The ameliorating effect of oxihumate on aflatoxicosis in broilers

by

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Submitted in partial fulfillment of the requirements for the degree

P.hD.

in the

Department of Animal and Wildlife Sciences

Faculty of Natural and Agricultural Sciences

University of Pretoria

Pretoria

April 2005

I declare that this thesis for the degree
P.hD.(Animal Science) at the University of Pretoria,
has not been submitted by me for a degree at
any other university.

Acknowledgments

Many people were involved in the successful completion of this project. I wish to express my sincere appreciation to the following people without whom this degree would not have been possible:

* My supervisor, Prof N.H. Casey, and co-supervisor, Prof J.B.J. van Ryssen, from the Department of Animal and Wildlife Sciences at the University of Pretoria, for their leadership and kind support during the study.
* Dr G.E. Rottinghaus from the University of Missouri for welcoming a complete stranger in his home and laboratory, his guidance and valuable inputs during the *in vitro* study, constructive review of this thesis, and, above all, showing me the great State Parks of Missouri.
* Prof C.E. Medlen, Department of Pharmacology, University of Pretoria, for her endless patience, encouragement and innovative ideas regarding this study, but also as mother during everyday life.
* Dr D. Cromarty from the Department of Pharmacology of the University of Pretoria for his valuable advice and assistance.
* Mr R Coertze from the Experimental Farm at the University of Pretoria for his management and help during the run of the trials and for conducting the statistical analysis.
* Mrs. E. Kreft for her assistance and meticulousness in the laboratory concerning the lymphocyte work, but also for her continuous support and friendship.
* All technical assistants for their valuable support and help in the completion of this project.
* Enerkom (Pty) Ltd. for funding this project and their positive attitude towards research.
* Finally, a special word of appreciation to my family and all my friends for their love, inspiration and prayers.

I pray that honor and glory will always be given to the only God, who lives forever and is the invisible and eternal King! Amen (1Tim 1:17, CEV).

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Abstract

Mycotoxins have become an important issue for the grain industry and animal producers with a growing interest in the decontamination and remediation of highly contaminated feedstuffs. Practical methods to detoxify mycotoxin-contaminated grain on a large scale and in a cost-effective manner are essential but not currently available. The most recent and promising approach to detoxify mycotoxin-contaminated grain is the use of non-nutritive adsorbents, which bind the aflatoxin and thereby reduce their absorption from the gastrointestinal tract.

Humic acids are products of chemical and biological transformations of animal and plant residues and are widely distributed in nature. Humic acids have some therapeutic characteristics and a strong binding affinity for several compounds. A South African company developed an effective large-scale regeneration process for humic acids from coal, called oxihumate.

This study evaluated the effectiveness of oxihumate to adsorb mycotoxins, for the purpose of developing it as a commercial mycotoxin binder to be used in the preventative management of contaminated poultry feedstuffs. The *in vitro* affinity and adsorption capacity of oxihumate to aflatoxin was evaluated and the efficacy of oxihumate as an aflatoxin binder in broiler feeds *in vivo* was determined. The data showed adsorptions of about 10.3, 7.4 and 11.9 mg aflatoxin B_1/g oxihumate at pH 3, 5 and 7, respectively. Oxihumate adsorbed 1.2, 2.6 and 8.5 mg aflatoxin G_2/g at pH 3, 5 and 7, respectively. Oxihumate supplementation at a concentration of 3.5 g/kg feed was

effective in diminishing the growth inhibitory effects of aflatoxin and apparent protection was noted for some of the organ, haematological and serum biochemical changes associated with aflatoxicosis. These results suggest that oxihumate could alleviate some of the toxic effects of aflatoxin in growing broilers, and when used with other sound mycotoxin management practices, might prove beneficial in the preventative management of aflatoxin-contaminated feedstuffs for poultry. The improvement observed during this specific study was, however, not satisfactory enough to recommend oxihumate as a commercially available product.

Key Terms: Mycotoxins; aflatoxins; adsorbents; humic acid; oxihumate; broilers

Summary

Fungi grow on most organic matter and produce mycotoxins that occur as food and animal feed contaminants worldwide, causing serious health problems and production losses in livestock. Aflatoxin is the most prevalent and economically significant mycotoxin consumed by poultry. Aflatoxin is stable once formed in grain, and is not degraded during normal milling and storage. The liver is the main target for aflatoxins, but they also affect other organs and the immune system.

Practical methods to detoxify mycotoxin-contaminated grain on a large scale and in a cost-effective manner are essential but not currently available. The most recent and promising approach is the use of non-nutritive adsorptive materials, which bind the aflatoxins and reduce their absorption from the gastrointestinal tract.

Humic acids are products of chemical and biological transformations of animal and plant residues and are widely distributed in nature. Humic acids have some therapeutic characteristics and a strong binding affinity for several compounds. A South African company developed an effective large-scale regeneration process for humic acids from coal. This technology can economically regenerate large quantities of pure, high quality humic acids, called oxihumate, by reversing the process whereby coal was formed.

This study evaluated the effectiveness of oxihumate to adsorb mycotoxins, for the purpose of developing it as a commercial mycotoxin binder to be used in the preventative management of contaminated poultry feedstuffs. This was done by (1) evaluating the *in vitro* affinity and adsorption capacity of oxihumate to aflatoxin by studying its Langmuir and Freundlich adsorption isotherms; and (2) investigating the ability of oxihumate as an aflatoxin binder in broiler feeds *in vivo* and (3) determining the efficacy of oxihumate to prevent the inhibiting effect of aflatoxin on lymphocyte proliferation *in vitro*.

Oxihumate showed a high *in vitro* binding affinity for a number of mycotoxins. The Langmuir and Freundlich oxihumate adsorption isotherm parameters were obtained for aflatoxin. The data show adsorptions of about 10.3, 7.4 and 11.9 mg AFB_1/g oxihumate at pH 3, 5 and 7, respectively. Oxihumate adsorbed 1.2, 2.6 and 8.5 mg AFG_2/g at pH 3, 5 and 7, respectively.

In vitro binding tests may not be a reliable indicator of a material's ability to bind a mycotoxin in the animal. The *in vivo* results from this study indicate that aflatoxin significantly reduced body weight and affected overall broiler health and performance. Oxihumate was effective in diminishing the growth inhibitory effects of aflatoxin and apparent protection was noted for some of the organ, haematological and serum biochemical changes associated with aflatoxicosis. The failure of oxihumate to alter the *in vitro* effects of AFB_1 exposure on lymphocyte proliferation supports the idea that the protective effect of oxihumate appears to involve the sequestration of aflatoxin in the gastrointestinal tract and a reduction in bioavailability of aflatoxin rather than a therapeutic effect at cellular level. Oxihumate might be beneficial in the management of aflatoxin-contaminated feedstuffs for poultry, but the improvement observed during this specific study was not substantial enough to recommend oxihumate as a commercially available product.

Opsomming

Fungi groei op die meeste organiese materiaal en produseer mikotoksiene wat voedsel en dierevoere wêreldwyd besoedel en gesondheidsprobleme en produksieverliese in vee veroorsaak. Aflatoksien is die algemeenste en ekonomies belangrikste mikotoksien wat deur pluimvee ingeneem word. Aflatoksien is stabiel wanneer dit in graan gevorm het en word nie deur normale voervermenging en stoor afgebreek nie. Die lewer is die hoofteiken vir aflatoksiene, maar beïnvloed ook ander orgaansisteme asook die immuunsisteem.

Huidiglik is daar geen praktiese metodes vir die detoksifisering van mikotoksien-gekontamineerde graan, op ‘n grootskaalse en koste-effektiewe wyse beskikbaar nie. Die mees onlangse en belowendste benadering is die gebruik van nie-voedsame adsorptiewe materiale wat die aflatoksien bind en die absorbering daarvan vanuit die spysverteringskanaal verminder.

Humiensure is stowwe wat algemeen teenwoordig is in natuurlike materiale as ‘n produk van chemiese en biologiese transformasies van dierlike en plantaardige residue. Humiensure toon sekere terapeutiese eienskappe en het ook ‘n sterk affiniteit om met verskeie verbindings te bind. ‘n Suid Afrikaanse maatskappy het ‘n effektiewe grootskaalse regenerasie proses ontwikkel vir die vervaardiging van humiensure vanaf steenkool. Hierdie tegnologie produseer baie suiwer en hoë kwaliteit humiensure, genoem oksihumaat, ekonomies en op grootskaal deur die proses wat verantwoordelik is vir die vorming van steenkool, om te keer.

Hierdie studie evalueer die doeltreffendheid van oksihumaat om mikotoksiene te adsorbeer, met die moontlikheid om dit as ‘n kommersieel-beskikbare mikotoksienbinder te ontwikkel vir die gebruik in voorkomingsbestuur van gekontamineerde pluimveevoere. Dit is gedoen deur (1) evaluering van die *in vitro* affiniteit en adsorbsie-kapasiteit van oksihumaat aan aflatoksien deur ‘n studie van die Langmuir en Freundlich adsorpsie isoterme; en (2) bepaling van die doeltreffendheid van oksihumaat as ‘n aflatoksienbinder in braaikuikenvoere *in vivo*. Die vermoë van oksihumaat om die inhiberende effek wat aflatoksien op limfosietvermeerdering het, te voorkom, is ook ondersoek.

Oksihumaat het ‘n hoë affiniteit vir ‘n aantal mikotoksiene getoon. Die Langmuir en Freundlich oksihumaat adsorpsie isoterme parameters is vir aflatoksien bepaal. Die data toon adsorpsies van

ongeveer 10.3, 7.4 en 11.9 mg AFB_1/g oksihumaat by pH 3, 5 en 7, onderskeidelik. Okishumaat het 1.2, 2.6 en 8.5 mg AFG_2/g by pH 3, 5 en 7, onderskeidelik geadsorbeer.

In vitro bindingstoetse is nie ‘n betroubare aanduiding van die vermoë van ‘n materiaal om ‘n mikotoksien in die dier te bind nie. Die *in vivo* resultate van hierdie studie wys dat aflatoksien liggaamsmassa betekenisvol verlaag en algemene braaikuikengesondheid en prestasie beïnvloed. Oksihumaat het die groei inhiberingseffekte van aflatoksien doeltreffend verminder en ‘n mate van beskerming was ook sigbaar vir sommige van die orgaan, hematologiese en serum biochemiese veranderinge geassosieerd met aflatoksikose. Die onvermoë van oksihumaat om die *in vitro* effekte van AFB_1-blootstelling op limfosietvermeerdering teë te werk, ondersteun die teorie dat die beskermende effek van oksihumaat eerder te wyte is aan die binding van aflatoksien in die spysverteringskanaal en ‘n vermindering in die biobeskikbaarheid van aflatoksien, as ‘n terapeutiese effek op sellulêre vlak. Oksihumaat mag voordelig wees in die bestuur van aflatoksien-gekontamineerde pluimveevoere, maar die verbetering wat tydens hierdie spesifieke studie waargeneem is, was nie bevredigend genoeg om oksihumaat as ‘n kommersieel-beskikbare produk aan te beveel nie.

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