

Influence of Ochratoxin A on the performance of broiler chicken

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Abstract

Among fungal metabolites, so called mycotoxins, ochratoxins are known to cause severe problems in animal production. Ochratoxins are mainly produced by *Aspergillus* and *Penicillium* species. Ochratoxin A (OTA), the most relevant and abundant mycotoxin belonging to this group, is known to decrease productivity, lower weight gain, decrease feed efficiency and increase disease incidence because of its immune-suppressive properties. Aim of this research project was the investigation of microorganisms capable of degrading Ochratoxin A in the intestinal tract of animals. Output of a comprehensive screening was a novel yeast strain, which was characterized thoroughly and successfully tested in several feeding trials.

Introduction

Mycotoxins are a large number of metabolites produced by many fungi under various conditions all over the world. Ochratoxins are a group of structurally similar metabolites produced by *Aspergillus* and *Penicillium* species. These toxins occur as contaminants of cereal grains and beans, especially in cool and moderate climates. Peak toxin production occurs between 20 and 25°C. The most harmful mycotoxin in this group is Ochratoxin A (OTA) with nephrotoxic-, hepatotoxic-, carcinogenic- and immunosuppressive-properties. In animal production regular consumption of a diet containing OTA causes economic losses mainly through a reduced growth rate, poor feed conversion and reduced immunity to infections by bacteria and viruses. Animal studies indicate that OTA is absorbed in the GI tract and in the proximal and distal tubules. It enters the enterohepatic circulation and can be excreted and reabsorbed. It can bind to the albumin fraction in the blood and thus can persist in animal tissues, even in meat, for extended periods of time (Marquardt et al., 1992). Thus strategies have to be developed to counteract this toxin and minimize the impact on animals and further on humans.

Detoxification of OTA can be achieved by hydrolisation resulting in non-toxic products. This reaction is well described and can be done enzymatically using Carboxypeptidase A (Pitout, 1968). Microbial degradation was also obtained using *Phenylobacter immobile* (Wegst and Lingens, 1982) or *Acinetobacter calcoaceticus* (Draughon et al. 1994).

Materials and Methods

Due to the fact that adsorptive materials do not work efficiently enough against ochratoxins (Huff et al, 1992), a project was realized with the aim of finding microorganisms with the capability to deactivate OTA in the intestinal tract of animals. This project was done in cooperation with the Institute for Agrobiotechnology (IFA) in Tulln, Austria, and **was sponsored by the Austrian national research fund** for 3 years. The project was separated in different working packages as following:

1. Development of analytical methods

To analyze screening samples 2 methods were used: HPLC (high performance liquid chromatography) and TLC (thin layer chromatography). Analytical methods were set up for the analysis of pure culture material, feeding stuffs, blood plasma, liver and kidney tissue samples as well as urine and faeces. "Degradation" was defined as positive (= degraded) when the metabolite ochratoxin α was detected.

2. Production of OTA

Solid state fermentation procedures were developed and optimized to yield a high amount of ochratoxin A. Crushed wheat kernels were infected with spores of OTA-producing fungi (*Petromyces albertensis* and *Aspergillus ochraceus*) and incubated for several weeks under optimal conditions regarding moisture and temperature. The contaminated wheat was harvested, dried and ground. After determination of the toxin concentration the material was mixed into the feeding stuff to give the desired final OTA-concentration.

3. Screening for microorganisms

Based on the results of several studies done during the previous years, several habitats like rumen fluid, intestinal contents and soil samples were screened for OTA-detoxifying microorganisms.

Different strategies were used in order to enrich the degrading strain and to isolate them: different culture media (modified), antibiotics (especially for anaerobic bacteria), dilution series, agar plating (pour plating and streak out cultures). Several strains with the capability of degrading OTA into the non-toxic ochratoxin α were identified and further investigated.

4. Investigation of microorganisms

OTA degrading aerobic strains were isolated out from habitats like soil and water. Based on a partial sequence analysis of the 16S rDNA these bacteria showed the closest relationship to the genera of *Sphingomonas*, *Stenotrophomonas*, *Ralstonia*, *Ochrobactrum*, *Rhodococcus*, and *Ralstonia*. Also anaerobic OTA-degrading strains were successfully isolated. Strains from culture collections were tested for OTA-detoxification as well. Positive yeast strains belong to the genera of *Trichosporon*, *Cryptococcus* and *Rhodotorula*.

5. Selection of most suitable strain

The screening process gave more than 20 positive (in terms of OTA-degradation) isolates and major focus was put on the evaluation of these microbes in order to identify the best suitable strain for the development of an OTA-degrading feed additive. Investigations comprised: degradation velocity, culture media, influence of phenylalanine, pH, redox potential, fermentation, stabilization, *in vitro* models, antibiotic resistance, physiological patterns, feeding trials, etc.

After this very comprehensive evaluation process the novel yeast strain *Trichosporon mycotoxinivorans* (Biomim[®]MTV) was identified as the most suitable and it was successfully used as feed additive in several feeding trials.

Results and Discussion

A feeding trial was conducted in cooperation with the Agricultural University of Athens, Greece.

200 broiler chickens (male; 1 day old, race: Arbor Acres) were randomly split into 4 groups. The animals were kept in cages on the floor (15 birds/cage) with litter from wheat straw. Animals belonging to the toxin group (i.e. receiving OTA but not MTV feed additive) showed a reduced weight gain of -4.3 % compared to the control group. Addition of Biomim[®]MTV could improve performance (in particular total weight gain) at an average of + 4.5 % compared to the toxin group.

Feed consumption and FCR showed significant differences between the groups. Animals belonging to the toxin group showed significantly increased feed consumption and a higher feed conversion compared to the other groups. By the addition of Biomim[®]MTV performance with regards to feed consumption and FCR could be significantly improved. Mortality was clearly increased in the toxin group and could be reduced about 50% by the addition of Biomim[®]MTV.

On day 10, 20, 30 and 40 one bird from each cage was euthanised and blood samples were collected for the determination of immunological parameters, especially parameters which play a major role in the bird's natural defense mechanism, for example macrophages.

Table 1: Performance parameters broiler trial

	Control	OTA	OTA + MTV1	MTV
OTA (µg/kg)	--	500	500	--
MTV (cfu/kg feed)	--	--	1 x 10 ⁴	1 x 10 ⁵
no. of animals	50	50	50	50
total weight gain (g)	1885.3	1804.7	1893.0	1884.9
total feed consumption (g)	3371 ^a	3533 ^b	3437 ^{ab}	3387 ^a
FCR (kg/kg)	1.79 ^a	1.96 ^b	1.81 ^a	1.80 ^a
mortality (%)	5	8.33	4.44	2.22

^{a, b}....different intercepts indicate significant differences (P ≤ 0.05)

The results for macrophages indicate that the animals were able to compensate the OTA induced negative effects on their immune system for approximately 4 weeks. After that period the viability of macrophages decreased rapidly and statistically significantly.

Conclusions

The results indicate a clearly negative influence of OTA on bird's performance and immunity, which could be alleviated by the addition of the feed additive Biomin[®] MTV. Losses due to reduced weight gain could be compensated. With respect to feed consumption and FCR statistically significant differences between the toxin group and the Biomin[®] MTV groups could be observed.

The results of the immunological investigations indicate that the amount of toxin included in the diets causes problems to the immune cells of the birds and the application of Biomin[®] MTV significantly improves the negative effects.

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