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# Polyclonal Antibodies against Zearalenone: Production, Characterisation, and Application in Food Safety Biosensors

**Nur Azura Mohd Said [1]*, Norhafniza Awaludin[1], Mohammad Rejab Ismail[2], Hazana Razali[3], Erna Mutiara Masdek[1], Sahira Akmar Zulkepli[1], and Syah Noor Muhammad Ramli[1]**

*[1]Biotechnology & Nanotechnology Research Centre, MARDI Headquarter, Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia*
*[2]MARDI Arau, Lot PT3747 Tambun Tulang, 02600 Arau, Perlis, Malaysia*
*[3]Biogenes Technologies, Jalan Maklumat, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia*

## ABSTRACT

Zearalenone (ZEA), a mycotoxin with estrogenic effects, poses significant risks to food and feed safety. This study reports the production and characterisation of polyclonal antibodies (pAb) against ZEA for potential biosensor applications. New Zealand White rabbits were immunized with a ZEA–protein conjugate emulsified in Freund's adjuvant, following approved animal ethics guidelines. Serum antibodies were purified using ammonium sulfate precipitation, dialysis and Protein A affinity chromatography on an ÄKTA Prime system. Antibody titers, determined by indirect ELISA, showed strong immune responses in both rabbits, with the second bleed demonstrating optimal concentration and stability. Cross-reactivity was evaluated via competitive ELISA against aflatoxins, ochratoxins, and fumonisins at 20, 50, and 100 ppb. The antibodies exhibited high specificity for ZEA (100% reactivity) with minimal cross-reactivity (13–19%) toward other mycotoxins. Preimmune serum showed negligible reactivity, confirming the specificity of the immune response. These findings highlight the potential of the generated pAb as reliable bioreceptor for electrochemical biosensors, offering sensitive and selective detection of ZEA in food safety monitoring. Future work will integrate these antibodies into sensor platforms to improve detection limits and extend applicability across diverse agricultural commodities.

*Keywords:* Biosensor, grain corn, mycotoxins, polyclonal antibody, zearalenone

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*E-mail addresses*:
nazurams@mardi.gov.my (Nur Azura Mohd Said)
hafniza@mardi.gov.my (Norhafniza Awaludin)
mdrejab@mardi.gov.my (Mohammad Rejab Ismail)
hazanarazali82@gmail.com (Hazana Razali)
emutiara@mardi.gov.my (Erna Mutiara Masdek)
sahira@mardi.gov.my (Sahira Akmar Zulkepli)
syahnoor@mardi.gov.my (Syah Noor Muhammad Ramli)
* Corresponding author

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## INTRODUCTION

Zearalenone (ZEA), a *Fusarium*-produced mycotoxin, contaminates grains such as corn, wheat, barley, rice and oats. Grain corn, a key component in animal feed, is particularly vulnerable. A Department of Veterinary Services report detected *Fusarium* mycotoxins—fumonisins (FUMs), zearalenone (ZEA), and deoxynivalenol (DON)—in all grain corn samples from Peninsular Malaysia (Syahidah et al., 2021). Similarly, a study conducted in grain corn plantations in Terengganu, Malaysia highlighted *Fusarium* species as major contributors to ZEA contamination, with tassels exhibiting the highest fungal loads due to airborne spores during the cropping season (Yazid et al., 2021).

Although less toxic than aflatoxins, ZEA's estrogenic effects pose serious risks to livestock, particularly swine- leading to reproductive disorders, infertility and hyperestrogenism, which can cause endometrial hyperplasia and malignancy. Reliable detection is crucial for feed safety. While gold-standard methods like HPLC, LC-MS and GC offer high sensitivity, they require extensive preparation, skilled personnel and lab-bound equipment, limiting field use. Enzyme-linked immunosorbent assay (ELISA), though widely used, is susceptible to light interference and remains laboratory-dependent.

Immunosensors offer a promising alternative, enabling rapid, on-site mycotoxin detection through antibody-based transduction. This study focuses on developing polyclonal antibodies against ZEA for biosensor applications in grain corn, with potential for broader food safety monitoring. Future innovationsincluding nanomaterials and IoT-based platformscould further enhance biosensor performance, improving accessibility and reliability in agricultural mycotoxin detection.

## MATERIALS AND METHODS

### Production and Purification of Polyclonal Antibodies against ZEA

Polyclonal antibodies against ZEA were produced by immunizing New Zealand White rabbits, following the guidelines outlined by Leenaars and Hendriksen (2007). Two rabbits (Z1 and Z2) were immunized with a ZEA-protein carrier conjugate mixed with Freund's adjuvant, following a protocol approved by MARDI's Animal Ethics Committee (Approval Number: 20230622/R/MAEC00139). Immunizations and blood collections were conducted fortnightly until the fifth booster. Post-immunization, rabbits were euthanized and incinerated per ethical guidelines. Serum purification involved ammonium sulfate precipitation, dialysis and protein A column chromatography using an AKTA-Prime system. IgG fractions were pooled, neutralized with Tris-HCl and dialyzed, yielding purified antibodies for further use (Kent, 1999).

### Characterisation of Polyclonal Antibodies against ZEA

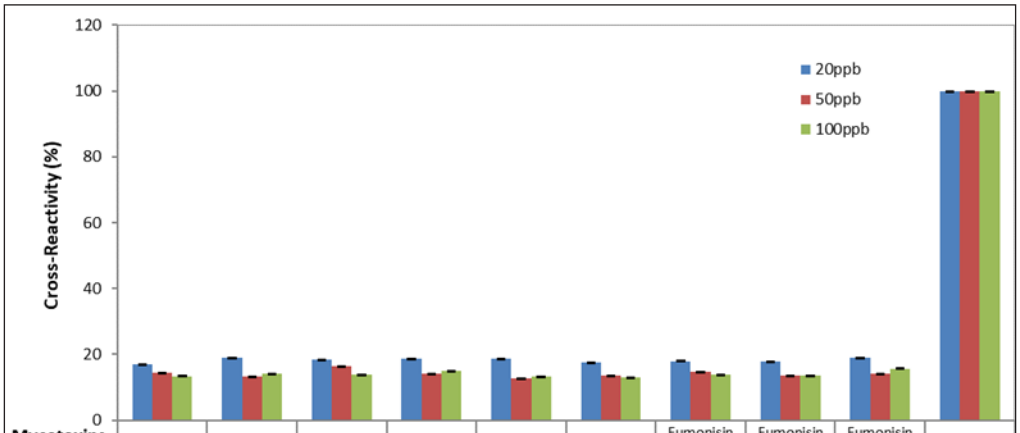
The antibody titer was determined using an indirect ELISA (Liu et al., 1985). Microtiter wells were coated with ZEA-KLH antigen, blocked with dry milk, and incubated with anti-ZEA antibody at varying concentrations. Anti-rabbit Alkaline Phosphatase (AP)-conjugated secondary antibody and p-Nitrophenyl Phosphate (pNPP) substrate were used, with absorbance measured at 405 nm.

Cross-reactivity of the anti-ZEA antibody was assessed via competitive ELISA against mycotoxins (Garg et al., 2022), including aflatoxins, ochratoxins, fumonisins and zearalenone, at concentrations of 20, 50, and 100 ppb.

## RESULTS AND DISCUSSION

The antibody titer analysis showed a sigmoidal decrease in absorbance with increasing antibody dilution for both Rabbit 1 (Z1) and Rabbit 2 (Z2), while preimmune serum controls exhibited negligible absorbance, confirming successful immunization and robust antibody production (Liu et al., 1985). The second bleed from both rabbits demonstrated the highest antibody titer, with Z2 showing slightly higher absorbance values at lower dilutions, suggesting a stronger immune response.

Cross-reactivity analysis of the anti-ZEA antibody from the third bleed of Rabbit Z2 demonstrated high specificity for ZEA (100% reactivity) with minimal cross-reactivity (13–19%) to other mycotoxins, such as aflatoxins, ochratoxins, and fumonisins (Figure 1). Although cross-reactivity with ZEA analogues (e.g., α-zearalenol, β-zearalenol) was




| Mycotoxins | Aflatoxin B1 | Aflatoxin B2 | Aflatoxin G1 | Aflatoxin G2 | Ochratoxin A | Ochratoxin B | Fumonisin B1 | Fumonisin B2 | Fumonisin B3 | Zearalenone |
|---|---|---|---|---|---|---|---|---|---|---|
| 20ppb | 17.07 | 18.97 | 18.47 | 18.67 | 18.62 | 17.52 | 17.95 | 17.77 | 18.93 | 100.00 |
| 50ppb | 14.30 | 13.33 | 16.29 | 14.23 | 12.76 | 13.50 | 14.63 | 13.67 | 14.07 | 100.00 |
| 100ppb | 13.42 | 14.08 | 13.73 | 14.91 | 13.39 | 12.90 | 13.75 | 13.48 | 15.68 | 100.00 |

*Figure 1*. Cross-reactivity analysis of anti-ZEA antibody (third bleed, Z2, 0.1 mg/mL) against various mycotoxins at different concentrations (20 ppb, 50 ppb and 100 ppb)

not assessed in this study, previous research suggest that polyclonal antibodies against ZEA can detect related compounds with up to 60% cross-reactivity (Thongrussamee et al., 2008; Wang et al., 2021).

Overall, the results confirm the high specificity and strong binding affinity of this antibody batch, making it a promising candidate for use in the development of a ZEA biosensor. Its minimal cross-reactivity with other mycotoxins ensures reliable detection, reinforcing its suitability for biosensor applications in food safety monitoring.

## CONCLUSION

This study successfully generated and characterized a high-affinity polyclonal antibody against ZEA. The antibody from the third bleed of Rabbit Z2 exhibited exceptional specificity for ZEA, with minimal cross-reactivity against other mycotoxins. These findings highlight its potential as a biorecognition element in ZEA biosensors for food and feed safety, particularly in grain corn monitoring. Its high specificity and strong binding affinity enable accurate and sensitive detection with minimal interference. Future research should focus on optimizing biosensor integration and evaluating performance in real-world food matrices to enhance practical applications in food safety systems.

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## REFERENCES

Garg, K., Villavicencio-Aguilar, F., Solano-Rivera, F., & Gilbert, L. (2022). Analytical validation of a direct competitive ELISA for multiple mycotoxin detection in human serum. *Toxins, 14*(11), Article 727. https://doi.org/10.3390/toxins14110727

Kent, U. M. (1999). Purification of antibodies using ammonium sulfate fractionation or gel filtration. In L.C. Javois (Eds) *Immunocytochemical methods and protocols* (pp. 11-18). Humana Press. https://doi.org/10.1385/1-59259-213-9:11

Leenaars, M., & Hendriksen, C. F. (2005). Critical steps in the production of polyclonal and monoclonal antibodies: Evaluation and recommendations. *ILAR Journal, 46*(3), 269-279. https://doi.org/10.1093/ilar.46.3.269

Liu, M. T., Ram, B. P., Hart, L. P., & Pestka, J. J. (1985). Indirect enzyme-linked immunosorbent assay for the mycotoxin zearalenone. *Applied and Environmental Microbiology, 50*(2), 332-336. https://doi.org/10.1128/aem.50.2.332-336.1985

Syahidah, H. W., Suhaimi, D., Hartini, Y., & Nurulhayati, A. B. (2021, August 3-4). *Screening of fungal contamination of Aspergillus and Fusarium mycotoxin in grain corn from Peninsular Malaysia, in Proc.* [Paper presentation]. 40th Malaysia Society of Animal Production Annual Conference (MSAP), Malaysia.

Thongrussamee, T., Kuzmina, N. S., Shim, W. B., Jiratpong, T., Eremin, S. A., Intrasook, J., & Chung, D. H. (2008). Monoclonal-based enzyme-linked immunosorbent assay for the detection of zearalenone in cereals. *Food Additives and Contaminants, 25*(8), 997-1006. https://doi.org/10.1080/02652030701823159

Wang, Y., Wang, X., Zhang, H., Jiang, J., & Fotina, H. (2021). Synthesis of zearalenone immunogen and comparative analysis of antibody characteristics. *International Journal of Analytical Chemistry, 2021*(1), Article 7109383. https://doi.org/10.1155/2021/7109383

Yazid, S. N. E., Ng, W. J., Selamat, J., Ismail, S. I., & Samsudin, N. I. P. (2021). Diversity and toxigenicity of mycobiota in grain corn: A case study at pioneer grain corn plantations in Terengganu, Malaysia. *Agriculture, 11*(3), Article 237. https://doi.org/10.3390/agriculture11030237