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Effects of Endophyte Infection of Grasses on the Chemical Composition, Quality and Stability of Silage

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The aim of the study was to determine the effects of *Neotyphodium uncinatum* endophyte infection of fescue forage on the chemical stability and nutritive value of the forages and silages and on the quality and aerobic stability of the silages. The content of water soluble carbohydrates (WSC) in both forages was similar. The infected fescue (E) had a higher buffering capacity than the control fescue (statistically significant difference). The experimental silage had significantly higher dry matter than the control silage. The dry matter of endophyte-infected silage (E) contained more organic matter than that of the control silage, with a highly significant difference. The endophyte-infected silage (E) had a significantly lower content of crude protein than the control silage. There were no statistically significant differences in the content of other nutrients. In 1 kg dry matter of the fescue silage there was 91.80 g crude protein on average. 1 kg dry matter of the E silage contained more milk feed units than the control silages, with a statistically significant difference. Acidity of the E silage was higher than that of the control silage (4.19 vs 4.08) and the difference was statistically significant. Lactic acid prevailed in the silages and their quality assessed on the Flieg-Zimmer scale was very good. The level of the alkaloid ergovaline in the *Neotyphodium*-infected forage was high (2.63 ěg/g). In this study the levels of infection were 52% (seeds) and 47% (forage). No ergovaline was found in the silages made from the infected forage.

Key words: Endophytes, fescue, ergovaline, silage, quality, stability.

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In many farms grass silage is the basic bulky feed used in cattle nutrition. Its nutritive value and thus its nutritional suitability depends on the silage preparation method and on the course of the fermentation process (GĄSIOR & BRZÓSKA 2000).

The infection of grasses with endophytic fungi is very common in natural habitats. It makes plants more resistant to stress factors (cold, drought, pests) but can also cause many livestock diseases such as toxicosis, mastitis and changes in the composition of blood serum (AULDIST & THOM 2000; VASQUEZ DE ALDANA *et al*. 2001). These diseases are caused by toxic alkaloids produced by endophytes. The alkaloid concentration depends on the grass species. Ergot alkaloids, similar to the compounds found in ergot, are ubiquitous in fescue. It is known that organic acids are lethal to fungal organisms, which means that the increasing concentration during the ensiling process should reduce the effects of endophyte infection.

The aim of the study was to determine the effects of fescue forage infected with *Neotyphodium uncinatum* endophytic fungi on the chemical composition and nutritive value of the forages and silages and on the quality and aerobic stability of the silages. The authors wanted to find out if ensiling would reduce the level of the alkaloid ergovaline, typically found in *Neotyphodium uncinatum*, in the infected forage.

Material and Methods

Meadow fescue v. Pasja in the second year of use, harvested from experimental plots of the Mochełek Research Station belonging to the Department of Animal Nutrition and Feed Management of the University of Technology and Agriculture in Bydgoszcz, was investigated. Forages from fescue infected with *Neotyphodium* (=*Acremonium*) endophytes (E) and endophyte-free fescue (K) were

used in the experiment. Ensiling was performed in May 2004 from first-cut fescue at an early flowering stage. Silages were made in tubular-shaped polyethylene microsilos (15 cm in diameter, 49 cm in height). The tube tops were capped with rubber stoppers which contained fermentation tubes filled with glycerine, allowing the release of gases. Prior to ensiling, the forage was cut into approx. 3 cm lengths.

The silage was made in two variants: control without endophytes (K) and experimental from forage infected with *Neotyphodium uncinatum* endophytic fungi (E). Each variant was made in four replicates (microsilos).

Microsilos were housed in a laboratory facility at approx. 18°C and were opened 140 days later in October 2004.

The forage intended for ensiling was assayed for basic nutrients, fibre fractions, water soluble carbohydrates (WSC) and buffering capacity. The fermentation coefficient was calculated from the formula WF = SM(%) + 8 * C/PB (PAHLOW & WEISSBACH 1999).

The silages were analysed for basic nutrients, fibre fractions, acidity, ammonium nitrogen, acids (lactic, acetic and butyric) and alcohol.

Aerobic stability of the silages was tested for 7 days in an air-conditioned facility at the ambient temperature of 20C±1°C, following a method described by HONIG (1990). Changes in silage temperature in aerobic conditions were measured using a Squirrel 2000 unit, which recorded, at hourly intervals, temperature as a mean of two measurements taken every half hour.

Ergovaline level in the forages and silages was determined using the HPLC method (CRAIG *et al*. 1994).

Significant differences between the groups were analysed using Duncan's multiple range test (SAS/STAT 1995).

Results and Discussion

Forage

Forage from meadow fescue was wilted prior to ensiling, resulting in a statistically significant difference in the dry matter content (Table 1). No significant differences were found in basic nutrients and crude fibre fractions between endophyte-infected fescue (E) and control fescue (K).

Crude protein accounted for 81.97 g of forage dry matter on average. Other authors (JARRIGE 1993; PODKÓWKA *et al.* 2003; ZARUDZKI *et al.* 1997) reported a higher crude protein content of fescue. Fescue dry matter contained an average of 330.31 g crude fibre. The authors of a collective work (JARRIGE 1993) reported that at an early flowering stage fescue contains 12 g more crude fibre in dry matter. Other authors (PODKÓWKA *et al.* 2003; ZARUDZKI *et al.* 1997) reported lower concentrations of fibre in fescue forage. In our study, forage dry matter contained an average of 621.63 g NDF and 351.30 g ADF. PODKÓWKA *et al.* (2003) reported higher contents of both fibre fractions in fescue (660 and 453 g respectively per 1 kg dry matter). JARRIGE (1993) also reported higher ADF content of fescue forage.

The WSC content of both forages was at a similar level (Table 2). Endophyte-infected fescue (E) showed a higher buffering capacity than the control fescue (statistically significant difference), but this did not negatively affect its suitability for ensiling. The high WSC content and the low buffering capacity make fescue an excellent ensiling material. PODKÓWKA and PODKÓWKA (2001) re-

Table 1

Chemical composition of meadow fescue forages

		Dry matter g/kg	Content (g) in kg dry matter							
			Organic matter	Crude protein	Crude fat	Crude fat	N-free extractives	NDF	ADF	ADL
K n=10	$\overline{X}$	291.59[a]	943.78	80.39	14.73	331.92	516.74	625.90	354.37	31.15
	SE	2.67	0.83	1.07	0.67	3.20	3.08	8.87	4.00	0.62
E(+) n=12	$\overline{X}$	302.75[b]	945.20	83.28	14.08	328.97	518.87	618.08	348.75	30.75
	SE	3.16	1.37	1.22	0.31	3.95	3.42	4.33	2.68	1.03
Average	$\overline{X}$	297.68	944.55	81.97	14.37	330.31	517.90	621.63	351.30	30.93
	S_x	11.34	3.98	4.12	1.67	12.29	11.00	22.26	11.29	2.96

Significant differences are marked in columns with the different letters (P≤0.01 – A, B; P≤0.05 – a, b)

Table 2

Suitability of fescue forages for ensiling

		WSC, g/kg dry matter	Buffering capacity, g lactic acid per kg dry matter	WSC/Buffering capacity ratio	Fermentation coefficient acc. to Weissbach
K n=10	$\overline{X}$	159.72	29.99[a]	5.51	73.24
	SE	4.91	1.30	0.43	3.55
E n=12	$\overline{X}$	169.55	33.07[b]	5.13	71.34
	SE	2.58	0.60	0.06	0.58
Average	$\overline{X}$	165.08	31.67	5.31	72.20
	S_x	13.31	3.52	0.95	7.77

Significant differences are marked in columns with the different letters (P≤0.01 – A, B; P≤0.05 – a, b)

ported a lower WSC content and a higher buffering capacity of this plant.

The nutritive value of the forages (Table 3) was at a similar level. Energy expressed as UFL and UFV in terms of dry matter showed 0.05 higher values than those reported elsewhere (JARRIGE 1993) for this stage of fescue vegetation. The content of PDIE protein was close to that reported in the literature, while the PDIN level (g/kg dry matter) showed approx. 20 g lower values in our study.

Table 3

Nutritive value of forages calculated according to the INRA system (content in dry matter)

		UFL	UFV	PDIN (g)	PDIE (g)
K n=10	$\overline{X}$	0.80	0.72	50.48 ± 2.13	76.06 ± 0.76
	SE	0.00	0.00	0.67	0.24
E n=12	$\overline{X}$	0.80	0.72	52.30 ± 2.64	76.70 ± 0.84
	SE	0.00	0.00	0.76	0.24
Average	$\overline{X}$	0.80	0.72	51.47	76.41
	S_x	0.01	0.01	2.59	0.86

Silages

Differences in the dry matter content of forages intended for ensiling resulted in the experimental silage having statistically higher dry matter than the control silage (262.96 g vs 278.37 g) (Table 4). In the dry matter of endophyte-infected silage (E) there was 10.36 g less organic matter than in the control silage, with a statistically significant difference. Silage from endophyte-infected fescue (E) contained significantly less crude protein (by 11.19 g) than the control silage. There were no statistically significant differences in the content of other nutrients.

In 1 kg dry matter of the fescue silage there was an average of 91.80 g crude protein, although PODKÓWKA *et al.* (2003) reported a higher protein content of this silage. Crude fibre accounted for 345.75 g of the silage dry matter. PODKÓWKA *et al.* (2003) reported a higher fibre content of fescue silage.

Silage made from the endophyte-infected forage (E) had a higher content of neutral detergent fibre (by 29.86 g/kg dry matter) and acid detergent fibre (by 18.82 g/kg dry matter), with highly significant differences. No significant differences were found in the lignin content of the silages.

Table 4

Chemical composition of the meadow fescue silages made in microsilos

		Dry matter, g/kg	Content (g) in kg dry matter							
			Organic matter	Crude protein	Crude fat	Crude fibre	N-free extractives	NDF	ADF	ADL
K n=4	$\overline{X}$	262.96[a]	935.25[A]	97.39[a]	32.83	335.49	404.79	593.77[A]	356.84[A]	29.79
	SE	3.18	0.30	2.52	1.30	10.43	7.52	4.08	2.29	1.02
E n=4	$\overline{X}$	278.37[b]	945.61[B]	86.20[b]	35.22	356.01	413.79	623.63[B]	375.66[B]	31.54
	SE	4.05	0.50	1.15	2.40	7.39	8.87	1.83	2.73	1.62
Average	$\overline{X}$	270.67	940.43	91.80	34.03	345.75	409.29	608.70	366.25	30.67
	Sx	10.60	5.25	6.83	4.04	20.79	17.06	16.22	10.67	2.84

Significant differences are marked in columns with the different letters (P≤0.01 – A, B; P≤0.05 – a, b)

In 1 kg dry matter, E silages contained more milk feed units than the control silages (0.81 vs 0.80), the difference being statistically significant (Table 5). The content of meat feed units was similar in both silages. The infected fescue silage had 6.5 g/kg dry matter PDIN less than the control silage. The difference in PDIE content between the silages was not statistically significant.

Table 5

Nutritive value of the silages from microsilos as calculated according to the INRA system (content in dry matter)

		UFL	UFV	PDIN (g)	PDIE (g)
K	$\overline{X}$	0.80[a]	0.72	56.64[a]	51.64
n=4	SE	0.00	0.00	1.47	0.68
E	$\overline{X}$	0.81[b]	0.73	50.14[b]	49.70
n=4	SE	0.00	0.00	0.67	0.19
Average	$\overline{X}$	0.81	0.73	53.40	50.67
	Sx	0.01	0.01	3.97	1.39

Significant differences are marked in columns with the different letters ($P \leq 0.01$ – A, B; $P \leq 0.05$ – a, b)

The acidity of silage made from endophyte-infected forage (E) was higher than that of the control silage (4.19 vs 4.08), with a statistically significant difference (Table 6). This is in line with the previous findings of PODKÓWKA *et al.* (2003) that silage from endophyte-infected forage has a higher pH than the silage from endophyte-free forage. Lactic acid prevailed in the silages and their quality assessed on the Flieg-Zimmer scale was very good. Differences in the content of particular acids between the groups were not significant. PODKÓWKA *et al.* (2003) reported that E silage contained less acetic acid than the control silage. A small amount of ammonium nitrogen in silages (0.78% in dry matter) is evidence that the ensiling process was normal and protein degradation low.

Ergovaline level

Ergovaline content of the analysed material is given in Table 7. The alkaloid level in the *Neotyphodium*-infected forage was high (2.63 μg/g). Other authors (VASQUEZ DE ALDANA *et al.* 2001) reported that the levels of this toxin in fescue samples from Europe, America and Australia ranged from 0.52 to 4.62 μg/g at an infection rate of 4-98%. In our study the levels of infection were 52% (seeds) and 47% (forage). No ergovaline was found in the silages made from the infected forage.

Stability

Figure 1 shows average daily temperatures of the silages stored in aerobic conditions at the ambient temperature of 21°C. The results reveal that both the silage prepared from endophyte-free plants (K) and the endophyte-infected silage (D) began to heat on the fourth day of incubation. However, the temperature rise was quicker for the K variant. Fig. 2 illustrates the average stability of the analysed silages by hours. The silage from endophyte-infected

Table 6

Quality of fescue silages made in microsilos

		pH	Content in dry matter					Score (points)	Quality
			Lactic acid (g)	Acetic acid (g)	Butyric acid (g)	Total N to NH_3-N (%)	Alcohol (%)		
K	$\overline{X}$	4.08[a]	148.61	27.07	0.19	0.79	2.14		
n=4	SE	0.01	6.54	1.72	0.09	0.10	0.08		
E	$\overline{X}$	4.19[b]	152.38	25.82	3.30	0.77	2.17		
n=4	SE	0.04	3.97	4.01	1.37	0.07	0.03		
Average	$\overline{X}$	4.13	150.50	26.45	1.75	0.78	2.16	100	Very good
	S_x	0.08	10.98	6.20	2.49	0.17	0.13		

Significant differences are marked in columns with the different letters ($P \leq 0.01$ – A, B; $P \leq 0.05$ – a, b)

Table 7

Results of ergovaline analyses (μg/ml)

Item		K	E
Forages n=22	$\overline{X}$	nd	2.63
	S_x		1.10
Silages n=8	$\overline{X}$	nd	nd
	S_x		

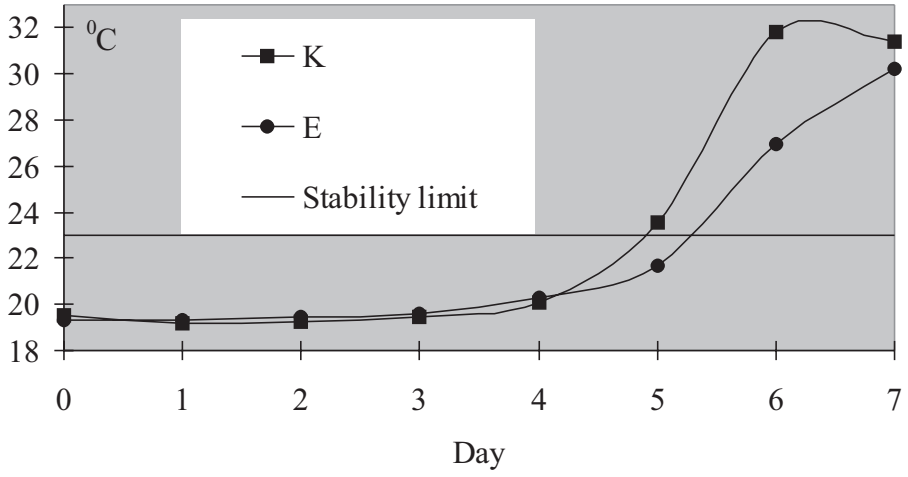

Fig. 1. Average daily temperature of the silages (ambient temperature 20°C).

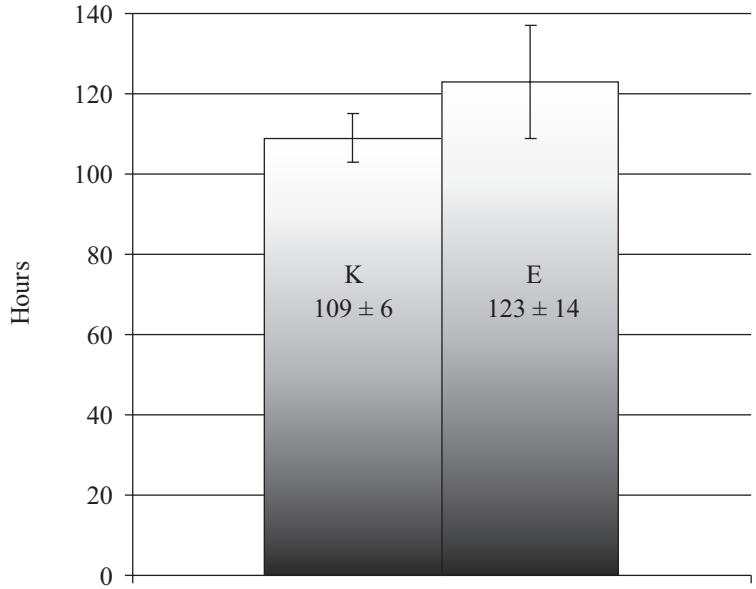

Fig. 2. Silage stability. No statistically significant differences.

grasses was slightly more stable than the control silage (without additives and endophyte free). However, the difference was not significant. PODKÓWKA *et al.* (2003) reported that the endophyte-infected silage showed poorer parameters of aerobic stability than the endophyte-free silage.

Conclusions

1. Infection with the *Neotyphodium uncinatum* endophyte had no effect on the chemical composition and nutritive value of meadow fescue forages.

2. The endophyte-infected silage was found to contain less crude protein, but there were no differences in the content of other basic nutrients.

3. The analysed silages were of very good quality.

4. Ensiling caused the alkaloid ergovaline in the endophyte-infected forage to break down.

5. Aerobic stability is greater for the silages from endophyte-infected grasses than for the silages from endophyte-free grasses, but the difference was not significant.

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