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INFLUENCE OF SPECIES AND PRESERVATIONS ON THE QUALITY AND SAFETY OF GRASS SILAGES

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Abstract

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The paper evaluates the quality of model silages made of wilted grass biomass and treated with silage additives. Grass species used for the production of silages were *Lolium perenne*, *Festulolium pabulare* and *Festulolium braunii* harvested in the first cut at the stage of earing. The assessed grass species were wilted after the cut for an identical time 36 hours (2008), resp. 24 hours (2009). The treatment was made either with a chemical preparation (formic acid, propionic acid, ammonium formate) and/or with a biological inoculant (*Enterococcus faecium*, *Lactobacillus plantarum*, *Pediococcus acidilactici*, *Lactobacillus salivarius*, cellulase, hemicellulase and amylase). The amount of the chemical ingredient was 4 l.t^{-1} and the amount of the biological additive was 10 g.t^{-1}.The biomass was after wilting ensilaged in containers whose diameter and height were 0.15 m and 0.64 m, respectively. After 60 days of ensilaging, the silages were assessed for pH, organic acids content, ethanol content and acidity of water extract (AWE); organic nutrients assessed in the silages were crude fibre (CF), neutral detergent fibre (NDF), acid detergent fibre (ADF), crude protein (CP) and digestibility of organic matter (DOM). Hygienic safety was assessed from the contents of zearalenon, fumonisin and aflatoxin mycotoxins. The high ($P < 0.05$) dry matter (DM) content in *Festulolium pabulare* silages indicates that the species tends to rapid wilting. The higher DM content reflected in lower biomass losses ($P < 0.05$). The lowest pH values ($P < 0.05$) were detected in silages made of *Festulolium braunii*. The fact relates to the higher content of lactic acid in the prepared microsilages. The use of ensiling additives affected the quality of extracts. Namely the application of the biological additive led to the increased content ($P < 0.05$) of not only lactic acid but acetic acid too. Titrable acidity was not affected by the ensiling additives. As to the emanation of ethanol, heterofermentative bacteria of lactic fermentation apparently took part in the fermentation process of the silages as well. While the evaluated forage species showed differences in the digestibility of organic matter ($P < 0.05$), the application of preservatives did not influence the content of organic nutrients. Fumonisin was not detected at all and aflatoxins were below the level of detection. None of the assessed factors had an effect on the content of zearalenon; in spite of the fact, an apparent tendency towards a higher content of zearalenon was recorded in *Lolium perenne*.

Lolium perenne, *Festulolium*, silage fermentation, organic nutrient, mycotoxins

Winter feed ration is based on the conserved fodder from the first cut of grassland. As compared with haymaking, ensiling represents a much lower weather risk, which reflects favourably in working costs and low conservation losses (Achilles *et al.*, 2002). Grassland management and weather conditions influence ensiling (Krizsan and Randby, 2007). Individual graminaceous species show great differences in their ensiling capacity (Holúbek *et al.*, 2007). Different ensiling techniques contribute to variation in fermentation quality (Krizsan and Randby, 2007). The variation in fermentation quality of grass silages affects the voluntary intake of cattle (Huhtanen *et al.*, 2002). Thanks to the suitable selection of lactic

acid bacteria, biological inoculants have a beneficial effect on the improvement of the fermentation quality of silages (Wrobel *et al.*, 2004; Jatkausas *et al.*, 2010). Similarly, Honig and Pahlow (1986) claim that the application of ensiling inoculants to partly wilted grasses with the DM content higher than 26% and lower than 45% improves the course of the fermentation process and decreases the total loss of nutrients. The conservation of wilted grass biomass by means of inoculants was also studied by Wyss (1999), Potkanski *et al.* (1999), Taina *et al.* (1999) and others.

Prerequisite for high-quality silage are not only the bacteria of lactic fermentation but also a clean and healthy phytomass (Holúbek *et al.*, 2007). Development of microscopic fungi may lead to the formation of mycotoxins (Opitz von Boberfeld *et al.*, 2006). These metabolites can cause economic losses in animal production and decreased meat quality (Opitz von Boberfeld, 1996). There are considerable differences among the species. Mould-resistant species include *Festuca arundinacea* and its hybrids (Opitz von Boberfeld and Banzhaf, 2006).

The goal of the work was to assess differences in the content of dry matter, quality of silage liquors and content of organic nutrients in silages made of *Lolium perenne*, *Festulolium pabulare* and *Festulolium braunii*. At the same time, the effect was established of ensiling additives on the quality of ensiled biomass and evaluated was also the incidence of zearalenon, fumonisin and aflatoxin mycotoxins.

MATERIAL AND METHODS

Experimental locality

The small-plot experiment was conducted at the Research Station of Fodder Crops in Vatín, Czech Republic (49°31'N, 15°58'E) and established in 2007 at an altitude of 560 m a.s.l. In 1970–2000, mean annual precipitation was 617 mm and mean annual temperature was 6.9 °C. Soil type used in our experiments was Cambisol as a sandy-loam on the diluvium of biotic orthogneiss. In the year of observation, the contents of soil nutrients were 89.1 mg.kg^{-1} P, 231.6 mg.kg^{-1} K, 855 mg.kg^{-1} Ca; pH was 4.76. The experimental plots were fertilized with 50 kg.ha^{-1} N in the spring (March). Dates of cuts were 9 June 2008 and/or 1 June 2009. A split plot design was used with plots of 1.5 × 10 m. The plots were harvested by the self-propelled mowing machine with an engagement width of 1.25 m. Harvested area was 12.5 m^2. Stubble height was 0.07 m. The grasses were harvested at the stage of earing.

Experimental design

The experiment was carried out in triplicates. The first evaluated factor was species for silages: *Lolium perenne* (cv. Kentaur), *Festulolium pabulare* (cv. Felina) and *Festulolium braunii* (cv. Perseus). Pure stands of each species were sown with 30 kg.ha^{-1} seeds. The assessed grasses were wilted for an identical length of time. The wilting time was 36 hours (2008) resp. 24 hours (2009). The second evaluated factor was preservative: untreated, chemical ingredient (formic acid, propionic acid, ammonium formate) and biological inoculant (*Enterococcus faecium, Lactobacillus plantarum, Pediococcus acidilactici, Lactobacillus salivarius*, cellulase, hemicellulase, and amylase). The amount of chemical ingredient was 4 l.t^{-1} and the amount of biological additive was 10 g.t^{-1}. Biomass was after wilting ensilaged in containers whose diameter and height were 0.15 m and 0.64 m, respectively. The observation lasted for two years 2008 (1st harvest year) and 2009 (2nd harvest year). The cumulative effect was observed.

Detected parameters

Green forage samples and silages dried at 60 °C and homogenized to a particle size of < 1 mm were analyzed for digestibility of organic matter (DOM), crude protein content (CP), crude fibre content (CF), neutral detergent fibre (NDF) and acid detergent fibre (ADF). Silages sampled 60 days after the beginning of conservation were assessed for pH, acidity of water extract (AWE), contents of lactic acid (LA), acetic acid (AA), (AOAC, 1980). The content of alcohol was established by a method described by Hartmann (1974). Analytical procedures including the preparation of water extract were in details characterized in our previous paper (Doležal, 2002). The content of nutrients was established according to the norm of the Czech Standard Institute (ČSN 467092). ELISA method was applied to estimate the content of mycotoxins zearalenone (ZEA), fumonisin (FUM) and aflatoxin (AFL), (Nedělník and Moravcová, 2006).

Statistical analyses

The data were processed using the STATISTICA. CZ Version 8.0 (Czech Republic). The results are expressed as means (x), which are supplemented with the standard error of mean (s.e.). The obtained results were further analyzed using the ANOVA.

RESULTS AND DISCUSSION

Silages from *Festulolium pabulare* had the highest dry matter (DM) content ($P < 0.05$). Although among harvesting DM contents were not significant difference. *Festulolium braunii* had DM content 19.3 % in 2008 and 24.4 % in 2009, *Lolium perenne* 20.4 % (25.0 %) and *Festulolium pabulare* 23.7 % (27.4 %). Regarding the fact that the assessed fodder species were wilting for an identical length of time, a higher wilting rate apparently exists in Festucoid hybrids. The higher DM content reflected in lower biomass losses ($P < 0.05$). The biomass loss was obviously affected also by the silage treatment. Silages treated with additives were observed to have lower biomass losses than untreated control silages (Tab. I). The finding is in line with Knický and Spörndly (2010) who claim that silages treated with additives exhibit lower DM losses. The lowest pH ($P < 0.05$) was ob-

I: *Effect of forage type and ensiling additive on DM content and biomass loss of grass silages*

Factor	DM (%)	Biomass loss (%)
Species (S)		
Lolium perenne	37.14[a]	0.88[ab]
Festulolium pabulare	47.80[b]	0.58[a]
Festulolium braunii	38.54[a]	0.96[b]
s.e.	1.053	0.101
Level of significance	0.000	0.044
Preservative (P)		
Untreated	41.07	0.99
Chemical ingredient	41.56	0.72
Biological inoculant	40.84	0.71
s.e.	1.555	0.110
Level of significance	0.945	0.154
Interaction S x P	0.961	0.590
Year		
2008	45.30[a]	0.87
2009	37.02[b]	0.75
s.e.	0.962	0.096
Level of significance	0.000	0.378

Indices ([a,b]) are to express significant differences at a level of $P < 0.05$

served in silages from *Festulolium braunii* (Tab. II). The fact also connects with a higher content of lactic acid in the prepared silages. These results correspond with the former findings of Doležal and Hejduk (2002), Jambor *et al.* (1995) or Novák and Škultéty (1995). The results show the pH value of silages sampled at the end of the experiment. The comparable pH between the untreated silages and the silages treated with a chemical preservative might have been due to the presence of epiphytic microflora in the ensiled biomass. Although the pH value rapidly decreased after the application of the chemical preparation based on a mixture of organic acids, the spared sugars would be used in the following phase by the epiphytic microflora (heterofermentative bacteria) and the pH value would increase again (Pieper *et al.*, 2010). In contrast, the stable pH value in silages is ensured by the homofermentative bacteria of lactic acid fermentation. These additives affected the content of lactic acid too ($P < 0.05$); the supplementation of bacteria and enzymes reflected in its increased content as well as in the increased content of acetic acid.

The increased contents of lactic acid or all fermentation acids represent a certain dietary risk related to rumen acidity. In this context, Wilkinson (1999) informs that a too high acidity of silages represents a certain excess connected with necessary buffer action on acids in the rumen of ruminants. Excessively acidic silages may also lead to a lower voluntary intake of silage DM by animals, namely if the content of acetic acid is high. A number of works deal with factors affecting the salivation of animals in dependence on feed mixture composition, DM content and fibre content in fermented feeds. Similarly, Cushnahan *et al.* (1996) informs about a change

II: *Effect of forage type and ensiling additive on pH, contents of lactic acid (LA), acidic acid (AA), LA/AA ratio and acidity of water extract (AWE) in grass silages*

Factor	pH	LA (g kg^{-1} DM)	AA (g kg^{-1} DM)	LA/AA	AWE (mg 100g^{-1})	Ethanol (g kg^{-1} DM)
Species (S)						
Lolium perenne	4.35[a]	78.6[a]	10.8	9.02[a]	1328.58[a]	22.5
Festulolium pabulare	4.31[ab]	70.8[a]	9.1	9.52[a]	1798.06[b]	16.8
Festulolium braunii	4.15[b]	110.2[b]	9.2	24.60[b]	1572.44[ab]	18.4
s.e.	0.057	11.08	1.22	3.795	104.841	2.750
Level of significance	0.044	0.036	0.522	0.007	0.010	0.331
Preservative (P)						
Untreated	4.16[a]	84.8[ab]	7.6[a]	16.75	1635.72	15.02
Chemical ingredient	4.23[a]	67.1[a]	9.5[ab]	11.49	1559.94	21.21
Biological inoculant	4.42[b]	107.5[b]	12.1[b]	14.89	1503.42	21.54
s.e.	0.055	11.12	1.15	4.148	113.933	2.717
Level of significance	0.003	0.044	0.029	0.664	0.714	0.171
Interaction S x P	0.007	0.307	0.800	0.918	0.241	0.908
Year						
2008	4.28	65.9[a]	13.2[a]	5.13[a]	1494.24	24.51[a]
2009	4.26	107.1[b]	6.2[b]	23.63[b]	1638.48	14.01[b]
s.e.	0.049	8.67	0.72	2.853	91.653	2.03
Level of significance	0.700	0.001	0.000	0.000	0.271	0.001

Mean values in the same columns with different superscripts ([a,b]) are significant at the $P < 0.05$ level

in the voluntary intake of grass silage dry matter in the course of 52 weeks in dependence on the change of pH, concentration of lactic and butyric acids and ammonia. The results suggest that a longer intake of dietetically less favourable diets the buffering capacity of animals becomes exhausted which subsequently leads to the decreased voluntary intake of dry matter, increased intake of alkaline mineral substances, urine drinking or preference of feeds with a higher concentration of fibre. With clinical forms of acidosis, the animals stop eating, milk production rapidly drops as well as rumination and the animals may even die.

An important indicator of fermentation process quality is acidity water extract (AWE), which corresponds with the concentration of fermentation acids. Tab. II shows however that the produced silages are of comparable AWE irrespective of their treatment.

In terms of ethanol emanation, the fermentation process of experimental silages can be valuated as successful only up to a certain limit. In spite of the fact that experimental silages treated with the biological additive exhibited the highest amount of lactic acid, it is evident that heterofermentative bacteria of lactic fermentation played also a great role in the production of ethanol in these silages. Alcohol as a minor fermentation product in silages is often generally put into connection with a higher dry matter content in the ensiled material and with a higher length of chopped straw. At the same time, it also signals a fact that the proper lactic fermentation could have been restricted (Driehuis *et al.*, 1999). Potential factors under conditions of our experiment appear to be the length of chopped straw and the DM content of green material. This is demonstrated particularly by the difference between the years 2008 and 2009 when a higher DM of ensiled biomass was recorded in the first experimental year and was followed by an increased content of ethanol.

Driehuis *et al.* (1999) can see prevention against alcohol fermentation in the suppressed activity of enterobacteria and particularly in the stimulation of the fermentation process. By contrast, Seija *et al.* (1999) observed a reduced production of alcohol but also of fermentation acids including lactic acid after the application of active chemical preservatives based on organic acids. Juráček (2002) states too that the use of microbial or chemical ensiling additives resulted in the reduced amount of alcohol in silages.

While the surveyed fodder species differed in the digestibility of organic matter ($P < 0.05$), the application of conservative additives did not affect the content of organic nutrients (Tab. III). *Festulolium pabulare* apparently exhibited a higher content of fibre and lower contents of crude protein (CP) and net energy of lactation (NEL) but a significant difference existed only in the digestibility of organic matter ($P < 0.05$). The lower quality of *Festulolium pabulare* as compared with *Lolium perenne* and *Festulolium braunii* (already in green matter) is documented also by other works. This indicates that the higher DM content of *Festulolium pabulare* at the beginning of the ensiling process does not necessarily have to be the reason for worse DOM values. Interesting is

III: *Effect of forage type and ensiling additive on digestibility of organic matter (DOM), crude proteins (CP), crude fibre (CF), neutral detergent fibre (NDF), acid detergent fibre (ADF) and net energy of lactation (NEL) in grass silages*

Factor	DOM (%)	CP (g kg^{-1} DM)	CF (g kg^{-1} DM)	NDF (g kg^{-1} DM)	ADF (g kg^{-1} DM)	NEL (MJ kg^{-1} DM)
Species (S)						
Lolium perenne	82.81^{a}	83.8	279.95	504.23	314.03	5.93
Festulolium pabulare	74.74^{b}	80.0	328.60	606.11	377.94	5.26
Festulolium braunii	81.74^{a}	73.2	301.11	526.99	322.13	5.85
s.e.	1.192	3.29	20.36	33.55	24.48	0.257
Level of significance	0.000	0.101	0.269	0.112	0.167	0.167
Preservative (P)						
Untreated	79.94	76.5	308.60	544.35	341.01	5.65
Chemical ingredient	79.07	79.5	303.33	545.41	331.88	5.74
Biological inoculant	80.28	81.0	297.70	547.57	341.21	5.65
s.e.	1.981	3.74	22.14	38.813	27.51	0.289
Level of significance	0.907	0.692	0.941	0.998	0.963	0.963
Interaction S x P	0.456	0.943	0.999	0.999	0.998	0.998
Year						
2008	77.87	73.3^{a}	347.17^{a}	616.98^{a}	390.49^{a}	5.13^{a}
2009	81.65	84.8^{b}	259.27^{b}	474.58^{b}	285.58^{b}	6.23^{b}
s.e.	1.428	2.24	8.20	17.553	11.467	0.120
Level of significance	0.080	0.002	0.000	0.000	0.000	0.000

Mean values in the same columns with different superscripts (^{a,b}) are significant at the $P < 0.05$ level

the look at the occurrence of mycotoxins. There was zearalenon detected in the silages (Tab. IV). Fumonisin was not detected at all and aflatoxins were below the level of detection. None of the studied factors had an influence on the zearalenon content but a tendency to the higher occurrence of zearalenon apparently existed in *Lolium perenne*. As to the preservatives, a lower zearalenon content in the silage was apparently found with the use of the biological inoculant. Statistical significance was not demonstrated namely with respect to a higher standard error of the mean. The fact may prove that the contamination of the green matter by mycotoxins was non-uniform.

IV: *Effect of forage type and ensiling additive on the content of zearalenon (ZEA), fumonisin (FUM) and aflatoxins (AFL) in grass silages*

Factor	ZEA (ppb)	FUM (ppb)	AFL (ppb)
Species (S)			
Lolium perenne	71.65	0	<LOQ
Festulolium pabulare	68.17	0	<LOQ
Festulolium braunii	59.67	0	<LOQ
s.e.	12.31	-	-
Level of significance	0.518	-	-
Preservative (P)			
Untreated	70.32	0	<LOQ
Chemical ingredient	74.40	0	<LOQ
Biological inoculant	54.77	0	<LOQ
s.e.	12.50	-	-
Level of significance	0.653	-	-
Interaction S x P	0.469	-	-
Year			
2008	62.29	0	<LOQ
2009	70.70	0	<LOQ
s.e.	9.56	-	-
Level of significance	0.166	-	-

LOQ = limit of quantifications

SUMMARY

The goal of the work was to assess differences in the content of dry matter, quality of silage liquors and content of organic nutrients in silages made of *Lolium perenne*, *Festulolium pabulare* and *Festulolium braunii*. At the same time, the effect was established of ensiling additives on the quality of ensiled biomass and evaluated was also the incidence of zearalenon, fumonisin and aflatoxin mycotoxins. Grass species used for the production of silages were *Lolium perenne*, *Festulolium pabulare* and *Festulolium braunii* harvested in the first cut at the stage of earing. The assessed grass species were wilted after the cut for an identical time 36 hours (2008), resp. 24 hours (2009). The treatment was made either with a chemical preparation (formic acid, propionic acid, ammonium formate) and/or with a biological inoculant (*Enterococcus faecium*, *Lactobacillus plantarum*, *Pediococcus acidilactici*, *Lactobacillus salivarius*, cellulase, hemicellulase and amylase). The amount of the chemical ingredient was 4 l.t^{-1} and the amount of the biological additive was 10 g.t^{-1}.The biomass was after wilting ensilaged in containers whose diameter and height were 0.15 m and 0.64 m, respectively. After 60 days of ensilaging, the silages were assessed for pH, organic acids content, ethanol content and acidity of water extract (AWE); organic nutrients assessed in the silages were crude fibre (CF), neutral detergent fibre (NDF), acid detergent fibre (ADF), crude protein (CP) and digestibility of organic matter (DOM). Hygienic safety was assessed from the contents of zearalenon, fumonisin and aflatoxin mycotoxins. The higher ($P < 0.05$) dry matter content found in *Festulolium pabulare* suggests the tendency of this species to more rapid wilting and faster lignification. The higher DM content was connected with lower biomass losses but did not affect the quality of silage extracts. The lower ($P < 0.05$) *in vitro* digestibility of organic matter in *Festulolium pabulare* is in contrast with the quality of *Lolium perenne* and *Festulolium braunii*. The high ($P < 0.05$) *in vitro* digestibility of organic matter in *Festulolium braunii*, but especially the higher content ($P < 0.05$) of lactic acid and the lower ($P < 0.05$) pH demonstrate the suitability of this hybrid for ensiling. Although was difference in the fibre content between species, the differences were not statistically significant. The use of ensiling additives affected the quality of silage extracts; particularly the application of the biological additive resulted in the increased ($P < 0.05$) content of not only lactic acid but also acetic acid. The content of organic nutrients was not significantly influenced by ensiling additives. Of mycotoxins, the silages contained zearalenon. The content of zearalenon was significantly affected neither by the species nor by the applied ensiling additive.

SOUHRN

Vliv druhu a ošetření na kvalitu a zdravotní bezpečnost travních siláží

Cílem práce bylo zhodnotit rozdíly v obsahu sušiny, kvality výluhů siláží a obsahu organických živin u siláží vyrobených z *Lolium perenne, Festulolium pabulare* a *Festulolium braunii*. Současně byl posouzen vliv silážních aditiv na kvalitu silážované biomasy a vyhodnocena přítomnost mykotoxinů zearalenonu, fumonisinu a aflatoxinů. Pro výrobu siláží byly využity druhy *Lolium perenne*, *Festulolium pabulare* a *Festulolium braunii* sklízené v první seči ve fázi metání. Hodnocené druhy trav zavadaly po seči stejnou dobu. Doba zavadání byla 36 hod (2008), resp. 24 hod (2009). Pro ošetření byl použit chemický přípravek (kyselina mravenčí, kyselina propionová, mravenčan amonný) nebo biologický inokulant (*Enterococcus faecium, Lactobacillus plantarum, Pediococcus acidilactici, Lactobacillus salivarius*, celuláza, hemiceluláza a amyláza). Chemický přípravek byl dávkován v množství 4 $l.t^{-1}$ a biologický přípravek v množství 10 $g.t^{-1}$. Po zavadnutí byla hmota silážována v kontejnerech o průměru 0,15 m a výšce 0,64 m. Šedesát dnů po začátku silážování bylo hodnoceno pH siláží, obsah organických kyselin, obsah etanolu a kyselost vodního výluhu (KVV), z organických živin byl hodnocen obsah vlákniny, neutrodetergentní vlákniny (NDV), acidodetergentní vlákniny (ADV), dusíkatých látek (NL) a stravitelnost organické hmoty (SOH). Zdravotní nezávadnost byla posouzena na základě obsahu mykotoxinů zearalenonu, fomonisinu a aflatoxinů. Vyšší ($P < 0,05$) obsah sušiny u *Festulolium pabulare* ukazuje na tendenci tohoto druhu k rychlejšímu zavadání a také k rychlejší lignifikaci. Vyšší obsah sušiny souvisel s nižšími ztrátami hmoty, ale neovlivnil kvalitu silážních výluhů. Nižší ($P < 0,05$) *in vitro* stravitelnost organické hmoty u *Festulolium pabulare* je v protikladu s kvalitou *Lolium perenne* a *Festulolium braunii*. Vysoká ($P < 0,05$) *in vitro* stravitelnost organické hmoty *Festulolium braunii*, ale zejména vyšší ($P < 0,05$) obsah kyseliny mléčné a nižší ($P < 0,05$) hodnota pH dokládá vhodnost tohoto hybridu k silážování. Mezi druhy byly sice zřejmé rozdíly v obsahu vlákniny, ale tyto rozdíly nebyly statisticky průkazné. Použití silážních aditiv ovlivnilo kvalitu výluhů; zejména aplikace biologického aditiva vedla ke zvýšení ($P < 0,05$) obsahu nejen kyseliny mléčné, ale také kyseliny octové. Obsah organických živin nebyl silážními aditivy výrazně ovlivněn. Z mykotoxinů byl v silážích detekován zearalenon. Obsah zearalenonu nebyl průkazně ovlivněn druhem ani použitým silážním aditivem.

Lolium perenne, *Festulolium*, fermentace, kvalita siláží, organické živiny, mykotoxiny

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