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To cite this version:

J-M Fremy, Imourana Alassane-Kpembi, Isabelle P. Oswald, B. Cottrill, H.P. van Egmond. A review on combined effects of moniliformin and co-occurring Fusarium toxins in farm animals. World Mycotoxin Journal, 2019, 12 (3), pp.281-291. 10.3920/WMJ2018.2405 . hal-02627929

HAL Id: hal-02627929

https://hal.inrae.fr/hal-02627929v1

Submitted on 2 Sep 2021

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World Mycotoxin Journal, 2019; 12 (3): 281-291

Wageningen Academic Publishers

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A review on combined effects of moniliformin and co-occurring *Fusarium* toxins in farm animals

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Received: 12 November 2018 / Accepted: 17 May 2019
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OPEN ACCESS

REVIEW ARTICLE

Abstract

Co-occurrence of mycotoxins in food and feed represents the rule rather than the exception. Information about combinatory toxic effects of co-occurring mycotoxins is scarce, in particular the effects that mixtures of mycotoxins in feed may have on farm animals. This review focusses on studies on the combined effects of moniliformin and co-occurring mycotoxins in feed on farm animals. Moniliformin is a mycotoxin of emerging scientific interest, which may co-occur with many other mycotoxins, especially *Fusarium* mycotoxins. Oral exposure to moniliformin reduces feed consumption and body weight gain in poultry, in pigs and catfish, and induces cardiotoxic effects and/or alterations in serum biochemical and haematological parameters. In this review only experiments comparing effects as a result of the exposure to a combination of mycotoxins with effects due to the exposure to single mycotoxins were considered. Identified published studies on combined toxicity have been limited to combinations of moniliformin with either fumonisin B_1 or deoxynivalenol, and were performed with poultry, pigs, and catfish. Most of the moniliformin/fumonisin B_1 investigations involved poultry and focussed on adverse effects on feed intake, weight gain and immune response, as well as organ lesions. These studies mainly reported an interactive toxicity of moniliformin and fumonisin B_1 but did not allow identification of the type of interaction. Likewise, no indication could be given for the interaction detected for both mycotoxins on weight gains of catfish. For the moniliformin/deoxynivalenol combination, only one study with broiler chickens was found relevant. This study concluded additive or less than additive toxicity, using kidney lesions and renal tubular epithelial degeneration as endpoints. While possible interactions between moniliformin and fumonisin B_1 or deoxynivalenol were identified, the conclusions are based on limited studies and experimental designs. Further studies on the combined toxicity of moniliformin with other mycotoxins and other animal species would be needed.

Keywords: deoxynivalenol, fumonisin B_1, adverse effects, feed, risk assessment

1. Introduction

The presence of multiple mycotoxins in food and feed represents the rule rather than the exception. Co-occurrence of mycotoxins may be due to the ability of certain fungal species to produce several toxins, and to the possibility of co-occurrence of different mycotoxin-producing species in the same substrate. The likelihood of co-occurring mycotoxins in food and feed is challenging for risk assessment, since recognised procedures of risk assessment on a chemical-by-chemical basis are in danger of underestimating the risk of chemicals to health where combined exposure occurs (EFSA, 2013, 2019; Kortenkamp *et al.*, 2009; Meek *et al.*, 2011). The scientific interest in the toxicity of mixtures of mycotoxins is increasing rapidly (Alassane-Kpembi *et al.*, 2017a; Assunção *et al.*, 2016). As far as mycotoxins are concerned, the simultaneous exposure to different compounds has been shown to result in antagonistic, additive or synergistic effects (Alassane-Kpembi *et al.*, 2017a; Grenier and Oswald, 2011). A

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demonstration of synergism would legitimately heighten concerns about health risks (Alassane-Kpembi *et al.*, 2017b), while the implications of additive combination effects have received only limited attention. Indeed, the threshold dose for toxic effects may sometimes be exceeded in the presence of exposure to a mixture, although the exposure to each single compound is unlikely to pose risk (Silva *et al.*, 2002).

The issue of combined toxicity of mycotoxins is very complex, since many mycotoxins may co-occur. Even restriction down to certain specific groups of fungi and the mycotoxins they may produce, poses a major problem in risk assessment. One of the most important groups of fungi known to produce multiple mycotoxins are the *Fusarium* species which can frequently infect small grain cereals and maize. Many toxigenic *Fusarium* species exist, and they may produce a wide array of mycotoxins. It was therefore considered appropriate to address the issue of the combined toxic effects of co-occurring *Fusarium* toxins, particularly since the importance of this was noted during the drafting of EFSA Scientific Opinions on the risks to human and animal health related to the presence of various *Fusarium* mycotoxins in food and feed. This was particularly the case for the Opinion on moniliformin (MON), a mycotoxin of increasing scientific interest (EFSA, 2018). During the preparation of these Opinions, information on the co-occurrence and the combined effects of MON and other *Fusarium* mycotoxins was gathered, although it was only marginally reflected in the EFSA Opinion because it was considered of minor importance for the derivation of the health-based guidance value. Therefore, this paper brings together and reviews the information available with the aim of contributing to and stimulating scientific debate on the combined toxicology of mycotoxins, with particular reference to MON. In doing so, the authors have limited the scope of the paper to co-occurrence in feed and combined effects in farm animals, since there were no data on the combined effects of MON in experimental animals.

MON was first described by Cole *et al.* (1973) while screening for toxigenic metabolites of *Fusarium moniliforme* from naturally infected southern leaf blight-damaged maize seed. Since then, MON has been reported to be produced by several *Fusarium* species (Sydenham *et al.*, 1996), including *Fusarium avenaceum, Fusarium subglutinans* and *Fusarium proliferatum*, and by *Penicillium melanoconidium* (Hallas-Møller *et al.*, 2016). Some of these *Fusarium* species are also known to produce mycotoxins other than MON, including beauvericin (BEA), enniatins (ENNs) and fumonisins B (FBs) (Gupta *et al.*, 1991). In addition, *Fusarium fujikuroi*, which is a complex of several *Fusarium* species, was reported to produce not only MON, but also FB_1, FB_2 and FB_3, BEA and fusaproliferin (Fotso *et al.*, 2002; Leslie *et al.*, 1996). Darnetty and Salleh (2013) analysed twenty selected strains of *Fusarium* species, including *Fusarium verticillioides*, *F. proliferatum*, *F. subglutinans* and *Fusarium konzum*; all strains were found to have the ability to produce FB_1 and/or MON and/or BEA.

A limited number of studies on MON which focus on co-occurring mycotoxins in food and feed grains (samples taken from 2000 onwards, focus on European studies) have been selected and the results were summarised in Supplementary Table S1. This table is not meant to be comprehensive, but it illustrates how MON may co-occur with other mycotoxins in grains.

Based on the evidence that MON can co-occur with other mycotoxins in cereal grains, and in particular *Fusarium* mycotoxins, this paper, after summarising the state of knowledge on toxicological data of MON, and specifically the adverse effects on farm animals, reviews toxicity studies on the combined effects of MON with other *Fusarium* toxins.

2. Toxicity of moniliformin

Two recent review articles compiled data regarding the mechanism of action of MON and its *in vitro* and *in vivo* toxicity for laboratory animals (Freayman *et al.*, 2017; Gruber-Dorninger *et al.*, 2017). By inactivating enzymes sharing thiamin as a common cofactor (e.g. pyruvate dehydrogenase and α-ketoglutarate dehydrogenase), MON impairs the Krebs cycle and compromises cellular energy supply.

The *in vitro* toxicity of MON is highly cell line dependent. It shows no inhibitory effects on the proliferation of human white blood cell progenitors or human platelet progenitors, nor to chicken primary chondrocyte and macrophage. In contrast, the mycotoxin is cytotoxic to human red blood cell progenitors and to chicken primary splenocytes, and both cardiac and skeletal myocytes. By the intraperitoneal route, the LD_{50} values of MON have been reported as 2.2-2.8 mg/kg body weight (bw) in 9-month-old female mink, and as 20.9 and 29.1 mg/kg bw in female and male mice, respectively, compared to 41.6 and 50.0 mg/kg bw by oral route in female and male rats.

In farm animals, the *in vivo* toxicity of MON targets the heart, causing acute heart failure, but the mycotoxin can also cause muscle weakness, respiratory distress and negatively affect immunity and animal performance. Accordingly, the massive increase of the number of mitochondria associated with the disruption of heart muscle fibres observed in Japanese quail and in broilers appears to be a compensatory event for the MON-induced decrease of cellular energy (Javed *et al.*, 2005; Sharma *et al.*, 2012; Thiel, 1978).

The addition of MON-contaminated material to diets from culture of toxigenic *Fusarium* strains, or purified MON from such culture material, has been used for the

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identification of adverse effects of MON in farm animals. Broiler chickens showed reduced body weight gain and cardiomyopathy, at exposure levels starting from 50 mg MON/kg feed during a 3-week experiment corresponding to a dose of 2.8 mg MON/kg bw per day (Broomhead *et al.*, 2002; Ledoux *et al.*, 1995). Reduced feed intake, reduced body weight gain, cardiomegaly and changes in some haematological parameters, including increased alanine transferase and aspartate transaminase activities, creatinine concentration and decreased mean corpuscular volume, and mean corpuscular haemoglobin concentration have been observed at a level of 100 mg MON/kg feed by several authors (Harvey *et al.*, 1997; Kubena *et al.*, 1999). Body weight reduction and cardiomegaly were also reported for turkeys receiving 100 mg MON/kg and 50 mg MON/kg feed, respectively (Bermudez *et al.*, 1997; Broomhead *et al.*, 2002), while 75 mg MON/kg feed induced cardiomegaly in fattening ducks (Broomhead *et al.*, 1996; Morris *et al.*, 1997). Reduced egg production and body weight gain in laying hens have been observed with a diet containing 100 mg MON/kg feed (Kubena *et al.*, 1999). In pigs, reductions in body weight gain and feed consumption, alteration of haematologic and serum biochemical values, and increases in heart weight and hydropericardium have all been associated with oral administrations of 100 mg MON/kg feed (Harvey *et al.*, 2001). No data on observed adverse effects were available for other farm animal species.

In summary, reductions of feed consumption and body weight gain, and/or cardiotoxicity, and/or alterations in serum biochemical analytes and hematologic values were the observed adverse effects of MON in some farm animal species. The lowest observed adverse effect levels (LOAELs) were determined at 100 mg MON/kg feed for pigs and laying hens, 75 mg MON/kg feed for ducks and 50 mg MON for broiler chickens and turkeys. No data on observed adverse effects were available for other farm animal species.

3. Combined effects of moniliformin with other *Fusarium* toxins

Selection of experimental studies

Experimental studies reported in the scientific literature focusing on the effects of diets simultaneously contaminated both with MON and other *Fusarium* toxins in farm animals have adopted different experimental designs. While the experimental studies based only on a design in which feed is naturally multi-contaminated provide, in principle, the evidence for the combined adverse effects under practical animal husbandry conditions, they have not compared the effects of feed naturally multi-contaminated with feed naturally contaminated with MON alone. Moreover, it should be noted that in such studies the naturally contaminated feed is also likely to have also contained several other mycotoxins. Consequently, studies reporting effects due to mixtures of MON with several mycotoxins or with another *Fusarium* toxin but without involving trials using contaminated diets with single mycotoxins have not been considered in this review. Moreover, publications available only as symposia abstracts, and not followed by a full description of experiment details in the associated proceedings, have been also excluded from this review. Using these criteria, the publications available in the literature on combined toxicity have been selected and their data are summarised in Table 1. The experimental studies focused on combinations of MON plus FB_1 and on MON plus deoxynivalenol (DON). They involved pigs and poultry, since toxicological and case studies had identified these species as being the most sensitive to FB_1 and DON (as compared to other animal species such as cattle). Only one experiment was performed with fish. The results from these experimental studies are summarised below.

Moniliformin and fumonisin B_1

Harvey *et al.* (2002) fed four groups of six barrows (mean bw 11.1 kg) with either a control diet or diets containing 100 mg MON/kg of feed, 100 mg of FB_1 per kg of feed, or 100 mg of FB_1 plus 100 mg of MON per kg of feed, for 28 days. Regarding the chosen levels, the authors reported that 100 mg MON/kg feed was identified to cause body weight gain reductions and feed consumption and led to alterations of haematologic and serum biochemical values for pigs (Harvey *et al.,* 2001), and levels of 100 mg FB_1/kg feed to induce kidney lesions, pulmonal oedema and hepatic disease and reduction of body weight gain in swine[1]. Ground culture materials from *F. fujikuroi* containing MON (at no more than 1.0% of the basal diet), and from *F. verticillioides* containing FB_1 (at no more than 2.5% of the basal diet) substituted part of the ground maize in the diets to achieve the appropriate concentrations of MON and FB_1. The total FB content determined by HPLC analysis was 100 mg FB_1 plus 30 mg FB_2 and 9 mg FB_3 per kg feed. Body weight gain, feed efficiency, serum biochemical analytes, and haematological values were adversely affected by the FB_1 and the combined MON/FB_1 diets. The MON diet decreased body weight gain. Two barrows in the MON diet group, and two barrows in the combined MON/FB_1 diet group died during the first 6 days of the study. Mild to moderate lesions were observed microscopically in heart and lung tissues of the groups fed MON and combined MON/FB_1 and in liver tissues of the groups fed FB_1 and combined MON/FB_1. Except for the mortality associated with the two diets containing MON, these effects remained unexplained despite investigations by the authors, which included necropsies. Clinical disease induced by the combined feeding of these two mycotoxins appeared to

[1] In the EFSA opinion on MON, a LOAEL of 5 mg FB_1/kg feed was identified for pigs (EFSA, 2018).

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Table 1. Selected studies on possible combined effects between moniliformin (MON) and other *Fusarium* mycotoxins in farm animals.[1]

MON/tested mycotoxin species (exposure period)	Concentrations (MON/ tested mycotoxin) (mg/kg feed)	Additive or synergistic combined effects[2]	Antagonistic combined effect[2]	Reference
MON/FB_1 chicken (14 days)	27 to 154 / 27 to 154	- alkaline phosphatase - gamma glutamyl transferase - aspartate aminotransferase - lactate dehydrogenase - cholesterol - sodium - red blood cell - haemoglobin - packed cell volume		Javed *et al.* (1993, 1995); Hall *et al.* (1995)
MON/FB turkey (21 days)	100 / 200	- feed intake (P) - relative weight (liver) - aspartate aminotransferase	- body weight gain - mortality - glucose - lactate dehydrogenase	Bermudez *et al.* (1997)
MON/FB pig (28 days)	100 / 100	- feed intake - creatinine (P) - number of red blood cells	- body weight gain - mortality - glucose - inorganic phosphorus - aspartate aminotransferase, alkaline phosphatase, lactate dehydrogenase, gamma-glutamyltransferase - total iron	Harvey *et al.* (2002)
MON/FB laying hens (420 days)	50 / 100	- relative weight (pancreas) - albumin - aspartate aminotransferase - mortality	- cytokine - relative weight (liver, kidney) - uric acid - egg production - egg weight	Kubena *et al.* (1999)
MON/FB chicken (21 days)	100 / 100	- relative weight (heart)	- feed intake - mortality - aspartate aminotransferase - body weight gain	Ledoux *et al.* (2003)
	200 / 100	- no parameter tested[3]	- relative weight (heart, kidney, liver) - albumin, total protein - mortality - aspartate aminotransferase	
	100 / 200	- relative weight (liver, kidney) - albumin, total protein	- relative weight (heart) - feed intake, body weight gain - mortality - aspartate aminotransferase	
	200 / 200	- relative weight (kidney, liver)	- relative weight (heart) - albumin - mortality - aspartate aminotransferase - total protein	

be additive or less than additive and due primarily to the toxic expression of FB_1. However, it should be noted that the total level of FBs was the sum of $FB_1 + FB_2 + FB_3$, i.e. 139 mg FBs/kg of feed.

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Table 1. Continued.

MON/tested mycotoxin species (exposure period)	Concentrations (MON/ tested mycotoxin) (mg/kg feed)	Additive or synergistic combined effects[2]	Antagonistic combined effect[2]	Reference
MON/FB quail (35 days)	100 / 200	- body weight - mortality	- total protein - cholesterol - alanine aminotransferase, aspartate aminotransferase - albumin - lactate dehydrogenase, cytokine - creatinine - delayed type hyposensitivity reaction	Sharma *et al.* (2008, 2012)
MON/FB fish (70 days)	40 / 20 40 / 40	- size of hepatocyte nuclei - body weight gain - serum pyruvate - size of hepatocyte nuclei - feed intake	- body weight gain, feed intake - Sa/So liver[4] - Sa/So liver[4]	Yildirim *et al.* (2000)
MON/DON turkey (21 days)	100 / 20	- feed intake - globulin - relative weight (kidney)	- body weight gain - relative weight (heart) - calcium	Morris *et al.* (1999)
MON/DON chicken (21 days)	100 / 16	- feed intake - relative weight (gizzard and heart) - epithelial degeneration		Harvey *et al.* (1997)

[1] DON = deoxynivalenol; $FB_{(1)}$ = fumonisin $B_{(1)}$.
[2] As concluded by the authors.
[3] No parameter tested: In a given study there was no parameter tested that demonstrated synergistic, additive or antagonistic combined effect.
[4] Ratio free sphinganine to free sphingosine.

The combined effects of MON/FB_1 were studied in chickens by Javed *et al.* (1993, 1995). Six groups of 10 day-old broiler chickens (Columbia × New Hampshire) were used up to 14 days of age: in addition to the control group, birds were fed diets containing: (a) purified FB_1 at 125 mg/kg feed; (b) purified FB_1 at 274 mg/kg feed; (c) purified MON at 27 mg/kg feed; (d) purified MON at 154 mg/kg feed; and (e) a diet containing both purified FB_1 and MON in combination at 137 and 77 mg/kg feed, respectively. No clinical signs or deaths occurred in the controls. Adverse health effects in birds treated with the combination occurred earlier (after 2 days) than in birds treated by MON alone at 154 and 27 mg/kg feed or FB_1 alone at 274 and 125 mg/kg feed after 5 and 7 days, respectively. These observed effects were ataxia, reduced feed intake, transient increase in water intake and seeking the heat source, and were followed in some birds by weakness and death. At day 10, mortality was 100% in birds treated with the combination. At day 7, mortality was 79% in the group with individual MON at 154 mg/kg, 50% with FB_1 at 274 mg/kg, 40% with MON-only at 27 mg/kg, and 20% with FB_1-only at 125 mg/kg. Birds surviving at least 10 to 14 days (the end of treatment) showed lowest weight gain in the combination group. The authors concluded that toxicity of MON and FB_1 was additive (Javed *et al.*, 1993). Regarding serohaematological parameters, increased values of alkaline phosphatase, gamma glutamyltransferase, aspartate aminotransferase, alanine aminotransferase, and lactate dehydrogenase activities were observed, and the authors interpreted these elevated activities as being indicative of both hepatic and renal damage. The authors also concluded that the decrease in glucose concentrations resulted from pancreatic damage (Javed *et al.*, 1995). It should be noted that in these studies, which were published in two separate papers (Javed *et al.*, 1993, 1995), the levels of both MON and FB_1 alone were different from the levels of MON and FB_1 in combination.

Since probable organ damage, as interpreted by Javed *et al.* (1995), was similar to that associated with vitamin A deficiency, serum vitamin A (retinol) status was studied by Hall *et al.* (1995) in a follow-up study. Depressed vitamin A concentrations were present in the serum of chicks given a diet supplemented with FB_1 and in combination

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with MON (at 137 and 77 mg/kg feed, respectively) when compared with those fed the MON-only supplemented diet (at 27 mg/kg feed), or the control diet. The authors concluded that vitamin A deficiency may be associated with organ damage as suggested by Javed *et al.* (1995) in their previous study. However, it should be noted that also in this study, which used on the same experimental design as those of Javed *et al.* (1993, 1995), the levels of both FB_1 and MON alone were different from the levels of FB_1 and MON in combination. While the levels were similar for FB_1 (125 mg/kg feed tested alone versus 137 mg/kg feed tested in combination), the levels for MON were very different (27 mg/kg feed when tested alone versus 77 mg/kg when tested in combination).

Consequently, due to this discrepancy in the toxin levels of the experimental design and in particular between the levels of MON tested alone and MON tested in combination, the results of these three studies and the conclusions of the authors of an additive effect in combination, should be interpreted with caution.

Ledoux *et al.* (2003) conducted a study on the individual and combined effects of MON and FB_1 in broiler chickens in four groups of five birds for 21 days. One group was fed an uncontaminated basal diet as the control. Two groups received a diet contaminated with single 100 mg MON/kg feed and 200 mg FB_1/kg feed, respectively, and a fourth group was fed the combination of 100 mg MON/kg and 200 mg FB_1/kg feed. The level of 100 mg MON/kg feed was chosen because it corresponded to the previously identified level which resulted in decreasing body weight gain for chickens (Ledoux *et al.*, 1995). Regarding the level of 200 mg FB_1/kg, the authors indicated that this was based on available literature data, where mild to moderate toxicosis was observed in chickens fed 75 to 400 mg FB_1/kg feed, with the primary changes being decreased body weight gain and liver lesions. Both MON and FB_1 contaminations were obtained by adding appropriate percentages of ground culture materials of referenced *Fusarium* strains producing MON or FB_1. Both feed intake and body weight gain were decreased in chickens fed diets containing 100 mg MON/kg feed and FB_1 as compared with controls. Chickens fed MON had heavier heart weights than control chicks or chicks fed FB_1. Compared to the controls, chickens fed diets containing 200 mg MON/kg had increased kidney and liver weights. Mild to moderate periportal extramedullary haematopoieses and mild focal hepatic necroses were observed in chicks fed FB_1 alone. An increased incidence of large pleomorphic cardiomyocyte nuclei, loss of cardiomyocytes, and mild focal renal tubular mineralisation were observed in chicks fed MON alone. Chickens fed diets containing the combination of 200 mg FB_1/kg and 100 mg MON/kg had increased kidney and liver weights and both cardiac and renal lesions were also observed. The authors concluded that MON is much more toxic to broilers than FB_1 at the levels tested and that the combined effects of MON and FB_1 were not synergistic and less than additive.

Kubena *et al.* (1999) investigated the combined effects of MON and FB_1 on White Leghorn laying hens, 24 weeks old at the start of the experiment. MON was supplied by *F. fujikuroi* culture material, and FB_1 was supplied by *F. moniliforme* culture material. Six groups of 6 birds were fed either a control diet, two diets containing MON (50 and 100 mg/kg feed), two diets containing FB_1 (100 and 200 mg/kg $FB_{1)}$ and one diet containing a combination of 50 mg/kg MON and 100 mg/kg FB_1, for 14 months. However, the 100 mg FB_1 diet also contained approximately 30 mg FB_2 and 9 mg FB_3, while the 200 mg FB_1/kg diet also contained 60 mg FB_2 and 18 mg FB_3 per kg feed. Adverse effects including mortality, reduced egg production and body weight gain were observed only in the group of hens fed 100 mg/kg MON. No synergy between MON and FB_1 was observed for any of the parameters measured when laying hens were fed the combination of 50 mg MON and 100 mg FB_1 per kg feed.

In an experiment carried out by Bermudez *et al.* (1997) four groups of 20-day old female turkey poults were fed a control ration, or rations containing 100 mg MON/kg, or 200 mg FB_1/kg, or a combination of 100 mg MON/kg and 200 mg FB_1/kg feed for 21 days. Regarding the chosen levels, the authors reported that 100 mg MON/kg feed was identified to cause body weight gain reductions for turkeys, and levels from 75 to 400 mg FB_1/kg feed were known to generate adverse effects to poultry species, such as body weight gain reduction. MON and FB_1 were supplied by *F. fujikuroi* and by *F. moniliforme* culture materials, respectively. Culture materials were included in the basal diet in percentages appropriate to provide the targeted levels of the respective toxins. No treatment-related mortality was observed. In comparison to the controls, turkeys fed FB_1 had increased liver weights. Both aspartate aminotransferase and lactate dehydrogenase were increased in poults fed FB_1. Turkeys fed MON had decreased feed intake and body weight gains and increased heart weights. Turkeys fed the ration containing both MON and FB_1 had all the above changes; however, no additive or synergistic effects were evident for any single parameter measured. No treatment-related morbidity or mortality was observed in the study (Bermudez *et al.*, 1997).

Sharma *et al.* (2008) performed an experiment on day-old quail chicks (*Coturnix coturnix japonica*) until 35 days of age. The control group involved 75 birds, fed quail mash alone, while in each of the treatment groups 105 birds were fed diets containing 100 mg MON/kg or 200 mg FB_1/kg feed, or a combination of 100 mg MON/kg and 200 mg FB_1/kg feed. The basal feed was found to contain aflatoxins at 6 µg/kg but not citrinin, FB_1, MON, ochratoxin A, T-2 toxin and zearalenone (ZEA). *F. verticillioides* culture material containing FB_1 (no information on the possible presence of

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FB_2 and/or FB_3 available) and *F. fujikuroi* culture material containing MON were used in appropriate proportions to achieve the contamination levels of the experimental diets. Birds fed a diet contaminated with 200 mg FB_1/kg feed showed ruffled feathers and poor growth. Those in the group fed the diet contaminated with 100 mg MON/kg feed and in the group fed the FB_1 diet exhibited signs of poor feathering and decreased feed intake. Clinical signs observed in the group fed a diet contaminated with 100 mg MON/kg and 200 mg FB_1/kg feed were similar to those observed in groups fed the MON or FB_1 diets alone. The mortality throughout the experiment was 7.6%, 12.4% and 21% for groups fed MON, FB_1, and MON/FB_1 diets respectively. The mortality for the group of birds fed with the combination diet (MON/FB_1) was higher than the mortality for the group of birds fed single toxin diets, which could indicate an additive effect. In the surviving birds, the reduction of feed intake was comparatively more severe in groups fed either MON diet alone or in combination with FB_1 relative to the group fed FB_1 diet alone, suggesting that MON was responsible for the reductions in feed intake. Although body weight gains in the MON diet alone and combination groups were lower than in the FB_1 alone group, no additive or interactive effects were evident between FB_1 and MON. Cell-mediated immune response was more or less comparable in the control and MON treatments. By contrast, throughout the study the presence of FB_1, in both the FB_1-only and MON/FB_1 groups, was associated with a gradual increase in skin thickness, and a lower mononuclear inflammatory cell response as compared with the control and MON groups. In conclusion, no additive or interactive effects were evident between MON and FB_1 except for mortality and serum aspartate transaminase, where additive effects were observed.

Building on this experiment, Sharma *et al.* (2012) undertook a subsequent study which focused on gross and microscopic observations reported. Thinning of the heart was the only gross lesion in the group fed FB_1 alone, whereas mild-to-severe cardiomegaly was observed both in groups fed MON alone and in the group fed the combined MON/FB_1 diet. Microscopically, thinning of cardiomyocytes was evident at the 7th day of the experiment in the group fed FB_1 alone. In the same study, hypertrophy of cardiomyocytes was noticed in the group fed MON alone. Similar – but more severe – lesions were observed in the group fed the combined MON/FB_1 diet. These included myocardial haemorrhages, vacuolar changes, hypertrophy of cardiomyocytes, focal myocarditis, and loss of myofibrils cross-striations. The authors concluded that the effect of MON on the heart is exaggerated in the presence of FB_1. Although the overall interactive effect of FB_1 and MON was less than additive, the interactive effects between the two toxins for cardiac lesions were at least additive and perhaps even synergistic up to the second week.

In a study reported by Yildirim *et al.* (2000) on fish, 35 Channel catfish (*Ictalurus punctatus)* were fed a basal diet as control (no information on the possible content of mycotoxins) and diets formulated to contain 20, 40, 60 and 120 mg MON/kg; 20 and 40 mg FB_1/kg, or two combinations of MON/FB_1, namely 40:20 mg/kg and 40:40 mg/kg, for 10 weeks. *F. proliferatum* culture material containing MON, and *F. moniliforme* culture material containing FB_1 (no information on possible co-occurrence of FB_2 and/or FB_3) were added in the experimental diets at appropriate proportions to achieve the desired contamination levels. Fish fed diets with contamination levels of 20 mg MON alone or 20 mg FB_1 alone per kg feed had statistically lower body weight gains than the fish fed the control diet, while the body weight gain of fish fed the FB_1 diet was even lower than that of fish fed the MON diet. An interactive effect from combinations of MON and FB_1 in diets was observed. Fish fed with both the combinations MON/FB_1 40:20 and 40:40 mg/kg had lower weight gains than fish fed MON alone and FB_1 alone at the same levels. Overall mortality of fish was 4% and not related to treatment effects. Fish fed diets containing MON (60 mg/kg feed) and FB_1 (40 mg/kg feed), and both combinations of MON and FB_1 at levels of 40:20 and 40:40 mg/kg resulted in significant reductions in haematocrit compared with the control fish. Increases in serum pyruvate concentration were observed with increasing concentrations of dietary MON and FB_1, and both combinations of MON/FB_1 as compared to the control. Dietary levels from 20 mg FB_1/kg feed caused a significant increase of the ratio of free sphinganine to free sphingosine in Channel catfish liver, while the diets with MON had no influence on this ratio. Also, no interaction due to both combinations of MON/FB_1 was observed since there was no change of the effect of FB_1 on this ratio. Altogether, from the five endpoints assessed in this unique study involving fish, only the combined effect of MON and FB_1 on the fish body weight gain gave some indication of an interactive toxicity, although the type of interaction was not indicated.

In summary, the typical experimental design in the publications reporting data on the combined toxicity of MON and FB_1 analysed the subacute toxicity induced by 200 mg FB_1/kg diet, or 100 mg MON /kg diet, or a combination of the two. These investigations mainly involved poultry and paid attention to the adverse effects on feed intake, weight gain and immune response, as well as organ lesions. Most of the publications concluded an interactive toxicity of FB_1 and MON, although for most of the studies the reported experimental design did not allow the type of interaction to be identified. The limited data available would suggest that the combined adverse effects exerted by FB_1 and MON are not synergistic.

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Moniliformin and deoxynivalenol

MON from culture material and DON from naturally contaminated wheat (no information on occurrence of other mycotoxins given) were used to prepare diets containing 100 mg MON/kg, 16 mg DON/kg and a combination of 100 mg MON/kg and 16 mg DON/kg fed to 36 one-day-old broiler chickens for 21 days (Harvey *et al.*, 1997). Body-weight gain and feed consumption were decreased by feeding MON and combined MON/DON diets. Relative heart weight was increased by the MON diet, whereas relative weights of proventriculus, gizzard, and heart were increased by the MON plus DON diet. The MON diet increased alanine transferase and aspartate transaminase activities and creatinine concentration. Histopathological lesions on the MON diet were limited to the kidney and consisted of extensive renal tubular epithelial degeneration plus luminal mineralisation. A moderation of the severity of lesions was seen in the tissues of chickens fed the MON/DON diet, consisting generally of mild tubular epithelial degeneration. None of the zootechnical and biochemical parameters studied, nor the examined organs and tissues were affected by the DON-only diet. Based on their results, the authors concluded that additive or less-than-additive toxicity was observed when chickens were fed a diet combining 100 mg MON and 16 mg DON per kg feed (Harvey *et al.*, 1997).

In a study reported by Morris *et al.* (1999) on growing turkey poults, 24 birds were fed a control diet, diets containing 20 mg DON/kg, or 100 mg MON/kg, or a combination of 20 mg/kg DON and 100 mg MON/kg, from 1 to 21 days of age. DON was semi purified from contaminated barley, and MON was obtained from culture material of *F. fujikuroi*. Compared with the control group, performance parameters such as feed intake and body weight gains were similar for the birds fed the DON alone diet but were decreased by dietary treatments containing MON alone and containing the combination of both DON and MON. The authors indicated that no interactive effects on performance were evident between MON and DON. Mean cell volume was decreased by the MON and MON/DON treatments, but the decrease was smaller in birds for the combination MON/DON treatment than for the other treatments, resulting in a significant DON × MON interaction. Mean cell haemoglobin and mean cell haemoglobin concentrations were not affected by any of the dietary treatments. No histological lesions were seen in the control poults or poults fed DON alone, whereas birds fed diets containing MON alone or the MON/DON combination exhibited an increased incidence of variable sized cardiomyocyte nuclei, with numerous large giant nuclei, and a generalised loss of cardiomyocyte cross striations. Isolated renal tubules in sections of kidney were noted to have mild diffuse mineralisation in birds fed MON and the combination MON/DON treatments. However, none of response variables measured were affected by DON alone. The authors concluded that the toxicity of the combination of MON/DON primarily reflected the toxicity of MON and not DON. No toxic synergy was observed when these toxins were fed simultaneously to turkey poults for 21 days (Morris *et al.*, 1999).

The two experimental studies identified for this review examined the subacute combined toxicity of MON and DON for two poultry species for a large panel of endpoints. The study on broiler chickens concluded an additive or less-than-additive toxicity. The study with turkeys did not conclude a toxic synergy effect. Clearly, the body of evidence for a strong interactive toxicity of both mycotoxins remains weak, and further investigations involving this combination of mycotoxins are necessary.

4. Discussion and conclusions

Many studies have identified the ability of several fungi belonging to *Fusarium* species and isolated from naturally contaminated cereal grains to produce both MON and one or several other toxin(s) including BEA, ENNs and FBs. Many of these grains are widely used in the diets of farm animals. Moreover, different fungal species producing different mycotoxins, such as DON and/or ZEA, can also contaminate grains and consequently these mycotoxins can co-occur with MON in the feeding stuffs. Surveys and monitoring programs have reported these co-occurrences and in particular the presence of MON together with BEA, ENNs, DON and FBs.

The scope of this review was limited to co-occurrence in feed and combined effects in farm animals, because there were no data on combined effects of MON in experimental animals. In the studies reported here the sources of the toxins were either the pure toxin (when it was commercially available or purified from *Fusarium* culture material) or the *Fusarium* culture material itself. It should also be noted that the presence of other mycotoxins in the culture material (i.e. that used as a source of MON) was, in most cases, not clearly investigated or reported. In addition, the available information often compiled the outcomes regarding specific endpoints for MON, and specific endpoints for the other combined mycotoxin in the mixture. There were no reports relating to adverse effects resulting from the presence of the two mycotoxins involved in the combination, which makes it difficult to even speculate about the type of interaction occurring for their joint toxicity.

The studies identified for this review from the scientific literature on the combined toxicity of MON and a second mycotoxin allowed only the investigation of the two combinations MON/FB_1 and MON/DON. No studies were available on the combination of MON and other *Fusarium* toxins, such as BEA and/or ENNs, although

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several *Fusarium* species are able to produce these toxins simultaneously. Supplementary Table S1 indicates the co-occurrence of these toxins in (feed) cereals. Clearly there is a discrepancy between the available toxicological studies concerning MON in combination with other mycotoxins and the available co-occurrence data of MON and other mycotoxins in the diets of farm animals. This could be due to the commercial availability of pure FB_1 and DON standards and the availability of referenced toxigenic *Fusarium* strains producing large amounts of FB_1 or DON in culture medium or on grains.

Table 1 indicates the selected publications available from the literature on swine, poultry and fish. Only two experimental studies with poultry examined the toxicity of the combination of MON and DON. In most of the experimental studies on poultry and pigs, the tested levels of MON were 100 mg/kg feed which corresponds to – or is above – the identified LOAELs in these species. While these MON levels are the most relevant from a toxicological point of view, they are higher than the levels identified from the occurrence database in the EFSA Opinion on MON (EFSA, 2018). It cannot be excluded however, that such high levels would occur in practice, given the fact that these levels were reached in the animal feeds in the reported studies using experimental cultures of toxigenic *Fusarium* strains such as *F. verticillioides* producing FB_1 and *F. fujikuroi* producing MON.

A rigorous assessment of the interactive toxicity of a mixture is complex; there is overall a methodological gap between the *in vitro* and *in vivo* studies exploring the combined toxicity of mycotoxins (Groten *et al.*, 1998; Lei *et al.*, 2013). The classical testing for a possible interaction requires the comparison of the actual effect of the mixture to a theoretically expected no-interaction effect, the so-called additive effect (Alassane-Kpembi *et al.*, 2017a). Prediction of the no-interaction pattern is made based on dose-response experiments for the individual mycotoxins, and some elaborated designs including the factorial designs and other theoretical biology-based approaches have been developed for the purpose. Stronger-than-expected effects indicate synergism, whereas lower-than-expected effects indicate antagonism. By contrast to the well-designed *in vitro* studies that have been published, the lack of an accurate definition of the additive effect is a frequent shortcoming of *in vivo* studies on the combined toxicity of mycotoxins, as illustrated by the MON combination studies reported in this review (Alassane-Kpembi *et al.*, 2017b; Grenier *et al.*, 2011).

This review has identified possible interactions between a few *Fusarium* mycotoxins and their effects on animal health and productivity, and these are summarised in Table 2. However, these conclusions are based on a small number of studies with limited experimental designs. Selection criteria were applied to consider the experimental studies with data providing useful information for the identification of interaction(s) between MON and another Fusarium mycotoxin. The typical experimental design in such studies would involve, in addition to a toxin-free control treatment, three groups of animals to determine the subacute toxicity induced by: (a) one level of MON; (b) one level of only one other *Fusarium* mycotoxin; and (c) a combination of the two toxins at the same levels as used in treatments (a) and (b). Of course, the feed used as a basal diet as well as the source of MON and the other toxin should be proven to be free of any other mycotoxin, to avoid any possible interfering activity. At present, the body of evidence for a strong interactive toxicity of studied mycotoxins, such as the combination of MON and FB_1 and the combination of MON and DON, remains weak. Further studies are needed on the combined toxicity of MON with other (*Fusarium*) mycotoxins, such as ZEA, T-2/HT-2 toxins, BEA and ENNs. Such studies should also be extended to other animal species, such as ruminants, for which cereal grains and their by-products are important feed ingredients.

Supplementary material

Supplementary material can be found online at https://doi.org/10.3920/WMJ2018.2405.

Table S1. Published European studies (samples taken after 2000) on occurrence of moniliformin in food and feed cereals, with information about other, co-occurring, mycotoxins.

Acknowledgement

The authors thank Dr. Mari Eskola (affiliated with EFSA until October 2017, currently working with the European Chemicals Agency, Helsinki, Finland), for the suggestion to draft this review, which was a spin-off of the work conducted during the preparation of the EFSA Scientific Opinions on '*Fusarium* Toxins'. The authors are also indebted to her helpful comments in the preparation of this paper.

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