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The search for ecologically safe means of mycotoxin detoxification in fodder

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A selection of yeasts detoxifying mycotoxins was carried out with the purpose to release or detoxify completely zearalenone, deoxynivalenole, aflatoxins and T-2 toxin in composite fodder. The investigation showed that the amount of yeasts introduced in the fodder and feeding stock studied depended on the fodder composition and on the adaptation abilities of yeast strains to substrata of this origin. Yeast development was more intensive in composite or full-value fodder than in feeding stock. Zearalenone and deoxynivalenole detoxifying effect is characteristic of *Saccharomyces cerevisiae* S.1.5 (T), *Kluyveromyces marxianus* K.7.1 (T), *Geotrichum fermentans* G.1, *Metschnikowia pulcherima* M.1, *Rhodotorula glutinis* Rh.2.1 and *Rhodotorula mulcilaginosa* Rh.6 strain yeasts when they are cultivated in the composite fodder of different composition. Besides, *Saccharomyces cerevisiae* S.1.5 (T) yeast has the ability to detoxify T-2 toxin, and *Rhodotorula mulcilaginosa* Rh.6 detoxifies aflatoxins.

Key words: mycotoxins, biological detoxification, yeasts

INTRODUCTION

The majority of feeding stock and fodder spoilers consist of various fungi which, developing on them, excrete secondary metabolites – toxins. Fungi are attributed to the main human and animal health risk factors.

Fodder damaged by various fungi becomes strongly toxic for livestock. Livestock fed with such fodder get toxicosis, the immunity to other diseases decreases, the productivity falls down, the production class changes, therefore agricultural companies suffer great losses (Bakutis, 1997; Brake et al., 2000; Drochner et al., 2001; Yiannikouris et al., 2002). Fusariotoxicosis is the most topical problem in Lithuania. Among fusariotoxins, zearalenone and deoxynivalenole are most frequent in fodder (Bakutis, 2002). Other toxins (ochratoxins, aflatoksins, patuline) are isolated as well. Incidentally, aflatoxins are detected in imported feeding stock (Lugauskas, 2005).

At present, fodder producers employ various means in order to avoid fungal growth and development and pollution by mycotoxins. Researchers propose various chemical, physical and microbiological means. Chemicals suggested for detoxification include absorbent carbon, ammonia, bentonite, zeolite and others (Phillips et al., 1988; Williams et al., 1994; Тремасов и др., 1997; Santin et al., 2002). Nevertheless, they only partly bind the mycotoxins excreted by fungi; moreover they absorb valuable nutrients.

Attempts are made to change the chemical structure of mycotoxins by physical methods. Therefore mycotoxin investigations often employ a study of the chemical composition and functional action of newly found toxic compounds (Hussein, Brasel, 2001).

Detoxification possibilities with the aid of microorganisms – various strains of bacteria and yeasts – are widely studied in various countries (Stanley et al., 1993; Santin et al., 2001, 2003; Schatzmayr et al., 2001).

Great attention is paid to the regularity of toxin accumulation in feeding stock and fodder in Lithuania (Bakutis, Januškevičienė, 1997; Lugauskas, 2005; Novošinskas et al., 2005; Paškevičius, 2005; Semaškienė et al., 2005). The search is carried out intensively and the methods and technologies are created to stop the distribution of toxin-producing fungi and suppress their negative effect on living organisms (Bakutis et al., 2005; Gaurilčikienė et al., 2005; Levinskaitė et al., 2005). However, in our opinion, too little attention is given to biological detoxification of mycotoxins as one of the most promising and safe ways of mycotoxin neutralization.

The main goal of the present work was to select yeast strains that are able to detoxify aflatoxins, deoxynivalenone, zearalenone and T-2 toxins excreted by fungi in fodder of plant origin. To this end, the following tasks were set: 1) to evaluate the growth intensity of yeasts on feeding stock and fodder; 2) to evaluate yeast

strains selected according to mycotoxin amount detoxified in composite fodder.

MATERIALS AND METHODS

The following kinds of composite fodder and their stock were used for the investigation: fodder for sows, for fat stock pigs, for 2–4-month pigs, for pigs, full-value fodder for sucker pigs, pea meal, corn-pea meal, barley meal, wheat meal, sunflower oilcake.

The following yeasts were used in the experiments: *Saccharomyces cerevisiae* Meyen ex E. C. Hansen, *Kluyveromyces marxianus* (E. C. Hansen) van der Wait, *Geotrichum fermentans* (Diddens et Lodder) von Arx, *Metschnikowia pulcherima* Pitt et M. W. Miller, *Rhodotorula glutinis* (Fresenius) F. C. Harrison and *Rhodotorula mulcilaginosa* Rh.6 (Jörgensen) F. C. Harrison. Yeasts were isolated from various substrata of plant origin (corn, fruits, vegetables and fodder) (Paškevičius, 2005).

The quantitative evaluation of yeast growth dynamics in feeding stock and fodder was carried out using them after sterilization, without additional supplements, moistening with sterile water only. Fedding stock and fodder were sterilized in autoclave at +112 ˚C temperature for 20 min. Feeding stock and fodder (100 g) were inoculated with yeasts (10^4 cells g^{-1}). Yeasts on feeding stock and fodder were cultivated in thermostat at 28 ± 1 ˚C for 10 days. Experiments were fulfilled in three replications. After yeast cultivation, the fodder samples (1 g) were washed and the suspension was sown on solid medium in Petri dishes. Yeasts were isolated using the surface method on standard malt extract medium (Liofilchem, Italija). Their growth intensity was estimated according to the amount of yeast colony forming unites (cfu) g^{-1}.

The amounts of zearalenone (ZON), deoxynivalenone (DON), aflatoxins (AFL) and T-2 toxin were fixed in fodder before yeast cultivation and after 10 days of cultivation. Three samples from every variant were analyzed. Fodder samples were analyzed by the ELISA (enzyme-linked immunosorbent assay) method (CHU, 1996). The VERATOX® DON 5/5 (Neogen, USA), VERATOX® T-2 toxin (Neogen, USA), VERATOX® Zearalenone (Neogen, USA), VERATOX® Ochratoxin (Neogen, USA), VERATOX® Aflatoxin (Neogen, USA) were used for the analysis. Mycotoxin extraction and tests were performed according to manufacturer's instruction.

The obtained data were processed using Microsoft Excel XP (mean, standard, deviation).

RESULTS

The results showed (Table 1) that the amount of yeasts introduced in the feeding stock and fodder depended on fodder composition and yeast strain adaptation abilities to substrata of this origin. The yeast developed more intensively in composed or full-value fodder that in feeding stock. *Saccharomyces cerevisiae* S.1.5 (T) strain yeasts developed more intensively in composed fodder for sows than corn-pea mixture meal and made, respectively, $(8.9 \pm 0.5) \times 10^4$ and $(3.4 \pm 0.3) \times 10^4$ cfu g^{-1}. *Kluyveromyces marxianus* K.7.1 (T) strain yeasts developed more intensively in composed fodder for fat stock pigs than in barley meal. Amount yeast was $(1.2 \pm 0.9) \times 10^5$ and $(4.1 \pm 0.1) \times 10^4$ cfu g^{-1} in these fodders. Among all fodders and feeding stocks the greatest yeast amount was fixed in composed fodder for 2–4-month pigs after *Geotrichum fermentans* G.1 yeast strain cultivation in them – $(6.4 \pm 0.2) \times 10^5$ cfu g^{-1} was estimated in fodder studied after 10 days of yeast cultivation. Yeasts of this species developed worse in wheat meal and made up 1.2 ± 0.04 cfu g^{-1}. The least amount of yeasts was noted in wheat meal after cultivation of *Metschnikowia pulcherima* M.1 strain yeasts – $(1.0 \pm 0.04) \times 10^4$ cfu g^{-1} and in pea meal after cultivation of *Rhodotorula mulcilaginosa* Rh.6 strain yeasts – $(0.2 \pm 0.01) \times 10^4$ cfu g^{-1}.

The yeast strains studied not only easily adapted to feeding stock and fodder but showed the ability to detoxify various toxins present there as well (Table 2).

With *Saccharomyces cerevisiae* S.1.5 (T) yeasts cultivated in composite fodder for sows, ZON toxin was fully detoxified and DON amount decreased from

Table 1. **The amount of yeasts (cfu g^{-1}) in feeding stock and fodder moistened with water after 10 days of cultivation at 28 ± 1 ˚C**

Treatment variant	Yeast amount (cfu $g^{-1} \times 10^4$)
Composite fodder for sows + *Saccharomyces cerevisiae* S.1.5 (T)	8.9 ± 0.5
Corn-pea meal + *Saccharomyces cerevisiae* S.1.5 (T)	3.4 ± 0.3
Composite fodder for fat stock pigs + *Kluyveromyces marxianus* K.7.1 (T)	12.0 ± 0.9
Barley meal + *Kluyveromyces marxianus* K.7.1 (T)	4.1 ± 0.1
Composite fodder for 2-4 month pigs + *Geotrichum fermentans* G.1	64.0 ± 0.2
Wheat meal + *Geotrichum fermentans* G.1	1.2 ± 0.04
Composite fodder for sows + *Metschnikowia pulcherima* M.1	3.1 ± 0.2
Wheat meal + *Metschnikowia pulcherima* M.1	1.0 ± 0.04
Composite fodder for pigs + *Rhodotorula glutinis* Rh.2.1	3.9 ± 0.3
Sunflower oilcake + *Rhodotorula glutinis* Rh.2.1	2.2 ± 0.1
Full-fledge fodder for sucker pigs + *Rhodotorula mulcilaginosa* Rh.6	3.2 ± 0.04
Pea meal + *Rhodotorula mulcilaginosa* Rh.6	0.2 ± 0.01

Table 2. **Changes of mycotoxin content in fodder after yeast cultivation**

Treatment variant	Mycotoxin amount (mg g^{-1})							
	Aflatoxins		Zearalenone		Deoxynivalenole		T-2 toxin	
	K[1]	B[2]	K	B	K	B	K	B
Composite fodder for sows + *Saccharomyces cerevisiae* S.1.5 (T)	–	–	0,3 ± 0.01	0	0.15 ± 0.01	0.13 ± 0.01	0.035 ±0.002	0.008 ± 0
Composite fodder for fat stock pigs + *Kluyveromyces marxianus* K.7.1 (T)	–	–	0.3 ± 0.02	0	1.5 ± 0.09	0	0.02 ± 0.001	0.002 ± 0
Composite fodder for 2-4 month pigs + *Geotrichum fermentans* G.1	–	–	0.7 ± 0.04	0	0.5 ± 0.02	0.1 ± 0.01	–	–
Composite fodder sows + *Metschnikowia pulcherima* M.1	–	–	0.67 ± 0.04	0	0.1 ± 0	0	–	–
Composite fodder for pigs + *Rhodotorula glutinis* Rh.2.1	–	–	0.3 ± 0.01	0	1.0 ± 0.05	0.07 ± 0.001	–	–
Full-fledge fodder for sucker pigs + *Rhodotorula mulcilaginosa* Rh.6	0.003 ± 0	0	0.4 ± 0.01	0	0.4 ± 0.02	0	–	–

[1] Control.

[2] After yeasts cultivation.

0.15 ± 0.01 to 0.13 ± 0.01 mg kg^{-1}. T-2 toxin amount decreased from 0.035 ± 0.002 mg kg^{-1} to 0.008 ± 0 in fodder as a result of the cultivation of the above yeasts.

A strong mycotoxin detoxifying effect was characteristic of *Kluyveromyces marxianus* K.7.1 (T) strain yeasts cultivated in composite fodder for fat stock pigs. ZON amount in control was 0.3 ± 0.02 mg kg^{-1} and DON 1.5 ± 0.09 mg kg^{-1}, but after 10 days of cultivation these toxins were detoxified completely. The amount of T-2 toxin decreased from 0.02 ± 0.001 to 0.002 ± 0 mg kg^{-1}.

With *Geotrichum fermentans* G.1 yeast strain cultivated in composite fodder for 2–4-month pigs ZON was fully detoxified, although 0.7 ± 0.04 mg kg^{-1} of this toxin was fixed in control. DON amount in this fodder decreased from 0.5 ± 0.02 to 0.1 ± 0.01 mg kg^{-1}.

Metschnikowia pulcherima M.1 strain yeasts fully detoxified ZON and DON toxins when cultivated in composite fodder for sows. *Rhodotorula mulcilaginosa* Rh.6 strain yeasts detoxified not only ZON, DON but also AFL when it was cultivated in full-value fodder for suckers. Positive results of DON and ZON detoxification were obtained by *Rhodotorula glutinis* Rh.2.1 strain yeast cultivation in composite fodder for pigs.

The selection of mycotoxin-detoxifying yeasts has shown the expediency of yeast use in order to decrease the effects or completely detoxify ZON, DON, AFL and T-2 toxins in composite fodders.

CONCLUSIONS

The results showed that *Saccharomyces cerevisiae, Kluyveromyces marxianus, Geotrichum fermentans, Metschnikowia pulcherima, Rhodotorula glutinis, Rhodotorula mulcilaginosa* strain yeasts isolated from plant origin substrata were able to develop in feeding stock and fodder moistened with water at a temperature of 28 ± 1 °C. The concentration of the yeasts introduced depended on fodder composition and specific yeast strain adaptation ability to substrata of this kind.

Zearalenone detoxification effect was characteristic of *Saccharomyces cerevisiae* S.1.5 (T), *Kluyveromyces marxianus* K.7.1 (T), *Geotrichum fermentans* G.1, *Metschnikowia pulcherima* M.1, *Rhodotorula glutinis* Rh.2.1 and *Rhodotorula mulcilaginosa* Rh.6 strain yeasts when they were cultivated in composite fodder of different composition. The strongest effect was detected after *Geotrichum fermentans* G.1 strain yeast cultivation in composite fodder for 2–4-month pigs.

The strongest deoxynivalenone detoxificating effect was characteristic of *Kluyveromyces marxianus* K.7.1 (T), *G. fermentans* G.1 and *Rhodotorula mulcilaginosa* Rh.6 strain yeasts. *Saccharomyces cerevisiae* S.1.5 (T) yeasts showed not only zearalenone, deoxynivalenole but also T-2 toxin detoxification ability, when they were cultivated in composite fodder for sows. *Rhodotorula mulcilaginosa* Rh.6 strain yeasts cultivated in full-value fodder for suckers completely detoxified deoxynivalenole, zearalenone and aflatoxins.

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EKOLOGIŠKAI SAUGIŲ PRIEMONIŲ, GALINČIŲ DETOKSIKUOTI MIKOTOKSINUS PAŠARUOSE, PAIEŠKA

Santrauka

Straipsnyje nagrinėjami augalinės kilmės pašaruose aptinkamų mikotoksinų biologinės detoksikacijos klausimai. Pagrindinis šio darbo tikslas buvo atrinkti mielių padermes, gebančias detoksikuoti mikromicetų išskiriamus aflatoksinus, deoksinivalenolą, zearalenoną ir T-2 toksinus augalinės kilmės pašaruose. Tyrimų rezultatai parodė, kad introdukuotų mielių skaičius tirtuose pašaruose ir pašarinėse žaliavose priklausė nuo pašaro sudėties ir kultivuojamos mielių padermės prisitaikymo prie šios kilmės substratų galimybių. Nustatyta, kad mielės intensyviau vystėsi kombinuotuosiuose arba visaverčiuose pašaruose, nei pašarinėse žaliavose. Išaiškinta, kad *Saccharomyces cerevisiae* S.1.5 (T), *Kluyveromyces marxianus* K.7.1(T), *Geotrichum fermentans* G.1, *Metschnikowia pulcherima* M.1, *Rhodotorula glutinis* Rh.2.1 ir *Rhodotorula mulcilaginosa* Rh.6 padermių mielės pasižymėjo zearalenoną ir deoksinivalenolą detoksikuojančiu poveikiu kultivuojant jas skirtingos sudėties kombinuotuosiuose pašaruose. *Saccharomyces cerevisiae* S.1.5 (T) padermės mielės, be minėtų toksinų detoksikacijos, pasižymėjo ir T-2 toksiną, o *Rhodotorula mulcilaginosa* Rh.6 – aflatoksinus detoksikuojančiu poveikiu.

Raktažodžiai: mikotoksinai, biologinė detoksikacija, mielės