International Livestock Research Institute

Current status of aflatoxin research and management at the International Livestock Research Institute (ILRI)

Report of a seminar held at ILRI Nairobi

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Contents

Acronyms and abbreviations

BecA	Biosciences eastern and central Africa
CAAREA	Capacity and action for aflatoxin reduction in East Africa
CSIRO	Commonwealth Scientific Industrial Research Organization
ELISA	enzyme-linked immunosorbent assay
IITA	International Institute for Tropical Agriculture
ILRI	International Livestock Research Institute
KARI	Kenya Agricultural Research Institute
NIR	Near infrared reflectance spectroscopy
ppb	parts per billion
QAAFI	Queensland Alliance for Agriculture and Food Innovation
UPLC	Ultra-high pressure liquid chromatography

Introduction

This seminar on the current status of aflatoxin research and management at the International Livestock Research Institute (ILRI) was held on Thursday 22 August 2013 in the Ndovu Conference Room, Biosciences eastern and central Africa (BecA) hub at ILRI. The seminar was jointly organized by the BecA-ILRI hub and the Food Safety and Zoonoses program, the two working groups at ILRI that are involved in research on aflatoxin. The seminar provided a forum for information sharing and discussion on progress made and future research plans. The agenda of the seminar is indicated in Annex 1. A total of 30 participants were in attendance (see Annex 2 for the list of participants).

Overview of the presentations

Johanna Lindahl, a postdoctoral scientist with the Food Safety and Zoonoses program, was the seminar's facilitator. She opened the meeting with a word of welcome to all present and then gave a brief overview of the day's agenda and the flow of the presentations. The presentations were grouped into three main categories: (1) assessment, (2) diagnostics and experiments and (3) mitigation. Summarized below are the key points that were discussed during the presentations.

Assessment

The Capacity and Action for Aflatoxin Reduction in East Africa (CAAREA) project

By Jagger Harvey

Jagger's presentation was a general overview of some of the activities and achievements of the first and second phases of the CAAREA project. The concept note for the third phase of the project is currently being developed.

CAAREA is a project of the BecA-ILRI hub and the Commonwealth Scientific Industrial Research Organization (CSIRO) and the flagship project on aflatoxins of the Australian International Food Security Centre. The project is working on methods to improve diagnosis of the occurrence of aflatoxin in maize. This will empower decision-makers with information to be able to deal with incidences of aflatoxin contamination based on reliable evidence from science.

The focus of the project is on detection of aflatoxin in pre-harvest maize in Kenya and Tanzania. One of the main activities is screening of maize germplasm that is resistant to aflatoxin accumulation in different environments. A shared platform for aflatoxin diagnostics has been set up at the BecA-ILRI hub. A platform for mycotoxin nutritional analysis has also been set up. There is a multi-disciplinary team of scientists plus a laboratory team that are working on developing new aflatoxin diagnostics, such as the electronic nose, that are suited to the African context. The project has worked with HarvestChoice and the Kenya Agricultural Research Institute (KARI) on risk mapping and prediction in the Rift Valley.

Aspergillus flavus survey by the CAAREA project

By Benoit Gnonlonfin

Benoit gave an overview of field work carried out to survey *Aspergillus flavus* in Kenya and Tanzania towards filling a knowledge gap in the type of fungi that exist in these two countries. The objectives of the survey were to (1) determine the population of microflora affecting maize and (2) select candidates for field inoculation in different agroecological zones.

Laboratory analysis of pre-harvest maize samples consisted of aflatoxin quantification and assessment of the genetic diversity of *A. flavus*. *A. flavus* was detected in all agroecological zones, namely, the lower midland, upper midland, highland and lowland zones. The survey found evidence of toxigenic *A. flavus* in pre-harvest maize in the region. There is need for more investigation on ways of reducing pre-harvest toxigenic *A. flavus* infection of maize and subsequent aflatoxin contamination. Sequencing is currently ongoing.

Risk assessment of aflatoxins in the Kenyan dairy value chain

By Anima Sirma

Anima presented her proposed research towards her PhD at the University of Nairobi. Her study is using a risk assessment approach to investigate the health impacts of aflatoxin in Kenya's informal dairy value chain. The study is premised on the knowledge that aflatoxin-contaminated dairy feed can result in contamination of the milk chain with the milk aflatoxin, M1. The objectives of the study are to (1) characterize the key risks of aflatoxin, (2) identify the best control options and (3) provide risk managers with information for decision-making. Risk assessment will be guided by the risk assessment model of the Codex Alimentarius Commission. The study will include dairy producers, feed manufacturers and retailers.

Assessing people's perceptions of aflatoxin: The case of milk consumers in Kenya

By Maria Walke

Maria presented an update of her research towards her Master's degree. The objectives of the study are to assess Kenyan consumers' perceptions and willingness to pay for aflatoxin-free labelled milk. Field work was carried out in Nairobi over a period of two months and involved 600 interviews of consumers of raw and processed milk. The study was a combination of descriptive and experimental design. A choice experiment was used to assess willingness to pay. Although data analysis is still not complete, some preliminary results are available. In regard to consumer awareness of aflatoxin, the results indicate that 55% of those interviewed were aware of this hazard. Indications are that there is still a long way to implement labelling of aflatoxin-free milk for the informal milk market in Kenya, something that is likely to be more important for consumers of processed milk.

Economic costs of aflatoxins in the Kenyan dairy value chain

By Daniel Senerwa

Daniel presented his planned research towards his PhD from the University of Nairobi. His study seeks to quantify the economic costs of aflatoxin contamination along the dairy value chain in Kenya. He outlined various types of costs associated with aflatoxin contamination, such as farm-level losses due to contaminated feed and milk, direct market costs, human health costs in terms of disability-adjusted life years, losses incurred by feed producers through destroyed produce, reduced milk productivity of dairy cows, animal loss, calf mortality, milk losses by traders and rejected milk destined for export. He noted that farmers are unlikely to throw away aflatoxin-contaminated feed because existing regulations that require the disposal of contaminated produce are poorly enforced. The study will examine the cost-effectiveness of various mitigation strategies, for example, dietary and food processing interventions and reduced consumption of high-risk foods. The expected outcome of the study is the identification of potential cost-effective intervention measures for the Kenya dairy value chain.

Diagnostics and experiments

Inoculum production and field inoculation

By Immaculate Wanjuki

Immaculate's presentation was a follow-up of the presentation by Benoit on the fungi survey in Kenya and Tanzania. She outlined the procedures that were used to produce the *Aspergillus* inoculum and the subsequent field inoculations. These activities also involved capacity development of research technicians in Kenya and Tanzania; training in field inoculation was carried out at four KARI research stations (Kiboko, Katumani, Mtwapa and Perkerra) and at research stations of the Mikocheni Agricultural Research Institute in Tanzania.

Mycotoxin diagnostics in whole grain and flour

By James Wainaina

James gave a presentation on the techniques being used at the BecA-ILRI hub for detection of mycotoxins in whole maize grain and maize flour. These techniques are the enzyme-linked immunosorbent assay (ELISA), VICAM and near infrared reflectance spectroscopy (NIR).

Ultra-high pressure liquid chromatography (UPLC) analysis

By Warwick Turner

Warwick's presentation was a continuation of James', but specifically focusing on UPLC as a method of quantifying aflatoxins. This method can be used where the levels of aflatoxin are over 7500 parts per billion (ppb). One advantage of UPLC is that it allows for the quantification of the individual aflatoxins in the samples (B1, B2, G1 and G2) whereas this is not possible with ELISA. However, ELISA could be used as a quick diagnostic method for initial screening of samples then UPLC can be used as the gold standard for testing of high-aflatoxin samples.

Will NIR predict aflatoxin?

By Glen Fox

Glen's presentation was on the use of NIR to predict the presence of aflatoxin in samples. He clarified that NIR is not a quantitative method per se. Rather, it is a modelling tool and predictive technology that uses the infrared spectral region.

NIR needs to be based on a good standard reference method and good calibration. Spectral imaging is used to detect different food components such as starch and protein. The data can then be used to build a model to detect aflatoxin level in maize. Models have been developed for whole and milled maize. NIR can also be used to screen for *Aspergillus* resistance; this is potentially useful for breeders to test maize as early as possible.

He noted that it would be challenging to apply NIR technology to build a calibration for milk, mainly because of the level of particulate matter in milk and light scattering. In addition, NIR has a lower limit of 200 ppb yet the maximum permitted limit for milk is 10 ppb in Kenya so the method might not be useful to detect levels down to the permitted level. For maize, it is better to use a homogenous product such as flour rather than the kernels. He concluded his presentation by answering the question posed in the title, "Will NIR predict aflatoxin?" The simple answer is: yes, but it is an ongoing development.

Predicting aflatoxin contamination with NIR to improve food safety
By Titilayo Falade

Titilayo's presentation was based on her PhD research which seeks to examine the potential to use NIR to detect aflatoxin in maize. This will improve food safety – both for domestic consumption and export – by improving prediction of contamination, excluding contaminated kernels and reducing the analytical cost of sampling.

Mitigation

Maize mycotoxin management by optical sorting
By Matthew Stasiewicz

The objective of Matthew's research is to use an optical sorter to screen contaminated maize kernels. This involves the use of a calibrated single-kernel maize sorter with maize from eastern Kenya, using NIR techniques. There is potential to scale up the method to screen bulk quantities of maize.

Creating a novel biological method to mitigate aflatoxin-induced risk
By Sara Ahlberg

Sara gave a brief overview of her project proposal for her PhD research. The objectives of her study are to (1) find mould growth inhibiting lactic acid bacteria in milk and (2) find aflatoxin-binding lactic acid bacteria and proteins. The main activities to be carried out are sample collection, isolation of lactic acid bacteria, mould growth inhibition tests, NIR calibration with milk samples and tests of aflatoxin binding.

Aflasafe: Aflatoxin management in Africa
By Charity Mutegi

Charity is the Kenya country coordinator for the aflasafe project for the International Institute of Tropical Agriculture (IITA). Her presentation gave an update on the use of aflasafe, a biocontrol method for managing aflatoxin in Africa. She began by presenting some statistics on the prevalence of aflatoxin contamination in Kenya. She cited a 2012 report by the Centers for Disease Control and Prevention which found that almost every Kenyan is chronically exposed to aflatoxin. Additionally, Kenya's Ministry of Health reports that one in every three Kenyan households is affected by cancer, either directly or through being connected to someone with the disease. The average Kenyan daily maize consumption is 400 grams per day.

Short-term approaches to the management of aflatoxin include education, decontamination, Good Manufacturing Practices and Good Agricultural Practices. Long-term interventions include screening and breeding of resistant crop varieties and biocontrol. Aflasafe is a biocontrol method for aflatoxin management. Already, there have been field trials in three countries -- Kenya, Nigeria and Senegal -- to test the efficacy of aflasafe. In Senegal, aflasafe has reported an efficacy rate of over 80%.

Currently, only Nigeria has been able to produce aflasafe commercially. The aflasafe plant in IITA Ibadan was set up through the Partnership for Aflatoxin Control in Africa. Both Nigeria and Senegal have products that are ready for registration, while Kenya and Burkina Faso have products that are under testing.

One challenge that has been recognized is how to incentivize the uptake of aflasafe by smallholder farmers in Africa. The World Bank's AgResults Initiative developed a pull mechanism to provide incentives for the adoption of the product. It is a private-sector driven initiative but focuses on smallholder groups.

Discussion and closing remarks

Following the presentations, the floor was opened for questions and any points of discussion. On the issue of aflatoxin diagnostics, the question was raised as to whether we will ever get the 'perfect' test for aflatoxin. The response was that ELISA remains a good method for quick screening of samples and UPLC can be used if further screening is necessary.

Sara's proposed investigation of mould-inhibiting and aflatoxin-binding properties of lactic acid bacteria in milk prompted a suggestion to also examine traditional methods of producing sour milk whereby the milk is placed in a smoked gourd and fermentation occurs through the action of mixed microflora. It was suggested that treating the interior of the gourd with smoke from a smouldering twig could have the effect of inhibiting the growth of moulds or binding aflatoxins. It was agreed that this is worth investigating, depending on the observations in the field.

It was suggested that it may be useful to map the aflatoxin hotspots and carry out soil analysis for toxigenic strains of *Aspergillus*. This may improve targeting of biocontrol interventions. It was also noted that experiments have shown that crop rotation and intercropping of maize with other crops can help reduce the build-up of toxigenic strains in the soil from season to season.

The final point was made that it is important to increase public awareness at all levels about the risks of mycotoxins and how to mitigate them.

Annex 1: Agenda

TIME	PRESENTATION	PRESENTER
0900 hours	Welcome and overview	Johanna Lindahl
0905–1015 hours	**Assessment**	
	The Capacity and Action for Aflatoxin Reduction in East Africa (CAAREA) project	Jagger Harvey
	Aspergillus flavus survey across Kenya and Tanzania	Benoit Gnonlonfin
	Risk assessment of aflatoxins in the Kenya dairy value chain	Anima Sirma
	Assessing people's perceptions of aflatoxin: The case of milk consumers in Kenya	Maria Walke
	Economic costs of aflatoxins in the Kenyan dairy value chain	Daniel Senerwa
1015–1030 hours	B R E A K	
1030–1130 hours	**Diagnostics and experiments**	
	Inoculum production and field inoculation	Immaculate Wanjuki
	Mycotoxin diagnostics in whole grain and flour	James Wainaina
	Ultra-high pressure liquid chromatography analysis	Warwick Turner
	Will NIR predict aflatoxin?	Glen Fox
	Predicting aflatoxin contamination with NIR to improve food safety	Titilayo Falade
1130–1145 hours	B R E A K	
1145–1300 hours	**Mitigation**	
	Maize mycotoxin management by optical sorting	Matthew Stasiewicz
	Creating a novel biological method to mitigate aflatoxin-induced risk	Sara Ahlberg
	Aflasafe: Aflatoxin management in Africa	Charity Mutegi
	Discussion and closure	
	Knowledge gaps and possibilities for the future	All

Annex 2: List of participants

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