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Aflatoxin B_1 Induced Systemic Toxicity in Poultry and Rescue Effects of Selenium and Zinc

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Abstract Among many challenges, exposure to aflatoxins, particularly aflatoxin B_1 (AFB_1), is one of the major concerns in poultry industry. AFB_1 intoxication results in decreased meat/egg production, hepatotoxicity, nephrotoxicity, disturbance in gastrointestinal tract (GIT) and reproduction, immune suppression, and increased disease susceptibility. Selenium (Se) and zinc (Zn), in dietary supplementation, offer easy, cost-effective, and efficient ways to neutralize the toxic effect of AFB_1. In the current review, we discussed the impact of AFB_1 on poultry industry, its biotransformation, and organ-specific noxious effects, along with the action mechanism of AFB_1-induced toxicity. Moreover, we explained the biological and detoxifying roles of Se and Zn in avian species as well as the protection mechanism of these two trace elements. Ultimately, we discussed the use of Se and Zn supplementation against AFB_1-induced toxicity in poultry birds.

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Aflatoxins, Aflatoxin B_1, and Their Impact on Poultry

Aflatoxins were first identified in early 1960s and since then have been the most studied mycotoxins. Being mainly produced by certain strains of *Aspergillus parasiticus*, *Aspergillus flavus*, and *Aspergillus nomius*, these mycotoxins are majorly found in agricultural products in tropical and subtropical regions [1–3]. Aflatoxins are often present in feedstuffs and cause some adverse effects, which can range from vomiting, weight loss, and acute necrosis of parenchyma cells to various types of carcinoma and immunosupression in large animals, pets, and poultry birds [4, 5]. Multiple reports are available about aflatoxins, their types, biotransformation, and toxicity in various animal species [6–10], but in avian species, data is scattered and needs to be summarized.

Aflatoxin B_1 (AFB_1), among the four major types of aflatoxins, is the most toxic and potent carcinogen in humans and animals [11]. AFB_1 causes series of pathophysiological changes in an organism such as lower growth rate, malnutrition, silenced immune response, and disturbed gastrointestinal tract. Also, AFB_1 can induce various histopathological manifestations of hepatocytes such as proliferation of the bile duct, centrilobular necrosis and fatty degeneration of the hepatocytes, and even hepatoma [12–15]. AFB_1 is shown to induce hepatocellular carcinoma in many species of animals including fishes (rainbow trout, sock eye salmon, and guppy), poultry (turkeys, ducks, and geese), non-human primates (rhesus, cynomolgus, African green, and squirrel monkeys), and rodents (rats, mice, and tree shrews) [4, 16]. In poultry, AFB_1 mainly affects the liver, kidney, immune organs (spleen, bursa of fabricius, and

thymus), and gastrointestinal system. Details of these effects are discussed in the later sections of this review.

Poultry industry is one of the largest, most organized, fastest-growing, and vibrant segments of agro-industries, generating direct and indirect employment and income for millions of people, in developing and underdeveloping countries [17–19]. However, the poultry industry is persistently facing many challenges [20], majorly being the diseases caused by virus, bacteria, protozoa, parasites, and fungus, among which mycotoxin contamination of feed is one of the most important aspects. According to an estimate by the Food and Agriculture Organization (FAO), 25% of the world's food crops are affected by mycotoxins, and the rate of mycotoxin contamination is likely to increase in line with the trend seen in preceding years [21–25]. A worldwide mycotoxin survey in 2013 revealed that 81% of around 3000 grain and feed samples analyzed had at least one mycotoxin, which was higher than the 10-year average (from 2004 to 2013) of 76% in a total of 25,944 samples. The most notorious mycotoxins are aflatoxins, which often result in low performance in poultry and decreased quality of egg and meat production and then cause significant economic losses [26–28]. In broilers, aflatoxins drastically affect almost all valuable production factors including weight gain, feed intake, and feed conversion ratio (FCR) and induce immunosupression, which is directly related to reduced effectiveness of vaccination programs, increased risk of infectious diseases, and high mortality. In layers, aflatoxins cause the decrease in egg production, egg size, and egg quality.

Biotransformation and Mechanism of AFB1 Toxicity in Poultry

Many controversies exist about the metabolism of AFB_1 in avian species [29] and still need extensive research to decipher the exact mechanism of AFB_1 biotransformation in poultry species. In general, AFB_1 passes through phase I and phase II metabolism. Phase I involves the addition of a small polar group, containing both positive and negative charges by any of the following reactions: epoxidation, hydration, hydroxylation, O-demethylation and reduction. Phase II involves the addition of another substance such as endogenous glutathione to the product of phase I (conjugation) to generate a polar or water-soluble product that can easily be excreted out by the kidneys [5, 6, 30]. Studies have shown that in phase I, AFB_1 undergoes microsomal and cytosolic biotransformation in the liver of poultry species [31] where it gets converted to toxic metabolites. Microsomal biotransformation results in AFB_1 exo-8,9-epoxide (AFBO), aflatoxin Q_1 (AFQ_1), aflatoxin B_{2a} (AFB_{2a}), aflatoxin M_1 (AFM_1), and aflatoxin P_1 (AFP_1) metabolites, and cytosolic biotransformation leads to formation of aflatoxicol (AFL) metabolite [29]. Studies showed that AFQ_1, AFB_{2a}, AFM_1, and AFP_1 are less toxic in comparison to AFBO [5]. There is a conflict on biological effects of AFL, and it largely remains unknown in humans and animals. Some authors believe it to be less toxic compared to AFBO and to be easily excreted out through conjugation reactions by kidneys [32], while others considered it not the detoxification product but toxic metabolite [29]. Among all metabolites, exo-AFB_1-8,9-epoxide (AFBO) is considered as the most toxic metabolite of AFB_1, but again, the controversy exists on the specific cytochrome P450 (CYP450) enzyme subfamilies responsible for biotransformation of AFB_1 into AFBO. More recently, a series of studies revealed that the avian CYP2A6 ortholog is the main CYP450 enzyme responsible for the bioactivation of AFB_1 into AFBO in all poultry species [29, 33, 34]. AFBO can bind with particular cellular compounds (proteins, DNA, and RNA) to influence normal cellular activities and is thought to be responsible for the carcinogenicity and mutagenicity of aflatoxins if not excreted from the body by phase II metabolic reactions [3, 5, 7, 30, 33]. Phase II metabolism of AFB_1 involves conjugation of AFB_1 with endogenous glutathione by a chemical reaction catalyzed by glutathione S-transferases; the most important family of enzymes, involved in protecting humans and most animals from potentially toxic chemicals such as drugs and carcinogens [35, 36]. Glutathione reduces the toxic effects of AFBO and helps to excrete it out from the body. Some evidences support the fact that it is the conjugation of epoxide by GSH, which is the major rate-limiting factor in species susceptibility to AFB_1, rather than the level of P-450-mediated bioactivation [37]. A schematic diagram of cytosolic and microsomal biotransformation of AFB_1 in the liver is presented in Fig. 1.

After biotransformation of AFB_1 by CYP450 enzymes in the liver, DNA binding is the most common mechanism of AFB_1 action [38, 39]. As a highly electrophilic intermediate, AFBO has an affinity for DNA, RNA, and proteins, resulting in toxigenicity, carcinogenicity, and mutagenicity [35] (Fig. 1). Another widely reported action mechanism of AFB_1 is the induction of ROS formation, which leads to oxidation of DNA bases, causing DNA damage [40]. The toxicity and carcinogenicity of AFB_1 are closely associated with the rate at which it is activated and the rate at which AFB_1 is detoxified and metabolized at primary and secondary levels.

Systemic Effects of AFB_1 in Poultry

Liver

As a major xenobiotic-metabolizing and xenobiotic-detoxifying organ in the body, the liver is mainly affected after the ingestion of AFB_1 in poultry species [32, 41]. AFB_1 is delivered to the liver through blood circulation after its absorption across the cell membrane. In almost all animal species including birds, AFB_1 is hepatotoxic, causing pallor discoloration, enlargement, congestion, and necrosis of the liver, along

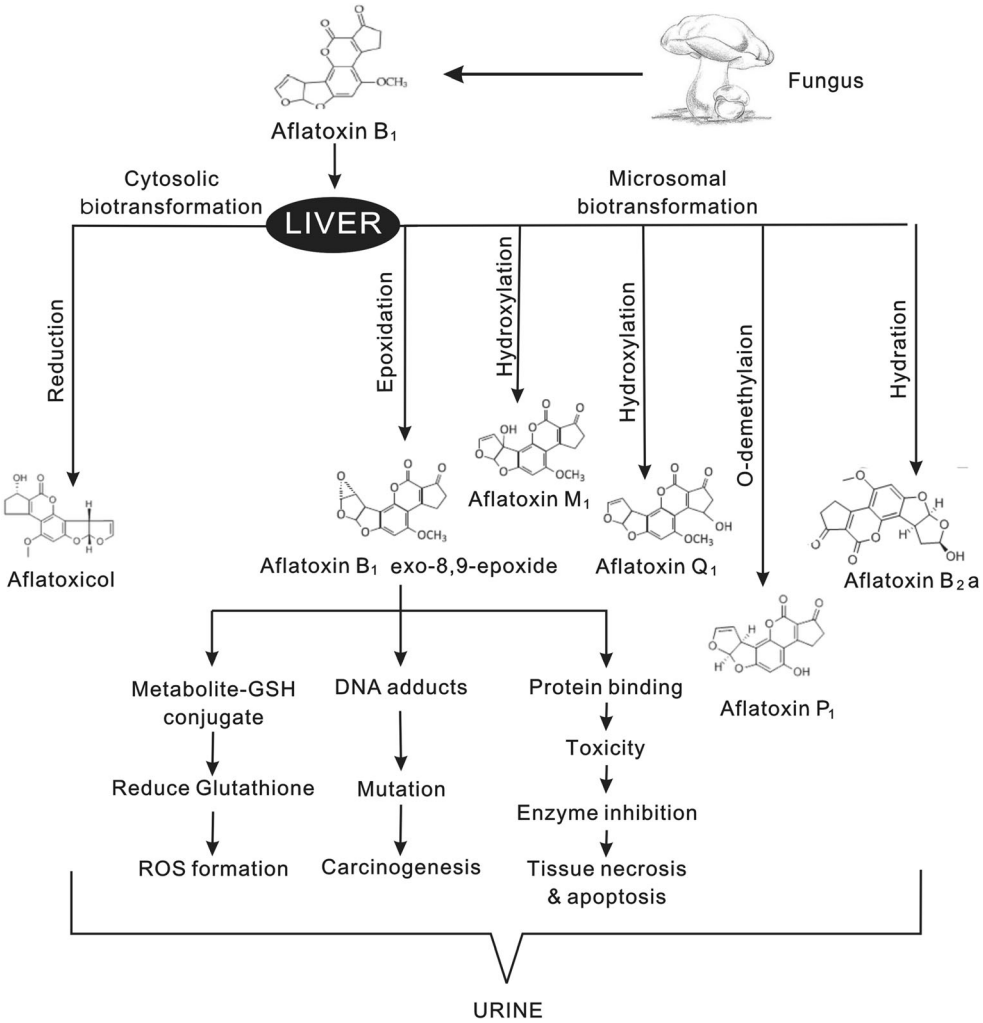

Fig. 1 Cytosolic and microsomal biotransformation of AFB_1 in liver coupled with mechanism of toxicity and carcinogenicity induction

with proliferation of bile duct and infiltration of mononuclear and heterophilic cells [42, 43]. Decreased activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and glutathione reductase (GR), and increased malondialdehyde (MDA) contents have also been reported in the liver of poultry birds while being fed with AFB_1 [44]. One study demonstrated that AFB_1 (0.1 mg/kg body weight) disturbed mitochondria and caused hepatocyte mitochondrial antioxidant dysfunction in duckling [44]. Another study documented that AFB_1 can provoke liver impairment by promoting hepatocyte apoptosis and disturbing cellular enzymatic activities in ducklings administrated with 0.1 mg/kg body weight of AFB_1 [45]. From previously discussed reports, it is clear that exposure to AFB_1 can alter liver functions on various cellular and molecular levels and provoke undesirable pathological effects in the liver of poultry birds.

Kidney

Second to the liver, the kidney is the most vulnerable organ due to its ability to filter large amount of blood and contribution to the body's homeostasis by eliminating metabolic waste products [46]. Different studies have shown that AFB_1 can disturb the renal functions via increasing the relative weight of kidneys and inducing congestion in renal sinusoids [47, 48]. In Africa, birds exposed to AFB_1 developed fatty and hemorrhagic kidney syndrome, characterized by thickening of the glomerular basement membrane, abnormal development of glomerular epithelial cells, degenerative changes in renal tubular cells, congestion, and parenchymal hemorrhage [47, 49]. AFB_1 induces the degenerative and necrotic changes in renal tubular epithelium and reduces the glomerular filtration rate, thereby altering the avian renal functions [50–52]. Also, AFB_1 causes impairment of blood biochemical parameters, including reduced concentrations of calcium, inorganic phosphate, sodium and potassium, and an increase in urea, creatinine, and uric acid [49, 53, 54]. Chickens fed on AFB_1 displayed excessive apoptosis, cell cycle blockage, and cell proliferation in renal cells and increased levels of Bax and caspase-3 messenger RNA (mRNA) [46].

Gastrointestinal Tract

The gastrointestinal tract (GIT) is the main route of entry after the ingestion of AFB_1. This mycotoxin affects the GIT in multiple aspects such as changes in gut morphology, digestive ability, activities of digestive enzymes, the intestinal innate immunity, and gut microbiota. There are very less reports available about the effects of AFB_1, and the results have been controversial [55, 56]. Some found that AFB_1 induced GIT pathological lesions, while others suggested that AFB1 did not induce any lesions of the GIT. This discrepancy is probably the result of different sections of GIT used for histopathological examinations, different exposure times, and the different strains of chickens used in various studies. AFB_1 could decrease digestive ability by affecting the active transport of nutrients across the intestinal membranes, and the effect is dose and time dependent. Three-week feeding of 1.25 to 5 mg AFB_1/kg diet had no effect on in vitro absorption of glucose and methionine in the intestine of broilers, whereas a higher dose of 10 mg AFB_1/kg diet, for more than 1 week, increased both the mediated and diffusion components of glucose and methionine absorption [57]. Few studies support the hypothesis that the decreased nutrient absorption in AFB_1-affected broilers is because of the effect of toxin on systemic metabolism and not an effect on digestive functionality [58, 59], which needs further studies. AFB_1 also exerts its effects on the GIT by interacting with digestive enzymes; however, controversy exists on this aspect too. AFB_1 has not been considered seriously for its effects on the intestinal innate immunity. Limited data related with intestinal health suggests that AFB_1 can only moderately affect transepithelial electrical resistance (TEER) during acute exposure to the toxin [60]. A recent study showed that 0.3 mg/kg concentration of AFB_1 in chicken feed can induce a decrease in T cell subsets and the mRNA contents of IL-2, IL-6, and TNF-α and also impairs ileum mucosa [61].

Immune System

The immune system is crucial for defense against invading organisms, and AFB_1 has shown to evoke and agitate immune responses in a number of studies conducted in past decade [62–64]. Exposure to AFB_1 provokes damaging effects on primary and secondary lymphoid tissues of birds including thymus, bursa of fabricius, spleen, and bone marrow. Enough evidences support that consumption of feed containing AFB_1 lowers the disease resistance and antibody-mediated function in avian species [31, 65–67]. In poultry, aflatoxin-contaminated feed generates a series of cell-mediated immune responses involving suppressed phagocytic efficiency of the phagocytes, delayed hypersensitivity reactions along with bursal involution, and depletion of cell populations of the thymus [66]. Studies have shown that AFB_1 negatively affected bursa and thymus by increasing the expression of caspase-3 and enhancing the apoptotic cell percentage in both immune organs. Furthermore, 0.3 mg/kg AFB_1 in the diet can induce histopathological changes, decrease mature lymphocytes, and increase apoptotic percentage of lymphocytes in broiler [68]. Poultry birds have shown decreased relative weight of spleen, histopathological changes, increased splenic apoptotic cell percentage, and decreased activities of GSH-Px, total SOD, GR, and CAT when exposed to AFB_1 [69]. Studies also showed that daily dietary exposure of AFB_1 could bring down antibody titers to vaccines for different diseases including Newcastle disease, infectious bronchitis, and infectious bursal disease [70, 71].

Effect of AFB_1 on Growth, Performance, and Production

A number of studies have focused on negative effects of AFB_1 on growth and performance in poultry. Susceptibility variation has been found among different age groups of birds in same species. Young birds are more susceptible and showed more damages as compared to older birds [72]. Lower growth rate, poor nutrient usage, decreased weight gain and egg production, and increased bruising after exposure to feed contaminated with AFB_1 have also been shown in many studies [73, 74]. A study [75] indicated that AFB_1 in feed can result in decreased growth performance and reduced body weight in duck and proposed that these changes are related to the decreased digestibility of nutrients. Despite of a scattered percentage of decreased body weights in different age groups and at different concentrations of AFB_1/kg diet in various reports [74, 76–80], there is a general agreement that dietary AFB_1 reduces weight gain and feed intake and decreases the FCR.

Along with producing hepatotoxic, nephrotoxic, gastrointestinal, and immunosuppression effects on liver, kidney, gastrointestinal, and immune systems, respectively, AFB_1 could induce adverse effects in other systems such as cardiovascular, nervous, respiratory, and endocrine systems as well. However, most of the studies about negative effect of AFB_1 on these systems were carried out on domestic animals and rodents, and deep understanding of AFB_1- produced effects on these systems in different poultry species calls for further investigation.

Detoxification of Aflatoxins with Nutritional Supplements

A successful detoxification process must be economical and capable of eliminating maximum traces of toxin without leaving harmful residues and without impairing the nutritional quality of the commodity. As a result, more efforts are focused on finding effective means of biological degradation of aflatoxins. Many detoxification methods of aflatoxin-

contaminated feedstuffs are recommended over last two decades and include various physical (mechanical separation of contaminated seeds and heat treatment), chemical (extraction using solvents, detoxification using chemical agents, and added sorbents), and biological treatments [81, 82]. In this context, a number of feed supplements have provided protection against the damage caused by AFB_1. A study [83] showed that vitamins A, E, K, and D could be used in protection against aflatoxins. Supplementation by vitamins A, E, and C has resulted in enhanced antioxidative effect in poultry birds and protects the immune cells from oxidative damage induced by AFB_1 [84]. Plenty of studies worldwide are compiled together in a comprehensive meta-analysis in poultry, where the nutrient supplements are used against AFB_1 in broiler, and some of them could be considered as cost-effective and useful to ameliorate the undesirable effects of AFB_1 [85].

Selenium (Se) and zinc (Zn) are two under studied trace elements for their protective roles against oxidative stresses and other adverse effects induced by AFB_1. A number of studies have documented the importance of Se and Zn in human and animal biology when used optimally.

Selenium, Its Detoxifying Effects, and Protection Mechanism

Selenium is an essential nutrient of fundamental importance in human and animal biology. It is crucial for the ideal functioning of the immune, cardiovascular, and reproductive systems. Also, it ensures defense against infections, tumors, and prevents inflammatory and allergic conditions in both humans and animals [86–90].

Se is a significant feed-derived natural antioxidant in poultry, and adequate level of Se is crucial for chicken health, productive and reproductive characteristics (embryonic development and sperm quality), and optimal functioning of immune system [91]. Two major Se sources, which are inorganic (selenite or selenate) and organic selenium (selenomethionine), are used in poultry [92]. The protective effect of Se against various toxic agents in various animal species along with poultry is well known [93–95]. Liao et al. investigated that AFB_1 exposure induced liver dysfunction by disturbing the tissue enzyme activity and enhanced apoptosis, but the Se coadministration protected liver tissues against AFB_1-induced toxicity [45]. A number of studies conducted on various organs in poultry birds demonstrated the protective effects of Se against AFB_1 [61, 69, 96]. The dietary sodium selenite in the feed of broiler has excellent effects on oxidative stress and apoptosis and can amend the immunosuppression effects induced by AFB_1 in spleen of broiler [69]. Se supplementation has improved AFB_1-induced apoptosis at a concentration of 0.4 mg/kg [96]. Also, Se supplementation in broiler diet provided protection against AFB_1-induced changes in the ileum and sodium selenite improved the cellular immune functioning of the AFB_1-affected ileum mucosa [61]. In their review, Galvano et al. mentioned several reports on in vitro and in vivo studies documenting that Se inhibits AFB_1-DNA binding and adducts formation, and sodium selenite and Se-enriched yeast extract protect cells from AFB_1 cytotoxicity [35].

Se exerts protective functions through its direct ability to enhance immunity, by affecting the metabolism of carcinogens, by playing a crucial role in protein synthesis, cell division, and the formation of anticancer metabolites, and last but not the least, its antioxidative abilities [97]. Out of all the functions, antioxidant and antitumor abilities are the most important roles played by Se. The anticarcinogenic action of Se is mediated by selenoproteins and smaller non-proteins, which could reduce metabolites through GSH-Px, modify carcinogen metabolism, modulate immune functions, inhibit enzymes that catalyze cell proliferation, and induce apoptosis [98]. Also, Se may prevent the binding of DNA with carcinogens as well as reactive Se metabolites can render the carcinogens into non-carcinogenic. Dietary Se has been shown to protect chicks from AFB_1-induced liver injury by inhibiting CYP450 enzyme, which is responsible for the activation of AFB_1 to toxic AFBO [99]. Alongside, the mitochondrial redox equilibrium is modulated by catalytic redox Se metabolites, which ultimately induce apoptosis in non-regulatory cancer cells [100].

Zinc, Its Detoxifying Effects, and Protection Mechanism

Essentiality of Zn for humans and animals has been known for many decades due to its principal role in individual's growth, development, and optimal functioning of various physiological processes. Indeed, the past two decades have seen a rapid growth in knowledge of the underlying mechanisms, whereby Zn exerts its ubiquitous effects on immune function, disease resistance, and general health [101–103]. Its involvement in such fundamental activities probably accounts for the essentiality of Zn for all forms of life.

Zn in avian species is necessary for normal growth and maintenance including bone development, feathering, enzyme structure and function, and appetite regulation in chicks [104]. Zn is important for proper functioning of the components of the cellular immune system including heterophils, mononuclear phagocytes, and T lymphocytes, which are important for disease resistance in poultry [105–108]. The deficiency of Zn in poultry results in suppressed immune system, poor shell quality and poor feathering, dermatitis, and infertility [109–111]. In poultry, Zn serves not only as a nutrient but can also be used as a dietary supplement to manipulate the reproductive system of the birds [112, 113]. Although a bunch

of studies had already been carried out, demonstrating protective effects of zinc against a range of noxious agents in human and different laboratory animal [114–118], only few studies focused on protective effects of Zn against AFB_1. Zinc amended growth retardation, thymic involution, and impaired peripheral immune efficiency in piglets exposed to AFB_1, and these protective effects were likely to play a key role in immune responses [119]. Another study showed significant enhancement in growth performance represented by improved relative body weight gain and feed efficiency in AFB_1-intoxicated birds when fed with Zn supplementation [119]. It is clear from all the aforementioned studies that Zn is a potential trace element which plays a crucial role against adverse effects induced by various agents. Also, its function in enhancing various systems of the body could be used as amending tool against AFB_1 intoxication.

Action mechanism of Zn involves its functions in the body in three different areas: catalytic, structural, and regulatory. In its catalytic role, Zn is a critical component of the catalytic site of more than 300 different metalloenzymes. These metalloenzymes play crucial roles in different biological processes [82], making it highly important cofactor for different protective mechanisms including protection against toxicity. In its structural role, Zn facilitates protein folding and produces "Zn fingers." When bound to a protein, Zn can either directly take part in chemical catalysis or support protein structure and stability [120]. Zn is involved in the regulation of nucleoproteins and the activity of various inflammatory cells, in its regulatory role [121]. For instance, Zn regulates the expression of metallothionein, which has multiple functions including intracellular Zn compartmentalization and antioxidant function [122].

Conclusion

Poultry is highly sensitive and susceptible to aflatoxins particularly AFB_1, which produces acute, chronic, mutagenic, and teratogenic toxicity along with causing millions of dollars per year damage to the poultry industry worldwide. Nutritional supplementation could be a cost-effective and efficient method to bring down the toxic effects of AFB_1, which has already been proved by various reports. Se and Zn are potential antioxidants that take part in enhancing various biological systems in humans and animals by being an important component of several essential enzymes. These two less studied yet important trace elements have been highly effective against the adverse effects produced by various noxious agents including AFB_1. A number of studies are being carried out on a global scale to optimize the concentration of these trace elements in feed, in order to gain the benefits against AFB_1. This comprehensive review will provide information about the harmful effects of AFB_1 in different organ systems of poultry birds, as well as the counter effects of various nutritional supplementations with a special focus on Se and Zn along with their protective mechanisms.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

References

1. Leszczynska J, Kucharska U, Żegota H (2000) Aflatoxins in nuts assayed by immunological methods. Eur Food Res Technol 210(3):213–215
2. Wu HC, Santella R (2012) The role of aflatoxins in hepatocellular carcinoma. Hepat Mon 12(10 HCC):e7238
3. da Rocha MEB, Freire FCO, Maia FEF, Guedes MIF, Rondina D (2014) Mycotoxins and their effects on human and animal health. Food Control 36(1):159–165
4. Robens J, Richard J (1992) Aflatoxins in animal and human health. In: Reviews of environmental contamination and toxicology. Springer, pp 69–94
5. Bbosa GS, Kitya D, Odda J, Ogwal-Okeng J (2013) Aflatoxins metabolism, effects on epigenetic mechanisms and their role in carcinogenesis. Health 2013
6. Mishra H, Das C (2003) A review on biological control and metabolism of aflatoxin
7. Rawal S, Ji EK, Coulombe R (2010) Aflatoxin B1 in poultry: toxicology, metabolism and prevention. Res Vet Sci 89(3):325–331
8. Yunus AW, Razzazi-Fazeli E, Bohm J (2011) Aflatoxin B1 in affecting broiler's performance, immunity, and gastrointestinal tract: a review of history and contemporary issues. Toxins 3(6): 566–590
9. Wogan GN, Kensler TW, Groopman JD (2012) Present and future directions of translational research on aflatoxin and hepatocellular carcinoma. A review Food additives & contaminants: part A 29(2):249–257
10. Eaton DL, Groopman JD (2013) The toxicology of aflatoxins: human health, veterinary, and agricultural significance. Elsevier, Amsterdam
11. Guzmán dPD (2007) Exposure to aflatoxin B1 in experimental animals and its public health significance. Salud Publica Mex 49(3):227–235
12. Ellis W, Smith J, Simpson B, Oldham J, Scott PM (1991) Aflatoxins in food: occurrence, biosynthesis, effects on organisms, detection, and methods of control. Critical Reviews in Food Science & Nutrition 30(4):403–439
13. Richard JL (2007) Some major mycotoxins and their mycotoxicoses—an overview. Int J Food Microbiol 119(1):3–10
14. Iheshiulor O, Esonu B, Chuwuka O, Omede A, Okoli I, Ogbuewu I (2011) Effects of mycotoxins in animal nutrition: a review. Asian J Anim Sci 5:19–33

15. Abrar M, Anjum FM, Butt MS, Pasha I, Randhawa MA, Saeed F, Waqas K (2013) Aflatoxins: biosynthesis, occurrence, toxicity, and remedies. Crit Rev Food Sci Nutr 53(8):862–874
16. Wogan GN (1992) Aflatoxins as risk factors for hepatocellular carcinoma in humans. Cancer Res 52(7 Supplement):2114s–2118s
17. Blake DP, Tomley FM (2014) Securing poultry production from the ever-present Eimeria challenge. Trends Parasitol 30(1):12–19
18. Islam MK (2014) Challenges and prospects of poultry industry in Bangladesh
19. Windhorst H-W (2006) Changes in poultry production and trade worldwide. World's Poultry Science Journal 62(04):585–602
20. Dagne A (2015) Challenges and prospects of poultry industry
21. Dalcero A, Magnoli C, Chiacchiera S, Palacios G, Reynoso M (1997) Mycoflora and incidence of aflatoxin B1, zearalenone and deoxynivalenol in poultry feeds in Argentina. Mycopathologia 137(3):179–184
22. Shetty PH, Bhat RV (1997) Natural occurrence of fumonisin B1 and its co-occurrence with aflatoxin B1 in Indian sorghum, maize, and poultry feeds. J Agric Food Chem 45(6):2170–2173
23. Fraga M, Curvello F, Gatti M, Cavaglieri L, Dalcero A, da Rocha RC (2007) Potential aflatoxin and ochratoxin A production by Aspergillus species in poultry feed processing. Vet Res Commun 31(3):343–353
24. Zinedine A, Juan C, Soriano J, Molto J, Idrissi L, Manes J (2007) Limited survey for the occurrence of aflatoxins in cereals and poultry feeds from Rabat, Morocco. Int J Food Microbiol 115(1):124–127
25. Murugesan G, Ledoux D, Naehrer K, Berthiller F, Applegate T, Grenier B, Phillips T, Schatzmayr G (2015) Prevalence and effects of mycotoxins on poultry health and performance, and recent development in mycotoxin counteracting strategies. Poult Sci: pev075
26. Bintvihok A, Thiengnin S, Doi K, Kumagai S (2002) Residues of aflatoxins in the liver, muscle and eggs of domestic fowls. J Vet Med Sci 64(11):1037–1039
27. Fink-Gremmels J, Malekinejad H (2007) Clinical effects and biochemical mechanisms associated with exposure to the mycoestrogen zearalenone. Anim Feed Sci Technol 137(3):326–341
28. Lizárraga-Paulín EG, Moreno-Martínez E, Miranda-Castro SP (2011) Aflatoxins and their impact on human and animal health: an emerging problem. InTech Open Access Publisher
29. Diaz GJ, Murcia HW (2011) Biotransformation of aflatoxin B1 and its relationship with the differential toxicological response to aflatoxin in commercial poultry species. InTech Open Access Publisher
30. Hussein HS, Brasel JM (2001) Toxicity, metabolism, and impact of mycotoxins on humans and animals. Toxicology 167(2):101–134
31. Grenier B, Applegate TJ (2013) Modulation of intestinal functions following mycotoxin ingestion: meta-analysis of published experiments in animals. Toxins 5(2):396–430
32. Rawal S, Kim JE, Coulombe R (2010) Aflatoxin B1 in poultry: toxicology, metabolism and prevention. Res Vet Sci 89(3):325–331
33. Diaz G, Murcia H, Cepeda S (2010) Bioactivation of aflatoxin B1 by Turkey liver microsomes: responsible cytochrome P450 enzymes. Br Poult Sci 51(6):828–837
34. Diaz G, Murcia H, Cepeda S (2010) Cytochrome P450 enzymes involved in the metabolism of aflatoxin B1 in chickens and quail. Poult Sci 89(11):2461–2469
35. Peng X, Yu Z, Liang N, Chi X, Li X, Jiang M, Fang J, Cui H, Lai W, Zhou Y (2016) The mitochondrial and death receptor pathways involved in the thymocytes apoptosis induced by aflatoxin B1. Oncotarget 7(11):12222–12234
36. Petit PX, Zamzami N, Vayssiere J, Mignotte B, Kroemer G, Castedo M (1997) Implication of mitochondria in apoptosis. Mol Cell Biochem 174(1):185–188
37. Hayes JD, Judah DJ, McLellan LI, Neal GE (1991) Contribution of the glutathione S-transferases to the mechanisms of resistance to aflatoxin B1. Pharmacol Ther 50(3):443–472
38. Tiwari RP, Dham CK, Bhalla TC, Saini SS, Vadehra DV (1985) Mechanism of action of aflatoxin B1 in *Bacillus megaterium*. Applied & Environmental Microbiology 49(4):904–907
39. Hamid AS, Tesfamariam IG, Zhang Y, Zhang ZG (2013) Aflatoxin B1-induced hepatocellular carcinoma in developing countries: geographical distribution, mechanism of action and prevention (review). Oncol Lett 5(4):1087–1092
40. Ibrahim ZS, Alkafafy ME, Ahmed MM, Soliman MM (2016) Renoprotective effect of curcumin against the combined oxidative stress of diabetes and nicotine in rats. Mol Med Rep 13(4):3017–3026
41. Grant D, Mendicino M, Levy G (2001) Xenotransplantation: just around the corner? Surgery 129(3):243–247
42. Hussain I, Anwar J (2008) A study on contamination of aflatoxin M1 in raw milk in the Punjab province of Pakistan. Food Control 19(4):393–395
43. Newberne PM, Butler WH (1969) Acute and chronic effects of aflatoxin on the liver of domestic and laboratory animals: a review. Cancer Res 29(1):236–250
44. Shi D, Guo S, Liao S, Su R, Pan J, Lin Y, Tang Z (2012) Influence of selenium on hepatic mitochondrial antioxidant capacity in ducklings intoxicated with aflatoxin B1. Biol Trace Elem Res 145(3):325–329
45. Liao S, Shi D, Clemons-Chevis CL, Guo S, Su R, Qiang P, Tang Z (2014) Protective role of selenium on aflatoxin b1-induced hepatic dysfunction and apoptosis of liver in ducklings. Biol Trace Elem Res 162(1–3):296–301
46. Yu Z, Wang F, Liang N, Wang C, Peng X, Fang J, Cui H, Mughal MJ, Lai W (2015) Effect of selenium supplementation on apoptosis and cell cycle blockage of renal cells in broilers fed a diet containing aflatoxin B1. Biol Trace Elem Res 168(1):242–251
47. Hussain Z, Khan MZ, Hassan Z (2008) Production of aflatoxins from Aspergillus flavus and acute aflatoxicosis in young broiler chicks. Pak J Agri Sci 45(1):95–102
48. Quezada T, Cuellar H, Jaramillo-Juarez F, Valdivia A, Reyes J (2000) Effects of aflatoxin B1 on the liver and kidney of broiler chickens during development. Comp Biochem Physiol C: Pharmacol Toxicol Endocrinol 125(3):265–272
49. Valchev I, Kanakov D, Ts H, Lazarov L, Binev R, Grozeva N, Nikolov Y (2014) Effects of experimental aflatoxicosis on renal function in broiler chickens. Bulgarian Journal of Veterinary Medicine 17(4):314–324
50. Mollenhauer H, Corrier D, Huff W, Kubena L, Harvey R, Droleskey R (1989) Ultrastructure of hepatic and renal lesions in chickens fed aflatoxin. Am J Vet Res 50(5):771–777
51. Glahn R, Beers K, Bottje W, Wideman R Jr, Huff W, Thomas W (1991) Aflatoxicosis alters avian renal function, calcium, and vitamin D metabolism. Journal of Toxicology and Environmental Health, Part A Current Issues 34(3):309–321
52. Yildirim E, Yalçinkaya L, Kanbur M, Cinar M, Oruc E (2011) Effects of yeast glucomannan on performance, some biochemical parameters and pathological changes in experimental aflatoxicosis in broiler chickens. Rev Med Vet (Toulouse) 162:413–420
53. Kubena L, Harvey R, Bailey R, Buckley S, Rottinghaus G (1998) Effects of a hydrated sodium calcium aluminosilicate (T-bind) on mycotoxicosis in young broiler chickens. Poult Sci 77(10):1502–1509
54. Sakhare PS, Harne SD, Kalorey DR, Warke SR, Bhandarkar AG, Kurkure NV (2007) Effect of Toxiroak® polyherbal feed

supplement during induced aflatoxicosis, ochratoxicosis and combined mycotoxicoses in broilers. Veterinarski Arhiv 77(2):129
55. Kana J, Teguia A, Tchoumboue J (2010) Effect of dietary plant charcoal from *Canarium schweinfurthii* Engl. and maize cob on aflatoxin B1 toxicosis in broiler chickens. Adv Anim Biosci 1(02): 462–463
56. Yunus A, Ghareeb K, Abd-El-Fattah A, Twaruzek M, Böhm J (2011) Gross intestinal adaptations in relation to broiler performance during chronic aflatoxin exposure. Poult Sci 90(8):1683–1689
57. Ruff M, Wyatt R (1976) Intestinal absorption of L-methionine and glucose in chickens with aflatoxicosis. Toxicol Appl Pharmacol 37(2):257–262
58. Verma J, Swain BK, Johri TS (2002) Effect of various levels of aflatoxin and ochratoxin A and combinations thereof on protein and energy utilisation in broilers. J Sci Food Agric 82(12):1412–1417
59. Verma J, Johri TS, Swain BK (2007) Effect of aflatoxin, ochratoxin and their combination on protein and energy utilisation in white leghorn laying hens. J Sci Food Agric 87(5):760–764
60. Yunus A, Awad W, Kröger S, Zentek J, Böhm J (2010) In vitro aflatoxin B1 exposure decreases response to carbamylcholine in the jejunal epithelium of broilers. Poult Sci 89(7):1372–1378
61. He Y, Fang J, Peng X, Cui H, Zuo Z, Deng J, Chen Z, Lai W, Shu G, Tang L (2014) Effects of sodium selenite on aflatoxin B1-induced decrease of ileac T cell and the mRNA contents of IL-2, IL-6, and TNF-α in broilers. Biol Trace Elem Res 159(1–3):167–173
62. Pier A, Heddleston K (1970) The effect of aflatoxin on immunity in turkeys. I. Impairment of actively acquired resistance to bacterial challenge. Avian Dis 14:797–809
63. Giambrone J, Ewert D, Wyatt R, Eidson C (1978) Effect of aflatoxin on the humoral and cell-mediated immune systems of the chicken. Am J Vet Res 39(2):305–308
64. Sharma RP (1993) Immunotoxicity of mycotoxins. J Dairy Sci 76(3):892–897
65. Thaxton J, Tung H, Hamilton P (1974) Immunosuppression in chickens by aflatoxin. Poult Sci 53(2):721–725
66. Bondy GS, Pestka JJ (2000) Immunomodulation by fungal toxins. Journal of Toxicology and Environmental Health Part B: Critical Reviews 3(2):109–143
67. Celik I, Oguz H, Demet O, Donmez H, Boydak M, Sur E (2000) Efficacy of polyvinylpolypyrrolidone in reducing the immunotoxicity of aflatoxin in growing broilers. Br Poult Sci 41(4):430–439
68. Peng X, Chen K, Chen J, Fang J, Cui H, Zuo Z, Deng J, Chen Z, Geng Y, Lai W (2015) Aflatoxin B1 affects apoptosis and expression of Bax, Bcl-2, and caspase-3 in thymus and bursa of fabricius in broiler chickens. Environ Toxicol 31(9):1113–1120
69. Wang F, Shu G, Peng X, Fang J, Chen K, Cui H, Chen Z, Zuo Z, Deng J, Geng Y (2013) Protective effects of sodium selenite against aflatoxin B1-induced oxidative stress and apoptosis in broiler spleen. Int J Environ Res Public Health 10(7):2834–2844
70. Azzam A, Gabal M (1998) Aflatoxin and immunity in layer hens. Avian Pathology 27(6):570–577
71. Gabal M, Azzam A (1998) Interaction of aflatoxin in the feed and immunization against selected infectious diseases in poultry. II. Effect on one-day-old layer chicks simultaneously vaccinated against Newcastle disease, infectious bronchitis and infectious bursal disease. Avian Pathology 27(3):290–295
72. Mariani G (1998) Desempenho produtivo de frangos de corte submetidos à intoxicação experimental com aflotoxina em diferentes idades. Santa Maria-RS
73. Scheideler SE (1993) Effects of various types of aluminosilicates and aflatoxin B1 on aflatoxin toxicity, chick performance, and mineral status. Poult Sci 72(2):282–288
74. Raju M, Devegowda G (2000) Influence of esterified-glucomannan on performance and organ morphology, serum biochemistry and haematology in broilers exposed to individual and combined mycotoxicosis (aflatoxin, ochratoxin and T-2 toxin). Br Poult Sci 41(5):640–650
75. Han X-Y, Huang Q-C, Li W-F, Jiang J-F, Xu Z-R (2008) Changes in growth performance, digestive enzyme activities and nutrient digestibility of cherry valley ducks in response to aflatoxin B1 levels. Livest Sci 119(1):216–220
76. Miazzo R, Rosa C, Carvalho EDQ, Magnoli C, Chiacchiera S, Palacio G, Saenz M, Kikot A, Basaldella E, Dalcero A (2000) Efficacy of synthetic zeolite to reduce the toxicity of aflatoxin in broiler chicks. Poult Sci 79(1):1–6
77. Valdivia A, Martinez A, Damian F, Quezada T, Ortiz R, Martinez C, Llamas J, Rodriguez M, Yamamoto L, Jaramillo F (2001) Efficacy of N-acetylcysteine to reduce the effects of aflatoxin B1 intoxication in broiler chickens. Poult Sci 80(6):727–734
78. Tedesco D, Steidler S, Galletti S, Tameni M, Sonzogni O, Ravarotto L (2004) Efficacy of silymarin-phospholipid complex in reducing the toxicity of aflatoxin B1 in broiler chicks. Poult Sci 83(11):1839–1843
79. Denli M, Blandon J, Guynot M, Salado S, Perez J (2009) Effects of dietary AflaDetox on performance, serum biochemistry, histopathological changes, and aflatoxin residues in broilers exposed to aflatoxin B1. Poult Sci 88(7):1444–1451
80. Zhao J, Shirley R, Dibner J, Uraizee F, Officer M, Kitchell M, Vazquez-Anon M, Knight C (2010) Comparison of hydrated sodium calcium aluminosilicate and yeast cell wall on counteracting aflatoxicosis in broiler chicks. Poult Sci 89(10):2147–2156
81. Pacheco IT (2011) Aflatoxins: detection, measurement and control. InTech
82. Piva G, Galvano F, Pietri A, Piva A (1995) Detoxification methods of aflatoxins. A review. Nutr Res 15(5):767–776
83. Wilson H, Douglas C, Harms R, Edds G (1975) Reduction of aflatoxin effects on quail. Poult Sci 54(3):923–925
84. Denli M, Celik K, Okan F (2003) Effects of vitamin A supplementary in the feed to reduce toxic effects of aflatoxin B1 on Japanese quails (*Coturnix coturnix japonica*). Int J Poult Sci 2(2):174–177
85. Oguz H (2011) A review from experimental trials on detoxification of aflatoxin in poultry feed. Eurasian J Vet Sci 27(1):1–12
86. Holben DH, Smith AM (1999) The diverse role of selenium within selenoproteins: a review. J Am Diet Assoc 99(7):836–843
87. Rayman MP (2000) The importance of selenium to human health. Lancet 356(9225):233–241
88. Surai P (2002) Selenium in poultry nutrition 1. Antioxidant properties, deficiency and toxicity. World's Poultry Science Journal 58(03):333–347
89. Ferenčík M, Ebringer L (2003) Modulatory effects of selenium and zinc on the immune system. Folia Microbiol 48(3):417–426
90. Fairweather-Tait SJ, Bao Y, Broadley MR, Collings R, Ford D, Hesketh JE, Hurst R (2011) Selenium in human health and disease. Antioxid Redox Signal 14(7):1337–1383
91. Surai P (2002) Selenium in poultry nutrition 2. Reproduction, egg and meat quality and practical applications. World's Poultry Science Journal 58(04):431–450
92. Surai P, Fisinin V (2014) Selenium in poultry breeder nutrition: an update. Anim Feed Sci Technol 191:1–15
93. Hurley W, Doane R (1989) Recent developments in the roles of vitamins and minerals in reproduction. J Dairy Sci 72(3):784–804
94. Weiss SL, Sunde RA (1998) Cis-acting elements are required for selenium regulation of glutathione peroxidase-1 mRNA levels. RNA 4(7):816–827
95. Surai P, Dvorska J (2001) Is organic selenium better for animals than inorganic sources? Feed Mix 9(4/5):8–10

96. Peng X, Zhang S, Fang J, Cui H, Zuo Z, Deng J (2014) Protective roles of sodium selenite against aflatoxin B1-induced apoptosis of jejunum in broilers. Int J Environ Res Public Health 11(12): 13130–13143
97. Wang T, Guo D, Dong X, Mu L (2014) Effect of linezolid on hematological and oxidative parameters in rats. J Antibiot 67(6): 433–437
98. Yang P, Hao Y, Feng J, Lin H, Feng Y, Wu X, Yang X, Gu X (1966) The expression of carnosine and its effect on the antioxidant capacity of muscle in finishing pigs exposed to constant heat stress. Breast Disease 20(8):137–143
99. Sun LH, Zhang NY, Zhu MK, Zhao L, Zhou JC, Qi DS (2016) Prevention of aflatoxin B1 hepatoxicity by dietary selenium is associated with inhibition of cytochrome P450 isozymes and up-regulation of 6 selenoprotein genes in chick liver. J Nutr
100. Ganther HE (2001) Selenium metabolism and mechanisms of cancer prevention. Adv Exp Med Biol 492(2):119–130
101. Prasad AS (2013) Essential and toxic element: trace elements in human health and disease. Elsevier, Amsterdam
102. Mills CF (2013) Zinc in human biology. Springer Science & Business Media, New York
103. Ho E (2004) Zinc deficiency, DNA damage and cancer risk. J Nutr Biochem 15(10):572–578
104. Batal A, Parr T, Baker D (2001) Zinc bioavailability in tetrabasic zinc chloride and the dietary zinc requirement of young chicks fed a soy concentrate diet. Poult Sci 80(1):87–90
105. Antoniou L, Shalhoub R, Schechter G (1981) The effect of zinc on cellular immunity in chronic uremia. Am J Clin Nutr 34(9):1912–1917
106. Bogden J, Oleske J, Lavenhar M, Munves E, Kemp F, Bruening K, Holding K, Denny T, Guarino M, Holland B (1990) Effects of one year of supplementation with zinc and other micronutrients on cellular immunity in the elderly. J Am Coll Nutr 9(3):214–225
107. Shankar AH, Prasad AS (1998) Zinc and immune function: the biological basis of altered resistance to infection. Am J Clin Nutr 68(2):447S–463S
108. Hirano T, Murakami M, Fukada T, Nishida K, Yamasaki S, Suzuki T (2008) Roles of zinc and zinc signaling in immunity: zinc as an intracellular signaling molecule. Adv Immunol 97:149–176
109. Mohanna C, Nys Y (1999) Effect of dietary zinc content and sources on the growth, body zinc deposition and retention, zinc excretion and immune response in chickens. Br Poult Sci 40(1): 108–114
110. Favero A, Vieira S, Angel C, Bess F, Cemin H, Ward T (2013) Reproductive performance of Cobb 500 breeder hens fed diets supplemented with zinc, manganese, and copper from inorganic and amino acid-complexed sources. The Journal of Applied Poultry Research 22(1):80–91
111. Dikmen BY, Sözcü A, Aydın İ, Şahan Ü (2015) Effects of supplementary mineral amino acid chelate (ZnAA-MnAA) on the laying performance, egg quality and some blood parameters of late laying period layer hens. Kafkas Üniversitesi Veteriner Fakültesi Dergisi 21(2):155–162
112. Brake J (1993) Recent advances in induced molting. Poult Sci 72(5):929–931
113. Hudson B, Dozier W, Wilson J, Sander J, Ward T (2004) Reproductive performance and immune status of caged broiler breeder hens provided diets supplemented with either inorganic or organic sources of zinc from hatching to 65 wk of age. The Journal of Applied Poultry Research 13(2):349–359
114. Smith B, Embling P, Towers N, Wright D, Payne E (1977) The protective effect of zinc sulphate in experimental sporidesmin poisoning of sheep. N Z Vet J 25(5):124–127
115. Hegazy S, Adachi Y (2000) Comparison of the effects of dietary selenium, zinc, and selenium and zinc supplementation on growth and immune response between chick groups that were inoculated with Salmonella and aflatoxin or Salmonella. Poult Sci 79(3):331–335
116. Powell SR (2000) The antioxidant properties of zinc. J Nutr 130(5):1447S–1454S
117. Prasad AS, Bao B, Beck FW, Kucuk O, Sarkar FH (2004) Antioxidant effect of zinc in humans. Free Radic Biol Med 37(8):1182–1190
118. Mansour SA, Mossa A-TH (2009) Lipid peroxidation and oxidative stress in rat erythrocytes induced by chlorpyrifos and the protective effect of zinc. Pestic Biochem Physiol 93(1):34–39
119. Chen T, Cui H, Cui Y, Bai C, Gong T, Peng X (2011) Cell-cycle blockage associated with increased apoptotic cells in the thymus of chickens fed on diets high in fluorine. Human & Experimental Toxicology 30(7):685–692
120. Varelas JB, Roy C, Hering TM (1997) A structural requirement of zinc for the folding of recombinant link protein. Archives of Biochemistry & Biophysics 347(1):1–8
121. Yamaguchi M (2004) Regulatory mechanism of trace elements in bone marrow-derived cells. Role of zinc in regulation of osteoclastogenesis. Biomedrestrace Elements 15:9–14
122. Hennigar SR, Kelleher SL (2012) Zinc networks: the cell-specific compartmentalization of zinc for specialized functions. Biol Chem 393(7):565–578