Introduction to Citrinin (CTN) and its Toxicity Status in Poultry Feeds: A Review Article

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Abstract: Citrinin (CTN) is a fungal secondary metabolite and polyketide nephrotoxic mycotoxin commonly present as a natural hazardous contaminant both in food and feed world wide. Its chemical formula is $C_{13}H_{14}O_5$ with exact mass 250.0841g. It occurs mainly in stored grains. It was first isolated from filamentous fungi i.e., *Penicillium citrinum*, but now produced by more than 5 fungal genera i.e., *Aspergillus*, *Clavariopsis*, *Monascus*, *Penicillium*, *Pythium* and also more than 29 fungal species in grains, foods, feedstuffs as well as in biological fluids. There are also many forms of CTN degradation products (derivatives), but the main 3 forms are CTN H (non-cytotoxic), CTN H (cytotoxic) and dicitrinin A (cytotoxic). The key ecological determinants for the synthesis of CTN during pre and post-harvest are temperature ($^\circ$C) and water availability (a%). Studies revealed that CTN production occurs at an optimum temperature of 20 – 30$^\circ$C and 0.75 – 0.85a, depending upon fungal species. However, based on the available limited knowledge few researchers stated that CTN is acutely nephrotoxic at relatively high doses in poultry feeds (500 mg CTN kg$^{-1}$ feed), causing interferences in the function and size of kidneys, liver, heart, pancreas, spleen and gall bladder of chickens. It also reduces body weight, egg weight and feed consumption, but increases water consumption. Therefore, on the basis of available limited data either submitted to EFSA (European Food Safety Authority) or collected from literature were not adequate to carry out poultry dietary exposure assessments for the general or specific groups of any country poults. At this stage it is difficult to establish wide acceptable limits for CTN concentration. Presently, there is no any specific legislation for CTN worldwide in general and Pakistan in particular. The main reason was either lack of suitable analytical routine techniques or its instability in various food and feedstuffs.

Key words: Citrinin (CTN) · Mycotoxin · Fungi · Poultry Feed · Toxicity

INTRODUCTION

The word “mycotoxin” is derived from the Greek word “Mykes” which means fungus and the Latin word “Toxicum” which means poison [1]. While mycotoxins are generally defined as secondary metabolites of fungi which can evoke pathological changes in man and other animals [2] and the disease caused by them both in human and animals are known as mycotoxicosis [3]. Many agricultural commodities are vulnerable to attack by a number of fungi that are able to produce toxic secondary metabolites termed as mycotoxins. There are more or less 400 known mycotoxins, but the most common ones are Aflatoxin, Amatoxin, Citrinin, Cytochalasin, Ergostamine, Fumonisins, Gliotoxin, Ibotoic Acid, Muscimol, Ochratoxin, Patuline, Phalloidin, Sterigmatocystin, Trichothece, Vomitoxin, Zeranol and Zearalenone etc [4].

Citrinin (CTN) is a nephrotoxic mycotoxin produced by a variety of fungal genera viz., *Aspergillus*, *Penicillium* and *Monascus* etc. CTN is generally found after harvest and occurs mainly in stored grains (e.g. maize; oat; rice; rye; sorghum; wheat etc), in other plant based products (like beans; fruits; vegetable juices; herbs; spices) and also occur in spoiled dairy products [5]. CTN is a structurally diverse family of naturally occurring fungal toxins, which directly or indirectly contaminate both the feed of livestock and poultry. The natural occurrence of CTN in poultry feeds causes major economic loss to the poultry sector too. In poultry mycotoxicosis causes reduced growth rate, lowered feed conversion, impaired resistance to infectious diseases and reduced vaccination efficiency with lesions in many organs viz., kidney, liver, heart etc [6].

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**Fungi Produces Citrinin (CTN):** Like ochratoxin A (OTA), citrinin is also one of the most important mycotoxin initially isolated by [7] from a culture of *Penicillium citrinum*. It has since been produced by a variety of other fungal genera found on human foods like grain, cheese, sake and red pigments. Research revealed that CTN is being produced now by a variety of fungal species including: *Aspergillus alabamensis*; *A. carneus*; *A. niveus*; *A. ochraceus*; *A. oryzae*; *A. terreus*; *Monascus aurantiacus*; *M. floridanus*; *M. luniisporas*; *M. pallens*; *M. pilosus*; *M. purpureus*; *M. ruber*; *M. sanguineus*; *Penicillium camemberti*; *P. citrinum*; *P. chraszczii*; *P. decaturense*; *P. expansum*; *P. gorlenkoanum*; *P. hetheringtonii*; *P. mangini*; *P. miczynskii*; *P. odoratum*; *P. radicicola*; *P. verrucosum* *P. westlingii*, *Pythium ultimum* and *Clavariopsis acquatica* [8, 9, 10, 11]. Though *Monascus purpureus* and its fermentation products have been used as dietary supplements, but on account of the presence of CTN such products become a potential threat to human health too [12].

**Physico-chemical Charateristics and Various Decomposed Types of Citrinin (CTN):** CTN is a polyketide nephrotoxic mycotoxin produced by a variety of fungal genera. Pure CTN is a crystalline lemon yellow compound with maximum UV-absorption at 250 and 333 nm (in methanol) and is a phenol derivative or quinonemethine mycotoxin. Its chemical formula is C_{10}H_{18}O with exact mass 250.0841g (Fig. 1). It is insoluble in cold water or slightly soluble in water, but fairly soluble in aqueous sodium hydroxide, sodium carbonate, ethyl alcohol, methyl alcohol and most other polar organic solvents [13, 14] and its melting point is 175°C. CTN crystallizes in a disordered structure, with the p-quinone and o-quinone tautomeric forms in a dynamic equilibrium in the solid state [15].

Besides CTN, there are a large number of CTN derivatives which have been isolated from different fungal species [16]. Citrinin is a heat sensitive and decomposes during heat treatment to form other complex compounds, such as citrinin H2 and citrinin H1 with higher and weaker cytotoxicity than the original citrinin, respectively. The chemical formula and exact mass of CTN H2 and CTN H1 are C_{12}H_{14}O_4 and 224.1049g and C_{13}H_{18}O_3 and 412.1522g, respectively. Several studies have been carried out on the degradation of CTN revealed that CTN decomposition occurs at temperature greater than 175°C under dry conditions and also at temperature greater than 100°C in the presence of water [17, 14]. There was also another decomposition product, the cytotoxic citrinin dimmer known as dicitrinin A, which has been reported by [18]. Their chemical formula and exact mass is C_{23}H_{55}O_{10} and 380.1624g. Isoquinoncitrinin A is also another derivative of CTN having chemical formula C_{14}H_{25}O_3 with exact mass of 219.0895g (Fig. 1).

**Diseases Caused by Citrinin (CTN):** Citrinin is a potent nephrotoxic with hepatic and teratogenic activity. It causes “Balkan nephropathy” and a form of “Cardiac Beriberi” often known as yellow rice fever in humans [19, 20, 21]. At the molecular level it exhibits a range of effects including free radical damage to DNA and also disruption of mitochondrial membrane-bound enzymatic activities as well as structural integrity. It inhibits specifically the electron transport chain (ETC) of mitochondria by interfering with complex III of the respiratory chain or by inhibiting the NADH dehydrogenase activity. It can also permeate through the human skin. Although non-significant health risk is expected after dermal contact [22]. Like ochratoxin A (OTA) it is also involved in the embryocidal and fetotoxic [23]. Citrinin and ochratoxin are the twin mycotoxins. Humans are much exposed to CTN, because it is usually produced by the same the fungi which are producing ochratoxin (another most common contaminant of human food throughout the globe). Till now there are no any specific regulations regarding the permissible limits of CTN either in Pakistan or in European Union concerning in any kind of food and food commodity (including poultry feeds).

**Factors Affecting Concentration of Citrinin (CTN):** There are so many external and internal factors which either increase or decrease the concentration of CTN production by the concerned fungal species. However, the key ecological determinants for the synthesis of CTN during pre and post-harvest are temperature (°C) and water availability (a_o). A very little is known about the effects of ecological factors on the production of CTN. However, there have been a very few studies where attempts have been made to amalgamate the available limited literature on these 2 key factors in relation to different feed and food processing raw materials, especially cereal grains. Information like this is very crucial for precisely focusing and monitoring the key critical control points in the feed and food chain to optimize prevention strategies [24]. CTN is generally produced after harvest and can be found chiefly in stored cereal grains viz., barley, wheat and rice [25].
1. Citrinin

Chemical Formula: C_{13}H_{14}O_{5}
Exact Mass: 250.0841
Molecular Weight: 250.2473

(3R,4S)-8-hydroxy-3,4,5-trimethyl-6-oxo-4,6-dihydro-
3H-isochromene-7-carboxylic acid

2. Citrinin H₂

Chemical Formula: C_{12}H_{16}O₄
Exact Mass: 224.1049
Molecular Weight: 224.2530

(2R,3S)-3-(3,5-dihydroxy-2-
methylphenyl)butan-2-yl formate

3. Citrinin H₁

Chemical Formula: C_{24}H_{26}O₇
Exact Mass: 412.1522
Molecular Weight: 412.4325

(2R)-1-((2R,3S)-5-hydroxy-2,3,4,10-tetramethyl-8,11-dioxo-3,8,11,11b-
tetrahydro-2H-pyrano[4,3,2-kl]xanthen-9-yl)propan-2-yl formate

4. Dicitrinin A

Chemical Formula: C_{23}H_{24}O_{5}
Exact Mass: 380.1624
Molecular Weight: 380.4337

(2R,3S,7R,8S)-5-hydroxy-2,3,4,7,8,9-hexamethyl-7,8-dihydro-
2H-furo[3,2-c]pyrano[4,3,2-kl]xanthen-10(3H)-one

5. Isoquinoncitrinin A

Chemical Formula: C_{12}H_{13}NO₃
Exact Mass: 219.0895
Molecular Weight: 219.2365

5,8-dihydroxy-3,4,5,7-tetramethyl
isoquinolin-6(5H)-one

Fig. 1: Citrinin (CTN) and its different decomposed form found in poultry feeds.
Studies revealed that CTN production occurs at an optimum temperature of 20 – 30°C and 0.75 – 0.85a, depending upon fungal species [26]. The said mycotoxin represent a severe problem especially in countries with a hot climate as under such circumstances it is a major source of food poisoning after fungal contamination [27]. In animals and humans the CTN toxin accumulates in the kidneys and can cause severe renal failure and also had adverse effects on gastrointestinal tract [25, 28, 29].

CTN Status in Poultry Feeds and Its Toxicities: Though no any reliable and concise data about CTN status of poultry feeds are available. However, based on the available scanty knowledge few researchers stated that CTN is acutely nephrotoxic at relatively high doses in poultry feeds, causing swelling and eventual necrosis of the kidneys and affecting the function of liver at a lesser extent [5]. Ames et al. [30] fed the mature laying hens by CTN @ 0, 50 and 250 mg kg⁻¹ diet for about 3 weeks. They found no effect on body weight; feed consumption; egg weight and quality of eggshell. But when diet comprised of 0, 62.5, 125, 250 and 500 mg CTN kg⁻¹ feed was given to them from hatching to 3 weeks, it resulted a significant decrease in body weight at 500 mg CTN kg⁻¹. While all dose levels also resulted in enlarged kidney and a slight dose resulted an increase in liver weight. In another set of the same experiment, they stated that CTN @ 250 and 500 mg kg⁻¹ feed resulted in a dose dependant increase in water consumption followed by acute diarrhea. Roberts and Mora [31] administered various amounts of CTN in per kg feed to broiler chicks for about 4-6 weeks. Diarrhoea was observed at the two highest CTN levels viz., 130 and 260 mg kg⁻¹ feed. At necropsy these experimental chicks had haemorrhages in the jejunum as well as enlarged livers and kidneys. They also interpreted their qualitative observations of anaplastic areas of the kidneys and pancreas observed at the highest concentration of CTN as of being suggestive that CTN could be a carcinogen in chicks. Wyatt [32] reported that nephrotoxicity and hepatotoxicity occurs in chickens at dietary levels of 250 μg g⁻¹ of CTN with liver and kidney enlargements of 11 and 22%, respectively. Serum sodium levels are also changed. He/She further stated that necropsy of affected birds revealed the presence of pale and swollen kidneys. However, toxic effects were not observed in broiler chicks fed with a diet containing 65 mg CTN kg⁻¹ feed [33]. Mehdi et al. [34] reported that CTN fed via the diet to ducklings @ 100, 250 and 500 mg kg⁻¹ feed was observed to be nephrotic both at 250 and 500 mg CTN kg⁻¹ feed. In another study Mehdi et al. [35] noticed decreased weight gain and feed consumption at both highest doses of applied CTN (250 and 500 mg kg⁻¹ feed). Abdelamid and Dorra [36] reported effects in laying hens when administering 125 g feed per day containing 0.1 μg CTN kg⁻¹ feed to hens of 2 kg b.w. which is equivalent to an intake level of 6.25 μg CTN kg⁻¹ b.w. per day. While other studies showed effects at higher doses of CTN only. They also examined the effects of 100 μg CTN kg⁻¹ feed in 6 weeks feeding study followed by 2 weeks recovery of thirteen months old Egyptian laying hens. Their results showed that CTN fed hens had enlarged spleen and reproductive organs. Changes in the relative organ weight of the adrenal glands and compositional changes in the liver, red and white muscle fibers were also recorded. Jewers [37] stated that like ochratoxin A (OTA), CTN is also a nephrotoxic mycotoxin. It reduces the growth rates and decreases both feed and water consumption by poultry. Decreased feed intake was observed with 62.5 μg g⁻¹ CTN, whereas body weight and water intake were affected by 500 and 250 μg g⁻¹ in chickens, respectively. Studies conducted by [38] on feed samples from chicken farms in Bulgaria had reported the incidences of nephropathy and about 96% of the analyzed samples contained 120.5 ± 43.3 μg CTN kg⁻¹. The biochemical studies of CTN fed (12.5 ppm) chickens blood samples showed hypocalcaemia, hypophosphoremia, hyponatraemia and hypokalaemia [39]. They also stated that CTN fed (12.5 ppm) birds showed congestion, enlargement, pallor or yellowish discoloration of liver with distended gall bladder, swollen and conjusted kidneys [40]. Kumar and Balachandran [41] found no mortality in the CTN (5 ppm) fed group of broiler chickens. They also found a significant (P<0.05) increase in the relative weight of the liver and spleen and decrease in the bursa of fabricius in CTN fed birds. They further stated that CTN fed chickens showed ruffled feathers, lethargy and stunted growth from the third week and onward of the experiment.

CONCLUSIONS

Keeping in view the above mentioned pivotal role of fungi in CTN production in poultry feeds, it is strongly recommended that seed lots before and after harvest should regularly be monitored through certain modern technologies in order to determine the health hazard status of seeds prior to be locally consumed by poultry.
The cereal grains contaminated by the fungal spores should be treated at pre-harvest level as a control measure of spread of disease caused by concerned nephrotoxic fungi. Present review also suggests that fungal growth needs to be controlled both in fields prior to harvest and post harvest at storage stage before to be consumed by poultry. The chance of CTN contamination in cereal grains increases on account of high moisture content and inadequate storage temperature. Therefore, cereal grains must be maintained at proper storage conditions to stop the fungal proliferation. Post-harvest agricultural practices also need to be properly improved by using the modern technologies in threshing, drying, storage and transportation of wheat grains. Detoxification and decontamination and cleaning are few other remedies which could be adopted in order to minimize or reduce the rate of CTN contamination. To ensure the health safety of chicks, the concerned regulatory authority of every country are suggested to take into consideration this serious issue of poultry feed contamination caused by fungal growth and controlling strategies should be practiced and quality control system of feed should also be adopted and needs to be improved.

Due to limitations and uncertainties in the current data base of CTNAM [EFSA (European Food Safety Authority) panel on contaminants in the food chain] panel concluded that the derivation of a health based guidance value for poultry was not appropriate. Based on the available data a concern for genotoxicity as well as carcinogenicity in poultries could not be excluded at the level of no concern for nephrotoxicity (Anonymous, 2012) [5]. Thus on the basis of available data no firm conclusion can be drawn regarding the poultry of exceeding the CTN level of no concern for nephrotoxicity on a daily basis over a prolonged period of time.

REFERENCES