Lucrări ştiinţifice **Zootehnie şi Biotehnologii**, vol. **41(1)** (**2008**), Timişoara

EVALUATION OF THE TOXINOGENOUS POTENTIAL OF THE *FUSARIUM GRAMINEARUM* STRAINS ISOLATED FROM THE COMPOUND FEEDS FOR PIGS

EVALUAREA POTENTIALULUI TOXINOGEN AL TULPINILOR DE *FUSARIUM GRAMINEARUM* ISOLATE DIN NUTRETURILE COMBINATE PENTRU PURCEI

TABUC CRISTINA
Institute of Biology and Animal Nutrition, Balotesti, Romania

Fusarium graminearum is a species of fungi with a wide geographical dispersal area which produces type B trychothecenes (deoxynivalenol mainly) and zearalenone. This species contaminates the cereals, corn mainly, the main ingredient for compound feeds.The mycotoxins produced by Fusarium graminearum (deoxynivalenol and zearalenone) produce intoxications, severe sometimes, in animals of economic interest, the pigs being the most sensitive to their action.The paper aims to evaluate the capacity of Fusarium graminearum strains isolated from compound feeds for pigs to produce deoxynivalenol and zearalenone.The study shows that 80% of the surveyed strains can produce deoxynivalenol in concentrations of 1.80-198.4 μg/g, and 66.66% produce zearalenone in concentrations ranging between 0.25-564.2 μg/g.

Keywords: feeds, Fusarium graminearum, toxinogenous potential, deoxynivalenol, zearalenone

Introduction

The *Fusarium* genus joins endoparasitic species, which grow on cereals, fruits and vegetables producing white-cream, yellow, pink, red to violet moulds present mainly on the soils from the temperate regions. Most *Fusarium* species are components of the soil microflora and are widely spread. They are phytopathogen species making the roots, stems and fruits to rot and they degrade the vascular system. Many species are pathogen for humans and animals. Many species are k own to produce mycotoxins belonging to the group of trychothecenes, to the families of zearalenone and fumosins, all with major implications in intoxications in humans and animals.

Fusarium graminearum is of the most widespread *Fusarium* species which contaminates the cereals, corn mainly. The stems of this species, isolated from cereals under optimal conditions of temperature and humidity are, in great part producers of deoxynivalenol and/or zearalenone.

A study in Argentina (Molto, 1997) which monitored the incidence of the *Fusarium* species in corn proved the presence of *Fusarium graminearum* isn 74%

of the analysed samples and all the isolated strains were producing large amounts of deoxynivalenol (13-20 mg/kg). Similar studies conducted at INCDBNA Balotesti have shown that *Fusarium graminearum* was present in 80% and 53% of the samples of corn used to manufacture compound feeds and in the compound feeds for poultry, and mycotoxin concentration (deoxynivalenol and zearalenone) reached concentrations of 0.44-109.7 μg/g and of 56.3-246.2 μg/g (Tabuc, 2004, 2007). Deoxynivalenol (DON or vomitoxin) determines intoxications characterized by feed refusal, nausea and vomiting, diarrhoea which cause the animal to loose weight and even to die by dehydration. These symptoms have been observed in pigs (Pollman 1985; Trenholm 1984; Harvey 1989), lambs (Harvey 1986), broilers (Hamilton 1985; Huff 1981; Huff 1986 Kubena 1985; Kubena 1989; Swamy 2002a), ducks (Boston 1996) and turkeys (Hamilton 1985; Morris 1999). Practically all the animal species are sensitive to the action of this toxin, but its toxicity differs among the species.

Zearalenone (toxin F2) determines intoxications in many animal species such as: pigs (Swamy 2002; Young, 1990; Green, 1990), cattle (Diekman, 1992), lambs (Hufstedler, 1996), broilers (Swamy 2002b; Chi, 1980), horses (Minervini, 2006), laboratory rodents (Yang, 2006; Perez-Martinez, 1997). The toxic effect of the zearalenone is characterized by hyperestrogenism and alterations of the reproducing apparatus. Zearalenon is involved in hepatotoxicity, hematotoxicity and immunotoxicity (Abid Essefi, 2004; Creppy, 2002; Maaroufi, 1996; Coulombe, 1993; Coe, !992; Hussein, 2001, Zinedine, 2007). Some toxic effects related to oesophagitis and esophagus cancer have been reported in humans after the intake of contaminated cereals in China and South Korea (Luo, 1990).

The paper evaluates the capacity of the *F. graminearum* strains isolated from piglet CF to produce DON and/or ZEA, because the pigs in general, and the piglets in particular, are very sensitive to the action of these two toxins.

Material and Methods

15 samples of piglet compound feeds, Ridascreen FAST kits to determine the mycotoxin concentration by ELISA – the competition variant.

Production of the F. graminearum strains

The CF samples under study were diluted with saline 0.5% tween 80 and were seeded in Petri dishes on malt medium (2% agar, 2% malt, 50 ppm chloramphenicol) and on Potato Dextrose Agar medium (0.4% potato extract, 2% dextrose, 1.5% agar). After the medium solidified, the Petri dishes were incubated for 5-7 days at 25^{o}C to allow the colonies develop.

The *Fusarium* colonies developed in the Petri dishes were picked again and again until pure cultures were obtained from which were kept only the *Fusarium graminearum* colonies based on the macro and microscopic characteristics. The selected strains, in pure cultures, seeded in tubes on malt medium, were preserved at +4^{o}C.

Evaluation of the toxinogenous capacity of the isolated strains

To evaluate the capacity of the *Fusarium graminearum* strains to produce DON and/or ZEA, they were seeded on a malt medium and incubated for 7 days at 25^{o}C. The obtained cultures were used as inoculums for the next stage.

To evaluate the mycotoxin production we used a solid medium starting from 50 g barley, introduced in 500 cm^{3}Erlenmeyer flasks, to which 50 ml distilled water were added and autoclaved for 30 min at 121^{o}C.

After the medium cooled, the inoculums consisting of 3 squares of 0.5 cm^{2} were introduced in the flasks and the medium thus seeded was incubated for 5 weeks at 25^{o}C, for the production of DON and at 20^{o}C to produce zearalenone; 3 flasks were seeded for each strain both for DON and for ZEA.

The cultures have been checked weekly to observe the absence of subsequent contamination with other fungal species by macroscopic examination. If suspect colonies developed the plate was examined microscopically. The suspect colonies have been identified with the methods described by Botton (1990). The flaks identified positive for foreign contamination have been removed.

After 5 weeks of incubation we determined the concentration of DON and ZEA mycotoxins.

Determination of mycotoxin concentration

The two mycotoxins wee identified with Ridascreen FAST kits, for dosing using the immunoenzymatic method ELISA – the competition variant.

The working instructions for each kit have been observed when the mycotoxin concentration was determined.

- the mycotoxins were extracted from the studied cultures with a solution of methyllic alcohol :distilled water 70:30 (for Zea) or with distilled water (for DON), stirring 3 minutes and filtration through filter paper Whatman nr. 1; the obtained extract was used as such and it was diluted to obtain convenient concentrations in accordance with the standardization curve of the kit; after the kit components (wells, standards, reagents) were brought to 20-25^{0}C, 50 µl of each standard were introduced in a well each and 50 µl of each sample in 2 wells each; 50 µl antigen solution were added on the bottom of each well, thereafter 50 µl antibody solution, the wells have been stirred and were left to rest at room temperature for 5 minutes; the wells were rinsed with distilled water (for ZEA) and with buffer solution delivered with the kit (for DON), in agreement with the instructions of each kit; the wells have been rinsed three times; 100 µl chromogen solution were added to each well (standard or sample), the wells have been stirred and were left for 10 minutes at room temperature in the dark; 100 µl stopping solution were added to each well, the wells have been stirred and after 10 minutes the optical density of the standards and samples was read with a microplate reader Uniskan II; the results were processed statistically to obtain the concentrations of the analysed mycotoxins.

Results and Discussions

Production of the F. graminearum strains

From the 15 CF samples under survey we isolated 15 *Fusarium graminearum* strains. The colonies have been identified with the macro and

microscopic characteristics described by Botton (1990): fast growing mycelium on malt medium, pink in the beginning, then purple red with pigment diffusion into the gelose, abundance of the fusiform macroconidia, curbed and septate, presence of the terminal cell, prolonged and ending in a sharp spur.

Capacity of mycotoxin production

After 5 weeks of incubation we determined the concentration of the surveyed mycotoxins using the immunoenzymatic method ELISA.

The analyses have shown that 12 (80%) of the studied strains produced deoxynivalenol in concentrations ranging between 1.80-198.4 μg/g (Fig. 1).

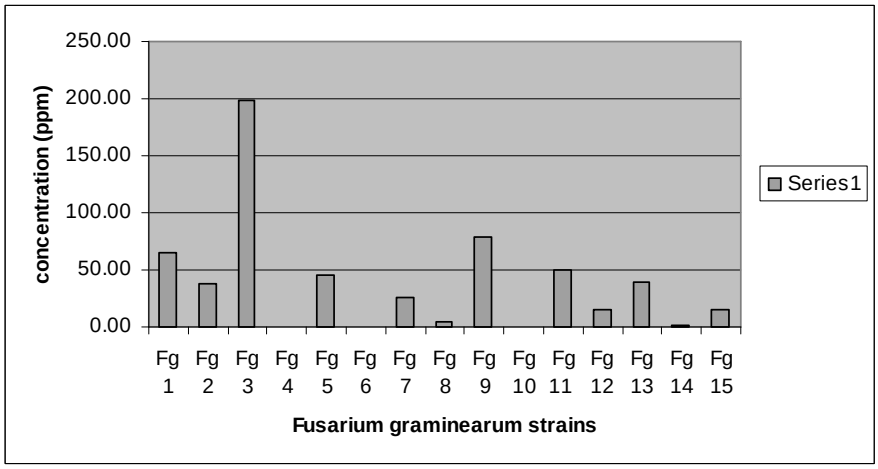

Figure 1. Deoxynivalenol production of the *Fusarium graminearum* strains

Zearalenone has been identified and quantified for 10 (66.66%) of the 15 studied strains. ZEA production ranged between 0.25-564.2 μg/g (Fig. 2)

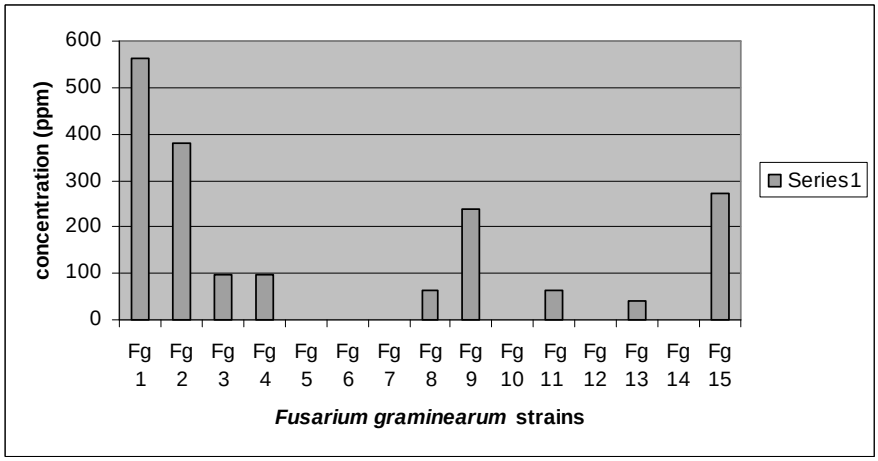

Figure 2. Zearalenone production of the *Fusarium graminearum* strains

Conclusions

1. *F. graminearum* was present in 100% of the analysed CF samples;

2. 80% of the surveyed strains are capable of producing large amounts of deoxynivalenol under optimal conditions of temperature and humidity;
3. 66.66% of the analysed *F. graminearum* strains also are able to produce zearalenone;
4. 46.66% % of these strains can produce both deoxynivalenol and zearalenone under optimal conditions of temperature and humidity.

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