Possible Nigella Sativa and Curuma longa Ameliorative Effect on the Hazardous properties of chicken broiler Aflatoxicosis

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Abstract

Study presented here was conducted to find out the possible positive effect and adding benefits for chicken ration supplement with Nigella Sativa (NS) and Curuma longa in reducing the aflatoxicosis negative effect on some physiological body parameters, antioxidant status. Two hundreds broiler chicks (Arbor Acres) were arbitrarily divided into 4 groups (5 replicates for each). During the experimental period (45 days), first group (negative control) received basal diet. Other experimental groups (second to fourth) during the first 25th days received basal diet containing 20ppm of aflatoxin (AFB1) then after (26th to 45th days) second group continue in AFB1 free basal diet, while group 3 received (AFB1) free plus Nigella Sativa (black seeds) at dose of 2% of basal diet, though the 4th group was fed a AFB1 free plus (Curuma longa) turmeric rhizome at a dose 1% of the basic ration. Results disclosed that AFB1 caused decreased body weight, increased morality, edematous carcasses, bleeding, liver surface appeared pale yellowish with reddish strikes, enlarged swollen kidneys, thymus, and bursa atrophy with necrosis. Serum analysis declared Curuma longa and Nigella Sativa significantly (P ≤ 0.5) reduced liver enzymes (AST and ALT), albumin compared to group 2 (aflatoxicated). As well as creatinine, urea, Cholesterol, malonaldehyde and triglycerides showed significant (P ≤ 0.5) improved. Addition of Curuma longa and Nigella Sativa to broiler chicks’ basal diets can ameliorate aflatoxins hazard effect and improve general health, growth rate, liver, and kidney’s function. Notably NS results were more promising

Keywords: Aflatoxicosis, Arbor Acres broilers, Nigella Sativa and Curuma longa.

Introduction

Aflatoxins (AF) are naturally occurring extremely toxic mycotoxins groups, these mycotoxins are the metabolites of Aspergillus parasiticus (Uysal and Agar, 2005). AF malignant, mutagenic, and teratogenic effects have been recorded (Mishra and Das, 2003). Aflatoxin B1 possess carcinogenic and genotoxic effect and can produce acute liver necrosis, cirrhosis, and carcinoma (Ehrlich et al., 2007). Aflatoxins negative effect may be attributed to their intermediate metabolic reactive, that bind to macromolecules results in transnational and transcriptional disruption (Diaz et al., 2005). Aflatoxin B1(AFB1) represent the most common form, although the aflatoxin intermittent occurrence, it represents a remarkable threat for human as well as livestock health (Abdelhamid, 2009). Poultry ration occasionally contaminated with one or more mycotoxin, climatic conditions (hot and humid) of the Mediterranean countries favors molds growth and infestation (Bacha et al., 1988). Contaminated food with AF ingestion considered as major source for AF exposure, it causes a lot of poultry industry economical and financial losses (Tedesco et al., 2004). Characteristics feature and cardinal signs of chicken aflatoxicosis are listlessness, reduced feed intake, negative feed conversions, decreased growth rates, fatty liver, decreased egg production, faulty pigmentation, mortality and reduced immune-response (Gholami-Ahangaran and Zia-Jahromi, 2013; Tedesco et al., 2004; Yarru et al., 2009). NS contains alkaloids, volatile, nonvolatile oils, and some active pharmacological substances such as carvacrol, Nigellicine-N-Oxide, Nelligidine, dithymoquinone, thymoquinone,and thymol (AlHomidan et al., 2002;
Several pharmacological uses for NS have been demonstrated, such as antioxidant, anti-neoplastic and anti-inflammatory effect (Aggarwal et al., 2003), hypolipidemic activities (Arun and Nalini, 2002), it is believed that NS is use is beneficial in management of these pathological conditions (Menon and Sudheer, 2007). From the biological point of view, NS possess several properties, authors (Mahmoud et al., 2002) have proven the anthelmintics rule of NS, others (Al-Hader et al., 1993) assured its ant-diabetic, while Zaoui et al. (2000) confirmed its diuretic like effects. Microbiologically few studies declared that NS possess anti-bacterial activities (Nair et al., 2005), likewise it is used for biliary diseases and hepatic disorder (Ammon and Wahl, 1991). Recently poultry rations were supplemented with antioxidants to decrease the aflatoxicosis negative effect (Surai, 2002). Curuma longa, dimethoxy-Curuma longa and bisdemethoxy Curuma longa are the active ingredients present in the turmeric Rhizonsand plant extracts, it is believed that this extract has antifungal (WuthiUdomler et al., 2000), and antioxidant properties (Osawa et al., 1995). Protective effects of food turmeric supplement against aflatoxin induced mutagenicity and hepatic-carcinogenenicity Soni et al. (1997). Pharmacologically, Curuma longa is tolerable, this fact is proven through human studies that stated no dose toxicity recorded administered at higher doses up to 10g/day (Aggarwal et al., 2003). Turmeric has protective effects against AFB1 (Soni et al., 1997). It is hypothesized that both Curuma longa and NS diet supplement possess beneficial effect on growth parameters and toxicity reduction, so the current study is conducted to show the possible beneficial effects and adding benefits of NS and Curuma longa on aflatoxicosis hazardous effect, general health, body weight, restoration of vital serum parameters closes to their normal values in chicken broilers.

Materials and Methods

Case History and clinical sings

Broiler chicks farm at 25-day old chicks present in Kalubia Governorate, Egypt was suffered from reduced feed intake, un-thriftiness, ruffled feather, decreased body weight and high mortality

Feed Examination

Samples from broiler feed were used for AFB1 extraction and quantification according to Matumba et al. (2009), High Performance Liquid Chromatography was applied according to (AOAC, 1995) in Agriculture Research Center, Egypt.

Diet

Basal diet contains (28.2% soya bean meal (44% CP), 63.1% grounded yellow corn, 4.3% corn gluten meal (60% CP), 0.6% vegetable oils, 1.8% dicalcium phosphate, 1.1% ground limestone, 0.4% common salts, 0.3% mineral and vitamin premix, 0.1% lysine and 0.1% methionine).

Medical Plants preparation

Nigella sativa seeds and Curcumin were purchased from commercial market in Kalubia governorate, purified, finally ground and added to the diet along the experimental period.

Experimental design

Two hundreds one day old chicks (Arbor Acres) were obtained from local hatchery, chicks were equally allocated into four groups (5 replicates for each). Stocking density initially was 15 birds/m2. All Experimental groups were subjected to continuous light thought out the experimental period, a stable brooding temperature (35-37°C), that gradually declined to reach the chicks comfort zone (24-26 °C) on the day 21. Sensitive thermo-hygrometer was used for regular temperature and relative humidity check. Through experimental period, feeding and water were ad libitum

Animal grouping

Group1 (control negative) received normal basic diet throughout the experimental period (45 days). Groups (2, 3, 4) at days 1 - 25 received basal diet containing aflatoxin (20ppb). At days 26 - 45, group2 just receive basal diet, group3 fed basal diet containing 2% black seeds (Nigella Sativa) according to Guler et al. (2006), while group4 fed on basal diet containing 1% Rhizomes of turmeric according to (Ayoub et al., 2011). The Ethics statement for Animal care and maintenance were in accordance with the guidelines of the Egyptian Research Ethics Committee and the guidelines for care and laboratory animals use by Benha University (BUFVTM02-05-22).

Samples collection

At the end of experimental period (45 days) blood samples and serum separation were done for biochemical analysis, 10 chickens from each group were dissected, careful examination for liver and kidneys abnormal macroscopic changes were done, small tissue specimens were collected from liver and kidneys of chickens, fixation was directly occurred using neutral buffered formalin (10%) and prepared for histopathology examinations according to (SK,
2019), liver samples were collected for antioxidant activity determination.

**Biochemical examination**

Serum biochemical analysis was performed, determination of both Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were done according to Reitman and Frankel (1957). Creatinine was determined according to Henry (1974). Urea determination occurred according to (Patton and Crouch, 1977). Determination of cholesterol (Zak et al., 1954) and triglyceride (Fossati and Prencipe, 1982), 0.5 gm of liver samples were taken for Malonaldehyde (MDA) and Total Antioxidant Capacity (TAC) owing to Ohkawa et al. (1979) and Erel (2004) respectively.

**Statistical analysis**

Statistical analysis was done using SPSS (Windows version 20, USA). Data (body weight, antioxidants, liver and kidney functions, cholesterol, triglycerides, MD and TAC) were assessed using one way ANOVA, Duncan (P value ≤ 0.05), followed by post hoc multiple comparison tests.

**Results**

**HPLC resolution**

Aflatoxins residues in broiler feed was 20ppb by HPLC apparatus (fig. 2).

**Body weight**

By the 6th week, average body weight of the experimental groups has shown variation (table:1) where group 2, 3, 4 have shown significant (p≤0.05) decrease in body weight compared to the group 1, while at the 6th week, groups 3, 4 showed significant (p≤0.05) increase in body weight compared to group 2. Notably group 4 has shown significant (p≤0.05) improvement in body weight compared to the group 3.

**Postmortem Examination**

Postmortem examinations have shown, low body weight carcass, abdominal ascites, friable discolored liver with reddish strikes on its surface, bile engorged gallbladder (fig:1a), enlarged congested kidney (fig:1b) and different body organs showed petechiae hemorrhage.

**Liver Function Test**

This study revealed that the activities of AST and ALT (table. 2) showed significant (P≤0.5) increase in group2 (729.00 ± 39.58 U/ml and 470.00 ± 30.61 U/ml) respectively in comparison with group 1 (257.80 ± 16.71 U/ml and 105.00 ± 6.0 U/ml) control negative ones, while these enzymes have shown a significant (P ≤ 0.5) reduction in group3, 4 (305.00 ± 23.13 U/ml and 264.00± 20.34 U/ml) and (381.00 ± 33.67 U/ml and 305.00± 25.72 U/ml) respectively when compared to group 2. In respect to plasma total protein (TP) and Albumin, they showed significant (P ≤ 0.5) decrease in group2 (2.68 ± 0.13 mg/dl) and 1.57 ± 0.04 mg/dl) respectively compering with group1.
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(6.28 ± 0.72mg/dl and 2.69 ± 0.17mg/dl) respectively, while they showed significant (P≤0.5) increased in groups 3, 4 (3.93 ± 0.30mg/dl and 2.17 ± 0.06 mg/dl) and (3.89 ± 0.42mg/dl and 1.92 ± 0.97mg/dl) respectively in comparison with group 2.

Urea and creatinine

Results obtained by the current study (table: 3) showed significant (P≤ 0.05) increase in both urea and creatinine in group2 (77.7 ± 0.43 mg/dl and 0.92 ± 0.09 mg/dl) respectively comparing to group1 (4.8 ± 0.18 mg/dl and 0.35 ± 0.05 mg/dl) concurrently group3 and 4 have shown significant (p≤0.05) improvement (16.44 ± 2.11 mg/dl and 0.49 ± 0.31 mg/dl) compared to group 2.

Lipid profile and antioxidant activities

Data obtained (table 4) revealed that cholesterol, triglyceride and malonaldehyde showed significant (p ≤ 0.05) elevation in group2 (193.8 ± 26.93 g/dl, 102.1± 64.01g/dl and 61.1± 3.2 nmol/g) respectively compared to group1 (91.27 ± 6.07 g/dl, 67.39 ± 5.39 g/dl and 61.1 ± 3.2 nmol/g) respectively. while TAC has shown significant (p ≤ 0.05) reduction (4.30 ± 0.51) in group 2 compared to group1 (7.20 ± 1.10). Concurrently groups 3, 4 showed significant decrease in cholesterol, triglyceride and malonaldehyde (101.63 ± 5.51g/dl, 70.49 ± 4.8g/dl and 40.3 ± 2.1 nmol/g) and (91.58 ± 4.53 g/dl, 56.11 ± 3.84g/dl and45.1 ± 5.3 nmol/g) in comparison with group2. While TAC was significantly decreased (4.3 ± 0.51Mm/g) in group 2 compared to other experimental groups.

Histopathological findings

Hepatic microscopic examination of group1 (control negative) chickens revealed parenchymal cells are arranged in plates with two cells thick and sinusoids between these hepatic plates (Fig. 3 A). The broiler chicks (group1) examined kidneys showed that glomeruli consist of capillary tuft, Bowman’s capsule and Bowman’s space, peritubular capillary sinuses that surrounded convoluted tubules (proximal and distal) (Fig.3B) The microscopic changes in the liver of broiler chicks(group2) with aflatoxicosis showed fatty degeneration and necrosis of hepatocytes (Fig.3C). The examined portal areas showed bile ducts hyperplasia with periductal fibrous tissue proliferation (2D). In addition, mononuclear cellular infiltration, and occasionally portal thrombosis with periportal fibrosis were also seen (Fig. 3E). While the examined kidney of chickens in the same group showed hemorrhagic areas (Fig. 3F), tubular epithelium multifocal necrosis (Fig. 3G), in addition, peritubular capillary sinuses congestion and extensive tubular degeneration were observed (Fig. 3H). Meanwhile, examined liver of aflatoxins intoxicated and Nigella Sativia supplemented chickens just showed central veins, hepatic sinusoids congestion and pyknosis of some hepatic cell nuclei (Fig. 4A). The examined kidneys tubules of same group chickens showed epithelial degeneration and nuclear pyknosis (Fig.4B). The microscopic examination of the liver of chickens intoxicated with aflatoxin and co-treated with curcumin revealed enhancement in the microscopic picture where congestion of sinusoids, portal and central veins with vacuolar degeneration of some hepatic cells and pyknosis of the nuclei of few hepatocytes (Fig. 4A). While the examined kidneys of chickens in this group showed cloudy swelling and vacuolar degeneration of the renal tubules with pyknosis of the nuclei of some renal tubular epithelium (Fig.4D).
Figure 4. Photomicrograph of liver (A) and kidney (B) of broiler chicks intoxicated with aflatoxin and co-treated with Nigella sativa, liver (C) and kidney (D) of chickens intoxicated with aflatoxin and co-treated with curcumin H&E stain X200.

Discussion

Liver is the principal aflatoxins detoxifying organ that, protects body against their deleterious effects, liver is one of vital assets handling metabolism and utilization of lipid, protein, and amino acid Fouad and El-Senousey (2014); Hiramoto et al. (1990), besides Shanmugasundaram and Selvaraj (2012) assured its rule in calcitriol (active vitamin D3) formation through hydroxylation of cholecalciferol to 25-hydroxycholecalciferol. Current study findings showed aflatoxicosis significantly increased liver enzymes (AST and ALT), these findings cope with the usual deleterious effects of AFB1 on hepatocytes result in high concentrations of ALT and AST in poultry blood after feeding diets containing AFB1 (Gómez-Espinosa et al., 2017; He et al., 2013), this may be attributed to hepatocytes damages caused by AFB1 that results in release of AST and ALT enzymes that present in the hepatocytes cytosol into blood stream. Adamson et al. (1976); Thabrew and Bababunmi (1980) revealed that the hepatocytes damage results from the 8,9 epoxide (produced by aflatoxin B1) union to protein forming DNA adducts that leads to disruption in cellular functions and division. Feed consumption and final body weight are negatively affected with liver malfunction, interestingly Curuma longa dietary supplement significantly improved final body weight, this may be attributed to its ability to increase the intestinal lining mitochondrial function and decrease the peroxidation (Ruan et al., 2019). Similar findings have been recorded by Rajput et al. (2012) who stated that overall performance and growth rate have been enhanced, when broilers diets were fortified with Curuma longa in rate of 200 mg kg⁻¹, he attributed that improvement to the increased intestinal segments villus heights. Congruently with this finding several studies declared that broiler diets Curuma longa supplement at 0.5-1% rate significantly managed to improve feed intake, efficiency and body gain (ALKassie et al., 2011; Gowda et al., 2009). Durrani et al. (2006); Yarru et al. (2009) reported that aflatoxin-exposed chicks body weights showed remarkable improvement when they received diet supplemented with 0.5% turmeric. In a recent study, Ruan et al. (2019) declared that Curuma longa diets supplement significantly enhanced feed intake, average daily gain and the end body weight of growing ochratoxin-exposed ducklings. Unlike, authors (Rajput et al., 2012) recorded Curuma longa supplement to broilers diets has no significant effect. some authors (Zhu et al., 2014) declared that blood biochemistry can indicate the nutritional, metabolic and health condition of broiler chickens. Blood biochemistry can be used for evaluating different physiological responses to different dietary supplements (Toghyani et al., 2010b). Curuma longa supplement for broilers diets subject to heat stress caused marked decrease in serum ALT and AST Concentration, this indirectly indicates decreased hepatocytes destructions (Polat et al., 2011; Zhang et al., 2018) Regarding to serum total proteins and albumin, Curuma longa supplemented diet showed significant elevations in total proteins and albumin this observation matches the ones reported by Zhu et al. (2014) who attributed this effect for the Curuma longa rule in improving protein metabolism, cellular protective effect through enzymatic and non-enzymatic mechanism. Study presented here showed that adding Curuma longa to basal diet restore the serum concentrations of TAC in which is indicator of scavenging capability for superoxide radicals. These findings matches with former studies done with various antioxidant supplements in the diet (Cai et al., 2012; Sahin et al., 2012). Many authors ((Cai et al., 2012; Sankar et al., 2012)) have proven that Curuma longa supplements have a potent capability for lipid peroxidation reduction. Similarly, Kalpana and Menon (2004) suggested that Curuma longa exerts its protective effect through modulating the biochemical marker enzymes and augmenting antioxidant defense system. In this sense Pita et al. (2004) suggested that Curuma longa possesses several antioxidant properties that reduce and neutralize the free radical.
injurious effect, consequently, improves vital process. *Curuma longa* is good antioxidant as it contains several phenolic compounds that minimize hydrogen peroxide formation (Farag et al., 1989). On the same aspect poultry fed *Curuma longa* treated diet, showed marked recovery (body weight, liver and kidney function). Similar findings have been revealed by Koul et al. (1994); Zhang et al. (2016) who declared the possible rule of *Curuma longa* in cytochrome toxin isozymes activities and transcription suppression, this leads to reduction in the level of 8-hydroxydeoxyguanosine (that destroy DNA). Furthermore serum total protein and albumin showed significant decreases in aflatoxicated group in compared to negative control one, on other wise significant increase of in albumin serum total protein were recorded in groups that fed on diet containing Nagella Sativia (group 3) and diets containing *Curuma longa* (group 4), this findings agreed the ones recorded by Cullen and Newberne (1994); Soliman et al. (2012) who recorded endoplasmic degranulation due to protein biosynthesis and DNA transcription deterioration caused by Aflatoxin B1, that manage to irreversibly binds with DNA forming DNA-adducts that inhibits DNA-RNA polymerase activity. Congruently authors (Al-Jishi and Hozaifa, 2003) revealed significant elevation in serum total protein and albumin in NS fed poultry, they justified this improvement to NS contain some enzymes that cause remarkable improvement to liver protein and albumin biosynthesis. Histopathological findings that the examined liver of broiler chicks intoxicated with aflatoxin and treated with Nigella saliva showed just congestion of sinusoids, portal, and central veins with vucular degeneration and pyknosis of some hepatocytes nuclear pyknosis (Fig. 4 A). Microscopic examination of chickens intoxicated with aflatoxin and co-treated with *Curuma longa* liver showed enhancement in the microscopic picture where just congestion of sinusoids, portal, and central veins with vucular degeneration and pyknosis of some hepatocytes (Fig.4 C). It is postulated that liver is site where creatine is synthesized, then after passes into circulation and almost entirely is taken up by skeletal muscle where it is converted into creatine phosphate, both creatine & creatine phosphate are spontaneously converted into creatinine (McLauchlan, 1988). Present study showed significant elevation of urea and creatinine in aflaxotin fed group in comparison with negative control one similar results obtained by Soliman et al. (2012); Verma and Raval (1997) they dosed male Sprague-Dawley rats with Aflatoxin B1 through single intraperitoneal injection (1.0mg AFB1 /kg body weigh), this significant increase in urea and creatinine concentration in serum could be due to increased release from muscles and/or decrease excretion from the kidney due to reduced glomerular filtration rate (GFR) caused by hazardous aflatoxin B1 renal functional unite (nephrons). Similarly, Damiano et al. (2021) showed that adding 150 mg/kg of *Curuma longa* to a broiler diet containing 100 µg AFB1/kg, may altering AFB1 metabolism to alleviate its toxicity and improve organs functions as renal functions, Aflatoxin B1 is metabolized to a highly reactive chemical compound, called the 8, 9-epoxide where it binds very rapidly to protein, DNA and other important constituents of living cells, forming ‘adducts’. Formation of these adducts disrupts the normal working processes of the cell, and in the case of DNA adducts, can ultimately lead to a loss of control over cellular growth and division. This toxic adducts causing cellular injury, which come in the same line of our present results showing significant elevation of MDA level in aflatoxicated group 2 comparing with negative control one. Concerning with TAC (antioxidant enzymes activity) in current work results have shown that aflatoxins caused significant reduction in TAC (group 2) compared to control negative (group 1) this finding matches the results obtained by Li et al. (2014); Liu et al. (2016). Congruently Abdel-Sattar et al. (2019) declared that giving broiler 100µg aflatoxin/kg feed resulted in MDA serum elevation, that indicates increased oxidative stress condition which leads to high serum level of ROS, the increased serum MDA led to variable decrease in serum level of TAC, that decrease varies according to the stress degree, lipid peroxidation and MDA formation levels. However, these levels of free radical molecules and lipid peroxidation are controlled by an antioxidant defense system of enzymatic components such as SOD, CAT, and GR, and non-enzymatic components such as GSH and vitamin E (Delles et al., 2014). On the opposite side NS and *Curuma longa* fed groups showed marked improvement of these results compared to aflatoxicated group, this may be due to NS can be used as antioxidant agent as it inhibited the non-enzymatic peroxidation which may increase the immune response (A.A., 2012), similarly, *Curuma longa* possesses antioxidant, anti-inflammatory, anti-apoptosis, and anti-cancer properties (Benzer et al.,
2018; Divya and Pillai, 2006). The antioxidant activity of Curuma longa may be due to it acts as oxygen free radicals’ scavenger and protect hemoglobin from oxidation, also Curuma longa protection of liver from inflammatory condition may be due to its anti-inflammatory effect through cyclo-oxygenase-2 inhibition and expression (Shakibaei et al., 2007). Curuma longa lowers the production of nitrite radicals and ROS like super oxide anions generation ( Chattopadhyay et al., 2004; Masuda et al., 2001). Reduced the increase in the lipid peroxidation, increased the antioxidant capacity and protein production, and offset the reduced average daily gain so enhance the efficiency of the antioxidant defense system. Moreover, our results revealed significant increased lipid profile (cholesterol a triglycerides) in aflatoxicated group in comparison with control negative one. Concerning lipid fractions, current work results showed that, NS supplementation significantly decrease total cholesterol and total lipids concentrations, this finding matches the one recorded by Tollba (2003) who declared that Nigella Sativa significantly decreased serum levels of triglyceride and total cholesterol, unlike Toghyani et al. (2010a) found that serum triglyceride and total cholesterol concentrations showed no significant changes with NS supplementation. The decrease in serum cholesterol level may be attributed to Nigella Sativa possess high contents from unsaturated fatty acids which may stimulate the cholesterol oxidation and excretion into the intestine ( Khodary et al., 1997). NS diet supplement decreases the total serum cholesterol, this may be to NS high contents of unsaturated fatty acids (linoleic and oleic) that interfere with cholesterol synthesis (Cheikh-Rouhou et al., 2007). While (Atta, 2003) assigned the reduction reason to the compound β-sitosterol of Nigella Sativa oil that may inhibit the cholesterol oxidation and excretion into the intestine (Khattab et al., 2011). Curuma longa supplementation significantly decreased total cholesterol and total lipids concentrations. However, triglycerides concentration showed no significant changes, these finding pass hand in hand with Khattab et al. (2011). Curuma longa significantly lowered serum of LDL, vLDL and total cholesterol levels, L-tocopherol level elevation in rats, this suggesting the possible interaction between Curuma longa and α-tocopherol, this fact may increase bio-availability of vitamin E decrease cholesterol levels (Patil et al., 1971; Rao et al., 1970).

Conclusion

Based on this study data one could conclude that both Curuma longa and Nigella Sativa diet supplement can reduce aflatoxins hazard effects, improve body weight, liver and kidneys functions. Wisely, to some extend Nigella Sativa is more effective than Curuma longa. Further studies with higher doses of NS and Curuma longa may be required for adjusting the optimal diet supplement level.

Conflict of interest

The authors haven’t conflict of interest to declare.

References


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