



FINAL YEAR DISSERTATION RESEARCH REPORT

**EVALUATION OF GROWTH PERFORMANCE OF WISTAR RATS FED ON AN
ENZYMATICALLY DETOXIFIED AFLATOXIN B1 MAIZE DIET.**

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**THIS FINAL YEAR RESEARCH DISSERTATION IS SUBMITTED TO THE
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PRODUCTION AND MANAGEMENT.**

DECLARATION

I declare that the information in this research dissertation was done by me and it has never been submitted to any institution for academic ward.

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APPROVAL

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DEDICATION

I dedicate this research desertion to the Aflatoxin project, Mr. Muyinda Robert and Dr. Zirintunda Gerald, my family and friends for the great support towards this research to see it come to accomplishment. may the good lord bless you.

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LIST OF ABBREVIATIONS

AFB1:-	Aflatoxin B 1
AFM1:-	Aflatoxin M1
AFQ1:-	Aflatoxin Q1
AFP1:-	Aflatoxin P1
DMP:-	2,6-dimethoxyphenol
ABTS:-	2,2'-azinobis
COVAB: -	Collage of Veterinary Medicine, Animal Resources and Bio-security
μ: -	micro
n: -	nano
L: -	liter
A.F: -	Aflatoxin
g: -	grams
mls: -	milliliters
S.I: -	international system of units.
M.F I: -	mean feed intake
M.F.C.R: -	mean feed conversion ratio
Hrs.: -	hours

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ABSTRACT

This research was aimed at evaluating the growth performance of wistar rats fed on enzymatically detoxified aflatoxin B1 maize diet.

In vivo research was carried on 20 male wistar rats, grouped into groups of five rats per cage for six weeks and fed on maize diet enzymatically detoxified at different time intervals of 0,4,12,24 hours, growth performance was determined using the parameters such as feed intake, weight gain and feed conversion ratio.

Results; mean feed intake of the control and treatment groups, Week 1 and week 4 was significantly different for the treatment and control groups with p values of 0.004 and 0.007 respectively. Week 2,3, and 5 had no significant difference among the treatment and control the groups with p values of 0.06, 0.19, 0.12 respectively that were greater than the 5%. In week one, group D had the highest feed intake of 0.027, followed group B 0.024, group C 0.023 and group A 0.017. In week two, group D 0.027, group B 0.024, group A 0.023, group C 0.022. In week three group D 0.024 group B 0.018, group C 0.017, group A 0.016. In week four, group D 0.023, group B 0.023, group C 0.018 and group A 0.016. In week five group D 0.021, B 0.019, group C and A group with 0.017. The mean weight gain of the control and treatment groups had no significant difference in all the five weeks of research with P values of 0.959, 0.994, 0.951, 0.821, 0.960 respectively. In week one A group had the highest mean weight gain of 0.068, group B 0.062, group C 0.059 and group D 0.057. In week two, group B 0.062, group D 0.059 and group C and group A had 0.058. In week three group D 0.059, group C with 0.0585, group B 0.058 and A group 0.052. In week four, group D and B 0.059 and group C and A 0.052. In week five group D 0.058, group B 0.057, and group C had 0.057 and group A had 0.049.

Findings of the study identified that, a group of rats fed on enzymatically detoxified AFB1 maize diet for 24hrs increased on the feed intake and weight gain growth, thus improving growth performance. Therefore, degradation of AFB1 contaminated maize by ligninolytic enzymes from spent mushroom substrate for time interval of 24hours is safe and effective.

Since this research was carried out on male wistar rats, I recommend a further study on the effect of enzymatically detoxified AFB1 maize diet on growth performance in other animal species.

CHAPTER ONE

1.1.0 Introduction

Globally aflatoxins are a worldwide health threat in both human and animals,(Mousavi Khaneghah et al., 2018), this affects maize quality along the production and consumption chain (Jallow et al., 2021) hence resulting into reduced consumption of maize as discovered in a research carried out among one population of China(F. Wu, 2015).Asia and Sub- Saharan- Africa

Maize contributes 63% to the world's feed industry (Kaul *et al.*, 2019). It is used as whole maize or processed into maize bran in livestock and poultry, and also maize flour for human consumption (Malhotra, 2017). The quality of maize feeds is lowered by AFB1 which are low- weight metabolites produced by *Aspergillus flavus* and *parasiticus*, that naturally contaminate feeds (Scarpari *et al.*, 2014). Aflatoxin contamination is as a result of; Poor storage methods, plant stress in the field, and environmental factors such as high humidity which favors formation of the toxic molds (Negash, 2018). Aflatoxicosis results in a number of health problems such as liver cancer, reduction in weight gain ,declining production in milk, and eggs, poor sperm quality in bulls, birds become prone to diseases, failure of vaccines (Godswill Awuchi *et al.*, 2020).These effects result into loses incurred during the production process(Frank, Matthew G. annis, Watkins, 2019)

Aflatoxicosis occurrence has remained high and prevalent due to condition such as hot and humid temperatures (Benkerroum, 2020b).In Uganda increasing aflatoxin contamination among the feed and animal products is because of limited awareness of farmers about the toxin molecule and the recommended toxin limit (Nakavuma et al., 2020).

Studies have shown, many approaches such as chemical, physical and biological have been used to detoxify aflatoxins, which requires the changing of the structure of the toxin molecule (Guo *et al.*, 2021) (Branà *et al.*, 2017) .most of this methods have shown a positive effect in detoxification of AFB1 but their use is practically limited due to safety concern, and loss of nutrients in the commodity, less knowledge on the toxicity of the feed (Karlovsy *et al.*, 2016). Enzymatically detoxified Aflatoxin B1 maize diet on liver function in wistar rats have been approved but have limited use (Tripathi & Mishra, 2009).In this study, evaluation of growth performance and feed intake of wistar rats fed enzymatically detoxified aflatoxin B1 maize diet was carried out.

REFERENCES

- Adebo, O. A., Njobeh, P. B., Gbashi, S., Nwinyi, O. C., Mavumengwana, V., Njobeh, P. B., Gbashi, S., Nwinyi, O. C., & Mavumengwana, V. (2017). *Review on microbial degradation of aflatoxins*. 8398. <https://doi.org/10.1080/10408398.2015.1106440>
- Ahamad, D. B., & Veterinary, T. N. (2019). *Aflatoxin B 1 Induced Carcinogenicity in Wistar Rats : Clinical Signs and Growth Performance Aflatoxin B 1 Induced Carcinogenicity in Wistar Rats : Clinical Signs and Growth Performance*. January.
- Asran, A. A. (2014). *FOOD PREFERENCE FOR ALBINO RATS AND ALBINO*. 92(4), 1279–1290.
- Assunção, R., Martins, C., Viegas, S., Viegas, C., Lea, S., Pires, S., Alvito, P., Assunção, R., Martins, C., Viegas, S., Viegas, C., Lea, S., & Assunção, R. (2018). Food Additives & Contaminants : Part A Climate change and the health impact of aflatoxins exposure in Portugal – an overview overview. *Food Additives & Contaminants: Part A*, 35(8), 1610–1621. <https://doi.org/10.1080/19440049.2018.1447691>
- Augusto, C., Oliveira, F. De, & Leandra, N. (2021). *Effects of Prenatal Exposure to Aflatoxin B1 : A Review*. 1–11.
- Benkerroum, N. (2020a). *Aflatoxins : Producing-Molds , Structure , Health Issues and Incidence in Southeast Asian and Sub-Saharan African Countries*.
- Benkerroum, N. (2020b). Aflatoxins: Producing-molds, structure, health issues and incidence in southeast asian and sub-saharan african countries. *International Journal of Environmental Research and Public Health*, 17(4). <https://doi.org/10.3390/ijerph17041215>
- Branà, M. T., Cimmarusti, M. T., Haidukowski, M., Logrieco, A. F., & Altomare, C. (2017). Bioremediation of aflatoxin B1-contaminated maize by king oyster mushroom (*Pleurotus eryngii*). *PLoS ONE*, 12(8), 1–14. <https://doi.org/10.1371/journal.pone.0182574>
- Cambaza, E. (2018). *Aflatoxins in Mozambique : Impact and Potential for Intervention*. 1–11.

<https://doi.org/10.3390/agriculture8070100>

- Cheng, Y.-L., Lee, C.-Y., Huang, Y.-L., Buckner, C. A., Lafrenie, R. M., Dénomée, J. A., Caswell, J. M., Want, D. A., Gan, G. G., Leong, Y. C., Bee, P. C., Chin, E., Teh, A. K. H., Picco, S., Villegas, L., Tonelli, F., Merlo, M., Rigau, J., Diaz, D., ... Mathijssen, R. H. J. (2016). We are IntechOpen , the world ' s leading publisher of Open Access books Built by scientists , for scientists TOP 1 % . *Intech*, *11*(tourism), 13. <https://www.intechopen.com/books/advanced-biometric-technologies/liveness-detection-in-biometrics>
- Chin, C. K., Iskandar, N., Samsudin, P., & Farawahida, A. H. (n.d.). *Molecular Characterisation of Aflatoxigenic and Non-Aflatoxigenic Strains of Aspergillus Section Flavi. 1*, 1–20.
- Chowdhary, P., More, N., Yadav, A., & Bharagava, R. N. (2018). Ligninolytic enzymes: An introduction and applications in the food industry. In *Enzymes in Food Biotechnology: Production, Applications, and Future Prospects*. Elsevier Inc. <https://doi.org/10.1016/B978-0-12-813280-7.00012-8>
- Dei, H. K. (2017). Assessment of Maize (*Zea mays*) as Feed Resource for Poultry. *Poultry Science*. <https://doi.org/10.5772/65363>
- El-shaer, H. F. A., Shoukry, A. A., & Youness, H. E. H. (2021). *Detection aflatoxin production by local isolates of Aspergillus spp . and molecular characterization. 4*(3), 45–63.
- Excretion, A. M., Yield, M., Gir, F. J., Olmedo, S., Herrera, M., Lor, S., Secundino, L., Benito, A., & Juan, T. (2023). *The Effects of Aflatoxin B1 Intake in Assaf Dairy Ewes on Aflatoxin M1 Excretion, Milk Yield, Haematology and Biochemical Profile*. 1–13.
- Fei, V., & Chiang, P. C. (2020). *The in vitro effects of aflatoxin B 1 on physiological functions of swine alveolar macrophages*. 919–925. <https://doi.org/10.1002/vms3.313>
- Fouad, A. M., Ruan, D., El-senousey, H. K., Chen, W., Jiang, S., & Zheng, C. (2019). *Harmful Effects and Control Strategies of Aflatoxin B 1 Produced by Aspergillus flavus and Aspergillus parasiticus Strains on Poultry : Review. 1*, 1–21. <https://doi.org/10.3390/toxins11030176>
- Frank, Matthew G. annis, Watkins, M. (2019). 乳鼠心肌提取 HHS Public Access. *Physiology &*

- Behavior*, 80, 678–687. <https://doi.org/10.1080/19440049.2016.1138545>. Potential
- Godswill Awuchi, C., Otuosorochi Amagwula, I., Priya, P., Kumar, R., Yezdani, U., & Gayoor Khan, M. (2020). Bulletin of Environment, Pharmacology and Life Sciences Aflatoxins In Foods And Feeds: A Review On Health Implications, Detection, And Control. *Env. Pharmacol. Life Sci*, 9(August), 149–155.
- Guan, Y., Chen, J., Nepovimova, E., Long, M., Wu, W., & Kuca, K. (2021). Aflatoxin Detoxification Using Microorganisms and Enzymes. In *Toxins* (Vol. 13, Issue 1). <https://doi.org/10.3390/TOXINS13010046>
- Guo, Y., Zhao, L., Ma, Q., & Ji, C. (2021). Novel strategies for degradation of aflatoxins in food and feed: A review. *Food Research International*, 140(November). <https://doi.org/10.1016/j.foodres.2020.109878>
- Hameed, R. M., Haider, H., Kadhim, W. A., Fatima, G., & Anwar, S. (2022). Toxicity of aflatoxin b1 towards the inducing alterations in the liver functions. *Era's Journal of Medical Research*, 9(2). <https://doi.org/10.24041/ejmr2022.27>
- Hassane, A. M. A., & Mwanza, M. (2017). *Influence of Different Moisture Contents and Temperature on Growth and Production of Aflatoxin B 1 by a Toxigenic Aspergillus flavus Isolate in Wheat Flour*. 83(3), 77–83.
- Huang, S., Zheng, N., Fan, C., Cheng, M., Wang, S., & Jabar, A. (2018). *Effects of aflatoxin B1 combined with ochratoxin A and / or zearalenone on metabolism , immune function , and antioxidant status in lactating dairy goats*. 31(4), 505–513.
- Jackson, L. W., & Pryor, B. M. (2017). Degradation of aflatoxin b1 from naturally contaminated maize using the edible fungus pleurotus ostreatus. *AMB Express*, 7(1), 1–7. <https://doi.org/10.1186/s13568-017-0415-0>
- Jallow, A., Xie, H., Tang, X., Qi, Z., & Li, P. (2021). Worldwide aflatoxin contamination of agricultural products and foods: From occurrence to control. *Comprehensive Reviews in Food Science and Food Safety*, 20(3), 2332–2381. <https://doi.org/10.1111/1541-4337.12734>
- Karlovsy, P., Suman, M., Berthiller, F., Meester, J. De, Eisenbrand, G., Perrin, I., Oswald, I. P., & Speijers, G. (2016). Impact of food processing and detoxification treatments on mycotoxin

- contamination. *Mycotoxin Research*, 179–205. <https://doi.org/10.1007/s12550-016-0257-7>
- Kaul, J., Jain, K., & Olakh, D. (2019). An Overview on Role of Yellow Maize in Food, Feed and Nutrition Security. *International Journal of Current Microbiology and Applied Sciences*, 8(02), 3037–3048. <https://doi.org/10.20546/ijcmas.2019.802.356>
- Krijgsheld, P., Bleichrodt, R., Veluw, G. J. Van, Wang, F., Müller, W. H., Dijksterhuis, J., & Wösten, H. A. B. (2007). Studies in Mycology. *Studies in Mycology*, 74, 1–29. <https://doi.org/10.3114/sim0006>
- Lagat, M. K., Toroitich, F. J., Makonde, H. M., & Amos, M. (2019). *Modulation of Aflatoxin Production by Interaction of Aspergillus Species from Eastern Kenya*. 13(11), 1–7. <https://doi.org/10.9790/2402-1311010107>
- Li, C., Liu, X., Wu, J., Ji, X., & Xu, Q. (2022). Research progress in toxicological effects and mechanism of aflatoxin B1 toxin. *PeerJ*, 10. <https://doi.org/10.7717/peerj.13850>
- Longe, L., Couvreur, J., Grandchamp, M. L., Garnier, G., Allais, F., Saito, K., & Accepted, J. (2018). *Importance of mediators for lignin degradation by fungal laccase*. <https://doi.org/10.1021/acssuschemeng.8b01426>
- Lou, H., Yang, C., Gong, Y., Li, Y., Li, Y., Tian, S., Zhao, Y., & Zhao, R. (2023). Edible fungi efficiently degrade aflatoxin B1 in cereals and improve their nutritional composition by solid-state fermentation. *Journal of Hazardous Materials*, 451. <https://doi.org/10.1016/j.jhazmat.2023.131139>
- Malhotra, S. K. (2017). Diversification in Utilization of Maize and Production Diversification in Utilization of Maize and Production. *Perspective of Maize Production and Value Chain - A Compendium*, 5(January), 49–57.
- Marshall, H., Meneely, J. P., Quinn, B., Zhao, Y., Bourke, P., Gilmore, B. F., Zhang, G., & Elliott, C. T. (2020). Novel decontamination approaches and their potential application for post-harvest aflatoxin control. *Trends in Food Science and Technology*, 106(September), 489–496. <https://doi.org/10.1016/j.tifs.2020.11.001>
- Monson, M. S., Coulombe, R. A., & Reed, K. M. (2015). Aflatoxicosis: Lessons from Toxicity and Responses to Aflatoxin B1 in Poultry. *Agriculture (Switzerland)*, 5(3), 742–777.

<https://doi.org/10.3390/agriculture5030742>

- Mousa, W., Jinap, S., & Radu, S. (2013). *Modeling Growth Rate and Assessing Aflatoxins Production by Aspergillus flavus as a Function of Water Activity and Temperature on Polished and Brown Rice*. 78(1). <https://doi.org/10.1111/j.1750-3841.2012.02986.x>
- Mousavi Khaneghah, A., Ismail, E., Raeisi, S., & Fakhri, Y. (2018). Aflatoxins in cereals: State of the art. *Journal of Food Safety*, 38(6), 1–7. <https://doi.org/10.1111/jfs.12532>
- Murokore, B. J., Masawi, A. N., Wacoo, A. P., Wangalwa, R., Ajayi, C. O., & California, P. V. (2023). Aflatoxin Susceptible Food Consumption Frequency, Prevalence, and Levels in Household Foodstuffs in Southwestern Uganda. *Journal of Food Quality*, 2023, 1–7. <https://doi.org/10.1155/2023/4769432>
- Nabwire, R. (2017). *Wangia R.N. /KESSA Conference Proceedings 2017 | 39-42* <http://www.kessa.org>. 39–42.
- Nakavuma, J. L., Kirabo, A., Bogere, P., Nabulime, M. M., Kaaya, A. N., & Gnonlonfin, B. (2020). Awareness of mycotoxins and occurrence of aflatoxins in poultry feeds and feed ingredients in selected regions of Uganda. *International Journal of Food Contamination*, 7(1), 1–10. <https://doi.org/10.1186/s40550-020-00079-2>
- Negash, D. (2018). A review of aflatoxin: occurrence, prevention, and gaps in both food and feed safety. *Journal of Nutritional Health & Food Engineering*, 8(2). <https://doi.org/10.15406/jnhfe.2018.08.00268>
- Pickova, D., & Ostry, V. (2021). *A Recent Overview of Producers and Important Dietary Sources of Aflatoxins*. 1–15.
- Pu, J., Yuan, Q., Yan, H., Tian, G., Chen, D., He, J., Zheng, P., Yu, J., Mao, X., Huang, Z., Luo, J., Luo, Y., & Yu, B. (2021). Effects of chronic exposure to low levels of dietary aflatoxin b1 on growth performance, apparent total tract digestibility and intestinal health in pigs. *Animals*, 11(2), 1–12. <https://doi.org/10.3390/ani11020336>
- Resources, M. (2012). *Research Article*. 1–8.
- Scarpari, M., Bello, C., Pietricola, C., Zaccaria, M., Bertocchi, L., Angelucci, A., Ricciardi, M. R.,

- Scala, V., Parroni, A., Fabbri, A. A., Reverberi, M., Zjalic, S., & Fanelli, C. (2014). Aflatoxin control in maize by *Trametes versicolor*. *Toxins*, 6(12), 3426–3437. <https://doi.org/10.3390/toxins6123426>
- Shabeer, S., Asad, S., & Jamal, A. (2022). *Aflatoxin Contamination , Its Impact and Management Strategies : An Updated Review*. 1–24.
- Suryadi, H., Judono, J. J., Putri, M. R., Eclessia, A. D., Ulhaq, J. M., Agustina, D. N., & Sumiati, T. (2022). Heliyon Biodeligni fication of lignocellulose using ligninolytic enzymes from white-rot fungi. *Heliyon*, 8(November 2021), e08865. <https://doi.org/10.1016/j.heliyon.2022.e08865>
- Teresa, M., Sergio, L., Haidukowski, M., & Logrieco, A. F. (2020). *Degradation of Aflatoxin B 1 by a Sustainable Enzymatic Extract from Spent Mushroom Substrate of Pleurotus eryngii*. 1–13.
- Ting, W. T. E., Chang, C., & Szonyi, B. (2020). Growth and Aflatoxin B 1 , B 2 , G 1 , and G 2 Production by *Aspergillus flavus* and *Aspergillus parasiticus* on Ground Flax Seeds (*Linum usitatissimum*). *Journal of Food Protection*, 83(6), 975–983. <https://doi.org/10.4315/JFP-19-539>
- Tripathi, S., & Mishra, H. N. (2009). Effect of feeding enzymatically detoxified aflatoxin-B1 diet on liver function test of wistar rats. *Nutrition and Food Science*, 39(3), 235–242. <https://doi.org/10.1108/00346650910957483>
- Usha, K. Y., J, P. S., Dileep, K., Shanti, B., B, R. R., & Praveen, K. (2020). Standardization of Methodology for Extracting Ligninolytic Enzymes from Standardization of Methodology for Extracting Ligninolytic Enzymes from Solid State Fermentation. *Research Journal of Biotechnology*, November.
- Valencia-quintana, R., Mili, M., & Juana, S. (2020). *Environment Changes , Aflatoxins , and Health Issues , a Review*.
- Wu, F. (2015). Global impacts of aflatoxin in maize: Trade and human health. *World Mycotoxin Journal*, 8(2), 137–142. <https://doi.org/10.3920/WMJ2014.1737>
- Wu, K., Liu, M., Wang, H., Rajput, S. A., Shan, Y., Qi, D., & Wang, S. (2021). *Review Article*

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