Harms of Mycotoxins to Animals and Research Progress of Detection Methods

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Abstract Mycotoxin is widely distributed in various feedstuffs and feeds of animals. Mycotoxin not only causes feed deterioration, but also leads to various diseases of animals, or even results in acute toxic death, causing great harm to the breeding industry. In this paper, the harm of mycotoxin to animals and its detection methods in feed and animals are summarized and analyzed, so as to provide the reference basis for further improving the detection methods of mycotoxin and reducing the harm of mycotoxin to breeding industry.

Keywords Mycotoxin; Harm; Detection method

Mycotoxins are secondary metabolites of mold, which are highly toxic and widely distributed in all kinds of food and feedstuffs. When mycotoxin enters animal body through various paths, it leads to decreased animal immunity, reduced function, occurrence of diseases and even death. There are a wide range of mycotoxins. At present, more than 300 kinds of mycotoxins that are toxic to humans and animals have been found, including aflatoxin (AF), ochratoxin A (OTA), sterigmatocystin (ST), *etc*. produced by *Aspergillus*, as well as deoxynivalenol (DON), zearalenone (ZEA), T-2 toxin, *etc*. produced by *Fusarium*. Among them, AF, T-2 toxin, DON, ZEA, OTA and fumitremorgin are the main toxins that are harmful to humans and animals[1]. Through sampling inspection of feed samples, the detection rates of mycotoxin in feed in China in 2013 were as follows: AFB 175.9%, ZEN 91.6%, DON 88.4%, FB1 95.2% and T-2 toxin 80.6%, respectively[2].

1 Harms of Mycotoxin to Animals

High concentration of mycotoxin will lead to acute poisoning and even death of animals, while long-term feeding with low concentration of mycotoxin leads to chronic poisoning of animals. The harms are mainly manifested in the following aspects: (1) destroy or reduce the nutrient composition of feed, affect the absorption and metabolism of nutrients; (2) cause the damage of liver, kidneys, lungs, intestines; for example, the target organ of AF is the liver, which can cause diseases such as cirrhosis and liver cancer; OTA, citrinin and sterigmatocystin have strong renal toxicity, which can cause renal tubule degeneration and necrosis, resulting in polyuria, hematuria and proteinuria, *etc*. (3) cause a variety of reproductive obstacles, leading to false estrus, anal prolapses, abortion and stillbirth of female animals; for example, ZEA has strong hormone-like effect, which can lead to animal estrus syndrome; (4) cause animal immune suppression, induce all kinds of chronic diseases, and reduce feed benefit.

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2 Detection Methods of Mycotoxin

2.1 Gold immune chromatography assay (GICA)

Immunochromatography is a solid-phase membrane immunoassay developed in the 1980s, which combines colloidal gold immunoassay and chromatographic assay. The principle is that the antibody is fixed to a specific area of nitrocellulose filter membrane (NC membrane); when one end of the NC membrane is submerged in the sample, the sample moves forward under capillary action; when the sample moves to the area where the antibody is present, the antigen binds to the antibody specifically; some labeling materials are colored, so the binding sites will show the corresponding color, thus achieving the effect of immunodiagnosis[3]. The method is featured by simple operation and short duration, and is suitable for rapid detection of various mycotoxins.

Under the optimal conditions, the polyclonal antibody of AFB1 was combined with colloidal gold to make a gold probe, and AFB1 was detected by immunochromatography and compared with the results of ELISA method; the LOD of GICA was as low as 2.5 μg/L, 1/2 of that

of ELISA method; the detection time could be controlled within 10 min, which was 6 –10 times shorter than that of ELISA method [4]. Xu *et al*.[5] established a GICA method for rapid and quantitative determination of AFB_1, B_2, G_1 and G_2 in corn, and the LOD and limit of quantity (LOQ) were 1.0 and 3.0 μg/kg, respectively. The method was simple, rapid, sensitive and reproducible, and could be used for rapid quantitative determination of total amount of four kinds of AF in corn. Deng *et al*. [3] established a GICA method for detecting AFB1, and the sensitivity of strips was up to 5 μg/L, which was suitable for rapid detection in the field. Lai *et al*.[6] established a rapid detection method of colloidal gold strip for OTA with a LOD of 10 μg/L, which could be completed within 10 min. In addition, the colloid gold strips/cards for T-2 toxin, vomitoxin, DON, AFB1, *etc*., have been successfully developed, playing a positive role in the rapid detection of mycotoxins[7–10].

2.2 Enzyme linked immunosorbent assay (ELISA) Enzyme-linked immunosorbent assay (ELISA) is a method of detecting target compound by utilizing the specificity between antibodies and antigens, which is featured by high specificity, high sensitivity and simple operation, and does not need expensive equipments in the process of testing. However, the preparation of antigens and antibodies requires high professional and technical level, and only one substance can be detected by this method. Therefore, it is mostly used for rapid screening and examination. Currently, a variety of ELISA kits have been developed, mainly including AF, OTA, ZEA, *etc*.

In 2013, Huang *et al*. [11] detected AFB1, OTA and ZEA in liver, kidney and feces of broilers via ELISA kit, respectively; the limit of detection (LOD) of AFB1 and OTA was 0.1 ng/g, and that of ZEA also reached 1.0 ng/g. Liu *et al*.[12] detected the content of ZEA in corn by indirect competitive ELISA with polyclonal antibody of ZEA, and the detection range was 0.5–50.0 ng/mL. Jiang *et al*.[13] established an indirect competitive inhibition ELISA method for the detection of OTA; the recovery was 79.0% –119.7% in the linear range of 2–500 μg/L, and the LOD reached 0.5 ng/L.

2.3 Thin layer chromatography (TLC) Thin layer chromatography (TLC) is featured by simple equipment, convenient operation, fast separation and great color selectivity when detecting mycotoxins. The principle of this method is to extract mycotoxins from samples with appropriate solvents according to different physical and chemical properties of samples, and then separate them with thin laminates. According to the fluorescence of mycotoxins, the strength of fluorescence spots was used for semi-quantitative detection. A variety of mycotoxins in feed are detected by TLC, such as determination of deoxynivalenol in compound feed (GB/T8381.6-2005), determination of oatoxin in feed (GB/T19539-2004), and determination of zearalenone in feed (GB/T19540-2004), *etc*.[14]. The disadvantages of the method are low accuracy and poor reproducibility, so it is only suitable for preliminary identification of toxins, small amount purification and optimized screening of column chromatographic conditions.

In 1982, Yin[15] simultaneously detected 14 mycotoxins such as AF, T-2 toxin and ZEA in grain using TLC method, among which the fluorescence absorption of AF was the strongest and the LOD reached 10.0 μg/kg. In 1985, Li[16] simultaneously extracted and analyzed 14 mycotoxins such as AF, T-2 toxin and sterigmatocystin from corn, wheat and other cereals by TLC; the LOD of AFB_1, B_2, G_1 and G_2 was the lowest, up to 10.0 μg/kg, and that of ZEA was up to 300 –500 μg/kg, so the method could be used for preliminary detection of mycotoxins in cereals.

2.4 Near infrared reflectance spectroscopy Near infrared reflectance spectroscopy (NIRS) is one of the fastest developing spectral analysis techniques since the 1990s, which is the organic combination of spectroscopic measurement technology and stoichiometry. The method has the advantages of fast analysis, high accuracy, low cost, no damage to samples, no need for reagents, and no pretreatment of samples, etc., and has been widely used in the fields of industry, agriculture, medicine, chemical industry, celestial bodies, *etc*.[17].

Chitin, also known as chitosan, is a linear amino polysaccharide that is widely found in fungal cell walls but not in higher plants, and the concentration of chitin increases with the increase of molds in grains. According to the above properties, some scholars have pointed out that chitin can be used to estimate the contamination level of mycotoxins in grains and other crops. Chitin is hydrolyzed into glucosamine by acid or alkali or enzyme, and the hydrolytic products can be measured by colorimetry, chromatography, microscopy, *etc*. Both colorimetry and chromatography require cumbersome pretreatment of samples. Fluorescence staining combined with microscopic detection is poor in reproducibility and the result is not ideal. NIRS method effectively avoids the disadvantages of the above detection methods, which is simple and rapid with accurate results[18].

2.5 Gas chromatography and gas chromatography-mass spectrometry Gas chromatography or gas chromatography-based GC-MS technology has the advantages of fast separation and high sensitivity. However, because gas chromatography is only suitable for the separation and detection of volatile substances with large volatility and high thermal stability, it also has some shortcomings in practical application. It is mainly used for the detection of fusarium mycotoxin and patulin.

In 1995, Chen *et al*.[19] established a gas chromatography method for simultaneous detection of DON, T-2 toxin and ZEN in feed. After extraction, purification and

derivation of samples, the three toxins were detected by meteorological chromatograph, capillary column and flame ionization detector (FID), the three toxins were well separated and the LOD of the three toxins was 50 μg/kg. Lin *et al.*[20] detected T-2 and HT-2 toxins in corn and other food crops by gas chromatography-tandem mass spectrometry (GC-MS-MS), and the linear relationship between T-2 and HT-2 toxins was good in the range of 0.5–100.0 μg/kg. The method was simple, sensitive and suitable for the detection of T-2 and HT-2 toxin in grain samples. In 2001, Wen *et al.*[21] tested nivalenol, DON and neosolaniol in grains; the samples were extracted with methanol aqueous solution, purified with Florisil column, and then derived with N-Heptafluorobutyrylimidazole before gas chromatography detection; the method was simple and rapid, and the LOD reached 10 –100 μg/kg. In 2006, Liang *et al.*[22] established a method for simultaneous determination of DON and nivalenol in feed by gas chromatography-mass spectrometry; the feed samples were purified by Mycosep 227 multifunctional purification column, derived with silanization reagent, and separated and detected with HP-5 MS capillary column. DON and nivalenol showed good linear relationship in the concentration range of 10 –1 300 μg/L and 8–1 300 μg/L, and the LOD was 9 and 7 μg/kg, respectively. The method had high sensitivity and good reproducibility.

2.6 Liquid chromatography and liquid chromatography-mass spectrometry Compared with ELISA, TLC, fluorescence spectrometry and other traditional methods, liquid chromatography-tandem mass spectrometry (LC-MS) method or the ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS) developed on this basis combines the advantages of high separation of liquid chromatography with the versatility of mass spectrometry detector, which provides molecular structure information, high sensitivity, good selectivity, and simple pretreatment, pointing the direction for simultaneous rapid detection of multiple mycotoxins[23].

In 2000, Jodlbauer *et al.*[24] established a LC-MS/MS method for the determination of ZEA in bovine and pig tissues; the samples were hydrolyzed by enzyme, purified by solid phase extraction on RP-18 column and determined by mass spectrometry; negative ions were detected by atmospheric pressure chemical ionization method under MRM mode, and the detection limits of pigs and bovine were 0.1 and 0.5 μg/kg, respectively. In 2007, Ren *et al.*[25] detected 17 mycotoxins including mycotoxin, fusarium toxin and penicillium toxoid in food and feed using ultra-high pressure liquid chromatography-ionization tandem mass spectrometry (UPLC-MS/MS); under ESI + ionization mode, the LOQ of 10 mycotoxins ranged from 0.01 μg/kg to 0.20 μg/kg, which were all lower than the minimum limits prescribed by the European Union and the United States; under ESI ionization mode, the LOQ of seven mycotoxins ranged from 0.2 μg/kg to 0.5 μg/kg. Ying *et al.*[26] adopted high performance liquid chromatography-ionization tandem mass spectrometry (LC-ESI-MS-MS) to establish a rapid method for the detection of 14 mycotoxins and their analogs in feed, including ZEA, AF and trichothecenes A, under the multiple reaction monitoring (MRM) mode; the LOD of samples was 0.1 –0.8 μg/kg after extraction with acetonitrile solution, degreasing with n-hexane and purification with purification column. In 2012, Zhu *et al.*[27] established a LC-MS method for simultaneous detection of AFB_1, B_2, G_1, G_2, OTA, fumonisin B_1, DON, T-2 toxin, HT-2 toxin and ZEA in peanut and its products; the LOD of various ingredients were AFB1 0.5 ng/kg, AFB_2, G_1 and G_2 1 ng/kg, OTA and ZEA 2 ng/kg, T-2 toxin and HT-2 toxin 10 ng/kg, fumonisin B1 20 ng/kg, DON 50 ng/kg; the added recoveries were all above 72%. Ren *et al.*[28] detected six components of AF (AFB1, AFB_2, AFG_1, AFG_2, AFM_1, AFM_2); the six components could be completely separated within 7 min, and the LOD was 0.012 – 0.040 μg/kg. Meantime, a method for simultaneous determination of eight mycotoxins (AFB_1, AFB_2, AFG_1, AFG_2, OTA, sterigmatocystin, ZEA, T-2 toxin) in different cereals was established within 8 min, and the LOD and LOQ were 0.012–0.160 μg/kg and 0.040 –0.533 μg/kg, respectively, so the method could be used for the detection of mycotoxins in grains. In 2013, Han *et al.*[29] established a UPLC-MS residue detection method for six kinds of zearalenone mycotoxins (α-zeranol, β-zeranol, α-zearalenol, β-zearalenol, zearalanone, zearalenone) in milk, and eight compounds were separated effectively within 6.68 –8.33 min, with the LOD of 1.0 μg/L. In 2014, Liu *et al.*[30] established a LC-MS-MS method for the detection of 12 mycotoxins such as ZEA and DON in wheat, corn and other grains. Under multi-reaction monitoring (MRM) mode, the detection limits of the method were as low as 0.04 –0.18 μg/kg, and the recoveries were above 90%. The method was suitable for rapid quantitative determination of mycotoxins such as ZEA and DON in grain.

3 Prospect

There are a variety of mycotoxins, causing serious harms to animals. As animal husbandry and veterinary science and technology workers, we should grasp the key points, especially AF, zearalenone and vomitoxin that cause serious harms to animals. According to their physiological and biochemical characteristics, effective detection methods must be screened, and long-term monitoring mechanisms of mycotoxin are established to effectively control mycotoxins and reduce the harms to livestock and poultry, thereby ensuring the healthy and orderly development of breeding industry.

References

[1] BINDER EM. Managing the risk of mycotoxins

in modern feed production[J]. Animal Feed Science and Technology, 2007, 133(1–2): 149–166.

[2] DU N. Investigation report on mycotoxin pollution of feed and raw materials in some areas of China in 2013[J].Pigs Today, 2014(3): 48–52. (in Chinese).

[3] DENG SL, LAI WH, XU Y. Study on Gold Immunochromatography assay for rapid detection of Aflatoxin B1[J]. Food Science, 2007, 28(2): 232–236. (in Chinese).

[4] XIU LS, XIAO L Z, JIAN T, *et al*. Preparation of gold-labeled antibody probe and its use in immune chromatography assay for detection of aflatoxin B1[J]. Int J Food Microbiol, 2005, 99 (2): 185–194.

[5] XU ZB, HU DQ, LI N, *et al*. Rapid determination of Aflatoxin in corn by immunochromatography[J]. Grain Science and Technology and Economy, 2013, 38(3): 24–26. (in Chinese).

[6] LAI WH, XIONG YH, CHEN GM, *et al*.Preparation of colloidal gold strip for rapid detection of ochratoxin A[J]. Food Science, 2005, 26(5): 204–207. (in Chinese).

[7] LI Q, CHEN XX, SANG LY, *et al*. Preparation of T-2 toxin immunocolloidal gold test strip and its application in feed detection[J]. China Feed, 2013(10): 33–36. (in Chinese).

[8] MA YS, ZHAO ZM, HAN JP, *et al*. Development of colloidal gold test paper card for detecting vomiting toxin[C]. Beijing: Material collection of 2013 academic report meeting on feed toxicology and the Fifth National Congress of feed toxicology Professional Committee, 2013. (in Chinese).

[9] WANG JS.Study on rapid immunoassay of deoxynivalenol colloidal gold in food [D]. Wuxi: Jiangnan University , 2008. (in Chinese).

[10] LU SY. Preparation of aflatoxin B1 colloidal gold immunochromatographic strip[D]. Yangzhou: Yangzhou University, 2010. (in chinese).

[11] HUANG XL, QU DF, HAN JZ. Study on mycotoxin residue in liver and kidney of table poultry and effect of mycotoxin adsorbent[J]. Journal of the Chinese Cereals and Oils Association, 2013, 28(8): 46–50. (in Chinese).

[12] LIU MT. Indirect enzyme linked immunosorbent assay for mycoloxin Zearalenone [J]. Journal of Applied Environmental Microbiology, 1985, 50(2): 332–336.

[13] JIANG T, LI FQ, LI YH, *et al*. Study on immunoassay for ochratoxin A[J]. Chinese Journal of Public Health, 2004, 50(5): 556–558. (in Chinese).

[14] National feed Work Office of the Ministry of Agriculture.Compilation of feed industry standards (2002 –2006)[M]. Beijing: China Standards Press, 2006. (in Chinese).

[15] YIN WS.Simultaneous determination of 14 mycotoxins by thin layer chromatography[J]. Journal of Zhengzhou Grain University, 1982 (4): 70–74. (in Chinese).

[16] LI H. Simultaneous extraction, partition elution and thin layer chromatography determination of 14 mycotoxins in cereals[J]. Guangdong Health and Epidemic Prevention Data, 1985 (5): 146–154. (in Chinese).

[17] XU QF, HAN JG, YU Z, *et al*. Applications of near infrared reflectance spectroscopy technique to determination of forage mycotoxins[J]. Spectroscopy and Spectral Analysis, 2010, 30(5): 1243–1247. (in Chinese).

[18] YI YY, LI DR, ZHANG WY, *et al*. Applications of near infrared reflectance spectroscopy in detecting chitin,ergosterol and mycotoxins[C]. Proceedings of 2008 China Grassland Development Forum, 2008. (in Chinese).

[19] CHEN BF. Determination of Fusarium toxin don, T-2 and Zen in feed by gas chromatography[J]. China Feed, 1995 (10): 32–34. (in Chinese).

[20] LIN Y, CHEN J, WU BD, *et al*. Determination of T-2 and HT-2 toxins in cereal grains by solid phase extraction and gas chromatography-tandem mass spectrometry[J]. Military Medical Sciences, 2013, 37(5): 381–384. (in Chinese).

[21] WEN ZX, YANG ZJ, SONG Y. Gas chromatographic method for detecting three kinds of fusarium mycotoxin in grains[J]. Acta Aacademiae Medicinae Qingdao Universitatis, 2001, 37(1): 35–36. (in Chinese).

[22] LIANG Y, ZHANG CH, LIU LW. Determination of trichothecenes B in feed by gas chromatography-mass spectrometry[J]. Feed Industry, 2006, 27(5): 20–21. (in Chinese).

[23] WANG SX, ZHANG Y, ZHENG CM, *et al*. Application of liquid chromatography-mass spectrometry in simultaneous detection of multicomponent mycotoxins in grain[C]. The 14th international grain technology and bread conference and intern ational oil and Oil Development Forum, 2012. (in Chinese).

[24] JODLBAUER J, ZOILLNER P, LINDNER W. Determination of zearalenone and its metabolites in urine and tissue samples of cow andpig by LC-MS/MS[J]. Mycotoxin Research, 2000, 16(2): 174–178.

[25] REN YP, ZHANG N, SHAO SL, *et al*. Determination of multicomponent mycotoxin residues in food and feed by liquid chromatography-mass spectrometry[C]. Abstracts of the 5th annual meeting of China society of food science and technology and the 4th East West food industry high level Forum, 2007. (in Chinese).

[26] YING YF, ZHU CY, WEI MY, *et al*. Simultaneous determination of 14 mycotoxins and analogues in feeds by liquid chromatography-tandem mass spectrometry[J]. Chinese Journal of Analytical Chemistry, 2010, 38(12): 1759–1764. (in Chinese).

[27] ZHU ZY, SONG H, FENG M, *et al*. Method for the Simultaneous determination of various mycotoxins residues in peanut and its products[J]. Chemical Analysis and Meterage, 2012, 21(6): 40–43. (in Chinese).

[28] REN BB. Detection of mycotoxins in food by liquid chromatography tandem mass spectrometry[D]. Shijiazhuang:Hebei Normal University, 2013. (in chinese).

[29] HAN JZ, TIAN XL, CONG X, *et al*. Determination of six zearalenone mycotoxins residues in milk by ultra high performance liquid chromatography tandem mass spectrometry[J]. China Animal Husbandry & Veterinary Medicine, 2013, 40(S): 46–49.(in Chinese).

[30] LIU HH, KANG L, MAO LS, *et al*. Determination of dichorodimethyl pyridol in poultry by high performance liquid clromatography-tandem mass spectrometry[J].Chinese Journal Of Health Laboratory Technology, 2014, 24 (5): 633–638. (in Chinese).