Ergovaline contents in grasses from semi-natural grasslands in Poland

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Abstract

The aim of following study was to investigate presence of endophyte (from *Neotyphodium* genera) and ergovaline contents in grasses from semi-natural grasslands in Poland. More than 610 ecotypes from grasslands in Central and North – Eastern Poland were tested for endophyte presence and ergovaline contents. More than 30% of tested ecotypes were colonised by endophytes (further marked as E+) and the majority of them (66%) produced ergovaline with different intensities.

All E+ tall fescue ecotypes, originating from Central Poland, were found to produce the highest amount of ergovaline (average = 1.86 ppm). Majority of perennial ryegrass and meadow fescue E+ ecotypes were also found to produce ergovaline. However, mean alkaloid contents in mentioned species were lower than for tall fescue (0.166 ppm and 0.122 ppm, respectively). Red fescue and sheep's fescue produced only traces of ergovaline (0.07 ppm, and 0.06 ppm, respectively). Ergovaline was not detected in E+ ecotypes of tufted hairgrass, italian ryegrass and Kentucky bluegrass. It can be concluded that due to multi-species nature of Polish semi-natural grasslands and rather low amount of ergovaline, current animal threat associated with alkaloid intake during feeding is rather low. Tall fescue in Poland is rarely consumed by animals as compared to meadow fescue or perennial ryegrass and probably has never been sown alone for feeding purposes or hay production. However, in case of highly productive short rotation meadows, especially were only a few species as perennial ryegrass or meadow fescue were sown, it is possible the high concentration of alkaloid in forage. Therefore, intensive research and monitoring of existing grasslands is still needed.

Introduction

Permanent grasslands in Poland cover ca. 3 mill ha (approx. 30% of the whole country area). The most important and valuable grass species in grassland swards are: perennial ryegrass (*Lolium perenne* L.), meadow fescue (*Festuca pratensis* Huds.), red fescue (*Festuca rubra* L.) and tall fescue (*Festuca arundinacea* Schreb.). Mentioned species are also known to host symptomless endophytic fungi, from *Neotyphodium* genera (Petroni *et al.* 1986). For agricultural practice, endophyte – plant symbiosis is both positive and negative. Endophyte colonized (E+) grasses express range of adaptations to abiotic (drought, mineral imbalance, soil acidity) and biotic (disease, pest or animals) stress (Funk *et al* 1994). As a result, E+ grasses are more compatible than non colonized grasses and thrive better in presence of limited resources. However, in certain

circumstances endophytes may produce toxic alkaloids (ergovaline, lolitrem B, etc.) that have been linked with animal production and health problems. "Ryegrass staggers syndrome" and "fescue toxicosis" are the most common animal diseases caused by E+ grass. Ryegrass staggers syndrome (decrease of milk production, weight loss, disorders of nervous system) is caused by presence of large quantities of lolitrem B in animal forage. Ergovaline production is connected with fescue toxicosis, which is manifested by reduced forage intake, excessive salivation and reduced reproductive performance. In case of both animal diseases described above, high mortality was also noted (Siegel *et al.* 1985; Reed *et al.* 2000).

In Poland, endophyte research is rather scarce; however endophytes were found both in seeds and in plants of common grass species in Polish grasslands (Pańka and Żurek, 2005; Wiewióra *et al.*, 2008). Endophyte colonization (from 20% to 60%) was also noted in Polish grasslands by Pfannmöller *et al.*, (1994) and by Lewis (2000). Positive effect of endophyte colonization on persistency and aesthetic value of *Festuca rubra* cultivars was noted (Pańka and Żurek, 2005; Prończuk and Prończuk, 2000). It was also concluded that endophyte colonization increase along with grass plantation age (Wiewióra *et al.*, 2006). However, nothing has been done so far, concerning ergovaline contents in Polish E+ grasses. The aim of current study was to identify endophyte colonization on semi-natural grasslands in different regions of Poland and to estimate potential sward toxicity due to ergovaline contents.

Materials and Methods

Ecotypes were collected from semi-natural grassland in a form of living plant. From 5 to 10 plants were collected per one ecotype in each locality. Plants were further planted in field collection in Radzików and analysed for endophyte presence with rapid staining method according to Saha *et al.* 1988. Further, during laboratory work, grass samples of ecotypes with endophytes were prepared for analytical treatment and HPLC analyses of ergowaline after descriptions given by Craig *et al* (1994) with Hovermale and Craig (2001) modifications. Jordi RP column (100A, 150 x 4.6 mm, 5 μm) with BRP-1 pre-column was used for mentioned toxin analyses.

Results and Discussion

Grass ecotypes were collected on 58 localities from semi-natural grasslands in different regions of Poland. Total number of 618 ecotypes were gathered in a form of living plant, mostly of fescues (red – 172 ecotypes, meadow – 130, tall – 31 and sheep's - 11) but also of perennial ryegrass (146 ecotypes), tufted hairgrass (*Deschampsia cespitosa* L. – 68) and Kentucky bluegrass (*Poa pratensis* L. – 56 ecotypes). Only few ecotypes of giant fescue (*Festuca gigantea* (L.) Vill.) and Italian ryegrass (*Lolium multiflorum* Lam.) were also collected.

The highest endophyte colonization was noted for meadow fescue – from 81 to 75% of all ecotypes collected (tab. 1). Tall fescue colonization ranged from 8.3% (ecotypes from Central East) to 47.4% (ecotypes from Central Poland). Red fescue endophyte colonization ranged from

11.8 % (Central East) to 40.0% (North East Poland). Perennial ryegrass ecotypes were colonised at lower level, despite of ecotype origin (range from 17.9 to 25.8%). Only traces of colonization were detected in tufted hairgrass ecotypes (4.4%) and in one ecotype of giant fescue. Kentucky bluegrass ecotypes were not colonized at all. Tufted hairgrass or giant fescue were infrequently reported as grass species hosting endophytes.

Ergovaline contents were not detected in all ecotypes colonised by endophytes. Only in 19.5% from all ecotypes collected, ergovaline contents ranging from 0.005 up to 2.63 ppm were detected. The highest ergovaline contents were found in ecotypes of tall fescue from Central Poland (range from 1.21 up to 2.63 ppm). Such amounts are claimed to be extremely toxic for animals (Bony and Delatour, 2000). Chronic toxic amounts causing weight loss, milk production decrease, daily weight increase reduction etc. are 0.2 – 0.4 ppm, disease symptoms may occur at 0.3 – 0.5 ppm for horses or at 0.4 – 0.7 ppm for cattle.

Generally, in more than one-third of ecotypes with ergovaline, toxic amounts (more than 0.2 ppm) were detected. However, due to specific nature of Polish grasslands, possible animal threat associated with ergovaline content in sward is rather low. In swards of majority of Polish grasslands up to 25 – 30 plant species as grasses with dicotyledonous herbs grow together. Therefore, even high concentration of toxins in only a few plants per grassland will be ‘diluted’ in green sward or in hay. Tall fescue is not very common on pastures and therefore it is rarely consumed by animals. However, due to increasing area of highly productive short rotation meadows, the possibility of toxic concentration of ergovaline or other alkaloids become quite real. Very important are also local stress factors as drought, which may induce significant increase of alkaloids in grasses (Zabalgogeza and Bony, 2005). Therefore, if our grassland will suffer from summer drought periods, percentage of endophyte infected plants and average amounts of alkaloids may increase. This will directly influence animal health.

Table 1. Endophyte presence and average ergovaline contents in grass ecotypes collected in Poland

Country region	Genus, species	Ecotypes collected	Frequency (%) of ecotypes with:		Ergovaline contents (ppm):	
			endophyte	endoph.+ ergovaline	mean	range (min. - max.)
Central East	*Desch. cespitosa*	68	4.4	0.0	-	-
	F. arundiancea	12	8.3	8.3	1.298	1,298
	F.pratensis	69	81.2	49.3	0.069	0,005 - 0,749
	F.rubra	102	11.8	7.8	0.144	0,026 - 0,545
	Lolium perenne	76	23.7	15.8	0.023	0,011 - 0,080
Average for region			25.9	16.2	0.383	
North East	*F.ovina*	11	27.3	9.1	0.076	0,076
	F.pratensis	29	75.9	58.6	0.289	0,044 - 1,545
	L. multiflorum	1	1.0	0.0	-	-
	F.rubra	25	40.0	8.0	0.028	0,016 - 0,040
	L. perenne	31	25.8	22.6	0.121	0,065 - 0,192
Average for region			34.0	19.7	0.129	
Central	*F. arundiancea*	19	47.4	47.4	1.924	1,213 - 2,630
	F.ovina	1	100.0	0.0	-	-
	F.gigantea	2	100.0	50.0	0.112	0,112
	F.pratensis	32	75.0	59.4	0.097	0,024 - 0,433
	F.rubra	45	20.0	13.3	0.042	0,050 - 0,089
	L. perenne	39	17.9	15.4	0.595	0,162 - 1,706
	Poa pratensis	56	0.0	0.0	-	-
Average for region			51.5	26.5	0.554	
Average for all ecotypes			38.5	21.4	0.37	

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