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REVIEW ARTICLE

Managing Mycotoxins in Animal/Poultry Feed Through Innovative Control Strategies: A Review

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Abstract

Mycotoxins are the secondary metabolites of certain toxigenic fungi that have deleterious effects upon the health of humans, animals, and poultry. More than 300 chemically different mycotoxins have been identified to date, among which the most important are aflatoxins, ochratoxins, fumonisins, trichothecenes, and patulins. Approximately 25% of global food crops are significantly affected by mycotoxins every year. Animals become exposed to the adverse effects of mycotoxins when fed mycotoxin-contaminated feed, and animal byproducts containing mycotoxin residues become a constant source of exposure to the human population. Once mycotoxins enter the food chain, their complete removal is inevitable; therefore, different control strategies are being adopted to minimize the adverse effects associated with them. This review encompasses various control strategies adapted to minimize mycotoxicosis.

Keywords: Mycotoxins, Animal feed, Poultry feed, Mycotoxicosis, Control strategies

INTRODUCTION

Certain toxic fungal species are ubiquitous in nature and have strong ecological link with human and animal food supplies. These fungi often produce certain chemical ingredients which are not necessarily required for their growth but play a crucial role in their survival and these chemical compounds are often termed as 'secondary fungal metabolites'. Mycotoxins are a diverse group of chemically different compounds originally produced as secondary metabolites by several toxigenic fungal species. Many fungal genera are predominantly involved in the production of these hazardous chemical compounds, but the most important among them are *Aspergillus, Fusarium, Penicillium, Alternaria* and *Claviceps* [1]. To date, about 400 chemically diverse mycotoxins have been identified which pose severe toxic effects in different animal species and human population in one way or another. Mycotoxin-associated toxicities and/or adverse effects are directly related to the dose, duration, mycotoxin type, and route of exposure to a specific mycotoxin [2]. Mycotoxins generally exhibit hepatotoxic, nephrotoxic, carcinogenic, and immunosuppressive effects in certain animal species, ultimately compromising the overall health of animals. When produced within the feed, mycotoxins form 'mycotoxin pockets' within the feed, which are generally rich in their concentration and are considered hotspots for mycotoxins, thereby ensuring an uneven distribution of mycotoxins within the feed.

The route of mycotoxin entry into the animal food/feed chain involves the use of contaminated agricultural byproducts during the formation of feed or production of different mycotoxins within the feed by storage fungi descending either from pre-harvest, harvest, or post-harvest durations of crops [3]. Contamination by different agricultural products also limits international trade, as certain countries have different regulatory measures regarding the levels of mycotoxins [4]. Entry of mycotoxins into the human food chain occurs either through the consumption of mycotoxin-contaminated agricultural products or through animal byproducts derived from animals fed mycotoxin-contaminated rations. The mycotoxin residues exhibit certain anomalies in humans, the details of which have been presented and elaborated in *Table 1* and *Fig. 1*.

Discussing about the classification of mycotoxins, some of the significant mycotoxins from the vast list are aflatoxins,

Table 1. *Mycotoxins associated diseases in human population along with details of specific mycotoxins involved in disease production*

Fungi Involved	Source of Contamination	Disease Production	Specific Mycotoxin Involved	Reference
Fusarium verticilloides, Fusarium proliferatum	Corn/maize	Oesophageal tumors	Fumonisin B2	[5]
Fusarium species	Toxic bread (cereal grains)	Alimentary toxic aleukemia	Trichothecene, Fumonisin B1	[6]
Stachybotrys atra	Contaminated dust from ventilation slits, ceilings and walls	Sick-building syndrome	T-2, Diacetoxyscirpenol, Verrucarol	[7]
Fusarium species	Wheat, oats, barley, rice	Akakabio-byo	Fusarium toxins	[8]
Aspergillus flavus and *Aspergillus parasiticus*	Cereal grains	Kwashiorkor	Aflatoxin M1, AFM2	[9]
Aspergillus ochraceus, Penicillium species	Cereal grains	Balkan endemic nephropathy	Ochratoxin A	[10]
Penicillium and *Aspergillus* species	Rice	Cardiac beriberi	Aflatoxins	[11]
Fusarium proliferatum, Fusarium verticillioides	Maize/corn	Neural tube defect	Fumonisin B1 and B2	[12]
Claviceps purpurea, Claviceps fusiformis	Cereal grains, rye	Ergotism	Ergotamine-ergocristine Alkaloids	[12]
Aspergillus flavus and *Aspergillus parasiticus*	Peanuts, cereal grains	Hepatocellular carcinoma	Aflatoxin B1	[13]
Stachybotrys atra	Grain dust	Stachybotryotoxicosis	Satratoxins, Trichothecene	[14]
Aspergillus species	Grain dust	Reye's syndrome	Aflatoxin B1, B2 and M1	[15]
Fusarium species	Wheat, rice, corn	Scabby grain toxicosis	Zearalenone, deoxynivalenol	

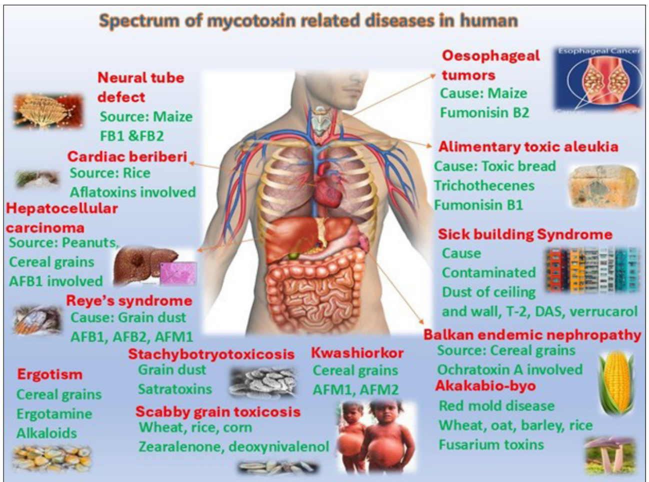

Fig 1. Spectrum of mycotoxin associated diseases in human

ochratoxins, zearalenone, trichothecenes, fumonisins, patulin, ergot toxins etc. However, keeping in view the one health perspectives, the most significant among all the mycotoxins are aflatoxins and ochratoxins. This review emphasizes the existence and after-effects of mycotoxins and control strategies adapted for these mycotoxins (in particular), along with the strategies adapted for the control of other mycotoxins in different livestock species.

Existence of Different Mycotoxins in Animal/Poultry Feed and Their Effects

Aflatoxins

Contamination of food with aflatoxins has remained a persistent issue for livestock and poultry feed, along with all processed food products. These chemical compounds were discovered accidently in 1961 when many turkey poults suddenly died in England due to an unrecognized disorder named as "Turkey-X disease." When investigated, it was revealed that a similar syndrome also appeared in farms where Brazil imported moldy peanut meal during feed formulation. Extraction using chloroform and detailed chemical analysis linked the extracted compound with "*Aspergillus flavus*." Scientists then gave the name "Aflatoxin" to this chemical compound by joining first three letters of both "*Aspergillus*" and "*flavus*." In the same year, this compound was also isolated in crystalline form in Netherlands and further fragmented as Aflatoxin B and G in United Kingdom based on color, they fluoresced under ultraviolet (UV) light. However, further investigations subdivided it into aflatoxins B1, B2, G1, and G2 based on minor differences in their chemical structures [16,17]. Furthermore, many other derivatives are linked to it from time to time, such as aflatoxin M1, aflatoxicol, AFP1, and AFQ1. There are approximately 20 different types of aflatoxins, but aflatoxin B1 (AFB1) *(Fig. 2)* is considered to be the most important and toxic among all types due to its toxicity [18].

Aflatoxins, belonging to the difuranocoumarin group, are secondary metabolites produced by a variety of toxigenic fungal species belonging to two important genera, *Aspergillus* and *Penicillium* [19] with *Aspergillus flavus* and *Aspergillus parasiticus* are considered major producers of aflatoxins [20,21]. Among all aflatoxins, aflatoxin B1 (AFB1) is the most toxic to animals, humans, and poultry, followed by AFB2, AFG1, and AFG2. Based on its extent of toxicity, the International Agency for Research on Cancer (IARC) has classified it as a group 1 carcinogen in humans and animals. Along with cancer, it causes hepatic disorders, metabolic diseases, vomiting, stunted growth, and diarrhea in human population.

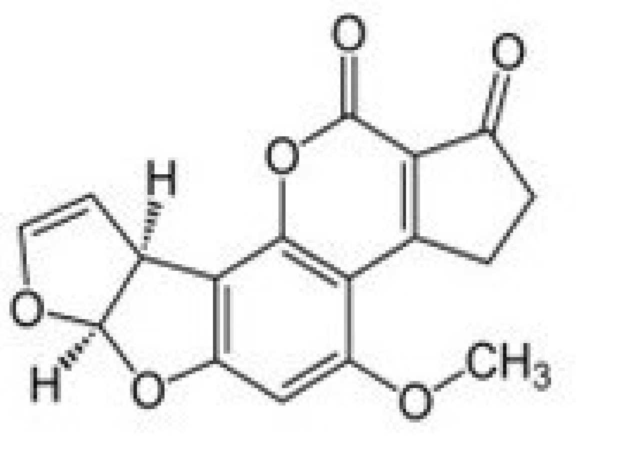

Fig 2. Structural presentation of Aflatoxin B1

Cereal crops, such as corn, wheat, sorghum, and rice, along with other feed ingredients, are readily contaminated with aflatoxins during their storage period when anaerobic conditions coupled with high humidity develop within the stored ingredients [6,22]. Aflatoxins have a tendency to easily infiltrate body tissues, muscles, and fatty tissues as residues, and when consumed, they become a potent source of contamination in the human population consuming such meat [23]. *Table 2* illustrates some studies on mycotoxins reporting the existence of tissue residues in animals. The problem of aflatoxins occasionally occurs in crops prior to harvesting, but they are also produced in the stored ingredients whenever the storage fungi get a favorable environment for their growth, ultimately producing them as their secondary metabolites [24]. There are different legislations for the maximum tolerable levels (MTL) for all foods, including feed/feed ingredients for poultry and large animals (sheep, cattle, and buffalo). The United States Food and Drug Administration (US FDA) recommends 20 µg/kg of feed as a worldwide range for maximum permissible and tolerable levels for the poultry sector, whereas the maximum tolerable level for cattle and buffalo is 100 µg/kg of feed [25,26]. However, many studies are available, particularly in developing countries, which report much higher levels in feed/feed ingredients than in the recommended MTL [23,27-32].

Table 2. *Occurrence of tissue residues of different mycotoxins in various animal species*

Mycotoxin	Specie	Level Used	Residues Detected	Reference
Aflatoxins	Broiler	100 µg/kg	0.32 µg/kg 0.08 µg/kg (Muscles)	[33]
	Broiler	1 mg/kg	0.166 µg/kg	[34]
	Broiler	1600-6400 µg/kg	6.97 ng/g 0.49-2.18 ng/g (Muscles)	[35]
	Broiler	Field Study	0.78-10.41 ng/g 0.23-5.67 ng/g (Kidney) 0.01-0.97 ng/g (Muscles)	[36]
	Broiler	600-1800 µg/kg	0.53-2.05 µg/kg	[37]
	Cattle	Field Study	0.36 µg/kg 1.37 µg/kg (Kidney)	[38]
Ochratoxins	Broiler	Field Study	0.58 µg/kg 0.51 µg/kg (Kidney)	[39]
	Broiler	100 µg/kg	1.92 ng/g 3.58 ng/g (Kidney)	[40]
	Broiler	2 mg/kg	1.79 ng/g 4.42 ng/g (Kidney)	[41]
	Beef sausages	Field Study	4.1-7.1 ppm	[42]

Table 3. Health impacts associated with aflatoxins in ruminants and poultry

Specie	Dose Tested	Duration	Effects	Reference
Cattle	300 µg/kg	133 days	No effects	[48]
Cattle	200-500 µg/kg	14 days	Severe pathological effects	[49]
Cattle	350-455 µg/kg	15-17.5 weeks	No effects	[50]
Cattle	60-300 µg/kg	155 days	No effects	[51]
Beef calves	1400 µg/kg	FS	Neurological signs, ataxia, depression	[52]
Lambs	2 mg/kg	37 days	Decreased body weight and immune responses	[53]
Lambs	350 µg/kg	150 days	Decreased serum parameters	[54]
Lambs	5.9-23.5 µg/kg	91 days	Decreased cellular immunity	[55]
Lactating dairy cows	96 µg/kg	7-12 days	Slight increase in serum proteins	[56]
Broilers	40 µg/kg	42 days	Reduced growth performance	[57]
White Leghorn cockerels	400 µg/kg	60 days	Hematological alterations	[58]
Broiler chicks	100-600 µg/kg	42 days	Immunosuppression	[16]
Broiler chicks	200 µg/kg	42 days	Serum biochemical and immunological alterations	[59]

Ruminants: Ruminants have a complex ecosystem of microflora and microfauna within the rumen [43,44] and nature has provided ruminants with diverse properties for detoxifying mycotoxins into their less toxic products through a diversified range of microflora and microfauna existing within their ruminal fluid [45]. As far as cattle are concerned, mature animals are less prone to the adverse effects of aflatoxins compared to growing, young, and pregnant animals. Aflatoxins are degraded in the rumen and converted to less toxic aflatoxicol [46]. Aflatoxins in feed bind with ruminal contents, and a lower quantity (only 2-5%) of ingested aflatoxin reaches the intestine. Feed levels of approximately 100 µg/kg are toxic to ruminants [47]. The health effects of aflatoxins in different species are listed in *Table 3*.

Aflatoxin B1 (AFB1), which escapes ruminal degradation, enters the liver and is converted into aflatoxin (AFM1), which is released in milk. The maximum tolerable level of AFM1 in milk is 0.5 µg/kg. This metabolite can be detected in milk 6 h after the ingestion of AFB1, whereas its peak level can be noticed 24-48 hours after continuous AFB1 ingestion. Its clearance from milk can be observed 3 days after withdrawal of a controlled diet [60]. It has been reported that AFM1 can cause pronounced aflatoxicosis in weaning calves often characterized by development of histopathological lesions in liver and kidney along with disturbance of hepatic enzymes [61]. About 1-2% of the total ingested AFB1 is released as AFM1 in milk [62]. The average transfer of aflatoxin from feed to milk is 1.7% while the maximum permissible level of aflatoxin for milk is 0.05 µg/liter in Asia [63]. Therefore, to avoid its residues in milk, dietary aflatoxin levels for ruminants must be as low as 25 µg/kg [64].

Poultry: In the poultry industry, chicks of all age groups are prone to adverse effects caused by aflatoxins, particularly AFB1. Apart from adversely affecting all organs of chicks, their residues infiltrate muscles and organs, making them a direct source of contamination for the human population consuming such meat. Aflatoxins in feed result in reduced feed intake and body weight gain, along with suppressed relative organ weights, and birds become dull and less attractive towards feed. Similarly, hematological and serum biochemical parameters are adversely affected, leading to anemic conditions, along with permanent damage to the kidney and liver [65,66]. In addition, the most prominent anomaly expressed by AFB1 in feed is immunosuppression, which makes birds susceptible to secondary bacterial infections [67,68]. Moreover, AFB1 forms DNA adducts, ultimately affecting the overall genome of organisms.

Ochratoxins

Ochratoxins are produced as secondary metabolites of certain species of *Aspergillus* and *Penicillium* with *Aspergillus ochraceus* being the major producer [30]. Based on minor structural differences, it has been further subdivided into ochratoxin A (OTA), ochratoxin B (Mohamed, #123), and OTC, with OTA being the most important and toxic among all. OTA is highly carcinogenic, and the International Agency for Research on Cancer has classified it as Group 2B [69]. *Penicillium verrucosum* is considered a major OTA producer in cold and temperate climatic zones, *Penicillium verrucosum* whereas *Aspergillus carbonarius, A. ochraceus, A. niger,* and *A. melleus* are considered major OTA producers.

Ochratoxins contaminate different crops, including corn, maize, sorghum, barley, and rice, resulting in a high chance of contamination in animal/poultry feed. Different regulatory levels are recommended for different raw materials and animal byproducts. According to the European Commission Recommendations (2006, 576), the MTL for complete feedstuff is 100 µg/kg feed [70]. Certain predisposing factors, such as hot and humid environments, poor pre-harvest/harvest conditions, and poor storage environments, enhance the production of OTA in feed.

Ruminants: OTA administered to young ruminants causes severe signs of depression, degeneration of the kidney, and polyuria, ultimately leading to the death of animals [71]. Ruminal microflora causes the degradation of amide bonds between ochratoxin-α and phenylalanine *(Fig. 3)*, ultimately producing less toxic compounds [72]. In the rumen, 50% of the total OTA is degraded within the first 15 min, while 95% of the total OTA is degraded into less toxic ochratoxin-α and phenylalanine moieties by ruminal microflora within 4 h of ingestion. Owing to this phenomenon, OTA does not affect or penetrate vital organs of the body [45].

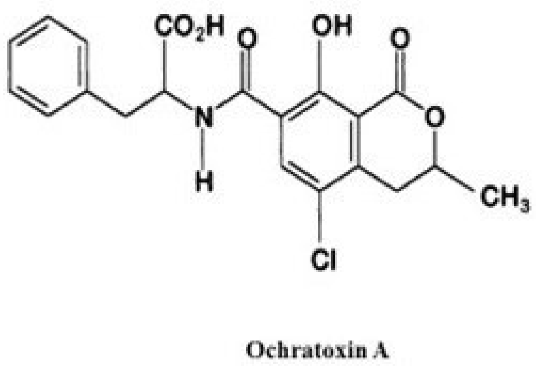

Fig 3. Structural presentation of ochratoxin A (OTA)

Poultry: Poultry chicks are highly sensitive to the effects of OTA, which affects almost all vital organs of the body. Toxicopathological effects include reduced feed intake, body weight gain, dullness, and reduced attraction to feed. Serum biochemical parameters and hematological indices are also severely affected by exposure to OTA [73,74]. Immunosuppression has been extensively observed in this regard. In this regard, our recent reviews elaborate on experimental ochratoxicosis in poultry [24,69].

Fumonisins

Fumonisins are secondary metabolites of *Fusarium proliferatum* and *Fusarium moniliformis*. Apart from these two species, some *Alternaria,* especially *A. alternata* have been found to produce fumonisins [75]. There are twenty-eight (28) chemically different types of fumonisins (FA1, FA2, FB1, FB2, FB3, and FB4), among which fumonisins B1 (FB1) is the most important and toxic form of fumonisins [76].

Ruminants: Regarding ruminants, fumonisins are tolerant to ruminal biodegradation; however, due to their low oral bioavailability, acute or chronic intoxication does not occur at the farm level [77]. However, the presence of very high levels in the feed can cause histopathological lesions in the kidneys and liver [78].

Poultry: The mechanism by which fumonisins cause toxicity in animals is thought to be the disruption of sphingolipid metabolism. It has been observed that fumonisins are the specific inhibitors of ceramide synthase enzyme which is needed for ceramide and complex sphingolipids synthesis. Because of this inhibition, a change in the sphingosine [7] to sphinganine [1] ratio occurs, and such an increased ratio has been observed in the tissues of turkeys, ducklings, and broilers exposed to FB1 in feed [79,80]. Turkeys and chicks were relatively resistant to the adverse effects of FB1, but mild to moderate toxicity was observed in turkeys and ducks fed FB1 at 75-400 mg/kg feed for 21 days. The observed changes included reduced body weight gain and hepatic damage, such as hepatic necrosis and biliary hyperplasia [81].

Trichothecenes

Trichothecenes are a diverse group of mycotoxins, the most important of which is deoxynivalenol [20] also known as vomitoxin. Other important members of this group are T-2 toxins, diacetoxyscirpenol (DAS), and scirpenol. They are produced by a variety of *Fusarium* species, while DON is produced primarily by *Fusarium roseum* and T-2 toxins are produced by *F. poae* and *F. sporotrichioides* under storage conditions [82].

Ruminants: Discussing about ruminants, microorganisms present within the ruminal fluid have distinct property of deacetylation and DAS is de-acetylated into monoacettoxyscirpenol [7] and scirpenetriol; and these products are less toxic as compared to their parent compounds [83]. DON occurs in excessive quantities within the concentrates, and it is also readily degraded within the rumen, but in animals suffering from ruminal acidosis, such degradation becomes incomplete, ultimately producing trichothecene-associated adverse effects in the animals [84]. However, in general, ruminants can easily tolerate 8.5 mg/g DON within the feed and is readily degraded within 6-24 h of its ingestion by the ruminants [85]. Furthermore, DON-associated lesions in animals include lesions within the gastrointestinal tract, vomiting, severe dermatitis, hemorrhage, and bloody diarrhea [86].

Poultry: Poultry chicks are also resistant to the adverse effects of DON to some extent, but the associated adverse effects include decreased feed efficiency, reduced body weight gain, and poor efficiency when fed extremely high doses [87].

Approaches/Strategies for Detoxification/Reduction of Mycotoxins

Three main approaches and strategies are generally adopted for the control and prevention of mycotoxins in animal and poultry feedstuff, which include physical, chemical, and biological methods.

Physical Methods

There are different physical methods for the control of mycotoxins, which unfortunately become impractical at a large scale, but such methods can efficiently help detoxify mycotoxins on a small scale. These methods include adsorption, irradiation, heating, solvent extraction, washing and separation.

Sorting and Separation

Grains are the major part of animal/poultry feed formulations [88] and when these grains are significantly contaminated with mycotoxins, they appear as moldy, broken, discolored and not distributed uniformly in the contaminated cereals rather they cluster together forming 'mycotoxin pockets' [89,90]. The separation and sorting of mycotoxin-contaminated grains are performed using sieving, aspiration, photoelectric separation, and image separation techniques [91]. Hand sorting, dehulling, and flotation alone can remove 93%, 63%, and 51% of mycotoxins from white-shelled maize, respectively, whereas using these three methods in combination can remove nearly 98% of mycotoxins [92]. Similarly, gravity separation and aspiration can reduce mycotoxins by 80%, but this also reduces wheat crop [93]. Moreover, visual sorting strategies (optical viscosity and near-infrared spectroscopy) have been used to remove mycotoxins from maize and wheat [90,94-96]. The limitations of these methods are that they are costly and limited to small-scale use.

Washing and Solvent Extraction

Mycotoxins can be easily removed by washing and solvent extraction because of their distinct fat-soluble and water-soluble properties. The floating method has the potential to decrease the concentrations of zearalenone, fumonisins, aflatoxins, and trichothecenes by 61%, 73%, 72%, and 69%, respectively [92,96,97]. The best results obtained by the floating method can be achieved by adding NaCl and sucrose to water to attain the maximum output [98]. When these physical techniques are used in combination to control mycotoxins, they provide better results than the individual techniques [99]. Commonly used solvents for mycotoxin extraction include hexane, methanol, ethanol, and aqueous acetone [100]. However, the major disadvantages associated with washing and solvent extraction are that they cause the loss of nutrients and are costly, which limits their application at larger scales.

Heating

This method for the control of mycotoxins has been extensively used for the removal of different mycotoxins; however, AFB1 and FB1 can tolerate heat and require high temperatures (probably more than 150°C) for their decomposition [101-104] ultimately making their decomposition difficult. Some studies have shown that thermal treatment up to 160°C for 20 min under a pressure of 10 MPa can reduce aflatoxins in rice by 80% from rice [105] while heating barley at 220°C can destroy 90% of zearalenone (ZEN) and DON [101]. Similarly, 150-200°C temperature can cause a 70% reduction in the concentration of FB1 in rice [106]. However, the disadvantage of this method is the production of the Maillard reaction, formation of certain carcinogens such as acrylamide, and reduction of the nutritive value, thereby limiting the use of this method at larger scales [105].

Decontamination by Irradiation

The irradiation process is usually divided into two forms: non-ionizing and ionizing. Non-ionizing processes involve microwaves, radio waves, visible light waves, and infrared waves, whereas the ionizing form includes ultraviolet rays, X-rays, electron beams, and gamma rays [107]. Extensive research has been performed by many scientists to evaluate the degradation of different mycotoxins using different irradiation technologies. *Table 4* presents different studies reporting the use of irradiation technologies for the degradation of mycotoxins in animal/poultry feed and their ingredients.

Using gamma irradiation: Gamma rays are electromagnetic waves produced as a result of decaying an unstable source such as radioactive isotopes. Gamma rays are preferred in the food industry because of their high reactivity and penetration power. However, certain factors are important in this regard as far as the usage of gamma rays for degradation of mycotoxin is concerned such as dose of radiation used, level of mycotoxin contamination, water content within feed and composition of matrix. Many studies have reported possible degradation of aflatoxins within feed at 5-10 KGy exposure to gamma rays [112,122,123].

Gamma irradiation is undoubtedly gaining popularity due to its excellent results in finished food products, whereas Di Stefano et al.[110] reported only up to 21% aflatoxin reduction from finished poultry feed, which suggests that this technique is not suitable for products containing high lipid and vitamin contents [107].

Using electron beam: Irradiation through electron beams has shown promising results in the degradation of certain mycotoxins because of their short processing time,

Table 4. *Degradation of mycotoxins by different irradiation techniques in animal/poultry feed and its ingredients*

Technique used	Feed type	Mycotoxin	Treatment Condition	Degradation Percentage	Reference
Gamma irradiation	Soybean	AFB1	10 kGy	62.20%	[108]
	Wheat	OTA	30.5 kGy	24%	[109]
	Commercial poultry feed	OTA	15 kGy	23.9%	[110]
	Commercial poultry feed	AFB1	15 kGy	18.2%	[110]
	Poultry feed	Aflatoxins	25 kGy	42.7%	[111]
	Maize feed	AFB1	10 kGy	94.5%	[112]
	Broiler feed	AFB1	6 kGy	89.53%	[113]
	Chicken liver	AFB1	10 kGy	25%	[114]
	Chicken liver	OTA	10 kGy	60%	[114]
	Poultry feed	OTA	4 kGy	100%	[115]
	Poultry feed	AFB1	6 kGy	100%	[116]
Electron beam	Corn	Zearalenone	50 kGy	71.1%	[117]
	Barley	Fusarium species	10 kGy	50-98%	[118]
	Wheat	DON	55.8 kGy	78.4%	[119]
Pulsed light	Rice bran	AFB1	0.52 J/cm per pulse for 15 sec	90.3%	[120]
Ultraviolet irradiation	Wheat	254 nm for 160 min	Aflatoxins	65-90%	[121]

dosage control, and low equipment costs. In the case of aflatoxins, this technology breaks down toxins into less toxic products, thereby reducing their toxicity potential of aflatoxins [124,125].

However, this technique has a lower degradation capacity than gamma irradiation. Moreover, Liu et al.[126] reported that this technique was not very efficient in degrading AFB1 from peanut meals, as this technique cannot be declared as a complete solution for degradation/decontamination purposes as far as mycotoxins are concerned.

Using ultraviolet irradiation: Over the past several decades, UV irradiation has been considered an effective technique for the destruction of several mycotoxins, particularly aflatoxins, owing to their photosensitivity. Being a non-thermal technique, UV irradiation depicts the benefits of being practical, cost-efficient and eco-friendly and it does not result in any toxic and/or waste product generation [127].

Ultraviolet (UV) irradiation has a strong penetration capacity through transparent and/or clear liquids, whereas its penetration capacity through solid materials is very limited, leading to its low decontamination ability for compact food products [128]. The disadvantage of this technique is that granular or opaque items should be presented in the form of a thin layer to achieve decontamination of mycotoxins by UV irradiation, thereby limiting its application at the field level for the degradation of mycotoxins in feed.

By photocatalysis: Recent advancements in the field have revealed that UV-visible irradiation, along with semiconducting photocatalysis, can efficiently degrade aflatoxins in a liquid matrix [129,130]. The most commonly used photocatalyst is titanium oxide (TiO_2), which is highly active under UV irradiation. Sun et al.[129] reported that AFB1 in methanol was efficiently degraded up to 95% within 120 min using this technique, while Xu et al.[130] reported up to 60.4% removal of AFB1 within 120 min through UV-vis irradiation. Similarly, DON degradation can also be achieved through photocatalytic techniques [131]. Although this is an efficient technique, less information is available on the safety and stability of photocatalysts.

By pulsed light: Pulsed light is an emerging non-thermal technique for decontaminating mycotoxins from foo and feed. Pulsed light is an FDA-approved technique for efficient and rapid decontamination of different food products. In this advanced technique, short- and high-intensity broadband emission light is produced, which includes ultraviolet, visible, and infrared rays [132]. Moreau et al.[133] reported a 92.7% reduction in AFB1 in water using eight flashes of pulsed light, while Wang et al.[120] reported 75% AFB1 and 39.2% AFB2 reductions from raw rice samples using this technique. Pulsed light technology can also be used for the efficient degradation of OTA, ZEN, and DON [133].

Despite the great outcomes of this technology, further studies are needed to investigate the breakdown products of pulsed light treatments. The design of cost-effective PL equipment is still needed, which can produce high UV output, so that this emerging technique can be effectively used at the industrial level.

By microwave heating: Electromagnetic waves with wavelengths ranging from 1m to 1 mm and frequencies of 300 MHz to 3000 MHz are used in microwave heating. It is a unique volumetric heating technology that efficiently converts electromagnetic field energy into thermal energy via the polarization effect of electromagnetic radiation [134]. Microwave heating is extensively used for heating, drying, extraction, and cooling of certain food products. Various studies have reported the use of this technology for non-thermal degradation of different mycotoxins. Flores et al.[135] studied the effects of microwave heating during alkaline cooling of aflatoxin-contaminated maize grains. They reported 36% AFB1 and 58% AFB2 reduction using microwave heating at 1650 W for 5.5 min.

Microwave manufacturers can customize equipment according to the needs of industrial and food product types. However, the non-uniform distribution of temperature during microwave heating is a challenge that could lead to hot/cold spot formation within food [136]. The presence of mycotoxins within the cold spot cannot be properly detoxified, whereas hot spots may lead to nutritional degradation of the feed. Further studies in this regard are required to ensure the proper distribution of temperature at all spots so that efficient degradation of mycotoxins can be achieved without disturbing the nutritional values of food and feed products.

By cold plasma: Plasma, often referred to as the fourth state of matter, is a highly energetic ionized gas that usually consists of ions, UV irradiation, electrons, and reactive nitrogen and oxygen species (RNS and ROS) [137]. Plasma is further categorized as cold or thermal. Additionally, it can be explained by the type of system generating it, such as dielectric barrier discharge (DBD), corona discharge (CD), and radio frequency plasma (RFP) and many others [138].

This latest technology has been used for the decontamination of different mycotoxins under ambient pressure and temperature conditions [139]. Aflatoxins were degraded using the DBD N2-plasma technique in hazelnuts, where 70% of AFB1 was detoxified under 1150 W plasma treatment within 12 min [140]. Similarly, cold plasma technology has been reported to efficiently degrade various mycotoxins including OTA [141], DON [142,143], T2 [144], fumonisins [145] and citrinin [140]. Cold plasma technology has been proven to be an efficient technique for the decontamination of mycotoxins. However, this technique is still in the early stages of development, and further advanced research is required to optimize various food products. Furthermore, the negative impact of plasma treatment on the nutritive value of different food materials needs to be addressed before the commercialization of this technology.

Adsorption

Some adsorbing substances have the potential to bind to mycotoxins and remove them from the gastrointestinal tract [146]. This technique is widely used and well understood, as it has fewer disadvantages than other available methods. The adsorbing agent used for the detoxification of mycotoxins should have some specific properties, including a high adsorption capacity for multiple mycotoxins, low binding efficacy for nutrients, and high safety and palatability [102].

The most commonly used adsorbents for mycotoxin detoxification are aluminosilicate minerals, including montmorillonites and aluminosilicates [146]. The adsorption ability depends on the structures of both the binding agent and the targeted mycotoxin [102]. Some studies have indicated that zeolite and bentonite clay can reduce AFB1 residues in the liver by 87% [147-150]. Similarly. Bentonite clay can decrease the bioavailability of AFB1 in the rumens of lactating animals [151,152]. Many studies have revealed that polar toxins, such as AFB1 and FB1, can be easily adsorbed by many aluminosilicates, which become ineffective against different non-polar mycotoxins [153,154]. Bentonites are considered good agents for the adsorption of mycotoxins because they are bioenvironment-friendly, have high adsorption efficiency, and are generally more economical than other agents [155-157]. Details of some of the adsorbing agents that are effective against mycotoxins are shown in *Table 5*.

Chemical Methods

Different chemical agents can be used to efficiently convert mycotoxins to less toxic or non-toxic compounds by destroying their structural makeup. Certain alkalines and ozone treatments are chemical methods that have proven beneficial in this regard [225,226]. *Fig. 4* shows a schematic flow of different chemical methods used for the control of mycotoxicosis.

Alkaline Treatment

Commonly used alkaline chemicals for the control of mycotoxins in moldy feed include sodium carbonate, potassium hydroxide, sodium hydroxide, and ammonia [226]. Base hydrolysis can open the lactone ring structure of AFB1, and the hydrolyzed product can then be removed by washing with water [227]. Treatment of various cereals with ammonia and hydroxide salts can remove almost 95% of the mycotoxins [228]. Although these treatments can nearly reduce the complete concentration of mycotoxins, the possible transformation of mycotoxins to other

Table 5. *Details of different In vitro and in vivo studies reporting the use of different adsorbents (binders) against mycotoxicosis*

Mycotoxin	Agent (Adsorbent)	Type of Study (and Efficacy)	Reference
Aflatoxins	Bentonite	*In Vitro*	[158]
	Activated charcoal	*In Vitro* Study	[159]
	Activated charcoal	Goats	[160]
	Activated charcoal	White Rock Chicks	[161]
	Activated charcoal	Broiler Chicks	[162]
	HSCAS	Chickens	[163]
	HSCAS	Broiler Chicks	[164]
	Activated charcoal	Chickens	[165]
	HSCAS	Chickens	[165]
	HSCAS	Chickens	[166]
	HSCAS	Pigs	[167]
	HSCAS	Pigs	[168]
	Zeolite	*In Vitro*	[169]
	HSCAS	Pigs	[170]
	Zeolite	Broiler Chicks	[171]
	HSCAS	Pigs	[172]
	HSCAS	*In Vitro* Study	[173]
	HSCAS	Turkey Poults	[174]
	Activated charcoal	Rabbits (Partial)	[175]
	Zeolite	Domestic Fowls	[176]
	Bentonite, HSCAS	Broilers	[177]
	HSCAS	Turkey Poults	[178]
	Activated charcoal	Minks	[179]
	HSCAS	Dairy Cows	[180]
	Aluminosilicate	Chicks	[181]
	HSCAS	Wethers	[179]
	HSCAS	Pigs	[182]
	Zeolite	Chickens	[181]
	Zeolite	Broiler Chicks	[183]
	Calcium Bentonite	Pigs	[182]
	HSCAS, Bentonite	Pigs	[184]
	Montmorillonite	*In Vitro*	[185]
	Activated Charcoal	Turkey Poults	[186]
	HSCAS	Turkey Poults	[186]
	Super-activated charcoal	Broiler Chicks	[187]
	Inorganic clay	Broiler Chicks	[188]
	Diatomaceous earth	*In Vitro*	[189]
	Clinoptilote	Quail Chicks	[190]
	Aluminosilicate	*In Vitro*	[191]
	HSCAS, bentonite	Rats	[192]
	HSCAS	*In Vitro*	[193]
	HSCAS	Broilers	[193]
	Zeolite	Broiler Chicks	[194]
	Bentonite	*In Vitro* Study	[195]
	Bentonite	Broiler Chicks	[196]
	Alumino silicate	*In Vitro*	[197]
	Sodium bentonite	Poultry Chicks	[147]
	Bentonite clay	Broilers	[149]
	Zeolite	Poultry Chicks	[198]
	Organo-clay composites	Broilers	[152]
Ochratoxins	Activated charcoal	Leghorn Chicks	[199]
	HSCAS	Chickens (Partial)	[200]
	Activated charcoal	*In Vitro*	[201]
	Activated Charcoal	Pigs	[201]
	HSCAS, Bento	Pigs	[201]
	Cholestyramine	Pigs	[201]
	Activated carbon	*In Vitro*	[202]
	Diatomaceous earth	*In Vitro*	[189]
	Bentonite	*In Vitro*	[195]
	Bentonite	Broiler (Partial)	[203]
Fumonisins	Activated charcoal	*In Vitro*	[204]
	Activated carbon	Rats	[205]
	Bentonite	*In Vitro*	[206]
Zearalenone	Divinylbenzene-styrene polymer	Rats	[207]
	HSCAS	Minks	[208]
	Cholestyramine	*In Vitro*	[209]
	Bentonite	Pigs	[210]
	Maifanite	Pigs	[211]
	Montmorillonite	Goat	[151]
	Activated charcoal	*In Vitro*	[212]
	Cholestyramine	*In Vitro.*	[212]
	Montmorillonite, Magnesium trisilicate, cholestyramine	*In Vitro*	[185]
	Organophilic montmorillonite	*In Vitro*	[213]
DON	Polyvinylpyrrolidone	Pigs	[214]
	Activated carbon	*In Vitro*	[202]
	Activated carbon	*In Vitro*	[215]
Trichothecenes	HSCAS	Turkey Poults	[174]
	HSCAS	Chicks	[153]
	HSCAS	Pigs	[216]
	Super-activated charcoal	Turkey Poults	[187]
	HSCAS	Broiler Chicks	[217]
	Inorganic clay	Broiler Chicks (Partial)	[188]
T-2 toxins	Bentonite	Rats	[218]
	Divinylbenzene-styrene polymer	Rats	[218]
	Super-activated charcoal	Rats	[219]
	Super-activated charcoal	Rats	[220]
	Activated charcoal	Swine	[221]
Cyclopiazonic Acid	Acidic clay, neutral clay, clinoptilolite	*In Vitro*	[222]
	Acidic clay, neutral clay, clinoptilolite	Broilers	[222]
Ergotamine	Montmorillonite	*In Vitro*	[223]
Patulin	Activated charcoal	*In Vitro*	[224]

forms, such as masked mycotoxins, along with harmful side effects on the environment and food (changes in nutritional quality, texture, or flavor) are some of the disadvantages that make this method less desirable at larger scales [225].

Ozone Treatment

Oxidizing agents, such as sodium and ozone, play a role in detoxifying mycotoxins by modulating the structures of these secondary metabolites [229,230]. Ozone can degrade the

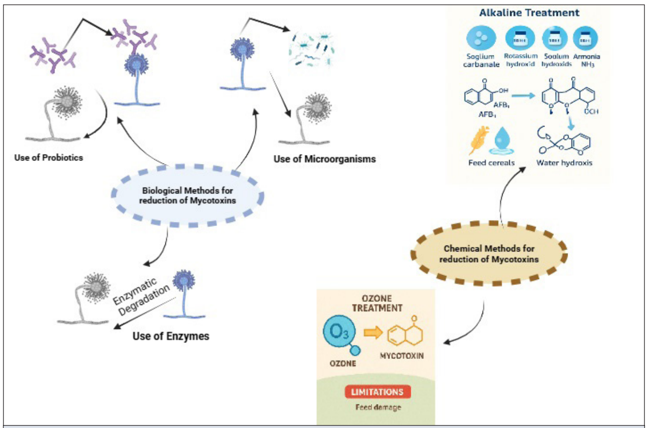

Fig 4. Flow diagram showing different chemical and biological methods used for the control of mycotoxicosis

FB1, AFs, ZEN and DON in various feeds of animals [231-234]. AFs can be reduced in corn and peanuts through ozone treatment [235-237]. Similarly, DON can be decomposed in corn and wheat using ozone [216,238-240]. Similarly, zearalenone can also be decomposed by treatment with varying concentrations of ozone [241]. Mycotoxins can also be degraded using other oxidizing agents, such as sodium hypochlorite [242,243]. However, the use of oxidizing agents for the detoxification of mycotoxins has some limitations, as these agents can change the physical and chemical composition of the feed, such as protein denaturation and lipid oxidation. Another disadvantage of using oxidizing agents is that they produce harmful chemicals during mycotoxin detoxification, making this method unsuitable for commercial use [230,235,236].

Biological Methods

Many physical and chemical techniques used to reduce the concentration of mycotoxins in feed have various limitations, as discussed above. However, the use of different biological methods for mycotoxin detoxification is necessary, as these methods have shown promising results in various studies [243,244]. *Fig. 4* shows a schematic flow of different biological methods used for the control of mycotoxicosis.

Microorganisms with Detoxification Activities

The use of different probiotics in animals promotes their development and growth and also improves the host's resistance against different diseases and metabolic disorders [245-249]. Certain beneficial microorganisms belonging to the category of probiotics are extensively used for the control of mycotoxicosis. These micro-organisms play a significant role in maintaining normal bacterial balance within the GIT and are often used to tackle certain pathological abnormalities, including fungal modifications [250,251]. These beneficial organisms can bind mycotoxins and eliminate them from the gastrointestinal tract [252]. In addition, they also possess

Table 6. *Details of different In vitro studies reporting the biological degradation/detoxification of certain mycotoxins*

Mycotoxin	Biological Strain/Type	Detoxification Rate [18]	Reference
AFB1	*Bacillus subtilis*	93.00%	[257]
	Pseudomonas putida	92.00%	[258]
	Bacillus licheniformis	95.70%	[259]
	Bacillus shackletonii	93.10%	[260]
	Bacillus subtilis	66.20%	[13]
	Bacillus velezensis	92.50%	[261]
	Escherichia coli	93.70%	[262]
	S. cerevisiae	68.00%	[244]
	Aspergillus niger RAF105	87.59%	[263]
	Stenotrophomonas sDOI:	99.00%	[264]
	Aspergillus niger FS11	97.64%	[265]
DON	Bacterial strain	100%	[266]
	Bacterial isolates	100.00%	[267]
	Aspergillus (NJA-1)	98.40%	[268]
	Eggerthella sDOI:	100.00%	[269]
	Pseudomonas sDOI: and *Lysobacter* sDOI:	100.00%	[270]
	Devosia insulae	85.00%	[271]
	Strain E3-39	100.00%	[272]
	Bacterial consortium C20	73.29%	[273]
	Bacillus subtilis	83.10%	[274]
ZEN	*Bacillus pumilus*	96.70%	[275]
	Bacillus natto/ pumilus	97.70%	[276]
	Bacillus subtilis	88.00%	[272]
FB1	*Bacillus* spDOI:	22%-50%	[277]
	Saccharomyces cerevisiae	100.00%	[278]
	Bacterial consortium	89.65%	[279]
	Strain NCB/ Bacterial consortium	100.00%	[280]

the ability to biologically degrade mycotoxins, ultimately converting them into less toxic metabolites, thereby protecting animals/chicks from mycotoxin-associated lethal damage [253]. Microorganisms, including various species of *Lactobacillus, Lactococcus, Streptococcus* and *Bifidobacterium* possess antimutagenic, antifungal, and immunomodulatory effects in this regard [254-256]. Many studies have been conducted in this regard, and *Table 6* and *Table 7* summarize some probiotics associated with different types of mycotoxins *in vitro* and *in vivo*.

Biodegradation/Biotransformation by Degrading Enzymes

In addition to the use of different bacteria, fungi, and their byproducts for the degradation of mycotoxins, the use of certain biological enzymes is also gaining popularity.

Table 7. *Details of different in vivo studies reporting biological degradation/ transformation of certain mycotoxins*

Mycotoxin	Biological Specie Used	Animal Model Used	Reference
Aflatoxins	*Sacchromyces cerevisiae*	Broilers	[281]
	Esterified glucomannan	Cows	[282]
	Manno-oligosaccharides	Wistar rats	[283]
	Nocardia corynebacteroides	Chicks	[284]
	Modified yeast extract	Cows	[285]
	Dried yeast culture	Sheep	[286]
	Lactobacillus casei Shirota	Wistar rats	[287]
	Modified yeast cell wall	Sheep	[288]
	Lactobacillus rhamnosus GAF01	Mice	[289]
	Lactobacillus plantarum MON03	Mice	[290]
	Yeast cell wall	Broilers	[291]
	Pichia kudriavzevii	Broilers	[292]
	Lactobacillus plantarum	Broilers	[293]
Ochratoxins	Esterified glucomannan	Broilers	[293]
	Yeast cell wall preparation (YCW)	Rats	[294]
	Saccharomyces cerevisiae	Ross male broilers	[295]
	Lactobacillus paracasei	Ross Broilers	[296]
	Saccharomyces cerevisiae	Ross Broilers	[296]
	Yeast sludge	Broilers	[297]
	Lactobacillus kunkeei	Male Rats	[298]
	Lactobacillus plantrum	Male Rats	[298]
T-2	*Saccharomyces cerevisiae lysate* (Masclaux-Daubresse, #1128)	Laying hens	[299]

These enzymes have been isolated from a wide range of microorganisms. These enzymes are also obtained from certain fungal species, but the isolation processes involving crushing of fungal mycelia are quite complicated, and their use on a larger scale is restricted. However, the use of enzymes isolated from different bacteria is promising for mycotoxin biodegradation [260]. *Table 8* shows the details of different degrading enzymes, along with the types of mycotoxins against which their efficacy has been reported.

Table 8. *Different experimental studies reporting the use of various degrading enzymes for the detoxification of mycotoxins*

Mycotoxin	Degrading Enzyme	Source	Reference
AFB_1	*Trametes versicolor Laccase enzyme*	*Aspergillus niger*	[300]
	F42H2-dependent reductase enzyme	*Mycobacterium smegmatis*	[301]
	Aflatoxin-Oxidase	*Aspergillus tabescens*	[302]
	Myxobacteria aflatoxin degradation enzyme	*Myxococcus fulvus*	[303]
	Manganese peroxidase	*Phanerochaete sordida* YK 624	[304]
	Manganese peroxidase	*Pleurotus ostreatus*	[305]
	Bacillus aflatoxin-degrading enzyme	*Bacillus shackletonii* L7	[260]
DON	Cytochrome P450 system	*Sphingomonas* sDOI: strain KSM1	[306]
	Peroxidase	Extract of rice bran	[307]
	Aldo-keto reductase DepA/DepB	*Devosia mutans* 17-2-E-8	[308]
	Quinone-dependent dehydrogenase, NADPH-dependent aldo/ keto reductases	*Devosia strain* D6-9	[309]
	Manganese peroxidase and *Lignin peroxidase*	*Flammulina velutipes*	[310]
ZEN	ZEN-specific *lactonohydrolase*	*Penicillium canescens* strain PCA-10	[311]
	Recombinant fusion enzyme (ZHDCP)	Zearalenone hydrolase (ZHD) and carboxypeptidase (CP)	[312]
FB_1	*Fumonisin carboxylesterase* FumD	*Sphingopyxis* sDOI: MTA144	[313]

Nutritional Strategies

Certain nutritional strategies have also been adapted to nullify the adverse effects of mycotoxins in animals. Similarly, the use of certain plants and their extracts has been practiced since ancient times for the rectification of different ailments in both humans and animals [314-317]. These plants contain a variety of flavonoids, alkaloids, essential oils, and tannins, which enhance the body's defense system by mitigating several pathological and management issues in animals and humans [318,319]. The mycotoxin detoxification system can be modulated by nutritional measures. In animals, detoxification systems include ketoreductase, CYP450s and α-glutathione transferase, which can efficiently degrade mycotoxins [239]. Therefore, nutritional regulators may enhance the detoxification potential of the body [320]. Cysteine, glycine, and glutamate synthesize glutathione and help detoxify mycotoxins by forming glutathione. The addition of

***Table 9.** Details of different experimental studies showing different nutritional strategies against the toxic effects of mycotoxins*

Mycotoxins	Nutritional strategy	Animal Model	Reference
AFB1	β-carotene, canthaxanthin, lycopene	Rats	[328]
	Dietary butylated hydroxytoluene	White Turkey poults	[329]
	Vitamin C	*Labeo rohita*	[330]
	Silymarin-phospholipid complex	Broiler	[331]
	Vitamin C & E	Rabbits	[332]
	Sea buckthorn berries	Broiler	[333]
	Alpha-lipoic acid	Broilers	[334]
	Resveratrol	Broilers	[335]
	Curcumin, quercetin, resveratrol	*In vitro*	[336]
	Selenium	Cobb male broilers	[280]
	Curcumin	Chickens	[337]
	Vitamin E	Leghorn cockerels	[58]
DON	Vitamin E & C, selenium	Wistar rats	[338]
	L-Arginine	Pig	[339]
	Selenium	*In vitro*	[276]
FB1	Isoflavones	Rats	[340]
	Vitamin E	rabbits	[341]
	Curcumin/silymarin	*In vitro*	[342]
OTA	Vitamin C	Mice	[343]
	Vitamin E & C	Broilers	[344]
	Retinol, ascorbic acid, α-tocopherol	Mice	[345]
	Coenzyme Q10, L-carnitine, Zn, Mg	Mice	[346]
	Vitamin C	Nile Tilapia	[347]
	L-carnitine	Leghorn cockerels	[66]
	Silymarin	Leghorn cockerels	[67]
	Curcumin	*In vitro*	[342]
	Ducks	Curcumin	[348]
T-2 toxin	Vitamin C & E, selenium	Wistar rats	[338]
	Vitamin E & C	Broiler	[344]
	Broilers	Lycopene	[349]
Zearalenone	Retinol, ascorbic acid, α-tocopherol	Mice	[345]
	Vitamin E	*In vitro*	[350]
	Vitamin C	Weaning piglets	[351]
	Silymarin	Rats	[352]
	Vitamin C	piglets	[353]

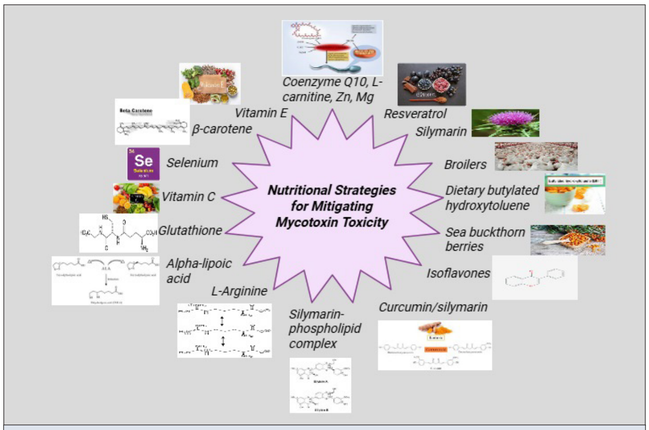

Fig 5. Schematic flow showing different nutritional strategies used for the control of mycotoxicosis

specific nutrients and prebiotics to mitigate the damaging effects of pathogens and toxins is another benefit [321-324]. Cytotoxicity occurs due to oxidants produced within the body by mycotoxins, and adding antioxidants can improve the ability of organisms to fight against mycotoxin toxicity. In this regard, selenium and vitamins (C and E) have proven beneficial as they act as superoxide anion scavengers [325-327]. *Table 9* describes some nutritional strategies reported in this regard while *Fig. 5* shows the schematic flow of use of different nutritional strategies used for the control of mycotoxicosis.

Conclusion

The presence of mycotoxins in feed is an unavoidable problem worldwide. This review summarizes a number of strategies to reduce mycotoxicosis, including physical methods (separation, washing, adsorption, heating, and irradiation), chemical strategies (oxidizing agents and basis), biological methods (enzymes and probiotics), and nutritional regulation strategies. Each of these approaches can be used practically, along with its own advantages and disadvantages. However, with the growing awareness of environmental protection, as well as feed and food safety, there is a growing expectation for more green and innovative technologies to control mycotoxin contamination.

Future Perspectives

More advanced techniques should be adapted for the control of mycotoxicosis in poultry. As there is a paradigm shift to the use of natural ingredients for the treatment globally, measures should be taken for the large-scale implementation of biological strategies to avoid physical and chemical strategies-associated disadvantages at farm levels. Widescale commercialization should be done to mitigate mycotoxicosis at farm (both dairy and poultry) levels.

Declarations

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