, maintained at ICAR-CARI, Izatnagar, India),

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which were harvested, characterized and diluted appropriately (BIS; AOAC, 1995). The feed-samples were evaluated for AFB1 content, using a standard Flurometer (VICAM®, Milford, MA, USA).

Raising of ducklings and generation of data

Juvenile growth

All the DODs were raised on floor and standard managemental practices were followed through-out the experimental period. Daily feed consumption and weekly live weight were recorded and the FCR was derived as feed consumed per unit live-weight gain.

Biochemical analysis of blood samples

Blood was collected from 5 ducklings per replicate on 21 and 42 days of age. Serum was separated out from blood and stored at -20°C till usage. Gama glutamyl transpeptidase (GGT), Serum glutamate oxalate transaminase (SGOT), Serum glutamate pyruvate transaminase (SGPT), Cholesterol and Triglyceride were analyzed using standard bio-chemical kits (Coral Clinical Systems®, Goa, India).

Necropsy analyses, morbidity and mortality profiling

Post mortem (P.M) was conducted routinely, immediately upon succumbing of ducklings. Necropsy was conducted for all the sacrificed ducklings (a proportion of survivors: 4 to 6 ducklings per treatment) at 6 weeks of age, upon termination. The gross pathology of the sacrificed ducklings were carried out, following histopathological analyses of vital organs (liver, Kidney and Spleen) from 5 randomly selected carcasses, as per standard procedures. All morbidities were characterized.

Ethical clearances and approval

The whole experiment was conducted, taking proper care of ducklings, under ethical clearances from Animal Ethics committee, vide letter OUAT-IAEC No.6/ Dt. 9th Mar 2018.

Statistical analysis

The data were subjected to Statistical analyses using SAS software package (version 9.2) for Analysis of variance (ANOVA) and tests of significance for various traits (Tukey's Tests).

RESULTS AND DISCUSSION

There was significant depression in weekly growth and feed conversion efficiency in both the Toxin-fed groups, at all ages. The Aflatoxin-B1 (AFB1) impacted the weekly growth of Pekins, where the T_2 had higher quantum of damage compared to the T_1 in terms of morbidity and resultant growth parameters.

Variations in Body weight of ducklings

The growth of ducklings over 2 to 6 weeks underwent significant variation ($p \leq 0.05$), where the variation remained erratic between T_1 and T_2 indicating differential debilitating impact from the AFB1 on different ages of Juvenile growth (Table 1). The live weight in both the Toxin groups (T_1 and T_2) was significantly poorer than control, at most juvenile ages of measurements, especially from 2 to 4 Weeks. The differential bodyweight between the control and both- Toxin groups, remained in 200 to 300% ranges, where in continued mortality and morbidity in both toxin groups rendered them to be with highest C.V, indicating impact of AFB1 exposure, on uniformity of ducklings. In general, the reduction in bodyweight was in a *dose-dependent manner*, where T_2 showed higher variation than T_1, where higher quantity of toxins consumed apparently caused the depression. To generalize, body weight was significantly ($p<0.05$) reduced for ducklings fed with diets containing AFB1 exceeding 200 ppb, resulting in wider Feed conversion ratio, similar to reports of earlier workers (Han *et al.,* 2008) where decrease in body weight of hens fed with 2.0 to 8.0 ppm levels of AFB1, were noted across juvenile stages.

Feed consumptions

There was significant ($p<0.001$) decrease in feed consumption (g/day) in AFB1- treated groups, throughout 1st to 5th week age compared to control (Fig 1). Depressing effect of toxins (AFB1) was noticeable in ducklings fed AFB1 @ 400 ppb, indicating that ducklings detested AFB1's

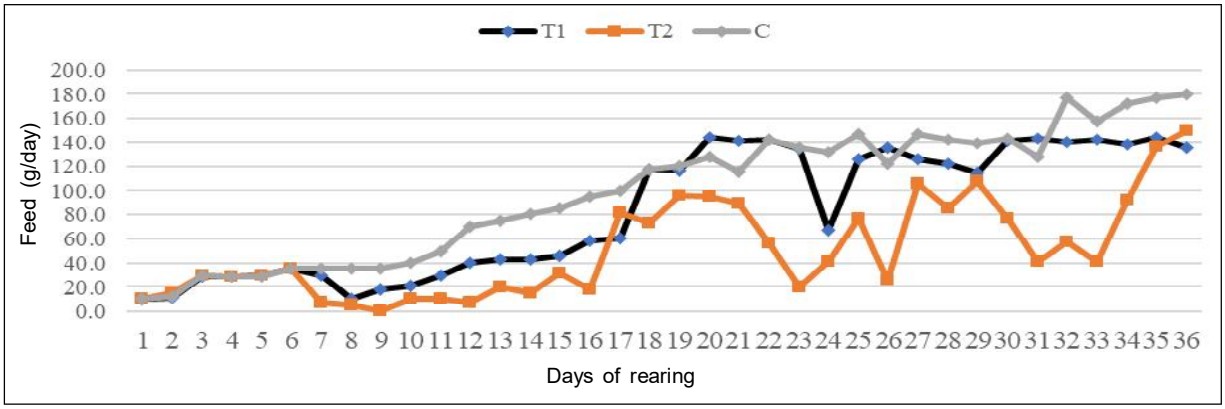

Fig 1: Daily feed consumption patterns (per duckling), in toxin-fed groups, *versus* control, (till 5 weeks age).

presence, with loss of appetite. The reduced cumulative feed consumption in both treated groups over control was probably due to impaired hepatic metabolism (Verma *et al.,* 2004).

Feed conversion efficiency

There was significantly-inferior feed efficiency ($p \leq 0.05$), in the toxin-treated ducklings (T_1 and T_2) during 2nd to 6th week of age (Fig 2). The poor feed conversion efficiency noted in both AFB1-groups in this study indicate that ducks are sensitive to mycotoxins with respect to feed efficiency that could be attributed to differences in source of contamination occurring in natural processes (Pandey and Chauhan, 2007). The inferior feed efficiency was observed specifically in higher-toxin diet (400 ppb) which indicated the negative effect from cumulatively-higher amount of toxin on vital organs, in supporting exuberant muscle growth and metabolism, as normally due in case of meat-type ducks (Pekins). The FCR ranged from 1.23 to 4.95 during 1 to 6 weeks. The FCR of ducklings tended to remain wider, upon increase in AFB1 level in feed: T_2 (@400 ppb) as compared to T_1 (@ 200 ppb) against control. Similar findings were also reported by earlier workers (Pandey and Chauhan, 2007; Chen *et al.,* 2016).

Live weight gain

The body weight gain of T_1 remained lowest, during the 2nd week of rearing, where as the control group gained the highest live weight, during 4th week of age (Fig 3). The T_2 remained lagging behind the other 2 groups, throughout course of study; where the growth performance remained lower in AFB1 treated groups, similar to patterns previously described by many authors (Han *et al.,* 2008; Khlangwis *et al.,* 2011; Murugesan *et al.,* 2015; Mahmood *et al.,* 2017).

Serum biochemical analyses

There was significant increase ($P<0.01$) in levels of liver-function enzymes namely, ALT, AST and GGT for the AFB1 treated ducks, compared to control. The T_1 group's liver enzyme status remained mid-way between control and T_2, showing

Table 1: Group-wise Means±S.E. for weekly live-weights along with C.V (%) through different weeks.

	1st week	2nd week	3rd week	4th week	5th week	6th week
Control	196.62±5.09^{a}	428.28±6.79^{a}	746.72±8.69^{a}	1231.45±24.2^{a}	1673.30±31.33^{a}	1954.8±38.38^{a}
(CV)	(13.2%^{x})	(10.8%^{x})	(10.2%^{x})	(8.8%^{x})	(8.4%^{x})	(8.8%^{x})
T_1	172.85±5.51^{a}	217.39±6.75^{b}	537.78±25.17^{b}	739.44±39.55^{b}	1101.91±65.92^{b}	1397.27±61.26^{b}
(CV)	(14.3%^{x})	(21.0%^{y})	(22.0%^{y})	(22.7%^{y})	(20.8%^{y})	(14.6%^{y})
T_2	152.58±4.88^{b}	175.68±3.89^{c}	202.33±7.36^{c}	470.40±60.55 ^{c}	855.50±57.83^{c}	1094±88.34^{c}
(CV)	(15.6%^{x})	(12.1%^{x})	(26.8%^{z})	(28.8%^{z})	(13.5%^{z})	(14.0%^{y})

N.B. Means bearing unequal superscripts column wise, within each estimate, differ significantly ($p<0.05$).

Table 2: Mortality percentages (Cumulative and [weekly]) of ducklings across experimental groups.

Weeks	1	2	3	4	5	6
Control	0	0	0	0	0	0
T_1 (%)	0 [0]	0 [0]	10^{c} [10]^{a}	10^{c} [0]^{a}	40^{c} [30]^{a}	45^{c} [5]^{a}
T_2 (%)	0 [0]	25^{d} [25]^{b}	60^{d} [35]^{b}	75^{d} [15]^{b}	80^{d} [5]^{b}	85^{d} [5]^{a}

N.B. Means (non-zero), within categories, bearing unequal superscripts columnwise, differ significantly ($p<0.05$).

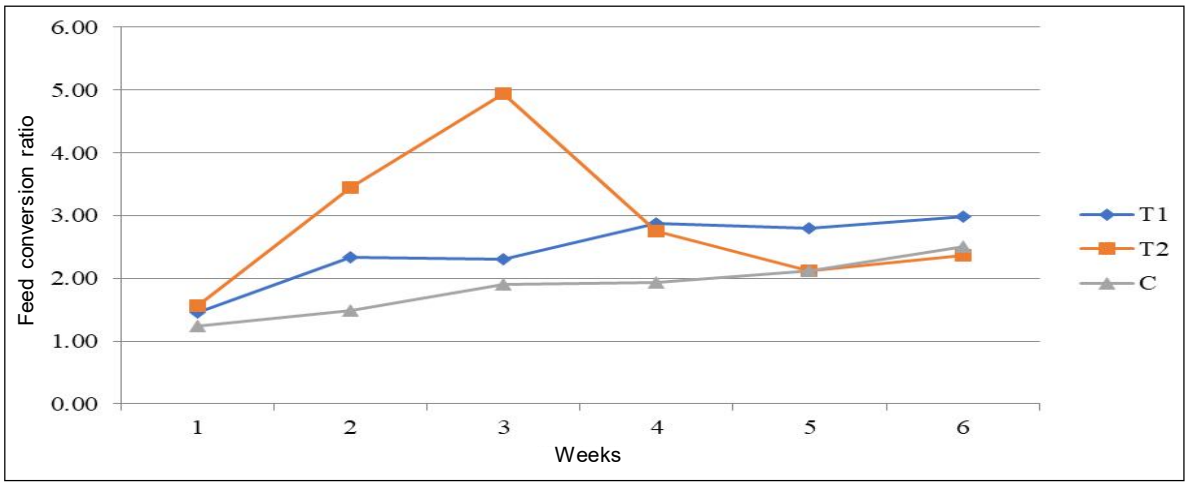

Fig 2: Mean feed conversion ratio across different weeks of growth, for the experimental groups.

a dose-dependent rise in these key enzyme's levels in liver, probably as a sequalae to hepatocyte's damage by Aflatoxin-B1 (Fernandez *et al.,* 1994). Significant increases (P<0.01) in the level of Triglyceride and Cholesterolat 3rd week and 6th week upon impact from AFB1, was recorded (Fig 4), parallel to the findings of earlier workers (Chen *et al.,* 2014).

Gross and histopathology

Significant gross-pathological changes were observed (Fig 5) primarily across 3 vital organs, *i.e.* liver, kidney and heart: but characteristic lesions were also visible on spleen too, which correlated well with available literature (Madheswaran *et al.,* 2005). There were changes in liver, signified by hepatomegaly with lesions, in ducklings fed with 400 ppb AFB1. Livers of T_1-ducklings revealed congestion with adjacent pale-patches and enlarged friable, loss of architecture with cloudy swelling and necrosis of hepatocytes; whereas in case of T_2 Liver, remarkable loss of architecture; necrosis with massive infiltration red blood cells (Fig 6) were prominent.

There was enlargement of kidney, at large, across all the specimens, with paleness evident in most samples drawn from T_1 (200 ppb) and T_2 (400 ppb). Nephrotic changes in kidney; including petechial hemorrhages, across whitish streaks (apparently from Uric acid deposits) were usual features of kidneys which were little-variant from those experienced across regular mortalities (Monson *et al.,* 2014). Similarly, against the normal histological factors observed in kidney from control groups, in case of T_1 kidney: slight degeneration of tubular epithelium with mild atrophy of glomerulus were noticeable and for T_2 Kidneys, distinct atrophy of glomeruli with necrosis of tubular epithelium were recorded. In T_1 spleen, mild congestion of red pulp with few nuclear debris and vacuoles were prominent; whereas across T_2 spleens, severe congestion of red pulp with Increase in Nuclear debris and vacuoles were evident.

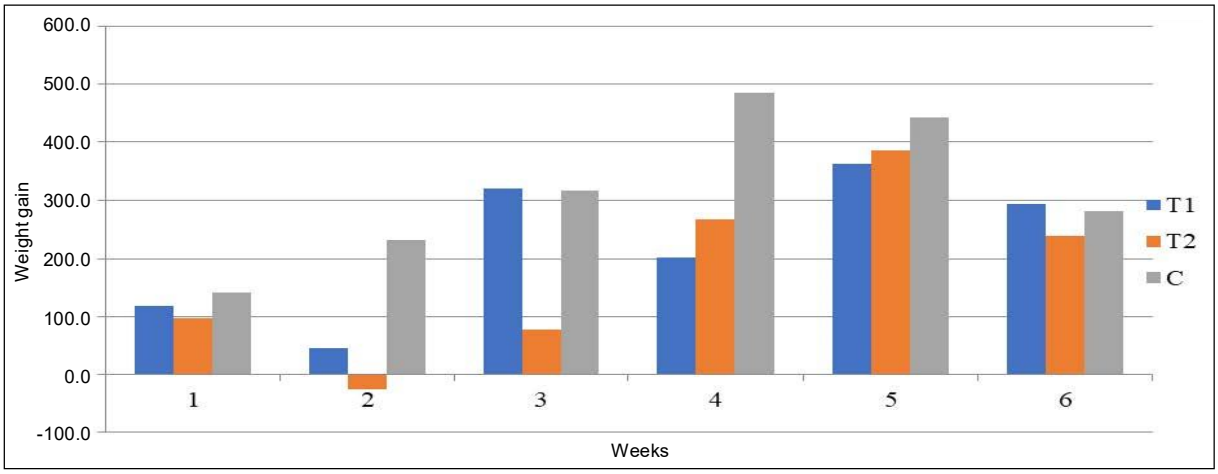

Fig 3: Average weekly weight gains per duckling, through weeks of growth, for treatment groups

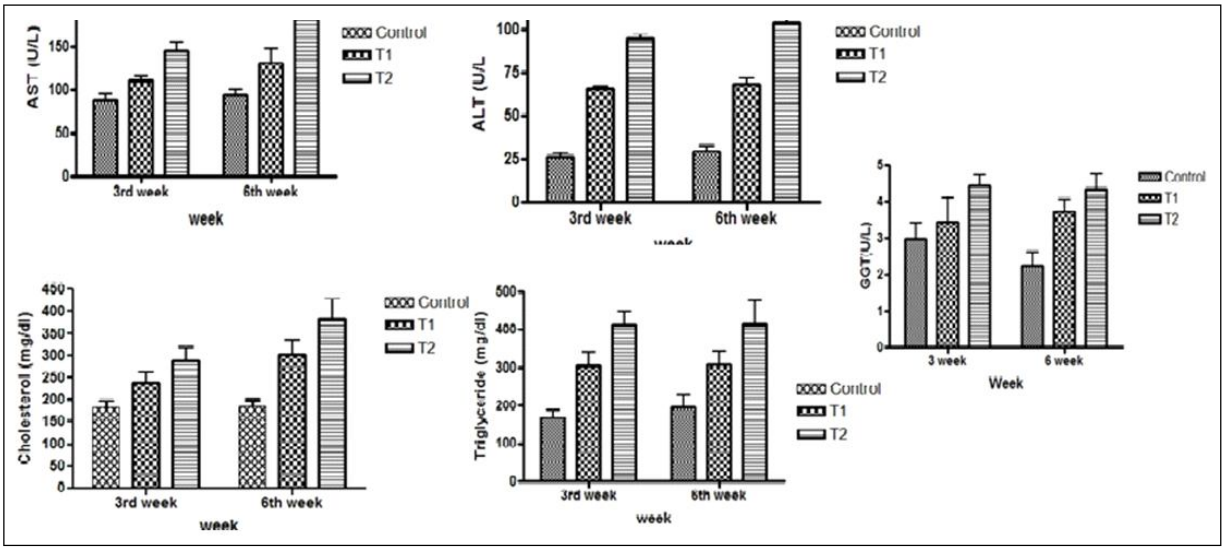

Fig 4: Biochemical parameters of different AFB1 groups [ALT, AST, GGT, Triglyceride and Cholesterol], at two time points.

The disturbed histopathological profiles across major necropsy specimens covering liver, spleen and kidney of the AFB1-treated pekin's profile, as realized in this study have also been documented by many authors (Chen *et al.,* 2014; Peng *et al.,* 2014), which concurs that: AFB1 at levels of 200 ppb, indeed constitutes a critical and vulnerable dose of AFB1.

Mortality and morbidity

The pattern of mortality in T_2 was in distinct contrast to that of T_1, where no mortality precipitated till 2nd week of age. There was 25% mortality in T_2 alone in 2nd week, apparently from poor-appetite and intense toxin-impact, which were similar to reports of Resanovic and Sinovec (2006). The

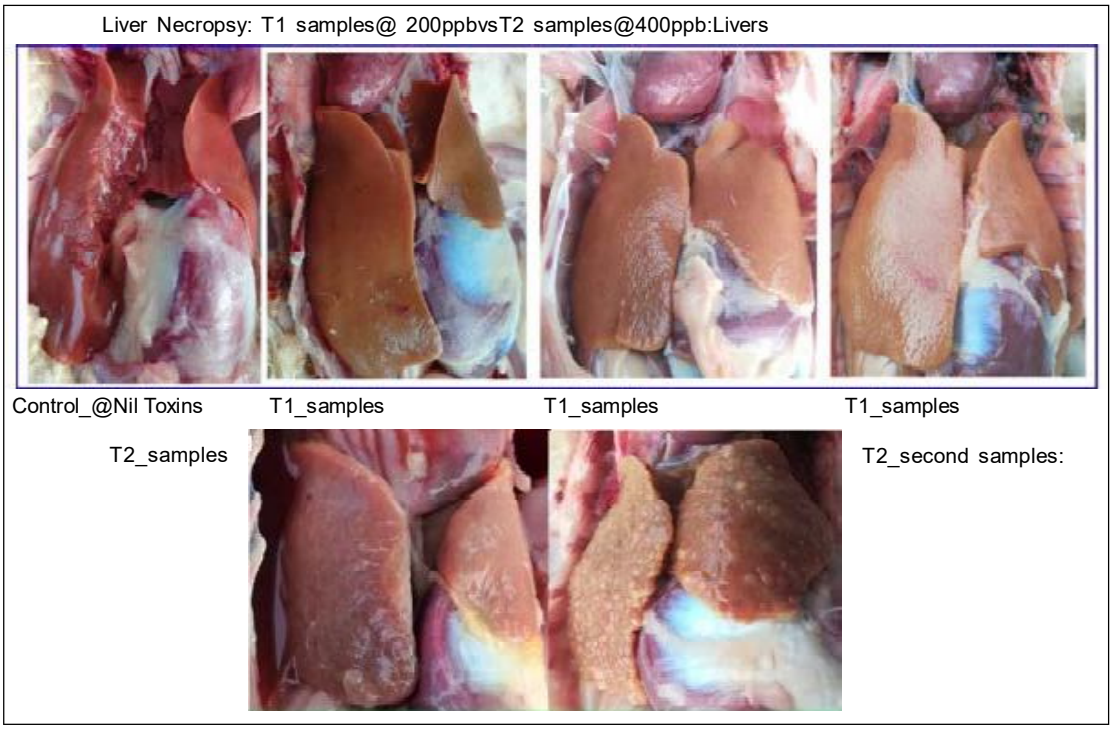

Fig 5: Necropsy profile of 42- day old White Pekin ducks: Control *Vs* T1 *Vs* T2, for selected liver samples.

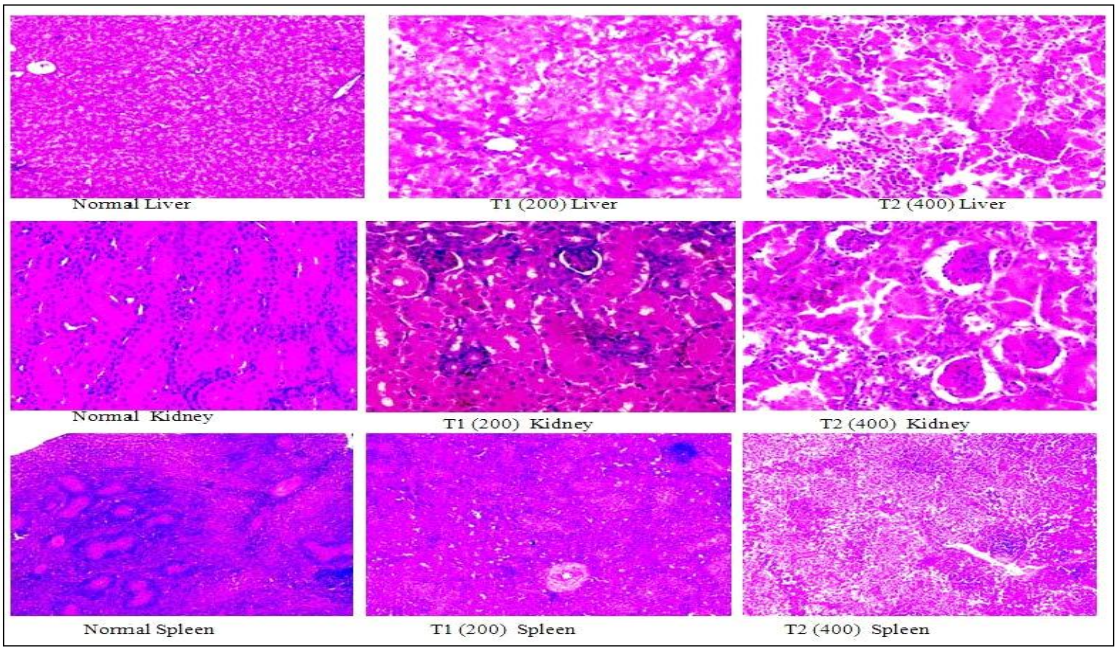

Fig 6: Histopathology of 3 vital organs of Pekins (42d) receiving varying dietary AFB levels.

mortality in T_1 occurred only after passage of 2nd week, where the peak-mortality became visible during 5th week of rearing. The T_1 had 10% mortality in 3rd week, followed by a spurt in same (30%) during 5th week, after which the mortality rate declined. Summarily, by 5th week of age, the T_2 had suffered nearly double the mortality of T_1 (cumulative mortality: 80% versus 40%, Table 2), whereas mortality in both groups came down by 6th week of age (5% in each group), which signified rising tolerance of ducklings to AFB1 intakes to some extent. The control group did not evince any mortality, throughout the 6 weeks of dietary toxicosis.

The control group which did not shown any morbidity and had the highest uniformity in live weight, as inferred from coefficient of variation (Table 1). The T_2 remained highly morbid, with display of non-specific problems in gait (difficulty in walking), which precipitated into distinct lameness; besides poor growth; wide variation in live weight with signs of flightiness and nervousness (Khlangwis *et al.,* 2011). After 3rd and 4th week, T_1 revealed more variability compared to T_2, where Poor uniformity remained a hallmark of both AFB1-fed groups (C.V ranging: 21-29%) during morbid period. The control group maintained the best live-body weight across the entire trial period, where differences in live weight between the control and both toxin-groups remained significant ($p<0.001$) from 3rd to 6th week period (Chen *et al.,* 2014). The liveweight in T_1 across all ages vis a vis Control and T_2 indicated that morbidity in AFB1-fed groups acted in a dose dependent manner (Murugesan *et al.,* 2015). The sensitivity of ducks to AFB1, at different levels, in the diets have been reviewed by Fouad *et al*. (2019), where AFB1-levels creating morbidity in ducks ranged from 20 to 1100 ppb. Our study reporting significant morbidity of ducks at 200 ppb AFB1 or above is well supported by the analogy from earlier authors (Fouad *et al*., 2019), though ours varied a bit for the threshold of AFB1 @ 300 ppb reported by Fin *et al*. (2012).

Aflatoxin induced leg deformities

It was observed beyond 3rd week of growth that many surviving Pekins developed lameness, across the hock joints, in both T_1 and T_2, in varying proportion. Typically, these lame ducks continued to remain live with slower growth, but without much agility, where most deformities were irreversible, despite medical amelioration. Such lameness resulted from feeding of AFB1, could plausibly be attributed to decline in resistance of long-bones (femur and tibiotarsus) to breakage (Maurice *et al.,* 1983) or to changes in cholecalciferol metabolism for hepato and nephrotoxic nature of AFB1 (Huff *et al.,* 1980), which are subject to further investigations.

CONCLUSION

It can be concluded that, while the AFB1 content of juvenile White Pekin ducks should be kept limited to the recommended safe levels (<10 ppb); exceeding a threshold of 200 ppb is sure to cause poor growth and FCR, with adverse blood biochemical changes, high mortality, morbidity and lameness.

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Conflict of Interest statement

The authors declare that they have no conflict of interest.

REFERENCES

AOAC. (1995). Official Methods of Analysis. 16th Ed., Association of Official Analytical Chemists, Washington, D.C.

Chen, X., Horn, N., Cotter, P.F., Applegate, T.J. (2014). Growth, serum biochemistry, complement activity and liver gene expression responses of Pekin ducklings to graded levels of cultured aflatoxin B1. Poultry Science. 93: 2028-2036.

Chen, X., Murdoch, R., Zhang, Q., Shafer, D.J., Applegate, T.J. (2016). Effects of dietary protein concentration on per-formance and nutrient digestibility in Pekin ducks during aflatoxicosis. Poultry Science. 95: 834-841.

Diaz, G., Cortes, A., Botero, L. (2009). Evaluation of the ability of a feed additive to ameliorate the adverse effects of AF in turkey poultries. Br. Poultry Science. 50: 240-250.

European Commission. (2006). Commission Regulation (EC) No 1881/2006 of 19 December 2006 Setting Maximum Levels for Certain Contaminants in Food Stuffs. Official J. European Union, Lex. 364: 5-24.

European Commission (2011). Commission Regulation of 16 June 2011. Amending Annex I to Directive 2002/32/EC of the European Parliament and of the Council as Regards Maximum Levels for Nitrite, Melamine, *Ambrosia* spp. and Carry-Over of Certain Coccidiostats and Histomonostats and Consolidating Annexes I and II thereto, 574/2011/EU. In: Official Journal, L 159, pp 7-24.

Fin, C.D., Mallmann, C.A., Dilkin, P., Giacomini, L.Z., Machado 1, L.V. and Mallmann, A.L (2012) Effects of Aflatoxins on the Performance of Pekin ducklings. Proceedings, XXIV World's Poultry Congress, 5-9 August, Salvador, Bahia, Brazil.

Fernandez, A., Verde, M.T., Gascon, M., Ramos, J.J, Gomez, J. (1994). Aflatoxin and its metabolite residues in edible tissues from laying hens and broiler chickens fed a contaminated diet. Journal Food Science Agriculture. 65: 407-414.

Fouad, A.M., Ruan, D., El-Senousey, H.K., Chen, W., Jiang, S., Zheng, C. (2019). Harmful effects and control strategies of aflatoxin B produced by *Aspergillus flavus* and *Aspergillus parasiticus* strains on poultry: Review. Toxins (Basel). 11: 176.

Han, X.Y., Huang, Q.C., Li, W.F., Jiang, J.F., Xu, Z.R. (2008). Changes in growth performance, digestive enzyme activities and nutrient digestibility of cherry valley ducks in response to aflatoxin B1 levels. Livestock Science. 119: 216-220.

Huff, W.E., Doerr, J.A., Hamilton, P.B., Hamann, D.D., Petersen, R.E. and Clegler, A. (1980). Evaluation of bone strength during aflatoxicosis and Ochratoxicosis. Applied Environmental and Microbiology. 40: 102-107.

Khlangwiset, P., Shephard, G.S., Wu, F. (2011). Aflatoxins and growth impairment: A review. Crit. Rev. Toxicol. 41: 740-755.

Madgwick, J.W., West, J.S., White, R.P., Semenov, M.A., Townsend, J.A., Turner, J.A., Fitt, B.D. (2011). Impacts of climate change on wheat anthesis and *Fusarium* ear blight in the UK. European Journal of Plant Pathology. 130: 117-131.

Madheswaran, R., Balachandran, C., MuraliManohar, B. (2005). Pathological effects of feeding aflatoxin and T-2 toxin in Japanese quail. Indian Journal of Veterinary Pathology. 29: 23-26.

Manafi, M. (2011). Effect of feeding different levels of aflatoxin in the diets on production performance of broiler breeders. Advances Environment Biology. 5: 3843-3848.

Maurice, D.V., Bodine, A.B and Rehrer, N.J. (1983). Metabolic effects of low aflatoxin B1 on broiler chickens. Applied Environmental Microbiology. 45: 980-984.

Mishra, S.K. (2016). Evidence of Genetic-background Impacting Duck's Susceptibility to Aflatoxicosis More than Climate in Terms of Mortality and Productivity-drops in Tropical Indian Conditions. S12-0053, Proceedings, XXV World's Poult. Cong., September, 5-9, Beijing, China.

Monson, M.S., Settlage, R.E., McMahon, K.W., Mendoza, K.M., Rawal, S., el-Nezami, H.S., Coulombe, R.A., Reed, K.M. (2014). Response of the hepatic transcriptome to aflatoxin B1 in domestic turkey (*Meleagris gallopavo*). PLoS One. 9: e100930.

Mahmood, S., Younus, M., Aslam, A and Anjum, A.A. (2017). Toxicological effects of aflatoxin B1 on growth performance, humoral immune response and blood profile of Japanese Quail. Journal of Animal and Plant Sciences. 27(3): 833-840.

Murugesan, G.R., Ledoux, D.R., Naehrer, K., Berthiller, F., Applegate, T.J., Grenier, B., Phillips, T.D., Schatzmayr, G. (2015). Prevalence and effects of mycotoxins on poultry health and performance and recent development in mycotoxin counteracting strategies. Poultry Science. 94: 1298-1315.

Pachauri, R.K. and Reisinger, A. (2007). Climate Change 2007 Synthesis Report: Summary for Policymakers. IPCC Secretariat, Cambridge, UK.

Pandey, I. and Chauhan, S.S. (2007). Studies on production performance and toxin residues in tissues and eggs of layer chickens fed on diets with various concentrations of Aflatoxin B1. British Poultry Science. 48: 713-723.

Peng, X., Zhang, K., Bai, S., Ding, X., Zeng, Q., Yang, J., Fang, J., Chen, K. (2014). Histological lesions, cell cycle arrest, apoptosis and T cell subsets changes of spleen in chicken fed aflatoxin-contaminated corn. International Journal of Environmental Research Public Health. 11: 8567-8580.

Rath, P.K., Mandal, K.D. and Panda, P. (2015). Backyard poultry farming in India: A call for skill upliftment. Research Journal Recent Science. 4: 1-5.

Resanovic, R. and Sinovec, Z. (2006). Effects of limited feeding of aflatoxin B1 contaminated feed on the performance of broilers. Mycotoxins Research. 22: 183-188.

Shabani, A., Dastar, B., Khomeiri, M., Shabanpour, B., Hassani, S. (2010). Response of broiler chickens to different levels of nanozeolite during experimental aflatoxicosis. Journalof Biological Sciences. 10: 362-367.

Verma, J., Johri, T.S., Swain, B.K. and Ameena, S. (2004). Effect of graded levels of aflatoxin, ochratoxin and their combinations on the performance and immune response of broilers. British Poultry Science. 45: 512-518.