R. Bras. Zootec., 52:e20220155, 2023
https://doi.org/10.37496/rbz5220220155

Non-ruminants
Short communication

Revista Brasileira de Zootecnia

Brazilian Journal of Animal Science
e-ISSN 1806-9290
www.rbz.org.br

Deoxynivalenol concentrations in feed ingredients and swine diets measured by enzyme-linked immunosorbent assay and high-performance liquid chromatography

Jong Young Ahn[1], Dahyun Cheong[1], Jin Young Jeong[2], Beob Gyun Kim[1]*

[1] Konkuk University, Department of Animal Science and Technology, Seoul, Republic of Korea.
[2] Rural Development Administration, National Institute of Animal Science, Animal Nutrition & Physiology Division, Jeollabuk-do, Republic of Korea.

***Corresponding author:**
bgkim@konkuk.ac.kr

Received: November 17, 2022
Accepted: March 8, 2023

How to cite: Ahn, J. Y.; Cheong, D.; Jeong, J. Y. and Kim, B. G. 2023. Deoxynivalenol concentrations in feed ingredients and swine diets measured by enzyme-linked immunosorbent assay and high-performance liquid chromatography. Revista Brasileira de Zootecnia 52:e20220155. https://doi.org/10.37496/rbz5220220155

ABSTRACT - The objective was to compare deoxynivalenol (DON) concentrations in feed ingredients and commercial swine diets measured by enzyme-linked immunosorbent assay (ELISA) and high-performance liquid chromatography (HPLC). Seventy feed ingredient samples consisted of corn, corn dried distillers grains with solubles, corn gluten feed, corn gluten meal, palm kernel expellers, rice bran, soy hulls, soybean meal, and wheat. Commercial swine diet samples (n = 92) were collected from 23 swine farms of varying regions in Korea and different growth stages of pigs. The DON concentration of all samples was determined in duplicate. Statistical comparisons were performed to compare the analytical methods (ELISA vs. HPLC), diet phases, and regions. The DON concentrations in most ingredients and all diets determined by ELISA method were greater than those determined by HPLC. The DON concentrations determined by the ELISA method were less than 1 mg/kg in all ingredients except corn dried distillers grains with solubles and corn gluten feed, and those determined by the HPLC were less than 0.5 mg/kg in all ingredients. The DON concentrations in complete diets did not vary by region or growth stages of pigs. The DON concentrations in most feed ingredients and commercial swine diets determined by ELISA method are greater than those determined by HPLC, but does not vary by regions in Republic of Korea or pig growth stages.

Keywords: analytical methods, mycotoxin, pigs, vomitoxin

1. Introduction

Deoxynivalenol (DON), naturally produced by the Fusarium species, is one of the mycotoxins commonly found in feed ingredients including cereal grains and byproducts (Tiemann et al., 2006; Rasmussen et al., 2012). Mycotoxins can be produced during the pre-harvesting period, as well as in the post-harvesting period. The environmental conditions of storage are one of the major factors contributing to the occurrence of mycotoxins (Homdork et al., 2000; Mannaa and Kim, 2017). A hot and humid environment, for example, can cause mycotoxin contamination during storage of feed ingredients and animal diets (Agriopoulou et al., 2020).

Dietary DON causes feed intake reduction, growth retardation, nutrient digestibility reduction, and even vomiting (Diekman and Green, 1992; Jo et al., 2016). In Korea, most feed ingredients used to produce commercial swine diets are imported from other countries. Feed ingredients are shipped for long distances, which can be a reason for the productions of molds and mycotoxins (Tang et al., 2019). In addition, the summer season in Korea is hot and humid, resulting in reduced feed intake for pigs, so the detrimental effects of dietary DON including feed intake reduction and growth retardation can be more critical during that period. Moreover, the recent climate change can increase occurrence risk of DON in the feed ingredients and diets.

Generally, DON concentrations in feed ingredients and diets are analyzed by using enzyme-linked immunosorbent assay (ELISA) and high-performance liquid chromatography (HPLC). However, large variations have been reported in values for DON concentrations among different analytical methods (Ghali et al., 2009; Ekwomadu et al., 2021). One of our hypotheses was that the analytical methods potentially affect DON concentrations in feed ingredients and diets. We also hypothesized that dietary DON concentrations may vary by region or growth stage of pigs. The objective of this study was to compare DON concentrations in feed ingredients and commercial swine diets by regions and growth phases measured by ELISA and HPLC.

2. Material and Methods

Feed ingredient samples (n = 70) and commercial swine diet samples (n = 92) were collected in Korea from May 2021 to July 2021. All samples were finely ground (< 0.1 mm) and stored at 4 °C in the refrigerator before analysis. A total of 70 feed ingredient samples consisting of corn (n = 6), corn dried distillers grains with solubles (DDGS; n = 8), corn gluten feed (n = 7), corn gluten meal (n = 9), palm kernel expellers (n = 8), rice bran (n = 9), soy hulls (n = 7), soybean meal (n = 8), and wheat (n = 8) were collected. The feed ingredient samples used in this study were collected at feed plants. Ninety-two commercial swine diet samples from 23 swine farms were collected from varying regions and growth phases. The regions were five provinces in Korea: Chungcheong (n = 19), Gangwon (n = 15), Gyeonggi (n = 19), Gyeongsang (n = 20), and Jeolla (n = 19). The growth phases of pigs were nursery (n = 23), growing (n = 21), gestating (n = 24), and lactating stages (n = 24). All diet samples were collected directly at a feeder in pig houses for each growth phase.

All experiments and analyses were conducted in a biosafety level 1 containment facility in Seoul, Republic of Korea (37°32'24.8" N, 127°04'27.5" E). The ELISA test kit (Romer Labs Inc., Singapore, Republic of Singapore) for DON with a quantification range of 250 to 5,000 ng/mL (AgraQuant® Deoxynivalenol; COKAQ4000) was used to determine DON concentrations in ingredient and diet samples. The analysis was performed according to the manufacturer's instructions. Briefly, 20 g of finely ground (< 0.1 mm) sample was moved into Erlenmeyer flask and shaken for 3 min with 100 mL of distilled water. The extract was filtered with Whatman #1 filter, and 10 mL of extract was diluted with 30 mL of distilled water. The DON concentrations in the prepared samples were measured by using the ELISA test kit and microplate reader at 450 nm. All samples and standards were analyzed in duplicate.

For the HPLC procedure, 25 g of ground sample and 5 g of sodium chloride were transferred into a 500 mL Erlenmeyer flask. Then, 200 mL of distilled water was added and homogenized for 2 min with a shaking incubator. After this, the 40 mL of supernatant extract was transferred into a 50 mL conical tube and centrifuged at 2,469 g for 10 min (Hettich Rotofix 32A, Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany), and adjusted to approximately pH 7.4 using 2 M sodium hydroxide. The whole supernatant extract was filtered through a microfiber filter paper. Two milliliters of filtrate were passed through the immunoaffinity chromatography (IAC) column (ZORBAX Eclipse XDB-C18 column, 4.6 × 250 mm, 5 μM, Agilent, USA) at a flow rate of 2 mL per min. The columns were washed with 10 mL of distilled water at a flow rate of 5 mL per min. The mycotoxins were eluted by passing 1.5 mL of 100% methanol through the column at a flow rate of one drop per second. The extracts were collected in a glass tube and dried with nitrogen at 60 °C. One milliliter of mobile phase (0.01% of

acetic acid in deionized water:methanol (85:15, v/v)) was added to the dried glass tube and vortexed for 1 min, and then 100 µL of solution was transferred to an HPLC vial for the deoxynivalenol analysis. Deoxynivalenol was determined using a mobile phase for 20 min at a flow rate of 0.8 mL/min under UV light at a wavelength of 220 nm at 45 °C. The DON peak has a retention time of 14.9 min. All samples and standards were analyzed in duplicate.

Data were analyzed by MIXED procedure of SAS (Statistical Analysis System, version 9.4) to compare the DON concentration by the analytical methods (ELISA vs. HPLC) or by the diet phases and regions. The statistical model used for the comparison of the analytical methods was:

$$Y_{ij} = \mu + A_i + b_j + \varepsilon_{ij}, \quad (1)$$

in which Y_{ij} is the response variable measured in the i-th analytical methods at the j-th replication block, μ is the overall mean, A_i is the fixed effect of i-th analytical method, b_j is the random effect of the j-th replication block, and ε_{ij} is the random error term.

The statistical model used for testing the influence of region and growth stage of pigs was:

$$Y_{ijk} = \mu + R_i + G_j + b_k + \varepsilon_{ijk}, \quad (2)$$

in which Y_{ijk} is the response variable measured in the i-th region and j-th growth stage at the k-th replication block, μ is the overall mean, R_i is the fixed effect of i-th region, G_j is the fixed effect of j-th growth stage, b_k is the random effect of the k-th replication block, and ε_{ijk} is the random error term. The experimental unit was each sample. The statistical significance was declared at an alpha less than 0.05.

3. Results

The DON concentrations determined by the ELISA method in all ingredients, except corn DDGS (2.614 mg/kg) and corn gluten feed (2.344 mg/kg), were less than 1 mg/kg (Table 1). The DON concentrations determined by the HPLC method in all ingredients were less than 0.5 mg/kg. The DON concentrations in corn DDGS, corn gluten feed, rice bran, soy hulls, soybean meal, and wheat determined by the ELISA method were greater (P<0.05) than those determined by the HPLC method.

The DON concentrations in commercial feeds determined by the ELISA method were greater (P<0.001) than those determined by the HPLC method (Table 2). The DON concentrations did not vary by farm region or pig growth stage regardless of the analytical methods (ELISA or HPLC).

Table 1 - Deoxynivalenol concentrations in feed ingredients measured by enzyme-linked immunosorbent assay (ELISA) and high-performance liquid chromatography (HPLC)[1]

Ingredient	No. of samples	ELISA (mg/kg)	HPLC (mg/kg)	SEM	P-value
Corn	6	0.093	0.013	0.043	0.222
Corn DDGS	8	2.614	0.411	0.439	0.004
Corn gluten feed	7	2.344	0.304	0.333	0.001
Corn gluten meal	9	0.099	ND	0.012	0.078
Palm kernel expellers	8	0.034	0.004	0.016	0.210
Rice bran	9	0.936	0.066	0.236	0.020
Soy hulls	7	0.244	0.001	0.045	0.003
Soybean meal	8	0.177	0.001	0.057	0.049
Wheat	8	0.069	0.003	0.017	0.018

DDGS - dried distillers grains with solubles; ND - not detected; SEM - standard error of the mean.
[1] All samples were analyzed in duplicate.

Table 2 - Deoxynivalenol concentrations in commercial swine diets measured by enzyme-linked immunosorbent assay (ELISA) and high-performance liquid chromatography (HPLC)[1]

Item	No. of samples	ELISA (mg/kg)	HPLC (mg/kg)	SEM	P-value
Region					
Chungcheong	19	0.918	0.052	0.051	<0.001
Gangwon	15	1.195	0.062	0.121	<0.001
Gyeonggi	19	0.884	0.052	0.067	<0.001
Gyeongsang	20	1.509	0.046	0.168	<0.001
Jeolla	19	1.205	0.061	0.132	<0.001
SEM	-	0.167	0.007	-	-
P-value	-	0.051	0.514	-	-
Growth phase					
Nursery pigs	23	1.149	0.046	0.125	<0.001
Growing pigs	21	1.162	0.050	0.120	<0.001
Gestating pigs	24	1.023	0.057	0.094	<0.001
Lactating pigs	24	1.244	0.064	0.101	<0.001
SEM	-	0.155	0.006	-	-
P-value	-	0.782	0.227	-	-

SEM - standard error of the mean.
[1] All samples were analyzed in duplicate.

4. Discussion

Dietary DON leads to harmful effects on growth performance in pigs, derived from reduced feed intake and weight gain (Mok et al., 2013; Kim et al., 2021). Moreover, ingested DON via contaminated diets can be accumulated in various organs of pigs (Goyarts et al., 2007). Most countries regulate mycotoxin concentrations in feed ingredients and complete diets, and regulatory limits or guideline values are provided (van Egmond and Jonker, 2004). In the current study, DON concentrations in all feed ingredients and diets were less than the recommended concentrations in the Republic of Korea (ingredients = 10 mg/kg, swine diets = 0.9 mg/kg regardless of growth stages).

Several analytical methods are available for determining DON concentrations in animal feeds (Turner et al., 2015). The HPLC method has been considered as a reference method for mycotoxin determinations (Zheng et al., 2003; Binder, 2007). The advantages of the HPLC method include high sensitivity and repeatability, but the HPLC method demands time, costs, and practiced skills (Wolf and Schweigert, 2018). In contrast, the ELISA method is one of most rapid techniques for detection of mycotoxins. Due to several advantages including simplicity, less time-consuming task, and multiple analyses, the ELISA method has been widely employed for mycotoxin analysis (Wolf and Schweigert, 2018). However, there has been a concern on the accuracy of measured values resulting from matrix effects in ELISA methods, which can cause underestimation or overestimation compared with the reference values determined by HPLC or gas chromatography methods (Janik et al., 2021).

The greater DON concentrations in the ELISA method compared with the HPLC method observed in the present study are in agreement with a previous study (Matić et al., 2011), indicating that the ELISA method potentially overestimates DON concentrations in feed ingredients given that the HPLC method provides true values likely due to the matrix effect and quantification of multiple DON precursors as DON in the ELISA method. In the present work, the direct ELISA method employing the antigen-antibody reaction was used for the analysis of DON concentrations in feed ingredients (Xu et al., 1988) and the HPLC method using ultraviolet detection was used for quantifying DON concentrations (Visconti and Bottalico, 1983). In DON-contaminated feed ingredients, DON precursors including DON-3-glucoside, 3-acetyl-DON, and 15-acetyl-DON can co-occur with DON (Pestka, 2007), and these precursors are sometimes analyzed along with DON due to their similar molecular structures in the direct ELISA method but not in the HPLC method (Cavaliere et al., 2005). Although analyzed values from the ELISA method were greater than those from the HPLC method, the first is considered as a comparable method

for screening the DON in feed ingredients due to a high correlation between analyzed values from these two methods (Zheng et al., 2003; Tangni et al., 2011). Continuous monitoring of DON values analyzed by the ELISA with the HPLC method, as the reference values, should be needed for accuracy and reliability from the ELISA method (Matić et al., 2011).

A previous study reported large variations in mycotoxin concentrations in different regions in China (Guan et al., 2011). In addition, it has been shown that differences in occurrence and concentration of mycotoxins in feed ingredients according to the countries and regions (Rodrigues and Naehrer, 2012; Schatzmayr and Streit, 2013) are likely due to different climates during harvesting crops. With the same token, the concentrations of DON in corn and corn DDGS in the US have been reported to have year-to-year and regional variations (Zhang and Caupert, 2012). However, the regional differences in the DON concentrations were not observed in this study. This discrepancy may be due to the fact that most feed ingredients used in Korea are imported from other countries.

Mycotoxin concentrations in diets may vary by pig growth stage likely due to the different DON concentrations in ingredients and the different inclusion rates of individual ingredients. A previous study reported variations in aflatoxin B1 and ochratoxin A concentrations in complete diets among pig growth stages in the Beijing region, China, but not in DON concentrations (Li et al., 2014). This indicates that DON concentrations may have not deviated much among feed ingredients. In the present work, similarly, DON concentrations were not affected by pig growth phase. However, if a specific ingredient such as corn gluten feed, corn DDGS, or barley is highly contaminated with DON (Kong et al., 2015; Leni et al., 2019), the DON concentration in complete diets can dramatically increase depending on the inclusion rate of high-DON ingredient.

5. Conclusions

Overall, deoxynivalenol concentrations in most ingredients and commercial pig diets determined by the enzyme-linked immunosorbent assay method are greater than those determined by high-performance liquid chromatography method, but do not vary by region in Republic of Korea or growth stage of pigs. The deoxynivalenol concentrations in feed ingredients and commercial pig diets are lower than regulatory levels in Republic of Korea.

Conflicts of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: J.Y. Jeong and B.G. Kim. Formal analysis: D. Cheong. Investigation: J.Y. Ahn and D. Cheong. Supervision: B.G. Kim. Writing – original draft: J.Y. Ahn. Writing – review & editing: D. Cheong, J.Y. Jeong, and B.G. Kim.

Acknowledgments

The authors are thankful to the Rural Development Administration, Republic of Korea (PJ015002), for the financial support. They are also thankful to Dr. Ah Reum Son, Mr. Jongkeon Kim, and Mr. Jeonghyeon Son, for technical support in manuscript preparation, and Hansol Kim, for the statistical support.

References

Agriopoulou, S.; Stamatelopoulou, E. and Varzakas, T. 2020. Advances in occurrence, importance, and mycotoxin control strategies: prevention and detoxification in foods. Foods 9:137. https://doi.org/10.3390/foods9020137

Binder, E. M. 2007. Managing the risk of mycotoxins in modern feed production. Animal Feed Science and Technology 133:149-166. https://doi.org/10.1016/j.anifeedsci.2006.08.008

Cavaliere, C.; D'Ascenzo, G.; Foglia, P.; Pastorini, E.; Samperi, R. and Lagana, A. 2005. Determination of type B trichothecenes and macrocyclic lactone mycotoxins in field contaminated maize. Food Chemistry 92:559-568. https://doi.org/10.1016/j.foodchem.2004.10.008

Diekman, M. A. and Green, M. L. 1992. Mycotoxins and reproduction in domestic livestock. Journal of Animal Science 70:1615-1627. https://doi.org/10.2527/1992.7051615x

Ekwomadu, T. I.; Dada, T. A.; Akinola, S. A.; Nleya, N. and Mwanza, M. 2021. Analysis of selected mycotoxins in maize from north-west South Africa using high performance liquid chromatography (HPLC) and other analytical techniques. Separations 8:143. https://doi.org/10.3390/separations8090143

Ghali, R.; Ghorbel, H. and Hedilli, A. 2009. Fumonisin determination in Tunisian foods and feeds. ELISA and HPLC methods comparison. Journal of Agricultural and Food Chemistry 57:3955-3960. https://doi.org/10.1021/jf803786h

Goyarts, T.; Danicke, S.; Valenta, H. and Ueberschar, K. H. 2007. Carry-over of *Fusarium* toxins (deoxynivalenol and zearalenone) from naturally contaminated wheat to pigs. Food Additives & Contaminants 24:369-380. https://doi.org/10.1080/02652030600988038

Guan, S.; Gong, M.; Yin, Y. L.; Huang, R. L.; Ruan, Z.; Zhou, T. and Xie, M. Y. 2011. Occurrence of mycotoxins in feeds and feed ingredients in China. Journal of Food, Agriculture & Environment 9:163-167.

Homdork, S.; Fehrmann, H. and Beck, R. 2000. Influence of different storage conditions on the mycotoxin production and quality of *Fusarium*-infected wheat grain. Journal of Phytopathology 148:7-15.

Janik, E.; Niemcewicz, M.; Podogrocki, M.; Ceremuga, M.; Gorniak, L.; Stela, M. and Bijak, M. 2021. The existing methods and novel approaches in mycotoxins' detection. Molecules 26:3981. https://doi.org/10.3390/molecules26133981

Jo, H.; Kong, C.; Song, M. and Kim, B. G. 2016. Effects of dietary deoxynivalenol and zearalenone on apparent ileal digestibility of amino acids in growing pigs. Animal Feed Science and Technology 219:77-82. https://doi.org/10.1016/j.anifeedsci.2016.06.006

Kim, J.; Jeong, J. Y.; Sung, J. Y. and Kim, B. G. 2021. Equations to predict growth performance changes by dietary deoxynivalenol in pigs. Toxins 13:360. https://doi.org/10.3390/toxins13050360

Kong, C.; Shin, S. Y.; Park, C. S. and Kim, B. G. 2015. Effects of feeding barley naturally contaminated with *Fusarium* mycotoxins on growth performance, nutrient digestibility, and blood chemistry of gilts and growth recoveries by feeding a non-contaminated diet. Asian-Australasian Journal of Animal Sciences 28:662-670. https://doi.org/10.5713/ajas.14.0707

Leni, G.; Cirlini, M.; Jacobs, J.; Depraetere, S.; Gianotten, N.; Sforza, S. and Dall'Asta, C. 2019. Impact of naturally contaminated substrates on *Alphitobius diaperinus* and *Hermetia illucens*: uptake and excretion of mycotoxins. Toxins 11:476. https://doi.org/10.3390/toxins11080476

Li, X. Y.; Zhao, L. H.; Fan, Y.; Jia, Y. X.; Sun, L.; Ma, S. S.; Ji, C.; Ma, Q. G. and Zhang, J. Y. 2014. Occurrence of mycotoxins in feed ingredients and complete feeds obtained from the Beijing region of China. Journal of Animal Science and Biotechnology 5:37. https://doi.org/10.1186/2049-1891-5-37

Mannaa, M. and Kim, K. D. 2017. Influence of temperature and water activity on deleterious fungi and mycotoxin production during grain storage. Mycobiology 45:240-254. https://doi.org/10.5941/MYCO.2017.45.4.240

Matić, J. J.; Jajić, I. M.; Šarić, B. M.; Mišan, A. Č.; Krstović, S. Z. and Mandić, A. I. 2011. ELISA and HPLC analyses of deoxynivalenol in maize and wheat. Zbornik Matice Srpske za Prirodne Nauke 120:25-32.

Mok, C. H.; Shin, S. Y. and Kim, B. G. 2013. Aflatoxin, deoxynivalenol, and zearalenone in swine diets: predictions on growth performance. Revista Colombiana de Ciencias Pecuarias 26:243-254.

Pestka, J. J. 2007. Deoxynivalenol: toxicity, mechanisms and animal health risks. Animal Feed Science and Technology 137:283-298. https://doi.org/10.1016/j.anifeedsci.2007.06.006

Rasmussen, P. H.; Nielsen, K. F.; Ghorbani, F.; Spliid, N. H.; Nielsen, G. C. and Jorgensen, L. N. 2012. Occurrence of different trichothecenes and deoxynivalenol-3-β-D-glucoside in naturally and artificially contaminated Danish cereal grains and whole maize plants. Mycotoxin Research 28:181-190. https://doi.org/10.1007/s12550-012-0133-z

Rodrigues, I. and Naehrer, K. 2012. A three-year survey on the worldwide occurrence of mycotoxins in feedstuffs and feed. Toxins 4:663-675. https://doi.org/10.3390/toxins4090663

Schatzmayr, G. and Streit, E. 2013. Global occurrence of mycotoxins in the food and feed chain: facts and figures. World Mycotoxin Journal 6:213-222. https://doi.org/10.3920/Wmj2013.1572

Tang, E. N.; Ndindeng, S. A.; Bigoga, J.; Traore, K.; Silue, D. and Futakuchi, K. 2019. Mycotoxin concentrations in rice from three climatic locations in Africa as affected by grain quality, production site, and storage duration. Food Science & Nutrition 7:1274-1287. https://doi.org/10.1002/fsn3.959

Tangni, E. K.; Motte, J. C.; Callebaut, A.; Chandelier, A.; De Schrijver, M. and Pussemier, L. 2011. Deoxynivalenol loads in matched pair wheat samples in Belgium: comparison of ELISA VERATOX kit against liquid chromatography. Mycotoxin Research 27:105-113. https://doi.org/10.1007/s12550-010-0081-4

Tiemann, U.; Brussow, K. P.; Jonas, L.; Pohland, R.; Schneider, F. and Danicke, S. 2006. Effects of diets with cereal grains contaminated by graded levels of two *Fusarium* toxins on selected immunological and histological measurements in the spleen of gilts. Journal of Animal Science 84:236-245. https://doi.org/10.2527/2006.841236x

Turner, N. W.; Bramhmbhatt, H.; Szabo-Vezse, M.; Poma, A.; Coker, R. and Piletsky, S. A. 2015. Analytical methods for determination of mycotoxins: An update (2009-2014). Analytica Chimica Acta 901:12-33. https://doi.org/10.1016/j.aca.2015.10.013

van Egmond, H. P. and Jonker, M. 2004. Worldwide regulations for mycotoxins in food and feed in 2003. Food and Agriculture Organization of the United Nations, Rome.

Visconti, A. and Bottalico, A. 1983. Detection of *Fusarium* trichothecenes (nivalenol, deoxynivalenol, fusarenone and 3-acetyldeoxynivalenol) by high-performance liquid chromatography. Chromatographia 17:97-100. https://doi.org//10.1007/BF02259348

Wolf, K. and Schweigert, F. J. 2018. Mycotoxin analysis: A focus on rapid methods. Partnership for Aflatoxin Control in Africa, African Union Commission, Addis Ababa, Ethiopia.

Xu, Y. C.; Zhang, G. S. and Chu, F. S. 1988. Enzyme-linked immunosorbent assay for deoxynivalenol in corn and wheat. Journal of AOAC International 71:945-949. https://doi.org/10.1093/jaoac/71.5.945

Zhang, Y. and Caupert, J. 2012. Survey of mycotoxins in U.S. distiller's dried grains with solubles from 2009 to 2011. Journal of Agricultural and Food Chemistry 60:539-543. https://doi.org/10.1021/jf203429f

Zheng, Z.; Houchins, D.; Ung, J. and Richard, J. L. 2003. Validation of an ELISA test kit for the detection of deoxynivalenol in several food commodities. JSM Mycotoxins 2003(Suppl3):295-302. https://doi.org/10.2520/myco1975.2003.Suppl3_295