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REVIEW ARTICLE

DEOXYNIVALENOL, A TRICHOTHECENE MYCOTOXIN: REVIEW OF ITS MASKED FORM, CONTAMINATION IN CEREAL-BASED FEED, AND MASS SPECTROMETRY ANALYTICAL METHODS

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Summary

Deoxynivalenol (DON) is a trichothecene mycotoxin regularly occurring in cereals. It is toxic to humans and inhibits DNA, RNA and protein synthesis. Moreover, its conjugated products, called masked mycotoxins, including DON-3-glucoside (DON-3G), is another "emerging" food safety issue in recent years. In this review, we first discussed the nature of contamination of masked DON in cereal-based food and feed; moreover, the new reported masked DON is summarized and we also focused on its toxicity and digestion in human and animal bodies. In addition, the contamination of DON in cereal-based feed in different countries was summarized and some regular patterns of DON occurrence are suggested. Finally, the LC-MS methods for the determination of DON and masked DON in food, feed and animal fluids were compared. This review will provide further information for DON and masked DON contamination and shed some light on the mycotoxin control strategies.

Key words: deoxynivalenol; DON; masked mycotoxin; DON-3-glucoside; contamination; food safety

INTRODUCTION

Deoxynivalenol (DON) is a type B trichothecene and one of the most common mycotoxins found in cereals. DON is produced mainly by fungi, such as *Fusarium graminearum*, *F. culmorum*, and *F. crookwellense*, which are common field pathogens of cereal crops, including wheat, barley, and maize [1]. DON can cause animal food refusal, vomiting, digestive disorders, decrease levels of serum protein and oxidative stress. This mycotoxin could also inhibit DNA, RNA and protein synthesis. Ribosomes and mitochondrion are major targets of DON action [2].

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Similar with the potential chemical warfare agent T-2 toxin, DON is able to induce cell apoptosis through activation of specific cell signaling pathways, including cell survival pathways [3, 4].

In general, DON is relatively stable during extrusion processing at high temperatures and high pressure; thus, DON persists in cereal-based foods and feeds [1]. In China, 89.3% of the wheat samples from Jiangsu and Anhui provinces were contaminated by DON (259 - 4975 μg/kg) [5]. A high frequency (94%) of DON contamination was found in bakery products in the Czech Republic [6]. In developing countries, DON contamination of feeds seems to be much more severe due to inadequate storage conditions. A very high percentage of DON contamination in poultry feed samples were reported in Kuwait, ranging from 79% in wheat bran to 100% in broiler finisher feed [7]. In China, DON was detected in feeds and feed ingredients, with an average level of 1670.2 μg/kg and an overall frequency of 95.2% among the analyzed samples [8]. DON contamination of feeds has important effects on livestock production in both economic terms and for the maintenance of the health and productivity of animals [9].

During the last decades, the so-called masked mycotoxins, plant metabolites of the parent mycotoxins, have caused increasing concern. Besides DON and DON-3-β-d-glucoside (DON-3G), other conjugates, such as DON-glutathione (GSH), DON-S-cysteine, and DON-Scysteinyl-glycine, have been detected in plants [10]. These masked mycotoxins have a very different chemical behavior; thus, they are often not detected during routine analyses. Moreover, these forms could be hydrolyzed to their precursors in the digestive tracts of animals or exert toxic effects comparable to those imputable to free mycotoxins. Therefore, it is crucial to understand the contamination in cereals and the fate of the masked DON in animals.

Analytical methods for rapid, sensitive, and accurate determination of DON and its derivatives in foods, feeds, human and animals are highly demanded for toxicological analysis and exposure risk assessment. For the confirmative determination of DON in food, a mass-spectrometric (MS) method would be the most suitable due to its unambiguous identification and accurate quantification possibilities [11]. Currently, many specific and sensitive HPLC-MS/MS methods for the simultaneous determination of DON and other mycotoxins in food and feed are well developed [12, 13].

In this review, we aimed at discussing the contamination of the masked form of DON in cereals and cereal-based food; the toxicity and the digestion fate of the masked DON in human and animals are also concerned. In addition, we discussed the contamination of DON in cereal-based feed in different countries and some regular patterns of DON occurrence are suggested. Finally, the LC-MS methods for the determination of DON and masked DON in food, feed and animal fluids were compared. We hope this review will provide further information for DON contamination and shed some light on the mycotoxin control strategies.

MASKED MYCOTOXIN DON

Currently, increasing studies have shown that the masked DON does not only exist in cereals but also could transfer to food and beer [55]. Lancova et al. (2008) have reported that DON and DON-3G could transfer from field barley through malt to beer [14]. In malt, the content of DON and DON-3G was higher compared with the original barley [14]. The most significant increase was found for DON-3G. Moreover, concentrations of this masked DON in final beer exceeded free DON. In another study, beer samples from 38 countries with a focus on Austrian and German beers were analyzed for the presence of DON-3G, DON and 3-acetyl-DON [15]. In total, 93% and 77%, respectively, contained DON-3G and DON at the levels above the limit of detection. Average concentrations of all beers were 6.9 μg/L for DON-3G and 8.4 μg/L in the case of DON.

In cereal-based products collected from the Czech retail market in 2010, DON was detected in 75% of samples with concentrations ranging from 13 to 594 μg/kg, but its masked form, DON-3G, had even higher incidence of 80% of samples, and concentrations ranging between 5 and 72 μg/kg were detected [6]. The occurrence of DON and DON-3G in durum wheat samples collected in 2010 from north-central Italy was evaluated [16]. A ubiquitous occurrence of DON-3G was found and 85% of the analyzed samples contained this masked mycotoxin at concentrations varying between 46 and 842 μg/kg. The DON-3G/DON ratio reached up to 30% in many samples.

In order to assess the levels of DON-3G on infected barley from the upper Midwest region of the United States, commercially grown barley

samples were selected [17]. Analysis of barley samples collected from 2001 to 2012 showed that DON-3G was present at levels of < 200 - 3110 μg/kg. In the case of the 2001 - 2011 crop, DON-3G was always at levels that represented less than 10 mol% of the DON present on the same samples. However, the overall average ratio was higher with the 2012 crop (19 mol%), and results of this study suggest that levels of DON-3-G might be impacted by environmental/cropping conditions. It was also observed that DON levels were a poor predictor of DON-3G.

The masked DON contamination is also found in Asian countries. In China, wheat and wheat-based products collected from 24 provinces during 2008 - 2011 were analyzed for DON, DON-3G, 3-acetyl-DON, and 15-acetyl-DON [18]. The natural occurrence of DON-3G is positively correlated with that of DON in all samples over the 4-year period. DON-3G was present at a level higher than 3-ADON and 15-ADON in all examined samples. DON-3G accounted for 30% (wheat kernel) and 13% (wheat flour), as well as 14%, 11% and 13% of the total 4 toxins tested in 2008, 2009, 2010 and 2011 samples, respectively. Zhao et al. (2014) first reported the co-occurrence of masked DON and type B trichothecenes in animal feed and raw materials in China and found that 90.2% samples were contaminated with DON-3G at the levels of 6.0 - 121.0 μg/kg [19].

In earlier studies, more researchers have focused on the degradation fate of DON during the food process. Currently, we have known that the masked toxins are also important contaminants and their

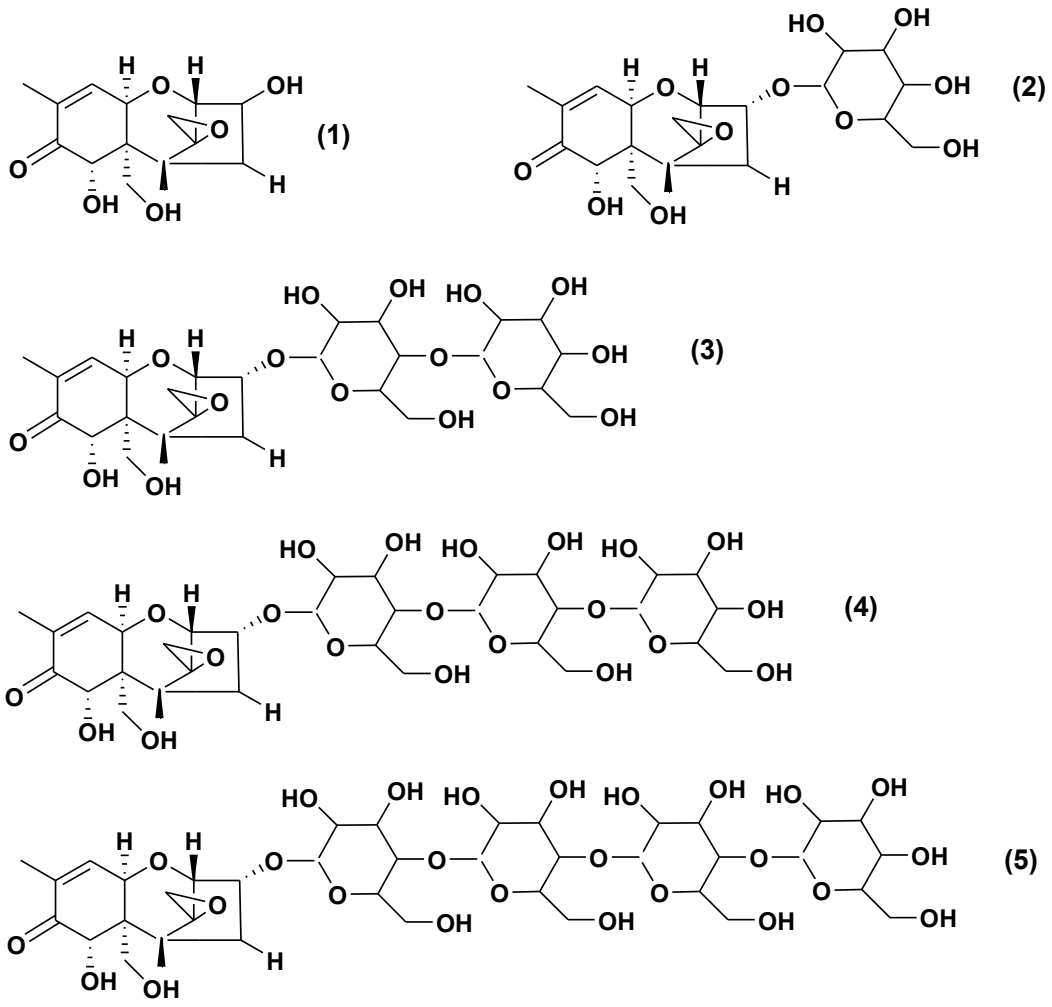

Figure 1. Structure of DON (1), DON-3-glucoside (2), DON-3-diglucoside (3), DON-3-triglucoside (4), and DON-3-tetraglucoside (5).

changes in food process were studied in recent years. The fate of masked DON within milling and baking technologies was studied by Kostelanska et al. (2011) [20]. A decrease of DON-3G (around 10%) took place during baking, and thermal degradation products derived from DON-3G were detected and tentatively identified as norDON-3G A, B, C, D, and DON-3G-lactone. Most of these compounds were located in the crust of experimental breads. Vidal et al. (2014) found that the level of DON-3G could be increased in the process of bread baking [21]. However, in the study of Zhang and Wang (2015), DON was increased during bread production and that DON-3G decreased [22]. DON and DON-3G concentrations in noodles decreased after cooking due to leaching of the mycotoxins into the cooking water. Very recently, DON-3G was shown to have extremely low toxicity to microbial cells, and the glucosylation of mycotoxins is a useful protective mechanism not only for host plants, but also for other species [58].

In addition to the most common DON-3G, some new masked DON, named oligoglycosylated DON, including DON-3-diglucoside, DON-3-triglucoside, and DON-3-tetraglucoside were detected and identified in malt beer and breadstuff using UHPLC-MS system [23] (Figure 1). Moreover, a substantial increase of DON and these conjugated DON levels occurred within the germination of barley during malting. Various mechanisms might be responsible for an increase of DON and its conjugates during malting. It can be assumed that additional DON originates due to its de novo synthesis during the germination phase, which is favorable for fungus growth. Origination of DON glycosides, both mono- and oligo-, might include both the glycosylation of DON by glucose and oligosaccharides after enzymatic hydrolysis of starch and their release from a "masked mycotoxins pool". In addition, DON-3-sulfate and DON-15-sulfate are some new masked mycotoxin found in wheat [24]. DON-sulfates can be regarded as detoxification products. DON-15-sulfate was about 44 × less inhibitory than the native toxin, and no toxicity was observed for DON-3-sulfate in the tested range.

Although considered as a detoxification product in plants, the toxicity of DON-3G in mammals is currently unknown. Berthiller et al. (2011) used *in vitro* model systems to investigate the stability of DON-3G to acidic conditions, hydrolytic enzymes and intestinal bacteria, mimicking different stages of digestion [25]. DON-3G was found resistant to 0.2 M hydrochloric acid for at least 24 h, suggesting that it will not be hydrolyzed in the stomach of mammals. While human cytosolic β-glucosidase had no effect either, fungal cellulase and cellobiase preparations could cleave a significant portion of DON-3G. Most importantly, several lactic acid bacteria such as *Enterococcus durans*, *Enterococcus mundtii* or *Lactobacillus plantarum* showed a high capability to hydrolyze DON-3G.

Similarly, in another study [26], the conditions in the GI-tract do not result in hydrolysis of the glucoside into the original mycotoxin DON. No evidence was found for the transformation of DON-3G to DON by the Caco-2 cells in both the apical or basolateral side in 24 h. Thus, no evidence was found in the *in vitro* experiments for significant elevated exposure of humans to DON, since DON-3G was not hydrolyzed to DON in the digestion model representing the upper part of the GI-tract and DON-3G was not hydrolyzed to DON by the intestinal epithelial Caco-2 cells. Bioavailability of DON-3G in humans may be low compared to DON since Caco-2 cells did not absorb DON-3G, in contrast to DON. However, in another study [27], conversion from DON to DON-3G is likely to occur during gastro-duodenal digestion of contaminated bread, since a decrease of DON and a remarkable increase of DON-3G was highlighted during the passage in the duodenal compartment.

Nagl et al. (2012) have evaluated whether DON-3G could be reactivated *in vivo* by investigation of its metabolism in rats [28]. After administration of DON-3G, only 3.7 ± 0.7% of the given dose were found in urine in the form of analyzed analytes, compared to 14.9 ± 5.0% after administration of DON, and only 0.3 ± 0.1% were detected in the form of urinary DON-3G. The majority of administered DON-3G was recovered as DON and 12,13-deepoxy-DON (DOM-1) in feces. Their study suggests that DON-3G is little bioavailable, hydrolyzed to DON during digestion, and partially converted to DOM-1 prior to excretion. In pigs, intravenously administered DON-3G was almost exclusively excreted in unmetabolized form via urine. Data indicate that DON-3G is nearly completely hydrolyzed in the intestinal tract of pigs, while the toxin seems to be rather stable after systemic absorption [29]. Recently, DON-3G is reported to be metabolized to DON-3G-sulfonate in rats. DON-3G sulfonates excreted in faeces accounted for 47% of the total amount of administered DON-3G [30].

To investigate whether or not the masked DON are excreted in human urine, unaltered DON-3G was monitored in all 24 h and spot urine samples [56]. 24 h samples were additionally analyzed after enzymatic hydrolysis. In none of the analyzed samples any masked form was detected. This might indicate its hydrolysis to free DON in the body.

In summary, DON-3G is the most common masked toxin in cereals and beer and other new masked toxins are also identified recently including DON-3-diglucoside, DON-3-triglucoside, DON-3-tetraglucoside, DON-3-sulfate and DON-15-sulfate. Currently there are relatively less investigations to study their toxicity. From the current data, we began to know that their toxicity is lower than DON, but it is important to understand that these masked toxins could be hydrolyzed to their precursors in the diges-tive tracts of animals and that they show much higher toxicity. Similar with DON, DON-3G is stable during food process, but some conflict results are reported from different studies, thus more investigations are needed to offer us more information in this field.

DON CONTAMINATION IN FEED

The problem of DON contamination affects animal feeds as well as human food, because livestock may be exposed to mycotoxins after ingesting mycotoxin-contaminated feed. DON is difficult to degrade during food and feed processing. In developing countries, DON contamination of feeds is much more severe due to inadequate storage conditions [8].

In the Asia-Pacific region, a 2-year study (2003 - 2005) evaluated the incidence of mycotoxins in feed and raw feed materials [31]. The DON contamination levels detected in feed samples from different regions of Asia and Oceania were 76 - 925 μg/kg, whereas the DON levels in Europe and the Mediterranean were 304 - 559 μg/kg. DON, ZON, and T-2 toxin were the major forms of mycotoxin contaminants. The materials collected from the Asia-Pacific region tended to be contaminated with DON, zearalenone (ZON), fumonisins, and aflatoxins.

Many countries in Europe reported the contamination of DON in feed. Pleadin et al. (2012) determined the DON contamination levels in pig feed samples from the northwestern part of Croatia and found that the levels of DON contamination in pig feed (817 ± 447 μg/kg) were higher than local regulatory limit in 40% of the analyzed samples [32]. In northern Italy, DON was detected in 38.9% of the raw feeds for horses, with levels ranging 200 - 1900 μg/kg. Maize samples contained the highest concentrations of DON, but barley was the most frequently contaminated grain (73.7%) [33]. In Germany, DON levels in horse feeds were found to be 16 - 4900 μg/kg (median 220 μg/kg), where the maximum level of DON was also found in maize [57]. In Poland, a 4-year survey (2006 - 2009) of mycotoxins in feed materials (cereal and corn grains) and feedstuffs (silages and mixed feeds) detected high concentrations of DON in these samples [34]. The levels of DON varied depending on the year, ranging among 835 - 7356 μg/kg in cereal grains, 3090 - 6817 μg/kg in corn grains, 409 - 2739 μg/kg in mixed feeds, and 1140 - 14470 μg/kg in silages. A recent study analyzed mycotoxin contamination in poultry feed mixtures from western Poland and DON was found to have the highest contaminant level (3.05 - 99.36 μg/kg) among the detected mycotoxins [35]. The levels of microbiological contamination in the feeds produced for broiler chickens in western Poland were within the requirements needed to satisfy the legal standards of EU.

Commercial swine feeds produced by feed mills in Portugal were analyzed to determine the presence of mycotoxins and 16.9% of the samples were positive for DON (100 - 864 μg/kg), while the co-occurrence of DON/ZON was also frequent [36]. In Burdur Province, Turkey, between 2006 and 2009, the most frequent mycotoxin was aflatoxin, followed by DON (48.3%) with concentrations of 18.5 - 500 μg/kg in cattle and calf feeds [37]. Streit et al. (2013) performed multi-myctoxin screening of feed and feed ingredients collected from markets in Hungary, Austria, and Denmark, where the masked form, DON-3G (75% positive), was detected frequently in addition to DON [38].

In commercial compound feeds from South Africa, the most common feed contaminants were fumonisins (104 - 2999 μg/kg), followed by DON (124 - 2352 μg/kg), but group A trichothecenes were not detected in these samples [39]. In China, DON was detected in feeds and feed ingredients, with an average level of 1670.2 μg/kg and an overall frequency of 95.2% among the analyzed samples [8]. The co-occurrence of mycotoxins was widespread and the majority of the tested samples (97.6%) were contaminated with more than one mycotoxin.

For the first time, Pietsch et al. (2013) reported the presence of DON in commercial fish feed samples designed for cyprinids, which were collected from central Europe [40]. The maximum DON concentration was 825 μg/kg feed in one sample and the average level was 289 μg/kg. The metabolite DOM-1 was not found in the samples. Thus, the DON values in fish feed did not exceed the guidance values for complete feeding stuffs. A high percentage of poultry feed samples were found to be positive for DON contamination in Kuwait, ranging from 79% in wheat bran to 100% in broiler finisher feed [7]. The maximum concentration was < 400 μg/kg in most commodities, except in layer mash and broiler starter feed, where the DON levels reached 1.5 mg/kg and 1200 μg/kg, respectively, in a few samples.

Zhang and Caupert (2012) analyzed various mycotoxins, including DON and T-2 toxin, in the distiller's dried grains with solubles (DDGS) produced by dry-grind ethanol plants in the Midwestern United States between 2009 - 2011 [41]. In two plants, the mean DON contamination levels in corn were 2200 μg/kg and 1300 μg/kg, where the mean DON levels in DDGS were 7900 μg/kg and 4400 μg/kg, respectively. The DON levels in DDGS were more homogeneous than those in corn. In Japan, DON, 3-acetyl-DON, and 15-acetyl-DON were detected in most DDGS and corn gluten feed samples, where the ranges of DON contamination in corn gluten meal samples and mixed feed samples were 50 - 640 μg/kg and 150 - 1200 μg/kg, respectively [42]. In addition, a typical order of the contaminant levels in contaminated feed or feed ingredients was: DON > 15-acetyl-DON > 3-acetyl-DON. Thus, corn-derived feed ingredients and feeds are extensively contaminated with trichothecenes in Japan.

In summary, DON, ZON, and T-2 toxin are the major contaminants of feeds, and feed materials from Asia and the Pacific tend to be contaminated with DON, ZON, fumonisins, and aflatoxins. Severe DON contamination is also found in European countries where the maximum level of DON is usually detected in maize, while the masked form DON-3G is frequent in these countries. In South Africa, fumonisins are the most frequent feed contaminants, followed by DON. In Japan, corn-derived feed ingredients and feeds are extensively contaminated with trichothecenes. China and Kuwait have the highest frequency of DON contamination in animal feeds. However, from the current data, the DON levels in fish feed seems to have not exceeded the guidance values for complete feeding stuffs in Europe. The different contamination levels of DON in cereals and feed in different countries and regions could be depending on the weather, climate, *F. graminearum* phenotype and storage conditions. The occurrence of DON in the cereal-based feed from different countries is summarized in Table 1.

MASS SPECTROMETRY ANALYTICAL METHODS

Liquid chromatography (LC) coupled with tandem mass spectrometric (MS) detection has gained more importance for mycotoxin determination in recent years. Unlike gas chromatograph-mass spectrometer (GC-MS), LC-MS samples do not need to go through derivatization. Atmospheric pressure chemical ionisation (APCI) and electrospray ionisation (ESI) have been successfully applied in LC-MS methods; however, ESI is preferred in the majority of DON quantification [43]. Currently, more specific and sensitive HPLC-MS methods for the simultaneous determination of DON and other mycotoxins in food and feed are well developed. These established methods for the determination of mycotoxin residues in food and feed will provide important insurance for human health.

The simultaneous determination of DON, ZON, T-2 and HT-2 toxins in foodstuff by HPLC-MS was investigated by Romagnoli et al. (2010) [44]. The low detection limit of DON was 60 μg/kg. In addition, they tested new multi-mycotoxin immunoaffinity columns (IACs) and showed that the combining IACs and LC–MS/MS technique has many advantages, for example efficient removal of matrix interferences, simple chromatographic outline, high selectivity, and low detection limits (DLs). Another IAC clean-up combined with UPLC-MS/MS method also showed high recoveries for DON in wheat and rice (61 - 103%) [45]. A limit of detection (LOD) and a limit of quantification (LOQ) were 0.3 and 0.8 μg/kg, respectively. Using this method, the authors found that 52.5% of the analyzed commercial cereal samples were positive at DON concentrations from 7 to 534 μg/kg.

Determination of mycotoxins in body fluids using HPLC-MS is a fast and convenient method for the monitoring of the mycotoxin residues in living animals. De Baere et al. (2011) have developed a specific method for the quantitative determination

Table 1. The occurrence of DON in cereal-based feed from different countries.

Country	Year	Samples (n)	Positive	Average/range (μg/kg)	Greatest level (μg/kg)	Analysis method	Reference
Asian and Pacific regions	2003-2005	Cereal based feed (1291)		76–925	18991	HPLC	[13]
Croatia	2011	Pig feed (30)	40%	817 ± 447	1864	ELISA test kits	[32]
Italy	2012	Raw materials for equine feed (maize (35), barley (15), oats (12), and rice bran (10))	38.9%	200–1900	1900	ELISA assay kits	[33]
Germany	2010	Commercial horse feed preparations (muesli and mash (n=39), pelleted feeding stuff (n=12), maize (n=5), oats (n=4), barley (n=2))	100%	410 ± 660	4900	Competitive direct enzyme immunoassays	[57]
Poland	2006–2009	Feed materials (cereal and corn grains) and feedstuffs (silages and mixed feeds)(1255)	74-100%	835–7356 (cereal grains), 3090–6817 (corn grains), 409–2739 (mixed feeds), 1140–14470 (silages)	14470	HPLC-MS/MS	[34]
Poland	2013	Poultry feed mixtures (45)	100%	33.58±26.97	99.36	HPLC	[35]
Portugal	2011	Commercial swine feeds (404)	16.9%	100–864	864	HPLC	[36]
Turkey	2006–2009	Cattle and calf feeds (180)	48.3%	59.76 ± 7.03/18.5–500	500	ELISA commercial kit	[37]
Hungary, Austria, Denmark	2010	Feed and feed raw materials (62): silage, maize, wheat and wheat by-products, barley straw	DON-3G (75%)	15	7764	LC-MS/MS	[38]
South Africa	2010-2011	Cereals (92) (mainly maize)	98.9%	696 ± 490	2352	LC-MS/MS	[39]
China	2009	Feed ingredients and feed compound sample (83)	95.2%	1670.2	13139.4	HPLC	[8]
Central Europe	2013	Fish feed (11)	>80%	289	825	HPLC	[40]
Kuwait	2006	Feed samples (34)	89-100%	170–290	1500	HPLC	[7]

of DON, DOM-1, T-2 and HT-2 toxins in animal body fluids (plasma and bile) using LC-ESIMS/MS [46]. LOQ were between 1 and 2.5 ng/mL for all compounds. LOD ranged from 0.01 to 0.63 ng/mL. The method has been successfully used for the quantitative determination of these toxins in plasma and the semi-quantitative determination of the same compounds in bile from broiler chickens and pigs, respectively.

DON and DOM-1 are suggested to be the urinary biomarkers for human exposure of DON [47]. A LC-MS method has been developed for simultaneous determination of DON and DOM-1 in human or animal urine. The LOD of DON and DOM-1 were 0.8 ng/mL [48]. Measurable concentrations of DON and DOM-1 were detected in most human urine samples. Moreover, a co-occurrence of ochratoxin A and DON in human urine was reported for the first time in this study. In addition, a GC-MS method was developed for the confirmation analysis of DON and its metabolites in urine samples using ^{13}C isotopic-labeled DON as internal standard [49]. SPE C18 cleanup procedure was applied for the first time in the analysis of DON and its metabolites in human urine. The achieved detection and quantification limits ranged from 0.06 to 0.30 ng/mL and from 0.2 to 1.0 ng/mL, respectively.

DON-3G is the main known DON metabolite in various processed cereal-derived products. The co-occurrence of DON and DON-3G in cereal-based products has already been reported but data about their absolute and relative concentrations are still insufficient. In order to contribute to a better understanding of the significance of DON-3G, the quantitative determination of DON and DON-3G has been carried out in cereal malt-based products by LC-MS/MS [50]. DON was detected in all cereal samples but only in 1 malt-based product whereas DON-3G was detected in 21 cereal samples (median: 19 μg/kg) and only in 1 malt-based product (6 μg/kg). The proportion of DON-3G in relation to DON concentration was within a 6 - 29% range with an average value at 12 ± 7% in the tested samples.

Currently, a LC/linear ion trap mass spectrometry method capable of determining DON-3G was developed [51]. The method adequate detection quantitation limits of 4 and 11 μg/kg, respectively,

were achieved. The reliability of the method was finally demonstrated in bread, cracker, biscuit and minicake commodities, resulting in relatively low levels of DON-3G, which were not higher than 30 μg/kg. Similarly, a simple and reliable method for simultaneous determination of DON-3G and major type B trichothecenes (DON, nivalenol, fusarenon X, 3-acetyl-DON 15-acetyl-DON and DOM-1) in animal feed and raw materials has been developed and validated by Zhao et al. (2014) [19]. The LOQ was 5.0 μg/kg for DON. This method showed that 90.2% of the corn samples were contaminated with DON-3G at the levels of 6.0 - 121.0 μg/kg. Recently, a LC-TOF-MS/MS method for the determination of DON and DON-3G in wheat was developed by Yoshinari et al. (2014) [12]. The LOQ for DON and DON-3G was 2.0 and 1.0 μg/kg, respectively.

Geng et al. (2014) have developed a new hydrophilic interaction liquid chromatography (HILIC) method for the determination of DON-3G in cereals [52]. DON-3G can be determined by chromatographic separation on a Syncronis HILIC column, and detected at 220 nm by UV detection. LOD and LOQ were 8 and 25 μg/kg, respectively. The method can be considered as an alternative to LC-MS/MS and could be adopted by nonprofessional analytical labs.

For the first time, Chen et al. (2013) have developed a new LC/ESI-MS/MS method to simultaneously determine 19 Fusarium toxins, including DON, 3-acetyl-DON, and 15-acetyl-DON in animal derived food [11]. The CCα and CCβ of the analytes in different samples varied 0.16 - 1.37 μg/kg and 0.33 - 2.34 μg/kg, respectively. The developed method was successfully used to monitor the contaminant exposure originating from different animal-derived foods. It will potentially be a useful tool for accurately evaluating the intake of mycotoxins in animal derived foods and protecting consumer health. In another study, a specific LC–MS/MS method for a simultaneous analysis of type-B trichothecenes (DON, 3-acetyl-DON, and 15-acetyl-DON and DOM-1) in chicken muscle, liver, kidney, and fat tissues was developed and validated [53]. The decision limits and the detection capabilities of the analytes in the chicken tissues ranged from 0.16 to 0.92 μg/kg and 0.68 to 2.07 μg/kg, respectively. A LC-ESI-MS/MS method was developed for the simultaneous determination of DON, ZEN and their metabolites in pig serum [13]. The analyte concentrations were determined by the use of isotopically labeled internal standards (IS). LOD and LOQ ranged 0.03 - 0.71 ng/mL and 0.08 - 2.37 ng/mL, respectively. The method has been successfully used for quantitative determination of ZON, DON and their metabolites in pig serum from a feeding trial with practically relevant ZON and DON concentrations.

A developed method is usually validated by different laboratories. For example, to validate an LC-MS/MS method for simultaneous determination of DON and its acetylated derivatives, 3-acetyl-DON and 15-acetyl-DON in wheat, an interlaboratory study was performed in 9 laboratories. The relative standard deviations for repeatability and reproducibility of DON were in the ranges of 7.2 - 11.3% and 9.5 - 22.6%, respectively [54].

CONCLUSION

The masked mycotoxin is another “emerging” food safety issue related to mycotoxins. Obtaining more knowledge concerning their natural occurrence, impact of processing, metabolic fate in animals and human, and toxicity has become increasingly important for assessing the potential health risks associated with mycotoxin contamination. DON-3G is the most common masked toxin in cereals and beer, but other new masked toxins are also reported recently. The toxicity of the masked DON is lower than DON. However, once transported into human or animal body, they are hydrolyzed to their precursors in the digestive tracts and show much higher toxicity. Thus the risk assessment of masked DON is an important issue for human and animal food safety.

The storage condition of feed is easily overlooked, especially in developing countries, and thus cereal-based feed are usually contaminated by DON and other mycotoxins. DON, ZON, and T-2 toxin are the major contaminants of feeds, and feed materials from Asia. Severe DON contamination is found in European countries where the maximum level of DON is usually detected in maize. Livestock may be exposed to DON from the consumption of contaminated feed and this will subsequently bring a potential risk to human health from the animal-derived food. The different contamination levels of DON in cereals and feed in different countries and regions could be depending on weather, climate, and storage conditions.

Analytical methods for rapid, sensitive, and accurate determination of DON and its derivatives

in foods, feeds, human and animal are highly demanded for toxicological analysis and exposure risk assessment. They are also needed to enforce regulatory requirements issued by governments and international organizations. Currently, the specific and sensitive mass spectrometry analytical methods are a very important control strategy of mycotoxins, and the established methods will provide critical insurance for human health.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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