# Food & Function

## REVIEW

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Cite this: Food Funct., 2024, 15, 8998

Received 12th April 2024, Accepted 17th August 2024 DOI: 10.1039/d4fo01705f

rsc.li/food-function

## 1. Introduction

Mycotoxins are secondary metabolites produced by various species of fungi, primarily belonging to the genera *Aspergillus*, *Penicillium*, and *Fusarium*. They are estimated to be present in up to 80% of food and feed. Animal feed contamination is particularly concerning, as in developed countries, up to 70% of cereal harvest—a primary source of mycotoxins—is utilized in the daily diet of animals. Mycotoxin regulation for feed is less restrictive than for food. These toxins pose significant public health risks due to their widespread occurrence and their toxic properties in both animals and humans. They can induce immunotoxicity, neurotoxicity, hepatotoxicity, nephrotoxicity, reproductive and developmental toxicity, and carcinogenicity.<sup>1</sup>

Currently, the legislative framework on maximum limits on mycotoxins is established by the recently updated Commission Regulation (EU) 2023/915 of April 25, for aflatoxin B1 (AFB1),

# Emerging mycotoxins and preventive strategies related to gut microbiota changes: probiotics, prebiotics, and postbiotics – a systematic review<sup>†</sup>

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Recent research has focused on the involvement of the gut microbiota in various diseases, where probiotics, prebiotics, synbiotics, and postbiotics (PPSP) exert beneficial effects through modulation of the microbiome. This systematic review aims to provide insight into the interplay among emerging mycotoxins, gut microbiota, and PPSP. The review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. In this review, unregulated yet highly recurrent mycotoxins are classified as emerging mycotoxins. The most frequently observed mycotoxins included those from the Fusarium genus—enniatins (n = 11) and beauvericin (n = 11)—and the Alternaria genus-alternariol monomethyl ether, altertoxin, and tentoxin (n = 10). Among probiotics, the most studied genera were Lactobacillus, Bifidobacterium, and the yeast Saccharomyces cerevisiae. Inulin and cellulose were the most found prebiotics. Data on synbiotics and postbiotics are scarce. Studies have shown that both the gut microbiota and PPSP can detoxify and mitigate the harmful effects of emerging mycotoxins. PPSP not only reduced mycotoxin bioaccessibility, but also counteracted their detrimental effects by activating health-promoting pathways such as short-chain fatty acid production, genoprotection, and reduction of oxidative stress. However, both quantitative and qualitative data remain limited, indicating a need for further in vivo and long-term studies. The formulation of PPSP as functional foods, feeds, or nutraceuticals should be considered a preventive strategy against the toxicity of emerging mycotoxins, for which, there is no established regulatory framework.

> aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2), aflatoxin M1 (AFM1), ochratoxin A (OTA), patulin (PAT), deoxynivalenol (DON), zearalenone (ZEA), fumonisin B1 (FB1), fumonisin B2 (FB2) and citrinin (CIT), the last one being applied only in food supplements.<sup>2</sup> In addition, there are other recent recommendations for emerging or unregulated mycotoxins such as alternariol (AOH), alternariol monomethyl ether (AME), tenuazonic acid (TeA) and the trichothecenes T-2 and HT-2 toxins (T-2 and HT-2).<sup>3,4</sup>

> The term "emerging mycotoxins" was first introduced in 2008 and initially referred primarily to *Fusarium* metabolites such as fusaproliferin (FP), beauvericin (BEA), enniatins (ENs), and moniliformin (MON). However, more recent scientific publications have defined emerging mycotoxins as those that are neither routinely detected nor regulated by legislation, despite growing evidence of their prevalence. This work is focused on the 22 emerging mycotoxins included in Fig. 1 and 2 that have been recognized for over 20 years, continue to be widespread, and are not yet subject to regulatory oversight, even though their toxic effects are increasingly documented. The mycotoxins covered in this review are: enniatin A (ENA), enniatin (ENB), enniatin A1 (ENA1), enniatin B1 (ENB1), BEA,



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<sup>†</sup>Electronic supplementary information (ESI) available. See DOI: https://doi.org/ 10.1039/d4fo01705f



Fig. 1 Chemical structures of the emerging mycotoxins of *Fusarium* included in the bibliographic search (n = 11).

fusaric acid (FA), MON, fusarenon X (FX), aurofusarin (AUR), FP, diacetoxyscirpenol (DAS), altuene (ALT), AME, TeA, altertoxin (ATX), tentoxin (TEN), CIT, sterigmatocystin (STE), neosolaniol (NEO), citreoviridin, rugulosin and phomopsin.

To better understand the relevance of these emerging mycotoxins, the occurrence and co-occurrence of most of them have been studied by Mihalache *et al.* (2023),<sup>5</sup> who evidenced 38 different combinations in foodstuff (maize, rice, wheat, and flours). These combinations changed from binary mixtures (AME + AOH or AME + TeA) to include 12 different emerging mycotoxins (AOH + AME + TEA + TEN + ENA1 + ENB + ENB + BEA + MON + DAS + NIV + STE). Furthermore, the rising of new data regarding their presence in foodstuff has been highly relevant to enhance multiple risk assessment methodologies,

## Alternaria



Alternariol monomethyl ether



Tenuazonic acid



Altertoxin



Tentoxin

Monascus



Citrinin



Sterigmatocystin



Neosolaniol

Penicillium

## Diaporthe



Fig. 2 Chemical structures of the emerging mycotoxins of Alternaria, Monascus, Penicillium and Diaporthe included in the bibliographic search (n = 10).

developing future evidence-based regulations and public health.<sup>5</sup> As shown in Table 1, these emerging mycotoxins are of significant concern due to their involvement in various severe health issues, including cell apoptosis, chromosomal abnormalities, acute cardiac distress, genotoxicity, hemorrhages in legs, skull, and feet, liver injury, cardiovascular collapse, chlorosis, embryotoxicity, teratogenesis, cancer, liver cirrhosis, hepatocellular carcinoma, Shoshin-kakke (acute cardiac beriberi), fatty degeneration, liver cell necrosis and cell death. Although not all emerging mycotoxins were considered in this research, the spectra covered are expected to encompass most of them.

Furthermore, climate change is anticipated to become a major environmental concern in the 21st century. Higher

temperatures combined with extreme precipitation or prolonged droughts increase the stress experienced by plants, making all plant-based foods more susceptible to fungal infection and mycotoxin contamination. Fusarium mycotoxins are expected to shift towards Northern Europe, while Aspergillus species will primarily affect southern and central Europe. Indeed, it is estimated that in Europe, over the next 50-100 years, mycotoxins will become a significant concern. This includes not only the most common mycotoxins, such as aflatoxins and OTA, but also emerging Fusarium mycotoxins like ENs and BEA.<sup>6,7</sup>

Recent research has focused on the involvement of the gut microbiota in a considerable number of diseases such as anxiety, depression, inflammatory bowel disease, dementia,

Table 1 Emerging mycotoxins, food sources, sample, dose administration, exposure time, target organ, toxicological mechanism, damage, and references

Mycotoxin	Food sources	Sample	Dose administration	Exposure time	Target organ	Toxicological mechanism	Damage	Ref
Fusarium ENA	Wheat,	Caco-2 and	0.66–66.70 μg mL <sup>-1</sup>	24–48 h	Intestine,	Generation of ROS	Apoptosis	8 and
	maize, and rice	cells	IIIL		livel	depolarization		9
ENB	Wheat, barley, maize, and rice	Caco-2 and SH-SY5Y cells	$0.63-63.60 \ \mu g \ mL^{-1}$	24-48 h	Intestine, liver	Generation of ROS and mitochondrial depolarization	Apoptosis	8 and 9
ENA1	Wheat, barley, maize, and rice	Caco-2 and SH-SY5Y cells	$0.66-66.70 \ \mu g \ m L^{-1}$	24–48 h	Intestine, liver	Generation of ROS and NADPH oxidase activation	Apoptosis	8 and 9
ENB1	Wheat, barley, maize, and rice	Caco-2 and SH-SY5Y cells	$0.63-63.60 \ \mu g \ mL^{-1}$	24-48 h	Intestine, liver	Generation of ROS and NADPH oxidase activation	Apoptosis	9
BEA	Wheat, maize, nuts, and coffee	Caco-2 cells	$1.17-19.6 \ \mu g \ mL^{-1}$	24-72 h	Intestine, liver	Generation of ROS	Apoptosis	10
FA	Wheat, barley, corn, and rice	<i>Allium</i> cepa L. bulbs and HepG2 cells	0.18–1.8 μg mL <sup>–1</sup>	24–168 h	Liver	Inhibition of cytochrome oxidase, decrease in ATP synthesis, change in the electrochemical gradient in the plasma membrane and increase in electrolyte loss	Chromosomal abnormalities	11 and 12
MON	Maize, wheat	Male Sprague- Dawley rats	500 $\mu g m L^{-1}$	28 days	Heart	Pyruvate substitution and carbohydrate metabolism interference	Death and acute cardiac distress	13
FX	Wheat, barley, and cereal-based products	Male and female mice	3–15 mg per kg b.w.	48 h	Liver, kidneys, spleen	Inhibition of protein and DNA synthesis	Apoptosis	14 and 15
AUR	Maize, barley, oats, and wheat	CHO-K1 cell line	$0.57-5.7 \ \mu g \ m L^{-1}$	48 h	Intestine	Enhanced levels of p53 protein	Genotoxicity	16
FP	Maize	Chicken embryo	0.45–2.22 µg mL <sup>-1</sup>	21 days	Chicken embryos	Reduction in cell viability	Hemorrhages on the surfaces of legs, skull, and feet	17 and 18
DAS	Wheat, corn, rice, and maize	Newborn chickens and Wistar rats	1–3 mg per kg bw	6–24 h	Liver, gallbladder, and small intestine	DNA synthesis and protein biosynthesis inhibitor and heterophil extracellular trap release	Liver injury	19 and 20
Apicidin	NR	HepG2 cells	0.06–62.3 μg mL <sup>-1</sup>	24 h	Liver	Cytotoxicity	Cell death	21
Alternaria AME	Wheat and its derivatives, rapeseed oil and peas	Male Sprague- Dawley rats	7.35 µg per kg bw per day	28 days	Liver, kidneys, spleen	DNA adducts and production of ROS	Genotoxicity	21
TeA	Tomatoes, apples, beer, and cereal foods	Mice	238 µg per kg per day	56 days	Liver, kidneys	Generation of ROS	Cardiovascular collapse and gastrointestinal hemorrhage	22
ATX	Cereals, oilseeds, fruits, and vegetables	Male Sprague- Dawley rats	5.51 µg per kg w per day	28 days	Liver, kidneys, spleen	Nuclear factor erythroid-derived 2-like 2/antioxidant response element (Nrf2/ARE)	Genotoxicity	16 and 23

#### Table 1 (Contd.)

Mycotoxin	Food sources	Sample	Dose administration	Exposure time	Target organ	Toxicological mechanism	Damage	Ref
TEN	Rice	Male Sprague Dawley rats	$0.4-41.45 \text{ pg} \text{mL}^{-1}$	10 min	Liver	ATP synthesis reduction	Chlorosis	24 and 25
Monascus CIT	Cheese, sake, and soy sauce	ProTox-II in humans	105 mg per kg weight	24 h	Kidneys	Inhibition of malate dehydrogenase, glutamate dehydrogenase and ATP synthase	Genotoxic, embryotoxic, teratogenic, carcinogenic	26
Aspergillus STE	Maize and peanuts	HepG2 cells	0.16–2.27 μg mL <sup>-1</sup>	24-48 h	Liver, kidneys	Generation of ROS	Liver cirrhosis hepatocellular	27
NEO	Maize, wheat, and oats	Porcine Langerhans cells	143.4 mg mL <sup>-1</sup>	24 h	Leydig cells	Decrease in the ATP content, overproduction of ROS, reduction in mitochondrial membrane potential	Cellular apoptosis	28
Emodin	NR	HepG2 cells	0.027–27 μg mL <sup>-1</sup>	24 h	Liver	Cytotoxicity	Cell death	21
Penicillium Citreoviridin	Maize, pecan nuts, and wheat	Mice	$1-10 \text{ mg L}^{-1}$	15 days	Heart	Inhibition of mitochondrial ATPase	Shoshin-kakke (acute cardiac beriberi)	29
Rugulosin	Rice	Mice	67 mg per kg b.w.	21 days	Liver	Inhibition <i>in vivo</i> and <i>in vitro</i> of DNA replication, transcription, and	Fatty degeneration and liver cell necrosis	30 and 31
Cyclopiazonic acid	Corn, peanuts and cheese	Caco-2 cells	$0.014-42 \text{ ng} \text{mL}^{-1}$	24–48 h	Intestine	reparation Cytotoxicity	Cell death	33 and 34
<i>Diaporthe</i> Phomopsin	Lupins	Sheep	$0.5 \mathrm{~mg~kg}^{-1}$	4 days	Liver	Cell cycle arrest	Cell death	32 and
Phoma Cytochalasin	Potatoes	FI 4	$2 \text{ mg mJ}^{-1}$	4 h	Plood	DNA fragmentation	Apoptosis	33
B	1 0101055	1	2 mg mL	7 11				50
Cytochalasin D	Potatoes	Mice	0.3 mg kg <sup>-1</sup>	17 days	Neural tube	Teratogenicity	Fetal death	37
Cytochalasin E	Potatoes	Brine shrimp	0.01–5 μg mL <sup>-1</sup>	16–24 h	NR	Cytotoxicity	Cell death	38

Deoxyribonucleic acid (ADN), alternariol monomethyl ether (AME), adenosine triphosphate (ATP), altertoxin (ATX), aurofusarin (AUR), beauvericin (BEA), body weight (b.w.), citrinin (CIT), diacetoxyscirpenol (DAS), enniatin A (ENA), enniatin A1 (ENA1), enniatin B (ENB), enniatin B (ENB), fusaproliferin (FP), fusarenon X (FX), fusaric acid (FA), moniliformin (MON), neosolaniol (NEO), nicotinamide adenine dinucleotide phosphate oxidase (NADPH), NF-E2-related factor 2/antioxidant responsive element (Nrf2/ARE), not reported (NR), reactive oxygen species (ROS), sterigmatocystin (STE), tenuazonic acid (TeA), and tentoxin (TEN).

Parkinson's disease, diabetes, obesity, and cancer.<sup>39–43</sup> To understand how important the gut microbiota is, it should be said that there are around 26 600 human genes, whereas bacterial genes are more than 4 000 000 in our body. What is more, the bacterial density in the colon is around  $10^{11}-10^{12}$ mL<sup>-1</sup>, being the highest density in any ecosystem on Earth.<sup>44</sup> On the other hand, there are some factors, such as diet,<sup>45</sup> stress,<sup>46</sup> exercise<sup>47</sup> and medication,<sup>48</sup> having a notable impact on gut microbiota modulation. Among these factors, this systematic review focuses on emerging mycotoxins and probiotics, prebiotics, synbiotics and postbiotics (PPSP); PPSP is a term recently coined by Li *et al.* (2021).<sup>49</sup>

PPSP has shown to regulate the abundances of some intestinal bacteria such as *Akkermansia*, *Bacteroidetes*, *Blautia*, *Bifidobacteria*, *Bifidobacterium* (B.) and gut microbial metabolites, such as lactic acid, acetate, butyrate and propionate, while suppressing the bile acid pools and decreasing the production of trimethylamine *N*-oxide and lipopolysaccharides.<sup>49</sup> The significance of PPSP, mainly probiotics and prebiotics, gave rise to the International Scientific Association for Probiotics and Prebiotics (ISAPP) in 2002. ISAPP is a non-profit organization that works to advance the science of probiotics, prebiotics and related substances, such as synbiotics, postbiotics and fermented foods,<sup>50</sup> contributing to a better understanding of how biotics are connected to health and disease. Regarding definitions, prebiotics are described as "a substrate that is selectively utilized by host microorganisms, conferring a health benefit".<sup>51</sup> Traditionally, they have been defined as "nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species already resident in the colon, thus attempting to improve host health".<sup>52</sup> Certainly, probiotics are defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host".<sup>52</sup> Recently, there has been an emerging trend to investigate synbiotics and postbiotics. Synbiotics are considered "a mixture comprising live microorganisms and substrate(s) selectively utilized by host microorganisms that confers a health benefit on the host", whereas postbiotics are described as "a preparation of inanimate microorganisms and/ or their components that confers a health benefit on the host", 53,54

It has been shown that there are three main strategies for alleviating the harm caused by mycotoxins transmitted by microorganisms: (I) directly degrading mycotoxins in food, thanks to microbial pretreatment, and decreasing toxin intake; (II) by boosting probiotic colonization, the creation of bacterial-toxin complexes is suppressed, consequently inhibiting toxin absorption in the intestinal tract; and (III) by modulating the intestinal microecology with probiotics or prebiotics, enhancing the intestinal barrier, remodeling the intestinal microflora, improving intestinal toxicity, and decreasing toxin penetration.<sup>55</sup> Previously, the interactions between the gut microbiota and regulated mycotoxins<sup>6,56</sup> and the detoxifying capacity of PPSP on some mycotoxins<sup>57-61</sup> have been studied. Notwithstanding, the interplay among emerging mycotoxins, gut microbiota and PPSP still remains a framework to systemize.

The aim of this systematic review is to shed light on the potential of PPSP in mitigating the harmful effects of emerging mycotoxins. In addition, it aims to enhance our understanding of the reciprocal relationship between emerging mycotoxins and the gut microbiota, elucidating how emerging mycotoxins can influence the gut microbiota and *vice versa*. Furthermore, this review aims to contribute to future research endeavors by proposing the development of new functional foods and/or nutraceuticals as a strategy for mitigating toxicity associated with emerging mycotoxins.

## 2. Materials and methods

#### 2.1 Search strategy

The scientific rigor of this systematic review and the minimization of potential bias were ensured through adherence to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement protocol for screening titles, abstracts, and full texts.<sup>62</sup> A comprehensive literature search was conducted in November–December 2023, utilizing three databases (PubMed, Web of Science, and Scopus). The search encompassed the period from January 2012 to December 2023, aiming to include recent data on emerging mycotoxins within the context of the gut microbiota and PPSP. In addition, other relevant terms, such as "bacteria", were considered in combination with "*in vitro*" or "*in vivo*" to capture additional relevant information

The systematic review was conducted using the following search strings:

(1) Refined research of Pubmed, Web of Science and Scopus. Period: all years.

("Enniatins" OR "Enniatin A" OR "Enniatin B" OR "Enniatin A1" OR "Beauvericin" OR "Fusaric acid" OR "Moniliformin" OR "Fusarenon X" OR "Aurofusarin" OR "Fusaproliferin" OR "Diacetoxyscirpenol" OR "Alternariol monomethyl ether" OR "Tenuazonic acid" OR "Altertoxin" OR "Citritin" "Tentoxin" OR OR "Sterigmatocystin" OR "Neosolaniol" OR "Citreoviridin" OR "Rugulosin" OR "Phomopsin") AND ("bacteria") AND ("in vitro" OR "in vivo").

(2) Refined research of Pubmed, Web of Science and Scopus. Period: 2012–2023.

("Enniatins" OR "Enniatin A" OR "Enniatin B" OR "Enniatin A1" OR "Beauvericin" OR "Fusaric acid" OR "Moniliformin" OR "Fusarenon X" OR "Aurofusarin" OR "Fusaproliferin" OR "Diacetoxyscirpenol" OR "Alternariol monomethyl ether" OR "Tenuazonic acid" OR "Altertoxin" OR OR "Citritin" OR "Sterigmatocystin" "Tentoxin" OR "Neosolaniol" OR "Citreoviridin" OR "Rugulosin" OR "Phomopsin") AND ("microbiota" OR "prebiotics" OR "probiotics" OR "postbiotics").

The criteria to include non-regulated mycotoxins in the list was based on Mihalache *et al.* (2023) and the reports issued by the Spanish Agency for Food Safety and Nutrition (AESAN).<sup>5,35</sup> It is well known that there are other non-regulated metabolites not been considered when conducting the research. Notwithstanding, it was supposed that the spectra covered would be enough to include all the non-regulated mycotoxins.

#### 2.2 Systematic review process

Among the 811 reports found through the research, 317 were retrieved from PubMed, 295 from Web of Science and 199 from Scopus. After an initial analysis, a total of 401 duplicate records were identified and removed. In addition, during the screening of titles and abstracts, another 381 records were excluded from the systematic review because of different reasons: 124 articles were discarded because they only focused on regulated mycotoxins (AFB1, OTA, fumonisins, etc.); 130 were rejected since they included emerging mycotoxins, but they were not assessed in combination with PPSP and 127 were discarded because they analyzed PPSP, but no emerging mycotoxins. Finally, a total of 29 were considered appropriate for inclusion in the present systematic review, assessed and classified based on: the gut microbiota, probiotics, prebiotics, synbiotics and postbiotics (Fig. 3). The selected articles are key to comprehend more deeply the role of the emerging mycotoxins in their combination with microbiota and PPSP.

**IDENTIFICATION** 

Refined research of Pubmed, Web of Science and Scopus



Refined research of Pubmed,

Fig. 3 Graphical representation of the total number of articles screened throughout the two strategies followed for the bibliographic research using the keyword combinations and range of years explained. Number of samples (n). Pubmed - PM, Scopus - SC and Web of Science - WOS.

#### 2.3 Data conversion

All data from the studies discussed in this review were converted to  $\mu g kg^{-1}$ ,  $mg kg^{-1}$ ,  $ng mL^{-1}$ ,  $\mu g mL^{-1}$  or  $mg L^{-1}$ .

#### 3. Results

#### 3.1 Mycotoxins and the gut microbiota

The bidirectionality between regulated mycotoxins and the gut microbiota has been previously described in mice, pigs, rats, broilers, turkey, and ducks. DON has revealed to up-regulate the relative abundance of pathogens highly related to chronic intestinal diseases at different taxonomical levels (phylum, family, and genus). OTA has disrupted the structure and diversity of gut microbial communities and AFB1 to alter gut microbiota-dependent organic acid metabolism. Meanwhile, some gut microbiota strains, such as probiotics *Lactobacillus plantarum* and *Bacillus shackletonii*, have also shown mitigation effects by metabolizing, binding, and eliminating these regulated mycotoxins.<sup>6,7,56</sup>

3.1.1 In vitro detoxification of emerging mycotoxins by the gut microbiota. Rumen microbiota has been found to completely detoxify NIV at normal pH after 24 h of incubation, whereas it partially degrades ENB up to 72% after 48 h. In both cases, this degradation depended on microbial activity and was influenced by pH.63 Hedman and Pettersson (1997)64 described that the capacity of cow gastrointestinal microflora to metabolize NIV has exerted the same trend. It was shown that 78-82% of anaerobically incubated NIV for 48 h was transformed into de-epoxy-NIV by gastrointestinal microbiota in the fluid rumen.<sup>64</sup> DAS, also produced by the genera Fusarium, has revealed to be deacetylated into 15-monoacetoxyscirpenol (15-MAS) and scirpentriol (SCP) as an additional metabolite by human fecal microbiota, being of utmost importance since deacetylation of mycotoxins results in reduced toxicity, for 15-MAS has been reported to be 8 times less toxic than both DAS and SCP.65

The metabolism of Alternaria mycotoxins such as AOH, AME and ALT by the microbiota present in feces from three human volunteers has also been studied. As a control for the activity of the fecal microbiota, the isoflavone daidzein was incubated with the fecal cultures and was transformed to its expected metabolites. In contrast, no metabolites of AOH, AME and ALT were detected in the fecal cultures from the same volunteers, showing that the gut microbiota did not possess the capacity to metabolize these compounds. Interestingly, AOH was noted to bind non-covalently to the surface of bacteria where the type and composition of this bacterial cell surface played a notable role in the magnitude of this binding.<sup>66</sup> An Alternaria alternata extract (5  $\mu$ g mL<sup>-1</sup>), containing among others, AOH, AME, altertoxin-II (ATX-II), stemphyltoxin III (STTX-III) and alterperylenol (ALP), all of which are known as genotoxic, was used to assess the impact of short-term fecal incubation on the DNA-damaging effects. It was shown that microorganisms suppressed the DNA-strandbreaking potential promoted by the applied extract. Besides, in fresh fecal samples, even before anaerobic incubation, the concentrations of most of the tested mycotoxins (AOH, AME, ATX-II, STTX-III, and ALP) were lower in those containing microorganisms, especially fecal matter, implying that microbes were engaged in mycotoxin detoxification.<sup>67</sup>

**3.1.2** Emerging mycotoxins and the gut microbiota *in vivo*. Novak *et al.*  $(2021)^{68}$  fed weaning piglets with different diets for a 14 day-period: DON, ENB + ENB1 + BEA and the four mycotoxins combined. Those piglets receiving the ENs + BEA diet were characterized by possessing a fecal microbiome with a lower diversity compared to the control group, and also compared to the ENs + BEA + DON diet. *L. amylovorus*, which is known to exhibit positive probiotic activities in weaning piglets, was reduced only in the ENs + BEA-fed group, but not in the ENs + BEA + DON one. Furthermore, the relative abundance of *L. reuteri*, which has shown to contribute to diarrhea treatment in piglets by reducing *Cryptosporidium parvum*, was increased in the ENs + BEA-fed group and, to a lesser extent, in the ENs + BEA + DON-fed group, compared to the control and the DON ones.<sup>68</sup>

The ability of the gastrointestinal microflora of pig and chicken to metabolize NIV was studied by Hedman and Pettersson (1997).<sup>64</sup> On one hand, before pigs were fed with NIV, this mycotoxin was not de-epoxidated or metabolized in any way. However, when the pigs received either 2.5 or 5  $\mu$ g g<sup>-1</sup> NIV for one week, 97.1% of NIV in feces from five of six pigs was in the de-epoxy-NIV form, while the concentration of unaltered NIV in feces was 0.08  $\mu$ g g<sup>-1</sup>. On the other hand, after three weeks on the NIV feed, even the pig that after one week could not metabolize NIV, was now able to form de-epoxides. In the feces from this pig, 90% of NIV was as de-epoxy-NIV, the concentration of it being NIV 0.17  $\mu g g^{-1}$ . Regarding chicken, it was revealed that de-epoxy-NIV was not detected in any samples of feces collected after three weeks of feeding with either 2.5 or 5  $\mu g g^{-1}$  NIV. Interestingly, another unidentified metabolite of NIV was found in all feces samples, except from one bird fed with 5  $\mu$ g g<sup>-1</sup> NIV.<sup>64</sup> All the data relating emerging mycotoxins and gut microbiota are shown in Fig. 4 and Table 2. These studies were primarily conducted in vitro using feces from humans, pigs, and chickens, or in cow rumen fluids. The most frequently encountered emerging mycotoxins were ENs, BEA, NIV, and Alternaria mycotoxins (AOH, AME, ALTX-I, and ALT).

#### 3.2 Emerging mycotoxins and probiotics

Studies *in vitro* and *in vivo* have shown the ability of probiotic cultures to bind to and/or even degrade many toxic substances, mitigating their toxicity.<sup>64,69</sup> Not only has it been reported that single strain or combination of lactic acid bacteria (LAB) are very useful for removal of heavy metals (copper, lead, cadmium, chromium and arsenic) and cyanotoxins (microcystin-LR, -RR and -LF), but also regarding mycotoxins (AFB1, AFB2, AFM1, aflatoxin M2 (AFM2), AFG1, AFG2, PAT, OTA, DON, FB1 and FB2), 3-acetyldeoxynivalenol, NIV, HT-2 and T-2, ZEA and its derivatives, *etc.*, and their harmful effects.<sup>58,61,70</sup>

**3.2.1 Emerging mycotoxins and probiotics** *in vitro*. The strains *L. johnsonii CECT 289, L. rhamnosus CECT 288,* 



Fig. 4 Graphical bars of the gut microbiota publications (n = 6) according to (a) the animal species and (b) emerging mycotoxins studied. Beauvericin – BEA, enniatins – ENs and nivalenol – NIV.

L. plantarum CECT 220, L. reuteri CECT 725, L. casei CECT 475, B. breve CECT 4839 T, B. adolescentis CECT 5781 T, B. bifidum CECT 870 T and B. longum CECT 4551 have exerted to degrade different types of ENs during a simulated gastrointestinal digestion process for 4 h. It was reported that all the bacteria reduced ENA, ENA1, ENB and ENB1 mean bioaccessibility (ranging from 21.0% to 31.2%) in comparison with the control (33.4%–39.6%).<sup>71</sup> The antimicrobial activity of *B. longum*, B. bifidum, B. breve, B. adolescentis, L. rhamnosus, L. casei-casei, L. plantarum, L. paracasei, L. ruminis, Streptococcus thermophilus, twenty-two strains of Saccharomyces cerevisiae and nine of Bacillus subtilis has been assessed against the minor Fusarium mycotoxin ENs (ENA, ENA1, ENA2, ENB, ENB1 and ENB4). The most active mycotoxins were ENA1 and ENB, mainly against B. adolescentis 5871 and S. thermophilus. Conversely, ENA1 and ENB1 have been reported to inhibit the growth of the cultivated B. subtilis strain with a minimum inhibitory concentration (MIC) of 16 and 8  $\mu$ g mL<sup>-1</sup>, respectively, and to be active against Staphylococcus aureus, Streptococcus pneumoniae, and Enterococcus faecalis with MIC values ranging from 2 to 8 μg mL<sup>-1</sup>.<sup>73</sup> Another nine bacterial strains characteristic of the gastrointestinal tract like B. longum, B. bifidum, B. breve, B. adolescentis, L. rhamnosus, L. casei-casei, S. thermophilus, L. ruminis, L. casei and twenty-two strains of S. cerevisiae have been studied to conduct EN metabolization. The fermentation processes were carried out in a liquid medium of De Man Rogosa Sharpe under anaerobic conditions and in a potato dextrose broth, and the degradation of the EN bioactive compounds was assessed in a food system made of naturally contaminated wheat flour. The results showed ENA, ENA1, ENB and ENB1 degradation ranging from 5.2% to 99.5%. The highest reduction was caused by *S. thermophilus CECT 986* in ENA and the lowest by *B. breve CECT 4839* in ENB. Furthermore, degradation products were identified for all the studied mycotoxins in both the liquid medium and the food system.<sup>74</sup>

Apart from ENs, the interaction between BEA and probiotics is also well documented. Nine yeast strains of S. cerevisiae (LO9, YE-2, YE5, YE-6, YE-4, A34, A17, A42 and A08) have shown a BEA mean degradation of 86.2%. The highest degradation activity (98.8%) was due to S. cerevisiae LO-9, whereas the lowest (39.7%) by S. cerevisiae LS100.75 The protective effect of L. acidophilus against BEA toxicity has been assessed on a Caco-2 cell line. It was revealed that, in the presence of BEA, L. acidophilus significantly increased cell viability at 12 h and 24 h.<sup>76</sup> L. paracasei CECT 277, L. casei CECT 4180, L. rhamnosus CECT 278T, L. plantarum CECT 220, L. ruminis CECT 4061T, L. casei casei CECT 277, B. breve CCT 4839T, B. adolescentis CECT 5781T, B. bifidum CECT 870T, B. longum CECT 4551, Corynebacterium vitaeruminis CECT 537, Eubacterium crispatus CECT 4840 and S. cerevisiae CECT 1324 have proven to exert a notable impact on BEA bioaccessibility. The highest bioaccessibility decrease was carried out by B. longum showing 45.4%, whereas the lowest data were revealed with the strain L. rhamnosus (27.5%).<sup>77</sup> Other 13 bacterial strains belonging to the gastrointestinal tract (B. longum, B. bifidum, B. breve, B. adolescentis, L. rhamnosus, L. casei-casei, L. plantarum, Eubacterium crispatus, Salmonella fecalis, Salmonella thermophilus, L. ruminis, L. casei and L. animalis) were fermented under anaerobic conditions in the liquid medium of De Man-Rogosa-Sharpe agar for 4 h, 12 h, 16 h, 24 h and 48 h at 37 °C. It was revealed that BEA reduction ranged from 66.5% to

Table 2 Collection of the most important characteristics of the studies found regarding emerging mycotoxins and the gut microbiota in vitro and in vivo. For each study, the studied mycotoxin, type of sample, mycotoxin dose administration, exposure time, experimental assay, results and references are described

Mycotoxin	Sample	Dose administration	Exposure time	Experimental	assay Results		Ref.
In vitro ENB NIV NIV	Cow rumen fluid Cow rumen fluid	1 mg kg <sup>-1</sup> ENB 60 mg kg <sup>-1</sup> NIV 2 µg mL <sup>-1</sup>	0–48 h 48 h	<i>In vitro</i> rumer simulation Anaerobic fec. culture	<ul> <li>ENB degradatio</li> <li>of incubation. I</li> <li>78–82% of incu</li> <li>the fluid rumen</li> </ul>	n up to 72% after 48 h and NIV completely detoxified at normal pH (6.8) after 24 h n both cases, the degradation was dependent of the microbial activity bated NIV was transformed into de-epoxy-NIV by the gastrointestinal microbiota in	63 64
DAS	Human faces	$0.7 \ \mathrm{ng} \ \mathrm{mL}^{-1}$	2-72 h	Fecal batch cı	Anaerobic incul Ilture DAS was deacet	pation of NIV with cow rumen fluid produced de-epoxide at a high proportion ylated into 15-MAS and SCP as an additional metabolite by human fecal	65
AOH AME	Human feces	12.9 µg mL <sup>-1</sup>	72 h	Anaerobic fec culture	al AOH, AME and bacteria in func	ALT were not metabolized by the gut bacteria. AOH bound non-covalently to the tion according to the type and composition of its surface	99
ADI AOH ATX-II STTX-III ALP	Human feces	50 µg mL <sup>-1</sup>	48 h	Fecal anaerob incubation	ic Microorganism AOH, AME, ATX especially fecal	s suppressed the DNA-strand-breaking potential promoted by the applied extract. -II, STTX-III, and ALP were lower in the samples containing microorganisms, matter, implying that microbes were engaged in detoxification	67
Mycotoxin	Sample	Mycotoxin dose administration	E>	xposure Expei	rimental assay	Results	Ref.
In vivo ENB	Weaning Diglets' feces	1.345 mg kg <sup>-1</sup> (1	ENB) 14	l days Meta, fecal	genomic analysis of the microbiome	<i>L. amylovorus</i> was reduced only in the BEA-fed group, but not in the BEA + DON group	68
ENB1 BEA		1.830 mg kg <sup>-1</sup> (] 2.570–3.578 mg (вел)	ENB1) kg <sup>-1</sup>			The relative abundance of <i>L. reuteri</i> was increased in the BEA group and, to a lesser extent, in the BEA + DON group compared to the control and the DON group.	
NIN	Pig and chicken feces	2.5-5 mg kg <sup>-1</sup>	÷	-3 weeks Chen	nical analysis of feces	After provide the faces from the develop on the faces from the of six pigs. After three weeks, this pig was now able to form develop des (90%) (90%) no develop of NIV, but another unidentified metabolite was found in the faces from chicken fed with 2.5 or $5 \ \mu g^{-1}$ NIV for three weeks	64
Alternariol (DON), enn DAS deacet	(AOH), alternaı iiatin B (ENB), <i>1</i> ylation.	iol methyl ether (A <sup>1</sup> <i>actobacillus (L</i> ), niv:	ME), alterpe alenol (NIV)	rylenol (ALP), alt( , scirpentriol (SCI	rttoxin I (ATX-I), alterpei ?), stemphyltoxin III (ST	ylenol (ALP), altuene (ALT), beauvericin (BEA), diacetoxyscirpenol (DAS), deoxynival IX-III), and 15-monoacetylscirpenol (15-MAS); both SCP and 15-MAS are the produc	enol s of

83.1%, being the highest decrease caused by the strain *L. casei casei.*<sup>78</sup>

Other Fusarium mycotoxins have been studied in combination with probiotics showing similar results. Escherichia coli (ATCC 25922), Salmonella enterica serovar typhimurium 3389-1 (DT12), Staphylococcus aureus (ATCC 29213), L. acidophilus (DSMZ 20079), L. acidophilus (20079), L. salivarius (20555), L. sobrius (16698), B. longum subsp. longum Reuter (20219) and B. breve (20213) received a fast digital time-lapse microscopic method to assess AUR, BEA, ENs and FA antibacterial effects after 6 h of cultivation. The most potent compound was AUR against L. acidophilus, whereas BEA, ENs and FA were characterized by having weak antibiotic effects.<sup>79</sup> Burkholderia ambifaria T16, a naturally beneficial bacterium with proven biocontrol properties but potential pathogenic risk, has shown to inhibit the growth of several Fusarium species and possess the unique ability to degrade FA as a carbon, nitrogen and energy source, as well as the capacity to detoxify FA in barley seedlings.<sup>80</sup> Different S. cerevisiae, Lactobacillus and Bacillus strains were tested for their ability to degrade different mycotoxins. Among the tested mycotoxins, DAS was proven to possess an inhibitory effect on the growth of almost all Lactobacillus and Bacillus strains, whereas no effect on yeast strains could be observed.<sup>81</sup> The human microbiome characteristic bacteria Prevotella copri has revealed to deacetylate 51.3% of DAS to 15-MAS after 48 h.<sup>82</sup>

The same trends have also been reported with Alternaria mycotoxins in Ge et al. (2017).<sup>83</sup> L. brevis 20023 (LAB-20023) cells have revealed to adsorb TeA from aqueous solution, demonstrating that polysaccharides and protein were important components of the LAB cell wall and were involved in TeA removal. The lowest and highest absorption rates were 12.5% and 90.1%, respectively.83 The effects of a complex extract of Alternaria mycotoxins (AME, ALT, TeA, TEN, ATX-I, ATX-II, ALP, STTX-III, altenusin and altersetin (AST)) were studied in combination with some typical bacterial strains of the gut microbiome such as Bacteroides caccae, Bacteroides eggerthii, **Bacteroides** thetaiotaomicron, Bacteroides vulgatus, Parabacteroides distasonis, E. coli, Clostridium innocuum, Alistipes finegoldii, Alistipes timonensis, Ruminococcus bicirculans, Akkermansia muciniphila, L. hominis, B. longum and B. sp. The results showed that both Gram types were able to adsorb AOH, AME, and AST, and it was seen that Gram-negative bacteria had higher adsorptive abilities. Notwithstanding, this absorption was not observed for neither ATX-I nor ALP. Interestingly, the mycotoxin tendency to accumulate within bacterial pellets, especially in those of Gram-negative strains, was connected to their lipophilic profile.<sup>84</sup>

**3.2.2** Emerging mycotoxins and probiotics *in vivo*. To study the influence of fermentation on feed acidity and microbiological parameters, two different diets were prepared: (i) non-fermented basal feed and (ii) fermented basal feed. A fermented basal diet was characterized by including some microbial starters (*L. uvarum LUHS245*, *L. casei LUHS210*, *Pediococcus acidilactici LUHS29* and *Pediococcus pentosaceus LUHS183*). Piglets were fed using these diets for 36 days and feces microbiota,

growth performance and health were evaluated. Mycotoxin biotransformation was quantified, including masked mycotoxins, in feed and piglet fecal samples. The analysis showed that AME and ALT were found in 61-day-old control piglets' feces and in fermented feed samples. However, AME was not found in treated piglets' feces. *Prevotella* was the most prevalent *genus* in both groups, but the prevalence of *Lactobacillus* was 6-fold higher in treated animals compared to the control ones (23.7 *vs.* 3.9%). After the exposure, in the fermented feed group animals' blood, triglycerides and serum high-density lipoprotein cholesterol were notably higher, but the levels of glucose, alkaline phosphatase, urea, thyroxine, glucose and potassium were significantly decreased ( $p \le 0.05$ ) in comparison with the control group.<sup>85</sup> Fig. 5 and Table 3 show all the data concerning emerging mycotoxins and probiotics.

#### 3.3 Mycotoxins and prebiotics

Prebiotics have been described as a potent agent to prevent metabolic disease through microbiome modulation.<sup>50</sup> K-carrageenan has revealed to be a promising fiber, thanks to ZEA bioaccessibility reduction, obtaining values lower than 20%. Lactose from fermented whey has shown to protect from AFB1 and OTA toxicity.<sup>86,87</sup> Pectin has exerted to decrease DON, HT-2, and T-2 (50–88%) bioaccessibility.<sup>59</sup> Garlic, whose prebiotic content is notable, has shown to reduce AFB1 cytotoxicity.<sup>88</sup> It has also been reported that flavonoids play an important role in protecting against the toxicity of mycotoxins like OTA, PAT, and DON in different experimental models.<sup>89,90</sup>

**3.3.1 Emerging mycotoxins and prebiotics** *in vitro*. As Fig. 6 shows, *Fusarium* mycotoxins, ENs and BEA are the most studied ones. Inulin (ranging from 1.5% to 10%) has revealed to decrease the bioaccessibility of ENA, ENA1, ENB and ENB1 in a dose-dependent manner in a digestion model.<sup>91</sup> Cellulose (1% and 5%) has exerted to reduce ENA, ENA1, ENB and ENB1 bioaccessibility at a mean of 26.9% and 44.7%, respectively. Meanwhile, in the case of BEA, the bioaccessibility decrease ranged from 60% to 80%.<sup>71</sup> Inulin and fructooligosaccharides (FOS) (1% and 5%), in a static gastrointestinal digestion model, have shown to reduce ENA, ENA1, ENB, ENB1 and BEA bioaccessibility. The lowest bioaccessibility was caused by adding the inulin 5% (5.7%) and FOS 5% enriched samples (3.9%) for ENA and ENA1, respectively.<sup>92</sup>

BEA bioaccessibility was decreased, ranging from 15.7% to 60.5%, compared to the control (93.2%), with the addition of the following prebiotics: galactomannan 5%, glucomannan high-molecular weight (HMW) 1%, glucomannan HMW 5%, glucomannan fine powder 1%, glucomannan fine powder 5%, citrus fiber 1%, citrus fiber 5%, bamboo fiber 1%, bamboo fiber 5%, carrot fiber 1%, carrot fiber 5%, pie fiber 1%, pie fiber 1%, β-glucan 1%, β-glucan 5%, xilan 1%, xilan 5%, cellulose HMW 1%, cellulose HMW 5%, cellulose medium-molecular weight (MMW) 1% and cellulose MMW 5%.<sup>72</sup> BEA has also shown to be decreased by β-1,3 glucan, chitosan LMW, chitosan MMW, FOS, galactomannan, inulin, and pectin at both concentrations (1% and 5%). Each sample was contaminated, reaching a BEA concentration of both 5 and 25 mg L<sup>-1</sup>.



**Fig. 5** Graphical bars of the probiotic publications (*n* = 15) according to the (a) microbial species and (b) the emerging mycotoxins studied. In the reports, *Bacillus, Bifidobacterium, Lactobacillus, Streptococcus, E. coli* and *S. cerevisiae* were the most recurrent probiotics, whereas enniatins, beauvericin, other *Fusarium* mycotoxins and *Alternaria* mycotoxins were the most used emerging mycotoxins. Beauvericin – BEA, enniatins – ENs, *Escherichia coli – E. coli* and *Saccharomyces cerevisiae* – *S. cerevisiae*.

While the mean control bioaccessibility was 92.6% and 90.0% for both BEA concentrations, respectively; when adding prebiotics, it was 13.2% and 50.4%, respectively, galactomannan being the most reducing one (5.2%).<sup>93</sup>

Similar results have been obtained with *Alternaria* mycotoxins. AOH, AME, ATX-II, STTX-III, and ALP have been found to be decreased, already before anaerobic incubation, in the samples containing particulate fecal matter. These samples were supposed to be also composed of indigestible fiber fractions.<sup>67</sup> The antifungal effects of the volatile compound 2-phenylethyl isothiocyanate (2-PEITC) against *A. alternata* and its mycotoxins TEN, AOH, AME, and ALT were assessed. 2-PEITC is found in some vegetables and contributes to gastrointestinal health.<sup>94</sup> After a 2 h treatment, these four mycotoxins were extracted and detected by high performance liquid chromatography-tandem mass spectrometry (HPLC-TOF-ESI-MS) in the mycelium of *A. alternata*. The concentrations of TEN, AOH, AME, and ALT at the MIC of 2-PEITC-treated groups were only 27%, 90%, 90%, and 88% of the corresponding control groups, respectively.<sup>95</sup> The anthocyanidin delphinidin (30.3  $\mu$ g mL<sup>-1</sup>) has exerted to strongly antagonize the genotoxic properties of ATX-II by significantly suppressing the level of DNA strand breaks by up to 75% in HT-29 colon carcinoma cells.<sup>84</sup> Conversely, AOH and AME degradation has been assessed after cinnamaldehyde treatment in centrifuge tubes under rotary shaking conditions (150 rpm) at 25 °C for 120 min. It was found that AOH and AME degradation rates were 16.8% and 7.3%, respectively.<sup>96</sup> The results are shown in Table 4.

#### 3.4 Mycotoxins and synbiotics

The use of synbiotics has been previously proved to be effective in the removal of PAT.<sup>97,98</sup> The deleterious effects of

Table 3 Collection of the most important characteristics of the studies found regarding the emerging mycotoxins and probiotics in vitro and in vivo. For each study, the studied mycotoxin, type of probiotic, mycotoxin dose administration, exposure time, experimental assay, results and references are described

Mycotoxin	Probiotic		Dose administration	Exposure time	Experimental assay	Results	Ref
In vitro							
ENA	L. johnsonii CECT 289	B. breve CECT 4839 T	Not reported	4 h	Dynamic gastrointestinal	ENA, ENA1, ENB and ENB1 mean	71
ENA1	L. rhamnosus CECT	B. adolescentis CECT 5781 T			in vitro digestion	bioaccessibility reduced ranging from 21% to	
ENB	L. plantarum CECT 220	B. bifidum CECT 870 T				31.2% compared to the control (33.4%–39.6%)	
ENB1	L. reuteri CECT 725 L. casei CECT 47	B. longum CECT 4551					
ENA	B. longum	L. ruminis	0.2-20.000 ng	24 h	Antimicrobial	ENA1 and ENB were the	72
ENA1	B. bifidum	Streptococcus thermophilus	per disc		analyses carried out the disc-diffusion	most actives against B. adolescentis 5871,	
ENA2	B. breve	22 strains of S. cerevisiae			method	<i>S. thermophilus</i> , 2 strains of <i>Lactobacillus</i> and 2	
ENB	B. adolescentis	9 strains of <i>Bacillus</i> subtilis.				other strains of Bifidobacterium	
ENB1	L. rhamnosus						
ENB4	L. casel-casel						
	L. pianiarum L. paracasai						
EN1	Bacillus subtilis 168	trnC2	$2-8 \ \mu g \ mL^{-1}$	3 weeks	Cocultivation media	ENA1 and ENB1	73
ENB1	Staphylococcus aureu	us ATCC 29213		5	_seathradon meala	inhibited the growth of	. 0
	Streptococcus pneum E. coli ATCC 25922	oniae ATCC 49619				<i>B. subtilis</i> and were also active against	
	Staphylococcus aureu	us 25697				Staphylococcus aureus,	
	Enterococcus faecalis	UW 268				Streptococcus	
	Pseudomonas aerugir	10sa B 63230				pneumoniae, and Enterococcus faecalis	
ENA	B. longum	Streptococcus thermophilus	Not reported	48 h	<i>In vitro</i> degradation under an anaerobic	ENA, ENA1, ENB and ENB1 degradation	74
ENA1	B. bifidum	L. ruminis			atmosphere	ranging from 5.2% to	
ENB END1	B. breve	L. casei				99.5%	
ENBI	B. adolescentes	S. cerevisiae					
	L. Mumnosus L. casei_casei						
BEA	S. cerevisiae LO9	S. cerevisiae YE-6	$5 \text{ mg L}^{-1}$ : 5 mg	48–72 h	Biological	BEA mean degradation of	75
	S. cerevisiae YE-2	S. cerevisiae A34	kg <sup>-1</sup> , 5g		degradations under	86.2%. The highest	
	S. cerevisiae YE5	S. cerevisiae A17	U		aerobic conditions	degradation activity	
	S. cerevisiae YE-6	S. cerevisiae A42			in the liquid	(98.8%) was due to the	
	S. cerevisiae YE-4	S. cerevisiae A08			medium of PDB	strain of <i>S. cerevisiae LO</i> -	
	S. cerevisiae YE5					9, whereas the lowest activity was due to $LS100$	
BEA	L. acidophilus		0.8–15.7 μg	24–72 h	Cell viability assay	L. acidophilus	76
			$mL^{-1}$			significantly increased	
						cell viability at 12 h and	
						24 n in the presence of	
BEA	L. paracasei CECT 277	B. adolescentis CECT 5781T	$5-25 \text{ mg kg}^{-1}$	48 h	<i>In vitro</i> dynamic digestion model	BEA BEA bioaccessibility highest reduction	77
	L. casei CECT 4180	B. bifidum CECT 870T			argestion model	(45.4%) was carried out	
	L. rhamnosus CECT 278T	B. Longum CECT 4551				by <i>B. longum</i> , whereas	
	L. plantarum CECT	Corynebacterium				bioaccessibility data were	
	220	vitaeruminis CECT 537				revealed with the strain of <i>L. rhamnosus</i> , with a	
	L. ruminis CECT 4061T	Eubacterium crispatus CECT 4840				27.5%	
	L. casei casei CECT	S. cerevisinas CECT					
	277	1324					
	B. breve CECT						

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### Food & Function

#### Table 3 (Contd.)

Mycotoxin	Probiotic		Dose administration	Exposure time	Experimental assay	Results	Ref.
BEA	B. longum B. bifidum B. breve B. adolescentis L. rhamnosus L. casei-casei	Eubacterium crispatus, Salmonella fecalis and Salmonella thermophilus L. ruminis L. casei L. animalis	5 mg L <sup>-1</sup>	4–48 h	Fermentations in the liquid medium of MRS	BEA reduction ranged from 66.5% to 83.1%, the highest decrease value being that caused by the strain of <i>L. casei–</i> <i>casei</i>	78
AUR	L. plantarum E. coli (ATCC 25922)	L. acidophilus (DSMZ 20079)	1.1–146.1 μg mL <sup>-1</sup>	6 h	Fast digital time- lapse microscopic	The most potent compound was AUR	79
ENs	Salmonella enterica serovar typhimurium 3389- 1 (DT12)	L. acidophilus (20079)	1.4–174.6 μg mL <sup>–1</sup>		method	against <i>L. acidophilus.</i> Meanwhile, BEA, ENs and FA were characterized by having	
BEA	Staphylococcus aureus (ATCC 29213)	L. salivarius (20555)	$1.6200.7~\mu g \\ m L^{-1}$			weak antibiotic effects	
FA	25210)	L. sobrius (16698) B. longum subsp. longum Reuter (20219) B. brave (20212)	$0.2-29.7 \ \mu g$ mL <sup>-1</sup>				
FA	Burkholderia ambifar	ы. ыеке (20213) ria T16	$0.06 \text{ mg mL}^{-1}$	72 h	<i>In vitro</i> detoxification	Burkholderia ambifaria has been shown to inhibit the growth of several Fusarium species and possess the unique ability to use the mycotoxin FA as the sole C, N and energy sources, as well as the capacity to detoxify FA in barley	80
DAS	L. acidophillus 1A L. acidophillus 4A L. helveticus 2A L. bulgaricus 3A	L. bulgaricus 5A Bacillus licheniformis Bacillus subtilis S. cerevisae	0.5 μg mL <sup>-1</sup>	48 h	Biodegradation <i>in vitro</i> in agar plates	DAS appeared to possess an inhibitory effect on the growth of almost all <i>Lactobacillus</i> and <i>Bacillus</i> strains, whereas no effect was observed on yeast strains	81
DAS	Akkermansia muciniphila DSM 22959 B. adolescentis DSM 20083 Bacteroides thetaiotaomicron DSM 2079 Prevotella copri DSM 18205 Faecalitalea cylindroides T2-87 Anaerobutyricum hallii DSM 3353 Anaerostipes hadrus SSC/2 Blautia obeum A2-	Butyrivibrio fibrisolvens 16/4 Coprococcus sp. ART55/1 Eubacterium rectale DSM 17629 (A1-86) Roseburia intestinalis L1-82 Lactiplantibacillus plantarum NCIMB 7220 Faecalibacterium prausnitzii A2-165 Enterococcus mundtii DSM 4838	0.7 ng mL <sup>-1</sup>	0-48 h	Anaerobic incubation	The human microbiome characteristic bacteria <i>Prevotella copri</i> revealed to deacetylate 51.3% of DAS to 15-MAS after 48 h	82
TeA	162 L. brevis 20023 (LAB	20023)	$0.5~\mu g~mL^{-1}$	12 h	Adsorption	<i>L. brevis 20023</i> ( <i>LAB-20023</i> ) cells have revealed to adsorb TeA from 12.5% to 90.1%	83

#### Table 3 (Contd.)

Mycotoxin	Probiotic		Dose admini	stration	Exposure time	Experimental assay	Results	Ref
AOH AME	Bacteroides caccae Bacteroides eggerthii	Alistipes finegoldii Alistipes timonensis	$0.5-50 \ { m mL}^{-1}$	ıg	24 h	Absorption assay	Both Gram types were able to adsorb AOH, AME, and AST. Gram-	84
AST	Bacteroides thetaiotaomicron	Ruminococcus bicirculans					negative bacteria showed higher adsorptive	
ATX-I	Bacteroides vulgatus	Akkermansia muciniphila					capacities. This tendency was not observed for	
ALP	Parabacteroides distasonis	L. hominis					neither ATX-I nor ALP	
	E. coli	B. Longum						
	Clostridium innocuum (CI)	<i>B.</i> sp.						
Mycotoxin	Probiotic	Mycotoxin dose administration	Exposure time	Experin	nental assay	Results		Ref
In vivo	-	40.4 <b>47</b> .05 <b>1</b> .71	ac. 1					
AME	L. uvarum	10.1–17.06 μg kg	36 days	Fermen	sformation	AME and ALT were	e found in 61-day-old	85
ALT	LUHS245 L. casei LUHS210			Diotran	sionnation	samples However	AME was not found in	
ALT	Pentosaceus					probiotic-treated r	piglets' feces	
	acidilactici					II	0	
	LUHS29							
	Pediococcus							
	pentosaceus							
	LUHS183							

beauvericin (BEA), *Bifidobacterium* (*B*), diacetoxyscirpenol (DAS), enniatin A (ENA), enniatin A1 (ENA1), enniatin A2 (ENA2), enniatin B (ENB), enniatin B1 (ENB), enniatin B4 (ENB4), enniatins (ENs), *Escherichia coli* (*E*, *coli*), fusaric acid (FA), lactic acid bacteria (LAB), *Lactobacillus* (*L*), minimal inhibitory concentrations (MICs), De Man-Rogosa-Sharpe agar (MRS), potato dextrose broth (PDB), *Saccharomyces* (*S*.), and tenuazonic acid (TeA).

OTA have also been reversed by using beneficial synbiotics possessing the ability to reduce the number of potential pathogens in the digestive tract and by increasing the activity of  $\alpha$ -glucosidase and  $\alpha$ -galactosidase, while decreasing the activity of potentially harmful fecal enzymes ( $\beta$ -glucosidase,  $\beta$ -galactosidase and  $\beta$ -glucuronidase).<sup>60,99</sup>

3.4.1 Emerging mycotoxins and synbiotics. The bioaccessibility of ENs (ENA, ENA1, ENB, and ENB1) and BEA was analyzed by using an in vitro static and dynamic simulated gastrointestinal digestion system, imitating the digestive physiological conditions until the colonic compartment. Inulin and FOS at two concentrations (1% and 5%) were first added, and afterwards, the colonic microbial fermentation was carried out by using L. paracasei CECT 277, L. casei CECT 4180, L. rhamnosus CECT 278T, L. plantarum CECT 220, L. ruminis CECT 4061T, L. casei casei CECT 277, B. breve CECT 4839T, B. adolescentis CECT 5781T, B. bifidum CECT 870T, B. longum CECT 4551, Corynebacterium vitaeruminis CECT 537, Eubacterium crispatus CECT 4840 and S. cerevisiae CECT 1324. The bioaccessibility of EN and BEA was shown to be higher in the synbiotic prebiotic static model (6.2%-44.9% and 46.7%-61.1%, respectively) than that found in the dynamic one (23.0%-68.9% and 76.2-91.0%, respectively).92 Similar results have been revealed in Meca *et al.* (2012),<sup>93</sup> where the fibers  $\beta$ -1,3 glucan, chitosan LMW, chitosan MMW, FOS, galactomannan, inulin and pectin (1% and 5%) were combined with the bacteria L. animalis

CECT 4060T, L. casei CECT 4180, L. casei rhamnosus CECT 278T, L. plantarum CECT 220, L. rhuminis CECT 4061T, L. caseicasei CECT 277, B. breve CECT 4839T, B. adolescentis CECT 5781T, B. bifidum CECT 870T, Corynebacterium vitaeruminis CECT 537, Streptococcus faecalis CECT 407, Eubacterium crispatus CECT 4840, and S. cerevisiae CECT 1324. In vitro mean bioaccessibility values of BEA (at both experimental concentrations: 5 and 25 mg L<sup>-1</sup>) by using synbiotics were 12.9% and 42.4%, respectively – 5.3 and 28.2-fold higher in comparison with the data evidenced with the prebiotic sample treated with duodenal digestion.<sup>93</sup>

#### 3.5 Mycotoxins and postbiotics

Postbiotics are considered to have positive effects on human health.<sup>50</sup> Yeast cell wall extract (YCWE) and a post-biotic yeast cell wall-based blend (PYCW) have revealed to reduce the adverse effects related to serum biochemistry, liver function, immune response, altered cecal short chain fatty acids (SCFAs), goblet cell counts and architecture of the intestinal villi. These results were found studying commercial broilers fed with a diet containing DON, T-2 and ZEA at 3.0 mg kg<sup>-1</sup>, 104  $\mu$ g kg<sup>-1</sup> and 79  $\mu$ g kg<sup>-1</sup>, respectively.<sup>57</sup> Some characteristic bacterial enzymes, which isolated are considered postbiotics, have exerted to remove AFB1, OTA, DON and ZEA.<sup>63</sup> Butyrate, one of the most studied gut microbiota metabolites, has shown to reverse the alterations caused by DON by blocking



Fig. 6 Graphical bars of the prebiotics (n = 9) according to (a) the prebiotic type and (b) the emerging mycotoxins studied. Beauvericin – BEA, enniatins – ENs and fructooligosaccharides – FOS. In the studies, inulin, cellulose,  $\beta$ -glucan, FOS and galactomannan were the most used prebiotics, whereas ENs, BEA and *Alternaria* mycotoxins were the most recurrent emerging mycotoxins.

some genes involved in reactive oxygen species and tumor necrosis factor alpha mediated pathways.<sup>100</sup> Likewise, AFB1 and ZEA have been reported to reduce the content of SCFAs (butyrate, propionate and acetate).<sup>101,102</sup>

**3.5.1** Emerging mycotoxins and postbiotics. The utilization of postbiotics (PYCW) for addressing emerging mycotoxins was first documented in Xu *et al.* (2023).<sup>103</sup> In contrast to inorganic clay-based adsorbents characterized by presenting some limitations, such as the possible contamination with other detrimental compounds (dioxins, polychlorinated biphenyls, and heavy metals), organic adsorbents such as PYCW have shown to be more efficient and safe product substitutes.<sup>104</sup> In this study, an absorption bioassay was conducted exposing BEA (0.5, 2, 5, 20, 40 and 63 µg mL<sup>-1</sup>) and CIT (0.5, 1, 10, 40, 50 and 68 µg mL<sup>-1</sup>) to PYCW at different concentrations for 2 h. The results showed that PYCW presented an overall mean adsorption efficiency of 70.1% for BEA and 35.1% for CIT.<sup>103</sup>

## 4 Discussion

The interactions along the gut are very complex due to the quantity of factors, which play a notable role in its physiology, so that there are times where it is so likely not to know completely which specific pathways or molecules are the consequence of a particular fact. However, there are some stablished correlations which help us to better understand how biological systems work. One of these strong correlations is the preventive role of PPSP and the gut microbiota in disease and its health-promotion effects.<sup>49</sup> As has already been said,<sup>62</sup> PPSP and the gut microbiota possess the capacity to bind and metabolize a vast quantity of substances entering the gastrointestinal tract. Therefore, despite theoretically reducing ben-

eficial compounds, PPSP can also decrease the exposure to detrimental ones, such as toxins, including emerging mycotoxins.

As presented, European regulations only encompass a small set of mycotoxins (AFB1, AFB2, AFG1, AFG2, AFM1, OTA, PAT, DON, ZEA, FB1, FB2, CIT, AOH, AME, TeA, T-2 and HT-2 and ergoalkaloids).<sup>2</sup> Due to their regulation, these mycotoxins tend to be more studied - also including their interactions with the gut microbiota and PSPP. In fact, studies show that studied mycotoxins disrupt the gut microbiota, while concomitant intake of PSPP mitigates the effects of the mycotoxins and contributes to maintain gut microbiota homeostasis.56,58,90,99,101 Although similar trends are expected with unregulated mycotoxins, it has to be carried out as a one-on-one mycotoxin analysis, since every mycotoxin possesses a specific chemical structure and a different response in the environment where it is located.<sup>62</sup> Actually, there are some 400 known mycotoxins, so the vast majority of them are not still regulated and need a more profound comprehension.<sup>1</sup> Furthermore, it should not be forgotten that mycotoxins tend to be found in combinations, so the most common mixtures found in food and feed are the ones interesting to be tested.

#### 4.1 Emerging mycotoxins and the gut microbiota

Regarding the interaction between the gut microbiota and emerging mycotoxins, studies have revealed a clear tendency: the gut microbiota contributes to emerging mycotoxin detoxification through binding and/or metabolization<sup>63–65,67</sup> and emerging mycotoxins alter the gut microbiota composition.<sup>68</sup> Despite being the trend confirmed by these studies, the difficulty stems from the heterogeneity of the analyzed samples. The microbiota exhibits significant variability, with the microbiome differing by species. In humans, microbiota changes are influenced by factors such as the level of urbaniz-

Table 4 Collection of the most important characteristics of the studies found concerning the emerging mycotoxins and prebiotics *in vitro*. For each study, the studied mycotoxin, type of prebiotic, mycotoxin dose administration, exposure time, experimental assay, results and references are described

Mycotoxin	Prebiotic		Dose administration	Exposure time	Experimental assay	Results	Ref.
In vitro ENA ENA1 ENB ENB1	Inulin (1.5%–10%	6)	1.02-2.04  mg mL <sup>-1</sup>	8 h	<i>In vitro</i> digestion model	ENA, ENA1, ENB and ENB1 bioaccessibility reduction in a dose-dependent manner at an inulin concentration ranging	91
ENA1 ENB END1	Cellulose (1% and	d 5%)	Not reported	4 h	<i>In vitro</i> digestion model	Cellulose (1% and 5%) decreased ENA, ENA1, ENB and ENB1 bioaccessibility a mean of 26.9% and 44.7%, respectively BEA bioaccessibility reduction ranged from 60% to 80%	71
ENB1 BEA ENA ENA1 ENB ENB1 BEA	Inulin and FOS (1	1% and 5%)	Not reported	4 h	Static gastrointestinal <i>in vitro</i> digestion model	Highest reductions carried out when adding enriched inulin 5% (5.7%) and FOS 5% (3.9%) for ENA and ENA1, respectively	92
BEA	Galactomanan 1–5% Glucomannan HMW 1%–5% Glucomannan FP 1%–5% Citrus fiber 1%– 5% Bamboo fiber 1%–5% Carrot fiber 1%– 5%	Pie fiber 1%– 5% β-Glucan 1%– 5% Xilan 1–5% Cellulose HMW 1–5% Cellulose MMW 1–5%	5–25 mg kg <sup>–1</sup>	52 h	<i>In vitro</i> digestion model	BEA bioaccessibility reduction ranging from 15.7% to 60.5% comparing to control (93.2%)	77
BEA	β-1,3 glucan Chitosan LMW Chitosan MMW FOS	Galactomannan Inulin Pectin (All 1% and 5%)	5 mg kg <sup>-1</sup>	52 h	<i>In vitro</i> digestion model	BEA mean bioaccessibility decrease when adding prebiotics (13.2% and 50.4%, respectively for 1% ad 5%), galactomannan being the most reducing one (5.2%)	94
AOH AME ATX-II STTX-III ALP	Particulate fecal r to be composed a indigestible fiber	matter supposed Ilso by fractions	50 $\mu g m L^{-1}$	48 h	Fecal incubation <i>in vitro</i>	AOH, AME, ATX-II, STTX-III, and ALP reduction by samples supposed to be also composed of indigestible fiber fractions	67
TEN AOH AME ALT	2-PEITC		0.1–1.38 mg kg <sup>-1</sup>	2 h	Qualitative analysis performed using a mass spectrometer after incubation for the MIC	TEN, AOH, AME, and ALT concentrations of 2-PEITC- treated groups were only 27%, 90%, 90%, and 88% of the corresponding control groups, respectively	95
ATX-II	Delphinidin		$0.35 \ \mu g \ mL^{-1}$	24 h	Coupled SRB/WST-1 cytotoxicity assays	ATX-II genotoxic properties were antagonized by delphinidin ( $30.3 \ \mu g \ mL^{-1}$ ) by significantly suppressing the level of DNA strand breaks by up to 75% in HT-29 colon car- cinoma cells	89
AOH AME	Cinnamaldehyde		$0.81-0.82 \ \mu g \ mL^{-1}$	2 h	Degradation assay	AOH and AME degradation rate was 16.8% and 7.3%, respectively, after 120 min of cinnamaldebyde treatment	96

Alternariol (AOH), alternariol monomethyl ether (AME), alterperylenol (ALP), altertoxin II (ATX-II), altuene (ALT), beauvericin (BEA), enniatin A (ENA), enniatin A1 (ENA1), enniatin B (ENB), enniatin B1 (ENB), fructooligosaccharides (FOS), high-molecular weight (HMW), low-molecular weight (LMW), medium-molecular weight (MMW), minimum inhibitory concentration (MIC), sulforhodamine B/water-soluble tetrazolium salt (SRB/WST-1), tentoxin (TEN), 2-phenylethyl isothiocyanate (2-PEITC), and stemphyltoxin III (STTX-III).

ation of the location and even the time of day within the same individual.<sup>105-107</sup> For example, rumen microbiome is characterized by possessing many enzymes involved in the *de novo* synthesis of vitamin B12.<sup>108</sup>

According to experimental feasibility, it has been shown that gut microbiota samples are obtained from feces in most of the experiments, since the fecal microbiota is more accessible than the colon one. Interestingly, it has been revealed that colon microbiota has a different profile than the fecal one, the microbiome in the large intestine being much more diverse than those of the small intestine and feces. For instance, while in the large intestine there were 4080 operational taxonomic units, in the feces only 443 were found.<sup>109</sup> Indeed, bacterial biomass also changes along the gastrointestinal tract and the feces: duodenum ( $10^3$  bacteria per g), jejunum ( $10^4$  g<sup>-1</sup>), ileum ( $10^7$  g<sup>-1</sup>), colon ( $10^{12}$  g<sup>-1</sup>) and feces ( $5^{10}$  g<sup>-1</sup>).<sup>110,111</sup> In addition, short-term trials are likely to hide that long-term mycotoxin exposure may lead to the development of the ability to degrade these mycotoxins despite being unable to do it in the first moment.<sup>64</sup>

There are other limitations that should be highlighted. Although in Debevere *et al.*  $(2020)^{63}$  the incubation time (48 h) was in line with other experiments studying mycotoxin degradation in vitro, it is considerably longer than the in vivo retention time of the digesta in the rumen, implying that under normal physiological conditions the results would be different. Despite Crudo et al. (2020)<sup>67</sup> having shown that AOH, AME, ATX-II, STTX-III and ALP were lower in the samples containing microorganisms than in those without them, it should be noted that the sample size was very small (n = 4). In this study, all donors shared some characteristics: they were healthy omnivorous volunteers (two males and two females) ranging from 26 to 34 years old and with a normal body mass index  $(18.5-24.9 \text{ kg m}^{-2})$ , none of them had been treated with antibiotics, probiotics, and prebiotics for the previous three months and they did not present previous intestinal diseases. However, the entire diet, playing a notable role in gut metabolism and in the interactions with mycotoxins, was not considered. Moreover, as mentioned in the same article, there are other limitations such as the short-term trial (3 h) and the fact that the growth media used might alter the initial microbial composition of the feces, contributing to the growth of some microorganisms at the expense of others. Conversely, in Novak et al. (2021),<sup>68</sup> only the mycotoxins ENB, ENB1, BEA and DON were assessed in weaning piglets' feces in contrast to the control. It was shown that L. amylovorus and L. reuteri, both beneficial microbes, were up-regulated and down-regulated, respectively, when adding BEA in comparison with DON and the control. Furthermore, the combination of ENs and BEA increased the total protein synthesis, but the addition of DON had an inhibitory effect.<sup>68</sup> Although it is important to know the interactions among these four mycotoxins, the real conditions are more complex, since a meal may contain a variety of contaminants, so the results under normal conditions are so likely to differ from these ones. While microbes could metabolize emerging mycotoxins, these toxins can also influence the composition of the microbiome, creating a potential

double-edged sword scenario. In addition, it is important to consider the varying doses of emerging mycotoxins used in experiments, ranging from nanograms to milligrams.

Viewing mycotoxins as toxic compounds or as potential drugs is indeed a question that arises. On the one hand, they can reduce beneficial microorganisms, such as Lactobacillus, S. ceverisiae and Bacillus,<sup>72,73,79,81</sup> whose depletion is generally connected to disease promotion.<sup>112</sup> On the other hand, they can play a role as antibiotics by eliminating microorganisms involved in diseases.<sup>112-116</sup> For instance, AOH and AME have exerted to be promising inhibitors of glycogen-synthase-kinase 3, being an important target in drug discovery. In fact, this enzyme has revealed to participate in signaling pathways of type 2 diabetes, cancer, neurological disorders, and other diseases.<sup>117</sup> In addition, ergotamine with its  $\alpha$ -adrenoreceptor-blocking mode of action has also been used for acute migraine treatments for over 50 years.<sup>118</sup> Further investigation is needed in the field to clarify the results and assess the real implications of these emerging mycotoxins towards health and diseases, also taking into consideration that the dose and the type of mycotoxin may be decisive in the health or disease-promoting outcomes.

#### 4.2 Emerging mycotoxins and probiotics

The bidirectional fluxes between probiotics and emerging mycotoxins have been widely confirmed. A vast quantity of probiotics (Lactobacillus, Bifidobacterium and S. cerevisiae) has shown to reduce emerging mycotoxins' bioaccessibility (ENs, BEA and Alternaria).<sup>74–78,92,93</sup> At the same time, emerging mycotoxins have exerted to alter probiotic compositions (B. subtilis and Staphylococcus aureus).<sup>72,73,79,81</sup> This bioaccessibility decrease may be due to the bacterial mycotoxin binding capacity<sup>83,84</sup> and/or their ability to contribute to detoxification by originating derivatives characterized by possessing less toxicity.<sup>82,83</sup> However, in most of these studies, probiotics are not assessed in combination, but one by one and using in vitro conditions. Only Vadopalas et al. (2020)85 assessed probiotics' effects on emerging mycotoxins under in vivo conditions. In this study, not only did the addition of some microbial starters modified some blood parameters, but also contributed to modulate the gut microbiota and detoxify AME. Despite these beneficial effects, it should be noted that the experiment only lasted two months and the piglets' organs were not fully mature. Combining probiotics and working in vivo would be a more realistic approach to the human environment, since there are a lot of microorganisms that form a part of the gut microbiota.<sup>119</sup> What is more, it is very well documented that the interplay along the gut concerns not only different bacteria, but also fungi, archaea, viruses, and helminths.<sup>120</sup>

#### 4.3 Emerging mycotoxins and prebiotics

It has been clearly shown that prebiotics reduce emerging mycotoxin bioaccessibility. Although the term prebiotic is very vast, the collected data cover a high quantity of these types of compounds at a different concentration.<sup>67,71,77,91–93,95,96</sup> As in the probiotic case, there are two mechanisms explaining this reduction. The first one is the capacity of prebiotics to bind

emerging mycotoxins, and the second one is the ability to metabolize them, and it is very likely to have both processes working at the same time. However, most studies do not consider an analysis of secondary metabolites of emerging mycotoxins, so this metabolization cannot be fully confirmed. Regarding mycotoxin reduction, if the metabolites have not been degraded, it is assumed that they have bound to the structures of the prebiotics, although they are also subject to metabolization anyway. Conversely, apart from reducing bioaccessibility, prebiotics can also directly suppress the detrimental effects of mycotoxins. As it is shown in Aichinger et al. (2018), the anthocyanidin delphinidin blocks significantly ATX-II genotoxic properties by suppressing the level of DNA strand breaks.<sup>89</sup> The action of these prebiotics may derive from blocking the mycotoxin, preventing its action or from having a beneficial agonist impact on some physiological pathways. Furthermore, it should also be noted that in all these experiments, prebiotics are evaluated only one by one. The reality of the human diet includes a wide combination of them, so more research needs to be carried out taking this complexity into account.

#### 4.4 Emerging mycotoxins, synbiotics and postbiotics

The role of synbiotics in the modulation of emerging mycotoxins has been assessed only in two studies.<sup>92,93</sup> In both, the results showed that the addition of synbiotics increased mycotoxin bioaccessibility when compared to prebiotics. It has been hypothesized that probiotics degraded prebiotics, so that the binding ability of the last ones was lost. Although it may be possible, it cannot be extended as a generality, since only small fractions of prebiotics and probiotics were used in these two trials, and they do not represent either all probiotics or complete prebiotics. Conversely, those synbiotics applied to non-emerging mycotoxins reveal opposite results. For instance, Zoghi et al. (2017)<sup>97</sup> showed that the removal of PAT by probiotic bacteria from apple juice depended significantly on the FOS content (as a prebiotic) and the addition of ascorbic acid. Under the best conditions, 91.3% of the initial patulin concentration was removed from juice for 6 weeks.97 Zoghi et al. (2019) also reported that by adding FOS, ascorbic and citric acid to apple juice inoculated with *L. plantarum*, the efficiency of PAT removal was improved to 95.9% for 6 weeks cold storage.<sup>98</sup> On the other hand, despite being supposed to enhance clinical outcomes compared to probiotics or prebiotics alone due to their synergistic effect, synbiotics are very difficult to formulate and few have shown to possess clinical efficacy. It is also worth mentioning that most clinical trials are not characterized by including relevant probiotic or prebiotic controls, and many have not performed relevant microbiota analyses.<sup>121</sup> Research needs to be expanded to more (emerging) mycotoxins in combination with synbiotics.

Finally, postbiotics have shown to bind, metabolize and alleviate the harmful effects of some emerging and non-emerging mycotoxins<sup>57,63,100,103</sup> as well as these mycotoxins have revealed to alter the composition of some postbiotics.<sup>101,102</sup> Notwithstanding, it is needed to extend these experiments to more (emerging) mycotoxins and to more postbiotics, since only the combination of PYCW with BEA and CIT has been assessed. In fact, it will be very interesting to focus on the interplay between (emerging) mycotoxins and SCFAs.

# 4.5 Interplay between emerging mycotoxins, the gut microbiota and PSPP

Although the results have revealed a clear trend, data on this interaction have only been found in 12 of the 20 emerging mycotoxins investigated. However, apart from these 20 ones,



**Fig. 7** Detoxification processes of the emerging mycotoxins by probiotics, prebiotics, synbiotics and postbiotics (PPSP) and the gut microbiota. Emerging mycotoxins are metabolized and bound by the gut microbiota. At the same time, PPSP contributes to colonize and modulate the gut microbiota, and also have an indirect impact on the metabolism of the emerging mycotoxins.

other emerging mycotoxins were found. The collected data encompassed a lot of experimental differences (mycotoxin/ biotic type and concentration, species, time exposure, assay type, etc.), making the comparisons difficult among them. As Fig. 7 illustrates, the interaction between the gut microbiota and emerging mycotoxins involves metabolization and binding processes. The role of PPSP in relation to emerging mycotoxins primarily revolves around colonization (probiotics) and modulation (all). Furthermore, there exists a two-way communication between microbiota and PPSP, where the gut microbiota can degrade and metabolize PPSP, activating secondary bioactive compounds that may contribute to the detoxification and/or alleviation of emerging mycotoxins. Similarly, PPSP can enhance certain gut microbiota strains involved in the removal of emerging mycotoxins. Indeed, this reciprocal relationship can also be applied to the entire spectrum of PPSP, as the addition of probiotics, prebiotics, and synbiotics may enhance the production of certain postbiotics (such as short-chain fatty acids), and vice versa.

## 5 Conclusions

Emerging mycotoxins have been implicated in numerous harmful biological processes. Despite their diverse nature, studies have demonstrated that the gut microbiota and PPSP can detoxify and/or alleviate the adverse effects resulting from exposure to these emerging mycotoxins. ENs, BEA, NIV, and Alternaria mycotoxins were among the most frequently encountered emerging mycotoxins. The genera Lactobacillus, Bifidobacterium, and Saccharomyces were the most extensively studied probiotics, while inulin, cellulose, β-glucan, FOS, and galactomannan were among the most evaluated prebiotics. Data on synbiotics and postbiotics were limited. Evidence clearly demonstrates the existence of interplay between the gut microbiota and emerging mycotoxins, as well as between PPSP and emerging mycotoxins, involving processes such as metabolization, colonization, modulation, and binding. In addition, connections between the gut microbiota and PPSP, as well as within the same PPSP, were identified, all contributing to the alleviation of the effects of the emerging mycotoxins.

However, quantitative and qualitative data remain limited. Further *in vivo* and long-term trials are necessary to better comprehend the complexity of these interactions in both animals and humans. The formulation of PPSP as functional food and nutraceuticals should be considered a preventive strategy against the toxicity of emerging mycotoxins in human diets and animal feed.

## Author contributions

Á. Lázaro: writing – original draft, methodology, investigation and validation. P. Vila-Donat: methodology, investigation, and review and editing. L. Manyes: investigation, review and editing, project administration, and conceptualization.

## Data availability

The data supporting this article have been included as part of the ESI.†

## Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

This publication is part of the project PID2022-140722OB-I00 funded by MCIU/AEI/AEI/10.13039/501100011033/FEDER, EU.

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