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Original Article

Ameliorative potentials of *Sida acuta* and vitamin C on performance and blood profiles of cocks exposed to aflatoxin B_1

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Abstract
This study aimed to assess the ameliorative effects of vitamin C and *Sida acuta* leaf meal (SALM) on the performance, haematology, and serum biochemistry of cocks fed dietary aflatoxin B_1 (AFB_1) contaminated diets. A total of 150 Isa White cocks of 24 weeks of age were distributed into five study groups: 1 (control), 2 (AFB_1 diet), 3 (AFB_1 diet + Vitamin C), 4 (AFB_1 diet + 2.50 g/kg SALM) and 5 (AFB_1 diet + 5.0 g/kg SALM). The inclusion of AFB_1 significantly ($P<0.05$) reduced the total weight gain despite significantly ($P<0.05$) increasing the feed intake, protein intake, energy intake and feed conversion ratio of birds in group 2. Also, a sharp ($P<0.05$) decrease in PCV and RBC as well as the differential white blood counts were noted among the birds in group 2. For serum biochemistry, a notable ($P<0.05$) decrease in serum proteins and abnormal elevations ($P<0.05$) in serum enzymes and creatinine with a reduction in the glucose were observed among birds in group 2. However, significant ($P<0.05$) restorations of these parameters were observed among the cocks fed vitamin C and varied inclusion levels of SALM. These findings underscore the potential of vitamin C and SALM as protective agents against AFB_1-induced toxicity in poultry diets.

INTRODUCTION

Aflatoxins, a group of mycotoxins produced by certain fungi, particularly *Aspergillus species*, have gained significant attention due to their detrimental effects on animal health and productivity. The pervasive contamination of animal feed with aflatoxins poses a serious threat to the poultry industry, resulting in impaired growth, compromised immune function, and alterations in biochemical parameters (Fouad *et al*., 2019). Research has shown that aflatoxin B_1 (AFB_1) contamination in animal feed can result in significant growth impairment in various livestock species, including poultry and swine. A study by Raju and Devegowda (2002) demonstrated that the inclusion of aflatoxin in poultry diets led to reduced weight gain and impaired feed conversion efficiency,

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ultimately impacting overall production performance. Furthermore, AFB_1 has been reported to suppress the immune system, rendering animals more susceptible to infectious diseases (Olarotimi *et al*., 2023). A report by Sarker *et al*. (2019) showed that AFB_1 exposure in poultry resulted in compromised immune responses, leading to increased susceptibility to viral and bacterial infections, thereby impacting the overall health and welfare of the birds. In another development, AFB_1 was widely recognized for its hepatotoxic properties. Continuous exposure to AFB_1 can lead to liver damage and dysfunction in animals (Olarotimi *et al*., 2023). In a study conducted by Hamid *et al*. (2013), the authors highlighted the hepatocarcinogenic effects of AFB_1, emphasizing its potential to induce liver tumour and other severe hepatic lesions in various animal species.
Also, AFB_1 contamination in animal feed has been associated with reproductive issues in livestock. El-Tarabany (2015) observed that cattle exposed to dietary AFB_1 exhibited reproductive disturbances, including reduced fertility rates and an increased incidence of stillbirths and abortions, emphasizing the impact of aflatoxins on reproductive performance.

To counteract the adverse effects of AFB1 on animal health and performance, researchers have explored various dietary interventions aimed at ameliorating the toxic impact of AFB_1 exposure on poultry. *Sida acuta*, commonly known as 'wire weed' or 'broom weed', is a plant with recognized medicinal properties, including antioxidant and hepatoprotective effects. Studies have shown that *S. acuta* possesses bioactive compounds such as flavonoids and alkaloids that can potentially mitigate the toxic effects of aflatoxins. For instance, research has shown that *S. acuta* possesses hepatoprotective properties due to its potent antioxidant activity. A study by Sreedevi *et al*. (2009) demonstrated the plant's ability to reduce oxidative stress and prevent liver damage, suggesting its potential as a protective agent against hepatotoxicity. Furthermore, *S. acuta* has been reported to exhibit anti-inflammatory activity attributed to its antioxidant components. Mah *et al*. (2017) highlighted the plant's capacity to inhibit inflammatory mediators and modulate oxidative stress, indicating its potential as an anti-inflammatory agent. *Sida acuta* has also been investigated for its neuroprotective effects, primarily linked to its antioxidant and free radical scavenging activities (Mah *et al*., 2017).

A study by Benjumea *et al*. (2016) indicated that *S. acuta* extract exhibited neuroprotective properties by reducing oxidative damage and preserving neuronal integrity, suggesting its potential in mitigating neurodegenerative conditions. *Sida acuta* has been associated with immunomodulatory properties that may indirectly contribute to growth enhancement. A study by Popoola *et al*. (2022) indicated that *S. acuta* extract exhibited immunomodulatory effects by enhancing immune response markers, which can potentially influence overall growth and development in animals.

In addition, Vitamin C, a well-known antioxidant, has been widely investigated for its ability to counteract oxidative stress induced by mycotoxin contamination. Vitamin C is recognized for its potent antioxidant properties (Gbore *et al*., 2020), which enable it to scavenge free radicals and reduce oxidative stress induced by aflatoxin B_1. Alpsoy and Yalvac (2011) suggested that vitamin C exhibited strong antioxidant activity, reducing the oxidative damage caused by mycotoxins in animals (Gbore *et al*., 2020; Olarotimi *et al*., 2023). Also, vitamin C has been shown to possess immunomodulatory effects, as highlighted in a study by Mousavi *et al*. (2019), where vitamin C supplementation enhanced the immune response and reduced the immunosuppressive effects of aflatoxin B_1 in animals. Vitamin C has equally demonstrated hepatoprotective effects, as indicated in a research by Attia *et al*. (2023), where vitamin C supplementation alleviated liver damage induced by aflatoxin B_1, reducing liver enzymes and preserving liver function. Hence, this study investigated the ameliorative potentials of *S. acuta* and Vitamin C on the growth performance, haematology, and serum biochemistry of cocks exposed to aflatoxin-contaminated diets. By evaluating key indicators such as weight gain, feed conversion ratio, haematological parameters, and serum biochemical profiles; this research aimed to elucidate the protective role of *S. acuta* with or without Vitamin C in mitigating the deleterious effects of AFB_1 exposure in poultry.

MATERIALS AND METHODS

Experimental site, animals, diets and materials

The research was conducted at the Teaching and Research Farm, Adekunle Ajasin University, Akungba-Akoko, Ondo State with 150 mature Isa White cocks model aged 24 weeks. The birds were divided randomly to five groups with each containing 30 cocks. The experiment lasted 12 weeks and the cocks received clean water and the experimental diet *ad libtum*. **For the preparation of *S. acuta*** leaf meal (SALM), fresh leaves of the plant were collected, air-dried for seven days and converted to powder. Aflatoxin B_1 (AFB_1) was produced by culturing autoclaved maize grains with a toxigenic strain of *Aspergillus flavus to* produce aflatoxin B_1. Then, the AFB_1-contaminated maize grains were included in the formulation of experimental diets containing 1mg AFB/kg. Briefly, 10g of the contaminated maize was mixed with 1kg of the basal diet and quantified for dietary contents of AFB_1. The result was 0.25 mg/kg AFB_1. Thus, 40g/kg of the maize was replaced for 40g/kg of pure maize to produce the experimental diet. This was quantified; the result was 1.005 mg/kg AFB_1. Five experimental diets were produced (Table 1) viz: Diet A (control/basal), Diet B (containing 1.0mg AFB_1/ kg), Diet C (containing 1.0mg AFB_1/ kg + 200mg vitamin C/kg), Diet D

Table 1. Ingredient composition of the experimental layer diets

	Inclusion levels of AFB_1 and SALM				
Ingredients (kg)	**1**	**2**	**3**	**4**	**5**
Maize	40	36	36	36	36
Soybean meal	6	6	6	6	6
Wheat Offal	22	22	22	22	22
Palm Kernel Meal	28	28	28	28	28
Bone Meal	1.4	1.4	1.4	1.4	1.27
Limestone	2	2	2	1.8	1.68
Salt	0.35	0.3	0.28	0.3	0.3
Contaminated Maize	0	4	4	4	4
Vitamin C	0	0	0.02	0	0
SALM	0	0	0	0.25	0.5
Premix	0.25	0.25	0.25	0.25	0.25
Total	**100**	**100**	**100**	**100**	**100**
Calculated Nutrients					
ME (Kcal/Kg)	2536.9	2536.9	2536.9	2540.8	2550.7
Crude Protein (%)	14.89	14.89	14.89	14.91	14.93
Calcium (%)	1.31	1.31	1.31	1.24	4.2
Phosphorus (%)	0.46	0.46	0.46	0.5	0.5
Lysine (%)	1.1	1.1	1.1	1.2	1.2
Methionine (%)	0.47	0.47	0.47	0.47	0.47
Crude Fibre (%)	5.06	5.06	5.06	3.77	3.76

Diets:
1 = Control/Basal,
2 = Basal + 1.00 mg/kg AFB_1,
3 = Diet 2 + 50 mg/kg Zn,
4 = Diet 2 + 2.50 g/kg SALM,
5 = Diet 2 + 5.0 g/kg SALM.

(containing 1.0mg AFB_1/ kg + 2.5g SALM/ kg) and Diet E (containing 1.0mg AFB_1/ kg + 5.0g SALM /kg).

Data collection

On weekly basis, the feed intake (FI) and weight gain (WG) were recorded. Feed conversion ratio (FCR) was calculated as the ratio of total feed intake (TFI) to total weight gain (TWG): **i.e. FCR = TFI/TWG.** Protein intake (PI) and energy intake (EI) were also determined as described by Olarotimi and Adu (2022) from the total feed intake, feed crude protein (FCP) and feed metabolizable energy (FME) contents: **i.e. PI= (TFI × FCP)/100 and EI = (TFI × FME)/1000.** Both protein and energy utilization indices were determined as the ratio of PI and EI to TWG respectively: i.e. **PU= PI/TWG and EU= EI/TWG.**

At the end of 12 weeks, 15 birds/ treatment were selected for blood sampling. Blood from each cock were collected into both haparinized and plain bottles for haematological and serum biochemical studies, respectively. Blood samples for serum biochemistry were centrifuged for 10 minutes at 3000 rpm to obtain clean supernatant serum. From the blood samples collected in heparinized bottles, haematological parameters such as red blood cells (RBC), white blood cells (WBC), haemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) were all determined according to standard procedures as highlighted by Olarotimi and Adu (2022). The harvested serum samples were used to determine the serum proteins (albumin, globulin and total protein), metabolites (creatinine and glucose) and enzymes (aspartate aminotransferase {AST}and alanine aminotransferase {ALT}) according to standard procedures reported by Olarotimi and Adu (2022).

Statistical analysis

Data collected were subjected to statistical analysis using one-way analysis of variance (ANOVA) procedure of SAS (2008). The significant treatments were compared using Duncan's multiple range test of the same software.

RESULTS

Growth performance

The results of the growth performance of cocks fed diets containing AFB_1 with or without vitamin C and *S. acuta* leaf meal are shown in Table 2. The inclusion of 1 mg/kg

Table 2. Growth performance of cocks fed diets with Aflatoxin B_1, Vitamin C and *Sida acuta* leaf meal (SALM)

	Diets						
Parameters	**1**	**2**	**3**	**4**	**5**	**SEM**	**P-Value**
Average initial Weights (kg/bird)	1.57	1.56	1.6	1.59	1.55	0.11	1.11
Average Final Weights (kg/bird)	2.30^a	2.01^c	2.16^b	2.26^{ab}	2.31^a	0.15	0.02
Total Weight Gain (kg/bird)	0.73^a	0.45^c	0.56^b	0.67^a	0.76^a	0.19	0.03
Total Feed Intake (kg/bird)	6.42^c	6.85^a	6.71^b	6.92^a	6.98^a	0.04	0.01
Feed Conversion Ratio	8.79^b	15.22^a	11.98^b	10.33^b	9.18^c	4.45	0.03
Protein Intake (kg/bird)	0.96	1.02	1.00	1.03	1.04	0.01	0.11
Protein Utilization	1.31^c	2.27^a	1.78^{ab}	1.54^b	1.37^c	1.00	0.02
Energy Intake (kcal/bird)	16.29^b	17.37^a	17.02^a	17.58^a	17.80^a	0.13	0.01
Energy Utilization	22.31^c	38.62^a	30.40^b	26.24^{bc}	23.43^c	13	0.01

Means in a row without common superscripts are significantly (P<0.05) different. SEM = Standard Error of Means, Level of significance = P<0.05. Diets: 1 = Control/Basal, 2 = Basal + 1.00 mg/kg AFB_1, 3 = Diet 2 + 50 mg/kg Zn, 4 = Diet 2 + 2.50 g/kg SALM, 5 = Diet 2 + 5.0 g/kg SALM.

AFB_1 in the diet significantly (P<0.05) reduced the average final weights (AFW) and TWG of the birds fed diet 2 while it caused a significant (P<0.05) increase in the TFI, FCR, PU, EI and EU of the cocks when compared with the cocks on the control diet (Diet 1). However, inclusion of vitamin C as inorganic antioxidant (Diet 3) significantly (P<0.05) improved AFW and TWG, and significantly (P<0.05) reduced the FCR, and EU of the birds. The PI, PU and EI values among the cocks fed diet 3 were not different significantly (P>0.05) when compared with the means of the birds on the control diet. Furthermore, the inclusion of SALM at 2.5 and 5.0 g/kg respectively enhanced the TWG, FI, FCR, PU and EI of the birds on diets 4 and 5 respectively with better performance recorded among the birds on Diet 5.

Haematology

Table 3 shows the results of the haematology of the cocks fed AFB_1 contaminated diets with or without Vitamin C and SALM. Significant (P<0.05) reductions in PCV, RBC, HB, heterophils, eosinophils, lymphocytes and monocytes values of the cocks fed Diet 2 were observed when compared with the values recorded among the cocks on the control diet.. Parameters such as MCV and MCH which were significantly (P<0.05) increased at the inclusion of AFB_1 in the diet was, however, reduced by the inclusion of vitamin C. Similarly, inclusions of SALM in the diets fed cocks in groups 4 and 5 brought about significant (P<0.05) positive enhancement of all the studied parameters when compared with the values recorded among the cocks fed diet 2.

Table 3. Haematology of cocks fed diets with aflatoxin B_1, Vitamin C and *Sida acuta* leaf meal (SALM)

Parameters	Groups					SEM	P-Value
	1	2	3	4	5		
Haemogram							
PCV (%)	40.08^{ab}	31.04^{c}	40.00^{ab}	41.01^{b}	42.11^{a}	1.80	0.04
RBC ($x10^6$ mm^3)	4.71^{a}	3.31^{c}	3.72^{b}	3.74^{b}	4.62^{a}	0.24	0.02
MCHC (g/dl)	33.03	32.44	33.00	33.00	33.00	0.01	0.79
MCV (fl)	80.12^{c}	110^{a}	100^{b}	90.81^{bc}	82.08^{c}	5.81	0.01
MCH (pg)	29.02^{b}	46.28^{a}	38.99^{ab}	30.16^{b}	30.01^{b}	1.90	0.03
Hb (g/dl)	13.09^{b}	9.17^{c}	13.10^{b}	13.97^{ab}	14.89^{a}	0.59	0.04
Differentials WBC (%)							
Heterophils	33.50^{a}	21.72^{c}	28.01^{b}	31.01^{ab}	32.79^{a}	1.75	0.01
Eosinophils	2.00^{a}	1.01^{c}	1.57^{ab}	1.41^{b}	1.99^{a}	0.26	0.01
Basophils	2.50	2.00	2.45	2.51	2.30	0.21	0.68
Lymphocytes	64.09^{b}	41.02^{c}	68.11^{b}	70.01^{ab}	74.11^{a}	2.72	0.02
Monocytes	2.35^{b}	0.82^{c}	2.21^{b}	3.52^{a}	3.68^{a}	0.66	0.01

Means in a row without common superscripts are significantly (P<0.05) different.
SEM = Standard Error of Means, Level of significance = P<0.05; PCV = Packed Cell Volume, RBC = Red Blood Cells, MCHC = Mean Corpuscular Haemaglobin Concentration, MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular Haemoglobin, Hb = Haemoglobin, WBC = White Blood Counts.
Diets: 1 = Control/Basal, 2 = Basal + 1.00 mg/kg AFB_1, 3 = Diet 2 + 50 mg/kg Zn, 4 = Diet 2 + 2.50 g/kg SALM, 5 = Diet 2 + 5.0 g/kg SALM.

Serum biochemistry

The effects of diets containing AFB_1 with or without SALM on the serum biochemistry of the experimental cocks are in Table 4. For inclusions of vitamin C and SALM, there were reductions (P<0.05) in the mean values of the parameters.

Table 4. Serum biochemistry of cocks fed diets with aflatoxin B_1, Zinc and *Vernonia amygdalina* leaf meal

Parameters	Diets					SEM	P-Value
	1	2	3	4	5		
Serum Proteins (g/l)							
Albumin	19.21^{c}	12.70^{d}	19.02^{c}	22.00^{b}	25.91^{a}	0.83	0.01
Globulin	20.03^{c}	12.30^{d}	18.15^{cd}	21.01^{b}	24.95^{a}	0.29	0.01
Total Protein	39.24^{c}	25.00^{d}	37.17^{b}	43.01^{a}	50.86^{a}	0.92	0.01
Serum Enzymes (U/L)							
Aspartate aminotransferase	110.15^{c}	160.01^{a}	111.00^{b}	116.10^{bc}	108.99^{c}	1.26	0.03
Alanine aminotransferase	18.91^{b}	48.12^{a}	18.01^{b}	21.94^{b}	19.00^{b}	0.62	0.02
Serum Metabolites (mmol/l)							
Creatinine	15.01^{c}	30.10^{a}	20.11^{b}	20.09^{b}	17.03^{bc}	3.4	0.04
Glucose	9.80^{a}	6.71^{b}	9.42^{a}	9.58^{a}	9.93^{a}	0.6	0.03

Means in a row without common superscripts are significantly (P<0.05) different. SEM = Standard Error of Means, Level of significance = P<0.05.

Diets: 1 = Control/Basal, 2 = Basal + 1.00 mg/kg AFB_1, 3 = Diet 2 + 50 mg/kg Zn, 4 = Diet 2 + 2.50 g/kg SALM, 5 = Diet 2 + 5.0 g/kg SALM.

the serum proteins and glucose, there were (P<0.05) reductions in the concentration of glucose, albumin, globulin and total protein respectively among the cocks fed diets containing AFB_1 contaminated diets. At the inclusions of vitamin C (diet 3) and SALM (diets 4 and 5), significant (P<0.05) improvements in serum proteins and glucose were observed among the cocks in groups 3, 4 and 5 when compared with the cocks in group 2 and the control. The cocks fed SALM presented better results than those on vitamin C with those in group 5 having the higher significant (P<0.05) means. However, the results recorded for glucose among the cocks fed vitamin C and varying inclusions of SALM were not different significantly (P>0.05). Conversely, significant (P<0.05) increases were observed in the serum enzymes (aspartate aminotransferase and alanine aminotransferase) and creatinine concentrations of the cocks fed diet 2. At the

DISCUSSION

The significant reductions in the average final weights (AFW) and total weight gain (TWG) of the birds in group 2 compared to the control diet indicates that AFB_1 has adverse effects on the growth performance of the birds. This has given credence to previous studies which highlighted the adverse effects of AFB_1 on growth (Dersjant *et al*., 2003), production (Ismail *et al*., 2020), feed intake and efficiency (Ditta *et al*., 2018) weight gain (Raju and Devegowda, 2002), immunity (Olarotimi *et al*., 2023). The significant improvements observed in AFW, TWG, feed conversion ratio (FCR) and energy utilization (EU) among birds in group 3, suggest that vitamin C supplementation helped mitigate the negative impacts of AFB_1 (Alpsoy and Yalvac, 2011) and improved the overall growth performance (Hieu *et al*., 2022) and nutrient utilization of the birds (Seven *et al*., 2012). The significant enhancement of various performance indicators emphasizes the potential benefits of using antioxidants and natural feed additives in poultry diets to counteract the negative impacts of mycotoxins and enhance growth and performance.

Furthermore, the significant reductions in packed cell volume (PCV), red blood cell count (RBC), hemoglobin (Hb), heterophils, eosinophils, lymphocytes, and monocytes levels among the cocks on diet 2 suggest that AFB_1 has adverse effects on the hematological profile of the birds. Our study strengthened the outcomes of Dönmez *et al*. (2012) who reported significant depressions in the RBC, WBC, Hb and PCV levels of Merino rams fed AFB_1 contaminated diets. These changes recorded in our study indicate potential disruptions in the birds' immune function and overall health due to AFB_1 contamination. Aflatoxin B_1 has the ability to predispose chickens to lymphocytopenia and monocytopenia as reported by the significant reduction in lymphocyte and monocyte by our study. The significant increases in the hematological parameters, which were reduced by the inclusion of 1 mg/kg AFB_1, upon the addition of vitamin C, highlight the potential protective effects of vitamin C against the hematological impacts of AFB_1. The ability of vitamin C to mitigate the negative effects of AFB_1 suggests its role in bolstering the birds' immune system and counteracting the detrimental impacts of mycotoxin contamination (Seven *et al*., 2012). The significant positive enhancements in all the studied parameters among the cocks fed diets with SALM (groups 4 and 5) compared to those on diet 2 underscore the potential benefits of SALM in improving the hematological status of the birds. These findings indicate the potential immunomodulatory effects of SALM, suggesting its potential as a natural feed additive for enhancing the birds' immune response and overall health (Popoola *et al*., 2022).

Also, the reductions in glucose, albumin, globulin, and total protein concentrations among the cocks fed diets containing AFB_1 suggest that AFB_1 contamination negatively influences serum protein levels and glucose metabolism. The present case supported earlier studies where AFB_1 had been reported to decreased serum protein concentrations (Denli *et al*., 2009; Chen *et al*., 2016) and Amiridumari *et al*. (2013) who documented significant decreases in serum total glucose, protein, albumin, total cholesterol and uric acid levels of broilers fed AFB_1. However, the significant improvements in serum proteins and glucose observed with the inclusion of vitamin C and SALM in diets 3, 4, and 5 indicate the potential of these dietary additives to counteract the detrimental effects of AFB_1 and improve serum protein and glucose profiles. This was not different from the opinion of Sahin *et al*. (2002) that supplemental vitamin C enhances serum protein and glucose levels in laying hens. The superior performance of SALM compared to vitamin C, particularly in group 5, suggests the beneficial impact of SALM on serum protein and glucose levels. Alagbe *et al*. (2020) previously highlighted the potentials of phyto-antioxidants in enhancing serum proteins and glucose. The significant increases in the concentrations of serum creatinine, aspartate aminotransferase and alanine aminotransferase among the cocks fed diet 2 suggest liver damage or dysfunction due to AFB_1 contamination (Olarotimi and Adu, 2022). However, the observed reductions in these parameters with the inclusion of vitamin C and SALM in diets 3, 4, and 5 indicate the potential hepatoprotective effects of these dietary components. These findings suggest that vitamin C and SALM may help mitigate the hepatic damage caused by AFB_1 and improve liver function (Sahin *et al*., 2002; Alagbe *et al*., 2020).

CONCLUSION

This study demonstrates the detrimental impacts of AFB_1 contaminated diets on the growth performance, hematological parameters, and serum biochemistry of the experimental cocks. The observed reductions in various growth and hematological parameters, as well as the alterations in serum protein and enzyme levels, underscore the potential health risks posed by AFB_1 in poultry diets. However, the dietary supplementation with vitamin C and *Sida acuta* leaf meal (SALM) exhibited promising mitigating effects, ameliorating the adverse impacts of AFB_1 on growth, immune function, and liver health. The notable improvements in growth performance, hematological profiles, and serum biochemistry parameters among the groups receiving vitamin C and SALM highlight the potential of these dietary interventions in bolstering immune responses, enhancing liver function, and improving overall health in poultry. This study underscores the significance of effective mitigation strategies to counteract AFB_1 contamination in poultry diets, with potential implications for enhancing immune responses, improving liver function, and safeguarding overall poultry health. Future research exploring optimal dosages and long-term effects of these interventions is warranted to develop comprehensive strategies for poultry health and product quality assurance.

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