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Protective action of N-acetyl cysteine in hepato-renal functions of aflatoxicated white pekin ducks

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Abstract

The study one was aimed to determine the haematological, clinical, biochemical, histopathological changes occurred due to aflatoxicosis in white pekin ducklings and its amelioration by n-acetyl cysteine (NAC). A total of 120, day old ducklings, of old white pekin variety were randomly selected and divided into four groups of 30 ducklings of in each group, having 3 replicas of 10 each. The group 1 was considered as control group and were fed with normal basal diet and water daily for 28days. Aflatoxin (AF) was added at the rate 100 ppb in remaining all group. NAC was added to feed of group 3 and group 4 ducklings at the rate 3.2 and 5.2 gm/kg body weight. The ducklings were found dull, depressed, anorectic and showed signs of lameness. At 28th day after the administration of aflatoxins, blood samples were collected for biochemical and haematological analysis. Group 2 ducklings showed increased serum enzyme levels like ALP, AST, ALT, creatinine and urea in comparison to control ducklings. In group 3 slight decrease in serum AST, ALT, ALP, Urea and creatinine levels as compared to group 2 that is aflatoxin treated group. There was significant increase in malondialdehyde (MDA) levels in different organs and decrease in Aflatoxin induced (ROS) along with marked decrease in CAT, GPx, and SOD levels. The increase in serum levels of ALT, AST, ALP, creatinine and urea levels were also noticeable. In group 2. Histopathological changes were observed like vacuolar degeneration in liver, mild congestion in liver, increased bowman space in glomeruli of kidney, necrosis and degeneration of tubular epithelial cells of kidney. But group 3 and 4 ducklings showed improvement in gross and histopathological changes are less severe. Present study revealed thr usefulness of NAC administration for the treatment of aflatoxicosis in ducklings.

Keywords: Aflatoxicosis, *White Pekin ducks,* N-acetyl cysteine (NAC), Hepato-renal protection

Introduction

Aflatoxins are the secondary metabolites of toxico-genic fungi *Aspergillus parasiticus, A. flavus* grows on animal feed and cause adverse biological effects when consumed in sufficient amount. There was reported decrease in appetite, weight gain, glutathione peroxidase activities, and superoxide dismutase concentrations in the liver (Abdel-Wahab *et al.*, 2006) [3]. It is one of the most common organismal metabolites in food and has one of the highest toxigenic activities (Richard, 2007) [90]. Aflatoxin activity induces formation of reactive oxygen species (ROS) that results in oxidative stress. ROS includes superoxide anion, hydrogen peroxide and hydroxyl radicals that are formed during the bioactivation of AF in liver by hepatic enzymes. It was diminished by the addition of N-acetyl cysteine (NAC) an anti- oxidant containing thiol group in animal feed (Hassan and Mansour 2016) [47]. The fungal toxins impact on animal health extends way beyond the end result of death. The monetary impact due to decreased productivity, deceased feed efficacy, reduced weight gain, reduction in meat and egg production, increased disease occurrence, hidden damage to vital organs with reproduction interference is much higher as compared to immediate morbidity and lethality (Venâncio and Paterson 2007) [99]. Administration of NAC, acetylated L-cysteine amino acid (C5H9NO3S), has been safely used in humans to mitigate oxidative stress, mitigation of liver injury and prevents plasma and liver GSH depletion due to aflatoxicosis with a daily dose as high as 500 mg/kg (Cam *et al.*, 2007) [97]. NAC has been previously used as a mucolytic medicine in plethora of respiratory diseases, heart diseases and neoplasia. Some researcher reported there is increase in serum triglyceride and cholesterol levels (Hassan and Mansour 2016) [47]. The serum levels of alanine transaminase

(ALT), aspartate transaminase(AST) and alkaline phosphatase (ALP) had been recognized widely as sensitive indicators in the hepatic tissues impairment and biliary system failure due to aflatoxin fed to the rat (Abdel-Wahhab and Aly, 2003) [2].There was increase in serum ALT, ALP and AST activities indicates primary damage to hepato-cellular structure resulting from AFB1treatments (Abdel-Wahab *et al*., 2006) [3]. Whereas, reduction in total serum protein and complementary activities may result in Aflatoxin to initiate immunotoxicity (Azzam and Gabal, 1998). The decreased total protein and albumin level and the increased urea levels indicated the protein synthesis inhibition, increase of protein catabolism and thus renal dysfunction (Jindal *et al*., 1994) [57].

The present study was conducted to evaluate the efficacy of NAC i.e. N-acetyl-L-cysteine and to ameliorate the aflatoxin toxic effects in ducklings.

Materials and Methods

The present investigation was conducted in the Department of Veterinary Clinical Medicine, Ethics and Jurisprudence, Odisha University of Agriculture & Technology, Bhubaneswar and in collaboration with RS-DPR Bhubaneswar during month of November to December of 2021. A total of 120, day old ducklings, of old white pekin variety were randomly selected and divided into four groups of 30 ducklings of in each group, having 3 replicas of 10 each. Aflatoxin (100ppb). added feed was given to birds of group 2, group 3, group 4 for 4 weeks of age. N-acetyl cysteine (NAC) was given as cotreatment to group 3 and group 4 ducklings at the dose rate 3.2gm and 5.2 gm/kg BW respectively. Ad Lib water and feed were made available to the birds throughout the experiment. The experiment duration was 28 days, which includes 14 days starter diet and grower diet from day 14 to 28.

Feed consumption was evaluated on a group basis every alternate day by weighing and weekly average of feed intake was determined. The initial and weekly body weights were analysed. The protocol for experimental was ethically conducted in college premise.

N-Acetyl Cysteine

N-Acetyl Cysteine was sourced from Sigma Chemical Co.

Measurement of body-weight

Ducklings were weighed at 7 days interval using a digitally calibrated weighing balance, starting from day 1 up until experiment completion. Recording of Feed consumption and Live-was done at varying ages, and average daily weight gain (ADG), average daily feed intake (ADFI) was calculated using the data.

Collection of tissue samples for study of histopathological examination changes in ducklings supplemented with aflatoxin.

After the completion of feeding trial for 4 weeks, the birds were sacrificed and organs were observed for any gross lesions. The tissue samples from liver, spleen, kidney, thymus and bursal tissue were collected in 10% formalin (buffered neutral) solution for histopathological change examination.

Investigation of clinical signs and symptoms in ducklings subjected to aflatoxin

Birds were constantly observed for behavioral alterations on daily basis up to the end of the trial. The clinical symptoms in ducklings were investigated daily which were exposed to aflatoxin. The investigation was done daily but noon period was specially observed when sun was in peak.

Study of hematological changes in ducklings exposed to aflatoxin

The peripheral venous blood was collected aseptically in sterile test tubes from medial tarsal vein by using 24 gauge needles. For haematological study, blood was collected using anti-coagulant sterile vial (EDTA) @1.0mg/5ml of blood as recommended by Jain (1986) [110]. The PCV value was estimated by Microhematocrit capillary tube method. Sahli's acid haematin method was used to estimate haemoglobin and differential count (Heterophils, Eosinophils, Basophil, Lymphocytes and Monocyte) by modified Giemsa stain.

Serum Biochemical study

The serum samples were timely subjected to different biochemical parameters assays in

UV biospectrophotometer from (EPPENDORF) company using commercial kits from (CORAL^R). These parameters get estimated total protein, albumen, globulin, Triglycerides, urea, cholesterol, uric acid, creatinine, GGT, Calcium, phosphorus, AST (SGOT), ALT (SGPT).

Histopathological Investigation

Liver samples were collected from both aflatoxin fed and control group for evaluation of abnormalities. Samples were collected from 5 ducklings from each group with intermediate weight and was preserved in 10% formalin (neutral buffered) solution, dehydrated using graded alcohol, and then implanted in paraffin. Sections of embedded tissue was obtained in the thickness of 3 to 5 μm and stained using hematoxylin eosin stain. A total of two liver tissue sections from each duckling was examined for aforementioned lesions like vacuolar hepatic degeneration, peri and interlobular inflammation and necrosis and bile duct hypertrophy or hyperplasia using light microscopy (Pandey and Chauhan, 2007) [80]. Sections was scored using 0,1,2 and 3 score depending on slight to moderate or intense lesion presence, respectively.

Study of various oxidative stress biomarkers

Assessment of oxidative stress was done by assaying of erythrocyte oxidant - antioxidant status (Jena *et al.*). The Placer method (1967) was used for lipid peroxides estimation in RBC haemolysate. DTNB (5,5'-Dithio-bis (2-nitrobenzoic acid)) method of Prins and Loos (1969) was used for estimation of Glutathione (GSH).

Bergmayer (1983) method of Catalase activity estimation using H_2O_2 as a substrate was used and Superoxide dismutase (SOD) was estimated by the method described in the studies of Madesh and Balasubramanian (1998) [111].

Ferric Reducing Antioxidant Power (FRAP) Estimation

FRAP analysis was done by ENZAssay^{TM} antioxidant activity estimation kit.

Statistical analysis

Control groups was found to be statistically significant at P<0.05 or lower. The biochemical parameters and oxidative parameters were subjected to unpaired student 't' test and ANOVA analysis (one way analysis of variance) along with

Tukey's post-test using the Graph Pad Prism software program version 4.03 (San Diego, California, USA), and the group differences was considered significant statistically at $P \leq 0.05$ and $P \leq 0.01$.

Results and Discussion

The environmental pollutions with their purging in to food chain have become a global menace and gained authorities attention all around the globe. Mycotoxins are the one of the most deleterious agents and causes a wide range of diseases with animal, and human health problems. Even though sensitivity of ducks toward aflatoxin is many folds more because of higher bioactivation activity than poultry, even then the research is extremely limited. The present evaluation was done to shed light on deleterious effects of AFs on internal organs and tissues, particularly the liver and kidney and its therapeutic management with NAC.

The correlation associated between mycosis, the environmental factors and mycotoxicosis in animals and their role in initiation of food borne infections was reported in details by Hassan *et al*., 2012 and 2014 [44]. The geographical location corresponding to specific climate qualities, land and vegetation are the important for the prevalence of fungi and common mould growth. Mould spores can disperse readily in air with light breeze or wind or in combination of both wind and rain. The climate and floral distribution also plays an important role in mould dispersion.

Species *Aspergillus flavus* known as a public health threat due to aflatoxin production causing varying degree of toxicity when consumed and is a potential carcinogenic compound. In developing countries, a direct correlation between hepatocarcinoma incidence and dietary aflatoxins intake was found. In addition, an estimated value of biosphere crop production contamination with aflatoxins is 25%. While, suspected that the primary source of infection was due to spore inhalation originating from mouldy hay or soil.

Effect of NAC on weekly body weight in ducklings exposed to aflatoxin (Table 1)

On completion of 28 day period, there was marked decrease ($p<0.05$) in body-weight in Group-II as compared to control group. However, body-weight was high ($p<0.05$) in Group-III and IV as compared to Group-II.

Effect on haematological parameters (Table 2)

It is evident that there was evident decrease ($P< 0.01$) in the haemoglobin levels, PCV (packed cell volume), TLC (total leucocyte count), lymphocyte count of mean in Group-II with aflatoxin as compared with control. These values show significant rise ($p<0.01$, $p<0.05$) in Group-IV (Aflatoxin 100ppb + 5.2 gm/kg BW NAC in feed), in comparison with group II. There is significant increase ($P<0.01$) in the levels of heterophil and heterophil, lymphocyte ratio from in Gr II in comparison to control group.

Effect on biochemical parameters (Table 3)

There was significant increase ($P<0.01$) in the biochemical parameters levels of liver enzymes such as ALP, AST, ALT, GGT and in the creatinine and BUN levels in Gr II in comparison to Gr I (control). There was a noticeable increase ($P<0.01$) in levels of triglyceride, cholesterol levels in Gr II as compared to Gr I (control), however the values of above parameters were significantly decreased ($P< 0.01$) in NAC treated group Gr IV (Aflatoxin 100ppb + 5.2 gm/kg BW NAC in feed) than Gr II.

Effect on oxidative stress parameters in liver (Table 4)

The aflatoxin has caused decrease ($P<0.01$, $P<0.05$) in SOD, GSH, catalase, and antioxidant levels in Gr II as compared to the control group however LPO value significantly increase ($P<0.01$) in Gr II as compared to the control group. But, in NAC treated Gr III, Gr IV SOD, GSH, catalase, antioxidant levels were found to be markedly increased ($P<0.01$,$P<0.05$). When compared with Gr II and LPO value showed significant decline in Gr III, Gr IV.

Effect on oxidative stress parameters in kidney (Table 5)

It was observed that aflatoxin has caused significant decline ($P<0.01$, $P<0.05$) in levels of SOD, GSH, catalase, antioxidant in Gr II when compared to control group, however LPO value showed increase ($P<0.01$) in Gr II when compared to control group. But, SOD, GSH, catalase, antioxidant levels were found to be increased ($P<0.01$, $P<0.05$) in Tulsi treated Gr-III, and Gr-IV, as compared with Gr-II and LPO value decreases significantly in Gr III and Gr IV, when compared with Gr II.

Gross and Histopathological changes

In group II (Aflatoxin 100ppb) liver was severely congested, enlarged, soft, and friable (Fig.1) and in some cases, liver was found with few petechiae along with fatty change (Fig. 2 and 3). Also in few of the cases there was presence of haemorrhage along with necrosis of the affected liver.

Grossly, in group II (Aflatoxin 100ppb), there was swollen and congested kidneys (Fig.4).

In group II (Aflatoxin 100ppb), spleen found to be pale (Fig.7) and some cases showed congested spleen (Fig. 18)

In group III (Aflatoxin 100ppb + NAC 3.2 gm/kg in feed) liver was found be slightly enlarged and pale. Similar changes were found in group IV (Aflatoxin 100ppb + NAC 5.2 gm/kg in feed) where liver was found to be less enlarged and pale.

In group III (Aflatoxin 100ppb + NAC 3.2 gm/kg BW in feed), kidneys showed mild congestion and slight enlargement (Fig.3). Similarly, with group IV (Aflatoxin 100ppb + NAC 5.2 gm/kg in feed) kidneys shows less congestion and enlargement.

The liver in group IV (Aflatoxin 100ppb + NAC 5.2gm/kg BW in feed) revealed apparently normal gross architecture without any congestion and enlargement.

Histopathological changes

Microscopically, liver of aflatoxin treated birds of Gr II showed massive loss of architecture, necrosis and mononuclear cell infiltration with hepatocyte vacuolation and condensed nuclei along with marked congestion with perivascular aggregation of inflammatory cells (Fig. 9), also focal infiltration of inflammatory cells with necrosis of hepatocytes and per lobular fibrosis.

Microscopically, kidneys showed Atrophy of glomerulus, and necrosis of tubular epithelium with interstitial congestion, interstitial haemorrhage and congestion with tubular epithelium showing degeneration and necrosis, glomerular degeneration and necrosis in aflatoxin treated group II (Aflatoxin 100ppb) with higher dose of 100ppb.

In group III (Aflatoxin 100ppb + NAC 3.2 gm/kg BW in feed), liver revealed diffused vacuolar degeneration of hepatocytes with mild sinusoidal congestion and perivascular inflammatory cells infiltration, hepatocyte necrosis with focal inflammatory cell infiltration.

In group III, where aflatoxin given at dose rate of 100 ppb along with NAC 3.2 gm/kg BW in feed some of the epithelial cells of tubular type showed desquamated basement membrane with pyknotic nuclei, interstitial congestion with increased Bowman's space, haemorrhages in glomerular tubules, atrophy and necrosis of glomeruli, cellular swelling of tubular epithelial cells with narrowing of lumen of the tubules.

In group III (Aflatoxin 100ppb + NAC gm/kg BW in feed, heart revealed sub-epicardial congestion and haemorrhage as well as intermycial oedema.

The group IV (Aflatoxin 100ppb + NAC 5.2 gm/kg BW in feed) birds revealed, marked hepatocytes vacuolation with pyknotic nuclei, focal mononuclear cells infiltration, mild sinusoidal congestion.

In birds of group IV (Aflatoxin 100ppb + NAC 5.2 gm/kg BW in feed), kidney revealed mild degeneration and desquamation from the basement membrane with pyknotic nuclei (Fig.31), diffused interstitial congestion along with degeneration of tubular epithelium and cellular swelling occluding the lumen of epithelium.

The group IV (Aflatoxin 100ppb + NAC 5.2 gm/kg BW in feed) birds showed mild myocardial congestion and disruption of muscle fibres at few places.

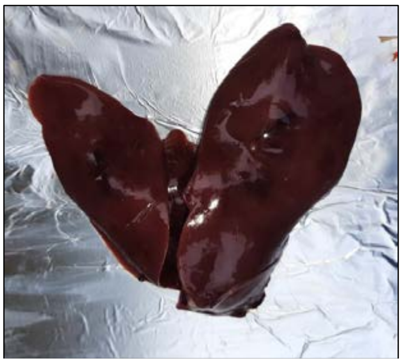

Fig 1: Group 2 showing improper margin of liver

Fig 29 Group 2 showing reticulated kidney

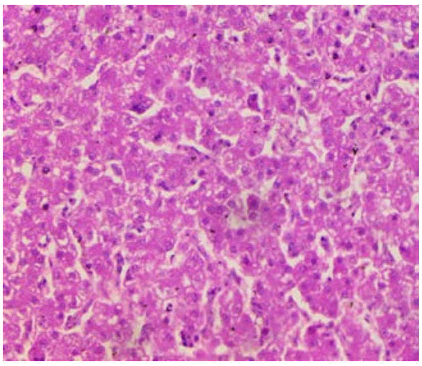

Fig 36: Vacuolar degeneration in liver

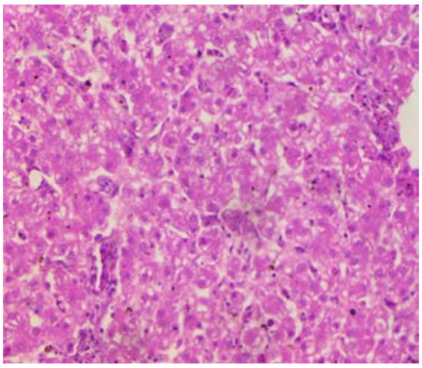

Fig 37: Mild congestion in liver

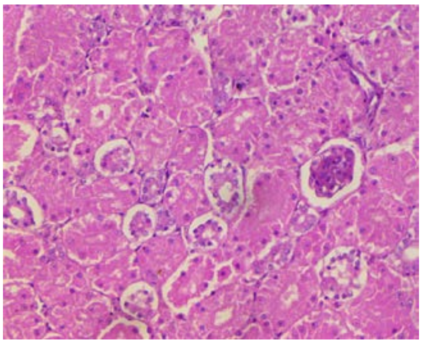

Fig 38: Increased Bowman space in glomeruli of kidney

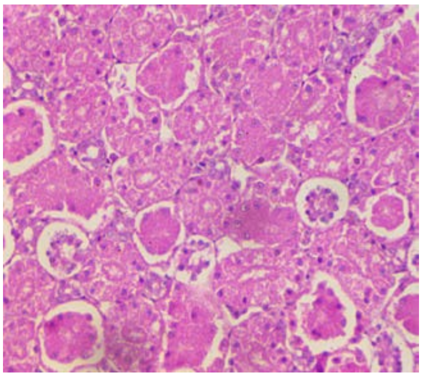

Fig 39: Mild degeneration and necrosis of tubular epithellial cell

Table 1: Effect of NAC on weekly body weight in ducklings exposed to aflatoxin.

Body - Weight	Group-1	Group-2	Group-3	Group-4
0 day	72.1±1.67^{a}	74.36±1.38^{a}	70.93±1.76^{a}	71.73±1.64^{a}
1st wk	125.56±2.65^{a}	111.73±6.42^{a}	126.56±10.81^{a}	118.5±2.50^{a}
2nd wk	445.2±11.32^{a}	329.1333±9.50^{a}	375.5±8.00^{a}	424.5±11.82^{a}
3rd wk	759.1±17.43^{a}	558.033±18.03^{bc}	590.5±18.69^{bc}	675.2±11.82^{bc}
4th wk	1140±27.90^{a}	775±24.10^{b}	810.9±18.36^{c}	923.63±22.31^{cd}

Table 2: Effect on haematological parameters.

Haematological Parameter	Group-1	Group-2	Group-3	Group-4
PCV	35.33±1.49[a]	26.13±1.42[b]	30.33±1.73[b]	32.25±1.38[bc]
Hb	10.45±0.440[a]	5.1±0.188[b]	8.2±0.259[b]	9.15±0.295[b]
Lymphocyte	58.17±1.28[a]	51.5±1.57[bc]	56±1.48[cd]	62.50±1.41[ac]
H:L Ratio	0.64±0.04[a]	0.84±0.033[b]	0.56±0.03[bc]	0.57±0.02[bc]

Table 3: Effect on biochemical parameters

Biochemical Parameters	Group -1	Group-2	Group-3	Group-4
GGT (U/ L)	42±2.33[a]	89.93±3.17[b]	70±6.20[b]	63.5±4.57[bc]
TG (mg/dL)	60±5.99[a]	83.16±4.64[b]	167.1±7.14[b]	140.83±8.2[bc]
ALP(U/L)	227.66±9.44[a]	378±7.87[b]	313.5±13.38[b]	315±16.60[b]
ALT(U/L)	28±1.97[a]	38±1.38[b]	35±2.14[b]	30±1.74[bc]
AST(U/L)	182.33±4.22[a]	200.5±4.46[b]	199.83±5.83[b]	182.83±5.40[bc]
BUN (g/dL)	26.76±3.15[a]	37.98±1.80[b]	31.5±0.53[b]	32.5±1.01[bc]
Cholesterol(mg/dL)	114.9±1.60[a]	249±9.38[b]	225.6±8.85[bc]	204±7.7[c]
Creatinine(g/dL)	0.645±0.072[a]	1.66±0.108[b]	1.348±0.061[b]	1.18±0.084[b]

Table 4: Effect on oxidative stress parameters in liver

Oxidative Parameters	Group-1	Group-2	Group-3	Group-4
LPO(nmol/milligram protein)	381.76± 11.66[a]	700.5±40.5[b]	666.18±31.54[b]	596.5±7.8[bc]
CATALASE(nmol/min/mg protein)	11.15±2.0[a]	4.7±045[bc]	5.6±0.69[bc]	7.09±0.52[bc]
ANTI-OXIDANT(mmol H2O2 Eqv/L)	359.444±17.85[a]	236.77±14.24b	265±15.64[b]	295.774±0.5124[ab]
SOD(mcmol MTT formazan/mg protein)	54.0127±5.6[a]	32.776±4.6[b]	35.66±4.9[ab]	44.761±5.7[ab]
GSH(nmol/mg protein)	11±0.66[a]	6.9±0.34[bc]	6±0.54[bc]	8.05±0.43[bc]

Table 5: Effect on oxidative stress parameters in kidney.

Oxidative Parameters	Group-1	Group-2	Group-3	Group-4
LPO in terms of MDA (nmol/milligram protein)	367.7±20.76[a]	621.3±24.21[b]	618.17±28.20[bc]	577.33±23.34[c]
CATALASE (nmol/min/mg protein)	16.17±1.06[a]	15.15±0.60[b]	5.24±1.40[ab]	13.53±0.63[ab]
Anti-Oxidant(mmol H2O2 Eqv/L)	355±18.17[a]	234.8 ± 25.97[b]	266 ± 26.55[bc]	303.8 ± 27.41[c]
SOD (mcmol MTT formazan/mg protein)	49.78±6.03[a]	28.67±4.61[b]	36.5±8.08[ab]	52.17±5.19[ab]
GSH (nmol/mg protein)	15.20±1.11[a]	8.8±0.679[bc]	9.8±0.41[bc]	5.4±0.36[cd]

Conclusion

Aflatoxin reduces duck productivity, affects performance, and changes blood and serum parameters as well as histology, all of which have a substantial impact on a farmer's bottom line. In the current investigation, it was discovered that including NAC in toxin-containing meals has a preventive action on the negative outcomes of aflatoxins in ducks.

As NAC is an antioxidant compound it will help in easy amelioration of aflatoxin induced hepatotoxicity and nephrotoxicity because aflatoxin induced toxicity is also caused by ROS (Reactive oxidative species). Aflatoxin-induced growth disruptions, haematological and biochemical changes, and oxidative stress can all be found in feed. Different therapeutic doses of NAC shows efficacious effect in 5.2 gm/kg body wt. incorporated in feed. As a result, the current study found that incorporating NAC in aflatoxin-intoxicated meals can help mitigate aflatoxin's detrimental effects in ducks, such as growth abnormalities, haemato-biochemical alterations, oxidative stress, and gross and histological changes.

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