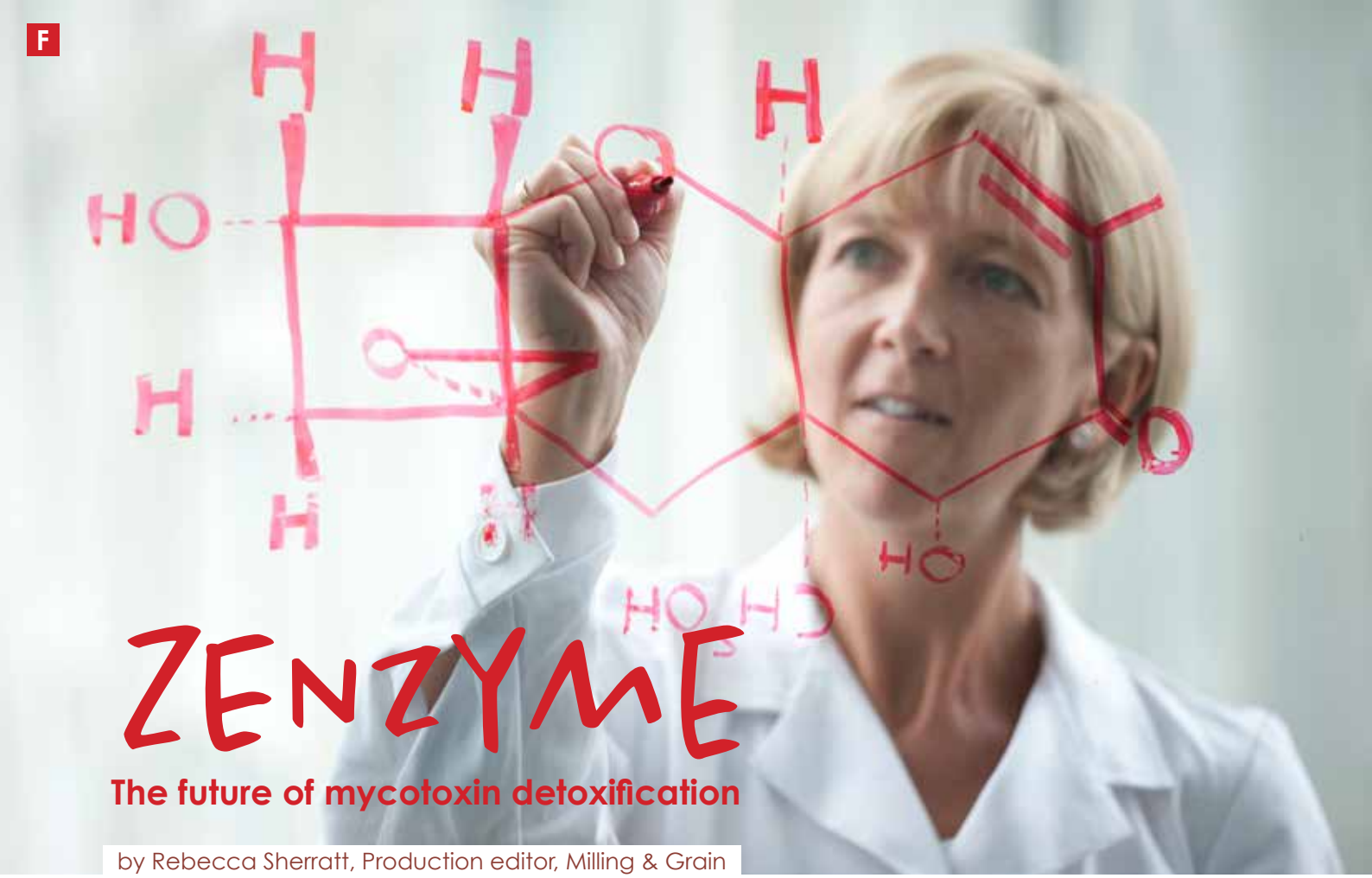


ZENZYME

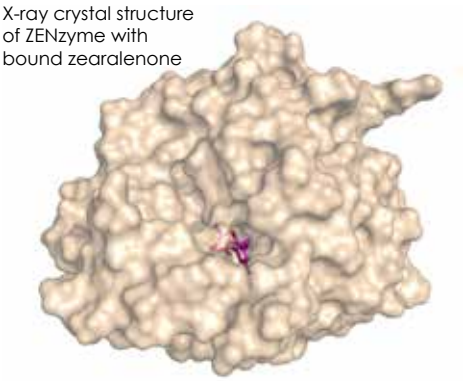
The future of mycotoxin detoxification

by Rebecca Sherratt, Production editor, Milling & Grain

As the population of the world only continues to increase, the need for a drastic increase in food production becomes only ever more vital to combat issues of world hunger. It is estimated that the Earth increases in population by a whopping 83 million people per year, and our population has increased seven-fold since the beginning of the nineteenth-century. Feeding our 7.7 billion people is no easy task when climate change and pollution are continuing to cause problems for our food supplies.

This is also a multi-layered problem, as this increase in food production we so desperately need also cannot afford to come at a cost to our natural ecosystem and biodiversity. This gap between resources we have and resources we need is what is known as a 'yield gap', and this gap only continues to expand as the human race continues to populate the earth at a drastic level.

Thankfully, Biomin have a solution. At the World Nutrition Forum in Cape Town, South Africa, their Austrian Research Team Leader, Dr Wulf-Dieter Moll, delivered a thrilling talk about the future of mycotoxins.



X-ray crystal structure of ZENzyme with bound zearalenone

The primary problem

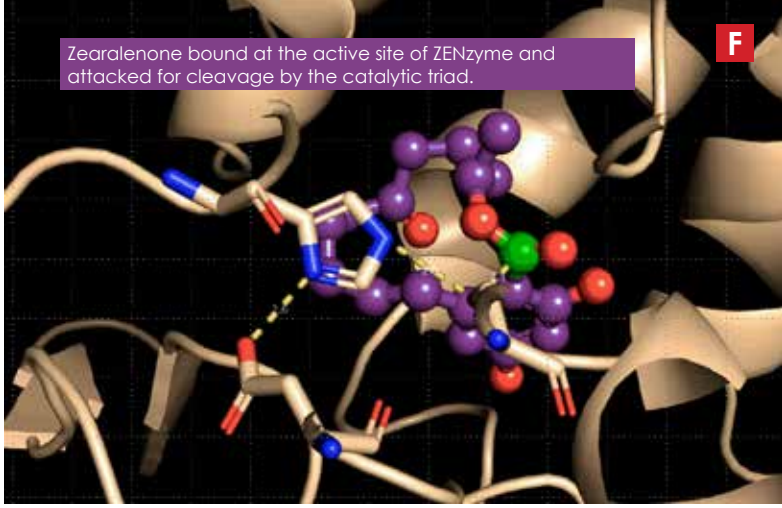
There are a myriad of reasons for the yield gap, such as failing crops caused by harsh winters and overwhelming droughts, but one key reason is the contamination of feed by mycotoxins, one of the primary ones being zearalenone. Dr Moll discusses the dangers of zearalenone, and the imminent need to resolve the problems it causes. This mycotoxin is an estrogenic metabolite, produced by Fusarium, a common fungi menace for many farmers. This pathogen is commonly found in wheat, maize and other crops.

Zearalenone binds itself to the oestrogen receptors in livestock, interfering with natural hormone signalling. This can result in a variety of reproductive problems, such as livestock infertility, abortion and other breeding problems. Farmers need only imagine the damage Zearalenone can do, if an entire herd of livestock becomes affected with such a contaminant.

Maize cob infested with Fusarium graminearum, a fungus which produces zearalenone

Animal nutrition innovators, Biomin, are working rapidly on a mycotoxin decontamination technology, which will work by utilising what we already know regarding detoxification and biological degradation. A discovery of a new family of enzymes for hydrolytic cleavage (the splitting of a compound into fragments via adding water), and detoxification of zearalenone seem to be the new answer to mycotoxin contamination, which Biomin is determined to make into an attainable product for the feed industry worldwide.

Biomin's Dr Moll also recently released a research paper


Zearalenone bound at the active site of ZENzyme and attacked for cleavage by the catalytic triad.

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discussing these recent technological advancements. His paper, entitled 'Enzyme technology against Zearalenone-induced hyperestrogenism' discusses the latest successes Biomin has held, in creating trials of a feed that eradicates zearalenone without any negative side effects, a prior unachieved concept.

Current feed additives sold commercially are noted to be especially ineffective at combatting mycotoxins. When subject to various trials, their levels of detoxification were remarkably low, especially where Zearalenone is concerned. Biomin have already released feed additives which combat various mycotoxins, and the world's only commercially available and registered recombinant enzyme product for detoxification of a mycotoxin, the fumonisin esterase FUMzyme for degradation of carcinogenic fumonisins, is also from Biomin. However, the current technology against Zearalenone-induced hyperoestrogenism, based on adsorption or degradation with the basidiomycete yeast Trichosporon mycotoxinivorans, is still not the ideal feed additive Biomin want to deliver to its consumers. They believe better is readily achievable.

Producing ZENzyme

Dr Moll proved to be the perfect man for the task. Moll joined Biomin in 2005, and immediately set his focus upon dealing with mycotoxins. He and his team have already developed several enzymes such as FUMzyme, which degrades and detoxifies another class of mycotoxins.

Dr Moll's research team in Austria continued their research into mycotoxin detoxification, beginning by raising multiple microbial cultures, sourced from different habitats. Subject to different conditions, each microbe culture was then given controlled zearalenone. Upon judging which strains proved able to metabolise the harmful mycotoxin, enrichment cultures and isolated strains were then made, with the aim of refining them into a mycotoxin degrading feed ingredient.

The successful microbe culture was named *Rhodococcus erythropolis*, (strain PFA D8-1). When in contact with PFA D8-1, zearalenone converted to a hydrolysed, (broken down) form of zearalenone, which Biomin isolated, used for structure verification, toxicity and oestrogenicity studies, and gave the abbreviation HZEN. BIOMIN identified the enzyme that catalyses hydrolysis of zearalenone, and, following the conventions of nomenclature for new microbial enzymes, called it ZenA. However, the enzyme had to be refined further, purified and made thermostable, so it withstands the heat exposure during animal feed pelleting.

The next step of the process was to finally use it in a trial, and




so the enzyme, now nicknamed ZENzyme, was tested in pig feed pellets. In vivo tests, or tests conducted in living organisms, needed to be conducted to ensure that ZENzyme works to the best of its ability before being put on the commercial market. These tests were carried out in one of Biomin's Centre's for Animal Nutrition (CAN).

Pigs in these tests received an experimental diet deliberately contaminated with zearalenone, and supplemented with various concentrations of ZENzyme. The results of these tests were positive, suggesting that the more ZENzyme that is placed into the feed, the more detoxification of zearalenone took place, resulting in healthier livestock. The feed fed to pigs with none or very minimal ZENzyme suffered the effects of zearalenone, such as enlarged vulvas and heavier reproductive systems, whereas the pigs who received ZENzyme in larger quantities showed no such effects. ZENzyme also proved remarkably resilient during feed pelleting, withstanding temperatures of up to 90 degrees Celsius, and only losing minimal activity at 95 degrees Celsius.

Biomin continued to soldier on, to ensure that ZENzyme is truly as efficient and useful as it can be. The next step was to examine the faeces and urine of the selected animals who ate the ZENzyme pellets. These results again proved that ZENzyme successfully broke down zearalenone in the artificially contaminated feeds. Tests then progressed onto broiler chickens and cows, who replicated the same positive results when exposed to ZENzyme.

Leaps and bounds

As tests continue to prove successful, and ZENzyme's role as the removal of mycotoxin worries for farmers begins to become reality, Biomin continue to revise and refine their product. At the World Nutrition Forum, Dieter Moll notes that optimising the ZENzyme to improve its efficiency and robustness, when in the gastrointestinal tract, is one of the many focusses Biomin are working on. Stopped-flow and quenched-flow instruments are also being used at the Biomin research centre to capture and measure enzymatic reactions, with precise accuracy.

Considerations are also being discussed regarding increasing the catalytic efficiency of the ZENzyme via enzyme engineering. To help with these ideas, X-ray crystal structures of ZENzyme are being obtained, to reveal details of molecular interactions between enzyme and substrate. As Biomin continue to work on this new technology, the future for feed looks bright.

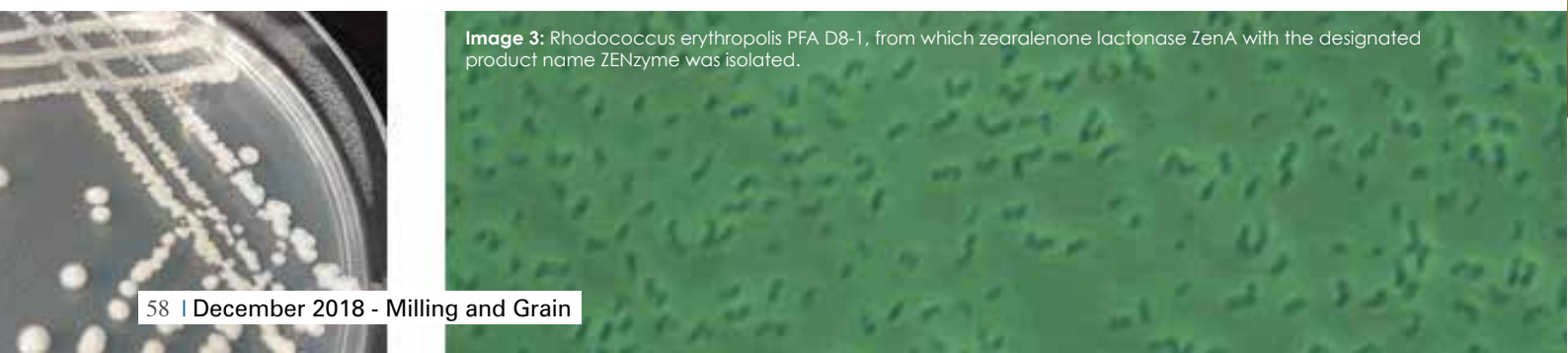

Image 3: Rhodococcus erythropolis PFA D8-1, from which zearalenone lactonase ZenA with the designated product name ZENzyme was isolated.