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Production and finalization of the milk in the EU

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The world cow milk production is constantly increasing and based on the estimations will reach in 2010 585 mil tons. However the trend of increase has been slowing down since 2005. In 2008 it only reached 1.6 %, there is the estimation for 2009 0.8 % only.

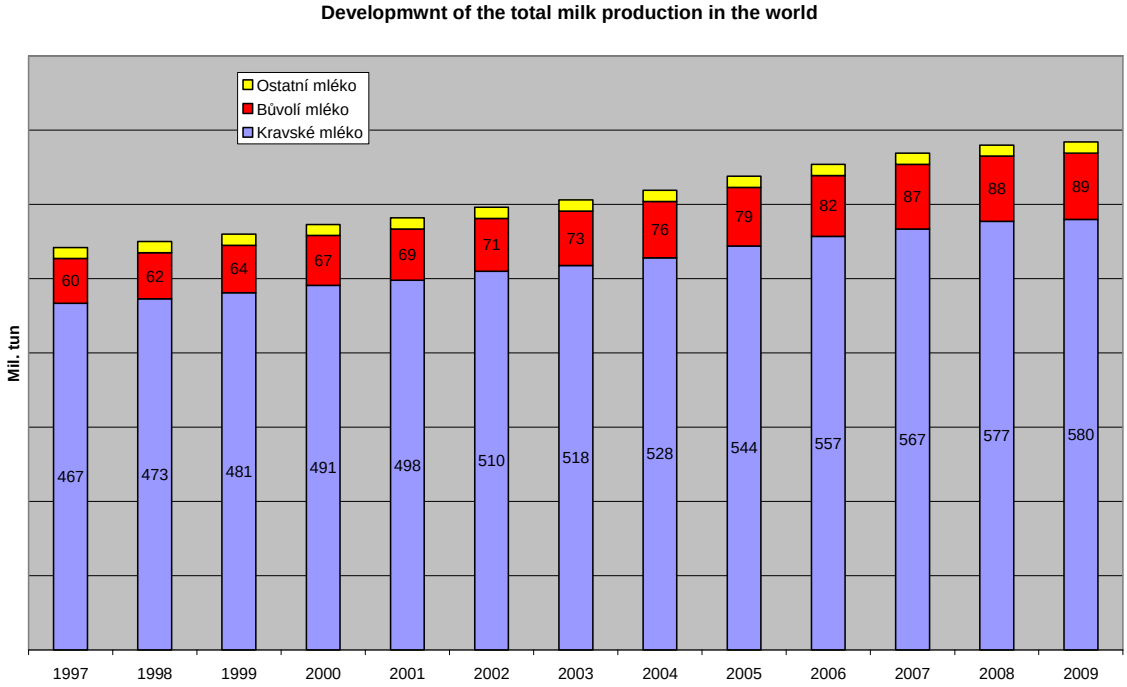
Rather low increase of the total milk production is expected for the year 2010. In the Oceania and especially in Australia this year's production is staying markedly below the expectations. In the US the increasing trend was interrupted during the fall 2009 and the prognosis for 2010 confirms a long-term interruption of the above mentioned trend.

In the most important parts of Latin America (Argentina, Brazil and Mexico) a stagnation of the milk production up to a small decline of the production is also confirmed for the year 2010.

India produced in 2009 more milk compared to the 2008, but the increased production is covering the increasing consumption of the milk in the country with permanent increasing number of inhabitants. There is an inconsistent information available from China According to the increasing volumes of imported SMP in China the decreasing trend of raw milk production is expected. In Russia the increasing trend in the self-sufficiency level in milk is expected thanks to state support schemes.

European Union is the biggest milk producers in the world with ca 148 mil tons of milk, followed by India, US and China.

In 2008 the decrease of the cows was stopped in the EU and Russia. The number of cattle in the EU (December 2008): 86,3 mil of heads, of which 23,7 milk dairy cows and 11,7 beef cows are registered.



However the additional milk quota was available on the EU market the total milk collections of the period jan-sep 2009 compared to jan-sep 2008 decreased by -0,32 %.

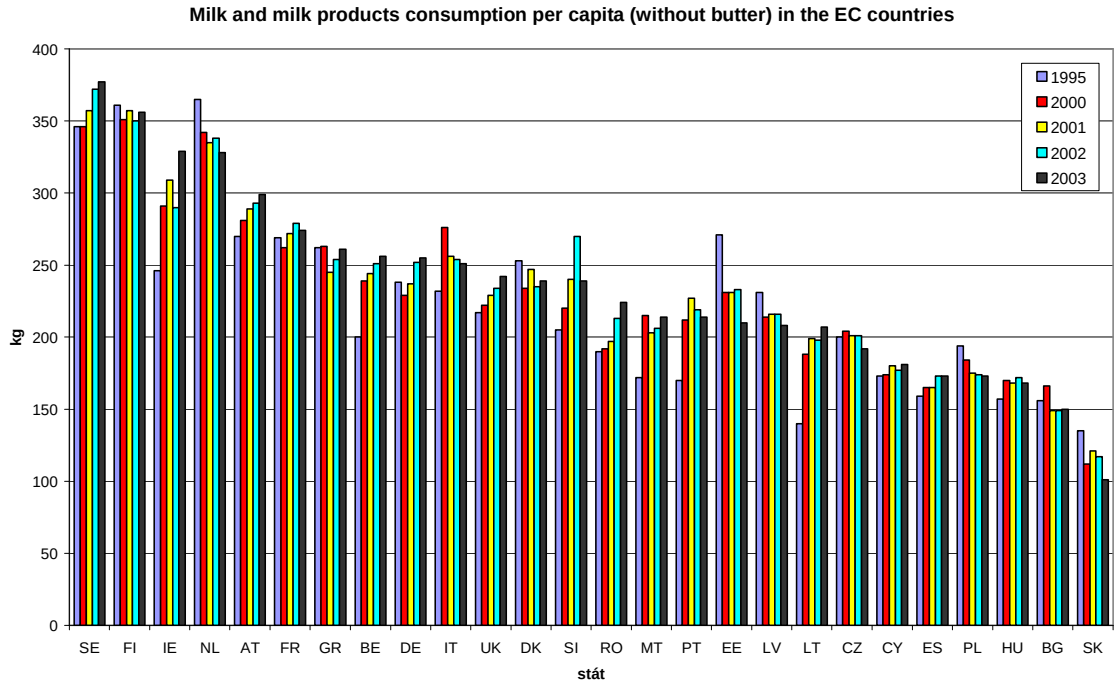
The international market in 2008 decreased, the USA were the only exception. More SMP were traded, less cheeses and butter. EU stayed in a position of the biggest exporter (12.5 mil tons of milk equivalent), followed by New Zealand (11.8 T MEQ) and the US (6 mil T MEQ).

During the summer 2009 the international trade turned up. Mainly due to higher demand for skin milk powder and butter.

The interventions stocks of the EU represent 76 353 tons of butter in intervention stock and 25 561 tons in private storages. In the intervention storages of the EU the biggest amount of SMP since 1990 has been stored – 257 788 tons (7.1. 2010).

Consumption and trade with the milk and milk products are more and more depending on the current economical situation and in the future higher volatility is expected. Consumption is highly sensitive on the retail-price level and is very different in the particular EU member states.

The total per capita consumption of milk products reached the average of 101,6 kg in the whole world in 2008. Cheese consumption per capita in the EU represent 17,9 kg, compared to 15.0 kg in US or 11.8 kg in Australia (data 2008).



The system of common agriculture policy after 2013 is crucial for the milk producers in the EU in the future. Especially for the new member states the abolition of the current dual and discriminating common agricultural policy is necessary.

Section 1: Forage Production - Yield, Quality, Fertilization

Sponzor of section

Forage conservation in mountainous regions – results of the Austrian silage monitoring project

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Introduction and field of problems

Grassland is the most important culture in Austria and covers up to 95% of the agricultural used area in mountainous regions. In these disadvantaged areas grassland and dairy farming represents the main source of agricultural production which is characterised by harsh conditions of climate, topography and infrastructure. In EU-27 Austria is still number one in organic farming (20,100 farms, 13% of AA) of which most are grassland farms. Another 40,000 grassland farms participate in a special measure of the Austrian agri-environmental program "ÖPUL" and abdicate yield increasing products like mineral nitrogen fertiliser or herbicides (PÖTSCH 2009). Most of grassland and dairy farms therefore follow a low-input strategy focussing on the efficient use of farm internal resources, namely farm manure and forage from meadows and pastures (PÖTSCH 2007).

Even disadvantaged areas are supported by the Program of Rural Development there is an increasing economical pressure on farms to reduce costs. It is evident that about two third of the total costs in livestock production are caused by feeding stuff (GREIMEL 2002, PÖTSCH et al. 2007, STOCKINGER 2009). Grazing is known the cheapest source of forage but is strongly limited by short vegetation and long winter periods (up to 7 months) in mountainous regions. Therefore sufficient forage conserves have to be produced for the indoor and winter feeding period. The total yield from Austrian grassland was 8.9 Mio. tons of dry matter in 2008 of which about 4.7 Mio. tons were conserved as hay, aftermath hay and silage. The proportion of silage production has increased continuously from 12% in the seventies to 72% nowadays. It has to be considered that in some specified regions of Austria silage production is not allowed for reasons of hard cheese production (10,235 farms running 115.400 ha grassland). The higher costs occurring on these farms are compensated by a special measure within ÖPUL.

Since forage conservation results in high costs all the more it is very important to obtain high quality of hay and silage. AREC Raumberg-Gumpenstein has therefore conducted a number of field studies and experiments on silage quality covering a wide range of different aspects (e.g. impact of vegetation, pre-wilting, harvesting techniques and additives on the fermentation process and on silage quality). a strong effort was given on the advisory service to introduce scientific findings into agricultural practice (BUCHGRABER et al. 2003; 2008). In 2003 a silage monitoring project was initiated by AREC Raumberg-Gumpenstein in cooperation with the agricultural chambers (STEINWIDDER 2003; RESCH and STEINWIDDER 2005, RESCH 2008a, RESCH 2008b). This project is aiming at the survey and analysis of silage quality in practice both to identify problems and to offer specific solutions. The present paper is presenting results of this project and points out weak points to be worked on in future.

Material and Methods

The Austrian silage monitoring project started first in 2003 and was repeated in the years 2005, 2007 and 2009. Seven of the nine Federal provinces of Austria participated in this project with at all 3,670 silage samples. In addition to the silage sampling a comprehensive collection of management data (e.g. farming type, grassland type, harvesting time, silage system, mowing system, chopping length, charging procedure, use of silage additives) was done by means of questionnaires. The silage samples were analysed for dry matter content, crude nutrients (WEENDER-analysis), minerals, energy concentration (GRUBER et al. 1997 according to DLG 1997) and fermentation quality. a drilling core was taken from

the different silo systems to determine the compaction level of the silages. Statistical data analyses were done by using Statgraphics-Plus (Version 5.1) for General Linear Model – procedures respectively SPSS (Version 12.0) for descriptive analysis. For the GLM-procedure fixed effects at different levels and quantitative factors were used (Table 1).

Table 1: Description of the fixed effects used in the Austrian silage monitoring project

fixed effects	variation/groups			
farming system	organic	ecopoint-system	abdication of yield increasing products	non-participation in ÖPUL
year	2003	2005	2007	2009
growth	1st growth	2nd growth	3rd growth	other
grassland type	permanent, red clover, clover-grass mixture, lucerne-grass, lucerne			
mowing system	cutter bar, drum mower, disk mower, mowing conditioner			
cutting height	< 5cm	5-7 cm	> 7cm	
tedding frequency	0	1	2	>2
cutting time	morning	midday	afternoon	evening
harvesting time (hours)	< 6, 6-12, 12-24, 24-36, > 36			
weather conditions	no rain	rain		
silo system	bunker silo	silo heap	tower silo	silo bales
harvesting technique	cutter forage harvester (2), self loading wagon (2), silo press (2)			
chopping length (cm)	< 3, 3-6, 6-10, 10-20, > 20			
compaction level (kg DM/m³)	< 100, 100-150, 150-200, 200-250, >250			
silage additives	no additives, acids and salts, bacteria additives, others			
sample packaging	vacuum package, non vacuum package			

Results and discussion

Silage quality is mainly characterised by nutritive values, fermentation parameters and by sensory properties which can additionally provide important information concerning hygienic and acceptance of feed intake. Table 2 presents target values for good quality grass silages which should be aimed at in practice. The content of crude fibre is indicating the vegetation stage that in general has a very strong impact on forage quality. In contrast to intensive grassland regions of Europe, most of Austrian grassland is permanent grassland with a high number of different species of grass, legumes and herbs. The content of crude protein of silages is normally ranging between 130 and 160 g/kg DM. Previous studies showed that the contamination of forage and silage with earthy material is a very crucial aspect in practice. a high content of ash not only decreases digestibility and energy concentration but very often also causes misfermentation resulting in high concentration of butyric acid. All samples of the Austrian silage monitoring project have been evaluated by means of the mentioned target values and were additionally rated in terms of colour, texture and olfactory in 2009 (results of sensory evaluation are not presented in this paper).

Table 2: Target values of silage and fermentation parameters

parameter/unit	target value
pre-wilting level (g DM/kg FM)	300-400
crude fibre (g/kg DM)	< 270
crude protein (g/kg DM)	> 120
ash (g/kg DM)	< 100
digestibility of organic matter (%)	> 70
energy concentration (MJ NEL/kg DM)	> 5.8
lactic acid (g/kg DM)	20 - 60
acetic acid (g/kg DM)	max. 30
butyric acid (g/kg DM)	max. 3
protein degratation (% NH_4-N of total N)	< 10
DLG (silage quality points)	> 70

Nutrient and energy concentration of grass silages

Data analyses presented in Table 3 show that a high proportion of the silages are well pre-wilted at an average content of 374g DM/kg FM. Nearly 60% of the samples met the given target range of 300 to 400 g DM per kg FM, 13% were below it. Whereas twenty years ago the production of wet silages was standard, a tendency to higher pre-wilting levels and even to the production of haylage can be noticed nowadays (BUCHGRABER et al. 2003). More and more farmers are handling forage conservation (especially production of big bales) with the assistance of machinery rings. Due to an accumulated demand at the main harvest period serious problems with timing occur and often result in a much higher dry matter content than aimed at. The three prior-ranking factors of the multivariate analyses for the DM-content were weather conditions at harvest (1), year (2) and growth (3). The average content of 262 g crude fibre and 148 g crude protein per kg DM indicate that most of the forage was harvested early enough at the time of ear and panicle emergence of the main grasses. But there are still a remarkable proportion of samples (38%) with a high content of crude fibre that causes problems in the fermentation process and leads to lower digestibility and energy concentration in forage. Some farmers are still hesitant especially harvesting the first growth for gaining higher yields and then they sometimes have to wait for even two or three weeks until the next fair weather period.

There was a significant impact of the cutting height on the content of ash in silages, which on average was at 104g/kg DM with a standard deviation of 22g. These results clearly show that the ash content in practice is still too high and some farmers seem not to be aware of mistakes in management.

Two third of the silage samples had an energy concentration between 5.6 and 6.3 MJ NEL per kg DM. Nearly 70% fulfilled the requirements of > 5.8 MJ NEL per kg DM which can be seen as a good basis for sufficient milk or fattening performance from forage. Energy concentration was mainly determined by crude fibre and ash content but also by the number of growth whereupon the first growth reached more than 6 MJ NEL/kg DM on an average. It has to be considered that using the GLM as fitted the coefficient of determination only explained up to about 40% of the variability of the different parameters (RESCH 2008). Even a number of influencing factors were used there are still lack of explanation respectively open questions. One problem could be the quality and reliance of information that is provided by questionnaires where sometimes differences between the real situation on the farm and the subjective perception of the farmers occur (PÖTSCH and GROIER 2005). Another black box is the botanical composition of the plant stand that cannot be provided in such detail which normally is available for exact field trials. It is well known that the botanical composition of grassland has a strong impact on the content of minerals and therefore on ash but there is also a wide but mostly unknown influence of secondary plant metabolites (GIERUS et al. 2007). Another weak point is the fact that forage contamination is not only determined by mineral, earthy substances but also by organic material from soil or dung which is not represented by the ash content (RESCH 2007). Grasslands on boggy or semi-boggy soils which are widespread in mountainous valleys very often show a high activity of moles and voles resulting in lots of earth heaps leading to forage contamination with organic material and clostridia.

Table 3: Impact of fixed effects and quantitative factors on nutrient and energy concentration of silages (GLM-analyses of data from the silage monitoring project in Austria, 2003/2005/2007/2009)

parameter	dry matter	crude protein	crude fibre	ash	energy
unit	[g/kg FM]	[g/kg DM]	[g/kg DM]	[g/kg DM]	[MJ NEL/kg DM]
mean value	**374.3**	**148.3**	**262.2**	**103.6**	**5.96**
standard deviation	74.1	19.6	26.7	21.6	0.34
R^2 in %	16.8	37.4	39.1	19.3	85.9
fixed effects (level)	P-value (significance if < 0.05)				
farming system (4)	0.227	0.000	0.000	0.000	0.327
year (4)	0.000	0.000	0.000	0.000	0.099
growth (4)	0.000	0.000	0.000	0.000	0.000
grassland type (5)	0.006	0.000	0.000	0.000	0.000
mowing system (4)	0.014	0.000	0.000	0.000	
cutting height (3)			0.339	0.000	0.003
tedding frequency (4)	0.028	0.159	0.025	0.008	
harvesting time (5)	0.000				
weather conditions (2)	0.000	0.248	0.004	0.137	0.819
silo system (4)		0.345	0.014	0.891	0.778
harvesting technique (6)	0.000			0.068	
chopping length (5)	0.535		0.732	0.645	0.246
compaction level (5)					0.036
silage additives (4)					0.329
Quantitative factors					
dry matter (p-value)		0.000	0.000	0.000	0.000
mean value[g/kg FM]		377.3	377.2	377.4	377.2
regressions coefficient [g/kg resp. MJ NEL]		-0.0024	-0.024	-0.028	-0.0002
crude protein (p-value)			0.000		0.000
mean value [g/kg DM]			148.7		148.9
regressions coefficient [g/kg resp. MJ NEL]			-0.705		0.001
crude fibre (p-value)	0.543	0.000		0.000	0.000
mean value [g/kg DM]	263.8	264.1		263.8	263.7
regressions coefficient [g/kg resp. MJ NEL]	0.033	-0.397		-0.251	-0.01
ash (p-value)		0.000	0.000		0.000
mean value[g/kg DM]		103.0	103.0		103.3
regressions coefficient [g/kg resp. MJ NEL]		-0.149	-0.385		-0.0093

prior-ranking factors

Fermentation properties of grass silages

Beside nutrient content and energy concentration of silage, parameters of fermentation also are of great interest. The analyses of these data are presented in Table 4. The quick reduction of the pH-value on a stable level is seen a basic criteria of lactic acid fermentation and of microbiological stability of silage (ADLER 2002; PÖTSCH und RESCH, 2002). The overall average pH-value of 4.48 corresponds well with the critical pH-value for silages pre-wilted between 30 – 40% DM (WEISSBACH und HONIG 1992; WEISSBACH 2002). Beside the package system of the samples the content of crude fibre (vegetation stage) and ash (contamination) were the strongest significant factors that influenced the pH-value. Whereas the content of dry matter had an unexpected slight impact on the pH-value of the silages the time between baling and wrapping showed a significant and strong influence.

Two third of all samples met the recommended range of the concentration of lactic acid and acetic acid which was strongly determined by the pre-wilting level but also by the year of investigation. The analyses for butyric acid showed that only 25% of the samples were below the given limit of 3g per kg DM! There was a significant and strong relationship between butyric acid concentration and the pre-wilting level as well as with crude fibre and ash content. By means of the used GLM-procedure at least 38% of the variability of butyric acid concentration could be explained. Concerning the fact that most of

the analysed silages showed disappointing high concentration of butyric acid, farmers have to be advised of management mistakes and weak points repeatedly (RESCH 2009).

The degradation rate of crude protein to ammonia can be seen as a quality indicator of the fermentation process (WEISSBACH und HONIG 1992). The proportion of ammonia related to total nitrogen should not exceed 10% and the analyses showed that this requirement was fulfilled by 75% of all silage samples. Nevertheless protein degradation can further be decreased by the reduction of chopping length (management) but also by optimal weather conditions. In the meantime protein degradation is no longer used as criteria for the DLG- silage classification.

For about 20% of the investigated grass silages additives were used to improve the fermentation process and to increase silage quality. In organic farming some special groups of silage additives are not allowed to be used, namely salts and most combined products which are in general recommended for unfavourable conditions (bad weather periods, contaminated and old plant material). Silage additives based on homo-fermentative and hetero-fermentative bacteria may also be used in organic farming. In Austria the use of silage additives is mostly related to the regulations of the DLG-quality label occasionally added by own national tests (RESCH 2008c). Results from silage experiments at AREC Raumberg-Gumpenstein have shown that under optimal conditions a successful fermentation process with high silage quality can be achieved without using any additives. From other field studies it is known that farmers often misuse silage additives and sometimes they are convinced that the use of additives can compensate mistakes in management.

Concerning energy concentration no significant effect of silage additives could be found in the Austrian silage monitoring project even a significant influence on the concentration of fermentation acids occurred. Silages with bacteria products had a higher content of lactic acid (+ 6.2 g/kg DM) and a significant lower concentration of butyric acid.

Table 4: Impact of fixed effects and quantitative factors on fermentation parameters and feed quality of silages (GLM-analyses of data from the silage monitoring project in Austria, 2003/2005/2007/2009)

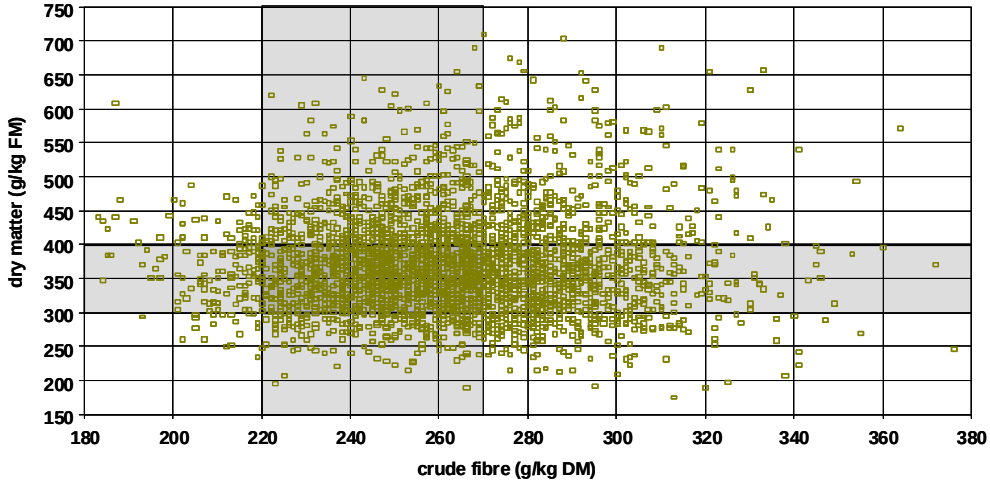
parameter	pH value	lactic acid	acetic acid	butyric acid	ammonia	DLG-value
unit		[g/kg DM]	[g/kg DM]	[g/kg DM]	[% of total N]	(0-100)
mean value	**4.48**	**43.8**	**11.6**	**10.9**	**8.4**	**75.8**
standard deviation	0.35	24.4	7.1	9.6	4.8	19.9
R^2 in %	23.1	14.3	14.6	38.5	20.2	40.1
fixed effects (level)	p-value (significance if < 0.05)					
farming system (4)	0.070	0.013	0.012	0.019	0.216	0.024
year (4)	0.000	0.000	0.000	0.033	0.000	0.000
growth (4)	0.001	0.168	0.101	0.000	0.067	0.000
grassland type (5)	0.006	0.001	0.000	0.001	0.024	0.021
cutting height (3)	0.094	0.007	0.912	0.043	0.539	0.006
weather conditions (2)	0.369	0.596	0.043	0.044	0.000	0.008
silo system (4)	0.000	0.000	0.269	0.000	0.051	0.000
chopping length (5)	0.001	0.046	0.000	0.000	0.000	0.000
compaction level (5)	0.006	0.004	0.532	0.027	0.457	0.003
silage additives (4)	0.000	0.004	0.000	0.000	0.083	0.000
sample packaging (2)	0.000	0.410	0.634	0.024	0.410	0.347
quantitative factors						
dry matter (p-value)	0.000	0.000	0.000	0.000	0.000	0.000
mean value[g/kg FM]	378.2	374.6	374.6	374.6	374.6	374.6
regressions coefficient [pH value, g/kg resp. MJ NEL]	0.001	-0.039	-0.018	-0.051	-0.015	0.073
crude fibre (p-value)	0.000	0.000	0.165	0.000	0.000	0.000
mean value [g/kg DM]	265.5	265.6	265.6	265.6	265.6	265.6
regressions coefficient [pH value, g/kg resp. MJ NEL]	0.003	-0.132	-0.009	0.089	0.048	-0.189
ash (p-value)	0.000	0.000	0.516	0.000	0.000	0.000
mean value[g/kg DM]	103.2	103.7	103.7	103.7	103.7	103.6
regressions coefficient [pH value, g/kg resp. MJ NEL]	0.004	-0.130	0.005	0.070	0.032	-0.136

prior-ranking factors

Classification of grass silages

Fermentation properties can also be used to evaluate silage quality by DLG-points (WEISSBACH und HONIG 1992) resulting in a classification system ranging from 1 = excellent to 5 = very bad). 58% of the silage samples reached > 70 DLG-points and can be judged good to excellent. Additionally the tested silages were classified by means of selected target values of silage and fermentation parameters presented in Table 2.

Figure 1: distribution of grass silages concerning pre-wilting level and crude fibre content expressing vegetation stage (data of the Austrian silage monitoring project, 2003/2005/2007/2009)



35% of all analysed forage samples (n=3,679) were harvested well-timed and pre-wilted between the recommended range of 300-400 g DM. When the criteria of forage contamination (ash content > 100 g) are added as a very important issue the percentage of optimal grass silages is reduced to 14%! Of course it can be discussed if the strictness of this classification is too high but we must not forget that the silage samples of this monitoring project are probably the premium third. No farmer would send in silage of bad quality for this monitoring project which is at the same time a silage competition. These results therefore very clearly show that there is both a lack of knowledge in practise respectively advisory demand and a high potential of improvement concerning forage and silage quality.

Conclusions

For grassland and dairy farmers following a low-input strategy it is essential to reduce farm-external feedstuffs and to optimise the quality of home-grown forage from meadows and pastures. The results of a comprehensive monitoring project organised and conducted by AREC Raumberg-Gumpenstein shows that there is a considerable potential in Austria to improve silage quality in practice. Apart from unfavourable natural weather conditions in mountainous areas the main reasons for unsatisfying silage quality are obvious in management mistakes. Too late harvest time resulting in high content of crude fibre, low concentration of easy fermentable sugar and serious problems with the compaction of such bulky material is still a big problem in practice. Forage contamination resulting in an increased risk of clostridia respectively butyric acid in the fermentation process is another serious problem that has to be faced with.

During the last years the mechanisation chain for silage production has improved a lot and a growing number of farmers make demands on the machinery rings to process ensiling. In many cases the charging of the silos on farms become the bottleneck and time is too short to ensure sufficient and proper compaction of the applied material.

Strong efforts have to be made to advise farmers specifically how to improve the ensiling procedure and to increase silage quality by means of field days, working teams, leaflets and articles.

Changes in management and avoiding mistakes mostly do not cause any extra costs, which is a clear and understandable argument to farmers.

How can science and research institutes contribute to the known issues? Exact silage experiments focus on specific questions, which can be worked on under controlled conditions and environments and are therefore still essential. Additionally field studies like the introduced monitoring project provide important data and findings reflecting the situation in practice. Such projects can identify weak points, show trends both negative and positive and provide a good basis to react precisely on the actual problems.

Beyond chemical and microbiological analyses which are generally used to evaluate forage and silage quality, sensory properties like colour, texture and olfactory could provide important additional information concerning feed intake and feed acceptance. Up to now there is no sufficient implementation of the sensory rating into the feed value system – this could be an interesting challenge.

References

[1] Adler A., 2002: Qualität von Futterkonserven und mikrobielle Kontamination. Bericht zum 8. Alpenländischen Expertenforum „Zeitgemäße Futterkonservierung“, HBLFA Raumberg-Gumpenstein, 17-25

[2] Buchgraber K., E.M. Pötsch, R. Resch und A. Pöllinger, 2003: Erfolgreich silieren – Spitzenqualitäten bei Grassilagen! Der fortschrittliche Landwirt (9), 2003, 29-37.

[3] Buchgraber K., L. Gruber, A. Pöllinger, E.M. Pötsch, R. Resch, W. Starz und A. Steinwidder, 2003: Futterqualität aus dem Grünland ist wieder mehr wert. Der fortschrittliche Landwirt (6), 2008, 16-19.

[4] DLG (Deutsche Landwirtschaftsgesellschaft), 1997: DLG-Futterwerttabellen für Wiederkäuer. 7. erweiterte und überarbeitete Auflage. Herausgeber: Universität Hohenheim-Dokumentationsstelle, DLG-Verlag, Frankfurt/Main.

[5] DLG (Deutsche Landwirtschaftsgesellschaft), 2006: Praxishandbuch Futterkonservierung. Silagebereitung, Siliermittel, Dosiergeräte, Silofolien. DLG-Verlags-GmbH, 7. Auflage, ISBN 3-7690-0677-1.

[6] Greimel M., 2002: Einsparungspotentiale in der Futterkonservierung. Bericht zum 8. Alpenländischen Expertenforum „Zeitgemäße Futterkonservierung“, HBLFA Raumberg-Gumpenstein, 77-80.

[7] Gierus, M., A. van Dorland, A. Elgersma, M. Kreuzer, R. Loges, E.M. Poetsch, H. Smit, H. Steinsham, F. Taube and M. Wachendorf, 2007: Influence of contrasting environments on forage quality of ryegrass and four legumes growing in binary swards. Grassland Science in Europe “Permanent and temporary grassland – plant, environment and economy”, Volume 12, 162-165.

[8] Gruber L., A. Steinwidder, T. Guggenberger und G. Wiedner, 1997: Interpolation der Verdauungskoeffizienten von Grundfuttermitteln der DLG-Futterwerttabellen für Wiederkäuer. Aktualisiertes Arbeitspapier der ÖAG-Fachgruppe Fütterung über die Grundlagen zur Berechnung der Verdaulichkeit und des UDP-Gehaltes auf der Basis der DLG-Futterwerttabellen für Wiederkäuer (7. Auflage 1997).

[9] Pötsch E.M. 2007: Low Input Farming Systems and livestock production - grassland and dairy farming in Austria. Proceedings of the Summer University at Ranco, Italy, JRC Scientific and Technical Reports, ISBN 978-92-79-08007-4, 33-38.

[10] Pötsch E.M. 2009: Grundfutterqualität im Konnex mit dem österreichischen Agrarumweltprogramm. Bericht zum 15. Alpenländischen Expertenforum zum Thema "Grundfutterqualität - aktuelle Ergebnisse und zukünftige Entwicklungen", 29-38.

[11] Pötsch, E.M. and M. Groier, 2005: Attitude of Austrian farmers towards agro-environmental programs and aspects of nature conservation. In: Proceedings of the 13th International Occasional Symposium of the European Grassland Federation (EGF), Volume 10: “Integrating Efficient Grassland Farming and Biodiversity”. Tartu, Estonia 29-31 August 2005, 120-12.3

[12] Pötsch E.M. und R. Resch, 2002: Einfluss von Futteraufbereitung und Erntetechnik auf den Gärverlauf und die Silagequalität von Grünlandfutter. Bericht zum 8. Alpenländischen Expertenforum „Zeitgemäße Futterkonservierung“, HBLFA Raumberg-Gumpenstein, 11-15
[13] Pötsch E.M., A. Adler and R. Resch, 2007: Stock conservation of total mixed feed rations on dairy farms. EGF-Symposium “, Gent, Belgien,
[14] Resch R. und A. Steinwidder, 2005: Silageprojekt 2003/05. Sonderdruck, HBLFA Raumberg-Gumpenstein.
[15] Resch R, 2007: Futterverschmutzung – Auswirkungen auf die Qualität von Grassilagen. Der Fortschrittliche Landwirt, Heft 7, 16-17.
[16] Resch R., 2008a: Ergebnisse Silageprojekt 2003/2005/2007. Bericht über die 35. Viehwirtschaftliche Fachtagung, LFZ Raumberg-Gumpenstein, 33-46.
[17] Resch R., 2008b: Abschlussbericht zur wissenschaftlichen Tätigkeit LFZ 073523 "Praxisorientierte Strategien zur Verbesserung der Qualität von Grassilagen in Österreich". Sonderdruck, LFZ Raumberg-Gumpenstein, 51 S.
[18] Resch, R., 2008c: Welches Siliermittel passt auf meinen Betrieb? Der Fortschrittliche Landwirt, (8) 2008, 18-21.
[19] Resch R., 2009: Description of significant influencing factors on butyric acid content of grass silage by means of a multi-factorial linear model. Proceedings of the 13th International Conference "Forage Conservation", Nitra, Slovakia Republic
[20] Steinwidder A., 2003: Silageprojekt 2003. Sonderdruck, HBLFA Raumberg-Gumpenstein.
[21] Stockinger C, 2009: Michproduktion in Zukunft – gewinnen wir den Wettbewerb? Bericht über die 36. Viehwirtschaftliche Fachtagung, LFZ Raumberg-Gumpenstein, 11-18
[22] Weissbach F. and H. Honig, 1992: Ein neuer Schlüssel zur Beurteilung der Gärqualität von Silagen auf Basis der chemischen Analyse. 104. VDLUFA-Kongress Göttingen, VDLUFA-Schriftenreihe 35, 489-494
[23] Weissbach F., 2002: Grundlagen und Praxis der Produktion guter Grassilagen. Bericht zum 8. Alpenländischen Expertenforum „Zeitgemäße Futterkonservierung“, HBLFA Raumberg-Gumpenstein, 1-5

Chemical Composition and Feeding Value of Maize Residual Plants in Different Varieties

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Abstract

The aim of the present study was to investigate the influence of genotypic variations and of physiological maturity of maize on chemical composition, *in vitro* digestibility and ruminal degradability of the residual maize plant. Fourteen different maize genotypes were assigned into three maturity groups and harvested at four different harvest dates (depending on the dry matter content of the kernels). Early maturing varieties were allotted into maturity group I (S210-S220), mid-early maturing varieties to group II (S230-S250) and mid-late varieties to group III (S260-S280). Chemical components of the cell wall as crude fibre (CF) and detergent fibre fractions (NDF, ADF, ADL) were quantified in the residual plant (RP). Ruminal degradability of DM and NDF in RP was determined by the in situ method. *In vitro* digestibility (ELOS) and gas production capacity (GP) were also determined. From the obtained data we observe that the evaluated maize varieties of maturity group I had lower cell wall contents and thus higher ruminal DM degradability as well as higher *in vitro* digestibility than maturity groups II and III at the same stage of physiological maturity. This indicates for all varieties of the present investigation a benefit for the early varieties due to a longer period of harvest in comparison to the later maturing varieties. Differences in cell wall contents did become more apparent with later stages of physiological maturity (later harvest dates). There was a strong negative interaction between NDF content and ruminal DM degradability of the plants stover (r=-0.81) whereas no interaction was found between NDF content and ruminal NDF degradability. Also, no significant relationship between lignin contents and ruminal degradability of DM and NDF could be investigated.

Keywords: Maize stover – carbohydrate composition – ruminal degradability – Maize silage maturity

Introduction

Maize silage is one of the most important forage crops in ruminant nutrition. During maturity of the maize plant a strong change in chemical composition and in ruminal degradability of residual plant appears. But the residual plant did not demand breeder's attention in the last years (Givens and Deaville, 2001). Thus the feeding value of maize silage could be massively influenced in a positive way by improving the residual plant of maize. The objective of this study was to examine the influence of different genotypes on the extent of the changes to the above mentioned factors. The results were sought to clarify which chemical components of the plant cell walls interact with the feeding value of maize and which traits are potentially important for plant breeders to improve the overall feeding value.

Material and Methods

Fourteen different maize hybrids (Monsanto Agrar Deutschland, GmbH) were included in the investigation to adequately cover genotypic variability. Varieties were separated into three maturity groups. The very early to early maturing varieties were assigned to maturity group I (MG I; S210-S220; n=4 varieties), mid-early maturing varieties to group II (MG III; S230-S250; n=5 varieties) and the mid-late maturing varieties to maturity group III (MG III; >S260; n=4 varieties). The cultivation of six hybrids was repeated for three harvest years (2004, 2005 and 2006) to exclude potential environmental effects on growth and cell wall development. All hybrids were harvested at four harvest dates which were decided by the dry matter content of the grain. Harvest period took about 6-8 weeks from beginning of September to end of October in all harvest years. The dry matter content of the grain for each harvest date was set at

to be 48-52% for the 1st harvest (HD 1), 54-58% for the 2nd harvest (HD 2), 60-64% for the 3rd (HD 3) and 65-70% in the last harvest date (HD 4). These criteria were kept for all varieties in all three years thereby reproducing the physiological development of the stover. The residual plant (RP) was separated. Chemical components of the cell wall as crude fibre (CF) and detergent fibre fractions (NDF, ADF, ADL) were quantified. Ruminal degradability of DM in RP was determined by the in situ method (Flachowsky et al., 1988; Madsen and Hvelplund, 1994). Degradation profile of DM was fitted to an exponential model to calculate the effective ruminal degradability assuming a passage rate of 6% h^{-1} (EDM6). Ruminal degradability of NDF at a passage rate of 6% h^{-1} (EDNDF6) was proven with the same method. *In vitro* digestibility (ELOS) and gas production capacity (GP) were also determined (see Zeller, 2009). Statistical evaluation was accomplished by Analysis of Variance (ANOVA), Tukey HSD, on 0.05 probability values using SAS ®.

Results and Discussion

Chemical components as crude fibre and NDF of residual plants for the different maturity groups at the various stages of maturity are shown in table 1. It is obvious that CF as well as NDF contents increase with later harvest dates. MG I shows also less cell wall contents for all harvest dates at the same stage of physiological maturity in comparison to MG II and III. With later harvest stage these differences become significant. Table 2 demonstrates the results of the ruminal degradability of DM and NDF as well as the ELOS and GP for the different maturity groups at the various stages of maturity. According to the lesser cell wall contents in MG I there is a higher EDM6 and ELOS and significantly higher GP in maturity group I. It is also found a strong negative interaction between NDF content and ruminal DM degradability of the plants stover r=-0.81 ($p<0.0001$) with $R^2= 0.72$. However NDF content and ruminal NDF degradability do not show any interaction. Lignin is observed as the major factor influencing the digestibility of cell walls (Méchin et al., 2005; Grabber, 2005). This could not be confirmed in the present study. There are no significant differences in the lignin contents between the maturity groups. Also no interaction is found between lignin contents and EDM6 or EDNDF6.

Table 1: Crude fibre and NDF contents (% of DM) in residual plant of the maturity groups at different harvest dates

		HD 1	HD 2	HD 3	HD 4
CF	MG I	32.2	33.8	33.8^{b}	34.3^{b}
	MG II	33.4	34.5	36.2^{ab}	37.6^{a}
	MG III	34.3	35.2	36.4^{a}	37.5^{a}
NDF	MG I	54.8	57.1	58.7	60.7^{b}
	MG II	56.0	58.3	61.3	64.4^{a}
	MG III	57.1	58.7	62.0	63.7^{ab}

a, b – differences significant on p<0.05

Table 2: EDM6 (%), ELOS (% of DM) and GP (ml/200 mg of DM) in residual plant of the maturity groups at different harvest dates

		HD 1	HD 2	HD 3	HD 4
EDM6	MG I	49.6	46.3	45.2	43.1
	MG II	48.6	46.0	42.6	39.4
	MG III	47.3	47.1	44.2	41.9
ELOS	MG I	49.6	45.9	45.0^{a}	43.4^{a}
	MG II	46.9	44.6	41.6^{b}	38.4^{b}
	MG III	46.1	45.0	41.8^{ab}	40.7^{ab}
GP	MG I	44.9	44.5	43.2^{a}	41.8^{a}
	MG II	41.6	41.7	37.9^{b}	37.5^{b}
	MG III	43.7	42.7	41.3^{ab}	39.5^{ab}

a, b – differences significant on p<0.05

Conclusion

The obtained data of the present work show, that genetic variation (here: maturity group) strongly influence the feeding value of maize residual plants at the same physiological maturity status. This applies to the chemical composition as well as to the ruminal degradability and the *in vitro* digestibility of the plants. There were significantly lesser contents of crude fibre and NDF in the early maturing varieties (maturity group I) in comparison to the later maturing varieties. Thus we found higher ruminal dry matter degradability and *in vitro* digestibility in MG I. This indicates for all varieties in the present investigation a benefit for the early varieties due to a longer period of harvest in comparison to the later maturing varieties.Furthermore, differences between maturity groups became more apparent with later harvest date (HD 3 and 4). The benefit of the early maturing varieties becomes stronger the later the harvest date proceeds. The study also shows that the NDF content strongly influences the ruminal degradability of the dry matter but not the ruminal degradability of the NDF itself. Also lignin content did not show any influence on ruminal degradability.

References

[1] Flachowsky, G.; Schneider, M.; Ochrimenko, W.I.; Richter, G.H.; Löhnert, H.-J. (1988): Methodische Hinweise zur Anwendung der Nylonbeutel-Technik beim Wiederkäuer. Schriftenreihe der Lehrgangseinrichtung für Fütterungsberatung Jena-Jemderoda 11, p. 20-26.

[2] Givens, D.I., Deaville, E.R. (2001): Comparison of major carbohydrate fractions and cell wall digestibility in silages made from older and newer maize genotypes grown in the UK. Anim. Feed Sci. Technol. 89, p. 69-82.

[3] Grabber, J.; (2005): How do lignin composition, structure and cross-linking affect degradability? a Review of cell wall model studies. Crop Sci. 45, p. 820-831.

[4] Madsen, J., Hvelplund, T. (1994): Prediction of in situ protein degradability in the rumen. Results of an european ringtest. Livestock Prod. Sci. 39, p. 201-212.

[5] Méchin, V.; Argillier, O.; Rocher, F.; Hébert, Y.; Mila, I.; Pollet, B.; Barrière, Y.; Lapièrre, Y. (2005): In search of a maize ideotype for cell wall enzymatic degradability using histological and biochemical lignin characterization. J. Agric. Food Chem. 53, p. 5872-5881.

[6] Zeller, F. (2009): Zum Einfluss von Genotyp und physiologischer Reife von Mais auf die chemische Zusammensetzung und ruminale Abbaubarkeit der Restpflanze. Dissertation, Technische Universität München.

Biotech Corn – trends and benefits for growers

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Crop Genetics Research and Development

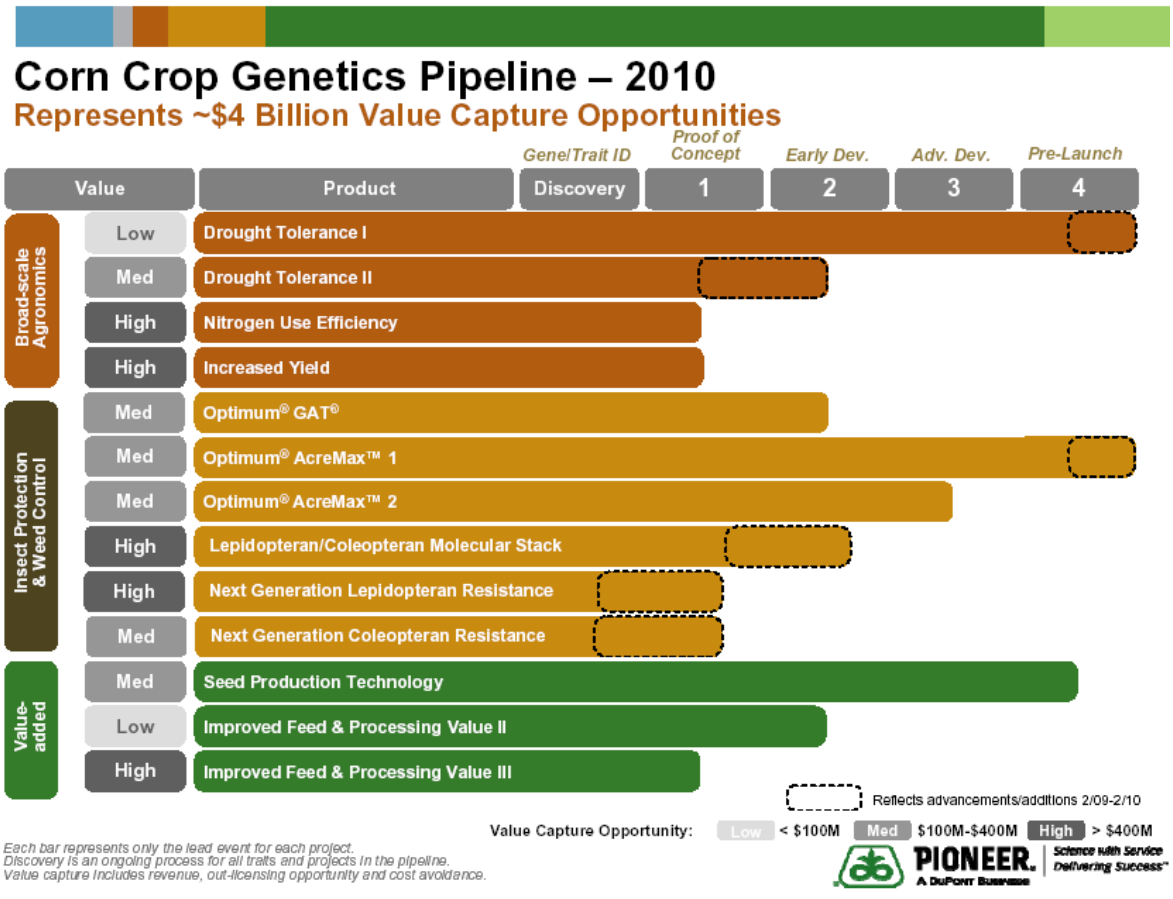
In today's complex and competitive agricultural environment, superior research is essential to achieving crop improvements. Agricultural research requires long-term commitment, investment, innovation and dedication. Crop Genetics researchers are world leaders in the discovery, development and delivery of elite seed genetics. Using both time-tested approaches and modern technologies for more than 75 years, we provide our customers with premiere agricultural products.

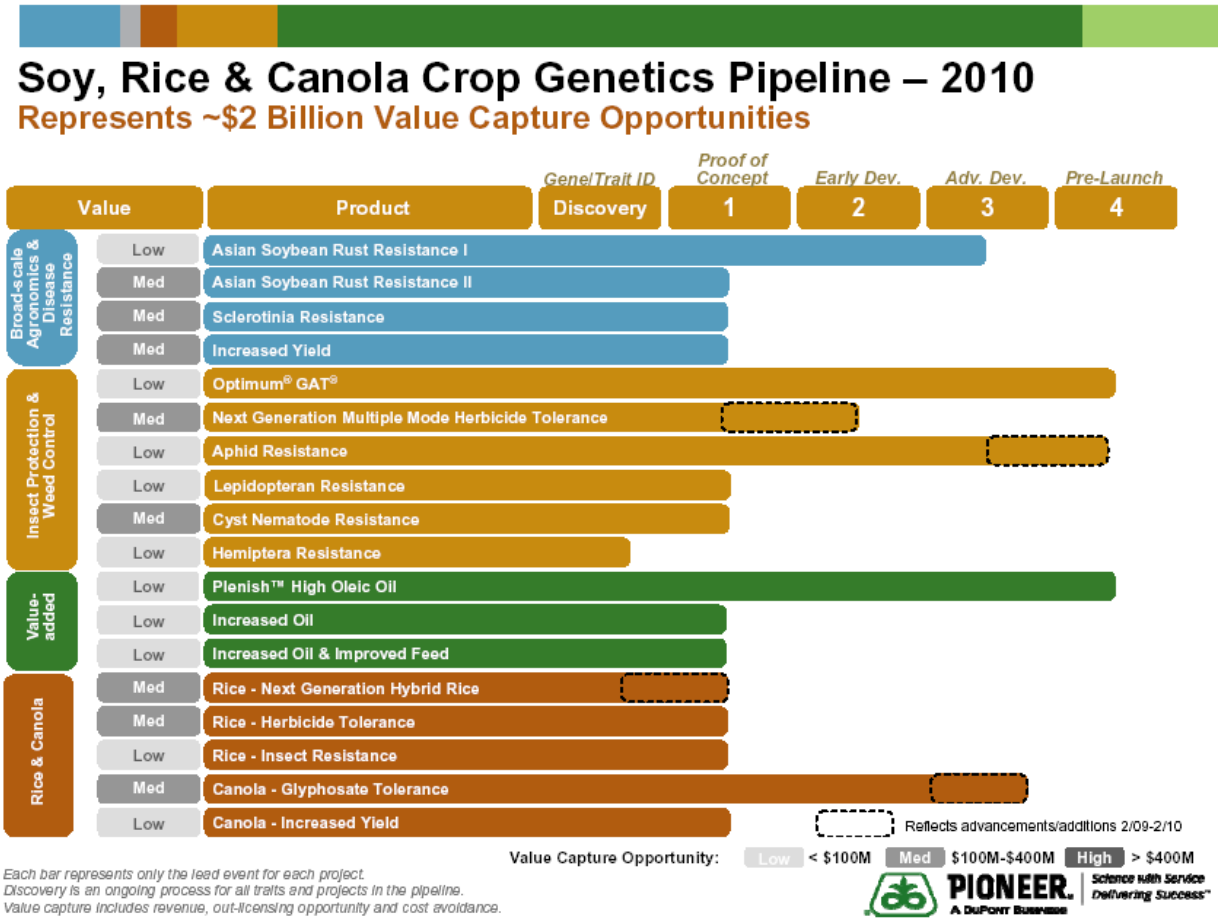
More than 1,800 Pioneer researchers in 25 countries work toward one common goal - bringing more value to the world's producers. They seek answers and develop solutions to unique challenges producers face today and will confront tomorrow. Our researchers are focused on results that introduce new uses for crops, new markets for farm products and farm efficiency improvements that increase farmer profitability.

The technology pipeline shows more information on our research efforts.

Technology Pipeline

The Technology Pipeline is a snapshot of the future traits and elite germplasm Pioneer is currently discovering, developing, testing and commercializing within its global research organization. This pipeline represents concepts and future products that we believe will bring farmers the greatest value and ultimately benefit the entire food and energy value chain.





The Technology Specification Sheets provide information on technologies in our product pipeline. From elite genetics to biotech traits and enabling technologies, these summaries provide clear definitions and outline the anticipated time to market for each technology.

Drought Tolerance

Pioneer Hi-Bred is developing drought tolerant corn that focuses on enhancing yield performance during water deficits with no yield penalty under optimal water conditions through a multi-phase approach.

Drought Tolerance - Corn

PIONEER
A DuPont Business

Trait at a Glance

Pioneer Hi-Bred is developing drought tolerant corn that focuses on enhancing yield performance during water deficits with no yield penalty under optimal water conditions through a multi-phase approach.

- Drought Tolerance I hybrids would protect yields in limited irrigation and arid land environments.
- Drought Tolerance II hybrids* would protect yields in a full range of environments during drought conditions.

- Projected Introduction: Drought Tolerance I - Conducting hundreds of on-farm system trials in 2010
- R&D Pipeline Phase:
  - Drought Tolerance I - Phase 4, Pre-Launch
  - Drought Tolerance II - Phase 1, Proof of Concept
- Target Markets: North America, Latin America, Europe, Asia Pacific, Africa
- Global Acreage Opportunity:
  - Drought Tolerance I - Narrow, <40 MM
  - Drought Tolerance II - High, 101 - 150 MM

Grower Value at a Glance = **Harvestable Yield** + **Risk Management** + **Expanded Corn Acreage**

Anticipating Needs

Farmers would realize more stable yield under drought stress and maintain maximum yield potential under optimal conditions by planting Pioneer drought tolerant hybrids. This benefits farmers in traditional corn growing areas, as well as areas where lack of water currently restricts profitable corn production.

Delivering Solutions

Conventional Pioneer® brand hybrids that have been developed with the industry's most diverse corn genetics to tolerate drought are performing well in the market today.

Drought Tolerance I would leverage native trait variation in elite germplasm and the AYT™ system to create hybrids that deliver significant improvements in grower return per acre in drought-stressed environments. Lead hybrids are consistently demonstrating more than 6 percent yield advantage under stressed conditions across multiple locations.

Drought Tolerance II would help protect yields even further by incorporating a combination of transgenic and native gene approaches that could improve yields across all Corn Belt environments in the U.S. Field trials of Drought Tolerance II have generated up to a 16 bushel improvement in stressed environments and significant yield increases in non-stressed environments.

Delivering Value

A globally devastating issue, drought causes losses in excess of $13 billion annually. Pioneer drought tolerant traits would stabilize grower income through higher yields and have the potential to reduce irrigation costs when water deficits occur.

Pioneer uses managed moisture stress environments throughout the U.S. Corn Belt, as well as at testing facilities in California and South America, to select drought tolerant hybrids.

Keosauqua, Iowa 2009. Corn ears with drought tolerant transgene produce more filled kernels than control plants under water-deficient stress conditions.

Limagrain, a Research Leader in Maize Silage

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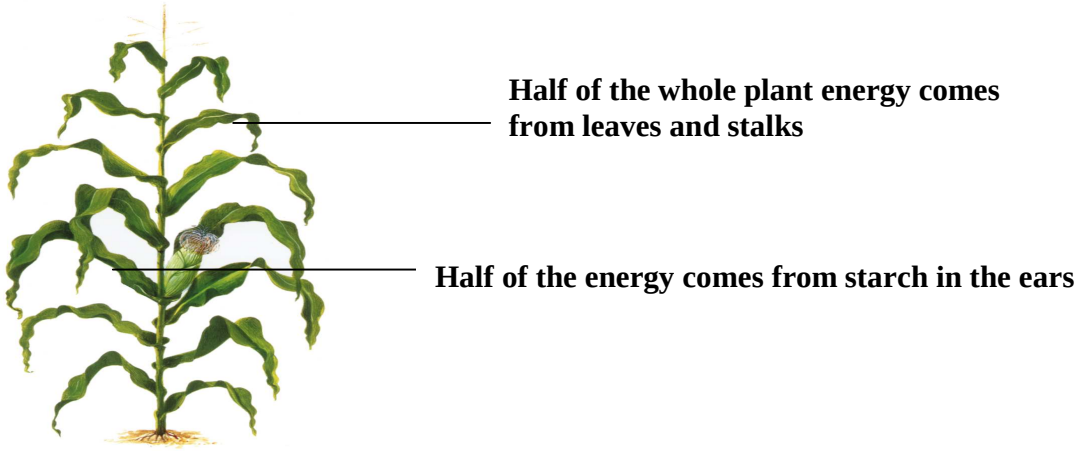
As the genetic potential for dairy cows to produce milk increases, maximum productivity and profitability of high-yielding dairy herds inevitably depend more on nutritional management. That logically begins by maximizing energy intake.

In Northern Europe, whole-plant silage maize is important in the diets of intensively managed ruminants. It can often represent between 2/3 and ¾ of the total forage intake.

This reliable roughage, with high energy content and high intake level, is today the aim of important quality breeding programs in Limagrain.

1) Silage maize: energy content and cell-walls



A whole maize plant, at classical harvest stage between 30 and 35 % dry matter, is composed of leaves, stalks and ears. More precisely, it means 56 % of cell content and 44 % of cell-walls.

All components of the cell content are totally digestible (table 1). These components can't be a limiting factor of the whole plant energy value.

Table 1

cell-content composition	Starch	Soluble sugars	Proteins	Lipids	Ash
% of the dry matter	30%	10%	8%	4%	4%
Digestibility	100%	100%	90-99 %	90-99 %	10-95 %

On the contrary, digestibility of the different components of the cell-walls is variable (table 2).

Table2

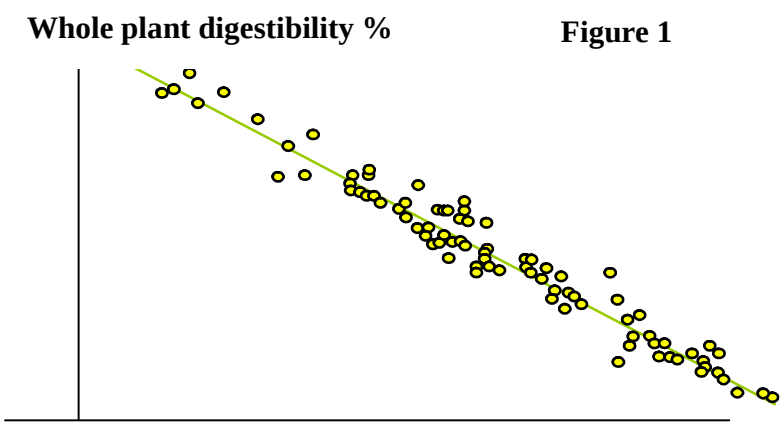
cell-wall composition	Hemicelluloses	Celluloses	Lignins
% of the dry matter	20%	20%	4%
Digestibility	20-100 %	20-100 %	0%

According to genetic variability of maize silage, in-vivo digestibility of the cell-walls can vary between 35 and 60 %.

It then seems clear that the limiting factor of the whole plant digestibility is the cell-wall digestibility.

A lot of animal trials have been done with different varieties of silage maize, they clearly show the interest of decreasing the non digestible cell-walls content. The results from Figure 1 are trials done by INRA, in 1993, on sheep.

Figure 1



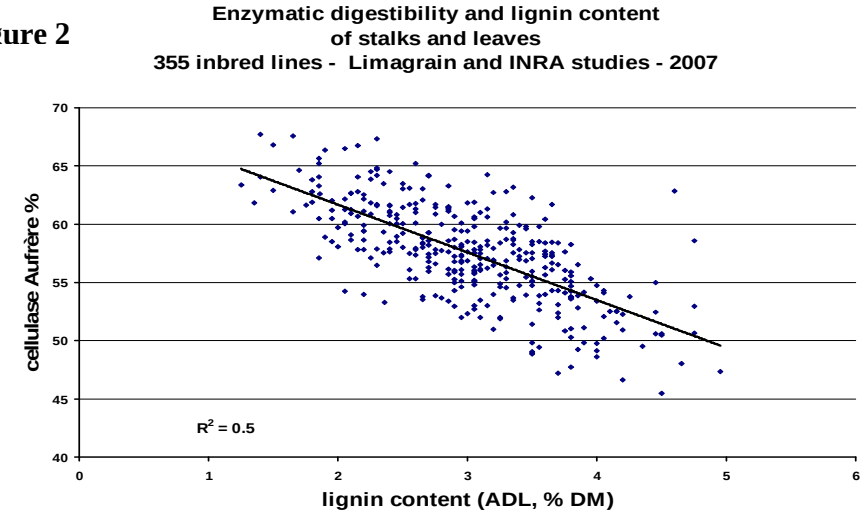
2) a specific /dedicated silage maize breeding program

25 years ago, Limagrain decided to separate the breeding programs of silage and grain maize, as it was obvious that silage maize needed specific quality criteria.

To increase the energy content of the cell-walls, or the energy content of the green part (leaves and stalks), which means the same, the easiest way at the beginning, for Limagrain breeders, was to measure and decrease the lignin content of the cell-walls.

We have seen, in Table 2, that lignin digestibility is null, so the less lignin the more digestibility (Figure 2), till a level we have to maintain, to keep a good standability.

Figure 2



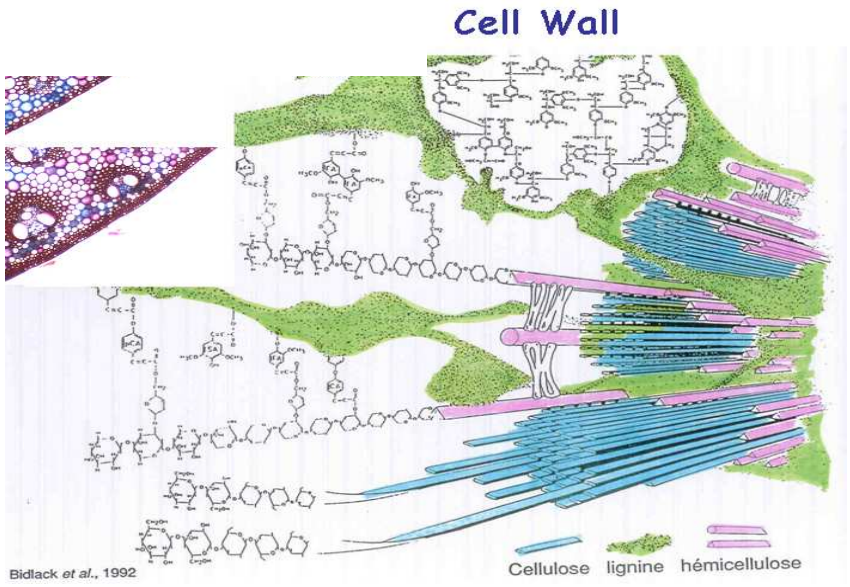
Limagrain research also did important studies, in collaboration with INRA in France, to better understand how the cell-walls are "built".

From a microscopic point of view, we have learnt that Lignin (Figure 3) is linked to hemicelluloses and celluloses with bridges which are not always the same according to the genotypes.

Some bridges seem easy to break by enzymatic methods or by microorganisms in the rumen; in this case, part of cellulose and hemi-cellulose can be digested in the same time that lignin is excreted.

Other bridges between lignin and celluloses or hemi-celluloses are too much difficult to break; we can easily imagine that in this case, cellulose and hemi-cellulose cannot be digested and are excreted in the same time as lignin…

Figure 3: composition of the cell-wall



So **quantity of Lignin** is an important criteria, necessary but not sufficient. We also need information about **quality of Lignin**, how it is linked with the rest of the cell-walls.

According to Lignin composition, cell-walls digestibility can vary in a very important way.

Limagrain research programs now take care of new criteria about quality of Lignin.

3) Cell-wall digestibility and silage maize intake

Numerous scientific works have shown that digestibility, or energy value of the whole plant, is linked with quantity of silage maize intake. The highest digestibility is the highest intake will be.

More specially, intake is correlated with cell-walls digestibility, and studies have shown that the more rapidly cell-walls are digested, the highest intake will be. It can be explained by the place that cell-walls occupy in the rumen. If the kinetic of degradation of the cell-walls is low, the rumen keeps filled a longer time, then no place to eat again…

On the contrary, if cell-walls are well and rapidly degraded, place will be free in the rumen to be filled again … The relation between cell-walls digestibility and intake is clear on the Table 3, trials were done on dairy cows by INRA.

Table 3

Tested hybrids	cell-walls digestibility %	intake (DM kg /cow /day)
H1	59.4	17.9
H2	51.4	16.8
H3	50.1	16.1
H4	46.9	15.6
H5	46.7	15.2
H6	46.6	14.4

When we work to increase digestibility of the cell-walls, we win on 2 tables, digestibility and intake.

4) The proof by trough …

The only way to be sure that we work in the good way is to do **animal trials**.

All research works, all results obtained, all new methods of evaluation of quality criteria (digestibility for example), are tested and verified with animals.

The majority of these trials are done with and under the control of institutes; we then constitute an important animal results data-base.

5) Economical interest to increase the cell-walls digestibility

Let us consider 2 hybrids with the same starch content at harvest stage, but with a little bit difference in the cell-walls digestibility.

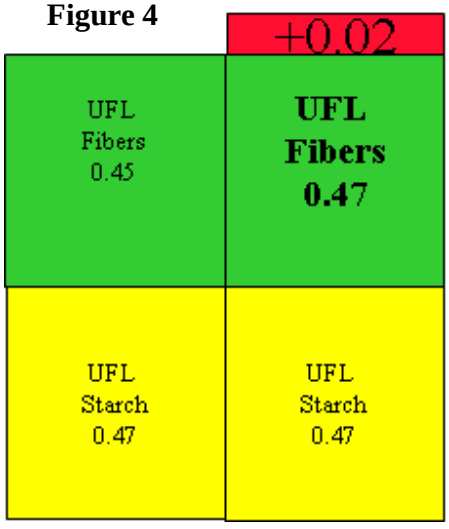
	Hybrid A	Hybrid B
cell-wall digestibility %	52	54
UFL /kg DM	0.92	0.94

As shown on the Figure 4, the energy /digestibility of Hybrid B is higher than the one of A. The energy coming from starch is the same, because starch content is the same between a and B. The fibers digestibility of Hybrid B is 2 points better than A.

As a consequence :

- **whole plant digestibility of B is 2 points better than A;**
- **this better energy content of B allows for 0.6 kg milk more per cow per day.**

Figure 4



Festulolium Hybrids from Breeding Station Hladké Životice and their Quality

HOUDEK I.[1], JAMBOR V.[2]
[1] Plant Breeding Station Hladké Životice, Ltd.
[2] NutriVet, Ltd., Pohořelice

Beginning cultivation hybrids in Plant Breeding Station Hladké Živořice fall in second halves sixieth years. Iniciator hybridism and primary objective author Festulolium was Ing. Antonín Fojtík, with him initially cooperated Ing. Světlík. From ends of 70. years with cultivation sharinged next future co - authors varieties above all RNDr. Jan Horák as well as engineers Ivan Houdek, Jan Orálek, Vladimír Černoch and on meanwhile last hybrid registrated in 2010 also Radomír Čapka. Already along cultivation were to be new coined materials examination partly in experiments foder teachers desk todays agricultural universities in Brno and České Budějovice, but also experimental workplaces , such as experimental station at Zubří and experimental station at that time research institute of meadow and pasture in Banská Bystrica.

Quality of fodder crossbreed are tested first in laboratory on Plant selection station on Větrov. There Ing.Václav Míka, DrSc. by Lampeter method screen out materials with worse digestibility. After obtaining higher volume seed for sowing was promising new breeding examinational on quality on workplaces research institute for nutrition of farm animals and production of silages. From RIAN at Pohořelice it were step by step in Ing. Petr Jakobe, CSc., Ing. František Barančic, CSc., Ing. Jaroslav Přikryl, CSc. and above all Ing. Václav Jambor, CSc. At RIAP Prague- Uhřiněves were to be half – breed at examinational by Ing. Radko Loučka, CSc. and Ing. Eliška Machačová, CSc. To expansion hybrid on Slovakia contributed significantly nutritionist Ing. Stanislav Knotek, CSc. a Ing. J. Žiláková from Research Institute at Banska Bystrica and experimenter from experimental plant station in Poprad Ing. D. Rataj and Ing. I. Ilavská, PhD.

In cultivation hybrid Festulolium were cooperated and with workplace abroad to the 1995 it was only workplace at Paulinenaue (D) and breader with HR Szelejewo and PAN Poznaň (PL), later with workplace at West Europe above all DLF - Trifolium and Limagrain.

Materials and methods

Since 1988 go down to the list of varieties in state verity book CZ meanwhile 10 hybrid Festulolium Plant Breeding Station at Hladké Životice from that is 8 verieties forage.

Come from crossing by **Italian Ryegrass** *(Lolium multiflorum Lam.)* x **Tall Fescue** *(Festuca arundinacea Schreb.)*; variety Felina, Hykor and Fojtan are festuca , hard graminoids grasses snášející hygric conditions from dry after as much as wet stand. Sow with in mixtures for perenial grassland and to the temporary growths also. Hybrid Felina and Hykor use also to the short-term mixture on arable soil. Lolium tetraploid variety Bačva and Lofa are exacting on sufficient number moisture and nutrients. Hybrid Bečva is 2-3- year old grass and same usage like **Italian Ryegrass,** multiannual Lofa with analouges to **Perennial reygrass** uses to the temporary mixture and to the short term mixture on arable soil. Next hybrid *(Festulolium braunii)* comes from crossing tetraploid female former **Italian Ryegrass** *(Lolium multiflorum Lam.)* x **Meadow Fescue** *(Festuca pratensis L.),* incurred veriety are lolium type how about vary earliness matutinal is Achilles, middle Perun and late Perseus. At 2010 year přibude next variety Hostýn. Similar as ryegrasses are these varieties more suitable for more wet conditions with enough of nutritions. In the central European conditions are predominantely grown with the red clover mixtures and alfalfa.

Table 1:

Variety	Crossing	Type	Ploidy	Persictency	Earliness	Year of listing
FELINA	LM x FA	festucoid	6n	perennial	early	1988
HYKOR	LM x FA	festucoid	6n	perennial	early	1991
FOJTAN	LM x FA	festucoid	6n	perennial	late	2005
LOFA	LM x FA	loloid (LP)	4n	jako LP	late	1997
BEČVA	LM x FA	loloid	4n	2, max. 3 years	late	1989
PERUN	LM X FP	loloid - intermed.	4n	max. do 5 years	medium	1991
ACHILLES	LM X FP	loloid	4n	max. do 5 years	early	2005
PERSEUS	LM X FP	loloid	4n	max. do 5 years	late	2004
HOSTÝN	*LM X FP*	*loloid*	*4n*	*max. do 5 years*	*medium*	*2010*

Experiments were based at Hladké Životice, each plot 10 m sowing to the blocks in three replication under separate festuca and lolium hybrid look at different earliness and persistence of growths. Dossage of nitrogen were to be follow: before 1. and 2. cut after 60 kg nitrogen per 1 ha and before next cut 40 kg nitrogen per ha. Yields introduced in tables come yield potential of varieties. Clear of-loss-making harvest was effected by harvestor Hege 12 obtained sample about weight 1 kg green matter from each plot. Immediately after harvest samples were taken to dry in oven at temperature 55^{o} C. Content of organic nutrients: dry matter (DM), water soluble carbohydrate (WSC), crude protein (CP), crude fiber (CF), neutral detergent fiber (NDF) was determinated by clasical analytical methods (Van Soest 1990). Digestibility, resp. degradability of organic matter (OMD), degradability crude fiber (CFD) and degradability neutral-detergent fiber (NDFD) was determinated by rest after 24 hours incubation at cow with rumen fistula - nylon bag (Ørskov and McDonald 1979).

Results

Yields mentioned in tables bear to high ability performance fodder hybrid Festulolium, however yields of lolium hybrid (tab. 2) are at middle european conditions above all in second and next add up contingents regularly distributed rainfall. At first cut with absence rainfall will not disply manifest so markandly, growth mostly will to proof with usage winter moisture serve with a notice fair yield high-quality fodder yields in line 3. cut are sum 3 - 5. cut Deeproots festulolium hybrid (tab. 3) with drought planish better . In the clear culture but used to be grown few and far between sow with above all to the mixture.

In laboratory fy NutriVet Ltd. were to be made analysis designes choice hybrid Festulolium, values are mentined in tables. Experiments at hygric favourable year were to be reaped at five add up to analysis were to be delivereds samples of those most significant 3. cut lolium and 2. cut Festulolium hybrid.

Table 2: Digestibility of organic matter, crude fiber and neutral detergent fiber, content of WSC, crude protein, crude fiber and NDF at DM lolium of hybrids and yields from 1. cropping year

Variety	cut	DM t/ha	WSC %	OMD v %	CF v %	CFD v %	NDF v %	NDFD v %	CP %
Perun	1.	9,74	14,9	73,24	26,66	67,35	51,2	61,08	17,4
	2.	5,0	11,78	67,27	27,86	57,79	64,81	61,33	14,4
	3.	3,2	14,52	67,99	26,25	62,5	56,19	55,12	14,5
	Σ	17,94							
Achilles	1.	10,2	16,94	66,6	27,71	58,96	61,65	58,45	15,9
	2.	4,8	17,93	65,41	28,87	56,35	56,18	51,51	14,3
	3.	3,6	16,75	70,78	29,42	69,43	52,1	58,47	13,3
	Σ	18,6							
Perseus	1.	10,23	14,83	77,18	26,92	71,66	66,93	73,17	16,8
	2.	5,3	14,5	67,61	30,61	63,48	64,56	60,55	13,6
	3.	3,5	14,36	66,92	25,7	64,16	52,29	58,91	13,1
	Σ	19,03							
Lofa	1.	10,27	16,67	70,56	29,04	65,7	50,5	54,54	17,1
	2.	5,3	15,15	73,24	29,04	69,24	61,71	65,97	17,3
	3.	3,26	14,75	65,49	25,52	60,29	53,5	54,24	13,3
	Σ	18,83							

Table 3: Digestibility of organic matter, crude fiber, neutral detergent fiber and content of WSC, crude protein, crude fiber and NDF at DM Feastu- lolium hybrids and yields from 1. Cropping year.

Variety	cut	DM t/ha	WSC v %	OMD v %	CF v %	CFD v %	NDF v %	NDFD v %	CP %
Felina	1.	8,61	12,18	64,01	28,54	57,96	61,34	53,24	14,8
	2.	2,67	9,83	73,08	27,26	6,03	52,89	70,04	16,6
	Σ	18,54	Sum of five cuts						
Hykor	1.	7,12	11,94	65,78	28,26	60,76	57,68	55,43	16,4
	2.	2,74	14,03	70,44	25,78	63,55	59,46	62,21	17,8
	Σ	16,79	Sum of five cuts						
Fojtan	1.	5,95	11,77	68,68	27,76	64,85	57,43	58,65	17,3
	2.	2,44	13,84	70,16	25,04	66,44	51,99	58,24	17,9
	Σ	14,53	Sum of five cuts						

References

[1] Ørskov E. R., McDonald I. 1979. The estimation of protein degradability in the rumen from incubation measurements weighed according to the rate of passage. J. Agric. Sci. Camb. 92, 499-503.

The Influence of Extensive and Intensive Use of Permanent Meadow Community on the Quality of Forage and Qualitative Production

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Abstract

The results relating to the influence of various levels of N+PK fertilization (without NPK, $P_{30}+K_{60}$ kg.ha^{-1}, N_{90}+PK kg.ha^{-1}, and N_{180}+PK kg.ha^{-1}) prove a marked decrease of N-substances, PDIN (protein supplied when nitrogen is limited in the rumen) and PDIE (protein supplied when energy is limited in the rumen) concentrations in the forage of sward without N-fertilization, and also with the application of low N_{90}+PK kg.ha^{-1} amount as compared with the intense level of N-fertilization variant, i.e. 180 kg.ha^{-1}. With other nutrients (fibre, NEL – Netto energy of lactation) no significant differences were ascertained. The influence of intensity of utilization had a marked influence on the increase of all nutrient concentrations with variants comprising 3 to 4 cuts in comparison to both variants of the use in 2 cuts (early and late first cut). For an objective evaluation it is necessary to carry out an assessment of the interactive influence of fertilization and intensity of use.

Keywords: permanent grassland – fertilization – intensity of utilization – forage quality.

Introduction

The level of NPK fertilization and the intensity of grassland utilization have a marked influence on species composition (KOHOUTEK, A. et al. 2009; OERLEMANS, J. 2006) and consequently on the quality and production of forage. GRUBER et al. (2000) carried out an assessment of their interaction with respect to area loading by cattle, production efficiency of forage (production of milk). Economic effectiveness of production (VELICH, 1986) and particularly the influence on the environment, esp. the so-called "greenhouse effect" (ŠIMEK, 2009) cannot be neglected.

Material and Methods

A permanent grassland with the dominance of *Festuca arundinacea* Schreb. was evaluated for the influence of four nutrition variants (without NPK fertilization, $P_{30}+K_{60}$ kg.ha^{-1}, N_{90}+PK kg.ha^{-1} and N_{180}+PK kg.ha^{-1}) and four variants of utilization intensities - 2-cut early (a), 2-cut late (b), 3-cut (c), and 4-cut (d) on differences in the concentrations of N-substances, fibre, PDIN, PDIE, and NEL/MJ.kg^{-1} of dry matter. The characteristics in question were determined by the NIRS method. The experimental area is situated in the potato growing area (545 m above sea level), on the plot operated by the Vatín Research Grassland Station in the Žďárské vrchy Hills Protected Landscape Area. The results are related to mean values (weighted average) from the individual cuts from 2004 to 2009. The statistic evaluation of differences was conducted using ANOVA and followed by Tukey's test (P □ 0.05).

Results and Discussion

Influence of trophism level on the concentration of nutrients

In accordance with a number of scientific statements, a high level of N-180 kg.ha^{-1} + PK (Tab. 1) manifested itself by a conclusively higher concentration of not only N-substances in forage dry matter (142.3 g.kg^{-1}), but also PDIN and PDIE, and also in their ratio (21.02). In line with the findings published in other communications (JANČOVIČ, 1994), the increasing concentration of the ballast component – fibre in forage is influenced by the progressive doping with N+PK (258.2 – 253.5 g.kg^{-1} of dry matter); however the difference as against other variants without N-fertilization is insignificant. a lower concentration of NEL with N+PK fertilized variants (5.14 – 5.15 MJ.kg^{-1} dry mat.) was insignificant in comparison with variant without N-fertilizing.

Influence of the intensity of use on the concentration of nutrients

As compared with the extensive 2-cut variants, the multicut harvest system of use, i.e. 3- to 4-cuts, conclusively increases the concentration of N-substances, PDIE, and PDIN, and markedly decreases the concentration of fibre (Tab. 2). The concentration of NEL (5.45 $MJ.kg^{-1}$ dry mat.) is also markedly higher, also in relation to N 90 $kg.ha^{-1}$ (5.30 $MJ.kg^{-1}$) fertilizing variant. In this case the resulting value representing the weighted average of all cuts is influences by the very early 1st harvest (15.5.); according to BUCHGRABER et al. (2004) the concentration of NEL in young and timely harvested forage from the first cuts is by ca 1.0 $MJ.kg^{-1}$ higher than in further cuts, or first cuts harvested later on.

The results prove that the cause of more marked differences in the quality of forage with respect to the N-complex (N-substances, fibre, PDIE, PDIN) rests in the sphere of a higher level of nutrition, namely with nitrogen. As to the intensity of use, i.e. the number of cuts, there is a marked difference in the quality of forage both in the sphere of N-components and NEL between 3- to 4-cut stands in comparison to double-cut stands. As concerns the suitability of forage quality for the respective production-breeding orientation, and also from the economic and ecological points of view, it is necessary to determine a suitable interaction variant based on a so-called net yield of nutrients (BUCHGRABER et al., 2004), or in relation to the productivity of stand (number of large cattle $units.ha^{-1}$) and its side externalities, i.e. the loads by nutrient (cycle) and production of greenhouse gases (GRUBER et al. 2000).

Conclusion

The high level of N-fertilization (180 $kg.N.ha^{-1}$) has conclusively increased (by 1/5) the concentrations of N-substances, PDIN, and PDIE in the dry matter of forage from the sward dominated by *Festuca arundinacea* Schreb. as compared with variants of up to the 90 kg $N.ha^{-1}$ amount and without N-fertilization. Differences in fibre concentration were insignificant with the increasing influence of N-fertilization and also NEL/MJ at a higher level of fertilization. As compared with 2-cut stands, the multicut system of harvest, i.e. 3 and 4 cuts, markedly increased (by 1/5) not only the concentration of N-substances, PDIN, PDIE, but also NEL/MJ (by 1/10), and markedly decreased the concentration of fibre (by 1/5).

Table 1: Concentration of nutrients in forage dry matter ($g.kg^{-1}$) of permanent grassland community in relation to N+PK fertilization level. Vatín, 2004 to 2009 (weighted average for cuts).

Variation of fertilization		Concentration ($g.kg^{-1}$) in dry matter				Relation PDIE/PDIN	NEL ($MJ.kg^{-1}$)
		N-substances	Fibre	PDIN	PDIE		
without NPK	$g.kg^{-1}$	118,1a	238,2a	69,5a	78,1	0,89a	5,28a
	rel. %	100,0	100,0	100,0	100,0	100,0	100,0
$P_{30}+K_{60}$	$g.kg^{-1}$	115,1a	250,5a	66,9a	77,7a	0,86a	5,16a
	rel. %	97,4	105,2	96,3	99,5	96,6	97,7
$N_{90}+PK$	$g.kg^{-1}$	122,4a	258,2a	71,9a	78,8a	0,91a	5,15a
	rel. %	103,6	108,4	103,5	100,9	102,2	97,5
$N_{180}+PK$	$g.kg^{-1}$	142,3b	253,5a	82,9b	80,9ab	1,02b	5,14a
	rel. %	120,5	106,4	119,3	103,6	114,6	97,3

Table 2: Concentration of nutrients in forage dry matter (g.kg^{-1}) of permanent grassland community in relation to the intensity of use. Vatín, 2004 to 2009 (weighted average for cuts).

Method of use		Concentration (g.kg^{-1}) in dry matter				Relation PDIE/PDIN	NEL (MJ.kg^{-1})
		N-substances	Fibre	PDIN	PDIE		
4-cut	g.kg^{-1}	145,8b	223,1a	86,1b	83,0b	1,04b	5,45c
	rel. %	100,0	100,0	100,0	100,0	100,0	100,0
3-cut	g.kg^{-1}	133,0b	236,8a	78,1b	80,8b	0,97b	5,30bc
	rel. %	91,2	106,1	90,7	97,3	93,3	97,2
2-cut early	g.kg^{-1}	112,8a	265,4b	65,8a	76,9a	0,86a	5,05ab
	rel. %	77,4	119,0	76,4	92,6	82,7	92,7
2-cut late	g.kg^{-1}	106,2a	275,4b	61,2a	75,0a	0,81a	4,92a
	rel. %	72,8	123,4	71,1	90,4	77,9	90,3

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References

[1] BUCHGRABER, K., DEUTSCH, A., GINDL, G. 1994. Zeitgemässe Grünlandbewirtschaftung. Graz, Stuttgart: Leopold Verlag, 199 s.

[2] GRUBER, L., STEINWIDDER, A., GUGGENBERGER, T., SCHAUER, A., HÄUSLER, J., STEINWIDDER, R. und STEINER, B. 2000. Einfluss der Grünlandbewirtschaftung auf Ertrag, Futterwert, Milcherzeugung und Nährstoffausscheidung. BAL Gumpenstein, 27. Viehwirtschaftliche Fachtagung, 6.-8. Juni 2000, pp. 41-88

[3] JANČOVIČ, J. 1994. Vplyv striedavej intenzity dusíkatého hnojenia na kvalitu poloprírodného trávného porastu. I. obsah vlákniny a dusíkatých látok. AGRICULTURE, sv. 40, č. 9, s. 674-683, ISSN 0551-3677

[4] KOHOUTEK, A., KVAPILÍK, J., CAGAŠ, B., HRABĚ, F., POZDÍŠEK, J. 2009. Selected indicators of productive and extraproductional management of grasslands in the Czech Republic. In Grassland Science in Europe: Alternativefunctions of Grassland. Brno: Organising Committee of the 15th European Grassland Federation Symposium 2009, s. 11–24. ISBN 978-80-86908-15-1.

[5] OERLEMANS, J. 2006. Langfristige Efekte abgestufter N-, P-, K-Gaben bei Mähweiden verschiedener Standorte auf die Zusammensetzung der Pflanzenbestände unter den Aspekten floristischer Diversität und Agronomie. Cuvillier Verlag Göttingen, s. 258. ISBN 3-86537-867-6

[6] ŠIMEK, M. 2009. Skleníkové plyny ze zemědělství, a co s nimi? Úroda: časopis pro rostlinnou produkci. sv. 57, č. 8, s. 65–68, č. 9, s. 67-70, č. 10, s. 56-58. ISSN 0139-6013.

[7] VELICH, J. 1986. Studium vývoje produkční schopnosti trvalých lučních porostů a drnového procesu při dlouhodobém hnojení a jeho optimalizace. VŠZ Praha, 162 s.

Production Ability Impact Of Temporary Grasslands On Forage Quality

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Abstract

Milk efficiency of dairy cows reached 6 548 kg of FCM with consumption of 0.30 kg of feed concentrates per a kg of FCM in 2007 in the Czech Republic (CR). Economic crisis makes the farm holdings reduce production costs by using quality forage from renovated grasslands instead of expensive feed concentrates. Therefore an accurate small-plot trial was established in the Jevíčko (elevation 342 m) site in 2008 in four replications with a selection of 15 grass species: hybrid ryegrass (1 variety), Italian ryegrass (1), perennial ryegrass (5), timothy (2), cocksfoot (3), tall fescue (3), Festulolium (6), tall oat-grass (1), meadow foxtail (2), red fescue (2) and mountain brome (1), smooth brome (1) and Alaska brome-grass (1), yellow oat-grass (1). The trial was fertilized with N 180 kg/ha in the form of ammonium nitrate with lime applied in three doses per 60 kg/ha (in spring, after the first and second harvests), 35 kg/ha P (superphosphate) and 100 kg/ha K (potassium salt); four-cut utilization, first cut on 29th April, then 45 days between cuts. The contribution evaluates dry matter production and forage quality.

Introduction

Milk efficiency of dairy cows reached 6 548 kg of FCM with consumption of 0.30 kg of feed concentrates per a kg of FCM in 2007 in the CR. Economic crisis makes the farm holdings reduce production costs by using quality forage from renovated grasslands instead of expensive feed concentrates. Generic hybrids (Frankow-Lindberg and Olsson, 2008; Gutmane and Adamovich, 2008) gain ground among the assortment of grasses due to their production abilities, good health condition and persistence.

Materials and Methods

The trial with the assortment of grasses was established at the site in Jevíčko in 2008 on gley fluvisoil with neutral soil reaction (pH/KCl 6.7). a quick renovation of permanent grassland was made after the first cut with herbicide Touchdown Quattro (glyphosate) in the amount of 8 litres/ha. Sowing was carried out at the beginning of August 2008. The fertilization was done with 180 (3 x 60 kg/ha N) in the form of ammonium nitrate and lime, 35 kg/ha P (superphosphate) and 100 kg/ha K (potash salt). The contribution evaluates the first yield year of 15 grasses species: hybrid ryegrass ´Odra´, Italian ryegrass ´Lubina´, perennial ryegrass varieties ´Algol´, ´Mustang´, ´Jaran´, ´Korok´ and ´Jaspis´, meadow fescue varieties ´Kolumbus´ and ´Pronela´, timothy varieties ´Bobr´ and ´Sobol´, cocksfoot varieties ´Niva´, ´Vega´ and ´Toscali´, tall fescue varieties ´Kora´, ´Probe´ and ´Prolate´, Festulolium varieties ´Felina´, ´Hykor´, new varieties HŽ 14 – DK and KL 26, ´Lofa´ and ´Perseus´, tall oat-grass ´Medián´, meadow foxtail varieties ´Talope´ and ´Vulpina´, red fescue varieties ´Tagera´ and ´Tradice´, smooth brome ´Tabrom´, mountain brome ´Tacit´, Alaska brome-grass new variety CD1 and yellow oat-grass ´Rožnovský´. The trial with the assortment of grasses is utilized in four cuts, the first cut is done at the stage of stalk shooting of cocksfoot, the second to fourth cut after 45 days. The contribution evaluates dry matter production and forage quality. The quality of forage dry matter was evaluated by NIR Systems 6500 fitted with a spinning sample module, in reflectance range 1100-2500 nm, band width 2 nm, measured in small ring cups, duplicate samples scanned twice. The parameters measured were crude protein (CP), fibre (CF), PDIE, PDIN, NEL (net energy of lactation), NEF (net energy of fattening), using software WinISI II, vers. 1.50.

Results and Discussion

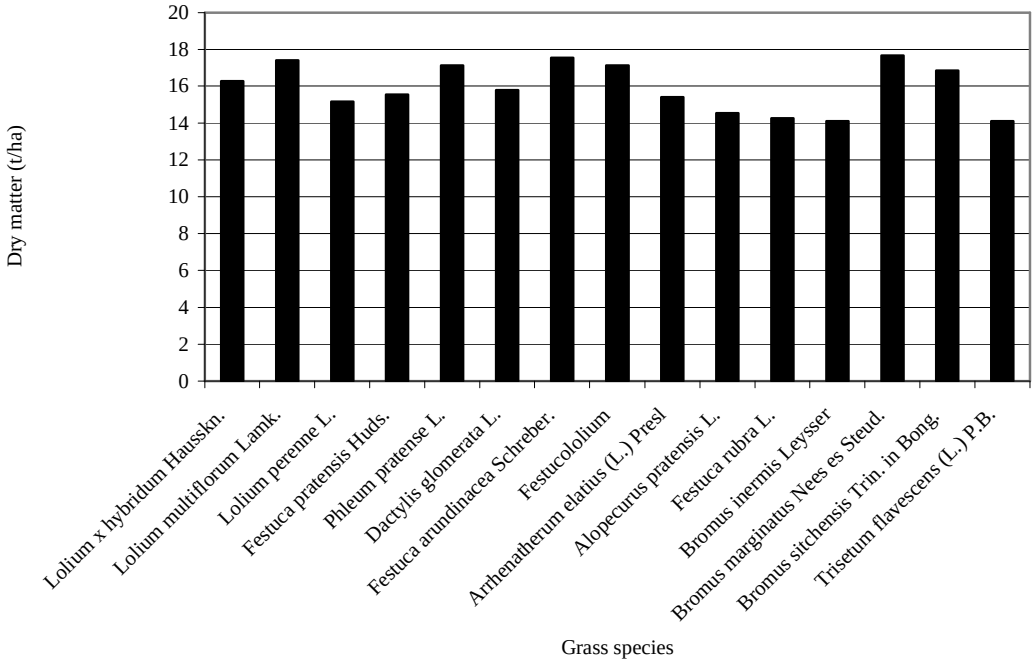
In 2009 the vegetation growth was accelerated by an early spring start and above-average temperatures in April (+5 °C) in the whole CR and the first cut was carried out on 29th April 2009. Among the evaluated assortment of grasses (Tab. 1, Fig. 1) tall fescue was the most productive with the yield of 17.54 t/ha DM, followed by Italian ryegrass 17.41 t/ha, festuloliums and timothy 17.12 t/ha, brome grasses 16.22 t/ha, hybrid ryegrass 16.27 t/ha, closely followed by other species, yellow oat-grass 14.11 t/ha and red fescue 14.25 t/ha had the lowest yield. This confirmed high and balanced production efficiency of the whole grass assortment in the first yield year. Festuloliums gain ground in other European countries as Gutmane and Adamovich (2008).

In the first yield year the forage shows high concentration of nutrients, especially CP (160.3 – 187.5 g/kg DM), PDIN and PDIE. The energy concentration (NEL) in the forage is the highest in ryegrasses (5.79 – 6.02 MJ/kg DM), the lowest in brome grasses (4.77 – 4.91 MJ/kg DM), red fescue (4.98) and meadow foxtail (4.98 MJ/kg DM). The concentration of CF was from 226.3 to 274.2 g/kg DM, the lowest was in ryegrasses, the highest in brome grasses.

Table 1: Dry matter production and quality of forage of grass species in 2009

Species	DM	Forage quality parametrs					
		CP	CF	PDIN	PDIE	NEL	NEF
	t/ha	g/kg	g/kg	g/kg	g/kg	MJ/kg	MJ/kg
Lolium x hybridum Hausskn.	16.27	160.3	232.5	94.2	83.2	5.79	5.65
Lolium multiflorum Lamk.	17.41	158.7	240.7	93.9	83.8	6.02	5.91
Lolium perenne L.	15.16	174.6	226.3	102.2	85.5	5.79	5.66
Festuca pratensis Huds.	15.55	178.7	235.1	102.5	85.9	5.64	5.46
Phleum pratense L.	17.12	165.5	248.8	95.9	85.2	5.69	5.52
Dactylis glomerata L.	15.78	179.2	243.0	104.6	86.0	5.51	5.32
Festuca arundinacea Schreber.	17.54	166.1	248.2	98.0	83.3	5.36	5.12
Festulolium	17.12	163.8	245.8	95.8	83.0	5.30	5.06
Arrhenatherum elatius (L.) Presl	15.42	176.0	243.7	102.3	84.2	5.45	5.25
Alopecurus pratensis L.	14.53	172.4	245.5	97.8	82.9	4.98	4.68
Festuca rubra L.	14.26	167.3	254.4	98.7	82.0	5.22	4.98
Bromus inermis Leysser	14.12	187.5	260.8	107.2	83.0	5.19	4.91
Bromus marginatus Nees es Steud.	17.67	168.0	274.2	96.3	83.2	5.22	4.94
Bromus sitchensis Trin. in Bong.	16.86	153.5	280.2	87.5	81.9	5.06	4.77
Trisetum flavescens (L.) P.B.	14.11	156.7	266.4	92.5	83.5	5.40	5.18
Average	15.73	168.5	249.4	97.9	83.8	5.44	5.22
$DT_{0.05}$	2.49	26.6	22.7	14.8	3.7	0.41	0.48
$DT_{0.01}$	2.90	30.9	26.4	17.3	4.3	0.47	0.56

Fig 1 Dry matter production of the grass species in 2009 at the Jevíčko site



Conclusions

The production abilities of grass species are high and they are the basis for effective forage production in monocultures, as well as in legume-grass mixtures. The forage harvested in time and well conserved is fundamental for high efficiency of dairy cows and suckler cows and allows reducing feed concentrates consumption.

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References

[1] Frankow-Lindberg B.E. and Olsson K.F. (2008) Digestibility and fibre content of leaves and straw of three Festulolium hybrids during spring regrowth. Grassland Science in Europe, 13, 456-458.

[2] Gutmane I. and Adamovich A. (2008) Analysis of Festulolium and hybrid ryegrass (Lolium x boucheanum) dry matter yield stability. Grassland Science in Europe, 13, 248-250.

Section 2: Fermentation Process of Forage - Harvest, Preservation and Storage

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Silage Inoculants - Where Next?

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Introduction

In a review considering the future of silage inoculants it is worth briefly examining where inoculants are now and where they have come from. The principle of silage inoculation with cultures of lactic acid producing organisms has been used for in excess of 100 years, the process being first applied to sugar-beet residues (Watson and Nash, 1960). The use of cultures such as 'lacto-pulp' were in common use in France, and Crolbois (1909) successfully developed methodologies to grow pure cultures of selected beneficial lactic acid producing bacteria. These studies also proved the benefits of applying these inoculants to beet-pulp silage compared to no treatment. Further research continued and Völtz, 1918 (cited by Watson and Nash, 1960), inoculated Italian ryegrass with *Bacillus cucumeris fermentati* and produced silage with a lactic to other volatile acids ratio of almost 3.5:1. Many silages on farms today fail to reach this ratio, which is as we know an excellent indicator of fermentation quality. Despite the work of these early pioneers showing the potential benefits of inoculation to silage production, inoculants were still viewed by many farmers as of little value to them until the mid to late 1980's. The reasons for this were predominantly due to the debatable efficacy of the commercial inoculants available at the time. However, microbiologists have risen to the challenge and a whole host of commercial silage inoculants have been developed over the intervening 25 years or so, and inoculants, for those farmers that use additives, have displaced acid additives across most of Europe as the additive type of choice.

Inoculant development until the mid 1990's focussed on homo-fermentative lactic acid bacteria species and most inoculants contained one or more strains from the species *Lactobacillus plantarum, Lactococcus lactis, Pediococcus pentocaseous, Pediococcus acidilactici* and *Enterococcus faecium.* Other species have subsequently been introduced to improve the fermentation process or to increase the aerobic stability of silage during feed-out these include species such as *Propionibacteria sp, Lactococcus salivarius, Lactobacillus paracasei, Bacillus sp.* and, probably most controversially, the hetero-fermentative species *Lactobacillus buchneri.* I am not going to review these recent developments as a number of excellent reviews have been published (Charmley; 2001; Muck, 1993; Spoelstra, 1991; Weinberg and Muck 1996). So homo-fermentative inoculants have been very successful in improving silage quality, reducing the concentrations of volatile fatty acids, ammonia-N and free amino acids, whilst increasing concentrations of water soluble carbohydrate and true protein (Cussen *et al.,* 1995; Davies *et al.* 1998; Jalc *et al.,* 2009; Merry *et al.,* 1995; Winters *et al.* 2001).

So Where Next for Silage Inoculants?

Before attempting to answer this question it is important to understand the situation in the ruminant farming sector. World-wide agriculture and particularly livestock farming, is facing it's biggest challenge to date. Increasing demand from developing countries for meat and milk products is competing with the fact that livestock are directly responsible for about 9% of the total anthropogenic greenhouse gas emissions and once all other associated emissions are accounted for this rises to 18% of the anthropogenic global emissions (Gill *et al.* 2009), resulting in the need to reduce emissions. Alongside this there is increasing pressure on land and water resources resulting in increased cost of protein and energy supplements for ruminant diets. However, ruminants have the ability to convert non-human-edible sources of feed into high value human food, but this also raises the health debate where an increased percentage of the world's population are becoming obese due partly to the over consumption of meat and milk products. There is also the increased consumer concern in certain quarters of the world of food safety, particularly with the rise of food borne illnesses such as those caused by verotoxigenic *E.coli.*.

Thus the challenge for the sector is to increase productivity of healthier and safe meat and milk products, whilst reducing emissions, or in other words improve production efficiency yet further.

With all of this in mind what opportunities are there for silage inoculants to rise to these challenges?

I would propose that silage inoculants have many opportunities in helping to provide some solutions to these global problems. So in the remainder of this paper I will focus on the role they could have in:- a) Reducing environmental impact of ruminant farming, both from the angle of nitrogen pollution and methane, which goes hand in hand with improved efficiency and reduction of losses from the system b) Improving both the biological and chemical safety of human food, and c) Improving the healthiness of human food.

The potential of inoculants to reduce environmental impact

The first key factor affecting environmental impact is overall efficiency of production, put simply to reduce waste. There is plenty of evidence already quoted in this paper to indicate that homo-fermentative silage inoculants by improving silage fermentation can reduce wasteful end products such as ammonia-N and volatile fatty acids, which result in poorer feed conversion efficiency and higher in-silo dry matter losses. However, it is also becoming increasingly evident that one of the consequences of improving silage quality is the negative impact this may have on aerobic stability. It is accepted that well-preserved, high quality silages, particularly those inoculated with homo-fermentative lactic acid bacteria, are more prone to spoilage than untreated silages (Weinberg *et al*., 1993), due both to the fact that more nutrients are preserved in the silo and there are fewer secondary end-products that inhibit the growth of spoilage microorganisms. This undoubtedly results in the potential for more waste.

The problem of aerobic instability has led to a shift away from homo-fermentative lactic acid bacteria towards hetero-fermentative lactic acid bacteria with the bacterium of choice being *Lactobacillus buchneri* (Oude Elferink *et al*., 1999; Driehuis *et al*., 2001). This approach has been shown to inhibit the aerobic deterioration of silages through the production of end-products other than lactic acid mainly acetic acid. However, whilst this approach can inhibit aerobic deterioration of silage (Driehuis *et al*., 2001), the effect is not always consistent (Kleinschmit and Kung, 2006). It is well documented (Woolford, 1990; McDonald *et al*., 1991) that the production of acetic acid results in a slower fermentation and thus will probably have a concomitant effect on the protein quality of the silage (Davies *et al*., 1998; Jones, 1998).

An understanding of the biochemistry of the silage fermentation shows that for every molecule of acetic acid formed an equivalent molecule of carbon-dioxide is produced (McDonald *et al.*, 1990). Thus for every 1 g of acetic acid in silage 0.733 g of CO_2 are also produced. Data taken from a large scale 75 tonne grass silage clamp study (Winters *et al.* 2001) shows that the acetic acid concentration in inoculated silage (10 g/kg DM) is significantly lower than that in untreated silage (27g/kg DM). Taking these figures and examining a farm producing 250 tonnes of silage dry matter the difference in CO_2 production for untreated versus inoculated silage in this case would have been 3.1 tonnes of CO_2. The reader may find my comparison between untreated and homo-fermentative inoculant treatment unfair, when arguing against the use of hetero-fermentative silage inoculants. However most published inoculant experimentation is conducted at small scale and usually no more than 1 kg. Many of these experiments show that treatment with hetero-fermentative inoculants produces more acetate than the untreated control silage. In one such study (Danner *et al* 2003) the *L. buchneri* treated silage had 55.3 g/kg DM acetate which was over 6 times greater than the *L. plantarum* treated silage and more than twice the untreated, and that was at laboratory scale! Furthermore upon entering the rumen acetate is an end-product of rumen fermentation, whereas lactate is converted to propionate which also requires $2H^+$ ions. Removal of hydrogen ions from the rumen is the sole reason for rumen methane production. The net result of this being a theoretical reduction in methane emissions from silages with a higher lactate:acetate ratio.

Whilst, for the reasons highlighted above, one can question the role of hetero-fermentative inoculants in silage production to control aerobic spoilage, what is beyond questioning is the fact that aerobic deterioration represents a significant loss to the industry and current homo-fermentative inoculants fail to guarantee aerobic stability on-farm. Much progress has been made in the use of

combination additives combining food preservatives with homo-fermentative inoculants to successfully control aerobic deterioration of silage (Owen, 2002; Rammer *et al.*, 1999; White *et al.*, 2002). However, to be more sustainable, I would argue that we need to focus further attention on a wholly microbial solution to the problem. This is not just a puritanical dream as there are a number of clues to a solution that do indeed warrant further investigation.

A few microbial approaches have been tried, such as natural end-products of microbial fermentation (Grinstead and Barefoot, 1992) or yeast killer toxins (Lowes *et al.*, 2000). The former is still worthy of further investigation. If we consider research published in a previous symposium here in Brno (Merry *et al.* 1997) these authors published work showing the benefits of adding pure diacetyl in controlling aerobic deterioration. Diacetyl is a produced by a number of lactic acid bacteria, particularly those used in the production of dairy food products. However, further work by this group was unsuccessful (Merry *pers comm.*) in realising the potential of this approach in silage inoculant bacteria. Despite this the improvements in rapid screening technologies and DNA methodologies (eg. Ennahar *et al.*, 2003) should be harnessed to investigate/isolate lactic acid bacteria that have the ability to produce natural end-products that could inhibit the growth of yeasts and moulds that cause aerobic deterioration, whilst maintaining fermentation quality. Whilst considering the role of fermentation end-products an interesting conundrum was posed when it was shown that lucerne silage was more stable than maize silage (Muck & O'Kiely, 1992; O'Kiely & Muck, 1992) but these authors further concluded that the factor causing stability was produced during ensilage, as the fresh crop was not stable. An investigation of the minor chemical constituents of lucerne that could be involved in the lactic fermentation could provide another microbial approach to reducing spoilage. Finally before leaving the important subject of aerobic deterioration it is worthy of note that whilst many believe fungi are responsible for deterioration the acetic acid bacteria have also been implicated (Spoelstra *et al.* 1988), these utilize either lactic acid or ethanol for the production of acetic acid and CO_2. However, more recently (Nishino *et al.*, 2009) *Acetobacter pasteurianus* was associated with aerobically stable silage with a 100 fold lower yeasts count than the untreated silage. Thus indicating another potential route to control aerobic spoilage solely through a microbial solution.

Careful selection of future silage inoculants also have the potential to help reduce total methane outputs from silage fed ruminants. This potential arises through their natural ability to produce bacteriocins or lantibiotics (a special group of antibiotics produced by the lactic acid bacteria). One such example of this is nisin, a lantibiotic produced by some strains of *Lactococcus lactis* (Matsusaki *et al.*, 1996). These strains are used in the dairy industry due to their activity against food bourne pathogens and spoilage organisms such as *Bacillus sp* and *Listeria monocytogenes*. However, studies with purified nisin (Callaway *et al.*, 1997) using *in vitro* rumen studies have shown the potential at 1µM in the rumen liquor to inhibit methane production. The obvious next step is to investigate the potential of introducing lactic acid bacteria as silage inoculants that both maintain silage quality characteristics but also that inhibit methanogenesis in ruminants when fed the resultant silages. As a result this approach could also reduce the incidence of listeriosis both in farmed livestock but also in human food. If nisin proves not to be the way forward, we must not loose faith in the lactic acid bacteria's ability to produce a range of bacteriocins/lantibiotics that could have beneficial effects in the rumen. Russell and Mantovani (2002) have already proposed that naturally occurring rumen bacterial bacteriocins could be used as feed additives, so why shouldn't the same be true of bacteriocin producing silage inoculant bacteria as forage additives with rumen modifying capabilities.

I would now like to focus on nitrogen use efficiency (NUE), improvements in whole farm NUE can have significant benefits to the economics of livestock agriculture. It is well documented that silage inoculants can improve the protection of protein from breakdown and thus the level of intact protein remaining in the silage and how this in turn improves animal performance and importantly whole animal nitrogen retention/utilisation (Davies *et al.*, 1998; Jones, 1998; Winters *et al.*, 2001). Whole animal nitrogen utilisation is not only important for farm economics but also to reduce outputs of waste nitrogen. There are two considerations with respect to nitrogen and climate change gaseous emissions. Firstly the better use of forage protein will result in reduced CO_2 emissions associated with fertilizer production and

fuel to transport concentrate nitrogen onto farms. Secondly nitrogen excretion from animals results in N_2O emissions so any reduction in N excretion positively reduces green house gas emissions. The exact contribution N_2O makes to total emissions is uncertain as there are considerable climatic effects causing variability (Gill *et al.* 2009), but N_2O is approximately 300 times more potent than CO_2 in it's global warming effect. As previously stated there is considerable evidence for NUE benefits from inoculants the benefits of amino acid supply have received far less attention. Winters *et al.*, (1999; 2001) have shown significant effects of inoculation on the overall silage amino acid profiles with significant increases in certain essential amino acids across a range of forage crops for example red clover had increases in lysine of 30% and histidine of 9.5% in inoculated compared to untreated silage. These are among the first limiting amino acids for production response and thus are a key reason why ruminants are fed supplementary protein which is often used inefficiently and thus supplied in quantities that are greater than required, resulting in increased nitrogen excretion. It is known that the amino acid profile of meat and milk protein is very different from that of plant protein, with microbial protein having a more similar amino acid profile to animal protein. Bach et al. (2000) suggested that if the amino acid profile of blood entering the mammary gland was the same as that of milk the crude protein content of the diet could be reduced to 15%. Little attention has focussed on the amino acid profiles of different strains/species of inoculant lactic acid bacteria. However, considerable diversity between different species of lactic acid bacteria has been identified as early as 1977 (Erdman *et al.* 1977). Attention should now be focussed on assessing both the variation amino acid profiles of silage inoculant bacteria and the effect silages treated with improved inoculants could have in reducing concentrate protein feeds in animal experiments.

Finally before moving on from the subject of reduced environmental impact, we should consider the CO_2 emissions associated with harvesting forage. The use of machinery on farms burns significant quantities of fossil fuels, any move to reduce this would give a positive impact on reducing GHG emissions. With the ensilage of grasses and legumes a 3 cut per season is common place across the temperate regions of the world, and for very good reasons. The relationship between forage quality measured either as digestibility or metabolizable energy content and animal production response is well documented. If poor quality forage is fed not only does production decline but there are significant increases in methane emissions per kg of meat or milk produced. Frequent cutting of perennial silage crops enables maximum forage quality to be attained, but, this does have consequences for on farm fuel consumption. One possible approach available to silage additives would be to increase digestibility of plant cell walls and thus enable delayed harvesting of forages without any resultant loss in quality, thus providing the potential to increase harvesting interval and reduce fuel usage. Considerable focus by additive manufactures on the use of enzymes to do just this has been made. However, scientifically the results have been poor. The reason for this was succinctly put by Stark and Wilkinson (1986) when they stated 'one of the major problems which has prevented the use of effective quantities of enzymes in silage additives is the cost:activity relationship.' Continuing they quoted Henderson and Mcdonald (1977) who suggested that about 4 kg cellulose/t of silage was needed to obtain meaningful improvements in silage quality. Whilst I can accept that enzyme technology has moved on in the last 30 years the cost:activity conundrum is one that in my view still holds true today. So what are the alternatives, as usual I fall back on my faithful friends the lactic acid bacteria. Inoculants as they grow in the silo produce all the enzymes that are required, so all that is required is for a candidate inoculant bacterium to be discovered that has cell wall degrading abilities. The key component of plant cell walls that reduces digestibility is lignification as the plant matures. The lignin becomes closely associated with the digestible cellulose and hemicellulose fractions rendering them more difficult for the rumen micoflora to degrade. Donaghy *et al.*, (1998) recently identified species of lactobacilli that produced ferulic acid esterase that would result in the solubilization of lignin and thus the potential to improve cell wall digestibility. Further work has been conducted (Nsereko *et al*, 2007) with potential silage inoculant species of lactic acid bacteria that produce the ferulic acid esterase. In these studies (Nsereko *et al*, 2007) using a *L. buchneri* ferulic acid esterase producing strain as part of the silage inoculant showed an increase in *in situ* ruminal neutral detergent fibre digestibility from 0.362 in the untreated to 0.422 in the inoculated silage. These indicate the encouraging potential of such an approach, but we must proceed with caution for 2 reasons.

To ensure the correct silage producing organism is utilized, and for the reasons outlined early in this manuscript, in my opinion, *L. buchneri* is not one of these.

There must be a guarantee that the cell wall that is digested in the silo is not subsequently fermented in the silo thus providing less available energy for utilization by the animal.

The potential of inoculants to improve food safety

Silages have the potential to act as a reservoir for both pathogenic microorganisms such as *Listeria* and *E. coli* and biochemicals such as mycotoxins. These undesirable components of silage have the potential on farms to reduce animal performance and cause disease, but they also have the potential to pass from farms via the food chain into man causing human disease. Silage inoculants have the potential to reduce these risks. Within this paper I have already set out the case for the role future inoculants should control aerobic deterioration of silage and reduce methane emissions by ruminants being fed silage. These or similar approaches can be targeted at both pathogen control and the reduction of noxious chemicals such as mycotoxins (the end products of fungal growth) in the silo. So other than highlight this important area and the potential inoculants have to control these processes, I intend to spend no more time discussing them.

There is one more area where inoculants could have a safety promoting function in both ruminants and man. If we take the situation in the food industry, where everyday we hear of the benefits of consuming this fermented milk product or that probiotic drink, it is surely time for those of us working in the field of silage inoculants to devote more effort to elucidate the health promoting attributes of silage inoculants. After all both rely on the fermentation of food/feed ingredients by selected species from the lactic acid group of bacteria. If we take one area of focus, animals particularly farmed animals are a major reservoir for the food borne pathogen group the verotoxigenic *E. coli* (Whipp *et al.* 1994). These pathogens reside in the gut of the ruminant animal with the main site being the hindgut and in particular the rectum (Naylor *et al.* 2003). However research has also shown that gut carriage and multiplication of this group of pathogens can be reduced by feeding probiotics (Zhao *et al.*, 1998). Species of lactic acid bacteria are common inhabitants of the hindguts of many animals including man and ruminants. Once established they compete for attachment sites on the gut wall and thus competitively exclude other bacteria from attaching to the gut wall thus preventing the incoming bacteria for proliferation. Species of lactobacillus have already been shown to be beneficial in species of farmed livestock to competitively exclude pathogens from the non-ruminant gut (La Ragione et al., 2004). Could silage inoculants provide a similar function? There is considerable research required before we could confidently claim success in this area, but it is not beyond the realms of possibilities. Firstly the inoculants would need to survive passage through the rumen. Weinberg *et al.* (2003; 2004) suggested that inoculants could have probiotic activities and have begun to assess the potential of them to survive passage through the rumen, their results look promosing. Ultimately only further research will answer whether the approach has future potential, but it is certainly a goal worth chasing.

The potential of inoculants to improve the healthiness of food

Governments and health practitioners are making the population as a whole increasingly aware of the importance of eating a healthy diet. Within this context, we need to take a holistic view of healthiness and not just focus on the commonly considered macro-nutrients of proteins, carbohydrates and fats but also examine the constituents of these particularly the fats and also the essential micronutrients such as vitamins and minerals. I would like to propose that silage inoculants again have the potential to influence silage quality to enhance both the concentrations of certain fatty acids with in silages. These would thus be available to the animal and from there on would be expected to be transferred into the human food chain.

Conjugated linoleic acid (CLA) has gained considerable attention due to it's many health promoting properties such as anticarcinogenic, antioxidative and cholesterol depressing. Fermented dairy products are known to contain higher levels of CLA than non-fermented milk equivalents (Lin, 2000), thus again indicating the potential lactic acid bacteria have to influence the outcome of the feed quality.

Studies (Kishino, *et al.*, 2002; Lin, 2000) have indicated that a number of species of lactic acid bacteria including *L. plantarum, L. acidophilus* and *L. lactis* have the potential to produce significant amounts of CLA in dairy based food production. Whilst ruminant food products are seen to be a good source of CLA in the diet of man, increasing the level with in these food products would be beneficial to the overall human diet. The developments in this technology are once again taking place in the food industry. However, future silage inoculant development should investigate the possibility of selecting bacteria that can enhance the concentrations of this health promoting fatty acid, as increasing the dietary intake of CLA by ruminants should ultimately lead to higher levels in human food. Silage inoculant microbiologists should in the future investigate the potential of lactic acid bacteria to enhance other health promoting nutrients with in animal feed, who knows one day we may have inoculants rich in vitamins and minerals.

Conclusions

Within this manuscript I have tried to highlight areas where future silage inoculants could enhance the quality of silage over and above the traditional nutritional measurements of silage quality that we currently work to. In this every increasing competitive world the farming community need to justify every expenditure they make. It falls upon us to meet this expectation; one way we can ensure this is to continue to develop silage inoculants not only to fulfil the farmer's needs but increasingly to meet the needs of the consumer. To do this we need to ensure that we keep pace with all relevant developments in other research arenas and in particular those involved in dairy/food microbiology.

References

[1] Bach, A., Huntington,G.B., Calsamiglia, S. and Stern. M.D. (2000). Nitrogen metabolism of early lactation cows fed diets with two different levels of protein and different amino acid profiles. Journal of Dairy Science. 83:2585-2595.

[2] Callaway, T.R., Carneiro, A.M.S., De Melo, C. and Russell, J.B. (1997). The effect of nisin and monensin on ruminal fermentations in vitro. Current Microbiology 35:90 – 96.

[3] Charmley, E. (2001). Towards improved silage quality - a review. Canadian Journal of Animal Science, 81, 157-168.

[4] Crolbois, J.(1909) Academy of Science 149:411 – 414.

[5] Cussen, R.F., R.J. Merry, A.P. Williams & J.K.S. Tweed (1995). The effect of additives on the ensilage of forage of differing perennial ryegrass and white clover content. Grass and Forage Science, 50, 249-258.

[6] Danner, H., Holzer, M., Mayrhuber, E. And Braun, R. (2003). Acetic acid increases stability of silage under aerobic conditions. Applied and Environmental Microbiology 69:562 – 567.

[7] Davies, D.R., R. J. Merry, A.P. Williams, E.L. Bakewell, D.K. Leemans & J.K.S. Tweed (1998). Proteolysis during ensilage of forages varying in soluble sugar content. Journal of Dairy Science, 81, 444-453.

[8] Donaghy, J., Kelly, P.F. and McKay, A.M. (1998). Detection of ferulic acid esterase production by Bacillus spp. And Lactobailli. Applied Microbiology and Biotechnology 50:257 – 260.

[9] Driehuis F., S.J.W.H. Oude Elferink, & P.G. Van Wikselaar (2001). Fermentation characteristics and aerobic stability of grass silage inoculated with Lactobacillus buchneri, with or without homofermentative lactic acid bacteria. Grass and Forage Science, 56, 330-343.

[10] Ennahar, S., Cai, Y. and Fujita, Y.(2003). Phylogenetic diversity of lactic acid bacteria associated with paddy rice silage as determined by 16s ribosomal DNA analysis. Applied and Environmental Microbiology 69:444 – 451.

[11] Erdman, M.D., Bergen, W.G. and Reddy, C.A. (1977) Amino acid profiles and presumptive nutritional assessment of single-cell protein from certain lactobacilli. Applied and Environmental Microbiology 33:901 – 905.

[12] Gill, M., Smith, P. and Wilkinson, J.M. (2009). Mitigating climate change: the role of domestic livestock. The Animal Consortium published on line by Cambridge University Press doi:10.1017/S1751731109004662.
[13] Grinstead, D.A. and S.F. Barefoot. 1992. Jenseniin G, a heat-stable bacteriocin produced by Propionibacterium jensenii P126. Appl. and Env. Micro. 58:215-220.
[14] Henderson, A.R. and McDonald, P. (1977). The effect of cellulose preparations on the chemical changes during ensilage of grass in laboratory silos. Journal of the Science of Food and Agriculture 28: 486 – 492.
[15] Jalč, D., Lauková, Simonová, M., Váradyová, Z. and Homolka, P.(2009). The use of bacterial inoculants for grass silage:their effects on nutrient composition and fermentation parameters in grass silage. Czech Journal of Animal Science 54:84 – 91.
[16] Jones, R., 1998. Bridging the protein gap: Potential of forage crops for UK livestock production. In Biotechnology in the Feed Industry, Proceedings of Alltech's 14th Annual Symposium p 119-133.
[17] Kleinschmit, D.H., and L. Kung. 2006. The effects of Lactobacillus buchneri 40788 and Pediococcus pentocaseous R1094 on the fermentation of corn silage. J. Dairy Sci. 89:3999-4004.
[18] La Ragione, R.M., Narbad, A., Gasson, M.J. and Woodward, M.J. (2004). In vivo characterization of Lactobacillus johnsonii FI9785 for use as a defined competitive exclusion agent against bacterial pathogens in poultry. Letters in Applied Microbiology 38:197 – 205.
[19] Lin, T.Y. (2000). Conjugated linoleic acid concentration as affected by lactic cultures and additives. Food Chemistry 69:27 – 31.
[20] Lowes, K.F., C.,A. Shearman, J. Payne, D. Mackenzie, D.B. Archer, R.J. Merry and M.J. Gasson. 2000. Preventioan of spoilage in feed and food by the yeast mycocin HMK. Appl. Env. Micro 66:1066-1076.
[21] McDonald, P., A.R. Henderson & S.J.E. Heron (1991). The Biochemistry of Silage. 2nd Edition Chalcombe publications, Marlow Bucks, UK, 340.
[22] Matsusaki, H., Endo, N., Sonomoto, K. and Ishizaki, A. (1996). Lantibiotic nisin Z fermentative production by Lactococcus lactis IO-1; relationship between production of the lantibiotic and lactate and cell growth. Journal of Applied Microbiology and Biotechnology 45:36 -40.
[23] Merry, R.J., M.S. Dhanoa & M.K. Theodorou (1995a). Use of freshly cultured lactic acid bacteria as silage inoculants. Grass and Forage Science, 50, 112-123.
[24] Merry, R.J., K.F. Lowes & A.L. Winters (1997). Current and future approaches to biocontrol in silage. In: V. Jambor, L, Klapil, P.Chromec & P. Prochazka (eds.) Proceedings of the 8th International Symposium on Forage Conservation, Brno. Research Institute of Animal Nutrition Ltd., Pohorelice, Czech Republic, 17-27.
[25] Muck (1993). The role of silage additives in making high quality silage. In: NRAES-67 Silage Production - from seed to animal. Proceedings from the national silage production conference. New York, 106-114.
[26] Muck, R.E. & P. O'Kiely (1992). Aerobic deterioration of Lucerne (Medicago sativa) and Maize (Zea mais) silages - Effects of fermentation products. Journal of Science and Food Agriculture, 59, 145-149.
[27] Naylor, S.W., Low, J.C., Besser, T.E., Mahajan, A., Gunn, G.J., Pearce, M.C., McKendrick, I.J., Smith, D.G., Gally, D.L. (2003) Lymphoid follicle-dense mucosa at the terminal rectum is the principal site of colonization of enterohemorrhagic Escherichia coli O157:H7 in the bovine host. Infection and Immunity 71, 1505-12.
[28] Nishino, N., Wang, C., Li, Y., Parvin, S. and Kan, K. (2009). Occurrence and survival in whole crop corn silage of Acetobacter pasteurianus. Pages 165 - 166 in Proc. XV Int. Silage Conf., 27 – 29 July. Madison, USA.eds G.A. Broderick et al.
[29] Nsereko, V.L., Smiley, B.K., Rutherford, W.M., Spielbauer, A., Forrester, K.J., Hettinger, G.H., Harman, E.K. and Harman B.R. (2008). Influence of inoculating forage with lactic acid bacterial strains that produce ferulate esterase on ensilage and ruminal degradation of fiber. Animal Feed Science and Technology 145: 122 – 135.

[30] O'Kiely, P, & R.E. Muck (1992). Aerobic deterioration of Lucerne (Medicago sativa) and Maize (Zea mais) silages - Effects of Yeasts. Journal of Science and Food Agriculture, 59, 139-144.
[31] Oude Elferink, S.J.W.H., F. Driehuis, J. Krooneman, J. C. Gottschal, and S. F. Spoelstra. 1999. Lactobacillus buchneri can improve the aerobic stability of silage via a novel fermentation pathway: the anaerobic degradation of lactic acid to acetic acid and 1,2-propanediol. Pages 266-267 in XII Int. Silage Conf., Uppsala, Sweden. T Pauly, ed.
[32] Owen, T. R. 2002. The effects of a combination of a silage inoculant and a chemical preservative on the fermentation and aerobic stability of whole crop cereal and maize silage. Pages 196-197 in Proc. XIII Int. Silage Conf., 11 – 13 September 2002, Auchincruive, Scotland. L. M. Mechie and C. Thomas, eds.
[33] Rammer, C., P. Lingvall, and I. Thylin. 1999. Combinations of biological and chemical silage additives. Pages 327-328 in XII Int. Silage Conf., Uppsala, Sweden. T Pauly, ed.
[34] Russell, J.B. and Mantovani, H.C. (2002). The bacteriocins of ruminal bacteria and their potential as an alternative to antibiotics. Journal of Molecular Microbial Biotechnology 4:347 – 355.
[35] Spoelstra, S.F., Courtin, M.G. and Van Beers, J.A.C. (1988). Acetic acid bacteria can initiate aerobic deterioration of whole crop maize silage. Journal of Agricultural Science Camb.111:127 – 132.
[36] Spoelstra, S.F. (1991). Chemical and biological additives in forage conservation. Landbauforschung Völkenrode 123:48 – 70.
[37] Stark, B. and Wilkinson, J.M. (1986). Developments in silage. Pp 90. Chalcombe Publications, Marlow Buckinghamshire, UK.
[38] Watson, S.J. and Nash, M.J. (1960). The conservation of grass and forage crops. Pp 758. Publ. Oliver and Boyd, Edinburgh. UK.
[39] Weinberg, Z.G., G. Asbell, Y. Hen & Azriela (1993). The effect of applying lactic acid bacteria at ensiling on the aerobic stability of silage. Journal of Applied Bacteriology, 75, 512-518.
[40] Weinberg, Z.G. & R.E. Muck (1996). New trends and opportunities in the development and use of inoculants for silage. FEMS Microbiology Reviews, 19, 53-68.
[41] Weinberg, Z.G., Chen, Y. And Gamburg, M. (2004). The passage of lactic acid bacteria from silage into rumen fluid, in vitro studies. Journal of Dairy Science 87: 3386 – 3397.
[42] Weinberg, Z.G., Muck, R.E. and Weimer, P.J. (2003). The survival of inoculant bacteria in rumen fluid. Journal of Applied Microbiology 94:1066 – 1071.
[43] White, J. S., C. J. Lin, M. K. Woolford, and K. K. Bolsen. 2002. a new solution to the old problem of cereal and maize silage stability. Pages 78-79 in Proc. XIII Silage Conf., Auchincruive, Scotland.
[44] Winters, A.L., Fychan, R., Davies, D.R., Lloyd, J., Merry, R.J. and Jones, R.(1999). Protein content of a range of ensiled legumes.P 170 – 171. in Proc. XII Silage Conf., Uppsala, Sweden.
[45] Winters, A.L., R. Fychan & R. Jones (2001). Effect of formic acid and a bacterial inoculant on the amino acid composition of grass silage and on animal performance. Grass and Forage Science, 56, 181-192.
[46] Whipp, S.C., Rasmussen, M.A. and Cray, W.C. (1994). Animals as a source of Escherichia coli pathogenic for human beings. Journal of the American Veterinary Medical Association 204, 1168-1175.
[47] Woolford, M.K. (1990). The detrimental effects of air on silage. Journal of Applied Bacteriology, 68,101-116
[48] Zhao, T., Doyle, M.P., Harmon, B.G., Brown, C.A., Mueller, P.O.E., Parks, A.H. (1998) Reduction of carriage of enterohemorrhagic Escherichia coli O157:H7 in cattle by inoculation with probiotic bacteria. Journal of Clinical Microbiology 36, 641-647.

The Efficiency of the Mixture of Sodium Nitrite, Sodium Benzoate and Potassium Benzoate in Aerobically Unstable Silages.

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Abstrakt

Four types of various crops were used in a specific study to examine the effect of silage additive based on sodium nitrite, sodium benzoate and potassium benzoate on silage quality and aerobic stability of silages in particular. Ensiling condition was challenged by weak packing density of forage and by air ingression into silos. Additive treated silages were found to have a lower pH, reduced formation of ammonia-N and 2.3 butanediol and ethanol compared with untreated control silages. Significantly eliminated yeast growth was reflected in considerably increased stability of additive treated silages. The use of the silage additive provides sufficient guaranty of a required ensiling process and prolonged aerobic stability even under difficult ensiling condition.

Introduction

A good fermentation process in a silo creates only one part in successful silage making. During another, feed-out phase, silo is opened and silage previously kept under oxygen-free condition is exposed to oxygen, which promotes the growth of undesirable microflora such as yeast and moulds. Main indications of their activity are the production of heat and carbon dioxide and simultaneously reduction in lactic acid concentration reflected in a pH increase (McDonald et al. 1991). This process degrades the nutritional value and hygienic quality of silages. The use of silage additives is one of ways to eliminate the aerobic degradation of silages. The antimicrobial properties of sodium benzoate, potassium sorbate and sodium nitrite in forage conservation were well characterised by Woolford (1975) and as such, they are widely used as additives in the conservation of a variety of foods. But to reveal a real capability of additive or other factors to stop spoilage of silages, it is recommended to conduct test under unfavorable silage-making conditions (Kwella et al., 1993).

Materials and Methods

Four types of various crops (mixtures of red clover and timothy) were harvested and chopped to approx. 5 cm particle length and divided into 2 fractions; one forage fraction was left untreated and was used as control and another treated with silage additive at the rate of 5 ml per kg FM. Crops were ensiled at densities of approx. 100 kg DM/m^3 in laboratory silos with a fermentation lock on lid for 49 days. Silos and lids were obtained with inlets with the rubber stoppers to allow air ingression into silos. This was performed twice during the storage period, 14 and 7 days before the end of the storage, for eight hours each time. Chemical and microbiological analyses were performed on silages, weight losses were monitored during whole storage period, and aerobic stability was determined by measuring temperature increase for 7 days.

Results and Discussion

Additive treatments in all crops were found to have significantly a lower both pH values, at end of storage and after stability test than in untreated control treatments (Table 1). The formation of ammonia-N and 2.3 butanediol and ethanol was significantly reduced in all additive treatments compared with untreated control treatments. On the other hand, concentrations of lactic acid were increased in all additive treatments in comparison with control treatments. Microbiological analyses revealed remarkably a lower count of lactate assimilation yeasts in all additive treatments than in control treatments. Additive treatments significantly reduced weight losses during whole ensiling period in all crops. Aerobic stability of silages, based on temperature measurements, showed that it took significantly less time for untreated

control silages to increase 3 ˚C than additive treated silages. Results give the presumption that tested silage additive guarantee a required ensiling process and stable silage even when ensiling condition is difficult.

References

[1] Kwella, M., Haaker, K., Reuter, B., 1993. About investigations on the efficiency of silage additives. Proc. X Int. Conference on Silage Research, Dublin City University, Ireland, pp. 99-100.

[2] McDonald, P., Henderson, A. R. & Heron, S. J. E. 1991. The Biochemistry of Silage. Chalcombe Publications, 13 Highwoods Drive, Marlow Bottom, Marlow, Bucks, UK.

[3] Woolford M.K. 1975. Microbiological screening of food preservatives. Cold sterilants and specific antimicrobial agents as potential silage additives. J Sci Food Agric 26: 229-237.

Table 1: Chemical and microbiological compositions of silages from aerobic stability experiments (n=3).

Treatment	DM	pH	pH after stability	NH_3-N*	Lactic acid	Acetic acid	Butyric acid	2.3-butan diol	Ethanol	Weight losses	Days until temp. aerated silages increased by 3˚C (max. temp)	Lactate yeasts
				% of TN	% of DM							lg cfu/g FM
Experiment I.											Ambient temp.: 19.7 ˚C	
Control	33.3	5.2	8.0	13.0	3.0	1.9	<0.05	1.7	0.9	4.5	0.4 (34.2)	7.1
Safesil	34.2	4.7	4.7	6.4	4.4	1.2	<0.05	<0.05	0.5	2.0	6.8 (18.0)	<1.7
$LSD_{0.05}$		**0.02**	**0.29**	**1.48**	**0.37**	**0.12**	**N.S.**	**0.08**	**0.08**	**0.24**	**0.10**	**0.14**
P value		0.001	0.001	0.001	0.001	0.003		0.001	0.01	0.001	0.001	0.001
Experiment II.											Ambient temp.: 19.7 ˚C	
Control	34.8	5.4	8.1	9.1	2.2	1.5	<0.05	2.2	0.7	4.6	0.3 (35.8)	7.0
Safesil	35.6	4.7	4.7	5.5	5.5	1.2	<0.05	<0.05	0.4	2.2	6.8 (19.1)	<1.7
$LSD_{0.05}$		**0.03**	**0.40**	**0.98**	**0.29**	**0.14**	**N.S.**	**0.15**	**0.06**	**0.21**	**0.03**	**0.11**
P value		0.001	0.001	0.001	0.001	0.004		0.001	0.001	0.001	0.001	0.001
Experiment III.											Ambient temp.: 19.7 ˚C	
Control	33.6	5.5	8.2	5.8	1.3	0.4	<0.05	0.8	1.8	4.0	0.4 (40.5)	7.0
Safesil	34.3	4.7	4.7	3.6	2.6	0.8	<0.05	0.1	0.4	1.5	6.8 (18.6)	<1.7
$LSD_{0.05}$		**0.03**	**0.16**	**1.43**	**0.20**	**0.06**	**N.S.**	**0.11**	**0.21**	**0.30**	**0.08**	**0.20**
P value		0.001	0.001	0.01	0.001	0.001		0.001	0.001	0.001	0.001	0.001
Experiment IV.											Ambient temp.: 19.7 ˚C	
Control	34.9	5.2	7.8	8.4	1.5	1.3	<0.05	1.2	1.1	4.1	0.4 (35.5)	6.9
Safesil	35.3	4.6	4.6	4.9	3.0	0.7	<0.05	0.1	0.4	1.7	6.8 (18.2)	<1.7
$LSD_{0.05}$		**0.04**	**0.08**	**1.29**	**0.27**	**0.11**	**N.S.**	**0.12**	**0.14**	**0.51**	**0.04**	**0.41**
P value		0.001	0.001	0.002	0.001	0.001		0.001	0.001	0.001	0.001	0.001

* The value is corrected for N added with the additive in form of NaNO2.
N.S. – Not significant

Storage of Wheat in Large Plastic Bags

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Introduction

Storing the grain in large plastic bags on the fields reduces labor time requirement in the harvest period. Additionally short-time storage of grain provides the chance to get along fluctuations in prices without investment in building operations. Experiences with high moisture grains in large plastic bags already showed, that there is a slight fermentation starting at 25% moisture content with losses of only 1% (Matthiesen 2006). The objective of this study was to compare the storage of grain with low moisture content in plastic bags with the conventional bulk storage of grain regarding quality parameters.

Materials and Methods

The experiments were undertaken in 2008/09. Newly harvested wheat (moisture content 12%) was stored during a period of six months in two plastic bags (9' diameter, 10 m length, AG BAG Profi Farmbagger, performance >300 t/h) and parallel in a granary on the same farm. After the plastic bags had been filled, temperature loggers were inserted into the centre of the silo at eight measuring points lengthwise on the right and left side of the bag (distance of 2 m each). After two weeks, one month, three and six months samples were collected at the same measuring points below the plastic film and in 0.80 cm depth (n=4). Parallel samples were taken in the granary in the same intervals and at the same measuring depths. The second bag was kept closed for the whole period to analyze the influence of the sampling in the first bag. The samples were analyzed for dry matter, starch, crude protein, pH-value according the German standard methods (VDLUFA, 2007) and the microbiological groups bacteria, yeast and mould according the German guideline (DGHM, 2007).

Results and Discussion

Only minor differences were revealed by investigations concerning the temperature development in the silos. There was a gradually decrease in temperature over the 6 months; it converges to the ambient temperature (Figure 1).Similarly, results demonstrated that there are no differences between the positions 'upper part' and 'centre' of the bag in parameters as ph-value, starch, crude protein, content of bacteria, yeast, mould and germination: no differences between the positions and no differences between the storage systems (Figure 2). It can be concluded from the very low differences, that the storage in a plastic bag has no influence on the baking characteristics of bread. Further investigations are concentrating on this parameter.

Conclusions

The results demonstrate that the temporary grain storage in plastic bags does not lead to any grain quality loss compared to conventional storage. Because of the very low costs of the flexible bagging system it represents an alternative to high investment in permanent storage structures for grain.

References

[1] Matthiesen, M., A. Wagner, W. Büscher (2006): Preservation of Crimped High Moisture Grain in a Plastic Tube Silo – Influence of the Moisture Content. XVI. CIGR World Congress „Agricultural Engineering for a Better World", 3.-7.09.2006 in Bonn. Book of Abstracts, VDI-Berichte 1958, S. 599-600.

Figure 1: Temperature data in the plastic bags during the storage in comparison with the ambient temperature

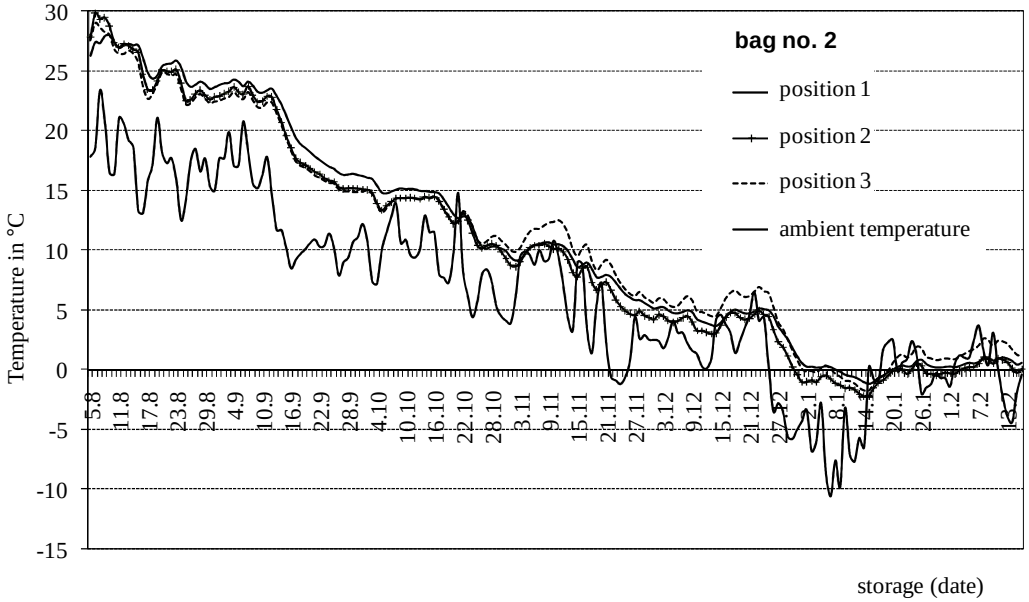
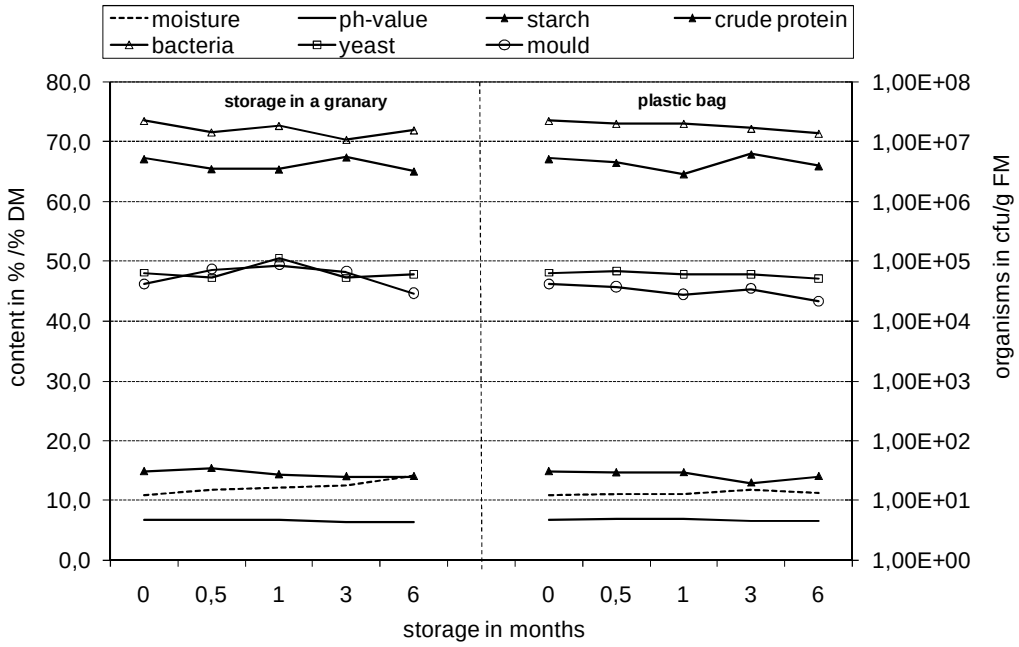


Figure 2: Effect of different storage systems of wheat on chemical and microbiological parameters.



Fermentation Quality and Nutritive Value of Grass-legume Silage Treated with Inoculant BioStabil Plus

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Abstract

The effect of adding the inoculant Biomin® BioStabil Plus (BIOMIN GmbH, Austria), a blend of Enterococcus faecium (BIO 34, DSM 3530); Lactobacillus brevis (IFA 92, DSM 19456) and Lactobacillus plantarum (IFA 96, DSM 19457), to medium wilted legume-grass silage was evaluated. Herbage was wilted to 320 g/kg/DM and had mean crude protein and water soluble carbohydrate concentrations at ensiling of 174 and 88 g/kg respectively.

Treatment with BioStabil Plus resulted in significantly higher (149.4 vs. 159 g/kg DM; ($P<0.05$) crude protein and (108.9 vs. 117.8 g/ kg DM; $P<0.01$) digestible protein concentrations. Inoculant treatment increased fermentation rate, resulting in a significant ($P<0.05$) pH drop and in a significant ($P<0.05$) increase of total fermentation acids concentration compared with control. The inoculant produce higher ($P<0.01$) lactic acid content and numerically higher acetic acid content compared with that of the control. Butyric acid and Ammonia N concentrations were significantly ($P<0.01$) decreased by application of BioStabil Plus. Dry matter loss values were significantly ($P<0.01$) lower for BioStabil Plus treated grass-legume silages. Inoculated silage had a higher by 2.1 % ($P<0.01$) digestible energy (DE) and a higher by 1.25 % ($P<0.05$) net energy lactation (NEL) concentration, when compared to untreated silage. The inoculation of silage with BioStabil Plus has shown to improve aerobic stability.

Introduction

Opportunities for promoting grassland utilisation are related to the positive health characteristics it gives to animal products. Obtaining good fermentation quality, digestibility of nutrients and high energy and protein value in silages, requires the regulation of the ensilage process, particularly for herbages with the higher values of buffering capacity (McDonald et al., 1991). The advantages of the use of biological inoculants, recently obtained bacterial additives, thanks to the suitable selection of lactic acid bacteria, have been stressed by many workers, and it is clear from the results that inoculants have a beneficial effect on the improvement of the fermentation quality of silages (Muck and Kung, 1997; Wrobel *et al*., 2004). The current study was designed to examine the effect of silage additive BioStabil Plus based on a bacteria strain mix (*Enterococcus faecium* BIO 34 (DSM 3530), *Lactobacillus brevis* IFA 92 (DSM 19456) and *Lactobacillus plantarum* IFA 96 (DSM 19457), BIOMIN GmbH, Austria on the fermentation parameters and aerobic stability of grass-legume silage.

Materials and Methods

In experiment mixed grass-legume sward (35% *Lolium perenne,* 15% *Phleum pretense*, 45% *Trifolium pretense* and 5% others) wilted to 320 g/kg DM was ensiled. The sward was cut with a mower conditioner *Kverneland 347* and was picked up with a precision chop forage harvester Massey Ferguson 5130 (chop length ≈ 30 mm) after a 6-8 – hour wilt. Herbage was either untreated (C-control) or treated (I) with inoculant (*Enterococcus faecium* BIO 34 (DSM 3530), *Lactobacillus brevis* IFA 92 (DSM 19456) and *Lactobacillus plantarum* IFA 96 (DSM 19457), BIOMIN GmbH, Austria). The inoculant was dissolved in water according inoculant usage recommendation (4 g /tonne of green forage) and was applied at rate of 4 liter solution per tone grass to give 2×10^5 colony forming units per gram of forage. Treatments were applied in

order of control and inoculant. After weighing, grass was transferred to one of two ferro-concrete trench (100-t capacity each). Five control bags (made from four layers cheesecloth) filled with 1 kg ensiling mass were putted in each silo to determine DM loss.

Results and Discussion

On average the herbage before ensiling had 320 g DM, 174. g/kg DM crude protein, 88.34 g/kg DM WSC and 0.4 g/kg DM nitrate. Buffering capacity of the grass- legume sward was 40 mequiv100/g DM. Therefore, the grass-legume sward characterized as moderate to ensile, because WSC/BC (water- soluble carbohydrates to buffering capacity) ratio was 1:2.22. Fermentation coefficient (FC) was 49.

There were no significant differences between untreated and treated silages in dry matter, crude fibre, NFE, ADF and NDF content. However, treatment with inoculant resulted in significantly higher (149.4 *vs.* 159 g/kg DM; P<0.05) crude protein and (108.9 *vs.* 117.8 g/kg DM; P<0.01) digestible protein concentrations. The results are shown in Table 1.

Bacteria strains *Enterococcus faecium* BIO 34 (DSM 3530), *Lactobacillus brevis* IFA 92 (DSM 19456) and *Lactobacillus plantarum* IFA 96 (DSM 19457 treatment increased fermentation rate, resulting in a significant (P<0.05) pH drop and in a significant (P<0.05) increase of total fermentation acids concentration compared with control.

Table 1: Chemical composition and fermentation parameters of ensiled grass- legume silage

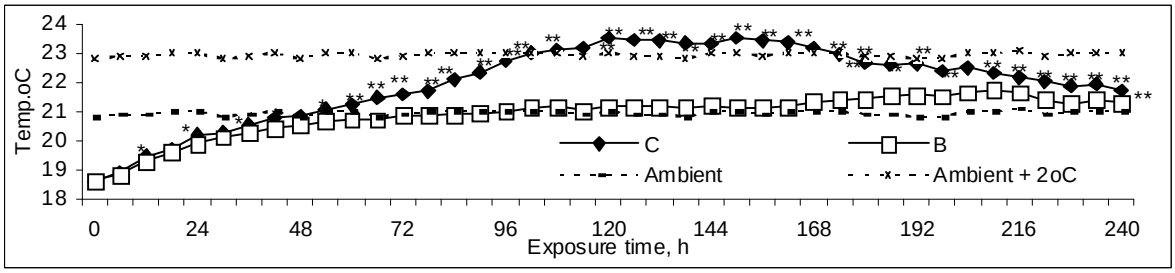
Measured parameters	Untreated control (C)	Treatment I	SE[1]	Sign[2].
Dry matter, DM, g/kg	315.4	319.2	1.072	0.079
Crude protein, g/kg DM	149.4	159.0	1.732	*
WSC, g/kg DM	9.4	10.7	0.433	0.139
Total organic acids, g/kg DM	67.16	76.62	1.970	*
Lactic acid, g/kg DM	36.74	44.15	1.419	**
Acetic acid, g/kg DM	28.23	32.17	1.021	0.051
Butyric acid, g/kg DM	2.15	0.23	0.362	**
Ethanol, g/kg DM	7.87	7.06	0.217	0.059
Ammonia N, g/kg total N	57.5	46.0	1.746	**
pH	4.38	4.25	0.023	*
DE, MJ/kg DM	13.05	13.32	0.035	**
NEL, MJ/kg DM	6.42	6.50	0.019	*
DM losses, g/kg DM	106.2	88.3	3.565	**

*[1]Standard error of a treatment; [2]Statistical significance where NS=Not significant, *=P<0.05and ** =P<0.01 respectively.*

The inoculant produce higher (P<0.01) lactic acid content and numerically higher acetic acid content compared with that of the control. The inoculated silage had higher lactate : acetate ratios (1.4) compared with that of the controls (1.3). Butyric acid and Ammonia N concentrations were significantly (P<0.01) decreased by application of inoculant. Dry matter loss values were significantly (P<0.01) lower for I treated grass-legume silages, as a consequence of better fermentation.

Inoculated silage had a higher by 2.1 % (P<0.01) digestible energy (DE) and a higher by 1.25 % (P<0.05) net energy lactation (NEL) concentration, when compare to untreated silage.

*Figure 1: Aerobic stability of BioStabil Plus (B) treated or untreated (C) grass-legume silages. (superscripts * and ** denote statistical differences of means at 0.05 and 0.01 levels, respectively)*



The control silage, which was not inoculated, started heating after 54 h (2.25 days) and reached temperature differences higher than 2^0C above the ambient temperature after 108 h (4.5 days). The maximum temperature (23.5^0C) in the control silage was reached within 120 h (5 days) from start of exposure to air. Increased concentration of acetic acid in BioStabil Plus treated silage had a positive effect on aerobic stability of the silage. The temperature rise of inoculated silage was slight. Inoculated silage started heating after 102 h (4.25 days) but no had a temperature rise of more than 2^0C above the ambient temperature during 10 days exposure to air (Figure 1).

Conclusions

Microbial inoculant based on a bacteria strain mix (*Enterococcus faecium* BIO 34 (DSM 3530), *Lactobacillus brevis* IFA 92 (DSM 19456) and *Lactobacillus plantarum* IFA 96 (DSM 19457 had a significant effect on legume-grass silage quality characteristics in terms of lower pH and shifting fermentation toward lactic acid with homofermentative LAB. The heterofermentative LAB *Lactobacillus brevis* added in microbial mix had a tendency to shift fermentation towards acetic acid. Inoculant treatment significantly decreased butyric acid content, $N\text{-}NH_3$ fraction and dry matter loss. As a consequence of better fermentation, inoculated silage had a higher by 2.1 % ($P<0.01$) digestible energy (DE) and a higher by 1.25 % ($P<0.05$) net energy lactation (NEL) concentration, when compare to untreated silage.

References

[1] McDonald, P., N. Henderson, and S. Heron. (1991). The Biochemistry of Silage. 2nd ed. Chalcombe Publ., Marlow, UK

[2] Muck R. E., and L. Kung, Jr. (1997). Effects of silage additives on ensiling. Pages 187–199 in Proc. Silage: Field to Feedbunk. NRAES-99. Ithaca, NY.

[3] Wrobel B. and Zastawny J. (2004). The nutritive value and aerobic stability of big bale silage treated with bacterial inoculants. In: Luscher A. et al. (eds.) Proceedings of the 20thGeneral Meeting of the European Grassland Federation, June 21-24, Luzern, Switzerland, pp. 978-980.

Description of a New Bale Forming Technology: Fermentation of Alfalfa and Grass Silage Bales

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Summary

The extent of wilting, harvester type and the effectiveness of excluding oxygen combine to create conditions within bales that are less effective to inhibiting the activity of undesirable microorganisms than occurs in precision-chop silage (*O'Kiely et al, 2007*). Technologies are needed to improve the fermentation in baled silage. Objective of the study was to evaluate a new baling system. Authors investigated the fermentation profile and bale parameters of the new-type alfalfa and sweet grass (*Lolium multiflorum)* silage bales. Baling was carried out by a Göweil LT Master baler-wrapper machine from chopped alfalfa (theoretical chop length: 20-30 mm, NDF: 445 g/kg) and grass (theoretical chop length: 20-30 mm; NDF: 451.5 g/kg). High density was carried out with the new technology: 213-278 DM kg/m^3 in fermented alfalfa and 246-261 DM kg/m^3 in grass silage compared to conventional bales (90-200 DM kg/m^3). It was confirmed that the new baling system is able to form bales in a wide range of dry matter content (290-520 g/kg).

Introduction

Conventional round baling systems provide a rather low density range (without and with cutting system in variable chamber round balers: 178.6-180.3 kg DM/m^3, without and with cutting system in fixed chamber round balers: 146.9 and 161.0 kg DM/m^3, respectively) stimulating undesirable fermentation process inside the bales. Aim of the study was to evaluate an ensiling method (a new bale-forming technology combined with wrapping system) and dry matter limitation of the new type round baler. Precision-chop wilted forage (alfalfa and grass) harvested by conventional self-propelled chopper-harvester machine was baled by the new technology. Authors investigated (i) the effect of short (290 g/kg DM - 4 hours) and long term wilting (520 g/kg DM - 12 hours) on bale parameters and fermentation profile in the new-type alfalfa wrapped bales and (ii) the effect of the new bale-forming system on bale parameters and fermentation profile of wilted and chopped 'sweet' grass (Italian ryegrass*)* baled silage.

Materials and Methods

Alfalfa (*Medicago sativa*) derived from the 2nd cut (July 2007) in a 2 years old alfalfa plantation (crude protein: 196 g/kgDM; NDF: 445 g/kgDM). It was mowed with rotary disk mower equipped with conditioner, spread and wilted on windrows for 4 hours (290 g/kg DM) and 12 hours (520 g/kgDM), respectively. Italian ryegrass (*Lolium multiflorum*) derived from the 1st cut (May 2008), tedded and wilted for 24 hours (crude protein: 146 g/kgDM; NDF: 474 g/kgDM). Pick-up was carried out by a self-propelled precision chopper-harvester (Claas Jaguar 840). The theoretical chop size range was: 20-30 mm. Baling was carried out by a Göweil LT Master baler-wrapper machine. Nominal size of bales was: 1.13 x 1,22 m. Pressurization: 150 bar. Film for wrapping was applied with thickness of 25 µm in 6.5 layers carried out by 26 turn and 60% pre-stretch. Output and efficiency were the following: 18-20 bales/hour. Fermentation profile was determined in three different stages (alfalfa:13th, 30th and 70th days, grass: 8th, 15th and 90^{th,} respectively). Crude nutrient-, carotene-, NDF- and fermentable carbohydrate content were analysed in the plant-, wilted forage and silages.

Results and Discussion

It was confirmed that the new baling system is able to form bales in a wide range of dry matter content (alfalfa silage: 290-520 g/kg, grass: 318 g/kg DM-content) in theoretical chop size of 20-30 mm with extremely high density (alfalfa silage: 213-278 DM kg/m^3; grass silage: 246-261 DM kg/m^3) due to high pressurization (150 bar) and small particle size (20-30 mm) compared to conventional bales (90-200 DM kg/m^3) in both treatments (Table 1). Recommended wet bale weight range of alfalfa (characterised by 350-400 g/kg DM, 20-30 mm chop size and 400-450 g/kg DM NDF content) is 750-800 (nominal bale size: 1.1 x 1.2m). Higher than 900 kg of bale weight due to low dry matter content (lower than 300 g/kg) can cause high challenge of effluent and bale deterioration. High density (effective and quick air exclusion) had beneficial effect on fermentation intensity and quality (Table 2). It was proven by fast pH-drop (pH in alfalfa silage (on the 13th day: 4.84a *vs* 4,87a, respectively), in grass silage (on the 15th day: 4.55) and early lactic acid dominated fermentation (LA:AA ratio in alfalfa silage on the 13th day: 2.83a and 4.46b, in 'sweet' grass silage on the 15th day: 6.52, respectively). Long term wilting of alfalfa (520 g/kg DM: 12 hours) reduced the acetic acid concentration ($p \leq 0.05$) and LA:AA ratio (LA:AA ratio on the 70th day: 2.83a *vs* 4.46b, respectively), but significantly reduced the carotene content of wilted alfalfa compared to un-wilted and short term wilted alfalfa (un-wilted alfalfa: 126.7a±6.7 g/kg DM, long term wilted alfalfa: 24.3b±2.0 g/kg DM, short term wilted alfalfa: 65.7c±8.5 g/kg DM).

Conclusions

It was confirmed that the new baling system is able to form bales in a wide range of dry matter content (290-520 g/kg). There is better homogeneity of the new bales compared to conventional bales, owing to the forage being chopped and mixed. a high density (alfalfa silage: 213-278 DM kg/m^3; grass silage: 255 DM kg/m^3) can be achieved due to high pressurization (150 bar), as well as a short particle size. High density results in good anaerobic conditions for fermentation.

Table 1: Comparison of new type of bales (n=15) and conventional bales (Forristal and O'Kiely, 2005)

	High density bales made from precision-chop forage			Conventional baled silage
	Baled alfalfa silage		Baled 'sweet' grass silage (*Lolium multiflorum*)	Typical grass bales in Ireland
	'Wet'	'Dry'		
Dry matter content (g/kg)	290.1±8.5	520.4±16.3	317,5±11.1	300
Nominal bale size (m)	1.13 x 1.22	1.13 x 1.22	1.13 x 1.22	1.25 x 1.25
Bale weight (kg)	904±25.1	657±13	987±13.4	650
Bale weight (kg DM)	262±7.3	342±6.6	310±4.1	195
Coefficient of variation of BW	1.4%	1.4%	1.3%	-
Wet density (kg/m^3)	734±10.3	534±7.9	813±12.1	425
Dry density (kg DM/m^3)	213±3.0	278±4.1	255±3.9	130

Table 2: Nutrient content and fermentation characteristics of the new type alfalfa- and 'sweet' grass baled silages (n=5).

Forage character		Alfalfa	Wilted alfalfa 'wet'	Wilted alfalfa 'dry'	Alfalfa silage 'wet'	Alfalfa silage 'dry'	Grass	Wilted grass	Grass silage
Dry matter, g/kg	mean	240.5a	300.6b	524.8c	290.1b	520.4c	188.8a	264.5b	317.5c
	ST	14.4	12.7	26.5	8.5	16.3	34.6	11.2	11.1
Crude protein, g/kg DM	mean	196.0a	192.7a	197.6a	190.6a	199.8b	146.0a	145.8a	146.9a
	ST	10.4	7.4	5.9	3.3	4.2	12.7	9.8	5.0
Total carotene, mg/kg DM	mean	126.7a	65.7b	24.3c	27.6c	10.9d	205.6a	126.2b	139.6b
	ST	6.7	8.5	2.0	11.9	0.7	25.8	15.1	19.1
NDF, g/kg DM	mean	444.7a	450.9a	450.5a	428.1b	441.0	474.4a	497.3a	451.5b
	ST	22.2	13.8	9.3	10.0	10.4	6.2	14.0	7.7
Total sugar, g/kg DM	mean	nd	nd	nd	nd	nd	169.6a	86.5b	25.1c
	ST						12.3	5.2	8.1

Fermentation stage		Baled alfalfa silage 13th day 'wet'	13th day 'dry'	30th day 'wet'	30th day 'dry'	70th day 'wet'	70th day 'dry'	Baled grass silage 8th day	15th day	90th day
pH	mean	4.84a	4.87a	4.60b	4.41b	4.49b	4.74c	5.08a	4.55b	4.34c
	ST	0.13	0.05	0.22	0.15	0.08	0.14	0.33	0.15	0.08
Lactic acid, g/kg DM	mean	71.26a	45.60b	89.77c	61.87d	84.70c	54.92b	68.01a	83.24b	99.77b
	ST	7.06	5.08	2.55	3.45	6.50	5.76	6.54	11.93	11.72
Acetic acid, g/kg DM	mean	25.29a	10.25b	26.13a	13.46b	25.59a	13.28b	9.56a	13.04b	16.27b
	ST	1.89	0.85	4.98	1.73	5.08	1.70	1.06	3.17	1.98
Propionic acid, g/kg DM	mean	0.24a	0.19a	0.40c	0.20a	0.52c	0.26a	0.22a	0.37b	0.12c
	ST	0.03	0.05	0.13	0.04	0.19	0.13	0.00	0.06	0.05
Butyric acid, g/kg DM	mean	0.000a	0.000a	0.00a	0.00a	0.09b	0.00a	0.000a	0.00a	0.54b
	ST	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Volatile acids, g/kg DM	mean	25.53a	10.44b	26.53a	13.66b	26.20a	13.54b	9.78a	13.41b	16.92b
	ST	1.90	0.86	5.09	1.72	5.14	1.67	1.06	3.22	3.02
Organic acids, g/kg DM	mean	96.79a	56.04b	116.29c	75.53d	110.89c	68.47d	77.79a	96.65b	116.70c
	ST	6.85	5.62	5.31	3.82	10.44	6.61	7.14	14.97	10.06
LA/AA	mean	2.83a	4.46b	3.54a	4.65b	2.83a	4.46b	7.16a	6.52a	6.24a
	ST	0.40	0.42	0.73	0.61	0.50	0.49	0.71	0.76	1.22
Total sugar, g/kg DM	mean	nd	nd	nd	nd	nd	nd	71.3a	51.5a	25.1b
	ST							17.75	14.27	8.05

abcd Means in the same row with different letters differ ($p \leq 0.05$)

References

[1] O'Kiely, P., D. P. Forristal, M. O'Brien, J. McEniry, C. Laffin, H. T. Fuller (2007): Technologies for restricting mould growth on baled silage. Report. Beef Production Series No. 81 Teagasc, Grange Beef Research Centre, Dunsany, ISBN 1 84170 491 X

[2] Forristal P. D. and O'Kiely P. (2005): Update on technologies for producing and feeding silage. In: Park R.S. and Stronge M. D. (eds) Silage production and Utilisation. The XIVth International Silage Conference, Belfast, Northern Ireland, UK. Wageningen Academic Publishers, pp. 83-96.

Economics of Sealing Maize and Alfalfa Silages in Bunker Silos and Drive-over Piles: An Excel Spreadsheet

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Introduction

In 2008, the USA produced 101.2 million tonnes of whole-crop maize silage (United States Department of Agriculture, 2008) and about 20 to 22 million tonnes of alfalfa haylage. Approximately 82 to 84% of this silage was made in bunker silos and drive-over piles. However, the failure to implement proper silage management practices, especially proper sealing technique, resulted in the unnecessary loss of approximately 16 to 20 million tonnes. Standard (std.) polyethylene, weighted with discarded full-casing tires or tire sidewalls, has been the most common method used to seal bunkers and piles, but organic matter (OM) losses in the original 0.91 metres can exceed 30.0 % (Berger and Bolsen, 2006).

The use of an oxygen barrier (OB) film (www.silostop.com) as an alternative to standard polyethylene for sealing bunker silos and piles was reported at the XII International Silage Conference in 1999 (Degano, 1999). Degano (1999) stated that the permeability of Silostop film was 0.025 that of standard polyethylene film of the same thickness. Oxygen transmission rate (OTR) through standard polyethylene film using 100% oxygen is 1812 $cm^3/m^2/24$ h (American Society for Testing Materials, ASTM D3985), while OTR through Silostop film using 100% oxygen is 65.5 $cm^3/m^2/24$ h (ASTM D3985). Thus, the permeability of OB film was 0.036 that of the std. polyethylene.

This paper presents an Excel spreadsheet, which estimates the economic benefit of sealing ensiled forage or high moisture grain in bunker silos and drive-over piles, and compares two sealing methods, std. polyethlene and OB film.

Materials and methods

The spreadsheet was developed from research conducted at Kansas State University from 1989 to 1995, and equations published by Huck et al. (1997). In the first section of the spreadsheet, the user enters values for the following: depth from the original surface to be evaluated; silage price; as-fed silage densities; bunker or pile deminsions; percent of the silage in the original depth lost during the storage and feedout phases; and cost of the sealing materials. The results are calculated and reported in the second section.

Results and discussion

Two examples from the spreadsheet, which compare bunker silos and drive-over piles sealed with either std. plastic or OB film, are presented in Table 1.

In a large, 18.3 m wide x 76.2 m long, bunker silo of maize silage, which has an average depth of 3.66 m, sealing would prevent the loss of 5.9 and 7.5 % of the original 3,637 tonnes of crop ensiled for the std. plastic-sealed and OB film-sealed bunkers, respectively. The OB film (bunker silo 2) would save an additional $2,714 of maize silage in the original top 0.91 m compared to std. plastic (bunker silo 1).

In a 27.7 m wide x 62.0 m long drive-over pile of alfalfa silage, which has an average depth of 1.98 m, sealing would prevent the loss of 9.4 and 12.2 % of the original 2,054 tonnes of crop ensiled for the std. plastic-sealed and OB film-sealed piles, respectively. The OB film (pile 2) would save an additional $3,870 of alfalfa silage in the original top 0.91 m compared to std. plastic (pile 1).

Conclusions

The economics of properly sealing bunker silos and drive-over piles makes it clear that farmers should pay close attention to the details of this troublesome task. Sealing with OB film has a greater economic benefit than sealing with std. polyethylene.

References

[1] Berger, L.L. and K.K. Bolsen. 2006. Sealing strategies for bunker silos and drive-over piles. In: Proceedings Silage for Dairy Farms: Growing, Harvesting, Storing, and Feeding. NRAES Publ.181. Ithaca. NY. Pg. 266-283

[2] Bolsen, K.K., J.T. Dickerson, B.E. Brent, R.N. Sonon, Jr., B. Dalke, C.J. Lin, and J.E. Boyer, Jr. 1993. Rate and extent of top spoilage in horizontal silos. J. Dairy Sci. 76:2940-2962.

[3] Degano L. 1999. Improvement of silage quality by innovative covering system. In: Proceedings XII International Silage Conference, Uppsala, Sweden (Ed. T. Pauly). Pg. 296-297.

[4] HUCK, G.L., J.E. TURNER, M.K. SIEFERS, M.A. YOUNG, R.V. POPE, B. E. BRENT, K.K. BOLSEN. 1997. Economics of sealing horizontal silos. Kansas Agric. Exp. Sta. Rpt. of Prog. 783:84.

[5] KUBER, R., K. K. BOLSEN, S. WIGLEY, M. WILKINSON, and R. E. BOLSEN. 2008. Preservation efficiency and nutritional quality of maize sealed in large pile silos with an oxygen barrier film or standard polyethylene film. In: Proceeding 13th Int. Conf. on Forage Cons. Nitra, Slovak Republic. Pg. 87-88 United States Department of Agriculture. 2008. National Agricultural Statistics Service. Corn for silage: 2001-2007. http://www.nass.usda.gov/ Accessed 17 April 2008.

Table 1: Profitability of sealing maize silage in bunker silos and alfalfa haylage in drive-over piles with either std. plastic or OB film.1

Inputs and calculations	Bunker 1 maize std. plastic	Bunker 2 maize OB film	Pile 1 alfalfa std plastic	Pile 2 alfalfa OB film
Silage value, $ per ton	**45.00**	**45.00**	**67.50**	**67.50**
Silage density in top 0.91 m, kg per m^3	**600**	**600**	**550**	**550**
Density below top 0.91 m, kg per m^3	**750**	**750**	**650**	**650**
Silo depth, m	**3.66**	**3.66**	**1.98**	**1.98**
Silo width, m	**12.2**	**12.2**	**27.7**	**27.7**
Silo length, m	**60**	**60**	**62.0**	**62.0**
Silage lost in the original top 0.91 m:[2]				
unsealed, % of the crop ensiled	**60**	**60**	**50**	**50**
sealed, % of the crop ensiled	**25**	**12.5**	**22.5**	**12.5**
Cost of covering sheet, ¢ per sq m	**50**	**140**	**50**	**140**
Total silage in the silo, tons	3,637	3,637	2,054	2,054
Total value of silage in the silo, $	163,685	163,685	138,646	138,646
Silage in the orig. top 0.91 m, tons	761	761	860	860
Value of silage in orig. top 0.91 m, $	34,262	34,262	58,020	58,020
Silage below the orig. top 0.91 m, tons	2,876	2,876	1,194	1,194
Value of silage below orig. top 0.91 m, $	129,423	129,423	80,625	80,625
Silage lost if unsealed, $	20,557	20,557	29,010	29,010
Silage lost if sealed, $	8,565	4,283	13,055	7,253
Silage saved by sealing, $	11,992	16,274	15,956	21,758
Sealing cost, $	2,371	3,940	2,920	4,852
Net value of silage saved by sealing, $	9,621	2,876	13,036	16,905
Net benefit from OB film, $	---	2,714	---	3,870

[1] Numbers in **bold** are user inputs.

[2] Values are from the data by Bolsen et al. (1993), Berger and Bolsen (2006), and Kuber et al (2008).

New Procedures for the Conservation of Nearly Un-fermentable Feedstuffs and High Moisture Grains

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Introduction

Successful silage fermentation depends on both anaerobic conditions and a low pH. The low pH is achieved through the conversion of sugars to lactic acid by epiphytic and/or added lactic acid bacteria (LAB). On the other hand, formic acid has been frequently used for effective feedstuff preservation, especially under conditions where ensiled crops had low dry matter content and low contents of water-soluble carbohydrates. Formic acid has been shown to reduce pH, and concentrations of lactic acid, acetic acid and butyric acid in different kinds of silages compared to untreated silages (Baytok and Muruz, 2003, McDonald et al.1991, Nadeau et al. 2000). The current study was conducted to ensile un-fermentable feedstuffs using a combination of homolactic acid bacteria (*Lactobacillus plantarum* DSM 8862 and 8866) and a partly neutralized FA with alkaline (FA NA).

Crop with dry matter content (DM) lower 85 % is conditionally storable and causes high costs with increasing DM. Crimped wet grain (DM < 75 %) can be inoculated with LAB and successfully ensiled (Fig. 1).

Materials and Methods

Nearly un-fermentable feedstuffs were successfully ensiled with a combination of homofermentative acid bacteria (LAB, *L. plantarum* DSM 8862 and 8866) and a partly neutralized formic acid (AmasilNA®) in lab-scale and in industrial-scale. The compounds were added separately to the harvested material. Using laboratory silos, alfalfa, *Lolium*, *Poa, Holcus* and red clover of different dry matter content (18-25%) and maturity were ensiled (Tab. 1) Plant materials were mixed with fresh soil to increase clostridial counts in the fresh material and increase the potential of butyric acid production during ensiling. Treatments were control, LAB, AmasilNA®, LAB+AmasilNA®, and LAB+molasses and the chemical preservative Kofasil liquid, respectively (Tab. 2).

Wet grain (DM < 75 %) was crimped immediately after harvest, inoculated with LAB and ensiled. At DM > 75% water was added before crimping. The trials were done with remoistened grain (model experiments) and harvest fresh grain (field experiments) with barley, triticale, wheat and corn at DM content of 75% and 65% with and without addition of LAB.

Results

The combination LAB+AmasilNA® led to lower concentrations of acetic and butyric acid, reduced dry matter losses and NH_3-N/N_{total} after a 90-day storage period as compared to the other treatments (Tab. 3). The additive effect of LAB+AmasilNA® was the result of rapid acidification to pH 4.4-5.0 caused by the formic acid with later lactic acid fermentation of fermentable carbohydrates by the LAB inoculum until a low and stable pH was achieved. Fermentation characteristics indicate less spontaneous but rather specific lactic acid fermentation of carbohydrates, which is mainly due to the sensitivity of detrimental bacteria to formic acid which improves the hygienic properties of the silage. Based on these results, the combined but separately added use of LAB+AmasilNA® and LAB+molasses can be recommended as reliable procedures. LAB+molasses is already a commonly used technology in Germany. Using LAB+AmasilNA®, has been shown to produce more than 30,000 t of silage of highly un-fermentable feedstuffs.

After 3 days the pH decreases faster and deeper caused by inoculation with LAB depending on the water content (triticale: DM 75%: control pH 6.5 – LAB pH 6.3; DM 65%: control pH 4.9 – LAB pH 3.9). In the fresh harvested triticale the numbers of aerobic spore forming bacteria and yeasts were drastically reduced by the LAB-inoculation after 50 days. Also dry matter losses caused by fermentation are very low (nearly 1%).

Summarizing the above ensiling of crimped remoistened or harvest fresh grain is safe and low-loss possible using homofermentative lactic acid bacteria. As shown in subsequent feeding experiments with pigs the digestibility of nutrients is not negatively influenced. The digestibility of organic substance tends to accompany with an increase. In consequence of the reduced phytate-phosphate as part of the whole phosphate content by ensiling the digestibility of phosphate in the moist silages was clearly increased (Pieper et al, 2007).

Grain is cost-efficient to ensile using water and homofermentative LAB. The low process-costs (Tab. 4) enable fully new possibilities for the production of hygienic cereals from bringing-forward the harvest or in rain periods, enhance the operating grade of harvest technique, for the cultivation of intertillages or undersown crops.

References

[1] Baytok, E., and H. Muruz. 2003. The effects of formic acid or formic acid plus molasses additives on the fermentation quality and DM and ADF degradabilities of grass silage. Turk. J. Vet. Anim. Sci. 27:425-431.

[2] McDonald, P., N. Henderson and S. Heron. 1991. The Biochemistry of Silage. 2nd ed. Chalcombe Publ., Marlow, Bucks, England

[3] Nadeau, E. M. G., D. R. Ruxton, J. R. Russell, M. J. Allison and J. W. Young. 2000. Enzyme, bacterial inoculant, and formic acid effects on silage composition of orchardgrass and alfalfa. J. Dairy Sci. 83:1487-1502

[4] Pieper, B., Korn, U., Pieper, R., Hackl, W. 2005. Eine neue Epoche der Getreidelagerung: „BIO-SIL® plus Wasser als Konservierungsmittel“, Tagungsbericht 9. Symposium „Fütterung und Management von Kühen mit hohen Leistungen“, Neuruppin, (B. PIEPER/S. POPPE/A. SCHRÖDER/U. KORN Hrsg.), 159-188, ISBN 978-3-00-019811-3

[5] Pieper, B., Pieper, R., Hackl, W., Korn, U. 2007. Ensiling high moisture triticale grain with lactic acid bacteria (Lactobacillus plantarum) - effect on fermentation characteristics and nutritive value in pigs. Proc. Soc. Nutr. Physiol. 16:91

Table 1: Characteristics of feedstuffs and factors affecting ensiling process.

Feedstuff / Parameter	Al-falfa 1	Al-falfa 2	Rough blue-grass	Red clover	Cocks foot	Creeping soft grass	Peren. rye grass 1	Peren. rye grass 2
DM (%)	25.5	18.8	20.5	18.6	19.1	20.8	20.1	18.1
Crude protein[1]	16.0	23.5	18.4	18.5	19.1	16.8	15.8	16.9
Sugar[1]	3.4	4.3	4.3	12.6	0.5	5.4	11.8	10.7
Buffering capacity[3]	5.5	7.9	5.3	4.4	5.3	5.0	6.0	7.2
Sugar/buffer. capacity	0.6	0.5	0.8	2.9	0.1	1.1	2.0	1.5
NO_3 (g/kg)	0.4	15.0	18.4	11.2	4.3	10.4	0.4	0.2
Epiphytic LAB (lg cfu/g)	n.d.[2]	0.4	4.7	5.9	4.8	6.4	4.9	5.0

[1] % of DM, [2] not determined, [3] g lactic acid/100 g DM

Table 2: Treatment scheme with additives. Controls obtained no additive, without clostridial spores in the mentioned case (Alfalfa 1).

Treatment \ Feedstuff		Al-Falfa 1	Al-falfa 2	Rough blue-grass	Red clover	Cocks foot	Creeping soft grass	Peren. rey grass 1	Peren. rey grass 2
Clost. spores[1]	lg cfu/g FM	0	3.3	3.3	3.3	3.3	3.3	3.3	3.3
LAB[2]	lg cfu/g FM	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5
FA NA[3]	liter/t FM	4,25	3.5	3.5	3.5	3.5	3.5	4	4
LAB[2]	lg cfu/g FM	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5
+ FA NA[3]	+ liter/t FM	+ 4.25	+ 3.5	+ 3.5	+ 3.5	+ 3.5	+ 3.5	+ 4	+ 4
LAB[1]	lg cfu/g FM	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5
+ molasses	+ liter/t FM	+ 35	+ 35	+ 35	+ 35	+ 35	+ 35	+ 40	+ 40
Chem. pres.[4]	liter/t FM	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0

[1] Clostridial spores, [2] BIO-SIL®, Dr. Pieper Technologie- und Produktentwicklung GmbH, [3] Amasil NA®, BASF AG, [4] Kofasil liquid, ADDCON Agrar GmbH.

Table 3: Effect of additives on the relative DM-losses, fermentation characteristics and energy content - average of all silages in comparison to control (%).

Parameter \ Treatment	Control	LAB[1]	FA NA[2]	LAB[1] + FA NA[2]	LAB[1] + molasses	Chem. preser-vative[3]
DM-loss	100^{a}	83^{ab}	51^{b}	37^{c}	63^{b}	68^{b}
Acetic acid	100^{a}	97^{a}	63^{b}	19^{c}	87^{a}	98^{a}
Butyric acid	100^{a}	105^{a}	9^{b}	4^{b}	4^{b}	42^{c}
Lactic acid	100^{a}	119^{a}	199^{b}	203^{b}	305^{c}	169^{b}
NH_3-N/N_{total}	100^{a}	88^{a}	30^{b}	19^{b}	27^{b}	59^{c}
Crude fibre	100^{a}	100^{a}	94^{a}	90^{b}	91^{b}	93^{b}
ME[4]	100^{a}	107^{a}	113^{a}	116^{b}	114^{b}	108^{a}
NEL[5]	100^{a}	104^{a}	110^{a}	112^{b}	111^{a}	106^{a}
DLG points	27	31	85	99	84	45

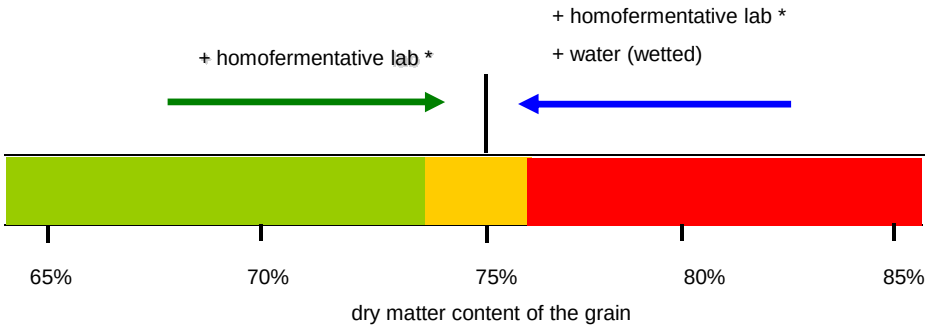
[a, b, c] Means in the same row with different superscripts differ ($P \leq 0.05$).
[1] BIO-SIL®, Dr. Pieper Technologie- und Produktentwicklung GmbH, [2] Amasil NA®, BASF AG
[3] Kofasil liquid, ADDCON Agrar GmbH.
[4] Metabolizable energy, [5] Net energy for lactation (calculated).

Table 4: Dosage of lactic acid bacteria and water to grounded grain for conservation

DM of the grain (%)	Moisture (%)	LAB[1] (g/t FM)	Water (l/t)	Costs[2] (€/t)
73	27	1	-	0,74
75	25	1	10	0,77
78	22	1	40	0,78
82	18	1	90	0,80

[1] BIO-SIL®, Dr. Pieper Technologie- und Produktentwicklung GmbH
[2] price basis 2010

Figure 1: Principle of the conservation of chopped grain with lactic acid bacteria and water (Pieper et al, 2005)



* homofermentative lab = BIO-SIL®
Lactobacillus plantarum DSM 8862 and DSM 8866

Section 3: Utilization, Nutritive Value and Hygienic Aspects of Pereserved Forages on Production Health of Animals

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Evaluation of Protein Value of Forages

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Introduction

The primary function of feed protein in the diet is to provide the ruminants with absorbed amino acids (AA), often denoted as metabolizable protein (MP) in the form of α-amino nitrogen. The MP requirement of ruminants is met from two sources: microbial protein synthesized in the rumen and feed protein that escapes microbial degradation in the rumen. In addition, endogenous protein is included in computing the MP supply and requirements in some protein evaluation systems, e.g. NRC (2001).

Protein nutrition of ruminants is complex, because the dietary supply of AA is modified both quantitatively and qualitatively by microbial fermentation in the fore-stomachs before digestion in the small intestine. Total CP and digestible CP are of little value in protein evaluation for ruminants, since dietary CP is only partly absorbed as AA. Meta-analysis of milk production trials (Huhtanen, 2005) indicated that metabolizable energy (ME), and even dry matter (DM) intake predicted milk protein yield responses better than CP or digestible CP intake, whereas MP intake was a better predictor of milk protein yield than ME intake. When high CP diets are fed a large proportion of apparent CP digestion is a result of ruminal ammonia production and absorption that does not provide absorbed AA for the host animal. In a recent meta-analysis (Broderick et al., 2010) zero rumen CP balance (=CP intake – omasal CP = 0) was obtained at dietary concentrations of CP and rumen degradable protein (RDP) of 147 and 106 g/kg DM, corresponding to ruminal ammonia-N and milk urea concentrations of 71 and 17.7 mg/100 ml, respectively. Above these concentrations a substantial proportion of CP is lost as net ammonia absorption and subsequently as urinary N.

Protein is usually the most expensive component of dairy cow diets. During the last decades there has been increasing concerns of N emissions from dairy farms, both in the forms of evaporative losses (ammonia N) to the atmosphere and leaching losses (nitrate) to ground waters. Feeding large amounts of supplemental protein is also often associated with increased phosphorus (P) intakes and emissions, since protein supplements contain more P than forages or cereal grains. Because of both economical and environmental concerns it is important to optimize protein feeding.

Accurate and precise evaluation of feed protein value is a prerequisite for optimizing production and minimizing environmental emissions from dairy operations. An ideal protein evaluation system should quantify accurately the supply of MP from microbial protein and undegraded feed protein (RUP) as well as the requirements RDP of rumen microbes and MP (amino acids) of the host animal. Accurate estimation of the distribution of manure N between faecal and urinary N would be useful in predicting environmental emissions. Considerable improvements in feed protein evaluation were made when CP or digestible CP were replaced with the MP-based systems that differentiate the RDP requirements of rumen microbes and absorbed AA requirements of the host animal. The concept of diving feed protein into RDP and RUP is theoretically a sound concept, but considerable problems exist in determining these protein fractions. In a meta-analysis based of North American (739 diets) and North European (998 diets) large datasets of milk production trials, prediction error of milk protein yield was only marginally improved by including effective protein degradability (EPD) in the model together with intakes of total digestible nutrients (TDN) and CP (Huhtanen and Hristov, 2009). a two variable model (microbial MP + feed MP) predicted milk protein yield markedly better than total MP; and interestingly, the regression coefficient of microbial MP was 5-fold compared to feed MP. Protein supplies were estimated according to NRC (2001) protein evaluation system. The lower coefficient for RUP suggests that its utilization was lower than that of microbial protein and/or differences in the supply of RUP were overestimated. This and other meta-analysis of production data have demonstrated that it is important to evaluate the

feed protein systems using the data from production trials. The ultimate aim of feed evaluation systems is to rank the nutritive values of feedstuffs correctly; not only qualitatively but also quantitatively.

With typical dairy cow diets the contribution of forages to the total CP intake is high, for example in the dataset of North European feeding trials with dairy cows the average proportion of forage CP was 56% (s.d. 11.9) of the total CP intake. Therefore understanding the factors that influence the protein value of forages is important in optimizing the supplementary protein feeding in order to minimize N emissions from dairy operations. The objective of this paper is to discuss factors influencing forage protein values and the strengths and weaknesses of the methodologies used in estimating feed protein value for dairy cows. Validation of different approaches will be made using meta-analysis of data from milk production trials.

Microbial Protein Synthesis

Quantitatively microbial protein is the major source of amino acids absorbed from the small intestine (generally MP) in feed protein evaluation systems. The mean contribution of microbial N to the total non-ammonia N (NAN) flow into the omasum was on average 71% for 96 diets (Broderick et al., 2010). Because microbial protein is the major component of the total MP supply, it is essential to predict microbial protein synthesis accurately and precisely. Reliable determination of microbial protein synthesis is technically demanding, since it is labour-intensive and it requires cannulated animals, digesta and microbial flow markers. Traditionally, measurement of compartmental nutrient flows within the digestive tract and microbial protein synthesis in the rumen have relied on sampling through simple T-cannulas fitted either in the abomasum or proximal duodenum (Harmon and Richards, 1997; Titgemeyer, 1997). Omasal sampling technique was developed (Huhtanen et al., 1997) and modified (Ahvenjärvi et al., 2000) to sample the digesta from the omasal canal. Omasal sampling method offers some advantages compared with duodenal sampling: it is less invasive - only rumen cannula is needed (i), smaller endogenous contribution to digesta, especially CP (ii) and sampling takes before the hydrolysis in the abomasums begins (iii). The method was evaluated using meta-analysis (Broderick et al., 2010; Huhtanen et al., 2010). The evaluation suggested that the prediction errors of the estimates of microbial protein synthesis were markedly smaller than in datasets based on duodenal sampling and a single marker (usually Cr_2O_3) method. Close relationships between ruminal and total NDF and OM digestion, and between omasal NAN flow and milk protein yield also provide credibility of the technique in rumen digestion studies. However, the authors attributed the accuracy and precision of the technique to the application of triple-marker method in estimating digesta flow rather than sampling site per se.

The analysis of omasal flow data (Broderick et al., 2010) indicated that microbial protein synthesis in the rumen was closely related to OM truly digested in the rumen (OMTDR). The amount of OMTDR can not be measured in practice, and therefore another parameter describing the supply of fermentable energy for rumen microbes is needed to compute MP concentration of a feed or diet. In feed protein evaluation models microbial N synthesis is usually expressed per kg digestible OM (DOM) that can corrected for substances that provide either no or less energy (ATP) for rumen microbes than digestible carbohydrates. For example, silage fermentation acids provide less (lactic acid) or no energy (VFA) for rumen microbes (Chamberlain, 1987). Restricting in-silo fermentation by gradually increasing the rate of formic acid application from 0 to 6 L/t increased the concentration of residual water soluble carbohydrates and decreased concentrations of fermentation acids (Jaakkola et al., 2006). These changes were associated with increased microbial protein synthesis and duodenal NAN flow. In contrast, the diets based on formic acid treated silages supported a greater microbial protein synthesis than diets based on hay harvested from the same sward (Jaakkola and Huhtanen, 1993). It could be expected that the absence of fermentation products in hay would increase microbial synthesis compared with silage. However, the microbes had a better access to substrate in fresh compared with dried forage that compensates for the smaller ATP supple from silage. In addition, a high proportion of soluble N is present as peptides (Nsereko and Rooke, 2000) in formic acid treated silage that may stimulate microbial synthesis.

Likewise silage fermentation acids, long-chain fatty acids, RUP or post-ruminally digested starch and NDF do not provide energy for microbial growth. Therefore, discounting DOM for these substances should result in more accurate MP concentration. To test this hypothesis mixed model regression analysis (St-Pierre, 2001) effect was performed to compare models predicting milk protein yield (MPY) using linear and quadratic terms of MP as independent variables ($MPY = a + bMP + cMP^2$). Quadratic term was included in the model to allow diminishing returns with increasing MP intake. The data consisted of 832 diets from 171 milk production trials with dairy cows. Microbial N synthesis was computed DOM calculated at maintenance level of feeding (DOM_m) as a basis. a value of 145 g microbial CP per kg DOM_m was derived from omasal sampling data (Broderick et al., 2010). When DOM_m was discounted for total acids (TA), fat expressed as ether extract (EE) and RUP or for combinations of these factors the efficiency value was modified to maintain the mean microbial supply constant for all models. The models were compared to the current system (MTT, 2006) that uses digestible carbohydrates (DCHO) + RDP as the basis computing microbial protein. The intake of RUP was estimated according to the Finnish protein evaluation system (MTT, 2006) that is a modification of the Scandinavian AAT-PBV system (Madsen et al., 1995). The goodness of the models was compared using residual mean squared error (RMSE) adjusted for random study effect and Akaike's information criterion (AIC).

Differences between the models predicting MPY were relatively small (Table 1), and prediction errors were small (about 18 g/d). Quadratic effects were highly significant ($P < 0.001$) indicating diminishing responses to increased MP supply. According to RMSE and AIC milk protein yield was most precisely predicted when microbial protein synthesis was calculated using DOM_m – RUP as a substrate. Discounting DOM_m for TA did not improve the model. Theoretically the model should have improved, since these fermentation acids provide no or very little energy for rumen microbes. This is in contrast with *in vivo* observations of reduced MPS with increased extent of silage fermentation (Harrison et al., 2003). This effect may be related to increased propionate production from silage lactic acid that has been demonstrated in the analysis of rumen fermentation data of cattle fed grass silage based diets (Sveinbjörnsson et al., 2006) with intraruminal lactate infusion study (Jaakkola and Huhtanen, 1992). Increased gluconeogenesis from propionate can improve the utilization of absorbed AA for milk protein synthesis thereby compensating for the reduced MPS with extensively fermented silages. Reduced plasma glucose concentration in cows fed restrictively fermented silages compared with those fed high lactate silages (Miettinen and Huhtanen, 1997; Shingfield et al., 2002) supports the hypothesis that glucose supply may be more limiting with restrictively fermented silages.

Although restricting in-silo fermentation has convincingly been demonstrated to increase microbial protein synthesis, MPY responses to restrictively fermented silages can almost entirely be attributed to reduced feed intake (Huhtanen et al, 2003). In addition to limited glucose supply amino acid profile of microbial protein may not be ideal for cows fed grass silage-based diets. Histidine is the first limiting amino acid in dairy cow diets based on grass silage and cereal grain supplements (Vanhatalo et al., 1999; Kim et al. 2001). Because the histidine concentration in microbial protein is markedly lower than in milk protein (20-21 *vs.* 26-27 g/kg amino acids), utilization of additional MP derived from microbial protein may be compromised by a sub-optimal amino acid profile. Changes in plasma amino acid concentrations in the study of Miettinen and Huhtanen (1997) support this suggestion. Compared with well-preserved high lactate silage restrictively fermented silage and post-ruminal casein infusion produced similar increases in plasma concentrations of lysine and branched-chain and total essential amino acids, whereas only casein infusion increased plasma histidine and it produced much greater MPY response (38 vs. 102 g/d).

Table 1: Prediction of milk protein yield from MP supply (MPY = a + bMP + cMP^2) computed using different models in estimating microbial protein synthesis (P-values for B and C all P < 0.001)

	A	SE	*P*-value	B	SE	C	SE	RMSE	AIC
DCHO + RDP	-131	50.6	0.01	771	63.0	-106	19.8	18.4	8145.5
DOM_m	-119	47.6	0.01	774	59.2	-112	18.5	18.3	8137.8
DOM_m - TA	-42	44.5	0.35	704	55.0	-100	17.3	18.0	8150.3
DOM_m - EE	-107	47.1	0.02	761	58.4	-109	18.3	18.3	8150.0
DOM_m - RUP	-128	49.7	0.01	767	61.9	-104	19.4	17.9	8107.6
DOM_m - TA -EE	-35	44.3	0.43	699	54.5	-100	17.1	18.2	8178.3
DOM_m - TA –RUP	-60	47.2	0.21	712	58.4	-98	18.4	17.9	8143.7
DOM_m - EE – RUP	-120	49.6	0.02	759	61.6	-103	19.4	18.1	8132.2
DOM_m - TA -EE - RUP	-55	47.3	0.25	713	58.3	-100	18.3	18.3	8189.7

SE = standard error
RMSE = Residual mean squared error, adjusted for random study effect.
AIC = Akaike's information criterion (smaller is better)
DCHO = Digestible carbohydrates
RDP = Rumen degradable protein
DOM_m= Digestible OM at maintenance level of feeding
TA = Total fermentation acids
EE = Crude fat (ether extract)
RUP = Rumen undegraded protein

Although fat does not provide energy for rumen microbes, discounting DOM_m for EE did not improve the prediction of MPY. However, many protein evaluation systems (Vérite and Peyrand, 1989; AFRC, 1992; Tamminga et al., 1994; Madsen et al., 1995) discount fat in calculating microbial protein. In contrast, in the NRC (2001) system fat has actually a very strong contribution to microbial protein synthesis because a factor of 2.25 is used for fatty acids [(ether extract – 10 (g/kg DM)] to calculate total digestible nutrients (TDN). In spite of being theoretically incorrect, standard error of prediction was not higher for TDN compared with total tract DOM (NRC, 2001). In a previous analysis (Huhtanen and Hristov, 2008) MPY prediction was only marginally poorer when the contribution of microbial protein to the total MP supply was estimated from TDN at maintenance than from DOM_m. It was discussed by NRC (2001) that improved efficiency of microbial protein synthesis may compensate for the reduced rumen fermentability with increased fat intake. Fat supplements have consistently decreased protozoal counts *in vivo* (Sutton et al., 1983; Hristov et al., 2004). Decreased protozoal populations in the rumen are usually associated with reduced intraruminal recycling of N as indicated reduced ammonia concentrations (Williams and Coleman, 1992), primarily as a result of a decrease in proteolysis of bacterial protein by ruminal protozoa (Broderick et al., 1991). Based on the relatively small variation in dietary fat content and possible increases in the efficiency of MPS with increased fat content, discounting fat from DOM_m may not affect the prediction accuracy of microbial protein synthesis. This is especially true for forages that have low variation in fat concentration.

Theoretically, postruminally digested starch should not be subtracted from DOM_m in calculating microbial protein, since it does not provide energy for rumen microbes. The current methods estimating ruminal starch digestibility are inaccurate (for discussion see Huhtanen and Sveinbjörsson, 2006). It is therefore possible that correcting the fermentable substrate for post-ruminal starch digestion would rather increase than decrease prediction errors in estimating MP. Starch digested in the small intestine is absorbed as glucose that can reduce the use of amino for gluconeogenesis. Increased glucose supply can therefore improve the utilisation of amino acids for milk protein synthesis and thereby compensate for the reduced energy supply for microbial

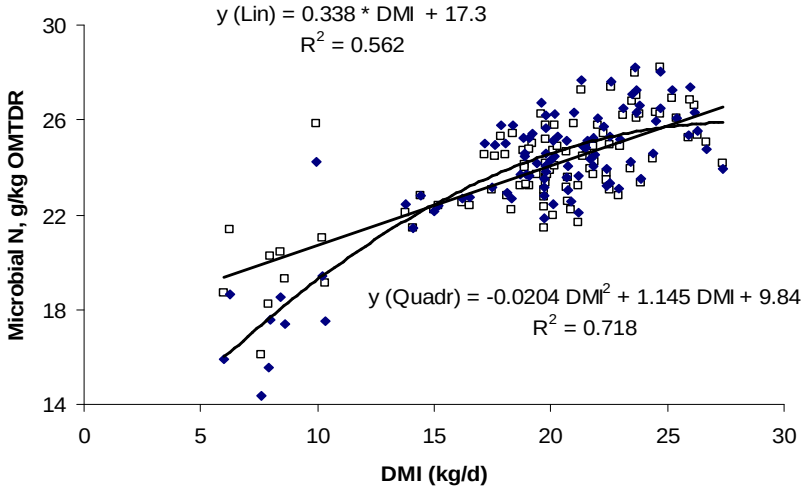
protein synthesis. Similarly, NDF digested in the hind-gut does support microbial protein synthesis and contribute to MP supply to the host animal. The analysis of omasal sampling data suggested that the contribution of the hind-gut to total NDF digestion is small (Huhtanen et al., 2010), and its true variation probably small.

Subtracting RUP from DOM_m from the fermentable substrate in calculating microbial protein synthesis improved the prediction of MPY from MP. This is logical, since by definition RUP is escaping fermentation in the rumen. Without subtracting RUP from DOM_m it would be double-counted; energy for microbial growth and escape of feed protein. There is also some evidence of negative effects of increased undegradability of dietary CP on microbial protein outflow from the rumen (Voigt and Piatkowski, 1987; Ipharraguerre and Clark, 2005). The latter authors reported a 7% reduction in microbial protein outflow from the rumen with increasing RUP intake. However, meta-analysis of omasal sampling data (Broderick et al., 2010) did not provide evidence for a lower efficiency of microbial synthesis with reduced ruminal protein degradability.

Increased feeding level can influence the supply of microbial protein by two different mechanisms: diet digestibility is decreased (NRC, 2001) and efficiency of microbial protein synthesis is increased (Volden, 1999; Broderick et al. 2010). Digestibility decreases with increased intake due to faster passage rate of feed particles from the rumen. Depression in digestibility with increased feeding level is greater for diets that have high digestibility at maintenance compared with poorly digestible diets (NRC, 2001; Huhtanen et al., 2009). Feeding level effects tend to be smaller for diets based on grass silage and small cereal grains (Huhtanen et al., 2009) compared with diets based on maize silage and maize grain based NRC (2001). Increased net efficiency of microbial protein synthesis with increased feeding level also results from faster passage rate of rumen fluid and particle phase. Consequently, microbial turnover time decreases allowing a greater proportion of fermentable energy (ATP) to be used microbial growth instead of maintenance of the cells. The positive relationship between DM intake and microbial efficiency in omasal sampling studies is shown in Figure 1. The efficiency increased significantly with increased intake, and the effect tended to be quadratic rather than linear.

Does adjusting the efficiency of microbial protein synthesis and DOM_m for actual intake level improve the accuracy of protein evaluation system? When DOM_m was adjusted for feeding level effects according to the model of Huhtanen et al. (2009) and microbial efficiency was adjusted using the linear relationship from Figure 1, prediction error of MPY with the quadratic MP model was slightly poorer (18.6) compared with the DOM_m and DOM – RUP models (see Table 1). The current analysis suggests that a more complicated system did not improve the accuracy of predictions of milk protein yield responses. This is probably because these opposite effects almost compensate for each other; on average; the positive effect of feeding level on microbial efficiency was slightly greater than the negative effect on digestibility. It is also possible that additional factors in the protein evaluation model generate errors. The NRC (2001) discounts TDN for the feeding level effects, but does not take into account increased efficiency of microbial protein synthesis with increased feed intake. This will result in greater requirement of supplementary protein at high intakes and milk production levels, and could partly explain the lower milk N efficiency in North American milk production trials compared with North European trials (Huhtanen and Hristov, 2009).

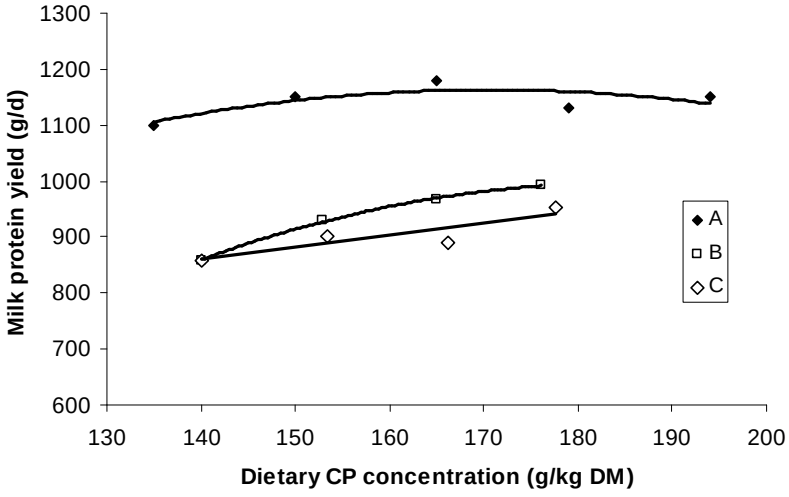
Figure 1: The relationship between DMI and the efficiency of microbial N synthesis in the rumen (n = 96 diets) estimated using a mixed regression model. The values are adjusted for random study effect (adopted from Broderick et al., 2010).



Undegraded Feed Protein

Ruminally undegraded protein is another major source of metabolisable protein. When the microbial requirements of RDP are met, the supply of MP to the host animal at given intake level can only be increased by feeding protein supplements high in RUP. However, ruminal protein degradability of the most common protein supplements is relatively high, and marginal MPY responses to supplementary protein remain rather low (Figure 2). With a diet based on lucerne and maize silage maximum protein yield was obtained at 165 g/CP kg DM, whereas with grass silage based diets positive responses were obtained at higher CP level. Better responses with grass silage based diet could be related to increased silage DM intake, whereas with lucerne-maize silage diet total DM intake started to decline above 165 g CP/kg DM. With soybean meal supplements the marginal MPY response was 0.10 – 0.11 g per g increase in CP intake and with rapeseed expeller it was 0.15, respectively. Because the true CP digestibility of protein supplements is high (soya close to 100%), 85-90% of incremental CP intake would be excreted in urine.

Figure 2: Effects of increasing dietary CP concentration on milk protein yield: a = soybean meal (Olmos Colmonero and Broderick, 2006); B = rapeseed expeller (Shingfield et al., 2003); C = soybean meal (Shingfield et al., 2003)



The value of additional forage protein is difficult to determine without confounding effects of digestibility, forage type etc. Marginal MPY response was 0.16, when forage CP concentration was increased by harvesting grass silage at four different maturities (Rinne et al., 1999). In contrast, manipulating silage CP concentration by increasing the rate of N fertiliser application had no effect on milk protein yield (Shingfield et al., 2001; Arvidson, et al., 2009). In the study of Shingfield et al. (2001) rapeseed expeller produced substantial production responses with both low and high CP silages. Differences in MPY responses to increased CP intake between maturity and fertilisation effects suggest that increased energy (and microbial protein) rather than feed protein supply was the influencing mechanism.

Increasing dietary CP concentration by replacing grass silage with red-clover silage has markedly increased protein flow the rumen (Dewhurst et al., 2003; Vanhatalo et al., 2009), but marginal milk protein responses to increased intestinal protein supply were minimal. Reduced ruminal protein degradability is attributed to the activity of polyphenol oxidase system that inhibits proteolysis. The lack of milk protein yield response to red clover despite increased protein supply suggests that forage RUP is of little value or other factors (e.g. energy) limit production.

Determination of protein degradability

In practical feed protein evaluation ruminal protein degradability is determined by incubation feeds in nylon bags in the rumen for different periods of time. The *in situ* method is also widely used determine NDF digestion kinetics. Several excellent reviews (e.g. Nocek, 1988; Stern et al., 1997; Nozière and Michalet-Doreau, 2000; Hvelplund and Weisbjerg, 2000) have been published. They provide a detailed insight into the sources of variation and methodological aspects of the procedure. Instead of discussing methodological details, this paper attempts to focus on the effects of forage degradability on protein yield responses observed in production trials in lactating dairy cows and discuss possible limitations of degradability models.

Kinetic models are then used to estimate degradation parameters that are subsequently used to calculate effective protein degradability (EPD) in the rumen. Disappearance of CP is most commonly described using Ørskov and McDonald (1979) model:

$$P = a + B \times (1 - e^{kd \times t}) \qquad [1]$$

where P = disappearance of CP at time = t, a = instantly degraded fraction, B =in time degradable fraction, k_d is the fractional rate of degradation of B fraction. Fraction that is completely indigestible in the rumen (C) is calculated as 1 - a - B, i.e. the sum of A, B and C is unity. Effective degraded protein is the calculated as:

$$EPD = a + B \times k_d / (k_d + k_p), \qquad [2]$$

where A, B and k_d are as defined above and k_p is fractional rate of passage. Proportion of undegraded or escape protein is given by the equation:

$$Escape = C + B \times k_p / (k_d + k_p) = 1 - EPD \qquad [3]$$

Ideally, fraction a is equivalent to forage soluble N and can be quantified as buffer soluble N. For silages soluble N can also be analysed as water soluble N, since it is strongly correlated with buffer soluble N (R^2 = 0.82; n = 24; unpublished data from MTT, Finland). This fraction consists mainly of non-protein N (NAN) and small amount of true protein. The basic assumption of the kinetic model (Ørskov and McDonald, 1979) is that faction a is degraded at infinite rate, i.e. the escape is zero. The same holds true also for Cornell Net Carbohydrate and Protein System (Sniffen et al., 1992) that use very high default values for the digestion rate of the A-fraction (NPN). However, there is a plenty of evidence based on different methodological approaches that this assumption is not correct.

Omasal flow measurements have shown a considerable flow of feed soluble NAN (SNAN = soluble N – ammonia N) with peptides being quantitatively the most important component (Choi et al., 2002; Reynal et al., 2007). Volden et al. (2002) fed a single dose of silage soluble N to cows and estimated that approximately 10% of the soluble non-ammonia N escaped rumen degradation in the liquid phase. a similar value can be recalculated from the data of Choi et al. (2002).

Hristov and Broderick (1996) estimated the flow of N fractions from rumen pool sizes and fractional passage rates of rumen solid and liquid phase. Outflow of alfalfa and corn silage SNAN in the liquid phase was approximately 24% of the dietary intake. Peltekova and Broderick (1996) estimated using inhibitor *in vitro* technique that 20% of silage SNAN escapes rumen fermentation. Hedqvist and Udén (2006) estimated using *in vitro* technique that proportionally 25% of soluble protein in ryegrass escapes ruminal degradation. In a recent *in vivo* study with dairy cows, proportionally 13% of SNAN in ^{15}N-labelled formic acid treated silage escaped ruminal degradation when estimated from ruminal ^{15}N kinetics (Ahvenjärvi et al., 2007). They labeled grass intrinsically with ^{15}N and isolated buffer soluble N that was pulse-dosed into the rumen. Variation in the estimates of SNAN flow probably reflects methodological and dietary differences between the studies in addition to contribution of microbial SNAN flow (Choi et al., 2002; Reynal et al., 2007).

Huhtanen et al. (2008) evaluated the effects of silage water soluble N on milk protein yield (MPY) and N efficiency (MNE) using data from milk production trials in which various forages factors (e.g. digestibility, fermentation quality, forage species) were studied (257 diets, 80 studies). Silage MP was calculated by using a constant EPD value irrespective the proportion of soluble N, i.e. assuming that solubility did not influence degradability. Various parameters were used in mixed model regression model in addition to the total MP supply to predict MPY responses.

Regression coefficient of soluble N was significantly negative (Table 2) when it was included as a second independent variable in the MPY and MNE models with MP intake or dietary CP concentration. This suggests that silage soluble N had a lower productive value than insoluble protein; however quantitatively the effects was small since 100 g/kg total N difference in soluble N corresponded 7 g/d difference in MPY. The negative effect of soluble N on MPY can almost completely be attributed to ammonia N, since regression coefficient for SNAN was non-significant and quantitatively minimal. One unit of standard deviation (88 g/kg N in the data) corresponded only to 1.7 g difference in MPY. Marginal effects of the SNAN fraction (free amino acids, peptides and soluble proteins) on MPY and MNE were consistent with the observations of considerable escape of this fraction from the rumen. In contrast, ammonia N has a negative effect on silage protein value. When proteolysis in the silo has proceed up to ammonia N, this fraction does not provide energy or preformed amino acids for rumen microbes and no escape of amino N in the liquid phase.

Table 2: Effects of silage N components on milk protein yield (g/d) and milk N efficiency (milk N/N intake; g/kg) when included in mixed regression models with total MP intake or dietary CP concentration (From Huhtanen et al., 2008).

X_1	X_2	X_3	Intercept	$Slope_1$	$Slope_2$	P-value	$Slope_3$	P-value	RMSE[a]	R^2
Milk protein yield (MPY)										
MP			111	0.424					15.7	0.982
MP	Soluble N		143	0.428	-0.070	<0.01			15.2	0.984
MP	NH_3-N		138	0.414	-0.190	0.004			14.5	0.985
MP	SNAN		111	0.432	-0.031	0.25			15.1	0.984
MP	NH_3-N	SNAN	143	0.416	-0.182	0.01	-0.019	0.46	14.6	0.985
Milk protein efficiency (MNE)										
CP			524	-1.51				0.01	6.2	0.932
CP	Soluble N		533	-1.51	-0.020	0.02		0.46	6.1	0.933
CP	NH_3-N		525	-1.49	-0.070	<0.01		0.14	6.0	0.937
CP	SNAN		523	-1.50	-0.003	0.81		<0.01	6.1	0.933
CP	NH_3-N	SNAN	525	-1.49	-0.070	<0.01	0.000	0.98	6.0	0.936

[a] *RMSE and R^2 values are adjusted for random study effect*
MP = metabolisable protein (g/d)
Soluble N, NH3-N and SNAN = concentrations of soluble N, ammonia N and soluble NAN (g/kg N)
CP = dietary CP concentration (g/kg DM).

The small effect of soluble N, and especially SNAN, suggests that N solubility does not markedly influence the true protein value of grass silages or mixtures of grass, whole-crop and legume (red-clover) silages. Although the degradability of silage SNAN is most likely higher than that of silage insoluble N, the difference is compensated by lower intestinal digestibility of insoluble N. True digestibility of SNAN fraction is completely, whereas indigestible fractions including ADF-found N are all associated with the insoluble fraction (Sniffen et al., 1992).

Broderick (1994) suggested a two-compartment kinetic model applying different degradation and passage rates for soluble and insoluble N fractions. Theoretically this model is more appropriate than the Ørskov and McDonald (1979) model, which is used in most protein evaluation systems to compute EPD values from degradation kinetic data. Equations for the two pool-compartmental model for EPD and escape are:

$$EPD = a \times k_{ds} / (k_{ds} + k_{ps}), + B \times k_{di} / (k_{di} + k_{pi}) \quad [4]$$

$$Escape = a \times k_{ps} / (k_{ds} + k_{ps}), + B \times k_{pi} / (k_{di} + k_{pi}) + C \quad [5]$$

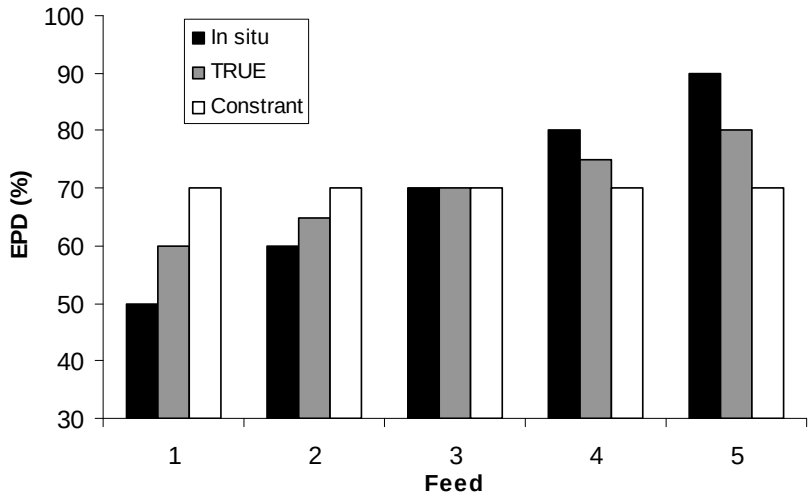
where *A*, *B* and *C* are as defined earlier, k_{ds} and k_{di} are degradation rates for soluble N fractions (*A*) and insoluble degradable fraction (*B*), and k_{ps} and k_{pi} are the corresponding passage rates. It should be noted that although the degradation rate of the *A*-fraction are much faster than that of the *B*-fraction, the liquid passage rate is also much faster than that of particles in dairy cows at high intake levels. Consequently, the differences in degradability of these fractions are much smaller than the large difference in degradation rates may suggest. Equations [4] and [5] are modifications of Broderick (1994) equations. He assumed that only soluble true protein could escape and included other SNAN fractions (free amino acids and peptides) in the completely degradable *A*-fraction. However, the later evidence from omasal sampling studies

(Choi et al., 2002; Reynal et al., 2007) indicates that also free amino acids and peptides escape from ruminal degradation. With this technique the samples are taken before the hydrolysis in the abomasum commences allowing a more detailed investigation of ruminal N metabolism. The model [4] can be modified to exclude ammonia from the *A*-fraction.

Although the two-compartmental model seems to be a satisfactory approach, the practical problem is how to determine the degradation rate of *A*-fraction. The method should be accurate and precise, if feed specific parameters values are going to be used in protein evaluation models.

One of the major problems of the *in situ* technique is its poor reproducibility (Madsen and Hvelplund, 1994). This was also demonstrated by Tuori et al. (1998) in evaluating feed protein systems. When MP supply was calculated using a constant EPD value for all feeds, residual predictions errors of production parameters were smaller compared with MP supply calculated using determined *in situ* values. The data consisted of 157 diets from two laboratories that had standardised their procedures of the *in situ* determination. The same conclusion can be made from a meta-analysis of large datasets of North American and North European production trials (Huhtanen and Hristov, 2009). Bacterial MP predicted MPY responses as well as the total MP; i.e. the predictions were not improved by including the feed MP to the total MP. Feed MP was calculated according to the NRC (2001) system, in which degradation parameters are based on a large *in situ* dataset. These findings do not imply that there are no differences in protein degradability between the feeds, but that the *in situ* values are not more accurate than constant values. This is demonstrated by a hypothetical example in Figure 3. The EPD values of five feeds *in situ* were 50, 60, 70, 80 and 90%, and the corresponding "true" values 60, 65, 70, 75 and 80%. In this case the mean square errors had been exactly the same (7.1%). For example, in situ underestimated EPD of Feed 1 by 10 %-units and overestimated that of Feed 5 by 10 %-units, whereas the reverse was true for constant EPD.

Figure 3: a hypothetical example of errors with the in situ and constant EPD values



Because of low reproducibility of the *in situ* method, using forage EP values that are estimated using empirical equations derived from other silage parameters. Yan and Agnew (2004) published models that predicted *in situ* EPD precisely (R^2 ~ 0.80). The most complete model based on concentrations of DM, CP, NDF, proportion of soluble N in total N (all expressed as g/g) and the ratio between lactic acid and VFA is given below:

EPD (g/g) = 0.758 + 0.701 × CP – 0.167 × DM – 0.191 × NDF + 0.251 × SN/N + 0.002 × LA/VFA [6]

According to the model silage EPD will increase with increased CP concentration, N solubility and lactic acid to VFA ratio and it will decrease with increased concentrations of DM and NDF. Rinne et al. (2009) calculated silage MP using EPD values calculated with

equation [6] or according the Finnish protein evaluation system (MTT, 2006) that uses a constant EPD for silages. Concentrate MP was calculated in both cases using the MTT (2006) system. The MTT (2006) system predicted MPY better compared with using EPD values computed using Yan and Agnew (2004) equations (Table 3). Residual analysis showed that productive value of low EPD silages according to Yan and Agnew (2004) model was overestimated and that of high EPD silages underestimated.

The Yan and Agnew (2006) model predicts higher EPD values for high CP and low NDF silages compared with low CP and high NDF silages. These differences may at least partly result from relative differences in the contribution of microbial contamination of undegraded feed residues. Incubation of intrinsically ^{15}N labeled feeds demonstrated that a large proportion of residual N is of microbial origin (Varvikko and Lindberg, 1985), especially for feeds of low CP and high NDF concentration. Since forages typically have relatively low CP and high NDF concentration, microbial contamination can severely influence forage EPD estimation. Michalet-Doreau and Ould-Bah (1989) developed two equations to correct the EPD values for microbial contamination; one was related to CP and the other to CP and NDF. Interestingly, their coefficient for correcting EPD values for the differences in NDF concentration was similar to those in the equations of Yan and Agnew (2004) for estimating EPD values from the laboratory measurements. This suggests that the variation in silage EPD values that is related to CP and from NDF concentration can reflect differences in the extent of microbial contamination of undegraded residues.

Table 3: The effects of different equations of effective protein degradability (EPD) estimation on prediction of milk protein yield from estimated metabolizable protein supply (Y = a + BX)

	N	A	s.e.	B	s.e.	RSME[1]
All data						
Basal model	397	92	19.4	0.437	0.011	16.16
Yan & Agnew	397	76	20.7	0.456	0.012	17.55
Soluble N data						
Basal model	248	103	22.1	0.442	0.012	14.53
Yan & Agnew	248	95	24.2	0.454	0.014	16.40

[1] *Root mean squared error*

Lower degradability of high NDF low CP silages could be associated with reduced intestinal digestibility of RUP. Nitrogen insoluble in acid detergent (ADIN) tends to increase with advancing maturity (e.g. Rinne et al., 1997; Elizalde et al., 1999) leading to reduced digestibility. Concentration of ADIN can be analyzed chemically, but it can also be predicted from iNDF concentration rather accurately (Figure 4). Concentration of iNDF is closely related to forage digestibility (Huhtanen et al., 2006), and is an essential parameter of mechanistic models predicting nutrient supply.

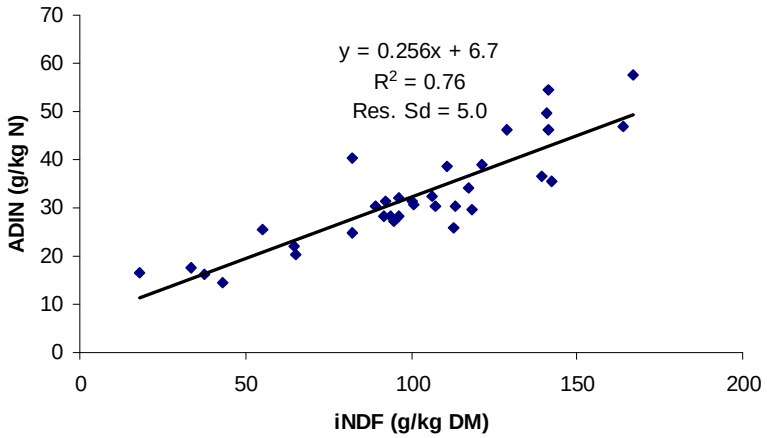
Prediction error of quadratic MP model remained unchanged (18.7) compared using a constant (0.82) RUP digestibility, when Yan and Agnew (2004) equations were used to predict silage EPD and digestibility of silage RUP was calculated as (RUP – ADIN) / RUP. Prediction error was smaller (17.8) when constant values were used both for EPD and RUP digestibility according to the Finnish protein evaluation system (MTT, 2006). This analysis was based on 822 diets in 170 studies. The results suggest that there is no such variation in ruminal degradability or intestinal digestibility of silage RUP that is predictable from other silage components and influence productive value of silage protein. Considering the methodological problems and low reproducibility of ruminal *in situ* and intestinal mobile bag technique it is even more unlikely that such differences can be detected by direct determining EPD and intestinal digestibility.

Passage model for insoluble protein (*B*-fraction)

Most feed protein evaluation systems use the Ørskov and McDonald (1979) model in calculating EDP from the *in situ* kinetic parameters. The model assumes that particle passage

from the rumen follows the first-order kinetics and

Figure 4: Relationship between iNDF and ADIN concentrations (unpublished data from MTT, Finland)



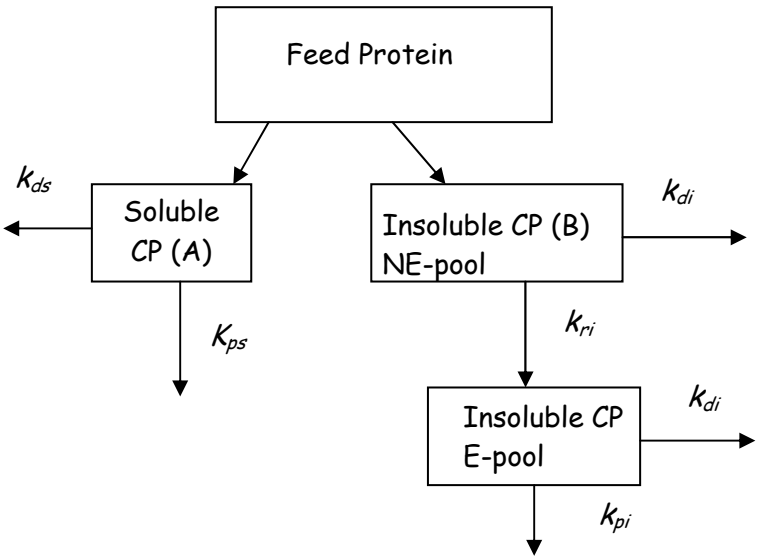
ignores the mechanisms of selective retention of feed particles. However, the marker kinetics data estimated from duodenal samples strongly indicate that the passage of feed particles cannot be described by assuming the rumen is a single first-order kinetic system (Ellis et al., 1994; Huhtanen et al., 2006). Both intrinsically (ADF-^{15}N) (Huhtanen and Hristov, 2001) and extrinsically (Lund et al., 2006) labeled forages showed an ascending phase of marker excretion curves. The passage rate estimated from the descending phase of marker curves clearly underestimates the residence time in the rumen during which the feed is subjected to degradation.

Allen and Mertens (1988) presented a model computing NDF digestibility for the two-compartment system including selective retention of feed particles. The same model can be applied for the *B*-fraction protein, since most likely the passage kinetic of insoluble protein follows that of NDF. Selective retention has even been demonstrated for concentrate particles or labelled faecal particles (for references see Huhtanen et al., 2006). Equation [4] can be extended to include selective retention of feed particles in the rumen as follows:

$$EPD = a \times k_{ds} / (k_{ds} + k_{ps}) + B \times [k_{di} / (k_{di} + k_{ri}) \times (1 + k_{ri} / (k_{di} + k_{pi})] \quad [7]$$

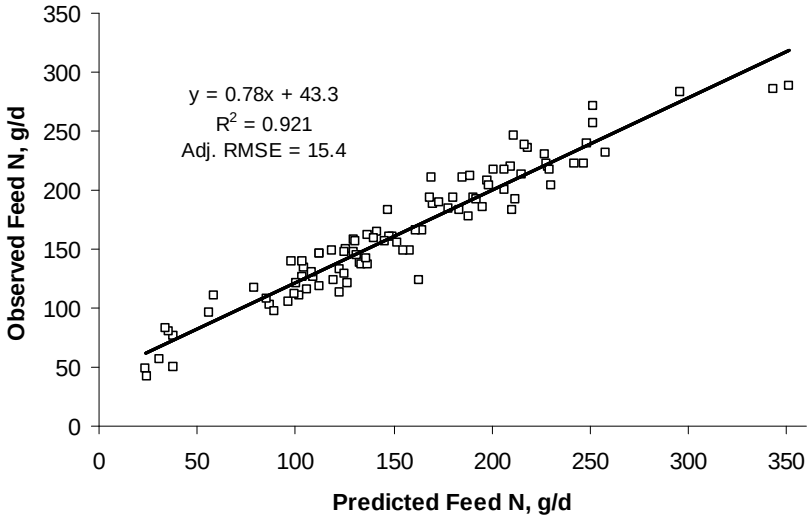
where k_{ri} is the rate of release of particles from rumen non-escapable pool (large particles) to escapable pool (small particles) and the other parameters as described before. The model is shown graphically in Figure 5

Figure 5. Schematic model of ruminal protein degradation considering escape of soluble NAN and selective retention of insoluble feed particles. a and B refer to protein fractions in the Ørskov and McDonald (1979) model.



The effect of the model assumptions have a strong influence to calculated supply on digestible RUP. Concentrations of *A, B* and *C* fractions for two example forages were assumed to be 400, 550, and 50 and 600, 350, and 50 g/kg, respectively. In the two compartment model the residence time of *B*-fraction was assumed to be 35 h [15 + 20 h; k_{ri} = 1/15 = 0.067/h and k_{pi} = 1/20 = 0.05/h] and assuming k_{ds} and k_{ps} values of 1.50 and 0.15/h for the *A*-fraction A. a value of 0.08/h was used as k_{di} for the *B*-fraction. The difference in the flow of digestible RUP between the two example forages decreased from 79 when the Ørskov and McDonald (1979) to 19 g/kg when the escape of *A*-fraction and selective retention of *B*-fraction were included in the model. This is consistent with the analysis of production data that strongly suggest that the variation in the RUP supply is overestimated. Analysis of omasal sampling data (Broderick et al., 2010) also indicated that the differences in the flow feed N are smaller than predicted by the NRC (2001) model (Figure 6). The slope of residual analysis was significantly negative indicating that the differences in feed N flow were smaller than predicted. Part of RDP was recovered as RUP and part of RUP was recovered as RDP in statistical analysis of the flow data. The former could be related to the escape of SNAN in the liquid and the latter to the underestimation of rumen residence time by one-compartment passage model. In omasal flow data the relative value of feed N was 0.68 of that of microbial N in two variable model predicting milk protein yield. This value is much higher than the corresponding ratio (0.2) in the meta-analysis of milk production data (Huhtanen and Hristov, 2009) when the protein supply was estimated according to the NRC (2001) system. Much smaller coefficient in production data indicates an overestimation of the range in the supply of RUP, i.e. true differences in RUP supply were smaller than predicted.

Figure 6: Relationship between predicted (NRC, 2001) and observed feed N flow (Brodrick et al., 2010).



Silage Analysis

For practical ration formulation it is important to know which chemical and biological parameters are important in describing productive protein value of forages. To determine which forage parameters are important in predicting milk protein yield responses a mixed model regression analysis was carried out. Forage parameters were included in the model in addition to MP supply from concentrate feeds. The data consisted of 527 treatments means from 98 milk production trials. Silage DM intake and D-value (concentration of digestible OM in DM) were clearly the most important parameters in determining MPY responses when expressed per unit of standard deviation (SD) of the parameter (Table 4). Relative silage DM intake potential can be predicted accurately from silage parameters with D-value and fermentation quality being the most important parameters (Huhtanen et al., 2007). Silage D-value can be reliably estimated by laboratory methods and NIRS provided that the methods are properly calibrated and validated with the *in vivo* digestibility data (Huhtanen et al., 2006). The strong effects of silage DM intake and D-value in predicting MPY responses indicate the microbial protein is the major component of MP in silage. This is obvious, since the supply of fermentable energy from silage for microbial synthesis is a function of these two variables. The contribution of silage CP concentration on MPY responses was surprisingly small and it only approached statistical significance. On average silage DM intake (11.1 kg/d) the marginal responses to increased silage CP concentration was only 0.018 (= 0.21 g per g CP/kg DM) / (11.1 kg/d × 1 g/kg DM). The low value can be due to the lower energy supply from CP compared with carbohydrates to rumen microbes (1), enhanced degradability with increased CP concentration (2) and/or poor amino acid composition of forage RUP (3). The low coefficient silage CP concentration suggests that forage CP is of minor value as a source of utilizable protein beyond the level of microbial requirements of RDP. It may be concluded that ideal forage to sustain high N efficiency in milk production would have a high digestibility and low CP concentration.

The effects of silage ammonia N and SNAN in this dataset were consistent with earlier study (Huhtanen et al., 2008) including data only from studies in which various forage factors were investigated. As discussed earlier, negative effects of ammonia are related to the loss of fermentable energy and other growth factors (e.g. free amino acids and peptides) during in-silo fermentation. In contrast, silage SNAN had no effect on milk protein yield. As discussed earlier, according to the assumptions of the widely used Ørskov and McDonald model this fraction should not escape from the rumen, but experimental evidence based on different methodologies has invalidated this assumption. The recovery of a pulse dose ^{15}N labelled silage SNAN was greater in dairy cows a grass silage-based diet than that of ammonia N (51 vs. 44%)

suggesting a better microbial utilisation of NAN rather than ammonia N (Ahvenjärvi et al., 2007).

Additional parameters [silage concentrations of DM and NDF, dietary concentrations of lactic acid, VFA, total acids and iNDF, proportion of legume (mainly red clover) in silage and harvest (primary vs. regrowth)] were neither statistically significant nor improved MPY predictions when included separately as an additional parameter into the model shown in Table 4). Because the concentrations of DM and NDF are negatively related to silage degradability (Yan and Agnew, 2004), degradability of red clover silage is lower than that of grass silage (Dewhurst et al., 2003; Vanhatalo et al., 2009), iNDF is positively related to ADIN concentration (Figure 4) and silage acids do not supply energy for rumen microbes (Chamberlain, 1987), it could have expected that at least some of these theoretically justified parameters had improved the model. Several reasons can be suggested for this: variation and consequently expected effects are small (i), the effects are compensated by other factors (ii), there are compensatory mechanisms in metabolism (iii), parameters can not be accurately analysed (iv).

Table 4: The model predicting milk protein yield (g/d) from concentrate MP intake and silage parameters (n = 527; RMSE adjusted for random study effect = 15.7)

Effect	Unit	Estimate	SE	P-Value	Mean	SD	Response per SD unit
Intercept		-270	42.2	<0.0001			
Concentrate MP	kg/d	492	19.4	<0.0001			
Silage DM intake	kg/d	28.6	1.39	<0.0001	11.1	1.54	44.0
Silage D-value	g/kg DM	0.64	0.075	<0.0001	675	41.1	26.3
Silage CP	g/kg DM	0.21	0.118	0.08	157	22.4	4.7
Silage ammonia N	g/kg N	-0.23	0.069	0.001	50	21.5	5.0
Silage SNAN[1]	g/kg N	-0.029	0.0239	0.23	517	87.6	2.5

[1] *SNAN = Water soluble N – Ammonia N*

Conclusions

Most of the variation in MP supply from forages appears to be related to microbial protein and therefore accurate and precise estimation of forage digestibility is essential also for accurate predicting of protein value. Variation in the supply of undegraded feed protein supply from forages appears to be small; at least when it concerns milk protein yield responses. It appears that the current experimental method, ruminal *in situ* nylon bag incubation, overestimates the differences between the feeds in ruminal protein degradation. This partly because of inherent problems of the technique and partly due to the false model assumptions in calculating degraded protein. Therefore it is unlikely that protein values based on determined degradability predict productive value of forage protein better than values calculated using constant degradability. This does not mean that there are not differences in ruminal degradability, but current techniques are not accurate enough to result better estimates than using a constant degradability. In is also possible that reduced forage degradability is associated with reduced intestinal digestibility of RUP. Proportion of soluble N in total N, especially SNAN, has relatively small influence on productive value of forage protein. However, it should be noted that when high solubility is related to extensive and/or poor fermentation milk protein yield will be decreased. Reduced performance can almost completely be attributed to reduced intake potential. Developing protein evaluation models and experimental methods to determine parameters required in the model it is essential that the models and methods are validated using the data from production trials. Complications and/or new parameters for practical protein evaluation models can only be justified if predictions of production responses are improved.

Due to complicated interaction both in digestion and metabolism adding new factors and parameters to factorial or semi-mechanistic protein evaluation models may increase prediction errors of milk protein yield unless for example the interactions between absorbed nutrients in tissue metabolism are taken into account. Mechanistic dynamic models such as the Nordic dairy cow model Karoline (Danfaer et al., 2006) may provide a tool a solution to handle the complicated interactions better than the current models.

References

[1] AFRC, 1992. Agricultural and Food Research Council. Energy and Protein Requirements of Ruminants. CAB International. Wallingford, UK, pp. 159.

[2] Ahvenjärvi, S., Vanhatalo, A. Huhtanen, P. and Hristov, A.2007. Ruminal metabolism of ^{15}N labelled ammonium-N and grass silage soluble non-ammonia-N. J. Dairy Sci. 90 (Suppl. 1.):232. (Abstr.).

[3] Allen, M.S. and Mertens, D.R. 1988. Evaluation constraints of fibre digestion by rumen microbes. Journal of Nutrition, 118, 261-270.

[4] Arvidson, K., Gustavsson, A-M. and Martinsson, K. 2009. The effect of crude protein level and fatty acid concentration in silage and on fatty acid composition of dairy cow milk. Manuscript.

[5] Ahvenjärvi, S., Vanhatalo, A., Huhtanen, P., and Hristov, A. 2007. Ruminal metabolism of ^{15}N labeled ammonium-N and grass silage soluble non-ammonia N. J. Dairy Sci., 90, Suppl. 1: 232.

[6] Ahvenjärvi, S., Vanhatalo, A., Huhtanen, P., and Varvikko, T.. 2000. Determination of forestomach digestion in lactating dairy cows by omasal or duodenal sampling. Br. J. Nutr. 83: 67-77.

[7] Broderick, G. A. 1994. Quantifying forage protein quality. Pages 200-228 in Forage Quality, Evaluation, and Utilization. G. C. Fahey Jr., D. R. Collins, D. R. Mertens and L. E. Moser eds. American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of America, Inc., Madison, Wisconsin, USA.

[8] Broderick, G.A., Huhtanen, P., Ahvenjärvi, S. Reynal, S.M., and Shingfield, K.J. 2010. Quantifying ruminal nitrogen metabolism using the omasal sampling technique in cattle - a meta-analysis. Accepted for publication in J. Dairy Sci.

[9] Broderick, G. A., Wallace, R. J., and Ørskov, E. R. 1991. Control of rate and extent of protein degradation. Pages 541–592 in Physiological Aspects of Digestion and Metabolism in Ruminants, T. Tsuda, Y. Sasaki, and R. Kawashima, ed. Academic Press, Inc., San Diego, CA, USA. .

[10] Chamberlain D.G. 1987. The silage fermentation in relation to the utilization of nutrients in the rumen. Process Biochem. 22:60-63.

[11] Choi, C.W., Ahvenjärvi, S. Vanhatalo, A. Toivonen, V. and Huhtanen, P. 2002. Quantitation of the flow of soluble non-ammonia nitrogen entering the omasal canal of dairy cows fed grass silage based diets. Anim. Feed Sci. Technol. 96:203-220.

[12] Danfær, A., Huhtanen, P., Udén, P., Sveinbjörnsson, J., Volden, H. 2006. The Nordic Dairy Cow Model, Karoline - Description. In: Kebreab, E. et al. (eds) Nutrient Digestion and Utilization in Farm Animals: Modelling approaches. CAB International, pp. 383-406.

[13] Dewhurst, R.J., Evans, R.T., Scollan, N.D., Moorby, J.M., Merry, R.J.and R. J. Wilkins, R.J. 2003. Comparison of grass and legume silages for milk production. 2. In vivo and in sacco evaluations ofrumen function. J. Dairy Sci. 86:2612–2621.

[14] Elizalde, J.C., Merchen, N.R. and Faulkner, D.B.. 1999. In situ dry matter and crude protein degradation of fresh forages during the spring growth. J. Dairy Sci. 82:1978-1990.

[15] Ellis, W. C., Matis, J. H. Hill, T. M. and Murphy, M. R. 1994. Methodology for estimation digestion and passage kinetics of forages. Pages 682-756 in Forage Quality, Evaluation and Utilization G. C. Fahey, Jr., M. Collins, D. R. Mertens, and L. E. Moser, ed. American Society of Agronomy, Madison, WI.

[16] Harmon, D. L. and Richards, C. J. 1997. Considerations for gastrointestinal cannulation in ruminants. J. Anim. Sci. 75:2248–2255.

[17] Harrison, J., Huhtanen, P and Collins, M. 2003. Perennial grasses. Pages 665-747 in Silage Science and Technology. D.R. Buxton, R.E. Muck, J.H. Harrison, ed. American Society of Agronomy, Madison, WI.
[18] Hedqvist, H. and Udén, P. 2006. Measurement of soluble protein degradation in the rumen. Anim. Feed Sci. Technol. 126:1-21.
[19] Hvelplund, T. and M. R. Weisbjerg. 2000. In situ techniques for the estimation of protein degradability and postrumen availability. Pages 233-258 in Forage Evaluation in Ruminant Nutrition. D. I. Givens, E. Owen, R. F. E. Axford and H. M. Omed eds. CABI Publishing, Wallingford, UK.
[20] Hristov, A.N., and Broderick, G.A. 1996. Synthesis of microbial protein in ruminally cannulated cows fed alfalfa silage, alfalfa hay, or corn silage. J. Dairy Sci. 79:1627-1637.
[21] Hristov, A. N., Ivan, M., and McAllister, T. A.. 2004. In vitro effects of individual fatty acids on protozoal numbers and on fermentation products in ruminal fluid from cattle fed a high concentrate, barley-based diet. J. Anim. Sci. 82:2693–2704.
[22] Huhtanen, P. 2005. Critical aspects of feed protein evaluation systems for ruminants. J. Anim. Feed Sci. 14, Suppl. 1: 145-170.
[23] Huhtanen, P., Ahvenjärvi, S., Broderick, G.A., Reynal, S.M., and Shingfield, K.J. 2010. Quantifying ruminal digestion of organic matter and neutral detergent fiber using omasal sampling in cattle - a meta-analysis.
[24] Huhtanen, P., Ahvenjärvi, S., Weisbjerg, M.R. and Nørgaard, P. 2006. Digestion and passage of carbohydrates. In: K. Sejrsen, T. Hvelplund, M.O. Nielsen (eds). Ruminant physiology: Digestion, metabolism and impact of nutrition in gene impression, immunology and stress. 'Proceedings of the X International Symposium on Ruminant Physiology', Copenhagen, Denmark. Wageningen Academic
[25] Huhtanen, P., Brotz, P.G., and Satter, L.D.. 1997. Omasal sampling technique for assessing fermentative digestion in the forestomach of dairy cows. J. Anim. Sci. 75:1380-1392.
[26] Huhtanen, P. and Hristov, A.N.. 2001. Estimating passage kinetics using fibre-bound ^{15}N as an internal marker. Anim. Feed Sci. Technol. 94: 29-41.
[27] Huhtanen, P, and Hristov, A. 2008. Evaluation of protein value of dairy cow diets. Proceedings of the Pacific Northwest Animal Nutrition Conference, Tacoma, WA, pp. 121-134.
[28] Huhtanen, P., Nousiainen, J.I., Khalili, H., Jaakkola, S., Heikkilä, T. 2003. Relationships between silage fermentation characteristics and milk production parameters: analyses of literature data. Livest. Prod. Sci. 81: 57-73.
[29] Huhtanen, P., Nousiainen, J., Rinne, M. 2006. Recent developments in forage evaluation with special reference to practical applications. Agric. Food Sci. 3: 293-323.
[30] Huhtanen, P., Rinne, M., and Nousiainen, J. 2007. Evaluation of the factors affecting silage intake of dairy cows; a revision of the relative silage dry matter intake index. Animal, 1, 758-770.
[31] Huhtanen, P., Rinne, M., Nousiainen, J. 2009. a meta-analysis of feed digestion in dairy cows. 2. The effects of feeding level and diet composition on digestibility. J. Dairy Sci. 92: 5031–5042.
[32] Huhtanen, P., Rinne, M., and Nousiainen, J. 2008. Effects of silage soluble N components on metabolizable protein concentration: a meta-analysis of dairy cow production experiments J. Dairy Sci. 91:1150-1158
[33] Huhtanen, P., Sveinbjörnsson, J. 2006. Evaluation of methods for estimating starch digestibility and digestion kinetics. Anim. Feed Sci. Technol. 130: 95-113.
[34] Ipharraguerre, I. R., and J. H. Clark. 2005. Impacts of the source and amount of crude protein on the intestinal supply of nitrogen fractions and performance of dairy cows. J.Dairy Sci. 88(ESuppl.):E22–E37.
[35] Jaakkola, S., and Huhtanen, P. 1992. Rumen fermentation and microbial protein synthesis in cattle given intraruminal infusions of lactic acid with grass silage based diet. J. Agric. Sci. 119:411-419.

[36] Jaakkola, S., and Huhtanen, P. 1993. The effects of the forage preservation method and the proportion of concentrate on nitrogen digestion and rumen fermentation in cattle. Grass Forage Sci. 48: 146-154.
[37] Jaakkola, S., Kaunisto, V., and Huhtanen, P. 2006. Volatile fatty acid proportions and microbial protein synthesis in the rumen of cattle receiving grass silage ensiled with different rates of formic acid. Grass Forage Sci. 61:282-292.
[38] Kim, C.-H., J.-J. Choung, and D.G. Chamberlain. 2001. Responses of milk production to the intravenous infusion of amino acids in dairy cows fed grass silage and cereal-based supplements. J. Anim. Physiol. Nutr. 85:293-300
[39] Lund, P., Weisbjerg, M.R. and Hvelplund, T. 2006. Passage kinetics of fibre in dairy cows obtained from duodenal and faecal ytterbium excretion. Effect of forage type. Anim. Feed Sci. Technol. 128: 229–252.
[40] Madsen, J. and T. Hvelplund. 1994. Prediction of in situ protein degradability in the rumen. Results of a European ringtest. Livest. Prod. Sci. 39:201-212.
[41] Madsen J., Hvelplund, T., Weisbjerg, M.R., Bertilsson, J., Olsson, I., Spörndly, R., Harstad, O.M., Volden, H., Tuori, M., Varvikko, T., Huhtanen, P., and Olafsson, B.L. 1995. The AAT/PBV protein evaluation system for ruminants. a revision. Norw. J. Agr. Sci. (Suppl. 19).
[42] Michalet-Doreau, B. and Ould-Bah, M.Y.. 1989. Estimation of extent of bacterial contamination in bag residues and its influence on in sacco measurements of forage nitrogen degradation in the rumen. Pages 909-910 in XVI International Grassland Congress, Nice, France.
[43] Miettinen, H.O., and Huhtanen, P. 1997. Effects of silage fermentation and post-ruminal casein supplementation in lactating dairy cows. 2. Energy metabolites and amino acids. J.Sci. Food Agric. 74:459-468.
[44] MTT. 2006. Rehutaulukot ja ruokintasuositukset (Feed tables and feeding recommendations, in Finnish). http://www.mtt.fi/rehutaulukot. Accessed Feb 1, 2008.
[45] National Research Counci (NRC). 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Sci., Washington, DC.
[46] Nocek, J. E. (1988). In situ and other methods to estimate ruminal protein and energy digestibility: a review. J. Dairy Sci. 71: 2051–2069.
[47] Nozière, P. & B. Michalet-Doreau (2000). In sacco methods. In: J.P.F. D´Mello (ed.) Farm Animal Metabolism and Nutrition. CAB International, Oxon, pp. 233-253.
[48] Nsereko, V.L., and Rooke, J.A. 2000. Characterisation of peptides in silages made from perennial ryegrass with different silage additives. J. Sci. Food Agric. 80:725-731.
[49] Olmos Colmenero, J.J. and Broderick, G. A. 2006. Effect of dietary crude protein concentration on milk production and nitrogen utilization in lactating dairy cows. J. Dairy Sci. 89:1704–1712.
[50] Ørskov E.R. and McDonald I., 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci., Camb. 92: 499-503.
[51] Peltekova, V.D. and Broderick, G.A.. 1996. In vitro ruminal degradation and synthesis of protein on fractions extracted from alfalfa hay and silage. J. Dairy Sci. 79:612-619.
[52] Reynal, S.M, Ipharraguerre, I.R. Liñeiro, M., Brito, A.F., Broderick, G.A. and Clark, J.H. 2007. Omasal flow of soluble proteins, peptides, and free amino acids in dairy cows fed diets supplemented with proteins of varying ruminal degradabilities. J. Dairy Sci. 90: 1887-1903.
[53] Rinne, M., Jaakkola, S. and Huhtanen, P. 1997. Grass maturity effects on cattle fed silage-based diets. 1. Organic matter digestion, rumen fermentation and nitrogen utilization. Anim. Feed Sci. Technol. 67:1-17.
[54] Rinne, M., Jaakkola, S., Kaustell, K., Heikkilä, T. and Huhtanen, P. 1999. Silage harvested at different stages of grass growth versus concentrate foods as energy and protein sources in milk production. Anim. Sci. 69:251-263.

[55] Rinne, M., Huhtanen, P. and J. Nousiainen, 2009. Effects of silage effective protein degradability and fermentation acids on metabolizable protein concentration: a meta-analysis of dairy cow production experiments. J. Dairy Sci. 92:1633–1642.
[56] Shingfield, K., Jaakkola, S., and Huhtanen, P. 2002. Effect of forage conservation method, concentrate level and propylene glycol on diet digestibility, rumen fermentation, blood metabolite concentrations and nutrient utilisation of dairy cows. Anim. Feed Sci. Technol. 97:1-21.
[57] Shingfield, K.J., Jaakkola, S. & Huhtanen, P. 2001. Effects of level of nitrogen fertilizer application and various nitrogenous supplements on milk production and nitrogen utilization of dairy cows given grass silage-based diets. Anim. Sci. 73: 541-554.
[58] Shingfield, K., Vanhatalo, A., Huhtanen, P. 2003. Comparison of heat-treated rapeseed expeller and solvent-extracted soya-bean meal protein supplements for dairy cows given grass silage-based diets. Anim. Sci. 77: 305-317.
[59] Sniffen, C. J., O'Connor, J. D., Van Soest, P. J., Fox, D. G. and Russell, J. B.. 1992. a net carbohydrate and protein system for evaluating cattle diets. 2. Carbohydrate and protein availability. J. Anim. Sci. 70:3562-3577.
[60] Stern, M. D., Bach, a and Calsamiglia, S. 1997. Alternative techniques for measuring nutrient digestion in ruminants. J. Anim. Sci. 75, 2256–2276.
[61] St-Pierre, N.R. 2001. Integrating quantitative findings from multiple studies using mixed model methodology. J. Dairy Sci. 84:741-755.
[62] Sveinbjörnsson, J., Huhtanen, P., and Udén, P.. 2005. The Nordic dairy cow model, Karoline – Development of volatile fatty acid sub-model. Pages 1-14 in Nutrient Digestion and Utilization in Farm Animals: Modelling approaches. E. Kebreab, J. Dijkstra, A. Bannink, J. Gerrits and J. France. ed. CAB International, Wallingford, UK
[63] Tamminga S., Van Straalen W.M., Subnel A.P.J., Meijer R.G.M., Steg A., Wever C.J.G., Blok M.C., 1994. The Dutch protein evaluation system: the DVE/OEB-system. Livest. Prod. Sci. 40, 139-155
[64] Titgemeyer, E. C. 1997. Design and interpretation of nutrient digestion studies. J. Anim. Sci. 75:2235–2247.
[65] Tuori, M., Kaustell, K.V. and Huhtanen, P.1998. Comparison of the protein evaluation systems of feeds for dairy cows. Livest. Prod. Sci. 55:33-46.
[66] Vanhatalo A., Kuoppala, K., Ahvenjärvi, S. and Rinne, M. 2009. Effects of feeding grass or red clover silage cut at two maturity stages in dairy cows. 1. Nitrogen metabolism and supply of amino acids. J. Dairy Sci. 92: 5620-5633
[67] Varvikko, T. and Lindberg, J.E.. 1985. Estimation of microbial nitrogen in nylon-bag residues by feed ^{15}N dilution. Br. J. Nutr. 54:473-481.
[68] Vérité R., Peyraud J.-L., 1989. Protein: the PDI system. In: R. Jarrige (Editor). Ruminant Nutrition. Recommended Allowances and Feed Tables. INRA, pp. 33-48.
[69] Voigt, J. and B. Piatkowski. 1987. Ruminal protein degradation and protein value of feeds. Arch. Anim. Nutr. 37:63–68.
[70] Volden, H. 1999. Effects of level of feeding and ruminally undegraded protein on ruminal bacterial protein synthesis, escape of dietary protein, intestinal amino acid profile, and performance of dairy cows. J. Anim. Sci. 77:1905-1918.
[71] Volden H., Mydland, L.T. and Olaisen, V. 2002. Apparent ruminal degradation and rumen escape of soluble nitrogen fractions in grass and grass silage administered intraruminally to lactating dairy cows. J. Anim. Sci. 80:2704-2716.
[72] Williams, A. G., and G. S. Coleman. 1992. The Rumen Protozoa. Springer-Verlag Inc., New York, NY
[73] Yan, T., and R.E. Agnew. 2004. Prediction of nutritive value of grass silages: II. Degradability of nitrogen and dry matter using digestibility, chemical composition and fermentation data. J. Anim. Sci. 82:1380-1391.

Mycotoxins, GMO and Bulk Feed

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Abstrakt

The work objective was to compare the content of mycotoxins entering the food chain from the maize silage. The paper includes three years observation. The content of mycotoxins was assessed in maize silages after four plant protection strategies against European Corn Borer GM hybrid MON 810, commercial isoline Monumental and two insecticide protection (chemical insecticide or biological protection using wasps). Mycotoxins assessed were deoxynivalenol (DON), fumonisin (FUM), and zearalenon (ZEA). The experiments have demonstrated very low or no contamination of GMO maize by ECB and subsequent analysis of Fusarium mycotoxins showed a correlation with insect resistance, i.e., mycotoxin content in GMO material was lowest compared to the highest content in the control untreated maize.
Keywords: maize, silage, mycotoxins

Introduction

One of the most important factors affecting the health of animals, their efficiency as producers, and the quality of livestock products is the feeding ration. Feedstuffs may contain harmful substances that negatively affect not only the health of animals, but also the safety and acceptability of their products. These harmful substances may contain contaminants that were created during the production, preservation and storage of these feedstuffs, or even during their technological processing. Often, this involves such common contaminants as fungi and their toxins. The occurrence of fungi on maize harvested for silage, which has higher stubble remaining after cutting, is partially eliminated as long as the upper part of the ear is not contaminated by European corn borer (ECB - Ostrinia nubilalis). Corn borer damage leads to fungal contamination which can subsequently spread to other parts of the plant. In the case of harvesting severely contaminated and older maize plants with high dry matter content, the material is usually contaminated with Fusarium spp., which leaves its toxins in the silage, decreasing its digestibility, reducing production efficiency, and negatively influencing animals' health. The appearance of fungi and their mycotoxins can also be expected if the silage production technique was not in accordance with proper standards. Especially problematic are slow and interrupted ensiling, contamination with soil, failure to cover the material, leakage of rainwater, insufficient sealing against air, etc. These factors lead to greater contamination with undesirable bacteria and fungi that might cause secondary fermentations and not only result in loss of nutrients but also put at risk the health and physiological functions of the animal consuming the silage.

Mycotoxins are secondary metabolic products from moulds belonging in particular to the Aspergillus, Penicillium and Fusarium genera. More than 300 secondary metabolites have been identified although only around 30 have true toxic properties which are of some concern. Toxinogenic moulds may develop under all climatic conditions on any solid or liquid supports as soon as nutritional substances and moisture (water activity Aw over 0.6) are present, hence the wide variety of contaminated foodstuff substrates. These toxins are found as natural contaminants in many feedstuffs of plant origin, especially cereals but also fruits, hazelnuts, almonds, seeds, fodder and foods consisting of, or manufactured from, these products and intended for human or animal consumption. Two groups of toxinogenic (mycotoxin producing) fungi can be distinguished. The first one consists of fungi (such as Fusarium) which invade their substrate and produce mycotoxins on the growing plants before harvesting: this is the category of field (pre-harvest) toxins. Aflatoxins and Fusarium toxins are included in this group. The other group contains fungi which produce toxins after harvesting and during crop storage and transportation. These toxins are named storage (or post-harvest) toxins and ochratoxin a belongs

to this group. Mycotoxins are small and quite stable molecules which are extremely difficult to remove or eradicate, and which enter the feed chain while keeping their toxic properties.

Issues regarding mycotoxins in forages have been much discussed in recent years. In considering the health risks connected with livestock consumption of corn silage contaminated by mycotoxins, we can divide this topic into three parts: 1) issues associated with the growing of maize; 2) issues relating to maize harvesting and silage production; and 3) the feeding process and the possible removal of dangerous contaminants from the feedstuffs. In the Czech Republic's soil and weather conditions, the main mycotoxin producers are soil fungi of the genus Fusarium.. In discussing this area attention has focussed on growers' interventions that may decrease the contamination of plants by these pathogens and so to create conditions for a lower content of toxic metabolites. From the entire spectrum of technological possibilities, the most important are: the cultivar and its type, dry matter content, and phytopathological and biotechnological steps toward reducing damage to plants by ECB. Among biotechnological steps, we refer mainly to the cultivation of GM maize with incorporated Bt toxin.

Material and Methods

Damage to plants by ECB allows infection by fungal pathogens, and is one of the factors that increase the possibility of contamination by mycotoxins. Experiments carried out over several years have compared protection of maize against ECB using 1) a genetically modified Bt-hybrid, 2) traditional protection using insecticides, 3) biological protection using wasps of genus Trichogramma, and 4) a control variant (isoline to Bt-hybrid Monumental). ELISA quantitative tests for mycotoxins analyses were used.

Results and Discussion

These experiments have demonstrated very low or no contamination of GMO maize by ECB. a 70-95% effectiveness was achieved using insecticides. The effectiveness of biological approaches was strongly dependent upon the weather conditions, but the average effectiveness was less than that using chemical protection. Subsequent analysis of Fusarium mycotoxins showed a correlation with insect resistance, i.e., mycotoxin content in GMO material was lowest compared to the highest content in the control untreated maize (tab. 1).

Table 1: Number of insect corridors (50 plants) (example from 2007)

	silage	**corn grain**	**DON**
Control	100 % (43)	100% (60)	100 % (540 ppb)
Insecticide	4%	8%	0
biological control	50%	50%	60%
BT hybrid	0%	0%	10%

It should be noted that the mycotoxin content in GMO material was not always zero. Even if this material was not attack by ECB, the material could still have been contaminated by fungi of the genus Fusarium, because the genetic modification is intended as a protection against damage done by insects and it does not increase the resistance against fungal pathogens. Notwithstanding all the questions that are related to the use of genetically modified plants, cultivation of GM maize can be recommended from the viewpoint of decreasing mycotoxine contamination. Thirty four studies about *Fusarium* mycotoxin contamination in isogenic BT maize and non-Bt maize hybrids grown in Europe, USA, South America and Asia were analyzed. Thirty out of total amount of studies on Bt maize came to the conclusion that Bt maize is less contaminated with mycotoxins (FUM, DON, ZEA) than the conventional control variety in each case (Ostry et al. 2009).

A list of mycotoxins of interest which are of some concern for the safety of animal feed in the European Union was published in an EU SCAN report (EU SCAN, 2003). It includes Aflatoxin B1 (AFB1) and Ergot sclerotia, which are subject to Commission Regulation (EC) No 1881/2006. Zearalenone (ZEA), Deoxynivalenol (DON), Ochratoxin a (OTA) and Fumonisins (especially Fumonisin B1, FB1), the maximum levels of which are now recommended (Commission Recommendation 2006/576/EC). This list also includes T-2 and HT-2 toxins

Bt-maize was cultivating on 8,300 ha in the Czech Republic in the year 2008 and making the country the second-largest grower of Bt-maize in the EU after Spain, with about 70,000 ha. In 2009 was a little smaller area about 7000 ha. Borer is afforded not only for maize grain production but also for silage production from maize. This protection is more important for locations with a higher occurrence of this pest in the past, and in later-maturing maize hybrids, for which the interval between the Corn Borer's invasion and the harvest is extended. The results from the subsequent experiments indicate the equivalence of the nutrient composition and feeding value between Bt maize and its near-isogenic control. The digestibility of crude fibre and nitrogen-free extracts of Bt silage determined on whether was higher than that of the control silage (Křížová et al. 2009)

A separate chapter relates to the harvesting of maize, its quality, the subsequent speed and quality of its ensiling, and the quickest possible sealing of the silage against air and its covering. Recommendations, for increasing the quality of silage are provided. There can be no exception in practice if there is not to be secondary contamination of the ensiled material by "storage fungi", which may be connected with mycotoxin production. The article's authors have analysed a wide range of samples collected during the ensiling processes from individual locations of trench silos, as well as samples that have been taken from the face of the silage when loading out the silage for feeding. The results confirmed that if the ensiled material contains a greater amount of mycotoxins, then these can be found across the entire profile of the final silage. If mycotoxins are present during the period of silage fermentation they are also present at the final opening of the silo. The conclusion is drawn, that if maize cannot be cultivated without the possibility of mycotoxins being present, then the ensiling process will not decrease the amount of those substances, because these are chemical compounds with high thermal and chemical stability.

Conclusion

Mycotoxins contamination of maize products could be important negative factor decreasing feed safety for farm animals. Plant protection strategies against European Corn Borer including GMO Bt-hybrid is good way to reduce not only insect incidence, but mycotoxin content too.

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References

[1] Křížová, L., Svobodová, J., Pavlok, J., Třináctý, J., Nedělník, J., Kocourek, F. (2009): Effect of insect-protected maize silage (Bt-MON 810) on feeding value and digestibility of nutrients estimated with wethers. Slovak J. Anim. Sci 42, (3): 118-123.

[2] Ostrý, V., Ovesná, J., Říha, K., Skarková, J., Ruprich, J. (2009): An occurrence of Fusarium mycotoxins in transgenic BT-maize vs. non- Bt maize hybrids – a review. In: 31th Mycotoxin Workshop, Munster, Germany, Society for Mycotoxin Research, p. 83.

Investigation of Clostridial Spores in Swedish Dairy Herds

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Introduction

Clostridium bacteria contaminate the forage at harvest by soil and manure that have high spore counts. Growth of *Clostridium* bacteria in silage is a problem in dairy production as Clostridial spores can be transferred from the silage to the milk through the faeces. Milk with high spore counts disturbs the cheese making process and results in cheese that cannot be marketed (Pahlow *et al.*, 2003). Therefore, spores are analysed at the dairy coops and too high of a spore count can result in a reduced milk price for the producer. This study aimed to investigate differences in management between dairy herds in south-west Sweden that had high or low spore counts in the milk and to advise the farmers on methods that potentially result in decreased spore counts in silage and milk.

Material and Methods

The study included 23 farms in south-west Sweden, of which half of the herds had high spore counts in the milk (> 600 spores/ml milk) during 65% of the time during the 16 month-period before the start of the project, whereas the other half of the herds had low spore counts for several years. The herds were further divided into the amount of manure applied on the leys; from no manure to 100 tonnes per hectare. Samples for analysis of Clostridial spores were taken of stored manure at spreading and of soil, growing grass-clover forage and of wilted forage at each harvest in the summer of 2007. Grass-clover silages from silos and round bales, silages or feed mixtures in the feed trough and faeces from the cows were sampled twice during the indoor period of 2007-2008 in all of the herds. Additionally, samples of wilted grass-clover forage were taken at each harvest and samples of stored silage from the silos were taken on farms with elevated spore counts in 2008-2009. Samples of silage or feed mixtures in the feed trough and of faeces from the cows were taken twice during the indoor period of 2008-2009 in all of the herds. The milk was analysed monthly for Clostridial spore counts by the dairy coops. Cleanliness of the cows was registered on a scale from 1 to 3, where 1 was clean, 2 was moderately dirty and 3 was very dirty, at the farm visits. The silages were stored in towers, bunkers, clamps, tubes or as round bales. Use of and type of silage additives were recorded. Samples for analysis of Clostridial spores were sampled in the middle of the silage surface or tube, 200-300 mm from the edge of the silage surface and from the silage that was going to be fed the same day as the sampling. The Clostridial spore counts in feed and faeces were determined at Eurofins laboratory, Lidköping, Sweden (Jonsson, 1990) whereas the Clostridial spore count in milk was analysed at Steins laboratory, Jönköping, Sweden (Cerf and Bergere, 1968). The freshly fed silage also was analysed for fermentation characteristics at Eurofins, Sweden. Results shown as log values are presented as medians with minimum and maximum values within parenthesis, otherwise the results are presented as means and standard deviations.

Results and Discussion

Clostridial spore counts in the soils were high (log 3.3 (2.5-3.9)/g) but unaffected by the application rate of the manure that had high spore counts (log 3.2 (2.0-4.9)/g). Growing and wilted forage contained very little spores (< log 2.0/g) and they were unaffected by manure application rates. Herds with low milk spore counts had no elevated spore counts in the silage in the middle of the silage surface but 14% of the samples from herds with high milk spore counts had a spore count of log 3.5 (3.5-5.8)/g in the middle of the silo surface. High Clostridial spore counts from silage near the edges of the silage surface were found in 64% of the samples (log 3.2 (2.3-4.7)/g) from normal herds and in 71% of the samples (log 3.9 (2.3-5.0)/g) from herds with elevated milk spore counts. Forty-one percent of the total number of samples from

round-baled silage had elevated spore counts (log 3.2 (2.1-6.1)/g). Fifty percent of the herds with high milk spore counts stored the silages as round bales only and 1/3 of these herds used silage additives, whereas the other 50% of the herds with high milk spore counts stored the silage in bunkers, clamps or tubes and almost all of these silages were treated with additives. Twenty-seven percent of the herds with low milk spore counts used round bales, most commonly without additives. The remaining 73% of the normal herds used towers, bunkers, clamps or tubes, usually with an additive applied to the silage. As expected, silage DM content affected fermentation characteristics and was confounded by the effect of additive and storage type (Table 1). The additives used were bacterial inoculants and acids in about equal proportions between herds. The inoculants were various mixtures of lactic acid bacteria and the acids were mixtures of formic acid and propionic acid. The silages generally had high ethanol concentrations, especially in the untreated silages in 2008-09, which also had high butyric acid concentrations, which basically originated from two samples with very high butyric acid concentrations (64 and 68 g/kg DM). However, the median Clostridial spore count in these untreated silages was log 2.0 (2.0-3.5), which was equal to the median spore count of all the other silages. The percentage of silage samples with spore counts > log 2.0 was higher for untreated than for treated silages in 2007-08 (25% vs. 10%) whereas the opposite was true for 2008-09 (8% vs. 25%) in problem herds. These results indicate that proper ensiling technique always is very important for a successful fermentation to occur, even when additives are used. It lso is important to use recommended application rates of the additives.

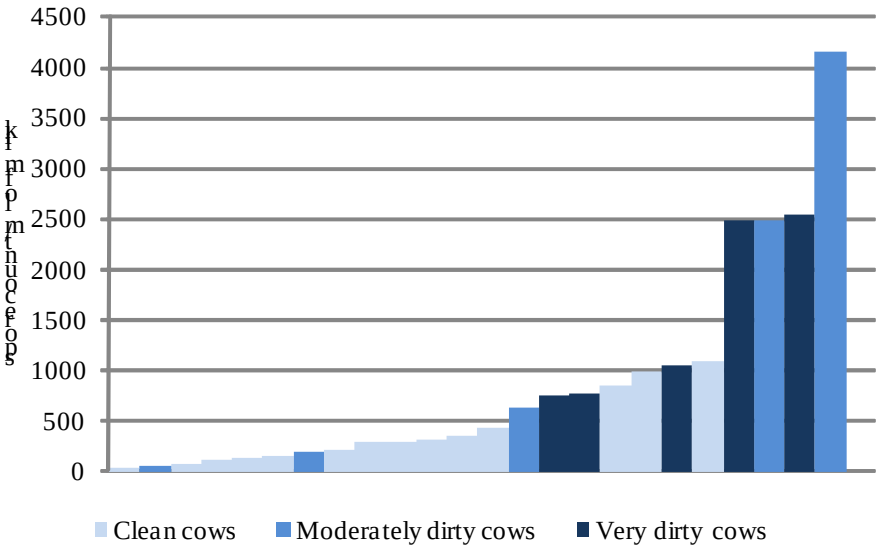
Table 1: DM, pH and fermentation products in grass-clover silages sampled fresh before being fed to the herds with low or high Clostridial spore counts in milk. Means and standard deviations within parenthesis expressed as g/kg DM, unless stated otherwise.

Fermentation characteristics	Herds with low spore count in milk 2007-08		Herds with high spore count in milk 2007-08		Herds with high spore count in milk 2008-09	
	No additive	Additive[1]	No additive	Additive	No additive	Additive
n (no. of samples)	12	18	16	10	24	12
DM, g/kg	393 (146)	299 (90)	380 (126)	307 (94)	444 (142)	404 (150)
pH	4.9 (0.5)	4.2 (0.3)	4.8 (0.5)	4.5 (0.4)	5.0 (0.6)	4.8 (0.6)
NH_3-N, g/kg total N	74 (44)	88 (23)	65 (34)	85 (38)	76 (56)	65 (43)
Lactic acid	51 (37)	120 (48)	60 (42)	88 (51)	65 (55)	58 (39)
Acetic acid	12 (9)	18 (14)	10 (11)	24 (28)	10 (11)	9.0 (9.3)
Butyric acid	0.4 (0.8)	0.8 (2.3)	0.9 (0.2)	0.3 (0.6)	6.1 (18)	1.1 (2.1)
Ethanol	7.3 (4.8)	5.4 (9.9)	8.5 (6.4)	5.5 (5.9)	20 (21)	13 (13)

[1]*Mean of silages treated with bacterial inoculant or acid in nearly equal proportions between herds.*

Silage and feed mixtures in the feed trough had spore counts > log 2.0/g in 50% of the samples from problem herds (log 3.0 (2.1-6.1)/g) and in 33% of the samples from the herds with low milk spore counts (log 3.3 (2.3-3.9)/g). Elevated Clostridial spore counts in the faeces from the cows were found in 73% of the samples (log 3.3 (2.2-5.3)/g) from herds with high spore counts in the milk whereas 63% of the samples from herds with no remarks on the milk had spore counts > 2.0 (log 3.1 (2.3-4.6)/g). The cleanliness of the cows generally was worse in herds with high spore counts than in herds with low spore counts in the milk (Figure 1).

Figure 1. Relationship between cleanliness of the cows and Clostridial spore counts in the milk from each of the herds averaged over the indoor periods from October until March 2007-08 and 2008-09.



Conclusion

Problems with high Clostridial spore counts in the milk start in the silage but can be resolved by keeping the cows clean. Silage stored close to the edges of the bunker silo or tube and round-baled silage are prone to Clostridial growth.

References

[1] CERF O. and BERGERE J.L. 1968. La numération des spores de Clostridium et son application au lait et aux produits laitiers. Numération des différents groupes de Clostridium. Le lait 48, 501-519.

[2] JONSSON A. 1990. Enumeration and confirmation of C. tyrobutyricum in silages using neutralred, D-cykloserine and lactate dehydrogenase activity. Journal of Dairy Science 73, 719-725.

[3] PAHLOW G., MUCK R.E., DRIEHUIS F., ELFERINK S.J.W.H. AND SPOELSTRA S.F. 2003. Microbiology of ensiling. pp. 31-93. Silage Science and Technology, Buxton, D. R., Muck, R. E. and Harrison, J. H., eds. Agronomy No. 42, ASA, CSSA, SSSA, Madison, Wisconsin, USA.

The Effect of T2 Toxin and Zearalenone on Health and Metabolic Parameters in Dairy Cows

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Mycotoxins are secondary toxic metabolites of filamentous fungi that adversely effect animal and human health. It is estimated that there are over 400 different mycotoxins. They are produced by a wide range of fungi and are contained in various foodstuffs and feedstuffs all over the world. Thus, mycotoxins represent a serious hygiene and health problem worldwide.

There is a high occurrence of mycotoxins in forage, and therefore there is a high incidence of health disorders caused by mycotoxins in cattle in this country. These are mainly mycotoxins produced by *Fusarium* sp. (Illek 2007, Moravcová and Nedělník 2007)

The aim of this study was to evaluate the effect of long-lasting increased intake of T2 toxin and zearalenone on production performance, health and metabolic profile in dairy cows.

Material and Methods

The monitoring was carried out in the autumn and winter season in the dairy herd C with average milk production 5,620 kg milk per 305 day lactation. Diets were adjusted to different phases of calving-to-calving interval and were based on maize silage, clover-grass silage, meadow hey, straw, concentrate and vitamin-mineral feed. Cows were fed total mixed ration twice a day, using a feeding cart. TMR provided levels of nutrients meeting the cow requirements. However, some cows in the herd had increased somatic cell counts in milk and there was poor conception rate (bulk tank somatic cell count 380,000-520,000; first-service conception rate 30-34%). At the farmer's request, the evaluation of nutrition and health status of the cows was performed with the aim to reveal causes of high SCC and reproductive failures.

The history of the herd, diet composition and feed ingredient quality were assessed. Concentrations of T2 toxin were measured in maize silage and clover-grass silage by ELISA at the State Veterinary Institute laboratory. Cows were physically examined by groups, and 10 cows were repeatedly withdrawn blood samples for blood chemistry and complete blood count (haematology). The first examination (A) was performed at the end of November in cows from 20 to 60 days in milk. The second examination (B) was performed in the same cows as (A) in mid December and the third examination (C) was performed in January. After the first examination (A), the feeding of the maize silage containing high mycotoxin levels (mainly T2) was terminated and a new silage with low mycotoxin levels was given. Haematology analyses were performed using the abc analyser. Blood chemistry was done with the analyser Hitachi 902, using standard sets from Roche and Randox. T3 and T4 hormones were determined by chemiluminiscence (Immulite).

Results and discussion

Clinical status – the cows showed lower appetite, optimum body condition score, dull hair coat. There was a sporadic occurrence of diarrhea. No other clinical signs were observed.

Total mixed ration had a favourable structure and did not show any sensory alterations. The laboratory examinations showed that the maize silage contained 486 ug/kg of T2 toxin and 2 452 ug/kg of zearalenone. Grass-clover silage contained only 32 ug/kg of T2 toxin and 26 ug/kg of zearalenone. The new maize silage contained only traces of T2 toxin, and zearalenone was not detected.

Table 1: Some blood and milk parameters.

Examination		A	B	C
Parameter	Unit			
Hb	g/l	82,6	84,3	90,5
HMT	1/1.	0,31	0,33	0,35
E	T/l	5,12	5,38	6,32
Le	G/l	5,83	6,62	7,24
Cb	gl/l	70,6	74,1	72,8
Al	g/l	30,8	32,6	34,1
Gl	mmol/l	3,34	3,28	3,36
Urea	mmol/l	4,86	5,12	5,34
Bil	umol/l	6,32	5,14	4,26
BHB	mmol/l	0,81	0,62	0,51
AST	ukat/l	1,74	1,52	1,44
GMT	ukat/l	0,53	0,55	0,42
CK	ukat/l	1,86	1,92	1,74
GSH-Px	ukat/l	722	834	886
B-carotene	umol/l	3,24	3,68	4,86
Vit. E	umol/l	4,12	4,52	5,33
T3	nmol/l	1,83	1,78	1,82
T4	nmol/l	58,62	61,35	80,21
Milk				
Milkyield	l/kus/den	16,2	16,8	18,2
SCC A	t/ml	476	422	296
SCC B	t/ml	420	380	280

SCC A = mean no. 30 cows
SCC B = bulk tank somatic cell count of the herd

The results showed that the cows had a high intake of mycotoxins via maize silage that adversely affected both milk production and quality (SCC) and some metabolic parameters. Decreased haemoglobin, marginal packed cell volume (hematocrit) and erythrocyte count values indicated impaired erythropoesis and blood losses due to haemorrhages in the digestive tract and mammary gland parenchyma. Reduced leukocyte counts were also due to the above-mentioned mycotoxins, as reported by many researchers (Roter et al.,1994; Gremmels 2005; Jouany and Diaz 2005). Long-lasting intake of mycotoxins and synergism between mycotoxins caused damages to the liver parenchyma which is indicated by high activities of liver enzymes and increased bilirubin concentrations. Similar results were demonstrated in laboratory animals (Rajmon et al. 2001), poultry and pigs (Smith et al. 2005, Wyatt, 2005) An interesting finding is low concentrations of antioxidants such as beta-carotene, vitamin E and GSH-Px and decreased concentration of T4. It is assumed that the above-mentioned mycotoxins increase the requirement for the antioxidants, or there could be a rapid degradation of beta-carotene and vitamin E inside silage before feeding. The change of the diet consisting in the replacement of the mycotoxin contaminated maize silage for the non-contaminated one resulted in a gradual increase of milk yield and a significant reduction in SCC and improvement of metabolic parameters under study. In approximately two months after the exclusion of the contaminated maize silage from the diet, the blood count improved, AST and GMT activity values decreased to physiological range, total bilirubin concentration decreased, and levels of beta-carotene, vitamin E, GSH-Px and T4 increased to the physiological range. Reproduction performance was evaluated 3 months later. First-service conception rate increased from 30–34% to 46%.

Conclusion

The monitoring of increased intake of T2 toxin and zearalenone in dairy cows under field conditions showed adverse effects on milk yield and quality, health status and fertility. A long-lasting intake of mycotoxins caused damage to the liver parenchyma, decreased milk

production, increased SCC and reduced fertility. The exclusion of the mycotoxin contaminated silage from the diet resulted in a gradual improvement of health status and metabolic profile of the dairy cows under study.

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References are available at the authors.

Silage Stored Crimped High-moisture Grain in View of Feed Nutritive Value and Hygienic

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Abstract

Trial were conducted to examine nutrient composition and hygiene of dry barley (DB) and ensiled barley (MB) and to compare the effects of DB and MB on voluntary feed intake and milk production of dairy cows. The barley crop from one half of a 30-ha field was combined when the grains were at the cheesy ripe stage of maturity (564 g DM/kg), and the grains were subsequently crimped and conserved in a plastic tube using Murska Crimper-Bagger. The barley crop from other half of the same field was combined when grains was at complete maturity (832 g DM/kg) and stored in aerated bin.

High-moisture fresh or preserved barley grains contain more ($P<0.05$) WSC and less ($P<0.05$) starch, however, other chemical composition parameters not differ compared with dried grains. The content of fermentation end products suggested that preserved crimped barley grains were of good fermentation quality. The concentration of the toxins and microbial contamination of the conserved high-moisture barley decreased in when compared with dried grains. Crimped preserved barley grains stimulated greater intakes of grass silage and total diet DM than those increased milk production by 6.5 %, improves milk quality and in addition, helps cut costs.

Introduction

Drying it is no longer an option for feed grain technology to protect cereals from spoilage due to significantly increased costs for energy and additional investments into drying capacities. a strategically desirable approach is the ensiling of early combined moist cereals to produce a highly digestible with easier and safer form of starch moist feed. High-moisture crimped grain preservation in hermetic plastic sleeve is an attractive and interesting alternative to drying and based on a procedure similar to ensiling grass (2009). An additional advantage that is becoming increasingly important is that feeding crimped grains allows the use of homegrown, traceable (source-verified) feeds instead of purchased concentrates This study was aimed to evaluate the effectiveness of ensiling high moisture barley (MB) grains and to compare the effects of dry barley (DB) and ensiled barley (MB) on feed intake and milk production of dairy cows fed grass silage.

Materials and Methods

MB was combine harvested at the cheesy ripe stage of maturity (dry matter (DM) 564 g/kg and DB at complete maturity (796 g DM/kg) from the same field. MB grains were rolled, treated with acid additive (3.5 l/t, AIV2000, Kemira Oyj) and conserved in a plastic tube using Murska Crimper-Bagger. DB was dried to the final DM content of 842 g/kg and stored in aerated bin. Eighteen dairy milking cows were selected for the experiment according to parity, lactation, date of calving, present milk yield and last year milk yield and live weight. Cows which were randomly assigned to one of two groups (n=9) were given concentrate (containing different treated barley grain) at fixed amount. Group D was fed concentrate mixture - DB-rapeseed meal-vitamin mineral supplement: 730-230-40 g/kg) and group M was offered concentrates based on MB (with fixed DM proportion of MB to DB) supplemented with equal value to group D of rapeseed meal and vitamin mineral supplement. Both groups were offered grass-legume silage *ad libitum.* Each forage was fed twice daily. The data were analysed using analysis of variance to test for the effect of silage treatments. For the feed intake, a group of 9 cows was considered as the experimental unit. For milk yield, milk protein yield and milk fat

yield respectively, each cow within a group was considered as the experimental unit. a probability of 0.05<P<0.10 was considered a near-significant trend.

Results and Discussion

The chemical composition of DB and ensiled MB is presented in Table 1. The results of the experiment show that ensiled MB has significantly higher basic nutrient content and significantly higher digestible energy concentration when compared with DB.

Table1: Chemical composition of dried and ensiled moisture barley grain

Treatment	Dry matter	Crude protein	Starch	Ash	Sugar	ADF	NDF	Dig. energy, MJ/kg DM
Dried barley (DB)	842	140	589	25	32	105	174	14.2
Ensiled barley (MB)	563	149	570	26	60	87	157	15.3
SEM	48.25	1.53	3.35	0.3	5.04	3.31	3.42	0.18
P	**	**	**	ns	**	**	**	**

*Significance: ** = P<0.01*

The fermentation quality of ensiled MB was good as indicated by optimal pH (4.09), ammonia-N (48.1 g/kg N), and volatile fatty acids (21.5 g/kg DM) content. Lactic acid, butyric acid and ethanol concentrations in ensiled MB were 12.9, 0.1 and 4.0 g/kg DM respectively. Similarly the quality of grass-legume silage was good with pH value (4.2), dry matter (320 g/kg), ammonia-N (46 g/kg N) and volatile fatty acids (32 g/kg DM) content. Lactic acid content was 44 g/kg DM and butyric acid was not detected. The results of a study confirmed good hygienic quality of anaerobically preserved MB grain. Fungal counts and yeast counts were lower in ensiled MB compared with DB. Aflatoxin and zearalenone concentrations tended to be lower in ensiled MB than in DB. (Table 2).

Table 2: Hygiene parameters evaluated in DB and ensiled MB

	DB	Ensiled MB
Deoxynivalenol (DON), mg/kg	0.063	0.0197
Zearalenone (ZEN), mg/kg	<0.0078	<0.00175
Aflatoxin B1, mg/kg	<0.0032	<0.001
Yeast, cfu/g	8.1x104	7.0x102
Fungal counts, cfu/g	2.0x105	<1.0x10
Contamination with dominant genera of moulds, %	Acremoniella – 3.3 Bipolaris – 16.7 Cladosporium – 1.7 Fusarium – 30.0 Ulocladium – 8.3	Cladosporium – 10.0 Others – 0.3
Dominant colonies of molds	Cladosporium cladosporioides, Fusarium soloni, F. Sporotrichiodes, F. tricinctum, Penicillium spp. Sporotrix schencki, Ulocladium chartarum	Cladosporium cladosporioides C. herbarum

The cows consumed large quantities of the silages (12.1 *vs* 11.8 kg DM per cow per day) when the ensiled MB diet was offered (Table 3). The increased silage intake and higher MB digestible energy value and crude protein concentration were reflected in higher milk yield, with higher milk fat concentration and a higher by 1.3 kg per cow per day ECM. Can be supposed that the cows fed moisture grain have slower starch digestion in the rumen and that results in improved utilisation and improved rumen function (Knowlton et al., 1998). However, a more recent dairy cow trial conducted by Jaakkola et al. (2005) did not detect any difference in dry matter take when dry barley was replaced with ensiled crimped barley in a total mixed ration.

Table 3: The effect of ensiled moisture barley on forage intake, milk yield and milk composition in cows

Treatment	Feed intake, kg DM/day/cow		Milk and constituent yield, kg/day/cow				Milk composition, g/kg		
	Silage	Conc.	Milk	ECM	Fat	Prot.	Fat	Protein	Lactose
DB	11.8	6.7	19,8	20.9	0.864	0.662	43.1	33.1	48.1
Ensiled MB	12.1	6.7	21.1	22.2	0.922	0.734	43.9	34.7	49.8
SEM	0.32	0	0.45	0.49	0.02	0.02	0.03	0.04	0.04
P	0.224	0	0.199	0.172	0.088	0.167	0.538	0.063	*

*Significance: * = P<0.05*

Conclusions

The results suggest that ensiled crimped moisture barley has higher nutritive value than dry barley and shows a tendency to increase grass-legume silage intake, milk yield and to improve milk quality. Ensiling of high moisture barley may reduce fungal counts and mycotoxins concentrations and can help to avoid the animal productivity problems potentially caused by the ingested mycotoxins.

References

[1] Jaakkola, S., E. Saarisalo, and R. Kangasniemi. 2005. Ensiled high moisture barley in the grass silage-based diet of dairy cows. Proc. 14th International Silage Conf. a Satellite workshop 20th International Gras. Congr., Belfast, Northern Ireland:184.

[2] Knowlton, K. F., B. P. Glenn and R. A. Erdman. 1998. Performance, Ruminal Fermentation, and Site of Starch Digestion in Early Lactation Cows Fed Corn Grain Harvested and Processed Differently, J. Dairy Sci. Vol. 81 No. 7 1972-1984

[3] Ensiled high-moisture grain: Better than its dry version. 2009. Feed Mix magazine Volume 17 No. 4

Effects of Variety Type and Maturity at Harvest on Whole Crop Maize Silage Characteristics

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Introduction

During the ensiling process, easily degradable carbohydrates are fermented to lactic acid, acetic acid, and alcohol (mainly ethanol). The formation of acids lowers silage pH, which has a preserving effect (Wilkinson, 2005). In practice however, sometimes very low pH-values are observed. Data from the laboratory of AVEVE (Merksem, Belgium) showed end-pH values for maize silage of 3.3 and even lower (Christiaenen, 2007). Intake of large amounts of acid silage combined with acid formation from high concentrate portions may depress rumen pH and provoke digestive problems (rumen acidosis, displaced abomasum) in high yielding dairy cows. No proof of this hypothesis was found in literature. Maize breeders for whole crop silage have developed different variety types, from early maturing tot late maturing types, from stay green-types to dry down-types (very rapidly maturing types) (Barrière et al., 2006). The aim of the laboratory experiment was to investigate the influence of variety and harvest time on whole crop maize silage characteristics. The experiment was supported by the Flemish Government.

Materials and Methods

Four variety types were involved in the laboratory experiment: Justina®[1] as dry down-, Lafortuna® as stay green- , Franky® as late maturing- and Aurelia® as early maturing type. Five airtight microsilos of 2.75l content and equipped with CO_2 –valve (5 per treatment) were made for each variety, striving at a density of 200 kg of dry matter per m³. Harvest took place at 5 times in order to finally obtain material within three distinct dry matter (DM) ranges 27-30%, 30-35% and higher if possible (see table 1). At harvest, dry matter content, sugar content and density were determined; particle size was measured by a sieve method (Melcion et al., 1981). At desiling after 50 days 4 silos per treatment were analysed for DM, crude protein (CP) and ash as well as the fermentation characteristics (pH, ammonia, lactic acid, volatile acids and alcohols). DM content was corrected for loss of volatile compounds during oven drying according to Dulphy and Demarquilly (1981. Data were statistically analysed by SAS. Normality was tested by Kolmogorov-Smirnov. Normally distributed variables were analysed by one-way ANOVA with Tukey as *post hoc* test. Crude fibre (CF), residual sugar and starch content were determined on a pooled sample per treatment. Finally, the physical structure of the silage was calculated using the formula of De Brabander (1999): SW=-0.1 + 0.009*CF.

[1] *mandatory for Belgium : Aurelia® : Clovis-Matton, Lafortuna® : KWS Benelux, Justina® : Pioneer Hi-Bred and Franky® : Scam.

Table 1: Characteristics of the chopped whole crop maize at harvest

variety	harvest	gADM[1]/kg	sugar (g/kg DM)	density (kg/m^3)	PL[3] (mm)
Aurelia	1	261	84	188	3.8
Aurelia	2	293	41	185	4.7
Aurelia	3	341	6	173	5.5
Lafortuna	1	254	46	160	4.7
Lafortuna	2	322	21	169	4.6
Lafortuna	3	342	12	174	5.7
Justina	1	267	50	184	4.6
Justina	2	322	67	182	nd
Justina	3	335	31	180	6.4
Franky	1	239	52	147	nd[2]
Franky	2	280	31	168	4.1
Franky	3	288	31	157	4.1

[1] ADM= g absolute dry matter/kg fresh weight at harvest, 2- not determined, 3 particle length

Results and discussion

Especially when the DM content at time of harvesting was low, it was difficult to obtain the desired density. For all varieties except for Justina , sugar content decreased with increasing DM content. Particle size tended to increase with DM content. As 2008 was a rather late harvest season, the highest DM range was not reached at ensiling. At desiling, distinct DM ranges were obtained for Aurelia, Lafortuna and more or less for Justina. For Franky, DM contents were rather low and differences were rather small in order to draw conclusions. The pH never reached very low levels and differences were small; pH significantly increased with higher DM content. There were significant differences among varieties within the same DM range, but these differences were rather small.. There were no significant differences in acetic acid content among treatments. Within the same variety there was a significantly lower lactic acid and alcohol content with higher DM content,but in absolute terms differences were small. Except for Franky, there was a tendency towards a lower crude fibre content and a higher starch content as DM content at harvest time increased. Finally, residual sugar contents were very low and quite similar for the different treatments.

Conclusion

In this laboratory experiment, variety type and harvesting date did not have a major influence on fermentation pattern and end-pH, as well as residual sugar content.

References

[1] Barrière Y., Alber D., Dolstra O., Lapierre C., Motto M., Ordas A., Van Waes J., Vlasminkel L., Welcker C., Monod J.P. (2006) Past and prospects of forage maize breeding in Europe, II History, germplasm evolution and correlative agronomic changes, Maydica 51, p435-449

[2] Christiaenen M. (2007), Personal communication, mc.veevoeding@aveve.be, AVEVE, Merksem, Belgium

[3] De Brabander, D. ,L. (1999) Ontwikkeling van een structuurwaarderingssysteem voor de melkveevoeding, Doctoraat proefschrift, Universiteit Gent, 193p.

[4] Dulphy J.P.,Demarquilly C. (1981) Problèmes particuliers aux ensilages. Prévision de la valeur nutritive des aliments, INRA Publications, Versailles, 577p.

[5] Melcio J.-P., Delort-Laval J., (1981). Méthodes objectives d'appréciation des caractéristiques physiques des aliments composés. In: Prévision de la valeur nutritive des aliments des Ruminants, INRA Publ., p. 307-323.

[6] Wilkinson, J.M. (2005) Silage, Ruddocks Prints, Lincoln

Table 2: Fermentation characteristics and chemical analysis of whole crop maize silage

Variety	Harvest	pH	CDM[1] (%)	CA[2] (g/kg DM)	CP[3] (g/kg DM)	AA[4] (g/kgDM)	LA[5] (g/kg DM)	NH_3 (g/kgDM)	alcohol (g/kg DM)	CF[6] (g/kg DM)	structure value	Sugar (g/kg DM)	starch (g/kg DM)
Aurelia	1	3.81 a[7]	279 a	42 a	72 a	17 a	60 a	0.8 a	34 a	234	2.0	3.9	295
Aurelia	2	3.83 b	313 b	45 a	69 a	17 a	58 a	0.9 a	23 b	232	2.0	2.0	317
Aurelia	3	3.92 c	361 c	41 a	70 a	16 a	46 b	0.7 b	13 c	201	1.7	4.0	394
Lafortuna	1	3.77 a	279 a	46 a	73 ab	16 a	64 a	0.1 a	31 a	210	1.8	3.9	320
Lafortuna	2	3.88 b	303 b	49 a	75 a	19 b	57 ab	0.9 a	17 b	216	1.8	2.0	367
Lafortuna	3	3.90 c	369 c	51 a	71 b	16 a	51 b	0.6 b	15 b	187	1.6	2.0	405
Justina	1	3.73 a	290 a	37 a	69 a	19 a	56 a	0.8 a	34 a	233	2.0	2.0	285
Justina	2	3.77 b	335 b	40 ab	66 b	17 a	48 b	0.7 b	23 b	220	1.9	2.0	334
Justina	3	3.84 c	347 c	44 b	71 c	17 a	49 b	0.7 b	18 c	214	1.8	4.0	365
Franky	1	3.80 a	277 a	45 a	66 a	17 a	57 a	1.0 a	20 a	264	2.3	2.0	277
Franky	2	3.85 b	284 a	46 a	76 b	18 a	52 b	0.8 a	16 a	242	2.1	2.0	300
Franky	3	3.82 c	315 a	61 b	61 c	17 a	52 b	0.5 b	20 a	255	2.2	2.0	272
Aurelia	1	3.81 a	279 b	42 b	72 ab	17 a	60 ab	0.8 a	34 a				
Lafortuna	1	3.77 b	279 b	46 a	73 a	16 a	64 a	1.0 a	31 a				
Justina	1	3.73 c	290 a	37 c	69 bc	19 a	56 b	0.8 a	34 a				
Franky	1	3.80 a	277 b	45 ab	66 c	17 a	57 ab	1.0 a	20 b				
Aurelia	2	3.83 a	313 b	45 ab	69 bc	17 b	58 a	0.9 ab	23 a				
Lafortuna	2	3.88 b	303 b	49 a	75 a	19 a	57 ab	0.9 ab	17 a				
Justina	2	3.77 c	335 a	40 a	66 c	17 b	48 c	0.7 b	23a				
Franky	2	3.85 d	284 c	46 a	76 a	18 ab	52 b	0.8 ab	16 a				
Aurelia	3	3.92 a	361 ab	41 c	70 a	16 a	46 b	0.7 ab	13 c				
Lafortuna	3	3.90 b	369 a	51 b	71 a	16 a	51 a	0.6 b	15bc				
Justina	3	3.84 c	347 b	44 bc	71 a	17 a	49 ab	0.7 a	18ab				
Franky	3	3.82 c	315 c	61 a	61 b	17 a	51 a	0.5 c	20a				

[1]corrected dry matter, [2]crude ash, [3]crude protein, [4]acetic acid, [5]lactic acid, [6]crude fibre, [7]means in the same column within the same variety or harvest moment followed by the same letter don't differ significantly P<0.05

Effects of Stage of Growth of Grasses and Conservation Method on Amino Acids Degradability in the Rumen and Intestinal Digestibility of Rumen By-pass Amino Acids

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Introduction

Feeding of modern dairy cows requires balancing the diets not only in terms of protein digested in the intestines but also intestinally absorbable amino acids [**AADI**; Lappierre et al., 2006]. In the models of calculation of AA absorbed in the intestines the assumption that ruminal degradability (**deg**) or intestinal digestibility (**dig**) of particular AA and protein are equal is still in use [Rulquin et al., 2001]. However, it has been shown in many experiments that it is not always true [e.g. Erasmus et al., 1994; Zebrowska et al., 1997]. Majority of these trials considered concentrates and only very few were conducted on forages. Stage of growth and conservation method can affect the content of NPN in total N in grasses, which in turn may have an impact on protein deg and dig. Little is known on the effect of such changes on AA deg and dig as well as on the relationship between AA and protein deg or dig. Thus the aim of a study was to determine the effect of stage of growth of grass and conservation method on protein and AA deg as well as on by-pass protein and AA dig and to determine the correlation between AA and protein deg or dig.

Material and Methods

First cut pasture sward was harvested in three different stages of growth of main grasses: early earing (EE), earing (E) and before flowering (BF). Green herbage was frozen (G), conserved as hay (sunny weather; H) or ensiled either after 24 h wilting (SW) or with formic acid (85%, 3 l/t; SFA). Silages were made in 60 l plastic silos. Representative samples of all forages were oven-dried (50°C/48 h) and ground (1.5 mm). The study was carried out on 3 bulls with rumen and duodenum cannulas, fed standard diet. In sacco and mobile nylon bag technique procedures were performed according to Polish standards [Kowalski et al., 2008]. The N content was determined in pooled residues after ruminal or intestinal incubations for each animal, whereas AA composition was determined in pooled samples for each incubation time (Beckman HPLC 126AA). Degradability rate constants and effective rumen degradability (ERD; k=6%/h) were estimated according to Ørskov and McDonald [1979], using MARQUARDT method [SAS, 2004]. The results were subjected to two-way analysis of variance and SNK test using GLM procedure [SAS, 2004].

Results and Discussion

Irrespective of conservation method, delaying of harvest decreased CP content, particularly between EE and E stages. It also increased NPN %total N in green herbage and hay, whereas in silages the tendency was the oposite. Silages made of older grasses contained less NPN %total N. Ensiling did not change the CP content but increased NPN %total N from 21.2 to 49.6%. Proteolysis was the most intensive in silages made of grass cut at EE. CP content and NPN % total N were similar in hay and green herbage. There was no clear treatment effect on AA composition.

Aging of grass decreased ERD of protein (77.3, 73.5, 70.8% for EE, E, BF respectively; $P<0.01$), which was particularly seen for silages. Such tendency was due to decreased b and c constants. The lowest by-pass protein dig was found for E forages (68.6, 54.9, 58.9%; $P<0.01$). Irrespective of stage of growth and ensiling method, ERD of silage protein was higher than of green herbage and hay (69.9, 77.4, 79.0, 69.3% for G, SW, SFA, H respectively; $P<0.01$). The highest differences between silages and other forages were found in EE stage of growth, and the lowest in BF. Among conservation methods, silages were also characterized by

the highest a and the lowest b fractions. On the other hand, by-pass protein of silages was significantly less digested in the intestines (64.5, 54.5, 54.9 and 69.4%; $P<0.01$). The highest by-pass protein dig was found for hay. Most systems use one single value describing either protein deg or dig for a particular forage [INRA, 2007]. However, the results of our study suggest that for grass silages the effect of stage of growth should be considered. The effect of ensiling method, formic acid vs. wilting, on protein deg and dig was lower than stage of growth.

Similarly as for protein deg, delaying of harvest, irrespective of conservation method, decreased significantly ERD of all AA studied, particularly between EE and E stages. On the other hand, ensiling, irrespective of the method, increased ERD of alanine, isoleucine, leucine, lysine and valine. Compared to other methods, ensiling with formic acid increased deg of phenylalanine, glycine, glutamic acid, histidine, methionine, proline, serine, threonine and tyrosine. Deg of valine from SFA was significantly higher than from SW, whereas there were no differences between SFA and H in proline deg. Above differences between forages were due to higher a and lower b fractions. The highest a values for majority of AA were found for SFA. There was no conservation method effect on deg of aspartic acid and cysteine. Moreover, there were no differences between green herbage and hay in deg of almost all AA studied. Generally, all above tendencies were found irrespective of stage of growth. The relationship between protein and AA deg depended on AA. The coefficient of correlation ranged from about 0.40 (for arginine and aspartic acid) to about 0.90-0.95 (for isoleucine, leucine and valine). Delaying of harvest, irrespective of the conservation method, increased the differences between protein and AA deg. For E and BF stages of growth, except for alanine and proline, AA deg was lower than protein deg. Moreover, protein and AA deg were similar for either green herbage or hay, whereas AA deg was lower than protein deg for both silages, particularly in the BF stage of growth.

Aging of grass, irrespective of conservation method, decreased all by-pass AA dig, which was particularly seen between EE and E stages. Moreover, in all stages of growth by-pass AA dig of almost all AA (except histidine, tyrosine and cysteine) was much higher than by-pass protein dig. The highest by-pass AA dig was found for hay, whereas the lowest for silages, particularly SW. Average by-pass AA dig was 73.6, 66.3, 72.7, 79.5%, respectively for G, SW, SFA, H, and it was higher than by-pass protein dig. Average coefficient of correlation between by-pass protein and AA dig was 0.80, with the lowest for alanine (0.69) and the highest for arginine and proline (0.91).

Conclusions

Stage of growth and conservation method affected protein and AA degradability in the rumen and by-pass protein and AA intestinal digestibility, which should be taken into the consideration in nutritive value tables. Since AA deg or dig differ from protein deg or dig it should also be considered in calculations of AADI.

References

[1] Erasmus, L. J., Botha P. M., Cruywagen C. W., Meissner H. H. 1994. Amino acid profile and intestinal digestibility in dairy cows of rumen-undegraded protein from various feedstuffs. J. Dairy Sci. 77, 541-551.

[2] INRA 2007. Alimentation des Bovins, Ovins et Caprins, Tables INRA 2007. Quae Éditions.

[3] Kowalski Z.M., Strzetelski J. A., Niwińska B., Nowak W., Pająk J., Szyszkowska A. 2008. Standardowe metody oznaczania rozkładu białka pasz w przewodzie pokarmowym zwierząt przeżuwających (in Polish). Wiadomości Zootechniczne, XLVI, 4, 53-58.

[4] Lapierre H., Pacheco D., Berthiaume R., Ouellet D. R., Schwab C. G., Dubreuil P., Holtrop G., Lobley G. E. 2006. What is the true supply of amino acids ? J. Dairy Sci. 89 (E Suppl.), E1–E14.

[5] Ørskov E. R., McDonald I. 1979. The estimation of protein degradability in the rumen from incubation measurements weighed according to the rate of passage. J. Agric. Sci. Camb. 92, 499-503.

[6] Rulquin H., Vérité R., Guinard-Flament J. 2001. Amino acids truly digestible in the small intestine: the AADI system for the dairy cows. INRA Prod. Anim. 14, 265–274.
[7] SAS 2004. SAS/STAT(r) 9.1 User's Guide. Cary, NC: SAS Institute Inc.
[8] Żebrowska T., Długołęcka Z., Pająk J.J., Korczyński W. 1997. Rumen degradability of concentrate protein, amino acids and starch, and their digestibility in the small intestine of cows. J. Anim. Feed Sci. 6, 451-470.

Section 4: Production of Biogass by Conserved Forages, Greenhouse Gases and Animal Agriculture, Ecology

Greenhouse Gases and Sustainable Animal Agriculture

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Summary

Major greenhouse gases (GHG) attributed to animal agriculture sector are methane (CH4) and nitrous oxide (N2O), though carbon dioxide (CO2) contributes almost half of total greenhouse effect. Rumen CH4 emission in an enteric fermentation can be accounted as the biggest anthropogenic source. The abatement mechanism of rumen CH4 emission may be divided to direct and indirect suppression to methanogens in the rumen. The most significant strategy to mitigate ruminal CH4 emission in indirect manner is to promote alternative metabolic pathway to dispose of the reducing power, competing with methanogenesis for H2 uptake. The efficient prebiotics and probiotics have been innovated to mitigate rumen CH4 emission instead of ionophores in respect to food safety. They are mostly propionate enhancers which consume metabolic hydrogen (H2) compete with methanogens and abate rumen methanogenesis in indirect manner. However, assimilate nitrate reduction in the rumen which shows strong redox potential is effective to mitigate rumen CH4 emission without toxic nitrite accumulation when L-cysteine is simultaneously administered. Furthermore, Escherichia coli modified genetically has been developed to promote nitrite reduction abating ruminal methanogenesis. One of protease-resistant antimicrobial substances (PRA) has been isolated from Lactobacillus plantarum as a direct suppressor of rumen methanogens. The suppressing effect of PRA on rumen methanogenesis were examined using the in vitro continuous methane quantification system.

The possible use of agricultural biomass consisted of non-edible parts of crop plants such as cellulose and hemi-cellulose and animal wastes was proposed as a renewable energy and nitrogen sources. The ammonia stripping from digested slurry of animal manure in biogas plant to apply three options of nitrogen recycling to mitigate N_2O emission. In the first option of the ammonia stripping the effect of ammonolysis on feed value of cellulose biomass was evaluated on digestibility, energy metabolism and protein utilization. Saccharification of the NH_3 treated cellulose biomass was confirmed in strictly anaerobic incubation with rumen cellulolytic bacteria, *Ruminoccous fravefaciens*, to produce bio-ethanol as the second option of ammonia stripping. In an attempt of NH_3 fuel cell, the reformed hydrogen from the NH_3 stripped from 20 l of digested slurry in thermophilic biogas plant could generate 0.12W electricity with proton exchange membrane fuel cell (PEM) as the third option.

***Key words*:** GHG, rumen methane, probiotics, ammonia stripping, biomass

The mitigation of anthropogenic four GHG, carbon dioxide (CO2), methane (CH4), nitrous oxide (N2O) and sulphur hexafluoride (SF6) and two groups of GHG, hydrofluorocarbons (HFCs) and perfluorocarbons (PFCs) have been established as legally binding commitments in The Kyoto Protocol (IPCC, 1996). Important GHG attributed to animal agriculture are CH4 and N2O. Rumen fermentation of ruminant livestock and anaerobic fermentation of agricultural organic waste including animal manures are major contributors of CH4 emission as anthropogenic sources (Moss, 1993).

To abate the GHG, the development of mitigation methods of rumen CH4 is the most significant issue in the world ruminant livestock production (Van Nevel and Demeyer, 1996).. The prompt increase of atmospheric N2O since last century is closely related to abrupt expansion of human and animal population after an innovation of Haber-Bosch process. Severe environmental pollutions were caused at the same time though the reactive nitrogen withdrawn from atmosphere as stable paired nitrogen brought about prosperous food production. To secure food production preventing environmental catalyses by global warming sustainable development of animal agriculture should be sought in not only developed but also developing countries as an alternative way. Inventories of emitters and their abatements are accurately

assessed in both GHG to develop “clean developing mechanism (CDM)” in Kyoto Mechanism. The CDM might give highly economical and environmental incentives for the implementation in developing countries. The key element of these recycling must be low-input for sustainable animal agriculture in developing countries. Carbon and nitrogen recycling in the agricultural biomass as renewable energy and nitrogen resources might contribute mitigation of CH4 and N2O (Takahashi, et al., 2003, 2004, 2007; Takahashi and Uemura, 2009).

The present paper deals with perspective on GHG control and possible uses of biomass towards sustainable animal agriculture.

Mitigation of rumen methane emission with prebiotics and probiotics.

In the rumen, metabolic H_2 is produced during the anaerobic fermentation of glucose. This H_2 can be used during the synthesis of volatile fatty acids and microbial organic matter. The excess H_2 from NADH is eliminated primarily by the formation of CH_4 by methanogens, which are microorganisms from the *Archea* group that are normally found in the rumen ecosystem (Baker, 1999). The stoichiometric balance of VFA, CO_2 and CH_4 indicates that acetate and butyrate promote CH_4 production whereas propionate formation conserves H_2, thereby reducing CH_4 production (Wolin, and Miller, 1960). Therefore, a strategy to mitigate ruminal CH_4 emission is to promote alternative metabolic pathway to dispose of the reducing power, competing with methanogenesis for H_2 uptake. As assimilate nitrate reduction in the rumen which shows strong redox potential is relatively higher affinity to H_2 than hydrogenotrophic methanogenesis, the administration of nitrate remarkably suppressed ruminal methanogenesis (Takahashi and Young, 1991, 1992).

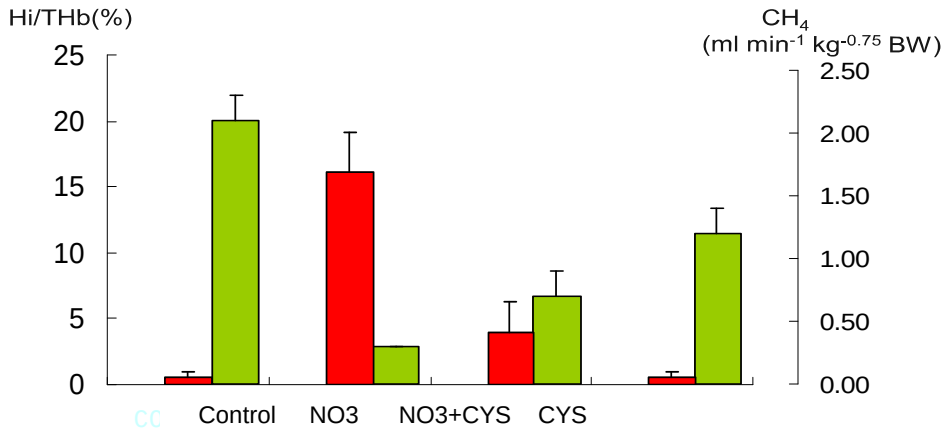
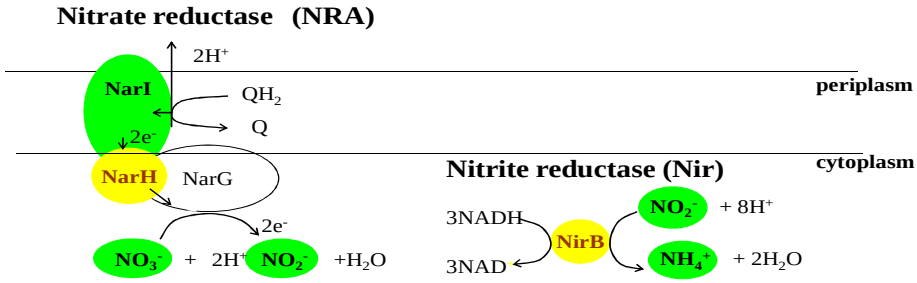


Fig.1. The suppressing effect of nitrate (1.3 g $NaNO_3$ $kg^{-0.75}$ body weight) on methane emission and prophylactic effect of L-cysteine (0.21 g S $kg^{-0.75}$ body weight) on nitrate-induced methemoglobinemia in sheep

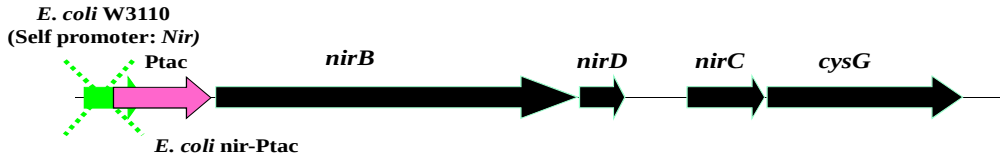
Fig.1 shows that the formation of toxic nitrite reduced from nitrate is successfully prevented by L-cysteine (Takahashi and Young, 1991, 1992; Takahashi et al., 1989, 1998, 2000, 2002), *i.e.* the effective mitigation of ruminal CH_4 emission is safely achieved by simultaneous administration of nitrate and L-cysteine without nitrate intoxication (Takahashi, 2001). Furthermore, *Escherichia coli* modified genetically was developed in an attempt to promote nitrite reduction abating ruminal methanogenesis (Sar et al., 2004a; 2005a; 2005b; 2005c;) (Fig.2).

Figure 2: Wild-type E.coli W3110 and the construction of E.coli nir-Ptac

1. Wild-type *E. coli* W3110



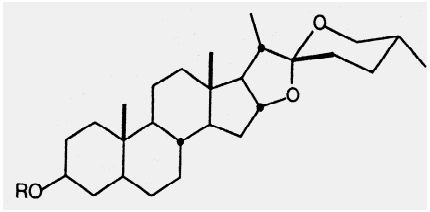
2. Construction of *E. coli* nir-Ptac by replacement of self-promoter (*nir)* in *E. coli* W3110 by *tac* promoter (Ptac) (Ajinomoto Co. Inc., Kawasaki, Japan)



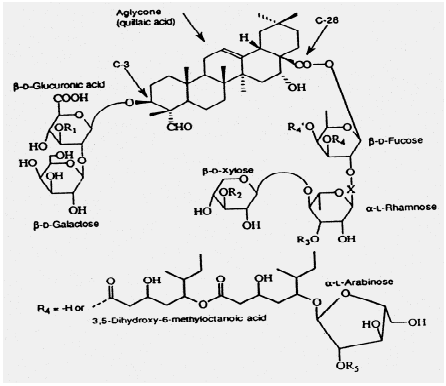
Rumen manipulation with ionophores such as monensin has been reported to abate rumen methanogenesis (Mwenya, et al., 2005), However, there is an increasing interest in exploiting prebiotics and probiotics as natural feed additives to solve problems in animal nutrition and livestock production as alternatives of the antibiotics due to concerns about incidences of resistant bacteria and environmental pollution by the excreted active-antibacterial substances (Mwenya et al., 2006). Nisin and saponin-containing extracts of Yucca schidigera and Quillaja saponaria have been categorized as 'generally recognized as safe (GRAS)' for human consumption by US Food and Drug Administration. Nisin produced by Lactococcus lactis subsp. lactis, antimicrobial activity against spectrum of Gram-positive bacteria is characterized bacteriocin and performed mitigating effect on ruminal methane emission (Mwenya et al., 2004a; Santoso et al., 2004b; Sar et al., 2006). Saponins are natural detergents found in variety of plants. Yucca saponins have a steroidal nucleus, whereas Quillaja saponins are triterpenoid in structure (Fig.3). Supplementation of saponin-rich plant extracts decreased ruminal protozoa counts and decreased methanogenesis accompanied by decrease in the ruminal acetate/ propionate (A/P) ratio in vitro and in vivo (Wallace et al., 1994; Wang et al., 2000, Takahashi et al., 2000; Santoso et al., 2004a; Mwenya et al., 2004a; Pen, et al., 2006). However, Pen et al. (2007; 2008) showed in recent detailed examination that Q. saponaria had no effect on ruminal methanogenesis and A/P ratio, although it suppressed protozoa number.

Figure 3: Chemical structure of Yucca schidigera and Quillaja saponaria

Yucca schidigera
(Steroidal saponins)

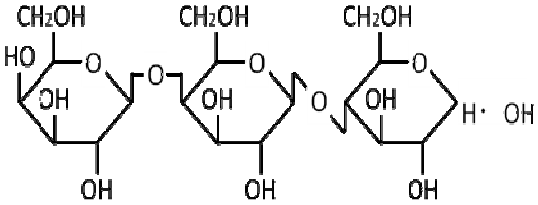


Quillaja saponaria
(triterpenoid saponins)



Galacto-oligosaccharides (GOS) are non-digestible carbohydrates in nonruminants and have a long history of research as a prebiotics food ingredient. GOS are resistant to gastrointestinal enzymes, but are selectively utilized Bifidbacteria (Sako et al., 1999). In the rumen, *Bifidobacteria* and *Lactobacillus* species utilize fructose, galactose, glucose and starch as substrates to produce lactate and acetate. Lactate is intermediate compound of a acrylate pathway during propionate production in the rumen. Meanwhile, propionate production is indirect competition with methanogens for available hydrogen. As *Bifidobacteria* and *Lactobacillus* species in the rumen can utilize GOS and produce more lactate,

Figure 4: Chemical structure of β1-4 galacto-oligosaccharides



ruminal methanogenesis have been suppressed by β1-4 galacto-oligosaccharides with or without direct-fed microbe yeasts and lactic acid bacteria (Gamo, 2001; Mwenya et al., 2004b; 2004c; 2004d: 2005; Santoso, 2004a; Sar et al., 2002; 2004b; 2004c; Takahashi et al., 2002; 2003). However, the efficacy of β1-4 galacto-oligosaccharides with the probiotics on different diets and animal species remains to be elucidated.

Figure 5: Effect of PRA on potential methane production. Control: Lactococcus lactis ATCC19435 (non-antibacterial substances), Nisin-A: Lactococcus lactis NCIMB702054, PRA-1: Lactobacillus plantarum TUA1490L (, and PRA-2: Leuconostoc citreum JCM9698. Vertical bars represent standard deviation (n = 4). Means with different letters differ significantly (p<0.01).

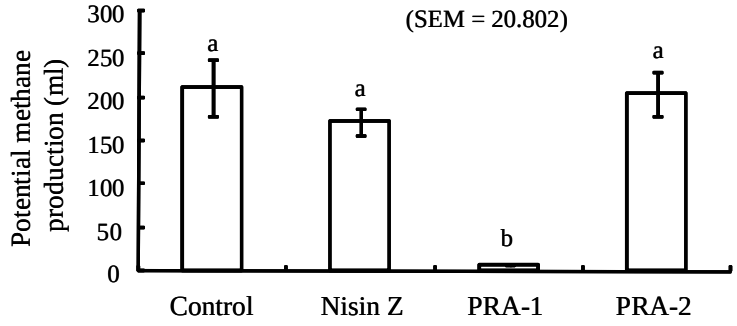
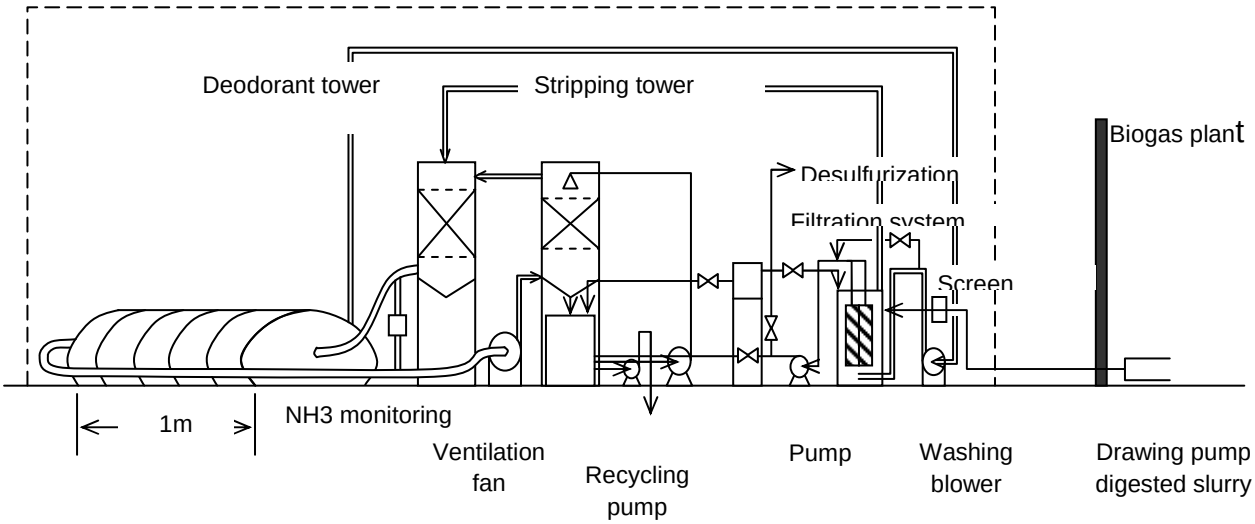


Fig.5 shows that effects of protease-resistant antimicrobial substances (PRA) produced by *Lactobacillus plantarum* and *Leuconostoc citreum* on rumen methanogenesis were examined using the *in vitro* continuous methane quantification system (Asa, 2010). Four different strains of lactic acid bacteria, Control: *Lactococcus lactis* ATCC19435 (non-antibacterial substances), Nisin-Z: *Lactococcus lactis* NCIMB702054, PRA-1: *Lactobacillus plantarum* TUA1490L, and PRA-2: *Leuconostoc citreum* JCM9698 were individually cultured in GYEKP medium. An 80 ml aliquot of each supernatant was inoculated into phosphate-buffered rumen fluid. PRA-1 remarkably decreased cumulative methane production. For PRA-2, there were no effects on CH_4 and CO_2 production and fermentation characteristics in mixed rumen cultures. The results suggested that PRA-1 reduced the number of methanogens or inhibited utilization of hydrogen in rumen fermentation.

Creation of renewable energy (biogas) from anaerobic fermentation (biogas plant) of animal manures and the innovative reuse of the digested slurry to mitigate N_2O

The increased emissions of CH_4 and N_2O from decomposing unmanaged and bio-based industrial wastes along with the expansion of human activities contribute climate change as GHG. The biogas plant produce biogas including combustible CH_4 as renewable energy using unused resources like animal manures, can provide fuel, heat and electricity (Takahashi et al., 2004;Umetsu et al., 2005, 2006; Komiyama et al., 2006), and minimize the impact on the environment thus reducing the amount of pollutants discharged. Biogas system and its application have been expanded in APEC member economies as a mitigation strategy with an economical incentive (Takahashi, 2009).

Figure 4: Ammonia stripping apparatus



The conventional biogas system based on anaerobic fermentation of the organic wastes, however, is not a nitrogen recycling but carbon recycling one. Therefore, isometric fertilization of the digested slurry after anaerobic fermentation may not be a solution of current issue on excess nitrogen abatement, although nitrous oxide emission is almost completely suppressed during anaerobic fermentation (unpublished data). It causes not only methane emission, but also nitrate leaching and N_2O emission from soil (Takahashi, 2006). The introduction of ammonia stripping from digested slurry of themophilic biogas plant might be a solution to reduce total nitrogen content of the slurry as a liquid fertilizer containing suitable nitrogen and eventually can contribute the mitigation of N_2O emission as a new concept of biogas system (Fig. 4). Furthermore, the stripped ammonia can be put to practical use as a low-input and renewable nitrogen resource without energy supply from outside, because abundant amount of organic wastes exist in developing countries and the energy required for ammonia stripping can be supplied from biogas plant attached to the ammonia stripping apparatus.

The following three options have been examined for future nitrogen recycling.

1. Production of high quality feed from cellulose biomass in agricultural waste with ammonia stripping process from digested slurry of biogas plant (Takahashi, 2006; 2007).
2. Saccharification of soft cellulose biomass to create bio-ethanol and hydrogen using ammonolysis by stripped ammonia from effluent and hydrolysis of rumen bacteria (unpublished).
3. Ammonia fuel cell with ammonia stripping from digested slurry (Takahashi and Uemura, 2009; FOCUS, April 14, 2009).

Reference

[1] Asa, R., A. Tanaka, A. Uehara, I. Shinzato, Y. Toride, N. Usui, K. Hirakawa and J. Takahashi. 2010. Effects of protease-resistant antimicrobial substances produced by lactic acid bacteria on rumen methanogenesis. Asian-Aust. J. Anim. Sci., (in the press).

[2] Baker, S.K., 1999. Rumen methanogens, and inhibition of methanogenesis. Aust. J. Agric. Res., 50: 1293-1298.

[3] IPCC, 1996. Revised 1996 IPCC Guidelines for National Greenhouse Gas Inventories. IPCC-NGGIP.

[4] Gamo, Y., M. Mii, X.G. Zhou, C. Sar, B. Santoso, I. Arai, K. Kimura and J. Takahashi, 2001. Effects of lactic acid bacteria, yeasts and galactooligosaccha-ride supplementation

on in vitro rumen methane production. In: Proceedings of the 1st International Conference on Greenhouse Gases and Animal Agriculture (GGAA), J. Takahashi and B.A Young (eds.) Obihiro, Japan, 7-11 Nov. 2001, Obihiro, Hokkaido, Japan. pp371-374.

[5] Komiyama, M., K. Umetsu and J. Takahashi, 2006. Biogas as a reproducible energy source:Its steam reforming for electricity generation and for farm machine fuel. R. C. Soliva, J. Takahashi and M. Kreuzer (eds.), In: Greenhouse Gases and Animal Agriculture: An Update, Elsevier Science, 234-237.

[6] Moss, A. R., 1993. Methane: Global Warming and Production by Animals. Cantebury. Chalcombe Publications.

[7] Mwenya, B., C. Sar, Y. Gamo, T. Kobayashi, R. Morikawa, K. Kimura, H. Mizukoshi, and J. Takahashi. 2004a. Effects of Yucca schidigera with or without nisin on ruminal fermentation and microbial protein synthesis in sheep fed silage- and hay- based diets. Anim. Sci. J., 75: 525 - 531.

[8] Mwenya, B., X., Zhou, B. Santoso, C. Sar, Y. Gamo, T. Kobayashi and J. Takahashi, 2004b. Effects of probiotic-vitacogen and β1-4 galacto-oligosaccharides supplementation on methanogenesis and energy and nitrogen utilization in dairy cows. Asian-Aust. J. Anim. Sci., 17: 349-354.

[9] Mwenya, B., B. Santoso, C. Sar, B. Pen, R. Morikawa, K. Takaura, K. Umetsu, K. Kimura and J. Takahashi. 2004c. Effects of yeast culture and galacto-oligosaccharides on ruminal fermentation in Holstein cows. J. Dairy Sci., 88: 1404-1412.

[10] Mwenya, B., B. Satonso, C. Sar, Y. Gamo, T. Kobayashi, I. Arai, and J. Takahashi, 2004d. Effects of including β1-4 galacto-oligosaccharides, lactic acid bacteria or yeast culture on methanogenesis as well as energy and nitrogen metabolism in sheep. Anim. Feed. Sci. Technol., 115: 313 – 326.

[11] Mwenya, B. C. Sar, B. Santoso, T. Kobayashi, R. Morikawa, K. Takaura, K. Umetsu, S.Kogawa, K. Kimura, H. Mizukoshi and J. Takahashi. 2005. Comparing the effects of β1-4 galacto-oligosaccharides and L-cysteine to monensin on energy and nitrogen utilization in steers fed a very high concentrate diet. Anim. Feed Sci. Technol. 118: 19 – 30.

[12] Mwenya, B. C. Sar, B. Pen, R. Morikawa, K. Takaura, S. Kogawa, K. Kimura, K. Umetsu and J. Takahashi. 2006. Effects of feed additives on ruminal methanogenesis and anaerobic fermentation of manure in cows and steers. In: C. Soliva, J. Takahashi and M. Kreutzer (eds), Greenhouse Gases on Animal Agriculture update. International Congress Series. Elsevier, Amsterdam. 1293: 209-212.

[13] Pen, B., C. Sar, B. Mwenya, K. Kuwaki, R. Morikawa and J. Takahashi, 2006. Effects of Yucca schidigera and Quillaja saponaria extracts on in vitro ruminal fermentation and methane emission. Anim. Feed Sci. Technol., 129: 175-186.

[14] Pen, B., K. Takaura, S. Yamaguchi, R. Asa and J. Takahashi, 2007. Effects of Yucca schidigera and Quillaja saponaria with or without β 1-4 galacto-oligosaccharides on ruminal fermentation, methane production and nitrogen utilization in sheep. Anim. Feed Sci. Technol., 138: 75-88.

[15] Pen, B., C. Sar, B. Mwenya, K. Kuwaki and J. Takahashi, 2008. Effects of Quillaja saponaria extract alone or in combination with Yucca schidigera extract on ruminal fermentation and methanogenesis in vitro. Anim. Sci. J., 79: 193-199.

[16] Sako, T., K. Matsumoto and R. Tanaka, 1999. Recent progress on research and application of non-digestible galacto-oligosaccharides. Int. Dairy J. 9: 69-80.

[17] Santoso, B., B. Mwenya, C. Sar, Y. Gamo, T. Kobayashi, R. Morikawa, K. Kimura, H.Mizukoshi, and J. Takahashi. 2004a. Effects of supplementing galacto-oligosaccharides, Yucca schidigera or nisin on rumen methanogenesis, nitrogen and energy metabolism in sheep. Livest. Prod. Sci. 91: 209 – 217.

[18] Santoso, B., B. Mwenya, C. Sar, Y. Gamo, T. Kobayashi, R. Morikawa and J. Takahashi. 2004b. Effect of Yucca schidigera with or without nisis on ruminal fermentation and microbial protein synthesis in sheep fed silage- and hay-based diets. Anim. Sci. J. 75:525-531.

[19] Sar, C., B. Santoso, X.G. Zhou, Y. Gamo, A. Koyama, T. Kobayashi, S. Shiozaki and J.Takahashi. 2002. Effects of ß1-4 galacto-oligosaccharide (GOS) and Candida kefyr on nitrate-induced methaemoglobinemia and methane emission in sheep. In: Takahashi, J., and B.A. Young (Eds), Greenhouse Gases on Animal Agriculture. Elsevier, Amsterdam.
[20] Sar, C., B. Santoso, B. Mwenya, R. Morikawa, N.Isogai, Y. Asakura, Y. Toride and J.Takahashi, 2004a. Effect of Escherichia coli W3110 or Escherichia coli nir-Ptac on in vitro ruminal methanogenesis and nitrate/nitrite reduction. Proceeding of 11th Asian-Australasian Association of Animal Production Societies (AAAP) Congress, 5-9th September 2004, Kuala Lumpur, Malaysia. pp316-318.
[21] Sar C., B. Santoso, Y. Gamo, T. Kobayashi, S. Shiozaki, K. Kimura, H. Mizukoshi, I. Arai and J. Takahashi, 2004b. Effects of combination of nitrate with ß1-4 galacto-oligosaccharides and yeast (Candida kefyr) on methane emission from sheep. Asian-Aust. J. Anim. Sci., 17: 73-79.
[22] Sar C., B. Santoso, B. Mwenya, Y. Gamo, T. Kobayashi, R. Morikawa,K. Kimura, H.Mizukoshi and J. Takahashi, 2004c. Manipulation of rumen methanogenesis by the combination of nitrate with ß1-4 galacto-oligosaccharides or nisin in sheep. Anim. Feed Sci. Technol., 115: 129-142.
[23] Sar, C., B. Mwenya, B. Pen, K. Takaura, R. Morikawa, A. Tsujimoto, K. Kuwaki, N.Isogai, I. Shinzato, Y. Asakura, Y. Toride and J. Takahashi. 2005a. Effect of rumianl administration of Escherichia coli wild type or a genetically modified strain with enhanced high nitrite reductase activity on methane emission and nitrate toxicity in nitrate-infused sheep. Br. J. Nutr., 94: 691-697.
[24] Sar, C., Mwenya, B., Santoso, B., Takaura, K., Morikawa, R., Isogai, N., Asakura, Y., Toride and Takahashi, J., 2005b. The effect of Escherichia coli W3110 on ruminal methanogenesis and nitrate/nitrite reduction in vitro. Anim. Feed Sci. Technol., 118: 295-306.
[25] Sar, C., B. Mwenya, B.Santoso, K. Takaura, R. Morikawa, N. Isogai, Y. Asakura, Y.Toride and J. Takahashi. 2005c. Effect of Escherichia coli wild type or its derivative with high nitrite reductase activity on ruminal methanogenesis and nitrate/nitrite reduction in vitro. J. Anim. Sci., 83:644-652.
[26] Sar, C., B. Mwenya, B. Pen, R. Morikawa, K. Takaura, T. Kobayashi and J. Takahashi. 2006. Effect of nisin on ruminal methane production and nitrate/nitrite reduction in vitro. Aust. J. Agric. Res., 56: 803-810.
[27] Takahashi, J., N. Johchi and H. Fujita, 1989. Inhibitory effects if sulphur compounds, copper and tungsten on nitrate reduction by mixed rumen micro-organisms. Br. J. Nutr., 61:742-748.
[28] Takahashi, J. and B. A. Young.1991. Prophylacticeffect of L-cysteine on nitrate-induced alteration in respiratory exchange and metabolic rate in sheep. Anim. Feed Sci. Technol., 35:105-113.
[29] Takahashi, J. and B. A. Young.1992. The modulation of nitrate-enhanced hypothermia by sulphur compounds in cold-exposed sheep. Anim. Feed Sci. Technol., 39:347-355.
[30] Takahashi, J., M. Ikeda, S. Matsuoka and H. Fujita, 1998. Prophylactic effect of L-cysteine to acute and subclinical nitrate toxicity in sheep. Anim. Feed Sci. Technol., 74:273-280.
[31] Takahashi, J., Y. Miyagawa, Y. Kojima, and K. Umetsu. 2000. Effects of Yucca schidigera extract, probiotics, monensin and L-cysteine on rumen methanogenesis. Asian-Aust. J.Anim. Sci., 13:499–501.
[32] Takahashi, J. 2001. Nutritional manipulation of methanogenesis in ruminants. Asian-Aust. J. Anim. Sci., 14:131–135.
[33] Takahashi, J., C. Sar, Y. Gamo, K. Kimura, H. Mizukoshi and I. Arai. 2002. Suppression of rumen methanogenesis by alternative reductions. Proceeding of 10th International Congress, 23-27th September, 2002, New Delhi, India. p55.
[34] Takahashi J., Y. Gamo, B. Mwenya, B.Santoso, C. Sar, H. Umetsu, H. Mizukoshi, K.Kimura, O. Hamamoto. 2003. Control and energetic recycling of methane emitted from ruminants. In: Proceeding of Progress in research on energy and protein metabolism,

W.B. Souffrant and C.C. Metges (eds.), EAAP publication, No. 109, 13-18th September 2003, Rostock-Warnemünde, Germany. pp371-374.
[35] Takahashi, J., B. Mwenya, B. Santoso, C. Sar, K. Umetsu, T. Kishimoto, K.Nishizaki, K.Kimura, O. Hamamoto. 2004. Mitigation of methane emission and energy recyclying in animal agricultural system. In: Proceeding of 2004 International Symposium on Recent Advances in Animal Nutrition, 7th September 2004, Kuala Lumpur, Malaysia. Page: 97-103.
[36] Takahashi, J. 2006. Sustainable manure management in the nitrogen recycling system FLRC/NZFMRA Conference on IMPLEMENTING SUSTAINABLE NUTRIENT MANAGEMENT STRATEGIES IN AGRICULTURE, Massey University, Palmerston North, New Zealand .
[37] Takahashi, J., 2007. Feed production from unused resources applied ammonia stripping from digested slurry of biogas plant. J. Agric. Food Technol., 3:3-5.
[38] Takahashi, J. and M. Uemura, 2009. Ammonia fuel cell modulated ammonia stripping from animal agricultural biomass. J. Fuel Cell Technol., 8: 93-96.
[39] Takahashi, J., 2009. Perspective on GHG control and agricultural biomass for sustainable animal agriculture. Proceedings of International workshop on sustainable green livestock agriculture confronting climate change in APEC member economies. RDA, Korea. pp1-265.
[40] Umetsu, K., Y. Kimura, J. Takahashi, 2005. Methane emission from stored dairy manure slurry and slurry after digestion by methane digester. Anim. Sci J. 76: 73-79.
[41] Umetsu,K., S. Yasmazaki, J. Takahashi, 2006. Anaerobic co-digestion of dairy manure and sugar beets. R. C. Soliva, J. Takahashi. And M. Kreuzer (eds.), In: Greenhouse Gases and Animal Agriculture: An Update, Elsevier Science, 307-310.
[42] Van Nevel C. J. and D. I. Demeyer, 1996. Control of rumen methanogenesis. Environ. Monit. Assess., 42:73-97.
[43] Walles, R.J., L. Arthaud, C. J. Newbold. 1994. Influence of Yucca schidigera extract in ruminal ammonia concentrations and ruminal microorganisms. Appl. Environ. Microbiol., 60: 1762-1767.
[44] Wang, Y., T.A. McAllister. L.J. Yanke, Z. J. Xu, Cheeke P.R. and K.J.Cheng, 2000. In vitro effects of steroidal saponins from Yucca schidigera extract on rumen microbial protein sysnthesis and ruminal fermentation. J. Sci. Food Agric., 80:2114-2122.
[45] Wolin, M.J. and T.L. Miller, 1988. Microbe-microbe interactions. In: The rumen microbial ecosystem. Elsevier Applied Science, London, UK, pp. 343-359.

Preservation of Sugar Beets in Plastic Bags for Biogas Production

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Introduction

The use of sugar beets has attracted significant attention as potential substrate for biogas production since dry matter (DM) yield per hectare and gas production per kg DM are high. However, storability of this crop is limited. The aim of this study was to evaluate the process of preservation of sugar beets by ensiling in AG BAG plastic bags.

Materials and Methods

Preliminary tests on the possibility to store whole beets in plastic tubes were carried out in 2007. Whole and crushed sugar beets, harvested in November 2008, were then stored in plastic drums (215 litres, 4 replications) and left untreated or treated with KOFASIL® STABIL (containing sodium benzoate and potassium sorbate) to inhibit fungal development. Crushed beets were treated with the additive at 2 l/t, whereas whole beets were dipped in the solution for about 2 min. Storage time of this trial was 4½ months. After opening the drums the beets (whole and crushed) were homogenized by garden chopper. The determination of DM and fermentation products was done by routine analytical procedures, fresh matter recovery was calculated based on individual weight of effluent and silage, and temperature change upon exposure to air was monitored by inserted data loggers in insulated polystyrol boxes for 15 days. Data were submitted to statistical analysis by ANOVA.

Results and Discussion

Also whole sugar beets underwent intensive fermentation as, under anaerobic conditions, beet cell tissue dies off and releases some juice which in turn is fermented. Expectedly, fermentation process starts in the peripherical areas of the sugar beet leading to production of high concentrations of fermentation products (Table 1). Since the vast proportion of DM in sugar beets silages is composed of soluble compounds (saccharose, fermentation products), DM (corrected for loss of volatiles during drying) of effluent is nearly as high as of silage. Therefore, all effluent must be collected and used in biogas facilities. Whole beets produce significantly less effluent whereby the risk is markedly reduced of nutrient losses through uncontrolled effluent leakage, which in turn makes it possible to store them in plastic tubes (Table 2). However, air ingress into the voids between whole beets causes a higher risk of aerobic losses upon emptying plastic tubes. Application of chemical additive significantly reduced fungal contamination at the end of fermentation (whole beets: $P = .07$, crushed beets: $P < .001$) but somewhat increased effluent production. Upon subsequent exposure to air, temperature of untreated beets significantly rose to about 40 °C indicating massive aerobic DM losses whereas temperature of treated material almost remained at ambient level (Figure 1).

Conclusions

Results of this trial should support and promote the future use of cost-efficient bagging technology in the preservation of sugar beets as substrate for biogas production. Further studies are necessary and have already been initiated.

Acknowledgement

This study was co-financed by NAWARO® BioEnergie AG (Germany).

Table 1: Concentration of saccharose and fermentation products in whole ensiled sugar beets (beets stored in a plastic tube from December 2007 to August 2008)

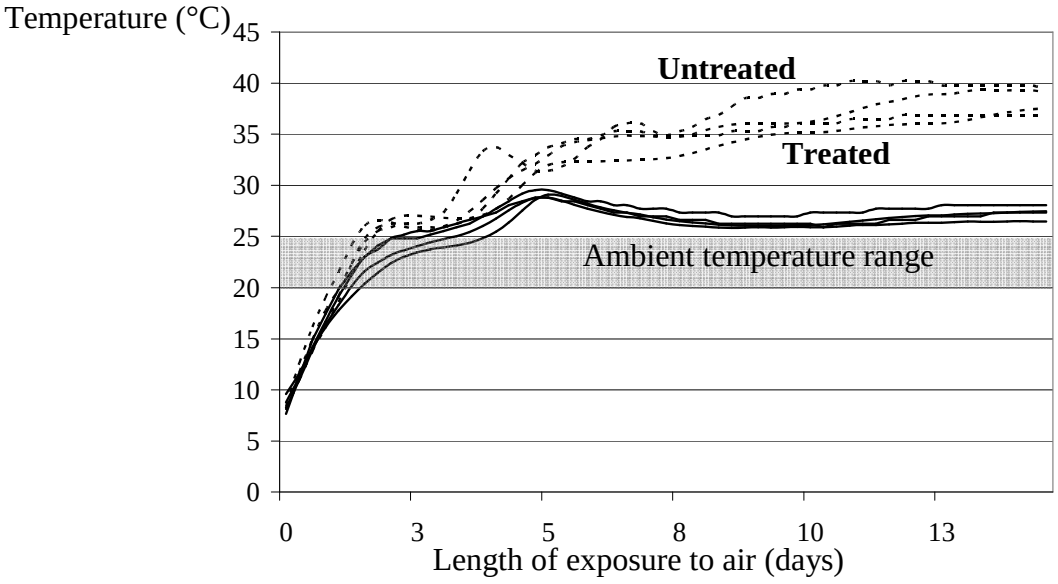
Parameter	Section of the whole sugar beet		
	Outer section	Mid section	Core section
$DM_{corrected}$, g/kg[1)]	250	231	242
Saccharose, g/kg FM	62.6	78.9	88.4
Lactic acid, g/kg FM	10.2	8.9	7.8
Acetic products, g/kg FM	10.6	9.6	7.9
Ethanol, g/kg FM	35.6	40.0	40.5
pH	3.85	3.85	3.85

[1)] *$DM_{corrected}$ (g/kg)=$DM_{uncorrected}$+0.95*volatile fatty acids (C_1-C_4, g/kg)+0.08*lactic acid (g/kg)+ 1.00*alcohols (C_1-C_4, including diols, g/kg); Weissbach and Strubelt, 2008, www.LANDTECHNIK-NET.com*

Table 2: Fermentation loss and fresh matter recovery of ensiled whole sugar beets (beets stored in plastic drums from December 2008 to March 2009)

Parameter	Crushed sugar beets			Whole sugar beets		
	untreated	treated		untreated	treated	
Fermentation loss, % of ensiled DM						
Mean	3.8	2.5	P =.134	9.4	1.8	P <.001
Standard deviation	1.5	0.3		0.5	0.4	
Fresh matter (kg) recovered from 1,000 kg of ensiled sugar beets						
Silage	605	566	P =.008	900	883	P =.01
Effluent	383	429	P =.009	78	113	P =.001
Total	988	995	P =.130	978	996	P =.001

Figure 1: Effects of additive treatment of whole sugar beets on temperature rise upon exposure to air (sugar beets stored before anaerobically in plastic drums from December 2008 to March 2009)



Forage Production and Quality of Short-term and Permanent Grassland with Festulolium Hybrids

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Introduction

With the ever reducing number of bred cattle, Festulolium hybrids proven in forage may also be used in biogas stations. Firstly, in the less favourable areas for growing maize which is still the main crop used in this respect. More winterhardy grass vegetation at higher locations and on more sloping grounds will efficiently prevent erosion. Secondly, it may very well interrupt the cropping pattern at lower locations, and its root system will supply a lot of organic matter to soil. Thirdly, it even tolerates higher fertilizer rates, utilizes nutrients very well and allows to balance collectors even when balancing is not allowed by maize. Much lower price of seed also plays its role.

Festulolium hybrids may also be sown into short-term grassland without clovers with other grass species and ryegrass and fescue hybrids may be combined – depending on the required time of cutting. For harvest with conservation already in the sowing year, they are sown together with annual ryegrass; this method is also used to establish clover-grass mixture.

Permanent grassland also provides a suitable source of biomass for biogas stations. Since many permanent grasslands do not yield quality forage, they may be improved by overseeding clover and grasses, amongst others, the very Festulolium hybrids, short-term ryegrass type as well as perennial fescue type cultivars such as Hykor or Felina. Overseeding is studied, amongst others, by VSTE researchers in Jevíčko (Kohoutek et al., 2007). For regeneration of permanent grassland, the above mentioned perennial hybrids contribute to long lasting, high and quality production of grass mixtures.

Material and Methods

Festulolium hybrid cultivars from the Plant Breeding Station Hladké Životice and its forage quality are described in the paper in Section 1; this paper will focus on mixtures – clover-grass stands and perennial meadow mixtures.

Both types of hybrids, ryegrass and fescue-type, are used for short-term 2-3-year use depending on moisture conditions of the site and silage requirement, i.e. sugar content in forage, which is much higher in ryegrass hybrids. In any case, fescue hybrids must be used for short-term mixture in addition to ryegrass to gradually fill up their cleared space and to prevent weed infestation and to maintain good yield of mixture in future years. Experiments with Festulolium mixtures have been conducted for 25 years in Hladké Životice, and sown into random blocks on land plots of 10 m^2 with four replications.

Trial with clover-grass mixture was established by summer sowing in 2006, with minimum nitrogenous fertilization prior to sowing and in autumn of each harvest year; no fertiliser is applied prior to cut. Felina hybrid was sown in c. 10.5 kg/ha and 11 kg of Vesna red clover. Ryegrass hybrids Achilles, Lofa and Perseus were sown in c. 6 kg and 16 kg clover per ha. Trial with meadow mixtures was established in 2001 using oats, nitrogenous fertiliser was applied prior to sowing, and low rates after oats harvest and in autumn. In crop years, nitrogen rates were as follows: 60 kg nitrogen per 1 ha prior to cut 1 and cut 2, and 40 kg N prior to cut 3.

Yields in the tables below show the yield potential of cultivars and mixtures; full harvest was conducted using a small-plot harvester Hege 212, and samples of fresh weight 1 kg taken from the land plot were dried immediately to harvest in kilns at the temperature 55^{o}C.

Samples were analysed using NIRS method in the Research Station of Jevíčko and selected samples were analysed in the laboratory of the company Nutrivet, s.r.o. in Pohořelice.

Results

Clover-Grass Mixtures

Table 1: Clover grass dry matter yields

Species and varieties in mixtures	Harvest year I	Harvest year II	Harvest year III	Average 3 years
TP Vesna + Fl Felina	15.05	22.33	20.64	19.34
TP Vesna + Fl. Achilles	19.72	22.02	20.12	20.62
TP Beskyd + Fl. Lofa	17.59	17.01	17.77	17.46
TP Beskyd + Fl. Perseus	16.7	16.91	18.68	17.43

The selected 4 variants include two early mixtures with red clover Vesna and two mid-early mixtures with Beskyd clover cultivar. The first early mixture with Felina fescue hybrid had the lowest yield (Table 1) in the first year after summer sowing, but highest yields in the next years. This mixture also had the lowest proportion of grass component in the first cut – 50% (slowly developing after sowing) while ryegrass hybrids in the other mixtures had 85-95% proportion in the first cut. The dominant grass proportion was caused by dry autumn after summer sowing where clover did not make to create branchy rosette. The grass proportion was gradually decreasing in the next cuts (and years).

The higher grass proportion also showed in lower crude protein (CP) in the first cut forage and higher content of fibre (Table 2). Despite of higher fibre content, organic matter digestibility (OMD) was similar to the next cuts.

It is a proven fact that NIRS method unfortunately underestimates water soluble sugar content (WSC) in forage, often by more than 50%. Nevertheless, there is a proven difference between the Felina fescue hybrid mixture and other mixtures with ryegrass hybrids.

Table 2: Forage quality of grass-clover mixtures in four cuts of the first crop year (NIRS results)

Cut 1	g/kg DM CP	Fibre	WSC	OMD %	NEL [MJ/kg]
Vesna + Felina	13.3	26.2	5.2	78.4	5.51
Vesna + Achilles	10.4	26.9	7.6	77.9	5.31
Beskyd + Lofa	11.3	26.1	7.7	78.5	5.32
Beskyd + Perseus	10.3	26.3	8.7	81.3	5.06
Cut 2					
Vesna + Felina	19.3	18.5	5.7	80.7	6.10
Vesna + Achilles	14.3	23.5	6.7	75.8	5.95
Beskyd + Lofa	13.7	23.2	7.7	80.8	5.92
Beskyd + Perseus	12.7	24.2	7.6	79.7	5.84
Cut 3					
Vesna + Felina	18.9	20.5	5.0	81.1	5.95
Vesna + Achilles	17.1	21.2	5.6	78.0	5.96
Beskyd + Lofa	13.9	24.6	6.2	75.8	5.65
Beskyd + Perseus	13.4	25.5	6.5	76.9	5.54
Cut 4					
Vesna + Felina	19.8	20.8	5.7	80.2	6.10
Vesna + Achilles	23.4	17.6	6.5	79.8	6.29
Beskyd + Lofa	21	19	7	79.0	5.99
Beskyd + Perseus	21.7	18.9	7.7	79.8	5.97

2. Grass mixtures

As an example of grass mixtures, I choose two from an extensive experiment with meadow mixtures – one for a humid stand and the other one for a dry stand. The seed mixture for the humid stand included dominant grasses Festulolium Felina, tall fescue *(Festuca arundinacea Schreb.)* variety Kora, and timothy *(Phleum pratense L.)* variety Sobol. The seed mixture for the dry stand included dominant grasses Festulolium Hykor and oat grass *(Arrhenatherum elatius (L.))* variety Median. The mixtures also comprised other grass species and red clover which disappeared by year four. a considerable drop of yield in the second year was caused by an extremely dry season. Once clover yield declines, annual yields varied depending on precipitation. Two cuts were evaluated in Year Eight.

Forage quality of meadow mixtures (already without clover) is expressed by values obtained from harvesting samples in the forth harvest year where two cuts were analysed for digestibility *in sacco.* More values were established using NIRS method (Table 3).

Table 3: Meadow mixture yields with dominant Festulolium

Mixture Name	DM yield t/ha in harvest years								%
	1.	2.	3.	4.	5.	6.	7.	8.	
dom. Felina	20.54	9.76	14.1	11.55	12.88	10.57	15.44	10.71	**100**
dom. Median and Hykor	19.89	12.93	15.96	14.18	14.95	11.73	15.09	12.25	110.8

Table 4: OMD in sacco, Fibre, Crude Protein, NEL, WSC of grass mixtures in the fourth harvest year

Mixture Name	Cut	OMD *in sacco* %	Fibre g/kg	CP g/kg	NEL MJ/kg	WSC g/kg
dom. Felina	1	67.3	276.6	134.5	5.83	56.7
	2	59.08	246.9	133.9	6.05	59.6
dom. Median and Hykor	1	60.3	298.9	133.3	5.83	43.9
	2	61.94	250.6	139	6.18	49.7

At the first cut harvest time, Festulolium was at the beginning of heading and oat grass heading was finished; meadow stands are mostly harvested in this stage. While oat grass heading showed in a higher content of fibre in the sample, crude protein content and NEL were not much affected. Oat grass heading was also at the second cut, but not deteriorating forage quality in comparison with the Felina hybrid mixture. Water soluble sugar content (WSC) is higher in the Festulolium Felina mixture.

Conclusions

High yields contributed by Festulolium in mixtures are documented in Table 1 and 3. Table 3 also shows fescue hybrids perennial and capacity to overcome dry years and regain a high production level even at older stands in the years with normal humidity.

Forage sample analyses prove very good quality and evolution for silage production not only to fodder livestock, but also to generate energy in biogas stations.

References

[1] KOHOUTEK, A., KOMÁREK P., NERUŠIL P., ODSTRČILOVÁ V. Strip-seeding of clover and grass into permanent grassland. Methodology for practice, Ref. 41752/2007-17220, VÚRV, v.v.i., 2007, 32 p. ISBN 978-80-87011-19-5.

Non-traditional crops for biomas

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Introduction to problem solving and grounds for the implementation

Post-revolutionary transformation changes in the Czech agriculture led to a drastic reduction in livestock production, accompanied by a significant reduction in livestock. E.g. cattle decreased from 3480 thousand in 1989 to 1402 thousand in 2008, i.e. more than 2-times. Correspondingly decreased the crop production of feed and fodder crops. Area for growing corn as green feed and silage has within the same period decreased from 384,867 ha in 1989 to 173,899 ha in 2008, the area of perennial feed crops on arable land - from 414,773 ha in 2000 to 188,246 ha in 2008, i.e. that there is a decline in area also more than 2-times. Similar trends apply as well for other feed and fodder. Decline of plantations of fodder and feed reaches tens to hundreds of thousands of hectares. Many farmers can not cope with such change – they simply do not know what they should cultivate in place of fodder and feed. On the other hand, the reduction in the representation of perennial fodder in crop rotation adversely affects the quality of agricultural land – it increases erosion, causes degradation of organic matter in the soil and reduces soil fertility. Therefore, the current issue of Czech agriculture is the application of new technologies for the production and use of plant biomass for non-food purposes, especially the extend of cultivation of perennial fodder crops, both conventional and new non-traditional crops.

Approval of the Act No. 180/2005 Coll. on the promotion of electricity from renewable energy sources opens for Czech farmers new opportunities in the area of production of plant biomass, because in terms of CR and the majority of EU states is biomass the most important source of renewable energy and occupies about 80% share of total energy from renewable sources. Long-term guarantee of feed-in tariffs for electricity produced from renewable sources evoked in the in the CR unprecedented interest in the construction of agricultural biogas plants, oriented along the lines of neighboring countries (Germany and Austria), grown specifically for processing plant biomass, particularly corn. Only during the last three years (2006-2009) were in CR built dozens of new agricultural and municipal biogas plants (BGP). Design and construction of biogas plants continues - currently are registered about 600 new projects for the construction of BGP. For example, the biggest domestic producer of electricity, ČEZ plans to build several BGP of the general power of 20 MWh of electricity, equivalent to annual consumption of 120-150 thousand tons of plant biomass. In the case of corn, which has in our conditions the average yield of green mass of about 30 t/ha, would require sowing area of 4-5 thousand ha.

The biggest biogas production is achieved from the fresh biomass of plants. Perennial nature of service in BGP is a counterpoint to the seasonal possibility of harvest of fresh green mass, which provides in summer about 2-3 months suitable for so-called green container for perennial fodder crops and about 1 month - for annual crops such as corn. Thatfor is its preservation a necessary requirement for the production of plant biomass as feedstock for the production of biogas. Most appropriate known method of preservation of biomass of plants for production of biogas is silage. Usefulness for purposes of for biogas second method for preserving of plants – heylage, is not sufficiently explored. Ideal conventional crop for silage is corn, which is the reason why this crop has reached the widest application for biogas production especially in Germany and then in Austria.

Following the models close foreign regions have as well the Czech designers of BGP focus on the use for the production of biogas a corn silage. Corn silage has for farmers one big disadvantage, which is the high production price. Overhead cost of production of corn silage at

32 % dry matter in terms of CR is equal to approximately 1650-1850 CZK/t, the threshold gain - 2100-2300 CZK/t of dry matter. There is therefore a danger, that if the farmer is not the owner or stakeholder of BGP, then he can expect a pressure from purchasers of plant biomass for the lowest possible price. Thus the farmer for the management of a profit has to produce and preserve an inexpensive crop biomass of the corn similar properties, of the yield of biogas. Therefore the main task of our project we see the development of technologies for the production of plant biomass suitable for production of biogas, which will be significantly cheaper (c. 1.5-2x) as corn silage. Only in this case, we can assume a rapid expansion of production of plant biomass as feedstock for the production of biogas in the CR, whose potential we estimate at dozens of thousands of hectares. We assume that this can be achieved only by developing new low-cost technologies for production and conservation of plant biomass, especially with the use of conventional and unconventional perennial-fodders.

Description of current implementation:

The project is supported by the Ministry of Agriculture of the Czech Republic and is registered under the number QH91170. Unfortunately, with regard to the lack of funds provided in the project period reduced to a minimum, that is 3 years (2009-2011). For the purposes of the project were selected 6 non-traditional forage crops - topinambour, girasole, rosin-weed, St. John's rye, mallow Lavatera and Schavnat alias sorrel Uteuša and for comparison 2 traditional forage crops - corn and multi-annual grass vegetation, i.e. a total of 8 crops. All crops except of reference corn are perennial crops. Given the short time of implementation (only 3 years), is the methodology of the project based on the use of existing multi-functional and experimental crops of selected traditional and nontraditional forage. Experiments are always made in at least 3 variants of nitrogen fertilization (usually 60, 120 and 180 kg N per 1 ha) and 3 repetitions, especially in the Chomutov region and at the agricultural biogas plants in Prosečná. The first spring fertilizing on existing or newly established experiments and the last withdraw of plant samples was accompanied by sampling of soils. Yield tests, sampling and harvesting of above-ground plant material for analytical assets and fermentation tests, were for most crops, except of rye, made in 3 stages of growth: 1) prior to flower, 2) in the flower, and 3) at the ripening of the fruit. Yield tests and chemical analysis of crops were made by different variants of fertilization, but for fermentation tests were always prepared composite samples of different variants of the same weight ratio. All the tests and experiments have been conducted in at least 3 repetitions. All the outputs from the fermentation experiments have been as well sampled and analyzed.

As this is the first year of implementation, for which no outcomes were planned, have the implemented activities been primarily focused on the acquisition of primary data applicable for further synthetic evaluation and preparation of documents for publications and application outputs. Nevertheless, it has been shown, that from all observed crops can despite the negative low dry matter (mostly below 28% and in some cases below 20%) be produced a sufficient quality silage. Wither has typically increased a dry matter of ensilaged phytomass and has improved the process of ensiling. The measured pH value of silage of crops ranged from 3.92 to 4.25, which corresponds to the requirements of a stable silage and satisfactory fermentation process. At all withered silage were detected, due to the higher dry matter, improved indicators of fermentation process.

Tests of biogas production are conducted on a unique 48-unitslaboratory equipment of CRI with adjustable precision heating and automatic timed stirring. Tests are carried out in mesophile regime 38-40 °C. During the experiments, an intensive stage of production of biogas during countdown since the end of the build-up (so-called lag-phase) usually lasts about 2-4 weeks, ramp phase lasts about 1-3 weeks. There is an ongoing implementation. With regard to the time demands of fermentation experiments are the planned experiments not yet fully completed and evaluated. However, the results obtained so far are very promising and suggest that most of the selected crops give very satisfactory results of biogas production and some of the selected crops (e.g. rosin-weed, Lavatera, Schavnat) show a similar or better biogas yield from 1 t of biomass and a similar or even a higher total production of biogas from 1 ha than a reference maize.

Silages as Feedstock for Biogas: Novel Perspectives For Silage Additives

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Introduction

Efficient storage of the substrate ensuring low losses, long aerobic stability and maximized digestibility are major criteria for profitable operation of biogas plants. During the biogas process biomass is converted through different steps into methane and carbon dioxide. Major intermediates are different acids mainly focused on acetic acid. Likewise acetic acid acts as an inhibitor of yeasts during ensiling and feed-out. Objective of this study was to evaluate whether adjusting the fermentation pattern of silage, by using microbial additives as silage-starter, will result in an enhanced methane production in biogas process. As well, the characteristic of silages under aerobic exposure was evaluated.

Materials and Methods

3 Maize silages from 2 harvest seasons (07/08) were ensiled untreated (UT) and treated (T) with a mixture of LAB (*L. buchneri*-LB, *L. plantarum*-LP, $2x10^5$ cfu/g; Lactosan Starterkulturen GmbH & Co.KG). Silage trials were performed in lab scale of 5 Liter capacity. Silage losses, chemical composition, aerobic stability and methane yield were determined after 90 days of ensiling. The influence of oxygen exposition on the chemical composition and the methane yield were determined at different time-steps of aerobic deterioration, evaluated in *HONIG* lab scale test. Methane yield was determined according to the German technical guideline VDI 4630. Dry matter of maize silages varied between 27 to 35%.

Results and Discussion

Silage losses (calculated according to *WEISSBACH)* were slightly higher in treated silages but not significant ($+0.3\%^{n.s.}$, $+0.7\%^{n.s.}$, $+0.8\%^{n.s.}$). Due to heterolactic fermentation treated silages resulted in significant higher acetic acid ($+5.1^{s}$, $+2.7^{s}$, $+6.5^{s}$ % of DM) and aerobic stability was enhanced (Table 1). The evaluation of LAB-concentrations after 90 days of ensiling showed clearly higher concentrations in treated silages (Fig. 1). Concentrations of LAB may indicate a higher survival rate and competitiveness of LB during ensiling in treated silages (Fig. 1).Yeast counts of harvest material varied only in a small range (2.5E+05; 2.7E+05; 1.8E+05). After 90 days of ensiling yeast counts of treated silages were below detection limit (< 1.0E+02). Untreated silages showed significant higher counts (1.2E+05; 1.3E+06; 4.1E+06).

Upon exposure to air treated silages showed no DM losses and methane yields remained stable (Table 1). The sum of fermentation products was preserved during exposure to air (Fig. 2). Deterioration of untreated silages started immediately. Degradation of all acids (Fig.2) and therefore a rise in pH resulted in significant methane losses (Table 1).

Table 1: Influence of exposure to air (t=0 without oxygen influence, 96 and 160 hours of exposure to air). [a,b,c]Means in the same column with different superscripts differ (P≤0.05), n.d. = not determined

	I		II		III	
	UT	T	UT	T	UT	T
Aerobic stability [hours]	23	118	131	>280	17	>256
t=0 [CH_4 m^3/t DM_c]	335[a]	334	304[a]	309	304[a]	319
app. 96 hours [CH_4 m^3/t DM_c]	n.d.	n.d	277[b]	304	256[b]	300
app.160 hours [CH_4 m^3/t DM_c]	272[b]	341	n.d.	n.d.	178[c]	299

Conclusions

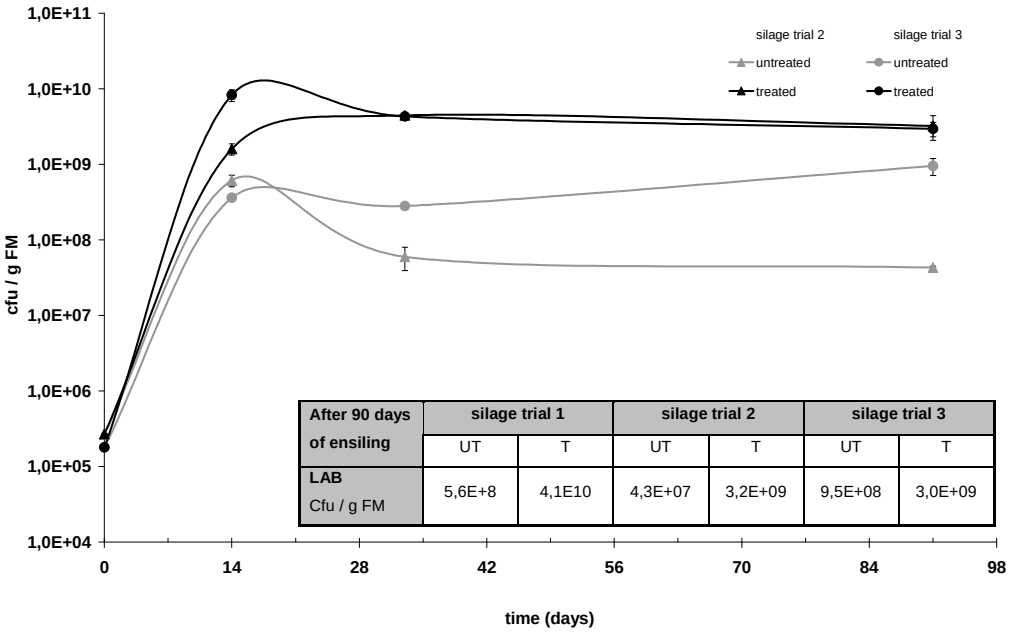
Aerobic stability is improved by heterolactic fermentation. Yeast counts of treated silages are significantly depressed due to rising acetic acid content. The methane yield varied without influence of oxygen between 335 and 304 m^3/t DM_c. Upon exposure to air methane yield of untreated silages was significantly lowered. Taking into account the special circumstances of agricultural biogas plants heterolactic fermentation improves the energy conservation of the harvested material and the plant efficiency can be increased.

References

[1] Honig, H., Evaluation of aerobic stability. Proceedings of the Eurobac Conference, 12-16 August 1986, Uppsala Schweden

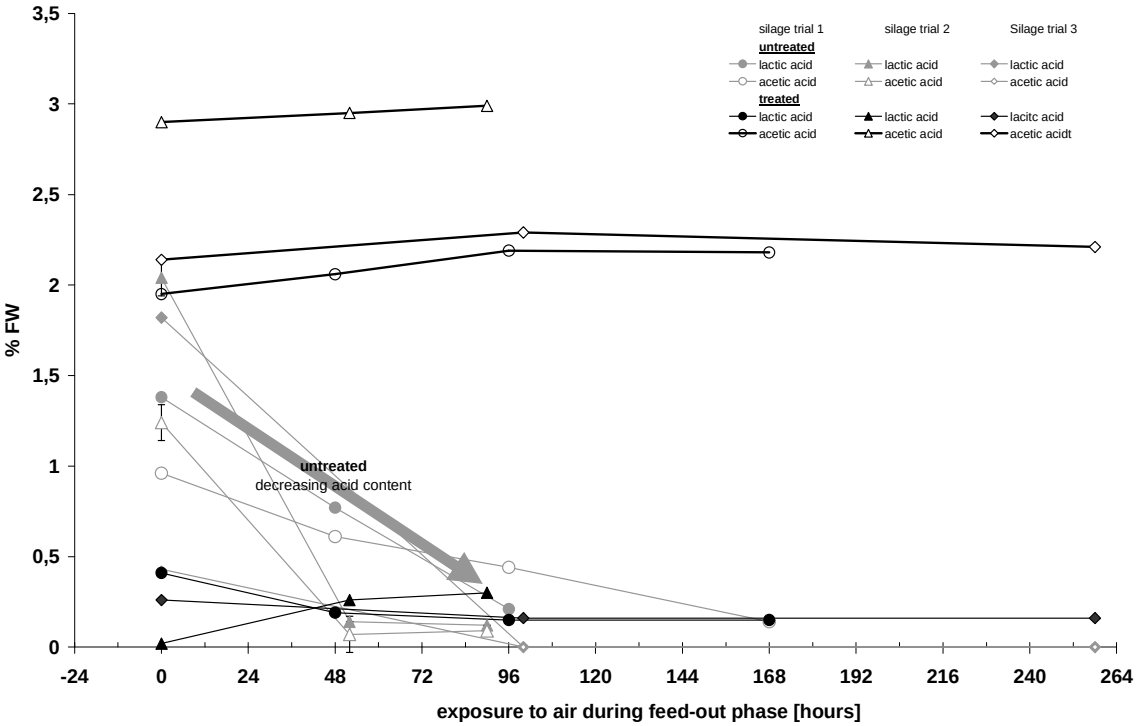
[2] Weissbach, F., Strubelt, C., Die Korrektur des Trockensubstanzgehaltes von Maissilagen als Substrat für Biogasanlagen. Landtechnik (63) 2/2008 S.82 – 83

Figure 1: Evaluation of total LAB concentration during ensiling. Silage trial 1 only after 90 days of ensiling (inserted table).



After 90 days of ensiling	silage trial 1		silage trial 2		silage trial 3	
	UT	T	UT	T	UT	T
LAB Cfu / g FM	5,6E+8	4,1E10	4,3E+07	3,2E+09	9,5E+08	3,0E+09

Figure 2: Changes in chemical composition (lactic and acetic acid) due to oxygen influence e.g. during feed-out phase. Means without Error-Bars are single samples.



Influence of Lactic and Acetic Acid in Corn Silage on Biogas Production and Conclusions for the Application of Silage Additives

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Introduction

Corn silage is a very common raw material for the biogas production in Germany. Beside the difficulty of accurate determination of the dry matter content of corn silage the impact of sugar and various fermentation products (lactic acid, acetic acid, butyric acid and ethanol) is controversial (Weißbach and Kuhla, 1995). Due to the specific role of acetic acid in the acetogenesis and while biogas formation, it is assumed that acetic acid enhances the methane yield.

Materials and Methods

To evaluate the influence of different fermentation products on biogas-formation, lactic acid and acetic acid were added to silage. 1% lactic acid or 1% acetic acid, resp. (relating to organic matter) were appended to corn silage with 31.5% dry matter and biogas slurry and fermented in 1.6 l laboratory batch-reactors under mesophilic conditions over 30 days (acc. to VDI 4630).

Results

It was demonstrated, that in presence of corn silage, added acetic acid and lactic acid produces the same amount of biogas and methane (Tab. 1).

Discussion

Nussbaum (2009) also showed, that these fermentation products added to maize silage, yielded not in different methane-formation. Homofermentative microorganisms reduce one molecule of either fructose or glucose via pyruvate to two molecules of lactic acid. There is no loss of dry matter as carbon dioxide and no loss of energy. The heterofermentative microorganisms ferment glucose and fructose to one molecule of lactic acid and either ethanol or acetic acid and mannitol with dry matter loss of 24% and energy loss of 1,7% regarding to the sugars (McDonald et al. 1991). Kung et al (2003) describe the advantages of homofermentative lactic acid bacteria with the rapid and dominant production of lactic acid, the improved energy and DM recovery and the decreased proteolysis. Untreated silage and silage manufactured with heterofermentative LAB have higher dry matter losses in comparison to silage, manufactured with homofermentative LAB and therefore produce less biogas. 3% higher DM corresponds to 5% higher biogas-yield, because DM losses are always energy losses. High amounts of acetic acid increase the aerobic stability. Accessorily the feed intake of cows decreases with increasing acetic acid in silages (Eisner et al, 2006). Aerobic deterioration of silage involves DM losses, caused by detrimental microorganisms, and can be avoided by potassium sorbate. The present potassium sorbate price of 1.50 €/t ensures about 3% biogas. On a price basis for corn silage of 50.00 €/t, the treatment with homofermentative LAB is comparable or better than with heterofermentative LAB and especially reasonable for dairy farmers due to the better feed intake.

References

[1] Eisner, I., Südekum, K.H., Kirchhof, S. 2006. Beziehungen zwischen Fermentationscharakteristika von Silagen und Futteraufnahme von Milchkühen, Übersichten Tierernährung (34) 197-221.

[2] Kung, L., Stokes, M.R., Lin, C.J. 2003. Silage additives, in: Silage Science and technology. Am. Soc. Agronomy No 42, USA. 305 -360

[3] McDonald, P., N. Henderson and S. Heron. 1991. The Biochemistry of Silage. 2nd ed. Chalcombe Publ., Marlow, Bucks, England.
[4] Nussbaum, H.-J. (2009): Effects of different fermentation products on dynamism and yield of biogas, In: Proc. 15. Int. Silage Conf., Madison, WI, USA, 435-436
[5] Weißbach, F., Kuhla, S. 1995. Substance losses in determining the dry matter content of silage and green fodder: arising errors and possibilities of correction. Übers. Tierernährung 23. 189-214

Table 1: Increase of the biogas-yield as influenced by organic acids

	Corn silage		Corn silage +1% ac. acid		Corn silage +1% lac. acid		Increase by 1% ac. acid	Increase by 1% lac. acid
	Mean	SD	Mean	SD	Mean	SD		
Biogas (Nm³/t FM)	190,5	5,5	198,5	2,5	201,8	4,9	7,95	9,29
Methane (Nm³/t FM)	105,8	2,7	110,2	1,6	110,9	1,8	4,35	5,08
Biogas (Nm³/t ODM)	636,4	18,4	663,0	8,4	674,1	16,5	25,56	31,02
Methane (Nm³/t ODM)	353,4	9,0	367,9	5,1	370,3	6,1	14,35	13,27
Methane content (%)	52,2	4,8	55,5	0,2	55,0	0,5	3,30	2,80

Development of biogas plants in the CR - potential and problems

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Introduction - the potential of biogas production in the Czech Republic

The production of biogas, including landfill gas, had been developing in the Czech Republic primarily through degassing of municipal waste landfills and stabilization of sewage sludge in wastewater treatment plants. This potential is largely (80%) used. The highest potential is in the processing of agricultural renewable raw materials, i.e. animal feces and plant biomass. Biodegradable waste was previously processed in biogas plants in co-fermentation with animal feces. New regulations concerning the operation of biogas plants, energy prices and digestate handling favor biogas plant, which will not be processing waste. Therefore, it is presumed the construction and operation of the BGP specialized in processing BDW. In particular, the processing of kitchen waste, including frying oils and in particular from cafeterias and restaurants, the grass from maintenance, distilling slops, waste from biodiesel production, and solid waste from the food industry including gastro- wastes and inedible food products and NGA (bone meal, rendering works fat and slaughter waste). Some of the waste gets lost in landfills of communal waste or in waste water (through the kitchen shredders) and green waste from a separated household biological waste is often preferable to use in composting. It must be assumed that the available potential of BGP processing only waste will not exceed 220 GWh and an installed capacity of 28 MWe. It can be calculated with about 125 BGP.

Data available on the potential of agricultural and waste BGP listed in tab. No. 1 are derived from the study of MUŽÍK, SLEJŠKA 2009 and the production data shows that agricultural BGP fill the available production potential of 485 million m^3 of biogas from 16.4 % and BGP processing only waste only from 5.5 %. The potential of sewage BGP is currently filled with about 80 % and at degassing facilities for landfill is almost completely filled. The total potential of all plants producing biogas is filled from 27.4 %.

Currently, is for the processing of BDW in case of necessity for hygienization (slaughterhouse waste, waste from restaurants and canteens), being used an anaerobic digestion. However as well the kitchen waste, including waste from kitchens, often disappears into the wastewater or municipal waste landfill, or are, in conflict with legislation, fed to livestock. Further development of anaerobic digestion of BDW depends on the separated collection of BDW, but even here exists the competition with on operation cheaper composting plants.

Barriers to further development of biogas plants in the CR and their removal

In 2002, in addition to sewage biogas plants have been operated only 6 other devices primarily processing animal feces and manure. Nowadays are already 131 facilities with installed capacity of 62 MWe in operation. This increase is due to legislative action. This is primarily a law 180/2006 Sb. "On the promotion of electricity from renewable sources" and in particular guarantee purchase of electricity, guarantee prices for 15 years and possible "Green bonus" for the use of electricity directly to the energy market. Furthermore this has been due to grant aid on financing projects BGP, particularly through operational programs and state program for the use of renewable and secondary energy sources with at least 40% cofinancing of the investor. The feed-in tariffs of electricity supplied to the network is intervened by the the State and varies by category of biogas plants. Highest feed-in tariff is for agricultural BGP (4120 CZK / MWh) and for BGP processing waste is only 3550 CZK / MWh. Price for electricity production in the BGP at WWT or landfill is at the facility built after 2005, only 2420 CZK / MWh. The same differences by category of BGP are at the price of green bonuses. Likewise the introduction of digestate from agricultural biogas plants as fertilizer does not required any registration, whereas for the digestate from waste biogas plants is such registration required even when it is for own use.

Categorization of BGP according to processed substrates was necessitated primarily because of the odor control at so-called co-fermentation of waste, animal feces biogas production with the addition of BDW on devices that have been adapted to other waste, particularly meat and bone meal rendering plant fats, paper sludge, etc. BGP designed as a one-stage device with uncovered reservoirs of digestate with a little delay time had been overloaded with waste by their operators in order to obtain high revenues for waste. During this has been shown that:

- lack of erudition of entities engaged with BGP;
- lack of discipline of BGP operators, obviously wrong processed operating procedures;
- lack of legislation on the operation of BGP;
- lack of control from the permitting and inspection bodies.

There are barriers despite the high societal support for the development of biogas plants in the Czech Republic. Civic associations against the construction of biogas plants in other new locations are getting formed based on the operation of newly built biogas plants co-fermenting BDW with animal feces inconveniencing the populations with odor.

Activities of these civil society groups have been successful in many cases and a number of socially beneficial investment projects were not implemented (Úholičky u Prahy, Tíšnov). On the basis of legitimate complaints by citizens it was necessary to terminate due to odor the activity of several biogas plants. Odor defects showed mostly BGP processing BDW and at BGP processing only agricultural renewable raw materials there is a minimum of complaints. This barrier is currently being successfully addressed through legislative amendments in particular the legislation of air, waste and fertilizers. Credit for this also has Guidance of the Ministry of Environment to conditions of approval for BGP before being put into operation. This guideline provides a uniform procedure of government at the approval and authorization for the BGP and imposes to optimize the operation conditions of BGP in term of environment. In particular, emphasis is placed on the permissible level of odors. On the BGP processing wastes including NGA are higher requirements for protection against leakage of odorous substances, both at the import and storage of waste, hygienization and at handling with digestate. Biogas plants processing waste should have plenty of hidden storage capacity for digest and that for minimum of 4 months. There is a need at each BGP address emission limits for odorous substances both in normal operation and during accidents. For this elimination is advisable to use bio-filters rather than filters with active carbon.

The barrier of resistance of the population against the BGP is being removed by good operation experience of some of the new BGP and by excursions to BGP in Germany and Austria. Since 2006, there have been about 50 new BGP put into operation in the CR. New knowledge from their operations show the possibility of other problems. It is a bad operation of BGP due to insufficient knowledge of their own fermentation process and operator inexperience, mistakes in the choice of technology with regard to the processed substrates, errors in construction projects and a huge efforts to save the investment and operating costs leading to violation of technological discipline. As a result of these errors occur operational difficulties manifested by decrease in power outputs and production of odor, which often ends up in a complete collapse of the fermentation process with the need to restart the process. These lacks are reflected in the service economy of BGP, in particular it prolongs the ROI.

Economic barriers for development of building BGP are gradually eliminated by the increasing societal support and the risk of construction of BGP decreases. Among agricultural BGP and BGP, which are devices for waste management, are some differences. Investment costs for BGP processing wastes are higher (210 to 230 thousand. CZK / kWe) than in agricultural BGP (110 - 130 thousand. CZK / kWe). This difference in investment costs is caused due to the need to build hygienization units and technical measures for the extraction and filtration of odorous gas and for digestate container closure at the BGP processing waste. Also, operating costs for these BGP are higher because of the consumption of heat and electricity for hygienization and crushing of BDW and in NGA, ensuring thermophil mode of fermentation, demonstrating hygienization and higher costs in the management of digest. Economic efficiency of BGP is affected not only by investment parameters, namely investment costs, costs of capital, the amount of grants, but as well operational characteristics, particularly income for waste

treatment, for the sale of electricity, heat and digestate. Feed-in tariff of electricity from BGP is set for 15 years ahead, but is lower than in neighboring Germany. Also price for waste treatment in the BGP is in comparison with Germany and Austria very low, however it can be assumed that it will increase with the price increases in landfill of waste. Sales of heat, which is at biogas plants still insufficient, can significantly increase the efficiency of BGP. The economic efficiency of BGPis as well given by the way of digestate treatment and its eventual sale as a registered organic fertilizer can be a significant for the operation of BGP. For the return of the investment costs incurred for the construction of BGP is primarily crucial the amount of non-repayable subsidies. In the case of grant of 40% of investment costs may reduce the return on investment in the good operation of the BGP to 9 to 10 years, which is about two thirds of the expected life of the equipment.

Conclusion

Proposal of Directives of the European Parliament and the Council "on the promotion of energy from renewable sources" puts great emphasis on the use of energy from bio-waste. Also the new document "Green Paper on handling of clinical waste in the European Union (COM 2008-811) prefers the energy use of BDW especially kitchen and food bio-waste. If the separate collection of kitchen waste is not guaranteed and these become components of communal waste landfills, mechanical - biological treatment using for stabilization of the biological compound anaerobic digestion producing biogas is recommended.

Environmental aspects of anaerobic digestion are relevant and can significantly reduce the increasing greenhouse effect and climate change. At the same time, the production of biogas ensure the substitution of fossil fuels, including motor fuels and enhances energy security. An example might be Germany, where is installed more than 4 thousand BGP. From this perspective, is provided a society support for the current projects by intervened price of electricity from BGP and investment subsidies completely justified. This support is significantly higher for agricultural BGP. In the conditions of the Czech Republic, where prices for the processing of biological waste are significantly lower than in Germany, I recommend to increase feed-in tariffs for electricity and the price of green bonuses for BGP processing BDW. This measure would lead to the installation of additional BGP processing waste, which would be reflected by reducing the amount of biodegradable municipal waste deposited in landfills.

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Table 1: Annual production potential and energy use of biogas in the CR and its implementation

Item	unit	WWTP municipal	WWTP industrial	BGP agricultural	BGP wastes	landfills	Total
Available potential:							
Biogas production	mil. m^3	69	7	485	140	69	780
Electricity production	GWh	89	7	753	218	100	1167
Heat production	TJ	870	110	2900	847	94	4821
Number of plants	pcs	110	27	365	125	60	687
Year 2008							
Biogas production	mil. m3	57,9	3,6	41,1	10,3	62,7	150,5
Electricity production	GWh	74	4	73,3	18,3	97,2	266,9
Heat production	TJ	690,2	62,2	181,3	45,2	86,4	1065,4
Number of plants	pcs	72	13	78	21	80	264
Year 2009 (estimation)							
Biogas production	mil. m^3	60,0	4,5	79,6	12,8	67,4	224,3
Electricity production	GWh	76,4	5,2	124,1	22,0	101,2	328,9
Heat production	TJ	702,7	70,4	454,0	46,5	95,2	1368,8
Number of plants	pcs	97	16	125	24	84	346

Posters

Competitiveness, Yield and Forage Quality of Soft and rough-Leafed Varieties of Tall Fescue (Festuca arundinacea Schreb.) in a Mountain Environment.

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Introduction

Tall fescue would be a desirable component of seed mixtures for permanent, intensively farmed meadows in mountain regions subjected to summer dry periods, such as it is the case of some areas of South Tyrol, as it is one of the few species well adapted to such conditions. However, there are two issues to be clarified, in order to allow for the development of such a seed mixture. Firstly, this species is known to be poorly competitive in the early phase because of slow establishment (Badoux, 1971). This may lead to an unsatisfactory share in mixed lawns and varieties with good competitiveness should be chosen. Secondly, under dry climate conditions tall fescue can become dominant in mixed plant stands. In pure plant stands there is indeed concern that the rapid deterioration of forage quality with increasing developmental stage may result in a poor intake of this forage when fed to dairy cows (Paoletti et al. 1998). For this reasons, a 5-year field experiment was conducted in a mountain environment to evaluate several tall fescue varieties for competitiveness, yield and forage quality.

Material and methods

The field trial was established on the 4th of April 2005 at the experimental farm Mair am Hof in Dietenheim (920 m a.s.l., Bruneck, South Tyrol, Italy). The soil had a pH of 6.3, a humus content of 51 g/kg, a P-content of 122 mg/kg and a K-content of 373 mg/kg. The mean yearly temperature and precipitation sum in the quinquennium 2005-2009 were on average 7.7 °C and 856 mm respectively. Five soft-leafed varieties (Barcel, Bariane, Barolex, Belfine, Molva) were compared to four rough-leafed varieties (Astico, Fawn, Hykor, Kora). Hykor, although registered as a tall fescue variety, is actually a festulolium (*Festuca pratensis* x *Lolium multiflorum*). The plots were mechanically sown with a plot seeder trm 2200 Plotmatic (Wintersteiger, Ried, A) at a seed rate of 40 kg/ha. The trial design was a Latin rectangle with three replications and a plot size of 6.4 x 4 m. In the first year, only cleaning cuts were made. Starting with the second growing season, the trial was harvested 4 times per year, following a harvest plan (22nd of May, 4th of July, 21st of August, 2nd of October). Adjustments of the harvest date were allowed up to 12 days in accordance with the weather conditions and with the management of the experimental farm. The trial was fertilised after each cut with about 20 m³/ha of 2:1-water-diluted slurry. Before each harvest date, the yield share of tall fescue was assessed in each plot. a 1.35 m-wide strip was harvested in the middle of the plot along its longest side and the fresh yield weighed with a field scale. a 500 g-mixed sample was used to determine water content after drying at 60°C for at least 4 days. a grass sample of 200 to 250 g fresh weight, containing tall fescue only, was obtained in the field trough manual separation from other species and used for forage quality analyses. Forage quality was determined from 2006 to 2008 according to Van Soest (Naumann et al., 1997). Digestibility in 2007 and 2008 was measured *in vitro* for the first cut and on a mixed sample of the following three cuts according to Tilley und Terry (1963). The tall fescue-net yield was calculated for each cut by multiplying the tall fescue share by the DM-yield of the mixed plant stand. Year summary variables were calculated for all traits but the digestible organic matter (DOM) as weighted means with respect to the tall fescue-net yield. Statistical analysis of data was performed with a mixed model taking into account the variety and design factors (lines and columns) as fixed

and the year as a repeated factor. The second order-interactions of the year with variety and design factors were included in the model. For the statistical analysis of DOM, the interaction cut*year was included as a repeated factor in the model, as well as the cut, the year and their interactions (up to the third order) with the other factors. Prior to analysis, data were checked for normality of residuals and homogeneity of variances. Post hoc comparisons were performed by LSD test. a probability of $P < 0.05$ was regarded as significant.

Results and discussion

All traits but DOM were significantly affected by both the variety and the year. Interactions between them were detected for tall fescue net DM-yield and crude protein. DOM was affected by variety, cut and by the interaction of cut and year. Results depending on the factor variety are shown and discussed.

Although pure sown, the vegetation of all plots quickly developed to mixed lawns, mainly due to the germination and establishment of legumes and forbs from the soil seed bank. The mean share of tall fescue decreased on average from 51% in 2006 to 35% in 2009, showing that other species rather tall fescue were advantaged by the given climatic conditions. Such conditions, not particularly dry in summer, provide valuable information about the competitiveness of tall fescue. The tall fescue-share was higher for rough-leafed than for soft-leafed varieties, with Barolex and Molva exhibiting intermediate features (Tab. 1). a similar pattern was observed for the tall fescue-net yield. The varieties showed in this respect a large variation, with Kora, the most productive variety yielding one third more than the least productive (Bariane). On the whole, rough-leafed varieties showed better competitiveness than soft-leafed varieties. In accordance with our findings, a lower competitiveness of Molva, Belfine and Barolex in comparison to Kora was reported by Suter et al. (2009). However, this is probably also caused by a different earliness of the varieties. As a matter of fact, among the investigated varieties, the rough-leafed have an earlier development than the soft-leafed, as shown by our observation in the field and by phenological surveys on these varieties reported by other authors (Jöggi et al., 1981; Paoletti et al., 1998; Suter et al., 2003; Suter et al. 2009). Concerning forage quality, higher crude protein content was found for soft-leafed varieties, while NDF and ADF were found in higher amount among rough-leafed varieties. Also these findings are in accordance with the expectations due to the different earliness of soft and rough-leafed varieties.

Table 1: Yield share, net tall fescue-yield and forage quality of the investigated varieties. ADF values were log-transformed for analysis; back-transformed means are shown. Means without common letters are significantly different.

Variety	Leaf type	Tall fescue-yield share [%]	Tall fescue-net DM-Yield [t/ha/year]	Crude Protein [g/kg]	NDF [g/kg]	ADF [g/kg]	ADL [g/kg]	DOM [g/kg DM]
Kora	rough	51.4 ^{A}	6.0 ^{A}	142 ^{CD}	592 ^{AB}	336 ^{AB}	50 ^{C}	641 ^{A}
Hykor	rough	51.1 ^{A}	5.8 ^{AB}	138 ^{DE}	591 ^{ABC}	339 ^{A}	53 ^{BC}	636 ^{A}
Fawn	rough	46.6 ^{AB}	5.9 ^{AB}	132 ^{E}	601 ^{A}	342 ^{A}	56 ^{BC}	601 ^{C}
Astico	rough	45.6 ^{AB}	5.7 ^{AB}	137 ^{DE}	592 ^{AB}	341 ^{A}	52 ^{C}	634 ^{AB}
Barolex	soft	44.3 ^{ABC}	4.7 ^{ABC}	154 ^{B}	573 ^{DE}	322 ^{D}	55 ^{BC}	636 ^{A}
Molva	soft	43.6 ^{ABC}	4.6 ^{BC}	149 ^{BC}	583 ^{BCD}	326 ^{CD}	51 ^{C}	616 ^{BC}
Belfine	soft	38.9 ^{BC}	4.2 ^{C}	153 ^{B}	578 ^{CDE}	331 ^{BC}	64 ^{AB}	625 ^{AB}
Barcel	soft	38.2 ^{BC}	4.3 ^{C}	153 ^{B}	573 ^{DE}	326 ^{CD}	51 ^{C}	628 ^{AB}
Bariane	soft	36.0 ^{C}	3.8 ^{C}	164 ^{A}	567 ^{E}	319 ^{D}	71 ^{A}	617 ^{BC}

On the contrary, the lignin content (ADL) was not found to be consistent with the leaf type. The highest value was observed for Bariane, which is reported to be very late in the development and exhibited in our experiment the lowest values of the NDF and ADF. On the opposite, the lowest lignin content was found for Kora, which had high values of NDF and ADF. Also the *in vitro*-digestibility varied depending on variety and was not consistently related

to leaf softness, with the highest values being found for the rough-leafed varieties Kora and Hykor and for the soft-leafed variety Barolex.

Conclusions

The choice of suitable varieties of tall fescue for a seed mixture for permanent, polyphyte meadows should take both competitiveness and quality traits into account. While competitiveness, protein content, NDF and ADL seems to be strongly related to the leaf type and to earliness, lignin content and digestibility seem to rather depend on the single variety. Kora among the rough-leafed and Barolex among the soft-leafed varieties can be considered a good compromise between competitiveness and forage quality. Further research should be devoted to the optimisation of such a seed mixture.

References

[1] Badoux, S. (1971): Observations sur la difficulté d'implantation de la fétuque elevée. Revue suisse d`agriculture, 3: 34-37.

[2] Jöggi, D., Lehmann, J., Briner, H.U. (1981): Essaix variétaux de fétuque élevée (Festuca arundinacea Schreb.). Revue suisse d`agriculture, 13: 285-290.

[3] NAUMANN, C., BASSLER, R., SEIBOLD, R. and BARTH, C. (1997): Methodenbuch Band III, Chemische Untersuchung von Futtermitteln. Darmstadt: VDLUFA-Verlag.

[4] Paoletti, R., Locatelli, C., Casadei, N. and Berardo, N. (1998): European cultivars of tall fescue (Festuca arundinacea Schreb.) in the Po Valley: adaptation, earliness, dry matter yield, quality and persistence. Grassland Science in Europe, 3: 829-834.

[5] Suter, D., Briner, H. and Bosshard, H.-R. (2003): Rohrschwingel und Wiesenfuchsschwanz: neue Sorten. Agrarforschung, 10: 270-275.

[6] Suter, D., Frick, R., Hirschi, H. and Chapuis, S. (2009): Rohrschwingel- und Timothesorten geprüft. Agrarforschung, 16: 250-255.

[7] Tilley, J.M.A. and Terry, R.A. (1963): a two stage technique for the in vitro digestion of forage crops. Journal of the British Grassland Society, 18: 104-111.

Forage of Perennial Grasses as a Source of Mycotoxins in the Food Chain

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Abstract

The work objective was to assess the content of mycotoxins entering the food chain from the fodder. The paper includes two years observation. The content of mycotoxins was assessed in fresh herbage. The evaluated species were Lolium perenne, Festulolium pabulare, Festulolium braunii, mixture these species with Festuca rubra and mixture these species with Poa pratensis. The content of mycotoxins was established by Elisa method. Mycotoxins assessed were deoxynivalenol (DON), fumonisin (FOM), aflatoxin (AFL) and zearalenon (ZEA). DON and ZEA were detected in summer and in autumn too. Lowest content ($P<0.05$) was in June (DON 32.5 ppb, ZEA <LOQ), highest content ($P<0.05$) was in October (DON 53 ppb, ZEA 173 ppb). AFL and FUM were under limit of quantifications (<LOQ).

Keywords: perennial grasses, zearalenone, deoxynivalenol, ELISA

Introduction

Microorganisms in the phyllosphere of grasses are influenced appreciably by changes in grassland management, particularly by transition from the intensive management to extensification due to reduced cutting frequencies and lower fertilizer applications (Behrendt et al., 1997). In late autumn, the vegetation of pasture plants gradually decreases and weather conditions stimulate the development of microscopic fungi (Giesler et al., 1996) which, in consequence, may lead to the formation of mycotoxins (Opitz von Boberfeld et al., 2006). These metabolites can cause economic losses in animal production and decrease meat quality (Opitz von Boberfeld, 1996). The issue of moulds is very topical, namely in connexion with forages from grass stands used at the end of the growing season. There are considerable differences amongst the species. Mould-resistance species include Festuca arundinacea and its hybrids (Opitz von Boberfeld and Banzhaf ,2006).

The goal of the paper is assess safety of selected grasses (Lolium perenne, Festulolium pabulare, Festulolium braunii) and their mixtures with Festuca rubra respectively Poa pratensis during the growing season.

Materials and methods

Experimental locality

The small-plot experiment was conducted in the Research Station of Fodder Crops in Vatín, Czech Republic (49°31'N, 15°58'E) and established in 2007 at the 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. Soil nutrient content was in year of observation 89.1 mg kg^{-1} P, 231.6 mg kg^{-1} K, 855 mg kg^{-1} Ca and pH was 4.76.

Experimental design

A split plot design was used with plots of 1.5 × 10 m. The main plots were species and the subplots were harvest dates. The experiment was carried out in triplicates. The first evaluated factor was species: Lolium perenne (cv. Kenatur), Festulolium pabulare (cv. Felina), Festulolium braunii (cv. Perseus), mixtures of these species with Festuca rubra (cv. Gondolin) and/or Poa pratensis (cv. Slezanka). The share of Festuca rubra and/or Poa pratensis in the mixture was 15 %. The second evaluated factor was harvest date. In summer was used grass

stand as double cut in June and July. Subsequently autumn harvest dates were October and/or November and/or December. The observation took place two years 2008 and 2009.

Pure stands of each species were sown with 30 kg ha^{-1} seeds and each mixture was sown at 37.5 kg ha^{-1}. The experimental plots were fertilized with 50 kg ha^{-1} N. Dates of cuts were in summer beginning of June and end of July. Subsequently cuts in autumn were beginning October and/or beginning November and/or beginning December. The plots were harvested by self-propelled mowing machine with an engagement of 1.25 m. Harvested area was 12.5 m^2.

Detected parameters

ELISA method was applied for estimated of content of mycotoxins deoxynivalenol (DON), zearalenone (ZEA), fumonisin (FUM) and aflatoxin (AFL).

Statistical analyses

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

Results and Discussion

The content of DON was in summer at evaluated species from 33.0 to 51.7 ppb (Tab. 1). The higher ($P<0.05$) content of DON was in June than in July. The content of ZEA was at Lolium perenne and Festulolium pabulare under limit of quantifications (<LOQ). Highest content of ZEA was at mixtures (102.1 ppb, respectively 112.5 ppb). Major different between species was not significant because are higher standard errors of means. The contents of AFL and FUM were zero or under limit of quantifications. This reality is valid for summer and autumn samples too. Samples of grasses in autumn contained comparable amount of DON as samples in summer. During of autumn decreased ($P<0.05$) content of DON (Tab. 2). Evident is different between year of observation ($P<0.05$), too. The content of ZEA was lowest at Festulolium braunii. Content of ZEA decreased from October to December ($P<0.01$) equally as DON. Reason for low production of mycotoxins can be decreasing temperatures when mycotoxins are not produce. Warm weather during autumn is the other way suitable for mycotoxins production. The effect of not only biotic but also abiotic factors on the production of mycotoxins mention DeNijs et al. (1996), Engels and Krämer (1996). The content of ZEA was in October considerable higher (173.0 ppb) then in summer (<LOQ, respectively 122 ppb). According to D´Mello (2003), a zearalenone concentration ranging from 0.2 – 1.0 mg kg^{-1} is even toxic for rodents. Forage with a zearalenone content higher than 0.5 mg kg^{-1} is not advised for feeding (Marasas et al., 1979).

Conslusions

During the growing season can be forage of grasses contaminate with mycotoxins. It is especially in July and in October deoxynivalenon (DON) and zearalenon (ZEA). Increase risk of mycotoxins input to food chain. Different in safety of individual grass species was not statistically proved. However was evident lowest content of ZEA at Festulolium pabulare.

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References

[1] Behrendt U., Müller T., Seyfarth W. (1997): The influence of extensification in grassland management on the populations of micro-organisms in the phyllosphere of grasses. Microbiological Res. 152, 75–85

[2] DeNijs M., Soentoro P., Asch E.D.V., Kamphuits H., Rombouts F.M., Notermans S.H.W. (1996): Fungal infection and presence of deoxynivalenol and zearalenone in The Netherlands. J. Food Protect. 59, 772-777

[3] D´MELLO J.P.F. (2003): Food safety contaminants and toxins. CABI Publishing, Wallingford, 452 p.

[4] Engels R., Krämer J. (1996): Incidence of fusaria and occurence of selected Fusarium mycotoxins on Lolium ssp. in Germany. Mycotoxin Res. 12, 31-40
[5] Giesler L.J., Yuen G.Y., Horst G.L. (1996): The microclimate in tall fescue turf as affected by canopy density and its influence on brown patch disease. Plant Dis. 80, 389-394
[6] Opitz von Boberfeld W. (1996): Changes of the quality including mycotoxin problems of the primary growth of a hay meadow – Arrhenatherion elatioris. Agribiol. Res. 49, 52-62
[7] MARASAS W.F.O., VANRENSBURG S.J., MIROCHA C.J. (1979): Incidence of fusarium species and the mycotoxins, deoxynivalenol and zearalenone, in corn produced in esophageal cancer areas in Transkei. J. Agric. Food Chem. 27, 1108-1112
[8] Opitz von Boberfeld W., Banzhaf K., Hrabě F., Skládanka J., Kozlowski S., Golinsky P., Szeman L., Tasi J. (2006): Effect of different agronomical measures on yield and quality of autumn saved herbage during winter grazing – 2nd communication: Crude protein, energy and ergosterol concentration. Czech J. Anim. Sci. 51, 271 – 277
[9] Opitz von Boberfeld W., Banzhaf K. (2006): Yield and forage quality of different xFestulolium cultivars in winter. J.Agron. Crop Sci. 192, 239 – 247

Table 1: The influence of species, harvest date and year on the content (ppb) of deoxynivalenon (DON) and zearalenone (ZEA) in summer

Factor	DON		ZEA	
	x	s.e.	x	s.e.
Species				
Lolium perenne	78,1	17,1	<LOQ	0,0
Festulolium pabulare	65,3	16,9	<LOQ	0,0
Festulolium braunii	35,1	13,6	91,0	90.9
Mixture with Festuca rubra	38,9	18,6	112,5	112,4
Mixture with Poa pratensis	42,4	25,2	102,1	102,0
Level of significance	0,433		0.732	
Harvest date				
June	32,5	12,3	<LOQ	0,0
End of July	71,4	7,7	122.0	62,5
Level of significance	0,015		0.066	
Year				
2008	54,6	11,6	122	62,6
2009	49,3	12,6	<LOQ	0,0
Level of significance	0,762		0.066	

<LOQ = under limit of quantifications

Table 2: The influence of species, harvest date and year on the content (ppb) of deoxynivalenon (DON) and zearalenone (ZEA) in autumn

Factor	DON		ZEA	
	x	s.e.	x	s.e.
Species				
Lolium perenne	41,4	11,1	56,7	51,0
Festulolium pabulare	36,2	12,1	16,3	7,4
Festulolium braunii	33,0	13,1	60,7	60,7
Mixture with Festuca rubra	51,7	15,7	78,0	77,9
Mixture with Poa pratensis	47,6	16,5	92,4	80,3
Level of significance	0.867		0.926	
Harvest date				
October	53,0	9,2	173,0	66,2
November	51,7	12,0	3,7	3,6
December	21,4	6,1	5,8	3,8
Level of significance	0.041		0.005	
Year				
2008	26	9,3	111	49,1
2009	58	4,7	10	3,9
Level of significance	0.005		0.049	

THE CHANGES IN GROSS ENERGY CONTENT IN LUCERNE LEAVES AND STEMS IN THE FIRST CUT

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Abstract

The aim of this study was to evaluate changes in calorific values of lucerne leaves and stems in dependence on stem length in the first cut. In 2007, the forage sampling was repeatedly realized at the late bud and at the late flower stage in the first cut with three replicates. Each sample was separated into two stem length category (0 – 40 cm; > 40 cm) and the maximum stem length, stems count, and dry matter weight of leaves and stems were assessed by each category. The calorific value (J/g) in dry biomass was consequently measured in each sample. The most significant effect was recorded for length category when leaves and stems > 40 cm had significantly higher calorific value in comparison with < 40 cm. Positive significant correlations were found between calorific value and count, length and dry matter weight for leaves and stems. It is possible to conclude, that stems tend to have higher calorific value than leaves. With regard to stage development, it was obvious that increase of calorific value during lucerne development is only in the stems whilst it stayed at the same level within the leaves. For future similar research, the assessment of calorific value primarily in lucerne stems should be recommended. This value represented gross energy content and could be connected with increasing ratio of energy rich materials (lignin) which are related to forage quality.
Keyword: forage, alfalfa, quality, calorific value

Introduction

The energy in plants is accumulated as an organic matter due to synthesis of energy rich materials originated from the photosynthesis. The energy content in plats is dependent on plant's compositions. According to Paine (1971), the lowest energy content is in mono or disaccharides (glucose 15.4 kJ/g, saccharose 16.5 kJ/g), polysaccharides have a higher energy content (starch 17.4 kJ/g, cellulose 17.6 kJ/g). From other plant substances, lignin has 26.3 kJ/g, proteins have around 23.7 kJ/g and fats have 39.7 kJ/g. The energy content is assessed as calorific value of dry matter of sample by calorimetric method (Sommer, 1994). If the ash is included in the weight of sample the resulted value would be called as gross energy and is a base for evaluation of energy value of feed.

Generally, the gross energy in plants is influenced by range of factors. According to Fuksa *et al.* (2006), statistically significant differences in calorific value among individual plant parts of maize as well as among level of weed infestation were observed. Hakl *et al.* (2009[A]) described significant effect of year on calorific value of lucerne forage in the first cut which was connected with sum of effect temperature as a preliminary result. In this experiment from vegetative to early flower stage, effect of stage of development was not significant in spite of changes in plant's composition. Similar results without substantive changes in calorific value during lucerne development were published by Homolka *et al.* (2009). This result could be explained by differences in calorific value of lucerne plant's part and also changes in leaf-stem ratio during lucerne development. For example, these changes were described by Hakl *et al.* (2009[B]) where stem length was observed as one of the most important parameter. Therefore the aim of this study was to evaluate changes in calorific values of lucerne leaves and stems in dependence of stem length in the first cut.

Materials and Method

The experiment was conducted in the experimental field of Czech University of Life Sciences Prague. In 2007, the forage sampling was repeatedly realized at the late bud and at late flower stage in the first cut with three replicates. Used variety was Jarka and experiment was in the third of vegetation year. Each sample was separated into two stem length category: 0 – 40 cm and > 40 cm. The maximum stem length stems count, and dry matter weight of leaves and stems were assessed by each category. The calorific value (J/g) in dry biomass in plant parts at each length category was assessed using an automatic adiabatic calorimeter IKA C 5000 control. Paired samples t-test, ANOVA and correlation analyses were performed using STATGRAPHICS Centurion XV.

Results and Discussion

Means of calorific value of lucerne forage parts in the bud and flower stage separated according to length category are presented in Table 1. Obtained calorific values correspond with Homolka *et al.* (2009). The most significant effect was recorded for length category when leaves and stems > 40 cm have significantly higher calorific value in comparison with < 40 cm. According to Hakl *et al.* (2009[B]), the stems with length < 40 cm represented in average only 20 % of total dry matter yield so did not influenced considerably total forage yield. Differences between calorific value of plant parts were significantly confirmed. However, this effect was highly irregular. Generally, it is possible to conclude, that stems tend to have higher calorific value than leaves. These observations were in agreement with the result of Fuksa *et al.* (2006) who showed this significant effect for maize crop. In our experiment, lower entry data set must be taken in the account. With regard to developmental stage, it is obvious that changes in calorific value during lucerne development are complicated. It is possible to expect that calorific value of lucerne forage would be increased during development because there are positive significant relations to dry matter weight, count, length and dry matter weight for stems and leaves, respectively (Table 2). Based on the Table 1, it is obvious that increase of calorific value during lucerne development is only in the stems whilst it stayed at the same level within the leaves. In this case for three replicates, the least significant differences for calorific value between stages were 511 $J.g^{-1}$ and 288 $J.g^{-1}$ at leaves and stems, respectively. It is possible to expect significant difference in calorific value at lucerne stems between developmental stages when the higher count of replicates will be used.

Table 1: Means of calorific value (J/g) of lucerne forage parts in the bud and flower stage in the first cut (n = 3; for all length categories n = 6; p-values for paired samples t-test are in Latin, significant values are in bold).

stage	length category	calorific value		p-value
		leaves	stems	
late bud	< 40 cm	16970	17267	0.0026
	> 40 cm	18264	17931	0.0034
		0.0064	0.0157	
late flower	< 40 cm	17159	17395	0.5425
	> 40 cm	18014	18246	0.0811
		0.1507	0.0091	
bud stage	all	17616	17598	0.9035
flower stage	all	17587	17821	0.1754
		0.9126	0.0842	

Table 2: Coefficient of determination between calorific value of lucerne forage parts and stems count and length and yield of stems or leaves (n = 12, significant values at α=0.05 are in bold).

	leaves		stems	
	R^2	p-value	R^2	p-value
count	-	-	0.39	0.0300
length	-	-	0.68	0.0009
weight	0.66	0.0013	0.79	0.0001

Conclusion

With limited data from one year, this study confirmed significant positive correlations between calorific value and stem length, count and weight as well as leave weight. With regard to plant parts, the stems tend to have higher calorific value than the leaves. It was obvious that increase of calorific value during lucerne development is only in the stems whilst in the leaves it stayed on the same level. For future similar research, the assessment of calorific value primarily in lucerne stems should be recommended. This value represented gross energy content and could be connected with increasing ratio of energy rich materials (lignin) which are related to forage quality. The experiment is continuing so results can not be definitive for the time being.

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References

[1] Fuksa, P., Kocourková, D., Hakl, J., Kalista, J. (2006): Influence of weed infestation on the calorific value and chemical composition of maize (Zea mays L.). Journal of Plant Diseases and Protection, Special issue XX, Stuttgart, p. 823 – 830.

[2] Hakl, J., Mášková, K., Fuksa, P., Šantrůček, J. (2009A): Změny v obsahu spalného tepla u vojtěšky seté v první seči. In. Aktuální témata v pícninářství a trávníkářství 2009. Česká zemědělská univerzita v Praze, p. 30 – 33.

[3] Hakl, J., Šantrůček, J., Fuksa, P., Krajíc, L. (2009B): Leaf-stem ratio of alfalfa in dependence on term of cut, stage of development, and stands structure. Výzkum v chovu skotu, LI, 2, p. 19 – 23.

[4] Homolka, P., Koukolová, V., Hladká, V. (2009): Effect of maturity stage on estimation of gross energy and digestibility of energy of alfalfa forage. In. Proc. of the 31st International Czech and Slovak calorific seminar. 25-29.5.2009, Vrátná dolina, Slovak republic, p. 67-69.

[5] Paine, R.T. (1971): The measurement and application of the calorie to ecological problem. Ann. Rev. Ecol. Systematics, 2, p. 145 – 164.

[6] Sommer, A. [ed] (1994): Potřeba živin a tabulky výživné hodnoty krmiv pro přežvýkavce. ČAZV, komise výživy zvířat, Pohořelice, 198 p.

[7] StatPoint, Inc. (1982-2007): Statgraphics Centurion XV, StatPoint, USA.

Fractions of Protein and Fibre of Alfalfa

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Introduction

Nitrogen substance (crude protein) region to decompose immediately after the harvest or chopping of green plants; this process is called proteolysis and shows a negative effect not only on the nutritional value of feed but also on the fermentation process in the course of ensiling. These changes concerned also fibre. Their course depends on the speed and method of harvesting and wilting and also on the speed and quality of feed preservation. In this study we tried to follow up changes taking place in individual fraction of nitrogen substances and in fibre in the course of wilting and fermentation of alfalfa preserved at lower dry matter content (i.e. 31.5 %).

Material and Methods

Experiments were performed with alfalfa variety 'SOCHA'. This is a medium-early variety of the *erectum* type (its plants are robust and upright). Alfalfa was sown from 5 to 7 April 2008 using a drill machine Vaderstat (6 m). The cover crop (a mixture of legumes and cereals - LCM) was sown thereafter, i.e. from 7 to 10 April. Green fodder (i.e. alfalfa plus LCM together) was harvested on 9 to 11 July and the yield of fresh matter was 317 q/ha. In 2009, the stand was repeatedly (twice) harrowed on 4 April. Agrochemicals were not applied neither in the first nor in the second year of this experiment. The first experimental cut from 95 hectares was harvested on 25 May and the average yields was 5 t/ha of DM. Samples of green matter from the first cut were collected immediately after cutting and after 24 hours of wilting on the fields of the experimental farm of the Institute of Animal Science in Prague-Uhříněves (cadastre Královice). Samples (1 kg) of chopped material with the theoretical length of cut (TLC) 40 mm were preserved with an additive based on formic acid (5 l/t) and put into plastic bags from which the air was sucked out. These samples were stored at a stable temperature ranging from 20 to 24 ^{o}C for the period of 60 days. Thereafter they were analysed using classical Weende methods (AOAC, 1995); NDF was determined according to Van Soeset et al., (1991) and Doane et al. (1997), CP fractions according to Licitra et al. (1991). Acid content and pH were determined by AOAC (1995). Nitrogen substances were estimated using the Kjeldahl method and (for comparison) also according to Dumas. Each variant was established in six replications. The results were analysed statistically using QC-Expert 3.0 (TriloByte Statistical Software, 2010).

Results and Discussion

During 24 hours of wilting, the DM content of alfalfa increased nearly twice (i.e. from initial 17.7 % to 31.5 %). At this DM content it was very risky to preserve the chopped material without a chemical preservative and for that reason an additive based on formic acid was used. After 60 days of fermentation the content of DM increased by 1 %; this indicates that DM losses were low and corresponded with results obtained under practical conditions. The differences between wilted fodder and silage were statistically insignificant.

The Cornell University in Ithaca, U.S.A. developed a system of estimation of individual fractions of N-substances and saccharides (Cornell Net Carbohydrate and Protein System, abbreviated as CNCPS). This system classifies crude protein (CP) as non-protein (A) and protein fractions. Proteins are further classified as soluble (B) and non-soluble (C) fractions. Although in our experiments the concentration of CP in ensiled material increased only insignificantly ($P>0.05$), there were significant changes in percentages of individual CP fractions. Fraction a was significantly higher in wilted forage than in fresh green matter (40.2 *vs.* 45.2 %); in silage, its percentage was significantly higher than in wilted fodder (45.2 vs. 61.3 %). An increase in the share of Fraction a took place to the detriment of Fraction B.

The content of CP in individual cells of plant tissue of fresh, wilted and ensiled alfalfa plants was studied for example by Makoni et al (1993). These authors found out similar changes in percentages of individual fractions. They concluded that the process of wilting increased the percentage of soluble CP fractions and simultaneously also that of chloroplasts. Proteolytic changes in CP fractions in silages were studied also by Lanzas et al (2008), Repetto et al. (2005), Guo et al (2008), Elizalde et al. (1999), and Ribeiro et al. (2001); their objective was to reduce degradation of protein and the obtained results were similar to ours.

In the course of wilting and fermentation of alfalfa with DM content of 31.5 % there were also changes in the fibre fraction. The highest content of NDF.was recorded in wilted fodder (446 g/kg DM); the difference between wilted and ensiled material (436 g/kg DM) was non-significant. However, a decrease in the content of hemicellulose due to fermentation was significant ($P<0.05$); in wilted fodder and silage, these contents were 102 g/kg DM and 62.2 g/kg DM, respectively. This indicated fermentation activities of lactic acid bacteria (LAB), which used hemicellulose for their growth and propagation.

Conclusions

During the process of alfalfa wilting on the field and during the subsequent fermentation under anaerobic conditions there are changes not only in the DM content but also in percentages of individual nutrients, above all of total crude protein and fibre (as well as in their fractions). Wilting and especially fermentation increase the percentages of soluble non-protein fraction (A) to the detriment of soluble protein fractions (B). Although the differences between contents of NDF in wilted and ensiled alfalfa were statistically insignificant, the content of hemicelluloses was significantly higher in wilted material than in silage. This indicates the fermentation activity of lactic acid bacteria, which use hemicelluloses for their growth and propagation.

Table 1: Fractions of protein and fibre of alfalfa, as green, wilted and ensiled

Index	Unit	Green	Wilted	Silage
DM	g	177.24 ± 2.11a	314.66 ± 0.93b	312.66 ± 6.69b
CP Dumas	g/kg DM	199.67 ± 6.12a	212.93 ± 3.61b	221.72 ± 6.14b
A fraction	%	40.17 ± 0.34a	45.23 ± 2.23b	61.28 ± 1.04c
B fraction	%	512.33 ± 6.34a	641.00 ± 22.06b	301.00 ± 11.76c
C fraction	%	88.87 ± 4.29a	87.00 ± 0.75a	86.33 ± 7.06a
NDF	g/kg DM	398.29 ± 12.63a	445.94 ± 21.41b	436.18 ± 16.06b
ADF	g/kg DM	321.92 ± 11.00a	343.86 ± 15.39a	374.02 ± 8.45b
Hemicelullose	g/kg DM	76.37 ± 21.37a	102.08 ± 8.47b	62.16 ± 9.93a
ADL	g/kg DM	68.24 ± 2.19a	75.72 ± 3.14a	85.25 ± 9.24b
CF	g/kg DM	259.00 ± 13.03a	288.01 ± 26.47ab	310.85 ± 7.16b

Values within the same row followed by different superscript letters are significantly different (P<0.05)

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References

[1] Doane, P.H., Pell, A.N., Schofield, P. 1997. The effect of preservation method on the neutral detergent soluble fraction of forages. JOURNAL OF ANIMAL SCIENCE, 75,4:1140-1148

[2] Elizalde, J.C., Merchen, N.R., Faulkner, D.B. 1999. Fractionation of fiber and crude protein in fresh forages during the spring growth. JOURNAL OF ANIMAL SCIENCE, 77,2:476-484

[3] Guo, X.S.; Ding, W.R.; Han, J.G., Zhou, H. Characterization of protein fractions and amino acids in ensiled alfalfa treated with different chemical additives. ANIMAL FEED SCIENCE AND TECHNOLOGY, 142,1-2:89-98

[4] Lanzas, C., Broderick, G.A., Fox, D.G. 2008. Improved Feed Protein Fractionation Schemes for Formulating Rations with the Cornell Net Carbohydrate and Protein System. JOURNAL OF DAIRY SCIENCE, 91,12:4881-4891
[5] Makoni, N.F., Vonkeyserlingk, M.A.G., Shelford, J.A., Fisher, L.J., Puchala, R. 1993. Fractionation of fresh, wilted and ensiled alfalfa proteins. ANIMAL FEED SCIENCE AND TECHNOLOGY, 41,1,:1-13
[6] Repetto, J.L.; Cajarville, C.; D'Alessandro, J., et al. 2005. Effect of wilting and ensiling on ruminal degradability of temperate grass and legume mixtures. ANIMAL RESEARCH, 54,2: 73-80
[7] Ribeiro, K.G., Pereira, O.G., Valadares, S.D., Garcia, R., Cabra, L.D. 2001. Characterization of the protein and the carbohydrate fractions, and the respective degradation rates of Tifton 85 bermudagrass hays at different regrowth ages. REVISTA BRASILEIRA DE ZOOTECNIA-BRAZILIAN JOURNAL OF ANIMAL SCIENCE, 30,2:589-595 AOAC. 1995. Official Methods of Analysis. 16th Edition. Association of Official Analytical Chemists. Washington, DC. Vol. 1: . 69-79.
[8] QC-Expert 3.0 TriloByte Statistical Software, 2010 (info www.trilobyte.cz)
[9] Van Soest, P.J., Robertson, J.B., Lewis, B.A. 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. JOURNAL OF DAIRY SCIENCE, 74, 3583-3597.

DYNAMICS OF PHYTOMASS GROWTH OF MULTI-CUT SORGHUMS DURING THE VEGETATION PERIOD

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Abstract

Field experiment with multi-cut sorghums was established in an experimental plot of Czech University of Life Sciences Prague in 2009. Four hybrids (sorghum x sudangrass) were evaluated in the dynamics of phytomass growth and yield. Two conventional hybrids (Nutri Honey, Latte) and two brown midrib (BMR) hybrids (Honey Graze BMR, Big Kahuna BMR) were used. Measurement of plant height and sampling of above-ground biomass was practiced in vegetation period in 7-days intervals (from 25 June till 30 July). BMR hybrids reached significantly ($\alpha = 0.05$) lower plant height during all evaluated period whereas plant weight of BMR hybrids was significantly lower only at harvest time. Among hybrids, there were no differences in dry matter content. At harvest time (73 days after seeding), the average plant height ranged from 1.57 m (Big Kahuna BMR) to 2.00 m (Latte). Conventional hybrids produced the yield 8.97 t/ha (Nutri Honey) and 9.08 t/ha (Latte). Yields of Honey Graze BMR was 7.42 t/ha and hybrid Big Kahuna BMR produced significantly the lowest value 5.11 t/ha.

Introduction

New hybrids of multi-cut sorghums (sorghum x sudangrass) are perspective annual forage crops. Phytomass can be used as green feed, silage, hay, for grazing or energy crop utilization. Multi-cut sorghums represent alternatives to traditional forage crop, above all the grasses, as they can tolerate the moisture deficit very well. They prefer warm conditions, but we can use them in areas with lower temperatures, as well (Pedersen and Fritz, 2000, Doležal *et al.*, 2009). Sorghum hybrids attract attention in the north-eastern USA because of its ability to produce acceptable forage yields on marginal maize ground (Kilcer *et al.*, 2005). Forage cultivars of sorghums are divided into conventional hybrids and brown midrib (BMR) hybrids according to digestibility of organic matter. BMR hybrids contain lower content of lignin in cell walls and show higher digestibility in later phase of vegetation. Stands of multi-cut sorghums could be harvested 2 – 3times in vegetation period (Doležal *et al.*, 2009). Our aim was to study the dynamics of phytomass growth and plant height of two conventional hybrids and two brown midrib hybrids in the period prior to first cut in conditions of sugar-beet growing region in the Czech Republic.

Materials and methods

Field plot experiment with four hybrids of multi-cut sorghums (sorghum x sudangrass) was established on 18 May 2009 on area of CULS Prague. The experimental plot is located in sugar-beet growing region in altitude 286 m and according to agrometeorological characteristics belongs to temperate warm and predominantly dry climatic region. Duration of vegetation period is 172 days. Average year temperature is 7.9 °C (14.0 °C per vegetation period) and long-term year sum of precipitation is 526 mm (364 mm per vegetation period).

Two conventional hybrids (Nutri Honey, Latte) and two brown midrib hybrids (Honey Graze BMR, Big Kahuna BMR) were tested in this experiment. Seeding rate was 800 000 seeds per ha (Nutri Honey: 19.0 kg/ha, Latte: 28.9 kg/ha, Honey Graze BMR: 22.4 kg/ha and Big Kahuna BMR: 27.5 kg/ha). Plot area was 10 m^2 (2 x 5 m) and distance between rows was 0.25 m. The experiment was established in Latin square. Fertilization with 130 kg of N/ha (ammonium sulphate), 45 kg of P/ha (superphosphate), and 130 kg of K/ha (potassium salt) was used before seeding.

Measurement of plant height and sampling of above-ground biomass was practiced in vegetation period in 7-days intervals (from 25 June till 30 July). Plant height was measured on 20 plants of each plot and 10 plants were sampled to determine the plant weight dynamics and dry matter content. The first cut was realized on 30 July (73 days after seeding). Sorghum plants were sampled from two rows of each plot in 3 m of length to determine the yield. Dry matter of sorghums was determined by drying to constant weight at 80 °C. Analysis of variance in Statistica 8.0 software ($\alpha = 0.05$; Tukey HSD) was used for statistical evaluation of the data.

Results and discussion

Monitoring of sorghum plants growth started on 25 June (38 days after seeding). Average plant height ranged from 0.21 m (hybrid Big Kahuna BMR) to 0.27 m (hybrid Latte) in this period (Figure 1). Initial phase of slow growth of plants up to 0.2 m height is succeeded by the period of intensive growth (Doležal *et al.*, 2009). Statistically significant difference in plant height was found in BMR hybrids during the monitoring period (except for first measurement), but no significant difference was found between conventional hybrids. Average plant height of traditional hybrids was higher than height of BMR hybrids for all the vegetation period. At harvest time, the average plant height ranged from 1.57 m (Big Kahuna BMR) to 2.00 m (Latte).

Plant weight of sorghums during the monitoring period is showed in Figure 2. Statistically, there was not found a significant difference in plant weight among tested hybrids in particular samplings in the period from 25 June to 23 July (1^{st} – 5^{th} sampling). Enhancement of plant weight was very slow up to the 4^{th} sampling (59 days after seeding). More intensive increase of phytomass was noted in 5^{th} sampling (66 days after seeding). Very rapid increase of plant weight occurred in the last 7 days prior to harvest. All tested hybrids showed approximately double increasing (1.8 – 2.3times) of plant weight in last week.

Dry matter content ranged from 102.7 to 126.1 g/kg in tested hybrids in the period of 1^{st} - 5^{th} sampling. At harvest time, the lowest value was found in BMR hybrids (Big Kahuna BMR: 142.7 g/kg and Honey Graze BMR: 150.3 g/kg) in contrast to conventional hybrids (Latte: 155.3 g/kg and Nutri Honey: 159.9 g/kg) with no significant difference (Table 1). However, almost significant difference (*p-value = 0.0504*) was found for Hybrid x Term of sampling interaction. According to Kilcer *et al.* (2005), the average dry matter content at harvest across their study was 160 g/kg with range from 80 to 220 g/kg.

Conventional hybrids produced the yield 8.97 t/ha (Nutri Honey) and 9.08 t/ha (Latte) at harvest of stand after 73 days of vegetation. Yields of Honey Graze BMR was 7.42 t/ha and hybrid Big Kahuna BMR produced significantly lowest value 5.11 t/ha (Table 1).

Figure 1: Plant height (m) of sorghums during the monitoring period in 2009 (p-value = 0.0000)

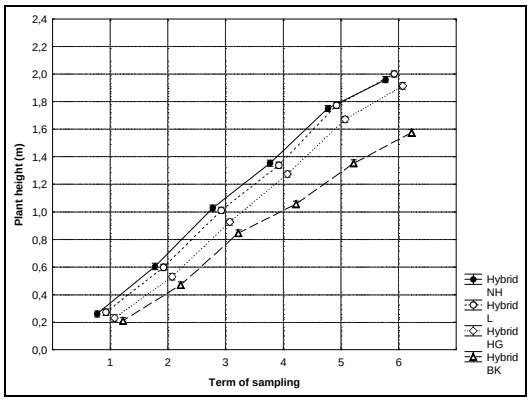
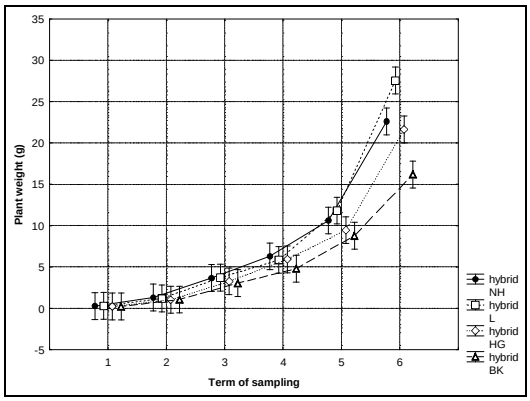


Figure 2: Plant weight (g) of sorghums during the monitoring period in 2009 (p-value = 0.0000)



(NH = Nutri Honey, L = Latte, HG = Honey Graze BMR, BK = Big Kahuna BMR, Term of sampling: 1 = 25 June, 2 = 2 July, 3 = 9 July, 4 = 16 July, 5 = 23 July, 6 = 30 July)

Table 1: Dry matter content (DMC; g/kg) during the monitoring period and yield of sorghums in green matter (GM; t/ha) and dry matter (DM; t/ha) in 2009

Hybrid	DMC (g/kg)						GM (t/ha)	DM (t/ha)
	25 June	2 July	9 July	16 July	23 July	30 July	30 July	
Nutri Honey	122.2	102.7	107.1	116.1	122.3	159.9	56.40[a]	8.97[a]
Latte	121.5	105.7	111.7	116.1	117.6	155.3	58.06[a]	9.08[a]
Honey Graze BMR	121.7	105.7	110.0	118.5	116.6	150.3	49.15[ab]	7.42[a]
Big Kahuna BMR	126.1	113.6	113.6	118.7	118.9	142.7	36.90[b]	5.11[b]
p-value							*0.0004*	*0.0001*

Conclusion

With limited one year results, it is possible to conclude that BMR hybrids reached significantly lower plant height during all evaluated period whereas plant weight of BMR hybrids was significantly lower only at harvest time. Among hybrids, there were no differences in dry matter content, but almost significant difference was found for Hybrid x Term of sampling interaction.

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References

[1] Doležal, P., Přikryl, J., Dvořáček, J., Podrábský, M., Vyskočil, I. (2009): Uplatnění vícesečných čiroků ve výživě a krmení zvířat (I. část). Krmivářství, 13, (2), 45-46.

[2] Kilcer, T. F., Ketterings, Q. M., Cherney, J. H., Cerosaletti, P., Barney, P. (2005): Optimum stand height for forage brown midrib sorghum x sudangrass. Journal of Agronomy and Crop Science, 191, (1), 35-40.

[3] Pedersen, J. F., Fritz, J., O. (2000): Forages and fodder. In: Smith, C. W., Frederiksen, R. A. (eds.): Sorghum: origin, history, technology, and production. John Wiley and Sons, 824 p.

The Effect of Organic Fertilizers and Cutting Frequency on Yield and Quality of Permanent Grasslands

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Introduction

The productive potential of permanent grasslands could be increased by different fertilization regimes and types of mineral/organic fertilizers. Organic fertilizers, in particular are the irreplaceable base for rational agriculture. As Samuil et al. (2009) point out, organic fertilizers (e.g. manure) has a positive effect on the plant nutrition regime and can improve the thermic and aeration regime of the soil, intensify the activity of the microorganisms in the soil and have a favourable effect on the development of vegetation. Organic fertilizers if applied rationally to grasslands can entirely replace chemical fertilizers. However, the level of fertilization and vegetation stage at harvest has effects not only on the dry matter yield but also on the fodder quality which is also greatly influenced by the floristic composition and morphological characteristic of the plants (Pozdisek et al., 2008). Further research to elucidate this issue is still necessary. This paper presents the results from 2005-2008, on permanent grassland sites, managed with different methods of organic fertilization (manure, dung-water, slurry) and intensity of use.

Materials and methods

The small-plot trial (plot size 12.5 m^2) was established in 2004 on permanent grassland sites in the locality Rapotin (the geomorphologic division Hrubý Jeseník) at 390-402 m a.s.l. Total annual rainfall is 693 mm, mean annual temperature is 7.2°C. The period of study was 2005-2008. The grassland vegetation on the stands was classified as *Arhenatherion*.

Experimental treatments simulating cattle grazing were as follows: M-0.9 - cow manure + dung water (model stocking rate 0.9 LU/ha), 2 cuts per year; M-1.4 - cow manure + dung water (model stocking rate 1.4 LU/ha), 3 cuts per year; M-2.0 - cow manure + dung water (model stocking rate 2.0 LU/ha), 4 cuts per year; S-0.9 - slurry (model stocking rate 0.9 LU/ha), 2 cuts per year; S-1.4 - slurry (model stocking rate 1.4 LU/ha), 3 cuts per year; S-2.0 - slurry (model stocking rate 2.0 LU/ha), 4 cuts per year. Unfertilized plots with three types of utilization were also observed – two (NF-2), three (NF-3) and four (NF-4) cuts per year as the control treatments.

The cow manure is applied in the autumn, dung water after the first cut; half of the slurry is applied in the spring and second half after the first cut. Nutrients in samples collected during the vegetation seasons 2005-2008 depending on the term of the cut (108 samples in total) were analysed according to Czech State Standard 46 7092 (Testing methods of feeding-stuffs). Crude protein (CP) was determined by the Kjeldahl procedure using the device Kjeltec Auto Distillation 2200 and ether extract (EE) by the Soxhlet method. The Fibertec System 2023 FiberCap (FOSS Tecator) was used to analyse crude fibre (CF). Ash content was measured gravimetrically by igniting samples in a muffle furnace at 450°C for 4 h. The *in-vitro* organic matter digestibility (OMD) was determined by the Tilley and Terry method (1963) modified according to Resch (1991). The energy value (ME - metabolisable energy; NEL - net energy of lactation) was predicted by means of the equations described by Petrikovic et al. (2000). This evaluation is officially used in the Czech Republic and Slovakia and this system of energy evaluation corresponds with the system INRA (Jarrige et al., 1989). The data were analysed by analysis of variance followed by the LSD test for pair-wise comparisons among means at the alpha level of 0.05.

Results and discussion

The results concerning dry matter yield and parameters of forage quality are shown in Table 1. The DM yields obtained were significantly influenced by the level of organic fertilization, confirming the results of e.g. Samuil et al. (2009). Influence of the used types of organic fertilizers on the DM yields was not confirmed. Nevertheless, the highest DM yields were achieved for slurry (8.23 t/ha; treatment S-3). The organic fertilizer rates determined changes in productivity by increasing the percentage of the dry matter yield by 41-68 % (for manure + dung water), and 56-94 % (for slurry), compared with the unfertilized control.

Further, there was a significant increase in crude protein content in connection with the increasing dose of slurry and increasing number of cuts per year. Significant differences were also found for organic matter digestibility (OMD), mainly in connection with intensity of utilization (60.8 %, 65.2 % and 67.4 % for two-, three- and four-cut regimes, respectively). The influence of type of fertilization was not significant as regards energy but it was found that reduced cutting frequency significantly decreased the energy (NEL) in the forage up to 4.60 MJ/kg DM. These results correspond with the previously published results. In agreement with the findings of Pozdisek *et al.* (2008), we can conclude that the treatment with three cuts per year and fertilized with a medium dose of fertilizers are most suitable.

Table 1: Dry matter yield and forage quality of grasslands at different levels of intensity of utilization and fertilization with organic fertilizers (2005-2008).

Treatment	DM [t/ha]	CP [g/kg DM]	CF [g/kg DM]	EE [g/kg DM]	A [g/kg DM]	OMD [%]	ME [MJ/kg DM]	NEL [MJ/kg DM]
M-0.9	6.07	107.9	285.9	26.4	98.0	60.5	8.09	4.60
M-1.4	6.94	124.0	255.2	31.5	106.7	65.1	8.75	5.07
M-2.0	7.11	151.5	230.2	35.3	112.7	67.5	9.08	5.30
S-0.9	6.71	113.0	287.5	27.5	100.3	60.8	8.13	4.63
S-1.4	8.26	130.2	262.5	31.0	107.3	64.9	8.70	5.03
S-2.0	8.03	159.4	241.8	33.1	121.7	67.6	8.98	5.23
NF-2	4.29	105.0	281.0	27.5	101.1	61.2	8.34	4.78
NF-3	4.24	118.4	257.3	31.8	115.5	65.7	8.95	5.21
NF-4	4.21	138.4	238.7	32.2	113.4	67.0	9.22	5.39
Mean	6.21	127.5	260.0	30.7	108.5	64.5	8.69	5.02
$LSD_{0.05}$	0.99	11.8	13.0	1.8	4.9	1.8	0.26	0.18

Fertilization: M…manure + dung water; S…slurry; NF…no fertilization;
Model stocking rate: 0.9…0.9 LU.ha^{-1}; 1.4…1.4 LU.ha^{-1}; 2.0…2.0 LU.ha^{-1};
Cutting regime: 2-4…number of cuts per year

Conclusion

Appropriate grassland management through e.g. number of cuts and fertilization makes it possible to increase the amount and quality of the fodder. It is also necessary, however, to take into account all other relevant factors that could influence these parameters. These findings are important for cattle nutrition and for assessment of efficient grassland management.

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References

[1] Jarrige, R. (1989) Ruminant nutrition. Paris-London-Rome: John Libbey Eurotext, 389 pp.

[2] Petrikovic P., Sommer, A., Ceresnakova Z., Svetlanska M., Chrenkova M., Chrastinova L., Polacikova M., Bencova E. and Dolejsova P. (2000) The feed nutritive value, part I. VÚZV, Nitra, Slovak Republic, 134 pp.

[3] Pozdisek J., Stybnarova M., Kohoutek A., Svozilova M. and Rzonca J. (2008) Forage quality by animal fertilizer applications and by different grassland management. Grassland Science in Europe 13, 498-500.

[4] Resch R. (1991) in vitro – Verdaulichkeitsuntersuchung nach Tilley und Terry, 1963. [In vitro – determination of organic matter digestibility by Tilley and Terry, 1963.] Bericht über die Tagung der ALVA Fachgruppe Versuchswesen. Innsbruck 22-23. Mai 1991.

[5] Samuil C., Vintu V., Iacob T., Saghin Gh. and Trofin A. (2009) Management of permanent grasslands in North-Eastern Romania. Grassland Science in Europe 14, 234-237.

[6] Tilley J.M.A and Terry R.A. (1963) a two stage technique for the in vitro digestion of forage crops. J. Brit. Grassl. Soc. 18, 104 – 111.

Nutritional Changes of Lucerne (Medicago Sativa) and its Mixture with Italian Ryegrass (Lolium Multiflorum) during Vegetation Period

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High buffering capacity and low content of water-soluble carbohydrates in forage of legumes, mainly Lucerne, make their ensilability difficult. Galik et al. (2008) reported also that feeds of protein character are a problem for intensive production of lactic acid and proper course of fermentation process because of deficit content of water-soluble carbohydrates.

We meet legume mixtures with grasses grown in our country as well as abroad in effort to improve ensilability (Merry et al., 2006, Loges and Taube, 1999).

The objective of our study was to compare dynamics of nutritional changes in stand of Lucerne and its mixture with Italian ryegrass during vegetation period.

Materials and methods

Pure stand of Lucerne and mixture of Lucerne with Italian ryegrass were grown in sub-montane cropping region, in the height above sea level 704 m.

Observations of stands were performed during the first year of production. The mixture contained 50 % of Lucerne during 1st cut and 54 % during 2nd cut. Average sampling was done immediately before small buds stage of Lucerne and at its beginning. Feeds were analysed for content of crude protein, fibre and its complex, sugars, fat and ash. Digestibility of organic matter and crude protein was assessed enzymatically, by means of *in vivo* method (Official Reports of MA SK, 2004). Concentrations of energy and PDI were calculated out of the measured values (Sommer et al., 1994).

Results and discussion

First cut of Lucerne stand was done in three terms: first one immediately before small buds stage, second one at the beginning of small buds stage and third one in the phase of small buds stage. Table 1 shows that the most favourable content of nutrients, energy as well as digestibility of organic matter and crude protein were in the first two terms of harvest. In the phase of small buds stage deterioration in the studied parameters occurred. Second cut was done in two terms; namely before and at the beginning of the phase of small buds stage. In the second cut, we noticed increase in content of crude protein and fat, decrease in concentration of fibre complex and ash. In connection with this finding was digestibility and energy value of stand higher in the second cut. Lucerne reaches the highest concentration of sugars in the first cut. Our experiment affirmed it.

Table 1: Content of nutrition, energy and digestibility of Lucerne

Parameter	First cut			Second cut	
	20.5.2009	25.5.2009	8.6.2009	30.6.2009	6.7.2009
Dry matter	158.5	178.2	235.5	135.5	152.5
Organic matter	891.3	888.2	908.5	895.4	900.4
Crude protein	216.9	216.2	194.7	291.3	271.9
Crude fibre	268.6	264.5	293.7	192.7	225.8
ADF	308.4	279.0	356.1	235.7	294.1
NDF	356.1	352.7	422.5	269.8	361.5
Hemicelluloses	47.7	73.7	66.4	34.1	67.5
Nitrogen free extract	383.7	382.9	394.3	384.5	376.3
Total sugars	66.0	62.2	57.6	55.3	41.3
Reducing sugars	38.2	35.3	44.4	30.7	23.7
Fat	22.2	24.6	25.8	26.9	26.4
Ash	108.7	111.8	91.5	104.6	99.6
ME in $MJ.kg^{-1}$ DM	9.43	9.46	8.73	9.93	9.50
NEL in $MJ.kg^{-1}$ DM	5.53	5.55	5.03	5.85	5.55
PDI in $g.kg^{-1}$ DM	81.79	81.50	74.56	93.25	88.36
OM digestibility in %	68.35	68.85	62.20	71.08	67.71
CP digestibility in %	89.66	88.93	85.83	89.25	89.47

Bíro et al. (2004) as well as Yu et al. (2004) confirm in line with our results that the stage of Lucerne maturity at harvest significantly influences the concentration of nutrients except crude protein, thus it is very important to choose a suitable date of harvesting.

Table 2: Content of nutrition, energy and digestibility of Lucernegrass mixture

Parameter	First cut			Second cut	
	21.5.2009	25.5.2009	8.6.2009	30.6.2009	6.7.2009
Dry matter	203.9	227.5	231.9	138.9	196.2
Organic matter	915.4	910.1	911.2	892.8	912.7
Crude protein	200.6	186.6	134.2	242.3	196.8
Crude fibre	225.7	256.2	305.0	210.9	289.5
ADF	245.6	266.2	339.0	277.9	352.1
NDF	372.8	391.5	468.4	347.6	450.9
Hemicelluloses	127.2	125.3	129.4	69.7	98.8
Nitrogen free extract	468.0	440.7	447.8	412.7	402.6
Total sugars	101.7	137.8	123.8	72.1	57.5
Reducing sugars	58.3	70.0	52.4	40.2	37.0
Fat	21.1	26.6	24.2	26.9	23.7
Ash	84.6	89.9	88.8	107.2	87.3
ME in $MJ.kg^{-1}$ DM	9.82	9.71	8.41	9.85	8.75
NEL in $MJ.kg^{-1}$ DM	5.78	5.71	4.81	5.86	5.02
PDI in $g.kg^{-1}$ DM	81.22	79.54	64.93	87.85	74.50
OM digestibility in %	69.54	69.27	59.73	72.19	61.62
CP digestibility in %	89.11	88.50	84.40	88.64	85.26

Stand of legumegrass mixture (Lucerne with Italian ryegrass) was harvested in the same terms as the Lucerne stand. Analyses of individual samples (tab. 2) showed slightly lower content of crude protein in the stand of mixture compared with the pure stand of Lucerne. Differences in concentration of fibre complex among individual samples of stands were more marked in the

mixture than in pure Lucerne. Increase in content of fibre in delayed term of harvest became evident in decrease of organic matter digestibility and concentration of feed energy. Content of total as well as reducing sugars was markedly higher in the mixture than in pure Lucerne stand. Decrease in concentration of sugars occurred in second cut. It is in line with the work of Wyss (2006), who dealt in detail with ensilability of grasses and legumes.

Conclusion

Results showed that early cutting is important with roughage. Content of nutrients and energy is higher in earlier terms of harvest of Lucerne as well as Lucerne-grass mixture than in later terms. Content of sugars, which are inevitable for the fermentation process and creation of lactic acid, was higher in the first cut than in the second one. Comparison of nutrients content in pure Lucerne with the Lucerne-Italian ryegrass mixture showed that there occurred slight decrease in concentration of crude protein, marked increase in content of total and reducing sugars under the influence of grass in the mixture. With regard to determined content of nutrients in stands, it is possible to suppose that the supplement of Italian ryegrass in Lucerne improved the ensilability of feed.

Differences in concentration of fibre complex among individual terms of harvest were more marked in the mixture than in pure Lucerne stand. Increase in fibre content became evident in decrease of digestibility and energy value in feed.

References

[1] Bíro, D., Juráček, M., Šimko, M., Gálik, B., Ulman, I. (2004): Zmeny výživnej hodnoty biologicky konzervovaných bielkovinových krmív. In Proteiny 2004. Brno: Mendelova zemědělská a lesnická univerzita, 2004, s. 27-30.

[2] Gálik, B., Bíro, D., Juráček, M., Šimko, M., Petránek, P. (2008): Kukurica – krmivárska charakteristika, význam a uplatnenie. In Krmivářství, roč. 12, 2008, s. 41-42.

[3] Merry, R. J., Lee, M. R. F., Davies, D. R., Dewhurst, R. J., Moorby, J. M., Scollan, N. D., Theodorou, M. K. (2006): Effects of high-sugar ryegrass silage and mixtures with red clover silage on ruminant digestion. 1. In vitro and in vivo studies of nitrogen utilization. In: J. Anim. Sci. 2006. 84:3049–3060.

[4] Loges, R., Taube, F. (1999): Ertrag und Futterqualität von Rotklee und Luzerne als Reinsaat sowie im Gemenge mit Gräsern. In: Beitrag präsentiert bei der Konferenz: 5. Wissenschaftstagung zum Ökologischen Landbau, Berlin, 23. - 25. Februar; p 501-504.

[5] Official Reports of MA SK (2004): Výnos Ministerstva pôdohospodárstva Slovenskej republiky č.2145/2004-100 o úradnom odbere vzoriek a o laboratórnom skúšaní a hodnotení krmív.

[6] Sommer, A. et al. (1994): Potreba živín a výživná hodnota krmív pre hovädzí dobytok, ovce a kozy (Nutrients demand and charts of nutrient value of feedstuff for beef-cattle, sheeps and goats), Nitra 1994, 113 s.

[7] Wyss, U. (2006): Silierbarkeit und Silagequalität von Gräsern und Leguminosen. In: AGRARForschung 13 (10): 442-447, 2006.

[8] Yu, P., Christensen, D.A., Mckinnon, J.J. (2004): In situ rumen degradation kinetics of timothy and alfalfa as affected by cultivar and stage of maturity. Canadian Journal of Animal Science, 84, 255–263.

Influence of Bacterial-enzyme Additive on Fermentation Process of Faba Bean, Alfalfa and Oat Mixture Silages

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Introduction

One of possibilities to ensure good fermentative process is silage additives application. The addition of exogenous lactic acid bacteria results in more intensive fermentation (Stokes, 1992; Sheperd, et al., 1995; Gallo et al., 2002). The main fermentative product of homolactic bacteria is lactic acid and theirs´ application lowers pH in silages and inhibits proteolysis (Fraser et al., 2001; doležal, 2002; Gallo et al., 2003). Some biological additives contain also enzymatic compound, mostly on fibrolytic enzymes base, which increases the proportion of fermentable carbohydrates in ensiling matter due to partial degradation of fiber (Mikolajczak et al., 1998). The aim of this study was to attest the influence of bacterial-enzyme additive on fermentation process in mixture silages of faba bean, alfalfa and oat with high dry matter content.

Materials and Methods

In farm experiment we ensiled mixture, which was sowed in two steps. In the first step was sowed common oat (*Avena sativa*) variety Flämingsstern, 15 $kg.ha^{-1}$, along with faba bean (*Faba vulgaris*) variety Inovec, 250 $kg.ha^{-1}$, and in the second step alfalfa (*Medicago sativa*) variety Palava, 20 $kg.ha^{-1}$. In ensiling matter was the ratio: oat 20%, faba bean 70% and alfalfa 10%. The mixture was harvested when bean was forming hulls. The experiment was realised in co-operation with VPP SPU, farm Kolíňany. The mixture fresh matter with average content of dry matter 156.53 $g.kg^{-1}$ was wilted to dry matter content of 528.43 (before ensiling control variant) and to 638.8 $g.kg^{-1}$ (before ensiling trial variant). The difference in dry matter content was caused by necessary time needed to filling the silage bags. Wilted ensiling matter was cut on the large of particles 20 mm and stuffed by press into silage bags with length 60 m, diameter 2.44 m and thickness 0.224 mm. We ensiled two variants: control variant (C) without additives and trial variant (A) with addition of bacterial-enzyme additive, which contains lactic acid bacteria: *Lactobacillus plantarum*, *Pedicoccus acidilacti*, *Lactococcus lactis lactis* (the bacteria concentration $2x10^{13}$ $cfu.g^{1}$) and enzymatic component: cellulase and hemicellulase, applicated in liquid state in dose 2 l per tone (after dissolving 2.5 g of powder in 2 l of water). After 3 months of fermentation we took the average samples of silages in which were determined the content of dry matter and indices of fermentation process. Contents of fermentation acids (lactic, acetic, butyric, propionic) we detected on analyzer EA 100 (Villa Labeco) by electroforetic method. Content of ammonia (NH_3) and alcohols we determined by microdiffusion method, acidity of aqua extract by alkalimetric titration to pH 8.5 and active acidity by electrometric method. The results were statistically processed using one-factorial variance analysis (ANOVA) of SAS. Means were separated using LSD multiple range test.

Results and Discussion

In mixture silages of faba bean, alfalfa and oat we detected after termination of fermentation process content of dry matter 501.0 $g.kg^{-1}$ (C) and 623.6 $g.kg^{-1}$ (A). Formic acid content was very similar in both variants of mixture silages. Content of desirable lactic acid we found from 48.49 $g.kg^{-1}$ of dry matter (A) to 72.58 $g.kg^{-1}$ of dry matter (C). In variant a was content of lactic acid statistically non-significantly lower. However, other researchers (Rizk et al., 2005; Bíro et al., 2008;

Filya et al., 2007) reported increase of lactic acid content after inoculation with LAB. Silages of both variants fulfilled condition of lactic acid content to classification as 1st quality class (content of lactic acid minimum 10 g in kg of original matter). The highest content of acetic acid (6.74 $g.kg^{-1}$ of dry matter) we detected in silages without additives (C). From point of negative influence to animal health and quality and nutritive value of silages is undesirable content of butyric acid. In tested silages of both variants we did not detect content of butyric acid, because the silages had higher content of dry matter. We detected the propionic acid only in silages of a variant. Content of total alcohols ranged from 3.69 $g.kg^{-1}$ of dry matter (A) to 3.93 $g.kg^{-1}$ of dry matter (C). Content of ammonia, which represents destruction of nitrogenous compounds, was the lowest in silages with bacterial-enzyme additive (C). Differences in ammonia content between variants of mixture silages were significant ($P<0.05$). a similar tendency we observed also in the content of NH_3-N of total N, with the lowest value in silages a ($P>0.05$). Szűcs et al. (2003) showed that biological additive inhibited ammonia production in alfalfa silage with dry matter content 53 %. Doležal (2002) confirmed positive influence of biological additive on content of N-compounds, too. Nutrient digestibility and nitrogen retention inoculated alfalfa haylage in sheep was described by Orosz et al. (2006). Titratable acidity of silage extracts ranged from 1165 mg KOH/100 g of silage (A) to 1751 mg KOH/100 g of silage (C). The highest TA ($P<0.05$) was in control silages, in which we detected the lowest pH (4.39). Silages with bacterial-enzymes additive had significantly ($P<0.05$) the highest value of pH. The highest content of fermentation products ($P<0.05$) we observed in control silages (C) with lower dry matter content.

Table 1 Result of fermentation process of faba bean, alfalfa and oat mixture silages

n=3		FA	LA	AA	PA	BA	NH_3	P	OH^-	TA	pHd	FP
C	$\bar{x}$	1.86	72.58	6.74	ND	ND	1.71^a	8.2	3.93	1751^a	4.39^a	79.76^a
	s	0.053	6.336	0.734	/	/	0.028	1.04	0.441	117.57	0.096	8.285
	v	2.847	8.730	10.887	/	/	1.640	12.680	11.221	6.72	2.197	10.387
A	$\bar{x}$	1.84	48.49	6.17	3.99	ND	1.42^a	6.71	3.69	1165^a	4.77^a	57.77^a
	s	0.274	4.844	0.419	0.867	/	0.105	0.450	0.469	74.35	0.072	4.083
	v	14.900	9.991	6.783	21.754	/	7.432	6.711	12.690	6.38	1.512	7.067

FA: formic acid, LA: lactic acid, AA: acetic acid, PA: propionic acid, BA: butyric acid, NH_3: ammonia, OH^-: alcohols, FP: fermentation products, $\Rightarrow$ content in $g.kg^{-1}$ of dry matter, P: NH_3-N of total N (%), TA: titratable acidity (mg KOH.100 g^{-1} of silage), pH: active acidity, ^{a}: the values with identical superscript in column are significantly different at $P<0.05$, ND: non-determination

Conclusion

Application of bacterial-enzymatic additive consisting of *Lactobacillus plantarum, Pedicoccus acidilacti, Lactococcus lactis lactis*, cellulase and hemicellulase positively influenced the fermentation process of mixture silages with high dry matter content by statistically significant lower content of ammonia and non-significantly lower value of NH_3-N of total N.

Acknowledgement

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References

[1] Bíro, D. – Juráček, M. – Gálik, B.– Šimko, M. – Petránek, P. 2008. Influence of biochemical additive on the quality of silage fermentation process. In Days of Animal Nutrition, Nitra: SAU, 2008. p. 36-40.

[2] Doležal, P. 2002. The effect of Lactobacillus plantarum 12771 on fermentation process of high wilted alfalfa and grass silages. In Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis. vol. 50, 2002, no. 5, p. 37-44.

[3] Doležal, P. - Vyskočil, I. - Kalhotka, L.- Skládanka, J.- Doležal, J. 2008. Effect of microbial inoculant on the quality of brewer's grain ensilage. In 13th International Symposium Forage Conservation. Nitra: RIAP. 2008, p. 118-119.

[4] Filya, I. – Muck, R.E. – Contreras-Govea, F.B. 2007. Inoculant effects on alfalfa silage: fermentation products and nutritive value. In Journal of Dairy Science. vol. 90, 2007, no. 11, p. 5108-5114.

[5] Fraser, M.D. – Fychan, R. – Jones, R. 2001. The effect of harvest date and inoculation on the yield, fermentation characteristics and feeding value of forage pea and field bean silages. In Grass and Forage Science. vol. 56, 2001, no. 3, p. 218-230.

[6] Gallo, M., Jambor, V., Mlynár, R., Rajčáková, L. 2002. Effect of the application of different silage preparations upon the fermentation process in red clover. In Proceedings of the XIIIth International Silage Conference, Auchincruive, Scotland, p. 110–111.

[7] Gallo, M. – Rajčáková, Ľ. – mlynár, r. 2003. Effect of application biological additive on fermentation quality of red clover silage. In 11th International scientific Symposium: Forage Conservation, Nitra: RIAP, 2003, p. 96-97.

[8] Mikolajczak, J. - Szejniuk, W. - Grabowicz, M. – Pilat, J. 1998. Chemical composition and quality of silages prepared with different additives on the farm. In Zeszyty Problemowe Postepow Nauk Rolniczych, 1998, 462, p. 363-368.

[9] Orosz, S. – Póti, P. – Szőcs, J.L. – Markovics, D. 2006. Environmental aspects of silage additives: Nutrient digestibility and nitrogen retention inoculated alfalfa haylage in sheep. In Cereal Research Communications, vol. 34, 2006, no. 1, p. 747-750.

[10] Rizk, CH. – Mustafa, A.F. – Phillip, L.E. 2005. Effects of inoculation of high dry matter alfalfa silage on ensiling characteristics, ruminal nutrient degradability and dairy cow performance. In Journal of the Science of Food and Agriculture, vol. 85, 2005, p. 743-750.

[11] Szűcs, J.P. – Avasi, Z. – Márki-Zay, K.I. – Mészáros, A. 2003. Dynamic of fermentation of hard wilted lucerne with biological preservative. In 11th International scientific Symposium: Forage Conservation, Nitra: RIAP, 2003, p. 120-121.

[12] Sheperd, A.C. – Maslanka, M. – Quinn, D. – Kung, Jr. L. 1995. Additives containing bacteria and enzymes for alfalfa silage. In Journal of Dairy Science, vol. 78, 1995, no. 3, p. 565-572.

[13] Stokes, M.R. 1992. Effects of enzyme mixture, an inoculant, and their interaction on silage fermentation and dairy production. In Journal of Dairy Science, vol. 75, 1992, p. 764.

Safety Issues in Managing Large-scale Bunker Silos and Drive-over Piles

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Introduction

Few farming operations invite as many different opportunities for injury or fatality as a silage program. From harvesting the forage in the field, transporting it to the farm, placing it into storage, and then feeding out the silage, employees are exposed to numerous serious risks (Murphy 1994). Silage-related tragedy knows no age boundary as workers and bystanders of all ages have been injured or killed during silage harvest and feedout (Murphy and Harshman, 2006). Countless stories of PTO and harvesting machine entanglements, highway mishaps between farm equipment and automobiles, entanglement in self-unloading wagons and blowers, and encounters with silo gas exist (Murphy, 1994). Increasingly, stories involve bunker silos and drive-over piles (Murphy and Harshman, 2006 and Bolsen and Bolsen, 2009). Consistently protecting employees, equipment, and property throughout harvesting, filling, and feeding does not occur without thought, preparation, and training. Presented in this paper are five major hazards involved with managing silage in bunker silos and drive-over piles, and the primary ways these hazards can be eliminated, reduced, or controlled.

Methods

The five hazards discussed include:1) tractor roll-over, 2) entangled in or run-over by machinery, 3) fall from height, 4) avalanche or collapsing silage, and 5) complacency.

Discussion

1. Tractor or truck roll-over. Roll-over protective structures (ROPS) create a zone of protection around the tractor operator. When used with a seat belt, ROPS prevent the operator from being thrown from the protective zone and crushed by the tractor or equipment drawn by the tractor. a straight drop from a concrete retaining wall is a significant risk, so never fill higher than the top of a wall. Sight rails should be installed on above ground walls. Lights should be added to the rail if filling occurs at night. Always form a progressive wedge of forage when filling bunkers or piles. The wedge provides a slope for packing, and a slope shallower than 3 to 1 minimizes the risk of a tractor roll-over. Backing up the slope can prevent roll backs on steep slopes. Use low-clearance, wide front end tractors equipped with well lugged tires to prevent slipping and add weights to the front and back of the tractors to improve stability. If using front-end loaders to move forage into the bunker or pile, do not carry the bucket any higher than necessary to keep the center of gravity low. When two or more pack tractors are used, establish a driving procedure to prevent collisions. Dump trucks can roll over on steep forage slopes, particularly if the forage is not loaded and packed uniformly. Raise the dump body only while the truck is on a firm surface.

2. Entangled in or run-over by machinery. Keep machine guards and shields in place to protect the operator from an assortment of rotating shaft, chain and v-belt drives, gears and pulleys, and rotating knives on forage harvesters, wagons, and silage feeding equipment. Keep non-workers away from traffic areas, and never allow people on foot (especially children) in or near a bunker or pile during filling or feedout. Adjust rear view mirrors on tractors and trucks and install back-up warning alarms.

3. Fall from height. It is easy to slip on plastic when covering or uncovering a bunker or pile, especially in wet weather. Standard guardrails should be installed on all above ground level walls. Use caution when removing plastic, tires, or pea gravel bags near the edge of the feedout face, and never stand on top of a silage overhang, as a person's weight can cause it to collapse. Where necessary, use equipment operating from the ground to remove spoiled silage from the surface of bunker silos and drive-over piles. Never allow a person to ride in the bucket of a front end loader!

4. Crushed by an avalanche/collapsing silage. a major factor contributing to injury or fatality from silage avalanche/collapsing silage is over-filled bunker silos and drive-over piles. a nutritionist had the following near miss, "I was taking a core sample at one of our large dairy customers and had just moved away from the face when a large section just fell off. This was a very well packed silo and had immaculate face management" (Bolsen and Bolsen, 2009).

In April 2007, an employee walked up to the feedout face in a bunker silo to take a sample. Approximately three tons of silage collapsed from the bottom, not the top, and engulfed the man causing his death (cited by Murphy and Harshman, 2007).

Avalanche/collapsing silage does not have to happen. Bunkers and piles should not be filled higher than the unloading equipment can reach safely, and typically, an unloader can reach a height of 3.5 to 4.5 meters. Use proper unloading technique that includes shaving silage down the feedout face and never "dig" the bucket into the bottom of the silage. Undercutting, a situation that is quite common when the unloader bucket cannot reach the top of an over-filled bunker or pile, creates an overhang of silage that can loosen and tumble to the floor. Never allow people to stand near the feedout face, and a rule-of-thumb is never stand closer to the feeding face than three times its height. When sampling silage, take samples from a front-end loader bucket after it is moved to a safe distance from the feedout face. Fence the perimeter of bunker silos and drive-over piles, and post a sign, "Danger: Do Not Enter. Authorized Personnel Only".

5. Complacency. a dairy nutritionist almost lost his life the day he took silage samples from a bunker silo with a 9-m high feedout face (Schoonmaker, 2000). "Even though I was standing 20 ft from the feedout face, 12 tonnes of silage collapsed on me. I did not see or hear anything. I had been in silage pits hundreds of times, and you just become kind of complacent because nothing ever happens. It just took that one time".

Here is another example cited by Bolsen and Bolsen (2009). "The accident happened on June 14, 1974 while making silage at Kansas State University's Research Farm. The blower pipe plugged for about the eighth time that afternoon, and I started to dig the forage out from the throat of the blower. The PTO shaft made one more revolution. Zap! The blower blade cut off the ends off three fingers on my right hand".

Conclusions

Even the best employee can become frustrated with malfunctioning equipment and poor weather conditions and take a hazardous shortcut, or misjudge a situation and take a risky action (Murphy, 1994). It is best to take steps to eliminate or control hazards in advance than to rely upon yourself or others to make the correct decision or execute the perfect response when a hazard is encountered. Only experienced people should be permitted to operate equipment associated with harvesting, filling, packing, sealing and feeding in a silage program. The correct sizing of bunkers and piles can reduce the risk of an accident. Spreadsheet software is available to assist producers and their silage team to better design and manage bunker silos and drive-over piles (Holmes and Bolsen, 2009). Think safety first. The silage industry has nothing to lose by practicing safety: it has everything to lose by not practicing it (Murphy and Harshman, 2006).

References

[1] BOLSEN, R. E., and K. K. BOLSEN. 2009. Bunker silo and drive-over piles safety reminders. Kansas State University

[2] Silage Team web site: http://www.ksre.ksu.edu/pr_silage

[3] HOLMES, B. J. and K. K. BOLSEN, 2009. What's new in silagemanagement?. In: Proceedings 15th International Silage

[4] Conference, Madison, WI. Pg. 61-76

[5] MURPHY, D. J. 1994. Silo filling safety. Fact sheet E-22. Agric. and Biol. Engineering Dept., The Pennsylvania State

[6] University, University Park, PA.

[7] MURPHY, D. J., and W. C. HARSHMAN. 2006. Harvest and storage safety. In: Proceedings Silage for Dairy Farms:

[8] Growing, Harvesting, Storing, and Feeding. NRAES Publ.181. Ithaca. NY. Pg. 171-187.

[9] MURPHY, D. J., and W. C. HARSHMAN. 2007. Horizontal silo safety. Fact sheet E-49. Agric. and Biol. Engineering Dept., The Pennsylvania State University, University Park, PA.

[10] SCHOONMAKER, K. 2000. Four ways to be safe around silage. Dairy Herd Mngt. October 2000.

Effect of BioStabil Mays on Aerobic Stability of Corn Silages

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Introduction

Traditionally corn silage ferments well. However, in contrast to green forages, it is usually prone to aerobic deterioration due to a long period of growth and accordingly a high natural contamination with moulds and yeasts. Therefore it is crucial to improve the content of stabilizing acids (e.g. acetic acid, propionic acid) in a way that secures effective suppression of aerobic spoilage organisms upon opening the silo.

This goal can be reached by directly adding stabilizing acids, which are usually expensive and/or corrosive, or by obtaining them during the fermentation process, using heterofermentative lactic acid bacteria. In the past numerous studies with Lactobacillus buchneri have shown successful improvement of aerobic stability. Lactobacillus brevis, while no less effective, has not yet come to its full recognition.

The trials used in this study apply Biomin® BioStabil Mays consisting largely of L. brevis to whole crop maize and crushed maize grains to improve aerobic stability.

Results and Discussion

Heterolactic fermentation yields a surplus of stabilizing acetic acid by using Biomin® BioStabil Mays thus ensuring aerobic stability. Contents do not surpass the max. recommended level of 35 g/kg DM.

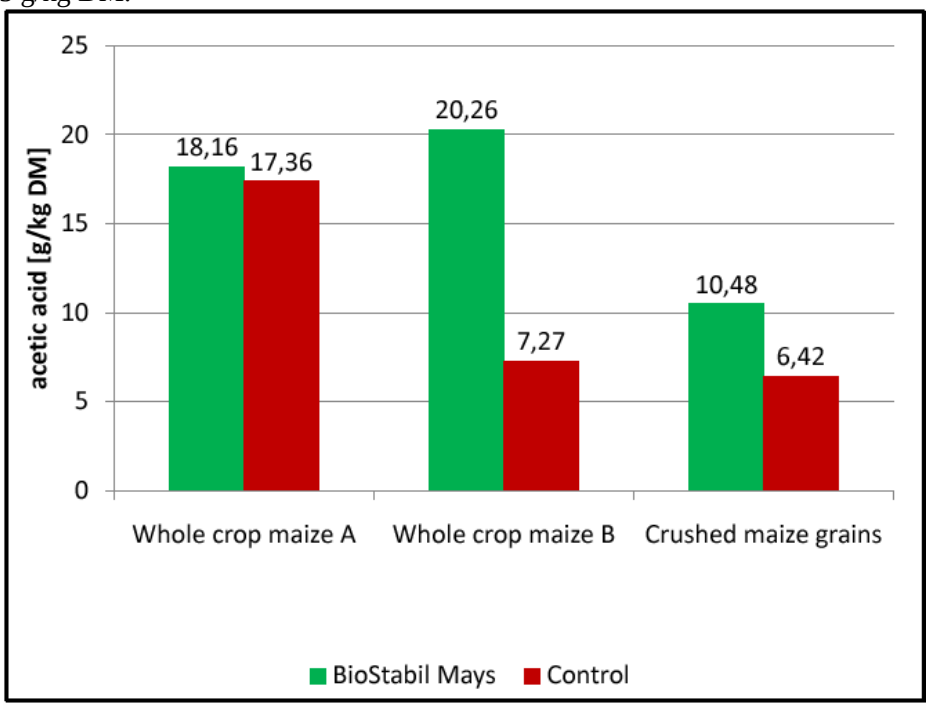

Figure 2 – Acetic acid contents after 90 days of ensiling with or without Biomin® BioStabil Mays

As a result of elevated acetin acid contents aerobic stability was improved (Fig. 2):

In all three trials a marked improvement of aerobic stability was obtained by use of Biomin ® BioStabil Mays. Silages treated with the inoculant were stable for at least 2,6 days longer than untreated silages.

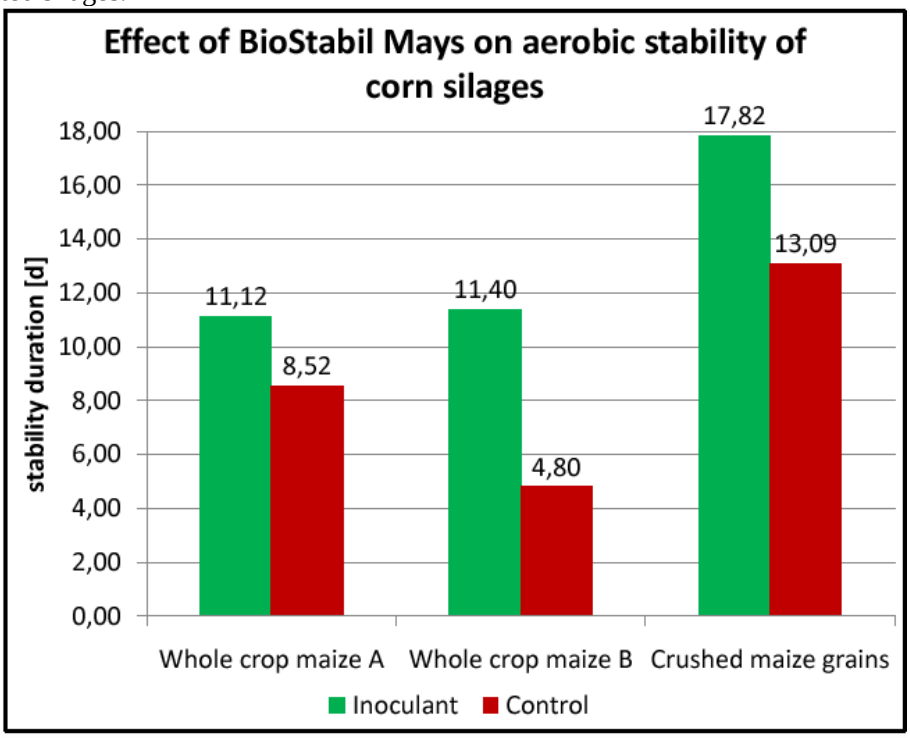

Figure 2 – Duration of aerobic stability after 90 days of ensiling with or without BioStabil Mays

Materials and Methods

Three lab scale trials (Whole crop maize A, Whole crop maize B, Crushed maize grains) were carried out ensiling maize with or without Biomin® BioStabil Mays (2 x 105 cfu/g fresh material).

Each trial group consisted of 12 model silos (six with 0,5 kg, six with 2 kg), of which three small ones were opened after 3 and 7 days, respectively and three large ones after 45 (+/-3) and 90 (-/+ 3) days, respectively.

Parameters analysed included pH, dry matter (DM) loss, organic acids (via HPLC) and aerobic stability (measurement of temperature rise according to Honig, 1990).

Conclusion

Biomin® BioStabil Mays was shown to effectively improve aerobic stability in whole crop maize and crushed maize grains!

Effects of Microbial Inoculants on Quality of Whole Crop Wheat and Maize Silage

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Introduction

Silage of whole crop cereals and maize were a good supplementation to legume-rich silage in the ration of lactating dairy cows. Maize silage has a good value of metabolizable energy, which is why it is cultivated even in the Nordic country where conditions are suboptimal for growth. The quality of silage depends on fermentation of the material, but the ensiling procsess can be optimised with the use of additives.

Several different biological additives can be used for making silage. The described research was aimed at finding silage additives suitable for treating whole crop wheat (WCW) and maize cultivated under Estonian climatic conditions. Their effects on silage fermentation characteristics, losses of dry matter, nutritive value and digestibility were also investigated.

Material and Methods

The whole crop wheat and maize were ensiled in 3-litre glass jars. The number of replicates was three. The first trial comprised six treatments (untreated control, four inoculants and chemical additive); and the second trial four treatments (untreated control and three inoculants). The additives were commercial products (inoculants 1 to 4; Table 1).

Table 1: Inoculants used in the trials

Number/Code	Inoculant	Source
Bonsilage(BO)	*L. plantarum, P. pentosaceus, L. rhamnosus, L. brevis, L. buchneri*	Schaumann Agri Austria GmbH & Co KCo KG
Lalsil MS01(LA)	*L. plantarum* MA18/5M, *P. acidilactici* MA 18/5M, *P. acidipropionici* MA	Lallemand Animal Nutrition, Blag nac, France
SilAll (SI)	*L. plantarum, E. faecium, P. acidilactici, L. salivarius*	Alltech Biotechnoligy Centre, Co. Meath, Ireland
Ecocorn (EC)	*L. plantarum* MTD1 + potassium sorbate	Ecosyl, Yorkshire, UK

After 90 days the jars were opened for analysis.

The pH value was measured with a Hanna Instruments Mikroprocessor pH meter 210, ammonia nitrogen was determined using an adjusted Kjeltec 2300 (FOSS) analyser. The ethanol, lactic acid and volatile fatty acids contents were determined chromatographically using a Agilent Technologies 7890A GC system with a column packed with 80/120 Carbopack B-DA/4% carbowax 20 M (Faithfull, 2002).

Samples were dried and analysed for the DM, crude protein, and crude fibre (AOAC, 2005). Crude protein was analysed by Kjeldahl method with Kjeltec 2300 analyser (FOSS Tecator Technology). *In vitro* digestibility of OM (IVOMD) were determined after incubating for 48 h using a DAISY II Incubators and NDF ANKOM Analyzer and to ashes in a furnace (ANKOM Technology, Fairport, NY USA). The NDF and ADF concentrations of the samples and digested residues were determined with amylase pretreatment using an ANKOM 220 Fiber Analyzer (ANKOM Technology) (Van Soest *et al*., 1991).

The content of DM of maize was 316 g/kg, CP 76 g/kg, NDF 443 g/kg and of WCW 359 g/kg, 90 g/kg, 619 g/kg respectively. The buffering capasity of maize was 30,3 g/kg LA in DM and wheat was 28,8 g/kg LA respectively. Statistical analysis was performed for each cut separately with the generalized linear model procedure of SAS. The effects of treatment were tested by means of orthogonal contrasts. Analysing the traits containing zero values, ranks of values were used; other traits were transformed to their logarithmic values.

Results and Discussion

Chemical compositions of the whole crop wheat and maize silage were significantly different.

Chemical composition and nutritive value of silages are given in Table 2. Maize silage contained more metabolizable energy than WCW silage. Organic matter digestibility of additives silages did not show any difference for the different silages in first and second trials (P<0.05).

Table 2: Chemical composition (in DM) and digestibility (OMD) and losses of whole crop wheat (WCW) and maize silages.

Treatment	Dry matter, g/kg		Crude protein g/ kg		NDF g/ kg		ADF g/kg		ME MJ/kg		OMD %	
	WCW	MAIZE	WCW	MAIZE	WCW	MAIZE	WCW	MAIZE	WCW	MAIZE	WCW	MAIZE
Control	313	303	95	79	622	442	346	242	9.1	10.8	67.7	69.3
BO	320	294	92	76	636	449	342	259	9.0	10.7	67.1	70.4
LA	318	–	92	–	629	–	357	–	9.0	–	66.5	–
SI	320	298	90	77	630	441	350	247	9.0	10.7	65.2	69.9
EC	326	300	86	78	618	447	339	257	9.0	10.8	65.6	69.7
CHEM	334	–	95	–	585	–	348	–	9.1	–	67.5	–

The positive effect of inoculants on silage fermentation but not on digestibility has been reported by Weinberg & Muck (1996).

Fermentation characteristics, pH, ammonia nitrogen in total nitrogen, organic acids and ethanol contents, and dry matter losses are given in Tables 3 and 4.

Table 3: Fermentation characteristics of the whole crop wheat silages in dry matter

Treatment	Dry matter losses, %	pH	Ammonia-N, % of total N	Lactic acid, g/kg	Acetic acid, g/kg	Butyric acid, g/kg	Ethanol, g/kg
Control	15.4	4.7	7.4	3.7	7.5	20.3	46.8
BO	11.9	3.8	6.0	98.2	29.5	1.3	24.0
LA	12.1	3.9	5.5	24.1	42.5	4.2	23.4
SI	11.7	4.0	6.5	26.7	11.6	8.6	46.5
EC	10.0	3.9	5.3	51.9	16.1	6.0	39.8
CHEM	7.5	4.5	8.8	37.9	24.4	0.9	26.6
Significant difference, *P*							
C vs BO	<0.001	<0.001	0.021	0.013	<0.001	<0.001	0.019
C vs LA	0.017	<0.001	0.005	0.011	0.003	<0.001	0.019
C vs SI	<0.001	<0.001	0.059	<0.001	0.019	<0.001	0.490
C vs EC	<0.001	<0.001	0.003	0.027	0.005	<0.001	0.252
C vs CHEM	<0.001	0.033	0.002	0.023	0.002	<0.001	0.159

Table 4: Fermentation characteristics of the maize silages in dry matter

Treatment	Dry matter losses, %	pH	Ammonia-N, % of total N	Lactic acid g/kg	Acetic acid g/kg	Butyric acid g/kg	Ethanol g/kg
Control	4.6	3.8	3.7	90.5	19.4	0.8	12.0
BO	7.4	3.8	3.7	97.2	31.7	0.0	3.3
SI	6.2	3.8	3.7	85.5	19.9	0.0	9.3
EC	5.3	3.8	3.7	99.1	20.2	0.2	12.1
Significant difference, *P*							
C vs BO	0.038	–	–	0.288	<0.001	0.029	<0.001
C vs SI	0.026	–	–	0.314	0.241	0.029	<0.001
C vs EC	0.124	–	–	0.223	0.149	0.069	0.445

DM losses during fermentation were the lowest in the WCW silages treated with CHEM additive (7.5%), in silages treated with biological additives these values were 10-12.1% and in untreated silage (15.4%) (Table 3).

In the first trial, the characteristics of silages treated with CHEM differed from those of silages inoculated with biological additives as well as from the uninoculated control silage by the lower content of organic acids (<0,01), but higher ammonia nitrogen (<0,01) concentration. AIV Pro contained ammoniumformiate (30.3%). This explains the high ammonia nitrogen concentration in the CHEM silages. This was predictable, as chemical additives have an inhibiting effect on fermentation. Compared to the control WCW silage, the lactic and acetic acid contents were higher in silages treated with additives, while the butyric acid content was lower (P<0.001). Ammonia nitrogen, pH, lactic and acetic contents of maize silages, were not different from those of the control silages (Table 4).

Conclusions

The use of inoculants or chemical additive at ensiling whole crop wheat material improved fermentation and silage quality: pH, the content of butyric acids, ethanol and ammonia nitrogen showed a decrease, whereas the lactic and acedic acids were increased. All commercial biological additives – Bonsilage, Sil-All, Lalsil MS01 and Ecocorn – improved the fermentation of whole crop wheat silage under the given conditions.

The maize fermentation was good whithout additive in Estonian conditions.

References

[1] AOAC Association of Official Analytical Chemists. 2005. Official Methods of Analyses. 18th ed. Urbana USA.

[2] Faithfull, N. T. 2002. Methods in Agricultural Chemical Analysis: a practical handbook, CABI Publishing, UK, 266 pp.

[3] Van Soest, P. J., Robertson, J. B., Lewis, B. A. 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. – J. Dairy Science, vol. 74, p. 3583–3597.

[4] Weinberg, Z.G. & Muck, R.E. 1996. New trends in development and use of inoculants for silage. FEMS Microbiol. Rev. 19, 53−68.

THE EFFECT OF SILAGE ADDITIVE ON THE QUALITY OF BREWER'S GRAINS ENSILED WITH THE SUPPLEMENTATION OF MOISTURE SORBENT AT LONG TIME STORAGE

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Introduction

Brewer's grains as a remainder after leaching of crushed malt at beer brewing represent an important protein feed. Dried brewer's grains are valuable raw material in the production of fodder mixtures and fresh grains with DM content of 200-220 g/kg are used either for the direct feeding of cattle and pigs, or for ensiling (Lohnert et al., 1996; Nishino et al., 2003 and others). Chemical composition and digestibility of brewer's grains were studied by many authors (Amari and Purnomoadi, 1996; Lohnert et al., 1996; Daccord et al., 1997 a.o.).

Net energy content ranges from 6.1 – 6.7 MJ NEL/kg DM (Lohnert et al., 1996; Spann, 1993). Costa et al. (1994) claim that 1 kg of brewer's grains DM contains 161.9 g/kg fibre, 386.3 g/kg BNLV, 486.0 g/kg NDF and 188.3 g/kg ADF. Brewer's grains have excellent dietary characteristics relating namely to the higher content of group B vitamins (Spann, 1993). a specific property of high-quality brewer's grains is their beneficial influence on the rumen environment in dairy cows, namely on microbial activity in the rumen and on the production of microbial protein.

Brewer's grains are fodder that readily deteriorates itself, especially in summer months. Gruber et al. (1997) and Doležal et al. (2006) report that fresh, non-conserved grains keep in feedable condition as a rule 48 hours at the longest. During storage, serious sensory, nutritional and particularly microbial changes occur in the grains. The low content of dry matter in the fresh grains causes extensive release and discharge of effluents. In order to prevent the discharge of silage effluents, Buchgraber and Resch (1997) recommend that fresh brewer's grains can be pressed to a higher DM content of 350-400 g/kg or ensilaged in combination with the addition of various absorbents (Pereira et al., 1998; Tanaka et al., 2001).

The objective of this model experiment was to establish the effect of the supplementation of various silage additives onto the quality of the fermentation process in brewer's grains with the addition of moisture sorbent.

Material and Methods

Material used in the model experiment was fresh brewer's grains at a DM content of 222 g/kg. Malt sprouts were used as moisture sorbent. The final dry matter content of ensiled material ranged from 320-330 g/kg. Established were three experimental variants in three repetitions: Variant a – control silage without the supplementation of silage additive, Variant B – treated with the ensilage additive based on organic acids (formic acid at 435 g/kg, propionic acid at 100 g/kg, ammonium formate 309, benzoic acid at 22 g/kg and water) at a dose of 3.5 litre/ton. Active substance in Variant C was bacteria of lactic fermentation (*Lactobacillus paracasei* (DSM 16245), *Lactobacillus lactis* (NCIMM 30160) and *Pediococcus acididlactici* (DSM 16243) at a dose of 2 g/ton.

Model silages were stored in the laboratory at average laboratory temperature of 26-28 ^{o}C for 112 days. Parameters assessed to establish the quality of the fermentation process after the 112 days were as follows: DM content of silage, pH, water extract acidity (KVV), amounts of lactic acid, acetic acid, propionic acid, butyric acid, contents of alcohol and ammonia. Analytical procedures

were described in our earlier work (Doležal, 2002). Results were statistically processed by using the analysis of variance and differences between individual groups were analyzed by Scheffe-test in program STATISTICA 8. Data in the text are presented as average ± standard deviation.

Results and Discussion

Dry matter of silages made from brewer's grains sampled after 112 days of storage ranged from 300.32 ± 8.46 g/kg in Variant C to 324.13 ± 5.1 g/kg in Variant B. Thanks to the use of moisture sorbent – malt sprouts, none of the model silages showed discharge of effluents.

The assessment of fermentation process quality corroborated the efficacy of silage additive on the pH value of silages. The lowest pH value (3.71 ± 0.01 pH) was found in the silage Variant B and the highest pH value (4.39 ± 1.64 pH) was detected in the control silage. The KVV value in variants treated with the silage additive correlated with the pH value. The highest value KVV (2124.2184 mg KOH/100 g) was found in Variant C. a statistically highly significant difference ($P<0.01$) was found between Variant B with the lowest KVV (1801.33 ± 45.34 mg KOH/100 g) and all the other variants. a statistically highly significant difference ($P<0.01$) was found between all the variants in the amount of lactic acid, the amount of acetic acid and in the total amount of fermentation acids.

The propionic acid was detected at an amount of 7.37 ± 0.74 g/kg DM only in Variant C. Nishino et al. (2003) observed that metabolization of lactic acid into acetic acid and propionic acid occurs with the storage time.

Statistically high significant differences ($P<0.01$) were found between lowest ratio in Variant C (1.51 ± 0.03) and the other studied variants in the assessment of the fermentation process quality with respect to the ratio of the amount of lactic acid to volatile fatty acids. The highest ratio was found in Variant B (4.23 ± 0.18).

Although the amount of ethanol in the respective silages was relatively equable, differences were between all variants statistically significant ($P<0.05$). The amount of ammonia was low in all variants and a difference between studied variants wasn't statistically significant.

Table 1: Quality of the fermentation process in brewer's grains silages (g/kg DM)

Variant	A		B		C	
Parameter	*Av. ±stand.dev.*	*Note*	*Av. ±stand.dev.*	*Note*	*Av. ±s tand.dev.*	*Note*
Dry matter [g/kg]	315.81 ± 3.4	A	324.13 ± 5.1	A	300.32 ± 8.46	B
pH	4.39 ± 1.64	a	3.71 ± 0.01	a	4.2 ± 0.01	a
KVV [mg KOH/100g]	2071.5 ± 51.27	A	1801.33 ± 45.34	B	2124 ± 21.84	A
Lactic acid	110.5 ± 3.32	A	68.12 ± 2.35	B	75.15 ± 2.47	C
Acetic acid	26.55 ± 0.45	A	16.1 ± 0.43	B	42.43 ± 1.33	C
Propionic acid	0 ± 0	A	0 ± 0	A	7.37 ± 0.74	B
Butyric acid	0 ± 0		0 ± 0		0 ± 0	
Sum of acids	137.04 ± 3.66	A	84.22 ± 2.39	B	124.95 ± 4.18	C
KM:KTM	4.16 ± 0.09	A	4.23 ± 0.18	A	1.51 ± 0.03	B
Ethanol	11.66 ± 0.5	Aa	10.65 ± 0.54	ABb	9.72 ± 0.47	Bc
Amonia	2.64 ± 1.17	a	3.14 ± 0.24	a	3.5 ± 0.21	a

KVV… water extrakt acidity, KM… lactic acid, TKM … volatile fatty acids; Statistically significant differences are among averages with by various index. Variants in capitals differ ($P<0.01$); variants in lower case differ ($P<0.05$).

Conclusion

The objective of the model experiment was to evaluate the effect of the supplementation of various silage additives onto fermentation process quality in brewer's grains with the addition of

malt sprouts as moisture sorbent. The results indicate that the dose of silage additive in Variant B was high because the fermentation process was suppressed, which corresponds with low pH level however with significant ($P<0.01$) lowest content the total amount of fermentation acids. But also nevertheless this silage we can evaluate very positively just for low value pH and lower value KVV and very good ratio of the amount of lactic acid to volatile fatty acids. Whereas at Variant C was pH relatively high, also amount acetic acids was high and it was only variant with detected propionic acid.

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References

[1] AMARI, M., PURNOMOADI, A., 1996: Chemical and digestive characteristics of brewers´grain for feed of cattle. Bulletin of National Institute of Animal Industry, No. 57: 39–46.

[2] BUCHGRABER, K., RESCH, R., 1997: Conservation of pressed brewers´grains and their utilization in cattle feeding. 1. The conservation of pressed brewers´grains with and without additives. Bodenkultur, 48: 1, 33–41.

[3] COSTA, J. M. B., MATTOS, W. R. S., BIONDI, P., CARVALHO, D. D., De-CARVALHO, D. D., 1994: Chemical composition of wet brewers' grains. Boletin de Industria Animal, 51: 1, 21–26.

[4] DACCORD, R., ARRIGO, Y., AMRHYN, P., 1997: Nutritive value of brewers´grains for ruminants. Revue Swisse d´Agriculture, 29: 3, 111–113.

[5] DOLEŽAL, P., 2002: Vliv přídavku Lactobacillus plantarum DSM 12771 na kvalitu siláží silně zavadlé vojtěšky a trávy (Effect of supplements of Lactobacillus plantarum DSM 12771 on the quality of ensiled alfalfa and grass with a high content of dry matter). Acta universitatis agriculturae et silvicultuarae Mendelianae Brunensis Brno, 5, p. 37–44.

[6] DOLEŽAL, P., VYSKOČIL, I., ZEMAN, L., KALHOTKA, L., DOLEŽAL, J., PYROCHTA, V., 2006: Kvalitativní změny čerstvého a silážovaného mláta. (Qualitative changes in fresh and ensiled brewer's grains.) Náš chov, no. 1, p. 32-34. ISSN 0027-8068.

[7] GRUBER, L., STOGERER, R., STEINWIDDER, A., LETTNER, F., 1997: Konservierung von Presstrebern sowie deren Einsatz in der Rinderfutterung. 2. Mitteilung: Einsatz von gepressten silierten oder getrockneten Biertebern in der Milchviehfütterung. Bodenkultur, 48: 3, 173–188.

[8] LOHNERT, H. J., RICHTER, G. H., OCHRIMENKO, W. I., FLACHOWSKI, G., KAMPHUES, J., 1996: Investigations on the storage and feeding value of fresh and preserved brewers´grains. Braunschweig–Volkenrode (FAL), 10–11th April, Landbauforschung–Volkenrode, Sonderheft, No. 169: 275–279.

[9] NISHINO, N., HARADA, H., SAKAGUCHI, E., 2003: Evaluation of fermentation and aerobic stability of wet brewers´grains ensiled alone or in combination with various feeds as a total mixed ration. Journal of the Science of Food and Agriculture, 83: 557–563.

[10] PEREIRA, J. C., CARRO, M. D., GONZALES, J., ALVIR, M. R., RODRIGUEZ, C. A., (1998): Rumen degradability and intestinal digestibility of brewers´grains as affected by origin and heat treatment and of barley rootlets. Animal Feed Science and Technology, 74: 2, 107–121.

[11] SPANN, B., 1993: Fűtterungsberater Rind. Verlagsunion Agrar, BLV Verlagsgesellschaft Műnchen GmbH, 183 pp.

[12] TANAKA, O., AKIYAMA, F., YAMADA , A. et al., 2001: Effect of gramma irradiation on microflora and fermentation quality of silages containing tofu cake or brewerś grain. Grassland Science, 47: 3, 274–282.

Fermentation Quality of Slightly Wilted Lucerne

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Introduction

Lucerne has high buffering capacity, which manifests itself in delayed onset of acidity in silage and enables development of undesirable microflora at the beginning of fermentation process, mainly with insufficient content of dry matter. It is known that dry matter in ensilaged feed at harvest influences markedly the quality of Lucerne silages (Biro and Juracek, 1999, Rajcakova and Mlynar, 2008). Wilting, which exceeds the critical value of dry matter is connected with high losses of nutrients and aerobic degradation during unfavourable weather (Owens et al., 2002). Cavallarin et al. (2000) studied the influence of wilting phase on fermentation parameters of Lucerne silages in dependence on stage of ripeness. More wilted Lucerne influenced positively the silage quality, namely by decreased fermentation, lower losses of dry matter and reduction of proteolysis. These factors influence also the variability of epiphytic microflora, which determines the first phase and result of fermentation. Application of ensilaging preparations is connected with increase in lactic acid content, quick decrease of pH and content of butyric acid, and in protein silages mainly with decrease of NH_3 content or percent of NH_3-N out of total N as parameter of proteolysis level.

The objective of this work was to assess the influence of different ensilaging preparations on quality of fermentation process in Lucerne silage with low degree of wilting.

Materials and Methods

In laboratory conditions was conserved the stand of Lucerne (*Medicago sativa*) at stage of budding. The matter was chopped after 6 hours of wilting; it was homogenized and filled into laboratory silos. We created four variants of silages. The first variant was the control silage, which was untreated with ensilaging preparation. The other three variants were treated with biological, microbiological-enzymatic and chemical additives:

T1 – containing as active substance life culture bacteria of lactic homofermentation (*Lactobacillus plantarum* DSM 3676 a 3677, *Propionic bacterium* DSM 9576 a 9577) in a total concentration of CFU $5x10^6$/g applied at a dose 2.0 litres per tonne ensilaged Lucerne

T2 – contained culture of lactic acid bacterium (*Pediococcus acidilactici* MA 18/5M, *Lactobacillus plantarum* MA 18/5U) and cellulase and hemicelullase enzymes too, the applied amount was 2.0 litres per tonne of ensilaged Lucerne

T3 – contained 24.4 % natrium nitride and 16.3 % hexamethylentetramine, the applied amount was 2.0 litres per tonne of ensilaged Lucerne

Each treatment consisted of six replicates. The model silages (silos 1.7 l) were stored in the laboratory at an average temperature 20 – 22 ^{0}C. Silage losses of dry matter were determined regularly at 21-day intervals. The experiment finished after 90 days of silage fermentation. Parameters measured to assess the fermentation process quality were: silage DM content, losses DM in %, crude protein, ADF, NDF, WSC, fat, pH electrometrically, lactic acid and volatile fatty acids were determined by gas chromatography, alcohol and NH_3 by the micro-diffusion method according to Conway. Analytical procedures were described in actual norm (MA SK, 2004). Energy and PDI concentrations in the silages were calculated as mentioned by Petrikovic and Sommer (2002). Results were statistically processed using the method of variance analysis and differences between the experimental groups compared by Student t-test.

Results and Discussion

Parameters of fermentation process are in table 2. Treated silages showed statistically highly significantly lower pH than the non-treated silage (4.76 vs. 4.33 to 4.45). Silage treated with microbiological-enzymatic preparation showed the lowest pH. Level of pH in our experiment was lower in treated silages than the one mentioned by Cavallarin et al. (2000), who found pH from 4.6 to 4.8. Different pH manifested itself also in the content of acids. Content of lactic acid was markedly lower in non-treated silage compared with its content in treated silages (49.90 vs. 63.89 to 81.87 $g.kg^{-1}$ dry matter). Differences among groups were statistically highly significant. Content of butyric acid decreased statistically highly significantly with application of ensilaging preparations, too. We noticed the lowest content of butyric acid in silage treated with life culture bacteria of lactic fermentation (0.37 $g.kg^{-1}$ dry matter).

Table 1: Lucerne – fresh matter

DM	OM	CP	ADF	NDF	WSC	Fat	ME	NEL	PDI
in g	in $g.kg^{-1}$ DM						$MJ.kg^{-1}$DM		$g.kg^{-1}$DM
328.10	902.97	218.79	362.76	416.48	73.27	18.76	9.46	5.54	81.53

Positive influence of ensilaging preparations became evident also in lower proteolysis. The lowest content of ammonia nitrogen expressed in NH_3-N out of total N was in silage treated with chemical preserver (7.62 %), and the highest content was in non-treated silage (9.16 %). The difference was statistically highly significant. Owens et al. (2002) came to similar results based on his experiments (2002).

Application of ensilaging preparations showed itself also in decrease of dry matter losses in ensilaged matter during the fermentation process and in higher content of nutrients compared with non-treated silage (Table 3). Markedly better effect was obtained with the chemical preserver compared with the biological and microbiological-enzymatic preparation. However, these differences were not statistically significant.

Table 2: Fermentation parameters in Lucerne silage in $g.kg^{-1}$ DM

Parameter n = 6	Untreated		T1		T2		T3		Statistical significance of differences
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	P < 0.01
pH	4.76	0.06	4.40	0.02	4.33	0.01	4.45	0.01	U : T1,T2,T3 T2 : T1,T3
Acids									
- lactic	49.90	1.87	72.07	5.62	81.87	3.88	63.89	3.61	U : T1,T2,T3 T3 : T1,T2
- acetic	13.79	0.58	15.19	1.50	15.84	1.47	20.08	0.95	T3 : U,T1,T2
- propionic	0.71	0.20	0.11	0.16	0.44	0.15	0.28	0.14	U : T1,T3
- butyric + isobutyric	1.36	0.68	0.37	0.12	0.78	0.37	0.68	0.15	U : T1,T2,T3
Alcohol	5.31	0.48	4.32	0.35	4.04	0.18	2.09	0.15	U : T2 T3 : U,T1,T2
NH_3-N of total N in %	9.16	0.27	8.87	0.29	8.13	0.76	7.62	0.53	T3 : U,T1

Positive effect of ensilaging additives was shown in decrease of ammonia nitrogen and higher content of crude protein in treated silages (211.04 to 215.32 $g.kg^{-1}$ dry matter) compared with non-

treated one (207.57 g.kg^{-1} dry matter). Higher content of nutrients in treated silages vs. non-treated silage manifested itself also in higher content of ME and NEL. Differences among nutrients were statistically highly significant in individual silages, except for fat.

Table 3: Nutrient composition in Lucerne silage in g.kg^{-1} DM

Parameter n = 6	Untreated		T1		T2		T3		Statistical significance of differences
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	P < 0.01
DM in g.kg^{-1} FM	320.14	2.50	324.67	1.23	324.29	1.88	324.97	2.77	
Losses DM in %	2.31	0.77	1.66	0.35	1.88	0.54	1.40	0.85	
Organic matter	898.69	0.84	899.10	0.87	899.94	0.67	899.97	0.52	
Crude protein	207.57	2.51	212.63	2.49	211.04	3.57	215.32	3.61	U : T3
ADF	381.04	7.72	364.99	7.95	373.54	9.64	350.84	6.11	U : T1,T3
NDF	424.58	7.61	414.91	11.08	423.32	7.28	406.04	18.78	U : T1T3
WSC	18.98	2.53	12.84	1.38	13.91	2.99	4.96	0.51	U : T1,T2,T3 T3 : T1,T2
Fat	23.86	1.21	24.21	1.15	25.41	0.45	25.82	1.58	
ME /MJ/	8.74	0.01	8.88	0.01	8.86	0.01	9.01	0.01	U : T1,T2 T3 : U,T1,T2
NEL /MJ/	5.04	0.01	5.13	0.01	5.12	0.01	5.22	0.01	U : T1,T2 T3 : U,T1,T2
PDI	73.34	0.58	75.58	0.30	76.36	0.57	78.76	0.40	U : T1,T2,T3

Conclusions

Results of the observations demonstrate that it is possible to use life culture bacteria of lactic fermentation, microbiological-enzymatic and chemical ensilaging preparation in conservation of wilted Lucerne with lower content of dry matter. Application of biological and microbiological-enzymatic preparation was less effective because of lower content of dry matter and higher buffering capacity in feed than the additive of chemical preserver composed of 24.4 % natrium nitride and 16.3 % hexamethylentetramine. The effect of chemical preserver became evident mainly in the lowest content of ammonia nitrogen expressed as NH_3-N out of total N, and the highest content of crude protein, which points out the low level of proteolysis and the lowest losses of dry matter in ensilaged matter related to it.

References

[1] BIRO, D. – JURACEK, M. (1999): Influence of chemical and biological additives on fermentation process and content of fiber fractions in the Lucerne silages. In: 9. Medzinárodné sympózium Konzervovanie objemových krmív, 6. – 8. Septembra, VÚŽV Nitra: SPU Nitra, 1999, s. 112-113, ISBN 80-88872-10-3.

[2] CAVALLARIN, L. – ANTONIAZZI, S. – BORREANI, G. – PEIRETTI, P.G. – TABACCO, E. – VALENTE, M.E. (2000): Effect of different levels of wilting on fermentation pattern of lucerne ensiled at two stages of maturity. In: Proceedings of the 18th General Meeting of the EGF, Aalborg, 22 – 25 May, Denmark, 2000, pp. 50-51.

[3] Official Reports of MA SK (2004): Výnos MP SR č.2145/2004-100 o úradnom odbere vzoriek a o laboratórnom skúšaní a hodnotení krmív. In: Vestník MA SK, XXXVI, Čiastka 22, Bratislava: MA SK, 2004, 342 p.

[4] Owens, V.N. – Albrecht, K.A. – Muck, R.E. (2002): Protein degradation and fermentation characteristics of unwilted red clover and alfalfa silage harvested at various times during the

day. In: Grass and forage science, Vol. 57, December, 2002, No. 4, pp. 329-341, ISSN 0142-5242.
[5] PETRIKOVIC, P. – SOMMER, A. (2002): Potreba živín pre hovädzí dobytok. II. aktualizované vydanie, VÚŽV Nitra, 2002, 62 s., ISBN 80-88872-21-9.
[6] RAJCAKOVA, L. – MLYNAR, R. (2008): Control of fermentation process by chemical additives at ensilaging of lucerne with low content of dry matter. In: 13th International Conference Forage Conservation Proceeding, 3rd – 5th September, SARC: RIAP Nitra, 2008, pp. 106-107, ISBN 978-80-88872-78-8.

THE EFFECT OF A BIOLOGICAL ADDITIVE ON ALFALFA SILAGE FERMENTATION AND IN VITRO OMD

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Introduction

Alfalfa (*Medicago sativa* L.) belongs amongst the oldest and most important perennial proteinous fodder plants with the highest content of N-substances, beneficial composition of amino acid, high protein value and lower incrustation by lignin. The steppe origin of alfalfa influenced its favourable agrotechnical characteristics (frost hardiness, drought resistance, favourable DM yield/ha, etc.). Due to the deep root system, it brings the nutrients up from deeper soil horizons, depositing them in the root system (VORLÍČEK, 2004) and enriching the soil with nitrogen. Alfalfa also contains large quantities of vitamins and minerals, especially calcium, phosphorus, potassium, magnesium, considerably more than for example grass stands (MITRIK, 2006). According to ZIMMER and HONIG (1987), differences in the quality and nutritive value of alfalfa and alfalfa silage are more determined by the fodder crop species because alfalfa is a typical fodder crop, which represents a very difficult problem in conservation by ensiling due to the insufficient content of fermentable sugars. Higher temperatures during the growing season cause a more intensive breathing of assimilates and thus reduction of water-soluble sugars. Higher temperature also influences the content of fructosans (MÍKA et al., 1997b). The highest content of fibre in the alfalfa dry matter is usually in the second harvest (BIRO et al., 1995; ŠIMKO et al., 2006). Because of the very low content of water-soluble sugars and the high content of N-substances and basic elements, alfalfa belongs amongst the worst ensilable fodder crops. To improve the ensilaging capacity and the quality of fermentation, it is necessary to leave the cut alfalfa stand to wilt intensively to a suitable content of DM or to use effective silage additives with respect to the content of dry matter. Several authors refer on the importance of adjusting the moisture content in alfalfa and application of suitable microbial inoculants during ensilaging (Dennis et al., 1999; Loučka et al., 1999; Savoie et al., 1999; Kung, 2009 and others).

Material and Methods

Material used in the model experiment was alfalfa from the second cut. The alfalfa stand was harvested after a short period of wilting by the cutting-machine Model Claas Jaguar 870. The average DM content was 287.15 g/kg. The theoretical length of shreddings was 15 mm. Five experimental variants in three repetitions were established for the preparation of model silages: Variant a – control silage without the supplementation of silage additive, Variant B – treated with the ensilage water-soluble microbial additive based on the bacteria of lactic fermentation *Lactobacillus paracasei* (DSM 16245), *Lactobacillus lactis* (NCIMB 30160), *Pediococcus acidilactici* (DSM 16243) at a dose of 2 g/ton; active substance in Variant C was monovalent bacteria of lactic fermentation (*Lactobacillus plantarum* LP 286 at a dose of 1 g/ton; variant D - treated with the ensilage water soluble microbial additive based on the lactic acid bacteria fermentation (*Lactobacillus buchneri* NCMB 40788) at a dose of 5 g/ton, and variant E - treated with the ensilage water soluble microbial additive based on the lactic acid bacteria fermentation (*Lactobacillus plantarum* MA 18/5 U, *Pediococcus acidilactici* MA 18/5 M, and enzyme cellulase (hemicellulase) 10 000 iu/g) at a dose of 10 g/ton. Model silages were stored in the laboratory at average laboratory temperature of 23-26 ^{o}C for 130 days. Parameters assessed to establish the

quality of the fermentation process after the 130 days were as follows: DM content of silage, pH, amounts of lactic acid, volatile faty acids, contents of alcohol and ammonia. Analytical procedures were described in our earlier work (Doležal, 2002). Results were statistically processed by using the analysis of variance and differences between individual groups were analyzed by Scheffe-test in program STATISTICA 8. Data in the text are presented as average ± standard deviation.

Results and Discussion

Results of experimental silages are in Table 1. Dry matter of silages made from alfalfa after 130 days of storage ranged from 304.87 ± 3,53 g/kg in Variant D to 307.85 ± 1,8 g/kg in Variant a (control silage). The higher content of dry matter in the control silage resulted from higher fermentation losses, which altogether represented 5.4%, while the lowest losses were found in the inoculated silage B (1.44%), resp. C (2.35%). Considerable differences were found in the pH values of which the lowest one was found in silage E (4.76±0.013), while in the inoculated silage D (5.04±0.021) the value exceeded the critical level for the given content of the dry matter (4.45). The explanation lies probably in the fact that the microbial inoculum contained only *Lactobacillus buchneri*, which typically produces acetate rather than lactic acid whose content is decisive for the pH value. The pH value of other inoculated silages was lower than that of the untreated control silage. All experimental silages showed a significantly higher ($P<0.05$) content of lactic acid (LA) than the control silage (2.222±0.079). Of the inoculated silages, the one with the lowest content of LA was the variant D silage (3.01±0.066). As compared with the untreated control silage, all experimental silages exhibited significantly lower values of acetic acid, which corroborates the fact that alfalfa with the the above-mentioned DM content was not biomass easy to ensile. From the dietary point of view, an important moment is the fact that the content of AA was in all cases higher than 1%, which in practice could have a negative effect on the intake of this silage. The fiery course of the fermentation is also indicated by the total content of fermentation acids in DM, which ranged from 13.31±0.186% to 16.17±0.47%. The lowest concentration was in the control silage, the highest one in the silage of the variant C. Differences between the other inoculated silages were insignificant. Great differences were found in the percentage of lactate within the total content of acids. The lowest proportion was found in the control silage (54.30%) and of the inoculated silages in silage D (63.14%) with respect to the pH value. The highest share (77.14%) of lactate in the total sum of acids was found in silage E, where the inoculant contained an enzyme component in addition to the bacterial one. Except for the control silage, ethanol contents did not show any significant differences.

Conclusions

The results of the experiment indicated that the used inoculants with the different contents of lactic acid bacteria regulated the fermentation process differently. Of the tested additives, the one best affecting the course of fermentation was that with the additional content of enzymes, which made it possible to attain a higher production of lactate aa well as the whole content of fermentation acids. Inoculants containing only one LAB species, namely *Lactobacillus buchneri*, did not have a positive influence on the quality of alfalfa silage fermentation. The untreated control silage exhibited the lowest proportion of lactic acid in the total sum of acids, the lowest total amount of fermentation acids, the lowest LA/AA ratio but the highest fermentation losses (5.40%).

Acknowledgement

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References

[1] BÍRO, D., PETRIKOVIČ, P., ŠIMKO, J., 1995: Lucerna siata, Vysoká škola polnohospodárská Nitra, 26 s.

[2] DENNIS, S., HENDRICK, C., MILLER, D., 1999: The effect of variety, cut, moisture lefel, and microbial inoculant on the terminal pH of ensiled alfalfa. In Conference Proceedings The XII th International Silage Conference, Uppsala: 87
[3] KUNG, L. jr. 2009: Potential factors that may limit the effectiveness of silage additives. In Vth International Silage Conference, Madison, Wisconsin:37-45.
[4] LOUČKA, R., TAKAHASHI, J., MASUKO, T., 1999: Fermentation quality of silage made from alfalfa, grass or alfalfa-grass mixture. In Conference Proceedings The XIIth International Silage Conference, Uppsala: 108-109
[5] MÍKA, V., KALAČ, P., 1997b: Přirozené škodlivé látky v rostlinných krmivech. Ústav zemědělských a potravinářských informací, Praha: 98−28 .
[6] SAVOIE, P., AGBOSSAMEY, R., SEOANE, J. R., 1999: Effect of three levels of maceration on lucerne silage, nitrogen fractions, rumen degradabiltiy and voluntary intake. In Conference Proceedings The XII th International Silage Conference, Uppsala: 155-156
[7] ŠIMKO, J. et al. 1998: Krmivárstvo, 1. vyd. Slovenská polnohospodárská univerzita, Nitra:24−25
[8] ZIMMER, E., HONIG, H. 1987: Bessseres Grundfutter für das Rindvieh. In AID Auswertungs und Informationsdienst für Ernährung, Landwirtschaft und Forsten, Bonn: 28 s.

Table 1: Fermentations charactersitics of alfalfa silages

	Specification	A	B	C	D	E
	Average	307,85	305,45	305,45	304,87	306,27
DM g/kg	s.e.g.	1,798	1,913	2,61	3,529	1,883
	Average	4,938	4,81	4,86	5,04	4,76
pH	s.e.g.	0,039	0,007	0,009	0,021	0,013
	Average	0,085	0,09	0,09	0,11	0,08
NH3 %	s.e.g.	0,005	0,005	0,005	0,005	0,005
	Average	2,222	3,37	3,53	3,01	3,57
LA %	s.e.g.	0,079	0,066	0,091	0,066	0,165
	Average	1,875	1,38	1,41	1,74	1,06
AA %	s.e.g.	0,051	0,018	0,062	0,025	0,033
	Average	1,187	2,44	2,51	1,73	3,36
LA/AA	s.e.g.	0,07	0,023	0,061	0,035	0,242
	Average	13,308	15,55	16,17	15,59	15,11
∑acids in DM	s.e.g.	0,186	0,242	0,474	0,192	0,496
	Average	0,038	0,04	0,02	0,06	0,02
Ethanol %	s.e.g.	0,007	0,007	0,006	0,01	0,007
IVOMD %	Average	83,1	86,59	83,57	85,19	84,21

Chemical Composition of Tifton 85 Grass Hay under Chemical Additives

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Abstract

In the winter of 2007, this study was conducted to evaluate the effect of additives on the chemical composition of Tifton 85 (Cynodon spp.) hay. The forage was harvested whit 45 days of vegetative growth. The treatments consisted of : 1 - hay with 70 to 80% DM without additive (HW), 2 - hay with 70 to 80% DM treated with propionic acid (HPA) 1% on the wet basis, 3 - hay with 70 to 80% DM treated with urea (HU) 1% on the wet basis and 4 – hay control with 85 to 90% DM (HC). Values of DM, CP, NDF, ADF, lignin, cellulose and DIVMO were evaluate. Treatments were distributed in a completely randomized design with four replications with a split plot scheme, considering the treatments as plots and periods of air exposure as split plot. The HC had DM levels higher than the others hays ($P<0.05$). Estimated in the HU higher values of CP than HPC in 5th and 9th day of evaluation ($P<0.05$). The HPA had lowest values of ADF and LIG than the others haylage ($P<0.05$). The additives change the chemical composition of Tifton 85 (Cynodon spp.)grass hay.

Keywords: urea, propionic acid, Cynodon spp.

Introduction

The tropical regions are generally well defined rainy season when the growth of forage plants is abundant, and a dry season, in which there is shortage of fodder. However, for animals to maintain good levels of production over the years, the use of forage quality also in the dry season is essential, since their nutritional requirement remains constant throughout the year. Looking around the low productivity of Brazilian herds in the dry season, various conservation techniques have been used fodder, among which stands out the hay. However, at the time these plants have high nutritional value, has high rainfall, leading to loss of nutrients in the field. Thus, the use of bulky, low-nutrient subjected to chemical treatment may be a viable alternative to meet the demand for good quality forage during the period of restricted availability of pasture for animals. The objective of this trial was to evaluate the effect of urea and propionic acid on the chemical composition of the Tifton 85 grass hays.

Materials and Methods

During the winter of 2007, the experiment was conducted in an area formed with Tifton 85 (Cynodon sp.) irrigated and fertilized with 120 kg N ha-1, 50 kg ha-1 P2O5 and 60 kg ha-1 K2O. Forage was harvested at 45 days of vegetative growth. The treatments consisted of four hays: HW - moist hay (70% DM) untreated; HPA - moist hay treated with propionic acid additive (1% on the wet basis); HU – moist hay treated with urea (1% on the wet basis) and HC - hay control (90% DM). The hays were stored in sealed plastic bags for 60 days, after this period of treatment were opened and samples were collected and evaluated at 0, 5 and 9 days after opening. Samples were collected to determine the dry matter (DM) levels, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), lignin (LIG) and in vitro digestibility of OM (DIVMO). The contents of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were evaluated according to the technique described by Robertson and Van Soest (1981). Lignin was determined after solubilization of cellulose in sulfuric acid at 72% (Van Soest, 1994). Treatments were distributed in a completely randomized design with four replications in a split plot scheme, considering the treatments as plots and periods of air exposure as split plots. The analysis of variance was

performed and comparisons between specific groups of treatments were made by orthogonal contrasts.

Results and Discussion

The table 1 presents estimates of the significant contrasts between treatment and evaluation time for the variables DM, CP, ADF and lignin. It is observed by the contrast test, applied to the averages in the fifth and ninth days of aerobic exposure, higher levels of CP ($p < 0.05$) was found in the hay with urea in relation to the acid and control. In the first evaluation time, higher levels of DM ($P<0.05$) were found in grass control compared to other treatments. However, the fifth day of evaluation, higher levels of DM were found only in the hay with urea, with no difference between the other treatments and times ($P>0.05$). The HPA had lowest values ($P<0.05$) of ADF and LIG than the others hays, in the first day of evaluation. No significant difference was confirmed ($P> 0.05$) in the values of cellulose and DIVMO. Lowest values of NDF were observed in HPA compared to Hay without additive and treated with urea (Table 2).

Table 1: Estimates of contrasts between groups of hays of Tifton 85 grass subjected to different treatments, with their standard errors and levels of significance for traits, during the aerobic exposition in the winter

Traits[1]	Day of air exposure	Contrasts[2]	Estimates[3]	Standard Errors
CP (% DM)	5	HPA × HU	-2.1634	0.0103
		HU × HC	-2.5409	0.0029
	9	HW × HU	-1.9050	0.0156
		HPA ×HC	1.7829	0.0327
		HU × HC	2.1425	0.0070
DM (%)	0	HW × HC	-15.2925	<.0001
		HPA × HC	-17.4550	<.0001
		HU × HC	-16.1500	<.0001
	5	HU × HC	-6.3050	0.0170
ADF (% DM)	0	HW × HC	-3.1100	0.0386
		HPA × HC	-3.4925	0.0208
	9	HW × HPA	5.7150	0.0003
		HPA × HU	-4.5425	0.0030
		HPA × HC	-3.7175	0.0141
Lig (% DM)	0	HU × HC	1.9204	0.0079
	5	HPA × HU	-1.7300	0.0099
		HPA × HC	-1.9025	0.0048

[1]CP: Crude Protein (%DM); DM: Dry Matter; ADF: Acid Detergent Fiber (%DM); Lig: Lignin (%DM);

[2] HW: Hay without additive; HPA: Hay treated with propionic acid; HU: Hay treated with urea; HC: control hay.

[3] Estimates of contrasts between the estimated averages of the first variable (+) and second (-).

Table 2: Estimates of contrasts between groups of hays of Tifton 85 grass subjected to different treatments, with their standard errors and levels of significance for contents of acid neutro detergent fiber, during the aerobic exposition in the winter

Trait[1]	Contrasts[2]	Estimates[3]	Standard Error Padrão	Pr > |t|
FDN	HW × HPA	4.1525	1.6037	0.0168
	HPA × HU	-4.2092	1.6037	0.0156

[1]*NDF: Neutro Detergent Fiber (%DM)*
[2] *HW: Hay without additive; HPA: Hay treated with propionic acid; HU: Hay treated with urea*
[3] *Estimates of contrasts between the estimated averages of the first variable (+) and second (-).*

Conclusion

The chemical additives used changed the chemical composition of the Tifton 85 grass hays, in the days after opening evaluated.

References

[1] Robertson, J.B.; Van Soest, P.J. (1981. The detergent system of analysis and its application to human foods. In: James, W. P. T.; Theander, O. (Ed.) The analysis of dietary fiber in food. New York: Marcel Dekker, p.123-158.

[2] Van Soest, P.J. (1994) Nutritional ecology of the ruminant. New York: Cornell University Press. 476p.

Short-term Storage (10 days) of Wet Brewery Grain: Effect of Organic Acid Treatment on Fermentation Products, Aerob Stability and Microbial Status

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Summary

Aim of the authors was to investigate the aerobic stability of wet brewery grain during 10 days aerobic storage treated by organic acids: propionic and formic acid mixture 1:1 in dose of 0.3% (i) and 0.5% (ii), and propionic-formic acid mixture buffered with ammonia in dose of 0.5% (iii). Aerobic storage was carried out (10 cm anaerobic and densed layer at the bottom and an upper soft, aerobic layer of 10 cm) in a newly improved model silo system (n=5). Temperature changes were detected in aerob and anaerobic layers with sensors of model mini silos during 10 days of storage by hours. Sampling was carried out on the 10th day of storage. Crude nutrients, pH, lactic- and volatile fatty acid, ethanol, ammonia-N, aerob mesophil bacteria and moulds were measured at the end of aerobic phase according to the Hungarian National Standards. Authors summarized that the dose of 0.5% (propionic-formic acid ratio 1:1) inhibited the deterioration process and improved the stability compared to the control-, the dose of 0.3%- and buffered organic acid mixture (0.5%). Application of 0.3% dose of propionic and formic acid mixture (1:1) is not recommended, because it presumably supplies substrate for aerobic micro-organisms causing accelerated deterioration compared to the control.

Introduction

Long-term storage of wet brewery grain (WBG) has not been preferred by the beer factories, therefore farms apply generally weekly deliver and short-term storage (7-10 days). Fast deterioration can be expectable above than 20°C and in humid weather circumstances. Aim of the authors was to investigate the aerobic stability of wet brewery grain during 10 days aerobic storage treated by organic acids: propionic and formic acid mixture 1:1 in dose of 0.3% (i) and 0.5% (ii), and propionic-formic acid mixture buffered with ammonia in dose of 0.5% (iii). The buffered propionic-formic acid mixture is not corrosive and not pungence for labours. Aerobic storage was carried out (10 cm anaerobic and densed layer at the bottom and an upper soft, aerobic layer of 10 cm) in a newly improved model silo system (n=5). The two layers (the top aerobic- and the lower densed anaerobic layer) imitated the natural layers in the temporary WBG pile stored on the farm.

Materials and Methods

Authors prepared different mixtures of acids 'A' mixture of acids and salts: propionic acid and formic acid mixture made from 96% propionic acid and Kemisile 2S mixed in ratio of 0.7: 0.3. Composition: 41,3 % formic acid, 3,01% ammmonium-formiate, 43,85% propionic acid, 1,75% K-sorbate and 10,09 % water. Dose of 0,3% acids can be provided by 0,33% mixture, dose of 0,5% acids can be provided by 0,55% mixture. 'B' mixture of acids: 50,4 % formic acid, 29,7% ammmonium-formiate, 18,1% propionic acid, 1% mono-propylene-glycol and 0,8 % water.
Treatments applied:

1. control: without any acid treatment
2. 0,3% dose: propionic acid and formic acid mixture (50:50), 'A' mixture in 0,33%

3. 0,5% dose: propionic acid and formic acid mixture (50:50), 'A' mixture in 0,55%
4. 0,5% dose: buffered (ammonia) propionic acid and formic acid mixture, 'B' mixture in 0,5%

Aerobic storage was carried out (10 cm anaerobic and densed layer at the bottom and an upper soft, aerobic layer of 10 cm) in a newly improved model silo system (n=5). Temperature changes were detected in aerobic and anaerobic layers with sensors of model mini silos during 10 days of storage by hours. Sampling was carried out on the 10^{th} day of storage from both layers, respectively. Crude nutrients, pH, lactic- and volatile fatty acid, etanol, ammonia-N, aerob mesophil bacteria and moulds were measured at the end of aerobic phase according to the Hungarian National Standards (Table 1 and Table 2).

Results and Discussion

Crude nutrient content and deterioration profile of WBG after 10 days storage is provided in Table1 and Table2.

According to ammonia-N content and the microbial status of the control WBG, it was confirmed that deterioration was more pronounced in the upper, aerobic layer compared to the lower anaerobic layer (moulds in aerobic layer: 4.88 $\log_{10}$ CFU/g *vs* moulds in anaerobic layer 2.72 $\log_{10}$ CFU/g). We have found in the anaerobic layer strong acetic acid fermentation and ethanol production compared to the upper layer.

The dose of 0.3% treatment in the upper soft and aerated layer did not inhibit the deterioration compared to the control (pH 6.36 *vs*. 5.97, respectively, $p \leq 0.05$). Moreover, the additional propionic acid (0,15%) concentration decreased by 96% in the aerobic layer compared to the initial concentration and was lower than in the anaerobic layer (aerobic layer: 5.9 g/kg DM *vs* anaerobic layer: 10.1 g/kg DM, $p \leq 0.05$). Presumably, undesirable aerobic bacteria and moulds are able to use the propionic acid as substrate in the dose of 0.3% in aerobic circumstances. The 0.3% dose (PA:FA 1:1) had beneficial effect on deterioration in the anaerobic layer compared to the control, but was not so effective than dose of 0.5% (AEMB 0.3%: 4.53 $\log_{10}$ CFU/g *vs.* AEMB 0.5%: 3.0 $\log_{10}$ CFU/g, $p \leq 0.05$).

Table 1: Crude nutrients in WBG after 10 days storage (anaerobic layer)

		Fresh	After 10 days storage (anaerobic layer)			
		wBG	Control	0,3%	0,5%	0,5% buff.
Dry matter	g/kg	221,8	229,2	213,2	221,1	230,0
Crude protein	g/kg DM	330,7	327,7	325,1	330,6	338,0
Crude fiber	g/kg DM	141,9	142,1	124,0	121,4	127,2

It was found that 0.5% of acid mixture (PA: FA 1:1) could inhibit effectively (at 20°C and 20% DM content WBG) the deterioration process compared to the control in both layers. There were not significant diffferences between the aerobic and anaerobic layer characteristics in the case of 0.5% acid treatment. The buffered acid mixture (0.5%, PA:FA 1:2.5) inhibited the spoilage compared to the control, but was not so effective as the dose of 0.5% acid mixture in the upper aerobic layer. The aerobic mesophil bacteria- and mould proliferation was more intensive in the case of buffered acid mixture than in the dose of 0.5% of acid treatment (PA: FA 1:1). The effect of buffered acid mixture (0.5%, PA:FA 1:2.5) on WBG profile was similar to the dose of 0.5% of acid mixture (PA: FA 1:1) in the lower, anaerobic layer

Conclusions

Acid mixture (PA:FA 1:1) in dose of 0.5% inhibited significantly the deterioration process both in anaerobic and aerobic layers, therefore it is recommended to use on farms. However, there can be technical problem with treatment of the whole amount (spraying and mixing of the whole pile). According to the results, that deterioration in the anaerobic layer is slower and not so intensive as in the aerobic top layer, treatment of the WBG pile surface with 0.5% acid mixture

(PA:FA 1:1) can be effective to inhibit undesirable aerobic micro-organisms during short storage (maximum 10 days) without treatment of the whole WBG pile. Impregnation of the top 10-20cm layer by 0.5-1 litre acid mixture/ 1 m^2 will inhibit effectively the deterioration during short storage. Application of 0.3% dose of propionic and formic acid mixture (1:1) is not recommended, because it presumably supplies substrate for aerobic micro-organisms causing accelerated deterioration compared to the control on the surface. The buffered acid mixture (dose: 0.5%, PA:FA 1:2.5) inhibited the spoilage, but was not so effective in protect the top layer than un-buffered acid mixture (0.5% dose, PA:FA1:1), therefore it is recommended to use just shorter period of storage at lower ambient temperature (< 20°C).

Table 2: Deterioration profile of WBG after 10 days storage

		Control		0,30%		0,50%		0,5% buffered	
		AE*	AN**	AE*	AN**	AE*	AN**	AE*	AN**
pH	Mean	5,97a	5,72a	6,36b	5,13e	4,44f	4,48f	4,62f	4,56f
	ST	0,16	0,06	0,06	0,24	0,04	0,05	0,02	0,04
Ammonia/N %	Mean	2,31a	0,85b	1,02b	0,93b	1,09b	1,04b	4,28c	4,32c
% of total N	ST	0,41	0,11	0,24	0,53	0,13	0,05	0,12	0,16
Lactic acid	Mean	1,31a	1,64b	1,41a	4,56c	5,33c	4,77c	4,84c	4,80c
g/kg DM	ST	0,00	1,11	0,00	1,47	0,45	1,08	0,96	0,71
Acetic acid	Mean	1,37a	6,66b	0,79a	3,20c	2,67a	2,61a	2,08a	2,35a
g/kg DM	ST	1,39	1,03	0,12	0,57	0,16	0,04	0,13	0,12
Propionic acid	Mean	0,35a	0,42a	0,23a	5,87b	10,09c	10,34c	4,18b	4,35b
g/kg DM	ST	0,24	0,09	0,00	0,42	0,24	0,40	0,19	0,12
Ethanol	Mean	0,00a	1,84b	0,00a	0,17a	0,00a	0,00a	0,00a	0,00a
g/kg DM	ST	0,00	0,60	0,00	0,06	0,00	0,00	0,00	0,00
Volatile fatty acids, g/kg DM	Mean	1,72	7,08	1,02	9,07	12,75	12,95	6,26	6,70
Organic acids, g/kg DM	Mean	3,03	8,72	2,43	13,63	18,08	17,72	11,10	11,50
Fermentation products, g/kg DM	Mean	3,03	10,56	2,43	13,80	18,08	17,72	11,10	11,50
LA:AA	Mean	0,96	0,25	1,79	1,43	2,00	1,82	2,33	2,04
AEMB***	Mean	6,48a	5,03b	6,48a	4,53b	3,00c	3,00c	4,12b	3,00c
log10 CFU/g	ST	0,00	0,05	0,00	0,70	0,00	0,00	0,76	0,00
Moulds	Mean	4,88a	2,72b	5,07a	2,95b	0,00b	0,00b	4,02a	0,00b
log10 CFU/g	ST	0,24	0,49	0,18	0,00	0,00	0,00	0,50	0,00

AE= aerobic layer **AN = anaerobic layer* aerobic mesophil bakteria abcd Means in the same row with different letters differ (p≤ 0.05)*

Long-term Storage of Wet CGF: Effluent, Optimal Density, Fermentation and Aerobic Stability

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Summary

Aim of the authors was to investigate the effluent production, fermentation profile and aerobic stability of wet CGF (wCGF) during 30 days anaerobic storage and 10 days aerob phase in model silos. Wet CGF was ensiled alone and mixed with corn grain (10%) or maize silage (20%), respectively. Samplings were carried out on the 4th , 14^{th,} and 30^{th,} day of fermentation and 10th day of aerobic storage. In the anaerobic phase crude nutrients, pH, lactic- and volatile fatty acid, ethanol, ammonia-N content were detected. The following parameters were measured after 10 days aerobic storage: ammonia-N, aerobic mesophil bacteria, moulds and aerobic stability according to the Hungarian National Standards.

Introduction

Aim of the authors was to investigate the effluent production, fermentation profile and aerobic stability of wet CGF (wCGF) during 30 days anaerobic storage and 10 days aerob phase in model silos. Wet CGF was ensiled alone and mixed with dry ground corn (10%) or maize silage (20%), respectively.

Materials and Methods

Ensiling was carried out in a model silo system (airtight sealing, air-valves, volume: 0,041 m^3). Applied treatments were the followings.

Experiment 1: Density of 1108 kg / m^3 =443 kg DM/ m^3

- control wCGF
- M1: wCGF and 20% maize silage (4,5 kg mixture: 0,9 kg maize silage and 3,6 kg wCGF).

Experiment 2: Density of 1192 kg / m^3=477 kg DM/ m^3

- control wCGF
- M2: wCGF and 10% dry ground corn (4,5 kg mixture: 0,4 kg dry ground corn and 3,6 kg wCGF.

Sampling was carried out on the 4th, 14th and 30th day of fermentation. Crude nutrients, pH, lactic- and volatile fatty acid, ethanol content and microbial status (moulds and aerob mesophil bacteria) were measured according to the Hungarian National Standards. Temperature changes were detected in aerobic phase after opening (240 hours) with sensors of model mini silos (in 110 mm depth).

Results and Discussion

Weight losses in wCGF and mixtures are shown in Table 1. It was found that the wCGF could be consolidated easily to high density, but density of 443 and 477 kg DM/m^3 caused high weight losses (8,8% and 8,4%, respectively) due to intensive gas and effluent production. Maize silage increased the weight losses compared to the control wCGF (+1,1%). Dry ground corn (10%) reduced the weight losses (-1,8%), gas and effluent production in the mixture compared to the control (8,4% *vs* 6,6%, p≤ 0,05). Tubular system (the proposed silotype to store wCGF) is sensitive for gas production, therefore it is recommended to reduce density to 370-390 kg DM/m^3. Crude nutrient content of the wCGF and the mixtures are shown in Table 2. Fermentation profile and aerobic stability of the wCGf and the mixtures are provided in Table3.

Table 1: Weight losses during the fermentation in wCGF and mixtures (n=5).

	Day 4	Day 14	Day 30
wCGF (exp 1)	8,3a	10,0b	8,8a
	0,28	0,40	0,57
wCGF + silage 20%	9,9b	15,3c	9,9a
	0,87	1,59	2,24
wCGF (exp 2)	6,7a	7,2b	8,4b
	0,45	1,10	0,44
wCGF+corn 10%	5,7b	6,7b	6,6c
	0,40	0,60	0,56

abcd Means in the same row with different letters differ ($p \leq 0.05$)

Table 2: Crude nutrients in wCGF and mixtures.

		Exp 1			Exp 2		
		wCGF	Mize silage	wCGF + silage 20%	wCGF	Dry corn	wCGF+corn 10%
Dry matter	g/kg	407,3	265,6	388,0	445,0	888,8	485,6
Crude protein	g/kg DM	186,2	56,2	164,6	211,7	97,0	192,6
Crude fat	g/kg DM	59,4	37,1	55,7	70,0	54,7	68,7
Crude fiber	g/kg DM	82,7	224,0	99,9	55,0	21,3	48,0

The wCGF had rather low organic acid content on the 30th day of fermentation (Exp 1 OA: 12.1 g/kg DM, Exp 2 OA: 8.5 g/kg DM), accompanied by high pH (Exp 1 pH: 4.8, Exp 2 pH: 4.7). However, LA:AA ration has been found appropriate (Exp 1 LA:AA: 9.7, Exp 2 LA:AA: 4.0). Ethanol production was considerable in the wCGF (Exp 1 ET: 19.0 g/kg DM, Exp 2 ET: 31.3 g/kg DM). Summarizing, low fermentation intensity, lactic acid dominating fermentation with very high ethanol concentration was found in wCGF. Deterioration was not found during the anaerobic phase. Maize silage improved fermentative intensity of wCGF (OA: 12.1 g/kg DM *vs.* OA: 18.2 g/kg DM), however significantly increased the acetic acid production and reduced the LA:AA ration (LA:AA: 6.1-9,7 *vs.* 2.2-2.3) compared to the wCGF control. Dry ground corn accelerated the ethanol production in initial phase of fermentation (4-14th day). The dry corn increased the lactic acid and acetic acid production between 14- 30th day. It was found that the wCGf aerobic stability is rather poor (Exp 1: 49 hours/1°C, Exp 2: 39 hours/1°C) after 30 days of fermentation. Maize silage (20%) increased the mould proliferation compared to the control wCGF during the aerob phase, while dry corn decreased the proteolyses (AmmoniaN%: 7.0 *vs.* 5.8). Maize silage had undesirable harmful effect on stability (49 hours/1°C, *vs* 28 hours/1°C), while dry ground corn significantly improved the aerobic stability of wCGF (39 hours/1°C, *vs* 84 hours/1°C).

Conclusions

According to the poor fermentation results, tubular system is recommended to use for store wCGF. However, this system is sensitive for gas production, therefore it is recommended to keep density between 370-390 kg DM/m^3. Dry ground corn in 10% is recommended to add to wCGF in order to reduce weight losses (gas- and effluent production) and improve aerobic stability. Maize silage (20%) quality has strong impact on losses and fermentation process in wCGF mixture and can not be excluded undesirable effects.

Table 3: Fermentation profile of wCGF and mixtures (n=5)

Fermentation stage		Day 4		Day 14		Day 30		Day 4		Day 14		Day 30	
		wCGF	M1*	wCGF	M1	wCGF	M1	wCGF	M2**	wCGF	M2	wCGF	M2
ANAEROBIC STAGE													
pH	mean	5,2a	4,6c	5,8b	5,0c	4,8c	4,5d	5,0a	5,2c	4,8b	4,9c	4,7b	4,7d
	ST	0,1	0,0	0,2	0,0	0,0	0,0	0,1	0,1	0,0	0,0	0,0	0,0
Lactic acid, g/kg DM	mean	7,0a	10,9b	9,1c	12,2b	10,8b	12,0b	4,3b	3,2b	5,0c	5,8c	6,6d	9,3e
	ST	0,7	0,4	0,7	0,6	0,4	1,0	0,4	0,1	0,8	0,2	2,1	1,0
Acetic acid, g/kg DM	mean	1,1a	5,0b	1,5a	5,2b	1,1a	5,6b	0,9a	0,9a	1,3b	1,2b	1,7c	2,9d
	ST	0,1	0,3	0,1	0,3	0,1	0,4	0,1	0,1	0,1	0,1	0,2	0,3
Propionic acid, g/kg DM	mean	0,0	0,5	0,1	0,5	0,2	0,5	0,0	0,0	0,0	0,0	0,0	0,1
	ST	0,0	0,1	0,0	0,0	0,1	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Ethanol, g/kg DM	mean	18,9a	17,2b	24,8c	23,9d	19,0a	21,6a	7,9a	13,9b	21,0c	26,8d	31,3e	23,4c
	ST	1,6	1,3	3,0	0,6	2,3	1,6	1,7	1,9	4,0	1,3	1,9	3,1
Organic acids, g/kg DM	mean	8,1a	16,3b	10,7a	17,9b	12,1a	18,2c	5,3a	4,1b	6,3c	7,0d	8,5e	12,3f
	ST	0,8	0,3	0,8	0,8	0,5	1,1	0,4	0,1	0,8	0,2	2,5	1,0
Ferm product, g/kg DM	mean	27,0a	33,6b	35,5b	41,8c	31,1b	39,8c	13,2a	17,9b	27,4c	33,8d	39,8e	35,8e
	ST	2,0	1,2	2,2	0,6	2,7	2,3	1,6	2,0	4,8	1,4	3,5	3,1
LA/AA	mean	6,1a	2,2b	6,1a	2,3b	9,7c	2,2b	4,7a	3,8b	3,8b	5,0c	4,0b	3,3b
	ST	0,4	0,2	0,4	0,1	1,2	0,3	0,6	0,5	0,5	0,4	1,1	0,5
AEROBIC STAGE (10 daerobic days = DAY 40)													
pH	mean	5,6a	5,9a					5,8a	5,8a				
	ST	0,5	0,3					0,4	0,3				
Ammonia, % total N	mean	6,4a	5,8b					7,0c	5,8b				
	ST	1,2	1,2					1,6	1,9				
AEMB***, log 10 CFU/g	mean	6,1a	6,2a					5,8b	6,0b				
	ST	0,2	0,2					0,6	0,1				
Moulds, log 10 CFU/g	mean	1,1a	3,9b					0,0c	0,0c				
		1,5	2,3					0,0	0,0				
Aerobic stability, Hours/1°C		49a	28b					39c	84d				
		4,1	1,1					2,5	4,2				

*M1= wCGF and maize silage 20% **M2 = wCGF and dry corn 10%, *** AEMB = aerob mesophil bacteria

abcd Means in the same row with different letters differ ($p \leq 0.05$)

The Influence of Biological and Chemical Additives on the Fermentation Process of Field Pea Silage

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Introduction

Pea (*Pisum sativum*) is an annual plant which is grown in many parts of the world. In the Czech Republic pea is grown on 22 800 hectares. Its positive attribute is a high crude protein content. Pea growing is very advantageous for agricultural engineering as it improves the soil fertility due to Root-nodule bacteria (Rhizobia) which are able to fix the atmosphere nitrogen into soil. Pea is an excellent break crop. It is used in the Czech Republic mainly for its seeds. However the whole plant can also be used for silage.

The aim of this experiment was to evaluate the effect of biological and chemical additives on the fermentation quality of pea silage. The bacterial inoculant contained selected bacterial strains of *Lactobacillus rhamnosus* (NCIMB 30121) and *Enterococcus faecium* (NCIMB 30122).

Keywords: field pea, fermentation quality, inoculant, chemical additive

Materials and methods

Whole plants of peas cv. Concorde were harvested when they were at the advanced pod filling maturity stage. Forage was wilted in the swath to ca 33 % DM and chopped by a conventional forage chopper to a length of 25 mm. The chopped matter contained 159.3 g/kg DM of crude protein, 295.75 g/kg DM of crude fibre, 100.01 g/kg DM of water soluble carbohydrates (WSC). At this time over 50 % of the silage was composed of pods with seeds. The first silage treatment was „control“ (without additive). As the second treatment the commercial bacterial inoculant (1 g/t) containing homofermentative lactic acid bacteria (*Lactobacillus rhamnosus* (NCIMB 30121) and *Enterococcus faecium* (NCIMB 30122) was used. The chemical additive containing formic acid (55 %), propionic acid (5 %), ammonium formiate (24 %) and benzoic acid (2.2 %) was used in the third group of silage at the amount of 4 l/t. Chopped forage (700 g) was packed into polyethylene bags. After samples vacuum sealing, the bags were stored at the temperature +18 to +20 °C. Silages were analysed for fermentation quality after 60 days of conservation.

Results and discussion:

Table 1: Fermentation characteristics of pea silage

	Control (n=7)	Bacterial inoculant (n=7)	Chemical additive (n=7)
Dry matter (%)	32.83	33.29	33.49
WSC (% DM)	1.30[a]	1.06[a]	2.28[b]
pH	4,08[a]	4.02[b]	3.94[a]
Lactic acid (% DM)	2.30[a]	3.29[b]	2.54[a]
Acetic acid (% DM)	0.54	0.44	0.52
Propionic acid (% DM)	0.07	0.06	0.08
Butyric acid (% DM)	0.00	0.00	0.00

[a,b]*Mean values in the same line with the different superscripts are significantly different (P<0.05)*

The use of bacterial inoculant significantly increased ($P<0.05$) the concentration of lactic acid. The same result was found by Borreani (2005). He examined pea silages containing pea harvested at four stages of growth. All silages with inoculant had increased contents of lactic acid

except for the pea silage harvested at the end of flowering maturity stage. Fraser (2001) reported that the fermentation was improved by applying of an inoculant with *Lactobacillus plantarum.*

The lactic:acetic ratio in the silage treated with bacterial inoculant was increased compared to control (bacterial inoculant 7.48:1 vs. control 4.26:1). This ratio is a good indicator of the efficiency of the silage fermentation.

Both bacterial inoculant and chemical additive positively influenced fermentative characteristic – pH. Silage treated with bacterial inoculant and with chemical additive had significantly ($P<0.05$) lower pH compared to control. The highest ($P<0.05$) content of water soluble carbohydrates (WSC) was retained in the silage treated with the chemical additive.

Conclusion

Generally the bacterial inoculant had a positive effect on pea silage characteristics. The pH was decreased and lactic acid was increased. Use of bacterial inoculant and chemical additive improved silage quality.

Acknowledgment

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References

[1] Borreani, G., Cavallarin, S., Antoniazzi, Tabacco, E. (2005). Effects of stage of growth and inoculation on fermentation quality of field pea silage. Silage Production and Utilisation. Satellite workshop of the XXth International Grassland Congress. Belfast: 205.

[2] Fraser, M.D., Frychan, R., Jones, R. (2001). The effect of harvest date and inoculation on the yield, fermentation characteristics and feeding value of forage pea and field bean silages. Grass and Forage Science. 56, 218-230.

An inoculant to improve the corn silage quality under field conditions

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Abstract

A field silage trial was conducted in the region of Torreon, Mexico, between July and October of 2009. The substrate corn whole plant was ensiled with a dry matter (DM) content of 27 – 30 %. The silages were treated with Product A (Biomin® BioStabil Mays; blend of *L. plantarum, L. brevis* and *E. faecium*) and a competitor (product B) (blend of *Lactobacilli* and enzymes) according to the dosages recommended by the producers (4 and 1 g respectively), guaranteeing 2 x 10^5 and 1 x 10^6 cfu/ g silage. The material was properly compacted and sealed. Samples of the raw material were sent to the laboratory to determine its nutrient content. The silages were opened 2 months later and sampled for nutrient content (DM, crude protein, energy and ash), silage quality (pH and lactic, acetic and butyric acid) and mycotoxin contamination (aflatoxins, ZON, DON and T-2). The aerobic stability was determined during 12 days after the opening of the silo, as the differences of the silages temperatures compared with the ambient temperatures (Honig, 1990). Losses of the different nutrients were calculated and compared at the end of the trial. The use of the Product A reduced the DM and metabolizable energy losses in 6.69 and 1.33 % compared with the treatment with Product B. The losses in protein were slightly higher for Product A (0.21 %), nevertheless the losses in ammoniac N were higher in 3.27 % when Product B was used. The pH value was similar for both treatments (around 4.02), as well as the acetic and butyric acid content (4.01 and 0.00 g/ kg DM respectively). Since the lactic content was higher for silages treated with Product A than that treated with Product B (7.58 *vs.* 7.98 g/ kg DM respectively), the proportion between lactic to acetic acid had better results (lower) for A than B (1.89 *vs.* 1.99). The temperatures in the silage were lower using product A (1- 5 °C). No contamination with aflatoxins was found in the silages. However, ZON, DON and T-2 were detected. The contamination with DON and T-2 was higher in B than in A.

Introduction

Corn is one of the most extended crops used for silages. Corn silages contain high amounts of energy which is essential for high yield animals.

A trial was conducted to test the efficacy of a biological silage inoculant (Biomin® BioStabil Mays; blend of *L. plantarum, L. brevis* and *E. faecium*) compared to a competitor product related to:

- silage nutrient content,
- sermentation quality and
- the aerobic stability

Materials and Methods

A trial under field conditions with inoculants for corn whole plant silage was conducted from July to September 2009 in a dairy farm in Torreón, Mexico. Two different inoculants (Biomin® BioStabil Mays or Product A, blend of homo- and heterofermentative bacteria, 286 tons ensiled) and Product B (blend of homofermentative bacteria and enzymes; 980 tons ensiled). The dosages used for the experiment were the recommended by the producers (4 and 1 g/ ton for products A and B respectively). The raw material as well as the silages was sampled and the samples were analyzed in an independent and certified local laboratory. After the opening of the silos, the temperature was measured in regular intervals during 10 days.

Results

The content of nutrients in the silages is shown in table 2. The use of the product A diminished the dry matter losses in more than 6.5 %, meaning more than 1.95 tons per 100 tons of silage (with 30 % DM). Considering a ME content of 2.2 Mcal/ kg DM and taking into account the requirement for the production of 1 liter of milk (1.16 Mcal/ liter milk), the use of Product A could decrease the losses in milk production (only because of dry matter losses) in about 3 700 liters/100 tons silage [(1 950 kg DM x 2.2 Mcal/ kg DM)/ 1,16 Mcal/ liter of milk].

The losses in protein in the silage treated with Product A were 0.21 % higher than the one treated with Product B. Nevertheless the role of the voluminous feed in the ration is mainly as energy supplier.

Table 2: Nutrient contents in the silages treated with two different inoculants

Treatment		Product A			Product B			Difference (B - A) (%)
Parameter	Unit	Raw material	Silage (n = 3)	Difference (%)	Raw material	Silage	Difference (%)	
Dry matter (DM)	%	29.81	28.49	4.43	27.44	24.39	11.12	6.69
Crude protein	% in	8.21	7.46	9.18	9.82	8.94	8.96	-0.21
Metabolizable energy (ME)	Mcal/ kg DM	2.17	2.12	2.30	2.20	2.12	3.64	1.33
Ash	%	10.12	9.33	7.84	9.59	8.82	8.03	0.19
pH	-		4.03			4.01		-0.02
Ammoniacal N	%		11.01			14.28		3.27
Lactic acid (LA)	% in		7.58			7.98		0.40
Acetic acid (AA)	% in		4.03			4.00		-0.03
Butyric acid	% in DM		0.00			0.00		0.00
Total acid	% in DM		11.61			11.99		0.38
Proportion LA : AA	-		1.88			1.99		0.10

Table 3: Calculation of the productive response with the use of the silage inoculant A

Parameter	Unit	Product A	Product B	Difference	
				(B - A)	%
Energy content in the raw material	Mcal ME/ kg DM	2.20	2.20	0	0
Losses of energy	%	2.20	3.64	-1.44	-40
Energy content in the silage	Mcal ME/ kg DM	2.15	2.12	0.03	1.49
Difference	Mcal ME/ kg DM	+0.03	-	-0.03	
Dry matter	%	28.49	24.39	4.10	
Energy plus per ton	Mcal	612.99	517.05	+95.94	19
Energy needed per liter milk	Mcal/ liter	1.16	1.16	0.00	0
Milk plus per treated ton	Liter/ ton	528.44	445.73	+82.71	19

The reduction in the ME content was higher for the silage treated with Product B (3.64 *vs.* 2.30 % compared with Product A). This difference of 1.44 % could have a marked productive response (table 3). The calculation was done for the same dry matter content present in the silages. As shown, this small quantity makes a difference of 95.94 Mcal/ ton for the treatment with the silage inoculant A. This amount of energy could represent 82 more liters of milk per ton of silage, taking only into account the energy factor.

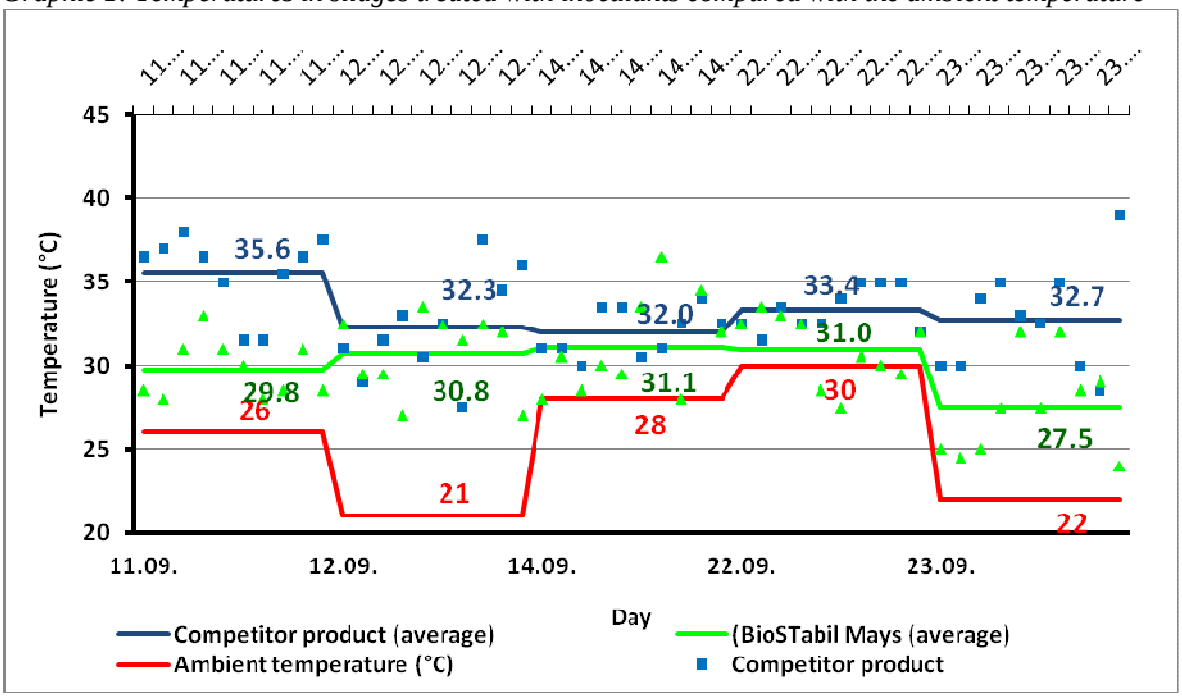
Considering the benefits of a higher dry matter and energy recovery together (calculations above) the use of the silage inoculant A could improve milk production in 119 liters per ton silage (37_ liters from table 2 + 82 liters_ from table 3) compared to Product B.

Other advantages in the use of Product A not discussed before from table 2, could be summarize as follow:

- lower content of ammonical N,
- higher content of acetic acid and
- better proportion between lactic and acetic acid

These two last parameters are essential for a better aerobic stability. The improvement in the aerobic stability is shown in graphic 1.

Graphic 1: Temperatures in silages treated with inoculants compared with the ambient temperature



As shown, the average temperatures in the silage treated with Product A were lower 1 to 5 °C than in the one treated with product B.

The aerobic stability (time in hours or days in which the silage remains stable without quantifiable losses) is better when the silage temperature does not increase. After the opening of the silo under aerobic conditions, yeasts mainly get active and degrade the silage nutrients in exothermic reactions, which means losses of energy and nutrients. Short chain organic acids, for instance, acetic acid stop the growth of yeasts (and moulds) and minimize these losses.

In case of silages of corn whole plant, the crucial aspect is to improve the aerobic stability, because the corn generally ferments well. Nevertheless due to its relatively high content of nutrients, very often the aerobic stability worsens due to the activity of the yeasts. For this kind of silages is common also to contain high quantities of lactic acid.

Conclusions

The use of the silage inoculant A has the following advantages:

- lower dry matter and energy losses,
- better fermentative parameters and
- longer aerobic stability

All this benefits mean a high productive potential for bettering the profitability in the use of silage inoculants and, finally, the profitability of the farm.

Effect of Rapeseed Meal and Ensiling Preparations on Fermentation Quality, Microbiological Composition and Aerobic Stability of Whole-Plant Maize Silages

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Introduction

Whole crop maize silage is particularly susceptible to an aerobic deterioration after opening a silo. It is caused by long vegetation period, plants exposure to yeasts and moulds contamination and a high level of lactic acid and water soluble carbohydrates (WSC), which are main nutrients for these microorganisms [Ohmomo et al., 2002]. During the deterioration process, rising temperature results in further growth of yeasts and thermophilic fungi [Ashbell et al., 2002]. Ensiling maize forage with heterofermentative lactid acid bacteria (LAB), homofermentative LAB in combination with organic acids salts or with organic acids and their salts improved the aerobic stability of silages obtained. These additives also decreased deamination and proteolysis as well as inhibited or even eliminated butyric acid fermentation in the silages [Doležal, 2004; Stryszewska and Pyś, 2006; Kania, 2007]. a better fermentation quality, a greater aerobic stability and much higher protein value of the silages can be obtained by ensiling maize forage with protein components [Kania, 2007; Pyś et al., 2008].

Material and Methods

Maize forage (cv. Eurostar, FAO 250, dry matter: 325.5 g/kg, particles size: 10-15 mm) was ensiled with no additives – M0 or with addition of: rapeseed meal (RSM) in an amount of 50 g/kg of forage – M1; RSM + BI (*Lactobacillus buchneri* bacteria - 1.0×10^6 cfu/g of a mixture) – M2; RSM + BCI (*L. buchneri* bacteria - 1.0×10^6 cfu/g of a mixture and potassium sorbate - 0.5 g/kg of a mixture) – M3; RSM + CC (59% of formic acid, 20% of propionic acid, 4.3% of ammonium formate, 2.5% of potassium sorbate; preparation dose - 4 ml/kg of a mixture) – M4. The whole crop maize was ensiled in 120 l polyethylene silos for 60.days, in an ambient temperature 17±2^{o}C. The silages were analysed for pH, ammonia-N (NH_3-N), ethanol, dry matter (DM), crude protein (CP) and true protein (TP) and organic acids: lactic (LA), acetic (AA), propionic (PA), butyric (BA) content. Samples of the silages were subjected to an aerobic stability (AS) test, which lasted for 7.days, at room temperature 20±1^{o}C (Völkenrode System; [Honig, 1990]. The AS was measured by the number of hours during which the temperature of the silages subjected to an aerobic exposure did not exceed the ambient temperature in the air-conditioned room by 2^{o}C. After opening the silos, as well as 7.days of an air exposure, *Clostridium* bacteria, yeasts and moulds counts were determined. The results were statistically analysed (one-way analysis of variance, Tukey's test), using the Statistical Analysis System Software (SAS, ver. 9.1., 2001-2003)

Results and Discussion

M1, M2, M3 and M4 silages had the greatest ($P<0.05$) dry matter content and a lower ($P<0.05$) NH_3-N, ethanol and lactic acid content, in comparison to M0 silages (Tab.1). Declined LA level and increased AA content in M2 silage was caused by an activity of *L. buchneri* bacteria, which degrade LA to AA. The lowest LA and AA content in M4 silage, resulted from restrictive influence of organic acids, on fermentative bacteria producing those acids. No butyric acid was found in M1, M2, M3 and M4 silages. Increasing DM level in M1, M2, M3 and M4 silages, inhibited the protein degradation to ammonia-N, as a result of decreasing access to water for protein-degrading bacteria. Ensiling maize with RSM, especially in combination with CC or BCI,

increased (P<0.05) CP and TP content in the silages (Tab. 1). The greatest resistance to an aerobic spoilage was characteristic of M3 silage and in sequence of M2 and M4 silages. M3 silage contained the highest level of acetic and propionic acids, which have strong antifungal properties (Tab. 1). No moulds were indicated in M1, M2, M3 and M4 silages, after opening the silos. (Tab. 2). The lowest *Clostridium* bacteria, yeasts and moulds counts, after opening the silos, as well as air exposure, was found in M3 silages (Tab. 2).

Table 1: Chemical composition and aerobic stability of maize silages

Item	Type of silage				
	M0	M1	M2	M3	M4
Dry matter g/kg	315.1 b	338.8 a	328.3 a	329.4 a	336.8 a
pH	3.90 c	4.02 b	4.05 b	4.08 b	4.25 a
NH_3-N g/kg of total-N	43.8 a	38.1 ab	33.2 bc	30.1 c	33.9 bc
	g/kg of dry matter				
Ethanol	16.0 a	10.3 b	8.2 b	7.9 b	8.6 b
Lactic acid	69.9 a	45.1 b	34.0 cd	38.2 bc	29.1 d
Acetic acid	14.4 bc	17.3 b	23.0 a	18.9 ab	10.2 c
Propionic acid	0.0 c	0.2 b	3.8 a	0.5 b	0.7 b
Butyric acid	2.6 a	0.0 b	0.0 b	0.0 b	0.0 b
Crude protein	80.2 b	110.6 a	111.4 a	113.0 a	117.6 a
True protein	50.3 c	72.6 b	74.5 b	80.3 ab	87.0 a
Aerobic stability h	33 d	59 c	85 b	131 a	81 b

Means in rows with different letters (a, b, c, d) differ significantly at P<0.05

Table 2:. Microorganisms (log_{10} cfu/g of fresh matter) recovered from maize silages

Time	Item	Type of silage				
		M0	M1	M2	M3	M4
After opening of the silo	*Clostridium*	2.68 a	1.59 b	1.09 c	1.01 c	1.41 b
	Yeasts	6.96 a	6.60 b	6.34 bc	6.12 b	6.44 b
	Molds	6.81 a	0.00 b	0.00 b	0.00 b	0.00 b
After 7 day of air exposure	*Clostridium*	2.10 a	0.81 b	0.60 bc	0,45 c	0.79 b
	Yeasts	9.11 a	8.60 b	8.44 b	7.89 c	8.51 b
	Molds	8.73 a	6.63 b	6.15 c	5.31 d	6.35 bc

Means in rows with different letters (a, b, c, d) differ significantly at P<0.05

Conclusion

Ensiling maize forage with rapeseed meal allowed to obtain silages of a very good quality, with a high resistance to an aerobic deterioration. The best fermentation profile, the longest period of aerobic stability and the lowest *Clostridium* bacteria, yeasts and moulds population, was characteristic of silages made with the combination of rapeseed meal, *L. buchneri* bacteria and potassium sorbate. The addition of rapeseed meal to the ensiled maize forage effected also with a much higher crude and true protein content of the silages.

References

[1] Ashbell G., Weinberg A.G., Hen Y., Filya I. 2002. The effects of temperature on the aerobic stability of wheat and corn silages. J. Ind. Microbiol. Biotech. 28, 261-263.

[2] Doležal P. 2004. Comparison on the effect of benzoic acid addition on the fermentation process quality with untreated silages. Acta Univ. Agric. Silvicult. Mendelianae Brunensis. 2, 15-22.

[3] Kania K. 2007. Wpływ dodatków białkowych i preparatów ki kiszonkarskich na jakość i skład chemiczny kiszonek z kukurydzy. Praca doktorska. Akademia Rolnicza w Krakowie, manuskrypt.
[4] Ohmomo S., Tanaka O., Kitamoto H.K., Cai Y. 2002. Silage and microbial performance, old story but new problems. Japan Agric. Res. Quart. 36, 59-71.
[5] Stryszewska K., Pyś J.B. 2006. Effects of different silage additives on the microbial population and aerobic stability of maize silage. J. Anim. Feed Sci., 15 Suppl. 1, 121-124.

Effect of Bacterial or Chemical Additives on Chemical Composition and Aerobic Stability of High-Moisture Maize Grain Silages

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Introduction

High-moisture maize grain silages are considered as one of the main high-energy roughages, which are used in dairy cows and cattle nutrition in Poland. Production of a good quality high-moisture maize grain silages with a low content of undesirable fermentation products, little nutrients losses and a high resistance to an aerobic deterioration, requires using of silage additives. A similar results were obtained by ensiling high-moisture maize grain with bacterial inoculants, containing homo- as well as heterofermentative lactic acid bacteria and propionic acid bacteria [Dawson et al., 1998; Taylor and Kung, 2002; Kung et al., 2004; Gálik et al., 2007; Pyś et al., 2009] or chemical preservatives (propionic and formic acids with their salts) [Kung et al., 2004; Bíro et al., 2006; Pyś and Karpowicz, 2008].

Materials and Methods

High-moisture maize grain (cv. Eurostar, FAO 250, dry matter: 659.9 g/kg) was grounded and ensiled with no additives – MWA or with: (*Lactobacillus buchneri* - 3.0×10^5 cfu/g of grain) – MB1 or (*L. plantarum* and *Propionibacterium acidipropionici* - 3.0×10^5 cfu/g of grain) – MB2 or (propionic acid - 50%, formic acid - 50%; preparation dose - 3 ml/kg of grain) – MC1 or (propionic acid - 90%, ammonium propionate - 4%, 1.2-propandiol - 4%; preparation dose - 3 ml/kg of grain) – MC2. Maize grain was ensiled in 120-l polyethylene silos and stored for 60.days in a room at 15±2°C. In the silages pH, ammonia-N (NH_3-N), ethanol, organic acids (lactic, acetic, butyric, propionic, formic), dry matter (DM), crude protein (CP) and true protein (TP), fiber fractions NDF and ADF, water soluble carbohydrates (WSC) and starch content was determined. The aerobic stability of the silages was tested for 7.days in an air-conditioned room in the ambient temperature of 20±1°C, according to the method of Honig [1990]. Aerobic stability was measured by the number of hours during which the temperature of the silages subjected to an aerobic exposure did not exceed the ambient temperature in the air-conditioned room by 2°C. The results were analysed statistically using one-way analysis of variance and Tukey's test [SAS, ver. 9.1, 2001-2003].

Results and Discussion

There was no significantly ($P>0.05$) differences in an ammonia-N and ethanol content between MWA, M1 and M2 silages. A considerable lower ($P<0.05$) protein to ammonia-N degradation and a great inhibition ($P<0.05$) of alcohol fermentation, was found in M3 and M4 silages. This fact resulted from a restrictive influence of components used as chemical preservatives, on yeasts and protein-degrading bacteria during fermentation process. Ensiling high-moisture maize grain with bacterial additives effected with intensive lactic and acetic acid fermentation of M1 and M2 silages. Whereas chemical additives decreased lactic and acetic acid amount in M3 and M4 silages. No butyric acid was found in M1, M2, M3 and M4 silages. A period of the aerobic stability of M1 and M2 silages, was two-fold longer, while of M3 and M4 silages three-fold longer, in comparison to MWA silages (Tab. 1). All the additives used did not influence ($P>0.05$) DM, CP, NDF, ADF and starch content in the silages obtained. The lowest ($P<0.05$) true protein as well as WSC degradation during fermentation process, was characteristic of M3 and M4 silages (Tab. 1).

Table 1: Fermentation parameters, nutrients content and an aerobic stability of high-moisture maize grain silages

Item	Type of silage				
	MWA	MB1	MB2	MC1	MC2
Dry matter g/kg	639.2	629.5	631.3	645.0	646.4
pH	4.49 b	4.39 c	4.32 c	4.60 a	4.63 a
NH_3-N g/kg of total-N	22.3 a	17.6 a	19.3 a	8.1 b	9.4 b
			g/kg of dry matter		
Ethanol	15.6 a	13.0 a	13.8 a	5.1 b	5.9 b
Lactic acid	11.0 b	14.8 a	16.9 a	5.6 c	4.7 c
Acetic acid	5.0 b	9.3 a	7.9 a	3.1 c	2.8 c
Butyric acid	0.3 a	0.0 b	0.0 b	0.0 b	0.0 b
Propionic acid	0.0 c	1.0 b	0.0 c	1.4 b	2.3 a
Formic acid	0.0 b	0.0 b	0.0 b	0.2 a	0.0 b
Crude protein	93.1	96.8	97.2	98.5	99.4
True protein	72.1 b	77.9 ab	75.5 ab	80.6 a	81.9 a
NDF	106.5	105.2	104.6	107.0	107.4
ADF	56.8	56.1	55.2	57.4	57.1
WSC	11.7 b	6.0 bc	5.3 c	19.3 a	21.5 a
Starch	733.8	32.7	731.9	735.2	735.7
Aerobic stability h	55 d	115 c	108 c	166 b	183 a

Means in rows with different letters (a, b, c, d) differ significantly at P<0.05

Conclusion

Ensiling high-moisture maize grain with chemical preparations allowed to obtain silages of the greatest resistance to an aerobic deterioration during air exposure. An increasing level of propionic acid in a chemical preservative, ensured a better silages resistance to an aerobic spoilage. Chemical preservatives used in the experiment resulted in the best inhibition of protein and water soluble carbohydrates degradation, during ensiling process of high-moisture maize grain.

References

[1] Bíro D., Juráček M., Gálik B., Šimko M., Kačániová M. 2006. Influence of chemical inhibitors on fermentation process and hygienic quality of high moisture corn. Slovak J. Anim. Sci., 39, 108-112.

[2] Dawson T.E., Rust S.R., Yokoyama M.T. 1998. Improved fermentation and aerobic stability of ensiled, high moisture corn with the use of *Propionibacterium acidipropionici*. J. Dairy Sci., 81, 1015-1021.

[3] Gálik B., Bíro D., Juráček M., Šimko M., Michálková J. 2007. Effect of combined biochemical additives on nutritive value and fermentation process of ensiled crimped high moisture corn. Slovak J. Anim. Sci., 40, 30-33.

[4] Kung L. Jr., Myers C.L., Neylon J.M., Taylor C.C., Lazartic J., Mills J.A., Whiter A.G. 2004. The effects of buffered propionic acid-based additives alone or combined with microbial inoculation on the fermentation of high moisture corn and whole-crop barley. J. Dairy Sci., 87,1310-1316.

[5] Pyś J.B., Karpowicz A. 2008. Konserwanty chemiczne - gwarantem wysokiej odporności na rozkład tlenowy kiszonek z wilgotnego ziarna kukurydzy. Kukurydza, 2(33), 16-18.

[6] Pyś J.B., Karpowicz A., Borowiec F., Ratych L.B. 2009. Chemical composition and aerobic stability of high moisture maize grain silage made with bacterial and chemical additives. Anim. Biol., 11, 1-2, 180-187.

[7] Taylor C.C., Kung L. Jr. 2002. The effect of *Lactobacillus buchneri* 40788 on the fermentation and aerobic stability of high moisture corn in laboratory silos. J. Dairy Sci., 85, 1526-1523.

Effect of Inoculation on Fermentation Process and Nutritive Value of Whole-Crop Oat Silages

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Close relation between degree of ripeness and concentration of water-soluble carbohydrates in whole crop cereals influences crucially the ensilability of these feeds. Proportion of glucose and fructose dominates in the content of cereals during the first phases of growth. Maximum content of water-soluble carbohydrates is during the phase of milk ripeness. Their proportion decreases with gradual ripening and there increases the proportion of higher sugars, which are worse available for the lactic fermentation bacteria.

The objective of this work was to observe the influence of biological inoculants on fermentation process and nutritive value of GPS silage from oats.

Materials and Methods

Stand of oats (*Avena sativa*) for ensilaging was cut in milk ripeness period of plants. After direct harvest (without wilting) was the matter chopped and ensilaged in 1.7 l laboratory silos. We created three variants of silages:

C – control silage, without silage additive,

E1 – inoculated silage, *Enterococcus faecium* M 74, *Lactobacillus plantarum*, *Lactobacilus casei*, *Pediococcus spp. (*Microsil), the application rate 1 ml.kg^{-1} feed,

E2 – inoculated silage *Lactobacillus casei*, *Lactobacillus plantarum*, *Pediococcus pentosaceus*, *Lactococcus lactis*, *Lactobacillus buchneri*, *Enterococcus faecium* and complex of cellulases and hemicellulases (Goldzym II Super), the application rate 1.5 ml.kg^{-1} feed.

In the course of fermentation were assessed weight losses in silages by weighing in regular 21 days intervals; dry matter losses in silages were calculated. Chemical analysis was done in samples of fresh feeds and silages (Official Reports of MA SK, 2004); results were statistically processed and evaluated.

The chemical composition of fresh oat before ensiling was as follows: dry matter 281 g.kg^{-1}, crude protein 114 g.kg^{-1} DM, crude fibre 298 g.kg^{-1} DM, ADF 326 g.kg^{-1} DM, NDF 578 g.kg^{-1} DM, starch 10 g.kg^{-1} DM, total sugars 141g.kg^{-1} DM, reducing sugars 78 g.kg^{-1} DM, fat 23 g.kg^{-1} DM, ash 61 g.kg^{-1} DM. Content of energy was: ME 8.76 MJ.kg^{-1} DM, NEL 5.07 MJ.kg^{-1} DM, PDI 67.54 g.kg^{-1} DM.

Results and Discussion

The results of analyses of fermentation process are presented in Table 1. It is obvious from them that the low content of dry matter in ensilaged matter caused intensive course of fermentation in all silages. We noticed marked butyric fermentation and heavy proteolysis in the control silage. Under the influence of inoculation occurred decrease in pH, increase of lactic acid content, marked decrease in butyric acid, alcohol and ammonia N of total N contents in both experimental variants of silages (E1, E2). The assessed differences were statistically highly significant.

Table 1 : Parameters of fermentation process in whole-crop oat silages

Parameters, n = 5	C		E1		E2		Statistical differences	
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	P < 0,05	P < 0,01
pH	4.40	0.16	3.73	0.03	3.70	0.02		C : E1,E2
Acids in g.kg^{-1} DM								
- lactic	79.64	10.36	113.35	3.04	118.63	8.86		C : E1,E2
- acetic	9.52	1.42	15.67	1.66	9.96	1.87		E1 : C,E2
- propionic	3.92	1.86	0.46	0.22	0.59	0.32		C : E1,E2
- butyric + i.b.	41.81	6.74	2.14	0.45	1.05	0.42		C : E1,E2
- valeric + i.v.	2.18	1.54	0.59	0.05	0.55	0.05		C : E1,E2
- capronic + i.c	0.57	0.11	0.29	0.16	0.27	0.14		C : E1,E2
VFA total	58.55	9.77	19.15	2.41	12.42	2.70		C : E1,E2
Acids total	138.19	6.47	132.50	3.64	131.05	10.80		
Alcohol in g.kg^{-1} DM	3.20	0.48	0.86	0.12	1.07	0.19		C : E1,E2
NH_3-N of total N in %	21.41	6.04	9.63	2.03	9.04	0.77		C : E1,E2

Losses in dry matter that arose during fermentation (tab. 2) were highly significantly lower in the inoculated silages. Dry matter losses were by 64 – 68 % lower in inoculated silages compared with the non-treated control silage. Differences among tested preparations were not statistically significant. Control of fermentation process by means of silage preparations increased the content of metabolizable energy by 0.3 MJ.kg^{-1} DM and content of NEL by 0.2 MJ.kg^{-1} DM in produced silages.

We came to similar results also in our previous experiments with ensilaging whole plants of barley and rye (Rajcakova and Mlynar, 2006, 2009). Other specialists (Taylor et al., 2002, Spann et al., 2002, Lingvall et a., 2005, Hargreaves et al., 2009) confirmed also the improvement in fermentation process and in quality of produced silages under the influence of biological silage preparations in silages from whole crop cereals in accordance with our findings.

Table 2: Content of nutrition and energy in whole-crop oat silages, in g.kg^{-1} dry matter

Parameter, n = 6	C		E1		E2		Statistical differences	
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	P < 0,05	P < 0,01
Dry matter	244.89	10.94	267.47	4.50	268.80	2.47		C : E1,E2
Losses of DM in %	15.06	4.36	5.38	1.61	4.84	0.90		C : E1,E2
Organic matter	928.28	6.78	937.54	1.13	935.16	2.13	C : E1	
Crude protein	113.06	11.87	116.51	19.86	115.93	4.70		
Crude fibre	349.83	21.90	315.77	7.97	310.05	5.63		C : E1,E2
ADF	385.89	15.78	355.53	11.09	347.17	6.45		C : E1,E2
NDF	644.75	35.80	607.97	20.57	603.11	26.92		
Hemicelluloses	258.86	21.29	252.44	9.77	255.94	24.04		
Nitrogen free extract	432.23	22.22	475.26	24.03	478.91	4.09		
Total sugars	13.80	8.37	29.00	2.50	51.65	2.10		C : E1,E2
Reducing sugars	8.53	6.49	32.98	8.09	45.04	5.95		C : E1,E2
Fat	33.15	2.74	29.99	1.92	30.27	1.62		
Ash	71.72	6.78	62.46	1.13	64.84	2.13		
ME in MJ.kg^{-1} DM	8.40	0.07	8.70	0.02	8.68	0.02		C : E1,E2
NEL in MJ.kg^{-1} DM	4.81	0.04	5.00	0.01	4.99	0.01		C : E1,E2
NEV in MJ.kg^{-1} DM	4.43	0.03	4.65	0.01	4.64	0.01		C : E1,E2
PDIN in g.kg^{-1} DM	67.93	7.13	70.00	11.93	69.65	2.82		
PDIE in g.kg^{-1} DM	51.93	5.41	52.03	2.27	51.93	0.83		

Conclusion

It is necessary to use biological silage preparations when ensilaging whole-crop oat (*Avena sativa*). They control and improve the fermentation process. It is manifested in decrease of pH in silages, increase of lactic acid content, and decrease in concentration of butyric acid, alcohol and ammonia nitrogen.

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Summary

In our work, we observed the influence of two biological inoculants on fermentation process and nutritive value in whole-crop silage. We found out that the inoculation of oat (milk stage, 281 g dry matter .kg^{-1}) controlled and improved the fermentation process as well as the whole quality of produced silages. In treated silages we found highly significant pH decrease from 4.40 to 3.73 and 3.70, increase in content of lactic acid from 79.6 to 113.3 and 118.6 g.kg^{-1} DM, decrease in VFA total from 58.5 to 19.1 and 12.4, as well as decrease in content of ammonia nitrogen from 21.4 to 9.6 and 9.0 %. Under the influence of better fermentation in inoculated silages decreased dry matter losses by 64 – 68 % and increased energy concentration (ME by 0.3 MJ.kg^{-1} DM and NEL by 0.2 MJ.kg^{-1} DM).

References

[1] Hargreaves, A., Hill, J., Leaver, J.D. (2009). Effect of stage of growth on the chemical composition, nutritive value and ensilability of whole-crop barley. In: Animal Feed Science and Technology 152(1-2), 50.

[2] Lingvall, P., Knicky, M., Frank, B., Rustas, B.O., Wallsten, J. (2005). The influence of crop maturity and type of baler on whole-crop barley silage production. Silage production and utilization. Proceedings of the XIVth International Silage Conference, a satellite workshop of the XXth International Grassland Congress, Belfast, Northern Ireland, July 2005, 180.

[3] Official Reports of MA SK (2004): Výnos Ministerstva pôdohospodárstva Slovenskej republiky č.2145/2004-100 o úradnom odbere vzoriek a o laboratórnom skúšaní a hodnotení krmív.

[4] Rajčáková, Ľ., Mlynár R. (2006): The influence of some biological additives on fermentation and quality of whole-crop barley silage.In: 12th International Symposium Forage Conservation, Brno, 3.-5.4.2006, VFU: Brno, s. 208 - 210, ISBN 80-7305-555-4.

[5] Rajčáková, Ľ., Mlynár, R. (2009): Zásady využívania potenciálu silážnych a konzervačných prípravkov pri výrobe kvalitných a hygienicky nezávadných konzervovaných krmív, Metodická príručka. Nehmotný realizačný výstup E 03 RÚVV Produkčne efektívne a environmentálne tolerantné využitie nutričných zdrojov, 47 s.

[6] Spann, B., Obermaier A., Maierhofer R. (2002): Nährstoffverdaulichkeit von Ganzpflanzensilage (GPS) aus Triticale bei Rind und Schaf bei unterschiedlicher Schnitthöhe der Triticale. In: Gruber INFO 3/02, p. 17 – 20.

[7] Taylor, C. C., Ranjit, N. J., Mills, J. A., Neylon, J. M., Kung, L. Jr. (2002): The Effect of Treating Whole-Plant Barley with Lactobacillus buchneri 40788 on Silage Fermentation, Aerobic Stability, and Nutritive Value for Dairy Cows. In: Journal of Dairy Science Vol. 85, No. 7, 2002, p. 1793 – 1800.

Aldehydes in Maize and Grass Silages

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Abstract

Aldehydes and ketones are volatile compounds of silages which probably affect feed preference, palatability and intake. a sensitive and fast method was developed to determine aliphatic aldehydes in maize and grass silages. The method is based on the solid phase micro-extraction and on-fibre derivatisation of aldehydes with *O*-(2,3,4,5,6-pentafluoro)benzylhydroxylamine hydrochloride (PFBHA). Aldehydes selectively react with PFBHA forming volatile oximes, which were desorbed in the injection port of a gas chromatograph and analysed by mass spectrometry. Using this method, eight aldehydes were detected in maize and grass silages being ethanal, propanal, butanal, 2-methylpropanal, pentanal, 3-methylbutanal, hexanal and heptanal, at levels ranging from tens to 200 mg/kg fresh matter.

Introduction

Carbonyl compounds, particularly aldehydes, are considered to be constituents of typical silage odour together with other volatiles, such as lower alcohols, volatile fatty acids, esters, terpenic and phenolic compounds. These volatile components may affect feed preference, palatability and intake by animals (Mo et al., 2001; Krizsan et al., 2007). Moreover, a possibility of their carry-over to milk and meat with an impact on sensory properties has been considered (Martin et al., 2005). Volatiles in silages can be produced during biochemical and microbial processes during forage fermentation and silage storage. Literature data on the occurrence of aldehydes in silages are very limited. Aldehydes and ketones in silages were selectively reported in only Langin et al. (1989) and other works have dealt with aldehydes merely as a group of determined volatiles. Similarly, very limited knowledge is available on aldehydes effect on ruminant health (Langin et al., 1988).

Materials and Methods

Samples of farm-scale silages of 8 maize (*Zea mays*) and 13 permanent grass swards were collected from several farms in South Bohemia during winter from large-scale vertical concrete silos (capacity of hundreds tone) using a core sampler. Grass silages were mostly produced from first-cut wilted forage. The silages were judged to be of good and medium quality. Silage from the central part of each silo was sampled and a vial of 7ml was filled with the sample (±2.0 g) to about two-third of its volume.

A 65-µm(polydimethylsiloxane)/divinylbenzene (PDMS/DVB) fibre coating (Supelco, Czech Republic) was used in this method. a water solution of PFBHA in a vial was loaded onto the solid phase micro-extraction (SPME) fibre (PDMS/DVB) at 25 °C for 10 min. Next, the fibre with PFBHA was exposed to sample vapours in the headspace of another vial filled with silage sample for 8 min at 60 °C. Finally, the oximes of volatile aldehydes formed on the fibre were desorbed at the GC injector at 270 °C.

Aldoximes were analysed using a gas chromatograph equipped with a mass-selective detector Finnigan GCQ (Supelco, Bellefonte, USA) fitted with a capillary column Zebron ZB-5 (Sigma Aldrich, Czech Republic). Analyses were carried out in Institute of Systems Biology and Ecology, Academy of Sciences of the Czech Republic, Laboratory of Environmental Analytical Chemistry, České Budějovice.

Eight aldehydes (i.e. ethanal, propanal, butanal, 2-methylpropanal, pentanal, 3-methylbutanal, hexanal, heptanal) were identified as aldoximes by comparison with external standards (Sigma

Aldrich, Czech Republic) which were prepared immediately for each run of analyses by the above described derivatisation procedure. Aldehydes content were calculated by comparison of peak areas of the standards and of the samples. The detection limit of all eight low-molecular aliphatic aldehydes was 0.1 mg/kg fresh matter.

Results and Discussion

The conservation process of all farm-scale maize silages was successful and the silages were judged to be of good quality. Farm-scale grass silages were judged to be of good and medium duality. Eight aldehydes were detected in silage samples, being ethanal, propanal, butanal, 2-methylpropanal, pentanal, 3-methylbutanal, hexanal and heptanal. All these aldehydes were found in all 21 farm-scale (Table 1).

Table 1: Aldehyde contents (mg kg^{-1}) in farm-scale silages sampled during winter period

Aldehyde	Maize silages			Grass silages		
	n = 8			n = 13		
	x	SD	Range	x	SD	Range
Ethanal	25.8	16.1	9.4-48.2	29.8	14.2	9.9-49.4
Propanal	70.2	17.8	51.3-99.1	76.6	12.0	54.4-97.6
Butanal	77.9	16.9	52.2-100	71.9	17.2	46.1-98.7
2-Methylpropanal	28.9	15.1	10.4-49.0	25.7	15.8	9.2-50.4
Pentanal	150	37.8	97.8-200	147	30.3	105-189
3-Methylbutanal	78.3	17.4	50.6-97.3	76.0	18.5	46.7-100
Hexanal	153	38.9	97.1-196	150	30.3	104-190
Heptanal	24.4	17.6	7.9-50.3	23.1	11.8	10.3-46.5

x ... mean value; SD ... standard deviation

Krizsan et al. (2007) determined the content of five aliphatic aldehydes in 24 Norwegian low-DM grass silages. Mean values of 2.67, 0.26, 4.96, 6.09 and 9.97mg/kg DM were reported for the contents of ethanal, propanal, 2-methylpropanal, 2-methylbutanal and 3-methylbutanal, respectively. Moreover, Krizsan et al. (2007) determined these aldehydes in two samples of initial herbage, with respective mean contents of 3.33, 0.89, 2.20, 14.70 and 3.14 mg/kg DM. Thus, herbages seem to vary considerably in levels of these aldehydes in their silages.

Nevertheless, more aldehydes were reported in some papers in ensiled forage than in the respective silages. In direct-cut Italian ryegrass forage, three aliphatic aldehydes, 3-methylbutanal, hexanal and heptanal, were detected at µg/kg levels while, in poor-quality silage prepared from the same grass, only ethanal of 28.2 mg/kg was reported (Kami and Ohsaki, 1988). No aliphatic aldehyde was detected among volatiles of red clover (*Trifolium pratense*) silage (Figueiredo et al., 2007), while low contents of nonanal and decanal were detected in cut green clover. Some aliphatic aldehydes in silages originate from plants as a class of so-called inducible volatile organic compounds (for an overview see Holopainen, 2004). Saturated and unsaturated aldehydes, preferentially with six-carbon chains, are produced immediately after plant damage via the lipoxygenase pathway of fatty acid oxidation. Such changes occur after forage crops are harvested.

Conclusions

A very sensitive method for the determination of aliphatic aldehydes in silages using on-fibre derivatisation with PFBHA was developed. The contents of several individual aldehydes in maize and grass silages ranged between tens and hundreds of mg/kg fresh matter.

Reference

[1] Figueiredo, R., Rodrigues, A.I., do Céu Costa, M. (2007): Volatile composition of red clover (Trifolium pratense L.) forages in Portugal: the influence of ripening stage and ensilage. Food Chem. 104, 1445–1453.

[2] Holopainen, J.K. (2004): Multiple functions of inducible plant volatiles. Trends Plant Sci. 9, 529–533.

[3] Kami, T., Ohsaki, K. (1988): Volatile constituents of the green forage and stack silage of Italian ryegrass. Jpn. J. Zootech. Sci. 59, 653–659.

[4] Krizsan, S.J., Westad, F., Adnoy, T., Odden, E., Aakre, S.E., Randby, A.T. (2007): Effect of volatile compounds in grass silage on voluntary intake by growing cattle. Animal 1, 283–292.

[5] Langin, D., Dumon, H., Nguyen, P., Malek, A., Fromageot, D. (1988): Carbonyl compounds in silages: toxic potential. a review. Revue Méd. Vét. 139, 407–415.

[6] Langin, D., Nguyen, P., Dumon, H., Malek, A. (1989): Aldehydes and ketones in silages: quantitative determination by high performance liquid chromatography. Ann. Rech. Vét. 20, 119–127.

[7] Martin, B., Verdier-Metz, I., Buchin, S., Hurtaud, C., Coulon, J.B. (2005): How do the nature of forages and pasture diversity influence the sensory quality of dairy livestock products. Anim. Sci. 81, 205–212.

[8] Mo, M., Selmer-Olsen, I., Randby, A.T., Aakre, S.E., Asmyhr, A. (2001): New fermentation products in grass silage and their effects on feed intake and milk taste. In: Proceedings of the 10th International Symposium on Forage Conservation, Brno, Czech Republic, 98–99.

THE INFLUENCE OF PRESERVE BY THE PREPARATE AGAINST EUROPEAN CORN BORER (OSTRINIA NUBILALIS) ON THE CONTENT OF YEASTS AND MOULDS IN THE MAIZE SILAGE

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Abstract

The aim of this work was the evaluation of influence of the preserving against the corn-borer ("ECB") on the content of yeast and moulds to state. In the experiment was used hybrid of maize with the number FAO 230. It was produced 6 variants of maize silage in one repetition (plants were or were not treated with against corn-borer, and each variant had three alternatives – control, by a microbial preparation, by a chemical preparation). It was monitored the content of yeast and moulds (CFU in 1g) in the day of the opening. The treated and untreated (against ECB) variants of corn silage did not show the statistical significant difference in the content of yeasts (treated – 16,9*104 ± 18,9*104 CFU; untreated – 24,4*104 ± 37,2*104CFU) and moulds (treated – 8,04*103 ± 15,5*103 CFU, untreated – 9,86*103 ± 13,7*103 CFU). The influence of used silage additive was not significant: yeasts (control – 27,9*104 ± 44,9*104 CFU; inoculated 18,6*104 ± 21,5*104 CFU; chemical preparation - 15,4*104 ± 11,7*104 CFU), moulds (control – 11,4*103 ± 13,9*103 CFU; inoculated 4,1*103 ± 4,7*103 CFU; chemical preparation - 11,3*103 ± 16,1*103 CFU). The differences were not evident between the same silage additives from different groups (within the treatment against the ECB).

Introduction

The maize silages form the main part of the feedstuffs. In the feed mixtures has the maize silage a yearly using. Thanks to favourable content of soluble carbohydrates (NFC) belongs the silage maize to the easily ensilable crops (DOLEŽAL et al, 2009). Its quality is given without a chemical composition and the characteristics of the fermentation process through a hygienic quality too. The main indicator is the content of moulds and yeasts. In this time is presented European corn borer ("ECB") as the biggest pest. It participates on the transfer of mycotoxins in the plants and on the destruction of the plants.

Yeasts contribute no acid from their fermentation activity. Being facultative anaerobes they can grow on the crop and in the silo by converting glucose to carbon dioxide and water in the presence of oxygenin the swath and during the vegetation phase of ensiling process (WILKINSON, 2005). Yeasts ferment the residual sugars on ethanol. However they can degrade the made lactic acid too and decrease the total acidity of silage. The activity of yeast is linked to the warming of silages. According to DOLEŽAL (in ZEMAN et al 2006) have the silages additive the inhibitive effect on the proliferation of yeasts, moulds and bacteria.

Material and Methods

The experiment was run in country Senice na Hané (above see level - 335 m.). In our experiment was used hybrid of corn with the number FAO 230. The plants were protected against the European corn borer by a chemical preparation (0,7l per ha, Integro). After the harvest was the

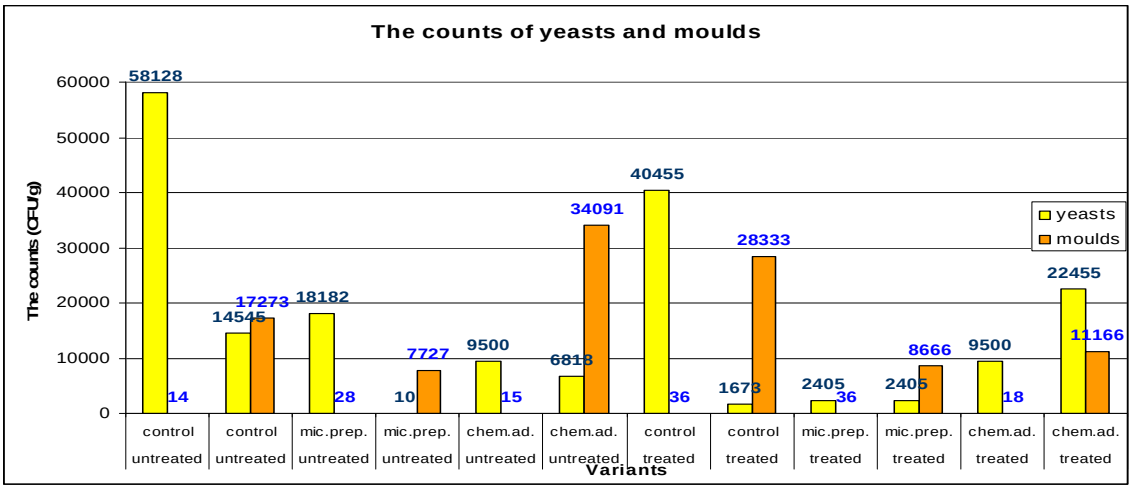
matter treated by a chemical preparation (organic acid blend) in the amount 2l/t and by a microbial inoculant (15g/t). As the control variant was the silage without the treatment. The size of the chopped forage was 1-2cm. The matter was compressed and conserved to the plastic tubs. After the opening (60 – 80 days) were taken the samples of silages and were measured the contents of moulds, yeasts and total amount of microorganism.

Subsequently followed the serial dilution by a factor of 10. 1 ml of respective dilutions was transferred on a Petri dish and overflown with culture medium. The counts of yeasts and moulds were estimated on Chloramphenicol Glucose Agar (Biokar Diagnostics, France) after 120 hours at 25 oC. It was used for the statistical analysis the t-test in the program Statistica CZ.

Results and Discussion

The treatment of maize against ECB did not significantly influence the amount of yeasts and moulds in the silages. The counts of yeasts and moulds are presented in the graph. Generally were the counts of yeasts and moulds lower in the treated variants (yeasts - treated – 16,9*104 ± 18,9*104 CFU; untreated – 24,4*104 ± 37,2*104CFU, moulds treated – 8,04*103 ± 15,5*103 CFU, untreated – 9,86*103 ± 13,7*103 CFU).

The using of silage preparation did not significant influence the counts of yeasts and moulds: yeasts (control – 27,9*104 ± 44,9*104 CFU; inoculated 18,6*104 ± 21,5*104 CFU; chemical preparation - 15,4*104 ± 11,7*104 CFU), moulds (control – 11,4*103 ± 13,9*103 CFU; inoculated 4,1*103 ± 4,7*103 CFU; chemical preparation - 11,3*103 ± 16,1*103 CFU). The differences were not detected neither between the silage additives from different groups (within the treatment against the ECB).



Conclusion

In the results is evident that the treatment against the ECB did not directly affect the content of yeasts and moulds in the corn silage. However, the effect of treatment against corn borer on the content of microorganism can be manifested when will used a silage additive.

Reference

[1] DOLEŽAL, Petr, et al. Vliv patogenních mikroorganismů a jejich sekundárních metabolitů na kvalitu a hygienickou nezávadnost objemných krmiv. In PROKEŠ, Karel. Kukuřice v praxi 2009. 1. vyd. Brno : Ediční středisko MZLU v Brně, 2009. s. 37-46. ISBN 9788073752637.

[2] ŠUK, J., et al. Kukuřice. 1. vyd. Kněževes : VP Agro, 1998. 131 s. ISBN 8086153991.

[3] WILKINSON, J.M. Silage. 1st edition. Lincoln : Ruddock print, 2005. 254 s. ISBN 0948617500.

[4] ZEMAN, Ladislav, et al. Výživa a krmení hospodářských zvířat. 1. vyd. Praha : Profi press, 2006. 360 s. ISBN 80-86726-17-7

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Maize Silage Quality on Swedish Dairy and Beef Farms

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Introduction

Land used for maize cultivation is increasing in Sweden. Maize was grown on 12 000 hectares in 2008 (SJV, 2009) and the number of acreages was presumably increased to 20 000 ha in 2009. As earlier maturing varieties are being developed and marketed, maize for silage production is grown as far north as to the Stockholm area (59º17´N, 18º3É). To gain knowledge regarding maize silage production in south Sweden, we performed a study aiming at 1) identifying problems related to harvest and conservation of maize silage and 2) investigating maize silage quality on dairy and beef farms in southern Sweden.

Materials and Methods

The study was performed 2007 on 19 dairy farms and 6 beef farms in south Sweden in collaboration with local advisors from the Swedish Dairy Association and The Swedish Rural Economy and Agricultural Societies. The farmers were interviewed regarding their maize. The maize was planted from April 15 in the south until May 14 further north with an average planting date of May 1. Twenty-eight different varieties of maize were used on the 25 farms. Both planting and harvest of the maize were done by machine stations. The farmers analysed the DM content of the plants and pinched the maize kernels to decide harvest date, aiming for a DM content of just above 30%. The maize was harvested from September 12 on the south farms until November 1 on the farms further north and yielded, on average, 11 tonnes of DM/ha (from 7 to 16 tonnes of DM/ha). The maize was precision chopped to 10-15 mm and kernel processors at 1-4 mm settings were used on the harvesters. The maize was stored in bunker silos on 21 of the farms and in tubes on the remaining four farms. a few of the farms that used bunker silos complemented with tubes. Only twelve of the farmers used additives to all of the harvested maize and six of the farmers did not use any additives at all. The salts and the acids were the most commonly used additives. The average storage time of the silage was 3.5 weeks before feed out and a new surface area of the silage was reached after an average time of 2.5 days. Silage samples for analysis of chemical composition and fermentation characteristics were taken from several spots of the surface area of the bunker silo or of the tube. Two samples for microbiological analysis were taken on each of 13 farms; one sample in the centre and one sample 300 mm from the edges of the surface area of the bunker silo or of the tube. As there generally were no differences in microbial growth between the two sampling sites, a mean value for each farm was used. Results are shown as means or medians with minimum and maximum values within parenthesis. Simple regressions between variables measuring silage quality were calculated in Microsoft Excel.

Results and Discussion

Silage DM concentration varied from 190 to 450 g/kg with an average of 300 g/kg being harvested from September 12 to November 1. The NDF concentration varied from 319 to 565 g/kg DM and was strongly correlated to the starch concentration ($r = -0.83$), which varied from 99 to 419 g/kg DM (Figure 1). The negative correlation between NDF and starch can be explained by an increased proportion of the starch-rich cob while the proportion of the fibre-rich stalk was

decreasing, which could be due to differences in the starch : NDF ratios between cultivars and between maturity stages of the maize at harvest as starch increases while the NDF decreases with advancing maturity (Jensen et al., 2005). The in sacco indigestible NDF (INDF) concentration varied from 132 to 254 g/kg NDF, which could be related to differences in INDF concentrations between cultivars and between maturity stages of the maize as NDF becomes less digestible as the crop matures (Jensen et al., 2005). Crude protein in the silages varied from 62 to 101 g/kg DM. Silages generally showed a good ratio between lactic acid and acetic acid (>3:1), a moderate ammonium-nitrogen content, a low pH, no or low butyric acid and clostridial spore counts (Tables 1 and 2). The large variations in DM concentrations of the silages from different farms caused large variations in fermentation patterns between silages treated with no or with the same type of additive.

Figure 1: Relationship between concentrations of NDF and starch in maize silages from 25 farms in south Sweden (Y = 708 – 1.03x, R^2 = 0.69, r = - 0.83).

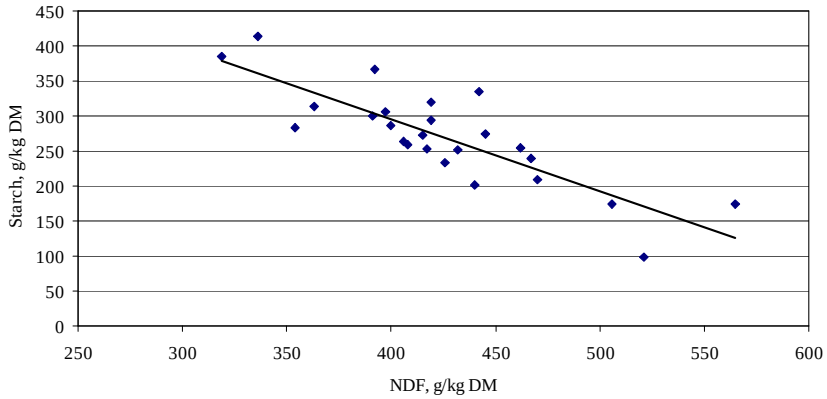

Table 1: The DM, sugar and fermentation products of maize silages treated with no additive on six farms or with additives applied to the whole harvest of the maize crop on 12 of the 25 farms in south Sweden. Mean values with minimum and maximum values within parenthesis; n = number of farms.

	Type of additive[1]			
	No additive (n = 6)	Salt (n = 5)	Acid (n = 6)	Inoculant (n = 1)
DM, g/kg	260 (190-370)	350 (260-450)	290 (230-330)	360
Sugar, g/kg DM	11 (8-15)	23 (5-68)	34 (2-83)	17
pH	3.9 (3.5-4.4)	3.9 (3.5-4.2)	4.0 (3.6-4.5)	3.9
NH_3-N, g/kg total-N	64 (18-94)	94 (82-106)	64 (51-80)	55
Lactic acid, g/kg DM	93 (30-173)	63 (45-98)	54 (10-110)	51
Acetic acid, g/kg DM	28 (3-49)	21 (9-36)	14 (7-21)	15
Butyric acid, g/kg DM	0.1 (0.0-0.7)	0.0	0.0	0.0
Ethanol, g/kg DM	6.0 (0.0-20.4)	2.2 (0-5.1)	10.5 (5.0-24.4)	5.8

[1]*Salt: Kofasil Majs (Addcon Nordic AS, Porsgrunn, Norway), Acid: Promyr, Proens (Perstorp Inc., Perstorp, Sweden), Inoculant: Lactisil Majs (Chr. Hansen A/S, Hørsholm, Denmark)*

There were yeasts in many of the silages and some silages contained moulds with P. roqueforti as the most common type, which explained the heating of silages at feed out, especially during the summer, reported by 15 of the farmers (Table 2). Yeast and mould growth in additive-treated silages could depend on insufficient packing of the chopped maize crop between each load during filling of the silo and on lower than recommended dosage of the additive.

Table 2: Spores and fungal growth (log cfu/g sample) in maize silage. Median with minimum and maximum values within parenthesis; n = number of samples with two samples per farm (one sample in the centre and one sample 300 mm from the edges of the surface area of the bunker silo or of the tube). See footnote for table 1.

	Type of additive			
	No additive (n = 4)	Salt (n = 8)	Acid (n = 10)	Inoculant (n = 2)
Clostridial spores	< 2.0	< 2.0	≤ 2.0 (< 2.0-2.3)	< 2.0
Bacilli spores	3.2 (2.0–4.3)	3.0 (< 2.0-5.1)	2.9 (2.3-5.2)	< 2.0
Enterobacteria	Not detected	< 1.0 (< 1.0-2.0)	< 1.0 (< 1.0 -2.0)	Not detected
Fusarium	< 2.0	< 2.0	< 2.0	< 2.0
Yeasts	6.2 (4.9-7.4)	6.4 (< 2.0-7.4)	3.3 (< 2.0-6.5)	7.4 (7.2-7.5)
Moulds	≤ 2.0 (< 2.0-2.3)	2.4 (< 2.0-4.9)	≤ 2.0 (<2.0-2.8)	< 2.0

Conclusion

This study shows large variations in DM and nutrient contents between maize silages produced at farms differing in locations and cultivars used. The variations in chemical composition of the maize silage probably were related to the maturity stage of the crop at harvest. There was heating in many of the silages at feed out, which was confirmed by the microbiological analyses.

References

[1] JENSEN C., WEISBJERG M. R., NØRGAARD P. and HVELPLUND T. 2005. Effect of maize silage maturity on site of starch and NDF digestion in lactating dairy cows. Anim. Feed Sci. Technol. 118, 279-294.

[2] SJV. 2009. Production of cereals, dried pulses, oilseed crops, potatoes and temporary grasses in 2008. Final statistics. JO 16 SM 0901. Swedish Board of Agriculture, Jönköping, Sweden.

Nutrition Value of Mountain Forage by Sheep

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Introduction

Grasslands are important in many parts of the world for their nexus between feed production and the environmental impacts and incorporated flexible combinations of cropping, pasture and forestry (Lemaire et al., 2005; Gibon, 2005). The ultimate objective in grazing management is to obtain the optimum balance between the animals and the pastures (Van Soest, 1994). Therefore, extensively exploited permanent mountain pasturelands can provide worthwhile animal performances (Troxler and Jans, 2000). Regular grazing of meadows has positive effect in increasing phytocoenosis and zoocoenosis biodiversity too.

Description of the specific mountains forages typical for the protected area of Krkonoše Mts. National Park is important for knowing of all nutritive factors of broad range of feeds as key information for understanding of all biochemical processes in the rumen of ruminants.

The proposed paper is based on current feed evaluation system, with focus on digestibility mechanisms in relations to forage quality of animal nutritional needs. The objective of this study was to estimate the specific mountain forages originated from Krkonoše Mts. National Park using chemical analysis and *in vivo* procedures.

Material and Methods

Samples description: *Individual plants of Deschampsia flexuosa.*

Representative (mixed) meadow sample. The experimental material (forage samples) were collected from the observed locality Krkonoše Mts. National Park, locality of Zadní Rennerovky in May 2008.

Forage samples were analyzed for:

Chemical composition: the samples were analyzed for content of dry matter, ash, ether extract, neutral-detergent fibre (NDF), acid-detergent fibre (ADF), acid-detergent lignin (ADL) and crude protein (CP). NDF, ADF and ADL were estimated according to Van Soest et al. (1991). CP was analysed according to the Kjeldahl method (Nitrogen × 6.25). Ether extract and CF were determined according to the AOAC (1990).

In vivo digestibility methods: the *in vivo* metabolic trials were performed on six weathers Merino breed (weighing 83±9 kg) stabled in balance separators. The forages were offered twice a ay (7.2 kg of pasture forage/animal/day and 5 kg of *Deschampsia flexuosa*/animal/day), at 6 a.m. and 6 p.m. The animals had free access to drinking water.

Calculations and statistical analysis: Statistical analysis of this experiment were performed using the statistical programme SAS (SAS Institute, 2003). Correlation coefficients between variables were computed using PROC CORR. Treatment means were compared by Scheffe test of multiple-comparison procedure at $P < 0.05$.

Results and Discussion

The original dry matter contents of the pasture forage and *Deschampsia flexuosa* were 20.3 % and 28.3 %, respectively. The pasture forage was consisted of 23.8 % of CP, 1.9 % of ether extract, 6.0 % of ash and 21.0 % of CF. Fibre fractions of pasture forage, i.e. NDF, ADF and ADL were 72.4 %, 30.9 % and 4.4 %, respectively. The chemical composition values of *Deschampsia flexuosa* of CP, ether extract, ash, CF, NDF, ADF and ADL were 22.0 %, 2.5 %, 5.2 %, 19.1 %, 64.1 %, 28.2 % and 2.9 %, respectively. The chemical composition of the estimated feedstuffs were generally in agreement with the Czech feed table (Sommer et al., 1994).

Values for *in vivo* sheep digestibility of individual nutrients of both forages are given in table 1. The *in vivo* digestibilities of DM and OM averaged 72.7 and 74.3 % for pasture forage and 73.6 and 75.8 % for *Deschampsia flexuosa*, respectively. The *in vivo* sheep digestibility of CP averaged from 79.0 % (pasture forage) to 84.5 % (*Deschampsia flexuosa*). As in the experiment of Ribeiro et al. (2005), the digestibility of CP tended to be higher than the digestibility of OM.

Statistical analysis of this experiment were performed using the statistical programme SAS (SAS Institute, 2003). The correlations were determined among the *in vivo* sheep digestibility of individual nutrients. Treatment means for pasture forage and *Deschampsia flexuosa* were compared by the Scheffe test at $P < 0.05$ (table 1). Significant differences ($P < 0.05$) between the pasture forage and *Deschampsia flexuosa* in the *in vivo* digestibilities of CP, CF, NFE and ADF were observed.

Table 1: *In vivo* sheep digestibility of individual nutrients and gross energy of pasture forage and *Deschampsia flexuosa* (units are in % of absolute dry matter).

Chemical composition	Unit	Pasture forage	*Deschampsia flexuosa*
Original dry matter	%	72.7	73.6
Crude protein	%	79.0	84.5
Ether extract	%	65.4	67.0
Crude fibre	%	64.1	49.7
Nitrogen-free extract	%	77.0	83.1
Organic matter	%	74.3	75.8
NDF	%	78.1	78.2
ADF	%	70.9	62.2

ADF = acid-detergent fibre, NDF = neutral-detergent fibre.
Note: Scheffe test of differences for pasture forage and Deschampsia flexuosa where "bold" letters in row are significantly (P < 0.05) different.

Conclusion

Feed estimation as a set of parameters of the chemical composition and *in vivo* sheep digestibility analysis are information important for feed quality evaluation. The evaluation of nutritive value of the specific mountain forages is necessary for reason of landscape conservation and cultivation, and interactions between plant/animal productions.

Acknowledgments

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References

[1] AOAC, 1990. Official Methods of Analysis, Association of Official Analytical Chemists. 15th Edition. Washington, DC.
[2] Gibon A., 2005. Managing grassland for production, the environment and the landscape. Challenges at the farm and the landscape level. Livest. Prod. Sci. 96 (1), 11-31.
[3] Lemaire G., Wilkins R., Hodgson J., 2005. Challenges for grassland science: managing research priorities. Agriculture ecosystems and environment. 108 (2), 99-108.
[4] Ribeiro C. V. D. M., Karnati S. K. R., Eastridge M. L., 2005. Biohydrogenation of fatty acids and digestibility of fresh alfalfa or alfalfa hay plus sucrose in continuous culture. J. Dairy Sci. 88, 4007-4017.
[5] Sommer A., Čerešňáková Z., Frydrych Z., Králík O., Králíková Z., Krása A., Pajtáš M., Petrikovič P., Pozdíšek J., Šimek M., Třináctý J., Vencl B., Zeman L. (1994): Potřeba živin a tabulky výživné hodnoty krmiv pro přežvýkavce. Česká akademie zemědělských věd, 198 p., ISBN 80-901598-1-8.

[6] Statistical Analysis Systems Inc., 2003. SAS; Statistic's Version 9.1 Edition. SAS Institute Inc., Cary, NC, USA.
[7] Troxler J., Jans F., 2000. Optimal management of forage on extensive mountain grazing pastureland: influences on vegetation and animal performance. In: Søegaard K., Ohlsson C., Sehesed J., Hutchings N.J., Kristensen T. (Eds.). Balancing environmental and economic demands. Grassland Science in Europe. 5, 319-321.
[8] Van Soest P.J., 1994. Nutritional ecology of the ruminant. Cornell University Press. pp. 476.
[9] Van Soest P.J., Robertson J.B., Lewis B.A., 1991. Methods for dietary fibre, neutral detergent fibre, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583-3597.

Effect of Harvesting Corn with Higher Dry Matter on Chemical Composition and Quality of Silage

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Introduction

In agricultural practice, harvesting of corn in an optimum stage of ripeness and its proper preservation are the basic predispositions when trying to assure a high productivity and good health of cattle. Unfortunately, it happens relatively often that the beginning of the harvest period is defined correctly, i.e. in the optimum stage of crop ripeness, but a great part of produced green matter is harvested much later on. This causes big problems due to an insufficient compaction of silage and the resulting final quality of produced silage. Such silage is then less palatable, its nutritional value is decreased and it contains various metabolites and mould toxins; it is also more vulnerable to degradation and decay.

According to Johnson et al. (1999), Andrae et al. (2001), Jensen (2005), and Ngonyamo-Majee et al. (2008, 2009) such factors as hybrid, maturity, and processing all affect digestibility and its nutritive value of produced corn silage. Di Marco et al. (2002) concluded that the *in vivo* digestibility of silage DM remained constant in the course of ripening because a decrease in NDF digestibility was compensated by the accumulation of starch in grain.

Cherney et al. (2004) monitored differences among individual maize hybrids. Russell et al. (1992) demonstrated that the differences in the nutritional value of silage were not influenced only by individual hybrids but also by methods of tillage and agro-technical measures. Meeske et al. (2002) and other authors emphasised that the quality of produced silage may be influenced by various additives. Cone1 et al. (2008) found out that the quality of silage was influenced also by early and late-ripening types of Dry Down and Stay Green cultivars.

Material and Methods

Experimental material involved results of chemical analyses of maize silages obtained in laboratories of the company EkoLab Žamberk in years 2002 – 2009. Analytical results were provided by the firma AgroKonzulta Žamberk. The experiment involved altogether 420 analyses of produced silage. In Group A, which consisted of samples of silage maize harvested in an optimum (or in a generally recommended) growing stage (when the so-called kernel milk line passed through the imaginary line of separation between the starchy tip and the milky base of individual grains in approximately two thirds of the grain), the results of 210 analyses ranged from 28 to 34 % of DM content. In Group B, the total number of analyses was also 210 and the crop was harvested in the maturity stage with a higher content of DM (34 to 40 %). In this case, the kernel milk line was visible above the separation line in more than two thirds of the grain and in some grains it was possible to see a black spot at the end of grain in the direction to the cob (i.e. towards its centre).

The chemical composition of silages was determined using the Weende (AOAC, 1995) and van Soest methods (Van Soest et al., 1991). Acid content and pH were determined by means if AOAC (1995). The results were analysed statistically using QC-Expert 3.0 (TriloByte Statistical Software, 2010).

The aim of these analyses was to evaluate the effect of a higher DM content on the quality of maize silage under practical conditions.

Results and Discussion

In Group A, which involved maize crops harvested in the optimum (and/or generally recommended) growing stage, the average content of DM was 317 g, while in Group B (harvested

later on) the average DM content was 363 g. Parameters of the nutritional value and fermentation of silages preserved at optimum and increased DM content are presented in Tab. 1. While statistically significant differences (P<0.05) between both groups were found out in contents of ash (A), crude fibre (CF), acid detergent fibre (ADF), starch, pH and lactic acid (LA), there were no statistically significant differences in contents of crude protein (CP), fat and neutral detergent fibre (NDF). There was a difference between our results and those published by Di Arco et al. (2002), who observed that the crop maturity decreased the content of NDF (P < 0.05). The decrease of NDF content in whole plants was only relative because the percentage share of grains increased to the detriment of green matter. This was also associated with an increase in starch content. The differences between both groups were statistically highly significant (P<0.01). In the control group of samples, the starch content was 302 g/kg DM, while in the experimental one it made as much as 322.5 g/kg DM. Also Di Marco et al. (2002) observed a high increase in starch content in course of ripening of maize crops. In Group B, an increase in the starch content did not influence values of net energy lactation (NEL), and/or net energy for fattening (NEF).

There were highly significant differences (P<0.01) in fermentation parameters (and/or in pH and LA content). Differences in fermentation parameters were also highly significant (P<0.01) and an increase content of LA was found out in silage maize harvested in an optimum growing stage, i.e. with the average DM content of 317 g.

Results obtained in Group a were significantly better than those recorded in Group B. similar conclusions were published also by Johnson et al. (1999), Andrae et al. (2001), Jensen (2005), and Ngonyamo-Majee et al. (2008, 2009).

Conclusions

A higher experimental (i.e. more than 34 %) vs. an optimum DM content (28 – 34%) showed a significantly (P<0.05) negative effect on the chemical composition and quality of fermented of corn silage. The obtained results indicate that under practical conditions is can be recommended to harvest the crop in the growing stage characterised by the milk line passing through two thirds of kernels.

Table 1: Effect of harvesting corn with higher dry matter

		Silage DM 280 - 340 g		Silage DM 341 - 400 g	
Index	Unit	AVG	SX	AVG	SX
DM	g	316.88a	15.13	363.36b	15.22
CP	g/kg DM	83.70a	9.46	81.97a	8.70
Fat	g/kg DM	32.49a	2.78	33.11a	3.70
A	g/kg DM	42.42a	6.26	40.32b	6.70
CF	g/kg DM	201.46a	26.75	191.56b	23.35
NDF	g/kg DM	454.59a	56.06	455.81a	57.15
ADF	g/kg DM	239.34a	38.24	230.66b	35.69
Starch	g/kg DM	301.96a	53.23	322.51b	50.63
OM	g/kg DM	957.58a	6.26	959.68b	6.70
NEL	MJ/kg DM	6.33a	0.17	6.32a	0.18
NEV	MJ/kg DM	6.31a	0,22	6.29a	0.22
pH		3.63a	0,14	3.70b	0.14
LA	g	20.79a	4.98	19.78b	4.14
AA	g	5.81a	2.514	5.48a	2.09
BA	g	0.12a	0.10	0.13a	0.10

Values within the same row followed by different superscript letters are significantly different (P<0.05)

Acknowledgement

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References

[1] Andrae, J. G., C. W. Hunt, G. T. Pritchard, L. R. Kennington, J. H. Harrison, W. Kezar, and W. Mahanna. 2001. Effect of hybrid, maturity, and mechanical processing of corn silage on intake and digestibility by beef cattle. J. Anim. Sci. 79, 9:2268-2275.

[2] AOAC. 1995. Official Methods of Analysis. 16th Edition. Association of Official Analytical Chemists. Washington, DC. Vol. 1: . 69-79.

[3] Cone1, J.W., A.H. Van Gelder, H.A. Van Schooten and J.A.M. Groten. 2008. Effects of forage maize type and maturity stage on in vitro rumen fermentation characteristics. NJAS - Wageningen Journal of Life Sciences. 55, 2: 139-154

[4] Di Marco, O. N., M. S. Aello, M. Nomdedeu, and S. Van Houtte. 2002. Effect of maize crop maturity on silage chemical composition and digestibility (in vivo, in situ and in vitro). Anim. Feed Sci. Technology. 99. 1-4:37-43.

[5] Cherney DJR, Cherney JH, Cox WJ. 2004. Fermentation characteristics of corn forage ensiled in mini-silos. Journal of dairy science. 87, 12: 4238-4246

[6] Jensen, C., M.R. Weisbjerg, P. Nørgaard and T. Hvelplund. 2005. Effect of maize silage maturity on site of starch and NDF digestion in lactating dairy cows. Animal Feed Science and Technology.
118, 3-4, 4: 279-294

[7] Johnson L, Harrison JH, Hunt C, Shinners K, Doggett CG, Sapienza D. 1999. Nutritive value of corn silage as affected by maturity and mechanical processing: a contemporary review. Journal of dairy science. 82, 12: 2813-2825.

[8] Meeske R, van der Merwe GD, Greyling JF, Cruywagen CW. 2002.The effect of the addition of a lactic acid bacterial inoculant to maize at ensiling on silage composition, silage intake, milk production and milk composition. South African journal of animal science. 32, 4: 263-270

[9] Russell JR, Irlbeck NA, Hallauer AR, Buxton DR. 1992. Nutritive-value and ensiling characteristics of maize herbage as influenced by agronomic factors. Animal feed science and technology. 38, 1: 11-24.

[10] TriloByte Statistical Software, 2010 (info www.trilobyte.cz)

[11] Van Soest P. J., Robertson J. B., Lewis B. A. 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. Journal of Dairy Science, 74, 3583-3597.

Voluntary Dry Matter Intake of Silage from Two Maize Varieties in Cows

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Introduction

Maize silage is an important component of the feeding rations of high-yielding cows. It is basic forage with a high concentration of energy in terms of dry matter. The nutritional properties of maize silage are significant for their use in feeding rations. We cultivated maize varieties that were suitable for growth appropriate for the high-quality feed production in Czech climatic conditions. Other varieties of maize in the Czech Republic are limited for environmental reasons. To ensure the necessary quantity of nutrients and energy in animal diets significant is their concentration in dry matter as well as the quantity of dry matter that animals receive through the free availability of feeds. In this paper we evaluated data on the voluntary dry matter intake of two silages composed of two maize varieties – CELIO (CE) and CELIVE (CV). Our question was whether there were differences in dry matter intake and in the feeding value of the two maize varieties.

Material and Methods

(2010) published by Oseva (Bzenec) Inc. Celio described as a double cross hybrid with FAO 250, use for silage, CCM and biogas production. The type of grain is horse tooth. This variety is characterized by very good initial development and fast growth. It is very adaptable to poor environmental conditions and it has a very good digestibility and concentration of energy. Celive is the single cross hybrid with FAO 250 and used for grain. The type of grain is horse tooth, as well. This variety is characterised by high grain yield. I n growing silage for production, superior quality of silage is assumed owing to its high content of starch and energy.

For our study we used the two maize varieties (CE and CV) grown in the holdings of the farm Bludovská Inc. in the locality near the Šumperk. Both varieties were grown on the same plot, same maize growing and the same field chopper on 15th September 2009. The chopped forage was stored in two small overground pit silos; every pit silo was 4x7x1.5m in size. The maize silage was stored for 6 wk. The feeding trial for voluntary dry matter intake evaluation was conducted on six cows – crossbreds of meat breeds with *Czech Fleckvieh* from a suckler cow rearing system (675 – 739 kg LW) in November and December 2009. After weaning, animals were in the middle of gestation. The group of six cows was divided into 2 groups (A and B) 3 cows pee group. Each cow in the group was fed ad-libitum using feeding troughs (RIC system from Insentec B.V). The feeding trial was divided into two experimental periods. Each experimental period was preceded by a 10 days habit-forming period. In the first experimental period (lasting 2 wks) cows in group a were fed by the maize silage made from the CE variety and the cows in group B were fed by maize silage made from the CV variety. In the second experimental period (lasting 2wk) the feeds were exchanged so that different maize varieties were offered to animals (group a – CV; group B – CE).

The nutrients (crude protein - CP, crude fibre – CF, ether extract - EE, ash – A, starch - ST) in samples and the water extract acidity (WEA) of maize silage were analyzed by the Czech State Standard (CSS) 46 7092 “Method for Feed Testing”. Nitrogen free extract (NFE) was calculated. Neutral detergent digestible fibre (NDFD) and organic matter (OMD) digestibility was determined

by *the in Sacco* method. Lactic acid (LA), acetic acid (AA), propionic acid (PA) and butyric acid (BA) were determined by gas chromatography. The NEL (net energy of lactation), PDIE (ingested digestive protein allowed by energy), PDIN (ingested digestive protein allowed by nitrogen) was predicted by means of regression equations (Pozdíšek *et al.,* 2001) and using the equations described by Sommer *et al.* (1994). The system NE, PDI for the evaluation of the nutritive value is officially used in the Czech Republic. This system corresponds to the INRA system (Jarrige *et al.,* 1989). The data were processed using descriptive statistics and General Linear Models with fixed effects of LW (live weight), method of utilization and between effect (day in test) and its interactions with fixed effects using the SAS® 2007 software package. Post-hoc Tukey´s HSD tests were done. The level of significance was set at $p < 0.001$.

Results and discussion

The maize silage made from both maize varieties was well fermented. The fermentation in the maize silage made from the variety Celio in fresh silage was: pH 3.96, WEA 1,012 (KOH mg /100 g of silage), LA 1.25 %, AA 0.65 %, PA 0.06 % and no BA. The fermentation in the maize silage made from the variety Celive in fresh silage was: pH 3.98, WEA 993 (KOH mg /100 g of silage), LA 1.28 %, AA 0.54 %, PA 0.04 % and no BA. The results for the nutritive value and the voluntary dry matter intake in hybrids Celio and Celive are shown in Table 1.

Despite the fact that the energy concentration (NEL) was higher in variety CV (6.35 MJ/kg DM) than in CE (6.27 MJ/kg DM), the total intake of NEL was higher in variety CE due to differences in the amount of the dry matter intake.

Table 1: Feeding value, digestibility and voluntary dry matter intake of maize silages made from the varieties CE and CV

V	DM	CP	EE	CF	NFE	A	OM	ST	OMD	NDFD	NEL	PDIN	PDIE	PDIN PDIE	VI**	STD
	a	b	b	b	b	b	b	b	c	c	d	b	b		e	e
C_1	300.3	77.3	32.8	205.2	638.6	46.8	953.2	267.8	70.0	38.2	6.27	47.52	68.33	0.696	18.27	14.49
C_2	323.5	84.3	32.9	178.3	667.1	37.3	962.7	332.9	71.2	35.7	6.35	51.79	71.28	0.726	17.51	11.67

*Legend: C1 (Celio), C2 (Celive); a = g, b = g/kg DM, c = %, d = MJ/kg DM, f = g/kg LW; VI (total voluntary dry matter intake), STD (standard deviation)/group; ** - high significant (p<0.001) total VI between varieties CE/CV*

Due to the use of feeding troughs using the RIC system (Insentec B.V.) we found a very significant ($p< 0.001$) difference in the total dry matter voluntary intake between the two maize varieties: CE 18.27 g/kg L.W. and CV 17.51 g/kg LW in our used animal breed. Our findings are in agreement with Ndwiga *et al.* (1990), who investigated a similar issue in dairy heifers Holstein (290 kg LW). Compared to our results, they found a higher level of voluntary dry matter intake of maize silage near the value 20.00 g/kg L.W. pH 3.7. In our trial the pH was near 3.96 for CE and 3.98 for CV. Shaver *et al.* (1984) found dry matter intake for maize silage was near 22.0 g/kg L.W. by pH 3.72 in dairy heifers Holstein (260 kg LW). Fernandez *et al.* (2004) also found significant difference in the dry matter intake in evaluating maize varieties.

The assessed dry matter intake was not influenced by OMD (CE - 71.0 % and CV - 71.2 %). The slightly higher value of OMD in the variety CV is probably caused by the higher content of starch (see Table 1). The difference in the dry matter intakes between two maize varieties could be related to the fibre composition and its digestibility (DNDF: CE 38.2 % a CV 35.7 %), and further, partly to the slight difference in the dry matter value of two different silages.

Sommer *et al.* (1994) state that for a milk yield of 1 kg (4% fat) an intake of 3.13 MJ NEL from feeding ration is necessary The theoretical feeding value from our results on the NEL intake was calculated for a cow weighing 700 kg LW. according to intake of dry matter and NEL for the tested varieties. Total intake of energy was divided by a value of 3.13 MJ NEL. The energy

received by one cow fed by the CE variety CE was higher by a value that corresponds to the production of 0.76 kg of milk (4% fat). a mixed feeding ration (about 40% maize silage made from the variety CE) creates a higher milk production 0.25 kg of milk (4% fat), than maize silage from hybrid CV.

Conclusion

Our results show that differences in the dry matter intake of maize cultivated from different maize varieties and types can be expected. The differences in dry matter intake can exceed the total energy intake the higher energy concentration in varieties with lower values of voluntary intake. Knowledge of the voluntary intake of maize silage is an important indicator for optimal inclusion to the diets of dairy cows. Our results confirm that the inclusion of Ce maize silage in cattle diets is an advantage due to the higher voluntary dry matter intake and theoretic higher milk production (+0.25 kg) than by the maize silage from the variety CV, although the variety CV has the better energy value and the better starch digestibility.

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References

[1] Maize variants. (2010), Oseva Bzenec Inc.: www.oseva.com/maize/maize.html (displayed on 28th January, 2010)
[2] Fernandez I., Martin C., Champion M., Michelet-Doreau B. (2004): Effect of Corn Hybrid and Chop Length of Whole-Plant Corn Silage on Digestion and Intake by Dairy Cows. Journal of Dairy Science 87:1298-1309.
[3] Jarrige R. (1989): Ruminant nutrition-recommended allowances and feed tables. INRA, Paris, France, 389 pp.
[4] Ndwiga C.A., Erdman R.A., Vandersall J.H., Sharma B.K. (1990): Effect of Type and Site of Acid Neutralization on Voluntary Intake of Corn Silage by Dairy Heifers . Journal of Dairy Science 73:1571-1577.
[5] Pozdíšek J., Kohoutek A., Jakešová H., Nerušil P. and Schuplerová-Němcová P. (2001) Changes of the nutritive value in the chosen species and grass and clover varieties during vegetation. Výzkum v chovu skotu, 43, 2: 9-14.
[6] Shaver R.D., Erdman R.A., Vandersall J.H. (1984): Effect of Silage pH on Voluntary Intake of Corn Silage. Journal of Dairy Science 67: 2045-2049.
[7] Sommer A., Čerešňáková Z., Frydrych Z., Králík O., Králíková Z., Krása A., Pajtáš M., Petrikovič P., Pozdíšek J.,
[8] Šimek M., Třináctý J., Vencl B. (1994): Nutrient requirement and tabeles of nutrition value of feedstuffs for ruminants. Czech Academy of Agricultural Sciences, Commision of nutrition of farm animals. Pohořelice, CZ, 33 pp.
[9] Testing methods for feeding-stuffs. Common regulations. (1985) Vydavatelství úřadu pro normalizaci a měření, Praha, 102 pp.

Fungal Species Contaminating Silages In Belgium

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Abstract

In Belgium, silages often show fungal contamination. Since maize and grass silages are the major components of ruminant diets during winter, good nutritive and hygienic quality of these silages is of great importance. Growth of moulds can reduce feed intake and cause animal health problems due to mycotoxins, leading to severe economical losses. At the University College Ghent, this problem has been investigated from 2006 to 2009. During these years, 46 maize silos and 22 grass silos have been investigated. The most prevailing fungal species were Penicillium roqueforti and P. paneum. P. roqueforti was present in 72.73% of the investigated grass silages and in 63.04% of the maize silages, while P. paneum was found in 45.45% of the grass silages and 78.26% of the maize silages. Thus, the animal health risks associated with toxigenic moulds and mycotoxin production in silages should not be underestimated.

Introduction

In Belgium, silages are the most important component of ruminant diets during winter (Flemish Authority, dept. Agriculture). Good nutritive and hygienic quality of silages is thus extremely important (Driehuis and Oude Elferink, 2000). Mould contamination of silage leads to reduced feed intake (because of changes in color, texture and flavour of the silage) and moreover, some moulds have the ability to produce mycotoxins, which can harm animal health (Barug et al., 2006; Fink-Gremmels, 2008; Scudamore and Livesey, 1998).

Infection of silages with moulds is quite often observed at Belgian farms, and is in many cases due to suboptimal ensiling practices (Reboux et al., 2006). Therefore, this issue was assessed by the University College Ghent in two projects : a first project "Characterization of fungal species and mycotoxins contaminating silages in Belgium", financed by the Belgian Coordinated Collections of Micro-organisms (BCCM), was started in 2005 and ended in 2008; the second project "Identification and control of mould growth in conserved roughages", which started in 2006 and ended in 2009, was financed by the University College Ghent. From 2006 to 2009, 46 maize silos and 22 grass silos were sampled by the University College Ghent to determine which fungal species were found in both visually mouldy and healthy samples. The aim was to find out what caused mould infection by observing silage characteristics and silo management, and to assess the risk for animal health due to mycotoxins, based on correct identification of the fungal species present in silages.

Materials and Methods

Sampling and mould identification. On Belgian farms, silages contaminated with moulds were sampled during winter storage / feed-out period from 2006 to 2009. Visually uncontaminated as well as mouldy silage samples were collected. a questionnaire was filled in to collect information about crop rotation, silo type, ensiling practices, use of silage additives, rate of desiling, animal health, … Over the years, 46 maize silos and 22 grass silos were examined. Silage samples were kept at 4 °C for a maximum duration of 48 hours. For mould isolation, silage subsamples were placed on Potatoe Dextrose Agar in Petri dishes at equidistant points, kept in the dark for 2-14 days at 25°C, and the developing moulds were isolated (with exception of *Zygomycetes*). Moulds were

sub-cultured and stored for later identification. The isolates were identified at the MUCL and/or at the laboratory of phytopathology of the University College Ghent, using appropriate identification keys (Samson et al., 2004). *Penicillium* isolates were subjected to further validation.

Validation of morphological identification of *Penicillium* species. *During project "Characterization of fungal species and mycotoxins contaminating silages in Belgium".* To validate the morphological identification of *Penicillium* species, the DNA sequence from the partial beta-tubuline gene was sequenced for terverticillate *Penicillium* isolates at the MUCL (Declerck et al., 2009).

During project "Identification and control of mould growth in conserved roughages". At the University College Ghent, *P. roqueforti* and *P. paneum* were distinguished by Random Amplification of Polymorphic DNA (RAPD) (Boysen et al., 2000) and by macro-morphological techniques (O'Brien et al., 2008).

Results and Discussion

Table 1 summarizes the identification results of the moulds isolated from grass and maize silages from 2006 to 2009. In 2009, only 4 mouldy maize silages and no grass silages could be sampled due to the weather conditions.

The most prevalent fungal species in grass and maize silages belonged to the *P. roqueforti* group (Boysen et al., 2000), being *P. roqueforti* and *P. paneum*. The ratio of these two species varied over the years. Mould growth could often be attributed to suboptimal ensiling practices (too high DM content, insufficient density, delayed sealing, …).

Overall, *P. roqueforti* was the most frequently isolated fungal species in grass silages (72.73%). *P. paneum* was isolated from 45.45% of the grass silages. Both *P. roqueforti* and *P. paneum* were present in 36.36% of the grass silages. Other *Penicillium* species, *Trichoderma* species and *Monascus* species were also regularly isolated from grass silages.

Table 1: Fungal species found in grass and maize silages from 2006 to 2009 (isolated from ... % of the sampled silages).

ENSILED CROP	SAMPLING YEAR	*Penicillium roqueforti*	*Penicillium paneum*	*both P. roqueforti and P. paneum*	other *Penicillium sp*	*Trichoderma sp*	*Geotrichum sp*	*Monascus sp*	*Byssochlamys nivea*	*Ulocladium sp*	*Cladosporium sp*	*Verticillium sp*	*Fusarium sp*
GRASS	all (n : 22)	72.73	45.45	36.36	22.73	18.18	9.09	13.64	4.55	4.55	0.00	0.00	4.55
	2006 (n : 10)	50.00	50.00	30.00	40.00	20.00	0.00	0.00	0.00	0.00	0.00	0.00	10.00
	2007 (n : 5)	80.00	20.00	20.00	20.00	20.00	40.00	0.00	0.00	0.00	0.00	0.00	0.00
	2008 (n : 7)	100.00	57.14	57.14	0.00	14.29	0.00	42.86	14.90	14.29	0.00	0.00	0.00
MAIZE	all (n : 46)	63.04	78.26	43.48	36.96	21.74	8.70	6.52	2.17	0.00	2.17	2.17	2.17
	2006 (n : 21)	42.86	76.19	23.81	33.33	14.29	0.00	4.76	0.00	0.00	0.00	4.76	0.00
	2007 (n : 15)	80.00	80.00	60.00	60.00	46.67	20.00	6.67	0.00	0.00	0.00	0.00	6.67
	2008 (n : 6)	66.67	66.67	33.33	16.67	0.00	0.00	16.67	16.67	0.00	16.67	0.00	0.00
	2009 (n : 4)	100.00	100.00	100.00	0.00	0.00	25.00	0.00	0.00	0.00	0.00	0.00	0.00

In maize silages, *P. paneum* was overall more frequently isolated (78.26%) than *P. roqueforti* (63.04%). These fungal species were simultaneously present in 43.48% of the sampled maize silages. Also other *Penicillium* species and *Trichoderma* species were quite frequently found.

Conclussions

P. roqueforti and *P. paneum* were the most frequently isolated fungal species, which was not surprising since these species are acid tolerant and can grow at low oxygen levels. Various studies have detected these fungal species as the predominant moulds in different types of silages (Auerbach et al., 1998; Nout et al., 1993; O'Brien et al., 2005). These fungal species have the ability to produce mycotoxins (Samson et al., 2004), and mycotoxins were detected in both unmouldy and mouldy samples (Declerck et al., 2009). The fungal species, the level of mould contamination, nor the absence of visible moulds on silage can dictate the type or level of mycotoxin contamination (Scudamore and Livesey, 1998). Caution is thus required when mould contaminated silages are being fed.

References

[1] Auerbach H., Oldenburg E. and Weissbach F. (1998). Incidence of Penicillium roqueforti and roquefortine C in silages, Journal of the Science of Food and Agriculture, 76, 565-572.

[2] Barug D., Bhatnagar, D., van Egmond H.P., van der Kamp J.W., van Osenbruggen W.A and Visconti A. (2006). The mycotoxin factbook – food and feed topics, Wageningen Academic Publishers, Wageningen, 384 p.

[3] Boysen M. E., Jacobsson K. and Schnürer J. (2000). Molecular identification of species from the Penicillium roqueforti group associated with spoiled animal feed, Applied and Environmental Microbiology, p. 1523-1526.

[4] Declerck S., Van Hove F., Bastiaanse H., Pussemier L., Tangni E., Haesaert G., Daemers E., Depoorter J., Blust R., Robbens J. and Nobels I. (2009). Action for the promotion of and the co-operation with the Belgian Coordinated Collections of Micro-organisms, BCCM – Characterization of fungal species and mycotoxins contaminating silages in Belgium, final report, 140 p.

[5] Driehuis F. and Oude Elferink S. J. (2000). The impact of the quality of silage on animal health and food safety: a review, Veterinary Quarterly, 22, p. 212-216

[6] Fink-Gremmels J. (2008). Mycotoxins in cattle feeds and carry-over to dairy milk: a review, Food Additives and Contaminants, 25, 172-180.

[7] Nout M. J. R., Bouwmeester H. M., Haaksma J. and Van Dijk H. (1993). Fungal growth in silages of sugarbeet press pulp and maize, Journal of Agricultural Science, 121, 323-326.

[8] O'Brien M., O'Kiely P., Forristal P. D. and Fuller H. T. (2005). Fungi isolated from contaminated baled grass silage on farms in the Irish Midlands, FEMS Microbiology Letters, 247, 131-135.

[9] O'Brien M., Egan D., O'Kiely P., Forristal P.D., Doohan F.M. and Fuller H.T. (2008). Morphological and molecular characterisation of Penicillium roqueforti and P. paneum isolated from baled grass silage, Mycological research, 112, 921-932.

[10] Reboux G., Reiman M., Roussel S., Taattola K., Million L., Dalphin J.-C. and Piarroux R. (2006). Impact of agricultural practices on microbiology of hay, silage and flour on Finnish and French farms, Annals of Agricultural and Environmental Medicine, 13, 267-273.

[11] Samson R.A., Hoekstra E.S. and Frisvad J.C. (2004). Introduction to food- and airborne fungi – seventh edition, Centraalbureau voor schimmelcultures, Utrecht, 389 p.

[12] Scudamore K.A. and Livesey C.T. (1998). Occurrence and significance of mycotoxins in forage crops and silage: a review, Journal of the Science of Food and Agriculture, 77, 1-17.

EFFECT OF USTILAGO MAYDIS ON THE QUALITY OF MAIZE SILAGE FERMENTATION

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Introductions

Maize smut (*Ustilago maydis*) can create from June until the harvest time greyish bulging galls with sticky and badly smelling, later strongly dusting mass of *teliospores* which can infect the stands, seeds and soil. The occurrence of maize smut has not been for a long time described as a disease of the stand edges only as its occurrence has significantly increased in the last five years. Bochowiak a Skorupska (2006) state that the distinctive temperature changes in June 2006 were the main reason behind the incidence of maize smut in Poland. Epidemiological study has shown that 20 – 50% of the maize stand was infected during this period. Spores are capable to infect only the following year if ploughed into the soil they can safely survive for one year and they survive for at least three years on the soil surface and in the plants remnants. The infection presents itself especially in the places of mechanical damage or injury (ŘÍHA, 2006) but the factor of mechanically damaged maize tissue due to the unfavourable climate (drought, dampness) resp. the lack of moisture followed by rainfall also plays an important part. The reduction of smut incidence is achieved in places where there is a consistent pest control as well as limited movement of mechanization in the fully grown stand and thus the plant damage is limited. As a consequence, the infection is limited (ŘÍHA 2006). Several authors studied the influence of smut in maize regarding the quality of silage and nutritional value. Many studies have shown that silages made from the infected maize had low content of mycotoxins and silages did not have a harmful effect on the production and health of cattle. On the other hand, Richter et al. (1994) state that plants infected with maize smut had lower content of DM, lower content of nutrients and i*n sacco* degradability of DM and OM did not differ from healthy plants. However, sheep ate 28% less of DM from sick plants than from healthy plants.

Material and Methods

In the model experiment there was used ensilaged maize from healthy stand with DM of original weight 358.95 gkg^{-1} (A) and maize plants naturally infected with smut(*Ustilago maydis*) from the same land with the weight of DM below 300 g. kg^{-1} (B). Established were two experimental variants in three repetitions: Variant a – control silage, Variant B – experimental treated – by natural contamination with *Ustilago maydis*. Model silages were stored in the laboratory at average laboratory temperature of 25-27 ^{o}C for 180 days. Parameters assessed to establish the quality of the fermentation process after the 185 days were as follows: DM content of silage, pH, water extract acidity (KVV), amounts of lactic acid, acetic acid, sume of acids in DM and contents of ethanol. Analytical procedures were described in our earlier work (Doležal, 2002). Silages also underwent microbial analysis and the total amount of microorganisms (CPM), moulds and yeast-fungi were established.. Results were statistically processed by using the analysis of variance and differences between individual groups were analyzed by Scheffe-test in program STATISTICA 8. Data in the text are presented as average ± standard deviation.

Results and Discussion

From the outcome of the model experiment in which the influence of smut (*Ustilago maydis*) infection of maize on the quality of fermentation (Table 1) was studied, it is apparent that the course of fermentation was different due to the differing DM content of silage biomass. This manifested itself especially in different pH values, KVV and fermentation acids. The silage infected with maize smut had, in comparison with control silage, higher ($P<0.01$) concentration of fermentation acids in 1kg of DM (12,15 %) as compared to 9.37 % and that was reflected in the higher value of KVV (1744.3 mg KOH) as compared to 1498.3 mg KOH in 100 g of silage.The higher value of titrate acidity corresponded statistically lower ($P<0.05$) with the lower average pH value of silage.There was found a statistically different ($P<0.01$) lower average alcohol content (0.87 %) in comparison with the control silage (2.385 %).

In the experimental silage even considering the statistically lower ($P<0.01$) DM content (249.63 $g.kg^{-1}$) there was found lower average content of lactic acid and higher ($P<0.05$) average content of acetic acid (0.838 %) in comparison with control silage (2.388 %, resp. 0.692 %), which had a higher DM content (328.70 $g.kg^{-1}$). Richter et al. (1994) also states lower DM content in infected silage as opposed to the silage from healthy maize plants. The experimental infected silage in correlation with lower DM content discharged silage liquids in ratio of 36.93 l. t^{-1} of silage matter while the control silage due to the higher DM content did not discharge any liguids. This confirmed that the infection of maize stand with smut (*Ustilago maydis*) results in lower DM content ($P<0.01$) of infected plants.

Table 1: Average characteristics of model maize silage from healthy and sick plants of Ustilago maydis

Maize silage	DM (g/kg)	pH	KVV mg KOH	LA %	AA %	∑ acids in D M %	LA/AA	Ethanol %	Ammonia %
Healthy plants	328.70 ±19.738^{A}	3.865 ±0.04^{a}	1498.3 ±37.333	2.388 ±0.00	0.692 ±0.261^{b}	9.370 ±0.755^{B}	3.605 ±0.936	2.385 ±0.203^{A}	0.040 ±0.00
Sick plants	249.63 ±5.797^{B}	3.692 ±0.124^{b}	1744.3 ±253.8	2.195 ±0.219	0.838 ±0.084^{a}	12.250 ±1.39^{A}	2.628 ±0.237	0.87 ±0.511^{B}	0.035 ±0.005

KVV… water extract acidity; LA ... lactic acid; AA .... acetic acid; Variants in capitals differ (P<0.01); variants in lower case differ (P<0.05).

Table 2: Average content of micromycetes i maize silages (in 1 g)

Maize silage	TAM	LAB	Micromycetes			
			Total	Yeast fungi	Moulds in total	Geotrichum
A- Controle	34 063 636	57 100 000	46 182	45 955	227	0
B - Sick plants	54 500 000	63 600 000	11 272	11 227	45	0

TAM: total amount of microorganisms; LAB:. bacteria of lactic fermentation

It is obvious from the results as shown in Table 2 that the smut (*Ustilago maydis*) infection of maize plants also influenced the change in microbial composition of silages. It was confirmed that the silage prepared from the infected stand was diagnosed with lower mould content than in the control silage which corresponds with the results of Richter et al. (1994). The silage from infected plants also contained higher amount of lactic fermentation bacteria ($63.6*10^{6}$) in comparison with control silage ($57.1*10^{6}$). The silage from infected plants had significantly lower yeast fungi content (24.4% share) from the content in control silage. The silage from infected plants had significantly higher total amount of microorganisms (TAM) than the amount diagnosed in control silage. From

the above stated it is apparent that the infection of maize plants with smut (*Ustilago maydis*) leads to the overall increase in the amount of microorganisms but at the same time it does not lead to the increase in the amount of micromycetes. This hypothesis can be also supported by the lower DM content in infected plants which is more convenient for bacterial microflora.

Conclusion

The results of the experiment indicated that the used sick plants of maize of *Ustilago maydis* has different effect on the contents of lactic acid bacteria and the quality of fermentation process. There were found significant differences in the important fermentation characteristics of the observed model silages. The silage from sick plants of maize had non significantly lower LA content, lower pH value, worse ratio of LA:AA but overall higher ($P< 0.01$) content of acids in the dry matter of silage. Ethanol fermentation was significantly ($P<0.01$) reduced in silage from sick plants in comparison with control silage. There was found a significantly lower content of moulds and yeast fungi but on the other hand significantly higher total amount of microorganisms. There was higher fermentation loss (4.30 %) in the experimental silage from sick plants of maize in comparison with control silage (3.17 %).

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References

[1] BOCHOWIAK, A., SKORUPSKA, M, 2007: Common smut of maize – a problem for breeders and farmers. Ochrona rosłin, 52:1, 23-26.

[2] DOLEŽAL, P., 2002: Vliv přídavku Lactobacillus plantarum DSM 12771 na kvalitu siláží silně zavadlé vojtěšky a trávy (Effect of supplements of Lactobacillus plantarum DSM 12771 on the quality of ensiled alfalfa and grass with a high content of dry matter). Acta universitatis agriculturae et silvicultuarae Mendelianae Brunensis Brno, 5, p. 37–44.

[3] RICHTER, G. H., FLACHOWSKY, G., SCHNEIDER, A., WIRTH, R., SCHWARTZE, J., JAHREIS, G., 1994: Investigations about the influence of blister smut (Ustilago zeae) on feed value of maize for silage making. Wirtschaftseigene Futter, 40:2/3, 161-169.

EFFECT OF LIMESTONE SUPPLEMENTATION ON RUMINAL DEGRADABILITY OF FIBER FRACTIONS FOR DIFFERENT KINDS OF SILAGE

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Abstract

Four fresh crops included whole corn plant, corn stover, fodder corn (Darawa) and fodder sorghum were harvested, chopped and ground limestone was added to the different crops at the levels of 0.0, 0.5, 1.0, 1.5 and 2.0% of wet weight and ensiled in plastic buckets with about 2 kg capacity for two months. Representative samples were taken for determination fiber fractions. The rate of ruminal degradation of fiber fractions were determined using Friesian cows fitted with rumen cannulate. Results indicated that fresh and ensiled corn stover showed significantly ($P<0.05$) the highest contents of all fiber fractions followed by fodder sorghum, and fodder corn, while the whole corn plant had the lowest values. Moreover, the contents of all fiber fractions increased after ensiling and tended to decrease with increasing the level of limestone supplementation. Corn stover silage (CSS) recorded significantly ($P<0.05$) the highest values of NDF, ADF, hemicellulose and cellulose disappearance followed by fodder sorghum silage (FSS) and fodder corn silage (FCS), while whole plant corn silage (WCPS) had the lowest values. The wishing loss (zero time) of NDF, ADF, hemicellulose and cellulose of different silages decreased significantly ($P<0.05$) with increasing the level of limestone supplementation more than 1%. While, NDF, ADF, hemicellulose and cellulose disappearance at the incubation times from 6 to 72 hours increased significantly ($P<0.05$) with increasing the level of limestone supplementation up to 1% and decreased significantly ($P<0.05$) afterwards. Corn stover silage recorded significantly ($P<0.05$) the highest degradability fractions (a and b) and effective degradability and the lowest undegradable fraction (u) of fiber fractions followed by FSS, while WCPS had the opposite trend. The rapid degradability fraction (a) decreased significantly ($P<0.05$) with increasing the level of limestone supplementation. While, the potential degradability fraction (b), degradability rate (c) and the effective degradability of fiber fractions at outflow rates 2, 5 and 8%/hour increased significantly ($P<0.05$) with increasing the level of limestone supplementation up to 1% and decreased afterwards. However, the undegradable fraction (u) showed the opposite trend.

Keywords: Silage, limestone, fiber fractions, in situ disappearance.

Introduction

In Egypt, the total planted area of corn crop was about 1 million feddans, the area of corn crop used as a silage was 250 thousand feddans and 22.53 thousand feddans are cultivated with corn fodder (National Campaign of Corn Crop Rising, 2007). The area of corn fodder should be cultivated with yellow corn hybrids for using as the silage, which the yield of digestible nutrients per feddan for corn fodder is very low compared with yellow corn silage as well as the two crops stay in the land nearly similar period (Bendary et al., 2003) and will added 0.78 million tons TDN and 99.91 thousand tons DCP to the feed resources in Egypt (Abou-Slim and Bendary, 2005). Ensiling fresh corn stover material reduces field losses and may produce a more palatable feeding (Colenbrander et al., 1971). Moreover, it may offer a significant reduction cost and use of concentrate feed mixture in Egypt for lactating cows (Bendary and Younis, 1997) and lambs (Ghanem et al., 2000). Since the acids produced eventually stop the fermentation, it was found through research that the addition of ground limestone would neutralize these acids. This makes

fermentation last longer with more total acids being produced. The addition of one percent of pulverized limestone approximately doubles the lactic acid content of the resultant silage (Perry and Cecava, 1995). They also, indicated that the addition of limestone corrects the normal calcium deficiency of corn silage. Addition of limestone to a corn silage-concentrate diet increased plant cell wall digestibility by lactating dairy cows (Wheeler, 1980). Ha et al. (1983) indicated that lambs fed limestone supplementation increased ($P<0.05$) fiber digestibility. Froetschel et al. (1991) reported that limestone additives increased fiber digestibility of wheat silage-based rations fed to Holstein heifers. Kinal and Pres (1995) reported that addition of limestone to the control diet increased digestibility of fiber. Wagner et al. (2004) found that the limestone treatment had a significant effect on the fiber digestibility. The objective of the present study was to investigate the effect of limestone supplementation on ruminal degradation of fiber fractions of whole corn plant, corn stover, fodder corn and fodder sorghum silages.

Materials and Methods

The current work was carried out at Sakha Animal Production Research Laboratories, belonging to Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture in co-operation with Department of Animal Production, Faculty of Agriculture, Kafr El-Sheikh University.

Four fresh cereal crops included whole corn plant, corn stover, fodder corn (Darawa) and fodder sorghum were used to evaluate the effect of limestone supplementation during making silage on ruminal degradation of fiber fractions. Whole corn plant was harvested at dough stage of maturity, corn stover was taken after harvesting the ears immediately. While, fodder corn and fodder sorghum were taken after 50 days from planting. Forage crops were chopped using harvester chopper machine to 1.5-2 cm of length. Ground limestone was added to the different crops at the levels of 0.0, 0.5, 1.0, 1.5 and 2.0% of wet weight, ensiled in plastic bucket with about 2 kg of weight capacity, pressed by hand to exclude the air from the silos and sealed with paraffin wax. Treatments were run in triplicates (three of each). At the time of ensiling (zero time) and after ensiling for two months, representative samples were taken and dried in a forced air oven at 60 oC for 48 hours and ground.

Three multiparous Friesian cows were used for studying degradability of different silages. Ruminal degradability of fiber fractions determined by in situ nylon bag technique (Mehrez and Orskov, 1977). The results of fiber fractions disappearance were fitted to the following exponential models of Orskov and McDonald (1979) and the degradation was calculated by using the NAWAY computer programme with the following exponential model:

$$P = a + b\ (1\text{-}e\text{-}ct)$$

Where, P = percentage disappearance at time t. a = rapidly soluble fraction. b = slowly degradable fraction. a + b = potential degradability. c = fractional rate constant at which b will be degraded. t = time. u = undegradable fraction.

Effective degradability of fiber fractions were calculated from the rumen outflow rate (K) and the constants a, b, P and c from the above model. K was calculated on P 0.05 per h. The effective degradability of fiber fractions were calculated using the following formula:

$$Effective\ degradability = a + bH\ c/(c + k)\ H\ EXP\ [-\ (c + k)\ H\ T]\ p$$

Where, k is the estimated rate of out flow from the p rumen and T is the time.

Fiber constituents, neutral detergent fiber (NDF) was determined according to Van Soest and Marcus (1964). While, acid detergent fiber (ADF) and acid detergent lignin (ADL) was determined according to Van Soest (1963).

The data were subjected to statistical analysis using general linear models procedure adapted by SPSS for windows (2008) for user's guide with one-way ANOVA. Duncan test within program SPSS was done to determine the degree of significance between the means.

Results and Discussion

Fiber fractions:

Fiber fractions of the different kinds of fresh forages and silages supplemented with limestone are presented in Tables 1&2. Fresh and ensiled corn stover showed significantly (P<0.05) the highest contents of all fiber fractions followed by fodder sorghum and fodder corn, while the whole corn plant had the lowest values, this may be attributed to its grain content. Moreover, the contents of all fiber fractions increased after ensiling and tended to decrease with increasing the level of limestone supplementation. These results are in accordance with those obtained by Hemken et al. (1971) and Joanning et al. (1981) who indicated that increasing grain content led to diluted the fiber components of corn crop. Valdez et al. (1989) indicated that cell wall constituents increased with age till maturity of corn plant. Byers (1980) and Campos and Huber (1983) reported that fiber fractions contents of silage decreased with limestone supplementation.

Table 1: Fiber fractions contents (% DM basis) of different kinds of forages.

Item	WCP	CS	FC	FS	±SEM
NDF	50.45^{d}	69.50^{a}	57.30^{c}	63.20^{b}	1.72
ADF	29.70^{d}	41.65^{a}	33.80^{c}	38.40^{b}	1.48
ADL	4.15^{d}	7.10^{a}	4.86^{c}	5.90^{b}	0.25
Hemicellulose	20.75^{d}	27.85^{a}	23.50^{c}	24.80^{b}	0.74
Cellulose	25.55^{d}	34.55^{a}	28.94^{c}	32.50^{b}	0.86

a, b, c, d: Means in the same row with different superscripts differ significantly at 5% level.

Table 2: Effect of limestone supplementation on fiber fractions contents (% DM basis) of different kinds of silages.

Items	NDF	ADF	ADL	Hemicellulose	Cellulose
Kind of silage					
WCPS	52.02^{d}	30.64^{d}	4.31^{d}	21.38^{d}	26.33^{d}
CSS	71.36^{a}	42.84^{a}	7.29^{a}	28.52^{a}	35.55^{a}
FCS	58.85^{c}	34.85^{c}	5.05^{c}	24.00^{c}	29.80^{c}
FSS	64.93^{b}	39.56^{b}	6.11^{b}	25.37^{b}	33.45^{b}
±SEM	1.64	1.06	0.26	0.59	0.81
Limestone %					
0.0	61.99	37.11	5.76	24.88	31.35
0.5	61.89	37.04	5.73	24.85	31.31
1.0	61.79	36.97	5.69	24.82	31.28
1.5	61.69	36.91	5.66	24.78	31.25
2.0	61.59	36.84	5.62	24.75	31.22
±SEM	1.64	1.06	0.26	0.59	0.81

a, b, c, d: Means in the same column with different superscripts differ significantly at 5% level.

NDF disappearance:

The disappearance of NDF of different silages is shown in Table 3. The results showed that corn stover silage (CSS) recorded significantly (P<0.05) the highest values followed by fodder sorghum silage (FSS) and fodder corn silage (FCS), while the whole plant corn silage (WCPS) had the lowest values. The wishing loss (zero time) of NDF of different silages decreased significantly (P<0.05) with increasing the level of limestone supplementation more than 1%. While, NDF disappearance of different silages at the different incubation times from 6 to 72 hours increased significantly (P<0.05) with increasing the level of limestone supplementation up to 1% and decreased significantly afterwards. Disappearance of NDF for the different silages increased with increasing its content, indicating a high positive correlation between them (r = 0.96). These results are in accordance with those obtained by El Tayeb et al. (1984) who found that ruminal neutral

detergent fiber (NDF) digestion was higher with the 1.5 than the .6 and 3.0% limestone diets. Wagner et al. (2004) found that the limestone treatment had a significant effect on the fiber digestibility.

Table 3: Effect of limestone supplementation on in situ NDF disappearance (%) of different kinds of silages.

Items	Incubation time (hours)					
	0	6	12	24	48	72
Kind of silage						
WCPS	3.07^{d}	9.31^{d}	14.04^{d}	21.68^{d}	31.73^{d}	37.41^{d}
CSS	4.54^{a}	13.43^{a}	20.43^{a}	31.77^{a}	46.69^{a}	55.11^{a}
FCS	3.28^{c}	9.92^{c}	14.97^{c}	23.15^{c}	33.91^{c}	40.00^{c}
FSS	3.89^{b}	11.62^{b}	17.63^{b}	27.41^{b}	40.14^{b}	47.28^{b}
±SEM	0.08	0.24	0.36	0.54	0.79	0.93
Limestone %						
0.0	3.75^{a}	10.07^{b}	15.55^{b}	24.46^{b}	36.27^{b}	43.00^{b}
0.5	3.75^{a}	11.34^{ab}	17.06^{ab}	26.32^{ab}	38.51^{ab}	45.32^{ab}
1.0	3.74^{a}	12.62^{a}	18.56^{a}	28.26^{a}	40.77^{a}	47.84^{a}
1.5	3.66^{ab}	11.32^{ab}	17.09^{ab}	26.41^{ab}	38.64^{ab}	45.52^{ab}
2.0	3.58^{b}	10.01^{b}	15.57^{b}	24.57^{b}	36.40^{b}	43.07^{b}
±SEM	0.08	0.24	0.36	0.54	0.79	0.93

a, b, c, d: Means in the same column with different superscripts differ significantly at 5% level.

Table 4: Effect of limestone supplementation on degradation fractions and effective degradability (%) of NDF for different kinds of silages.

Items	Degradation fractions				Effective degradability		
	a	b	c	u	K=0.02	K=0.05	K0.08
Kind of silage							
WCPS	3.05^{d}	41.72^{d}	0.0238	55.23^{a}	26.11^{d}	17.06^{d}	13.25^{d}
CSS	4.51^{a}	61.51^{a}	0.0238	33.98^{d}	38.33^{a}	24.92^{a}	19.27^{a}
FCS	3.26^{c}	44.65^{c}	0.0238	52.09^{b}	27.89^{c}	18.19^{c}	14.13^{c}
FSS	3.87^{b}	52.82^{b}	0.0238	43.32^{c}	32.96^{b}	21.48^{b}	16.63^{b}
±SEM	0.08	1.03	0.0002	1.10	0.65	0.43	0.34
Limestone %							
0.0	3.79^{a}	48.08^{b}	0.0234^{b}	48.13^{a}	29.71^{b}	19.11^{b}	14.66^{b}
0.5	3.73^{ab}	50.71^{ab}	0.0238^{ab}	45.56^{ab}	31.69^{ab}	20.73^{ab}	16.10^{ab}
1.0	3.67^{ab}	53.22^{a}	0.0241^{a}	43.11^{b}	33.67^{a}	22.35^{a}	17.56^{a}
1.5	3.61^{ab}	50.74^{ab}	0.0240^{ab}	45.65^{ab}	31.77^{ab}	20.77^{ab}	16.13^{ab}
2.0	3.55^{b}	48.14^{b}	0.0239^{ab}	48.32^{a}	29.76^{b}	19.12^{b}	14.65^{b}
±SEM	0.08	1.03	0.0002	1.10	0.65	0.43	0.34

a, b, c, d: Means in the same column with different superscripts differ significantly at 5% level.

The degradability fractions and outflow rates of NDF of different silages kind are presented in Table 4. CSS recorded significantly (P<0.05) the highest degradability fractions (*a*&*b*) and effective degradability and the lowest undegradable fraction (*u*) followed by FSS and FCS, while WCPS had the opposite trend. This may be due to the NDF content of different silages (Table 2). The rapid degradability fraction (*a*) decreased significantly (P<0.05) with increasing the level of limestone supplementation. While, the potential degradability fraction (*b*) and degradability rate (*c*) increased significantly (P<0.05) with increasing the level of limestone supplementation up to 1% and decreased afterwards. However, the undegradable fraction (*u*) showed the opposite trend. On the other hand, the effective NDF degradability at outflow rates 2, 5 and 8%/hour increased

significantly (P<0.05) with increasing the level of limestone supplementation up to 1% and decreased afterwards. These results are in agreement with those obtained by Froetschel *et al.* (1991), Kinal and Pres (1995), Resende *et al.* (2003) and Wagner *et al.* (2004).

ADF disappearance:

The ADF disappearance of different silages are shown in Table 5. CSS recorded significantly (P<0.05) the highest values followed by FSS and FCS, while WCPS had the lowest values. The wishing loss (zero time) of ADF of different silages decreased significantly (P<0.05) with increasing the level of limestone supplementation more than 1%. While, ADF disappearance of different silages at the different incubation times from 6 to 72 hours increased significantly (P<0.05) with increasing the level of limestone supplementation up to 1% and decreased significantly afterwards. The disappearance of ADF for the different silages increased with increasing its content, indicating a high positive correlation between them (r = 0.95). These results are in accordance with those obtained by El Tayeb et al. (1984) who found that ruminal acid detergent fiber (ADF) digestion was higher with the 1.5 than the .6 and 3.0% limestone diets. Wagner et al. (2004) found that the limestone treatment had a significant effect on the fiber digestibility.

Table 5: Effect of limestone supplementation on in situ ADF disappearance (%) of different kinds of silages.

Items	Incubation time (hours)					
	0	6	12	24	48	72
Kind of silage						
WCPS	2.45^{d}	7.61^{d}	11.38^{d}	17.48^{d}	25.57^{d}	30.10^{d}
CSS	3.68^{a}	11.03^{a}	16.71^{a}	25.90^{a}	38.06^{a}	44.82^{a}
FCS	2.86^{c}	8.78^{c}	13.19^{c}	20.39^{c}	29.82^{c}	35.14^{c}
FSS	3.34^{b}	10.11^{b}	15.27^{b}	23.63^{b}	34.63^{b}	40.84^{b}
±SEM	0.06	0.21	0.30	0.45	0.65	0.76
Limestone %						
0.0	3.15	8.40^{b}	12.96^{b}	20.38^{b}	30.21^{b}	35.88^{b}
0.5	3.13	9.65^{ab}	14.43^{ab}	22.18^{ab}	32.38^{ab}	38.15^{ab}
1.0	3.12	10.91^{a}	15.88^{a}	23.99^{a}	34.64^{a}	40.51^{a}
1.5	3.05	9.62^{ab}	14.45^{ab}	22.25^{ab}	32.57^{ab}	38.23^{ab}
2.0	2.98	8.33^{b}	12.96^{b}	20.46^{b}	30.31^{b}	35.86^{b}
±SEM	0.06	0.21	0.30	0.45	0.65	0.76

a, b, c, d: Means in the same column with different superscripts differ significantly at 5% level.

The degradability fractions and outflow rates of ADF of different silages are presented in Table 6. CSS recorded significantly (P<0.05) the highest degradability fractions (*a*&*b*) and effective degradability and the lowest undegradable fraction (*u*) followed by FSS and FCS, while WCPS had the opposite trend. This may be due to the ADF content of different silages (Table 2). The rapid degradability fraction (*a*) decreased significantly (P<0.05) with increasing the level of limestone supplementation. While, the potential degradability fraction (*b*) and degradability rate (*c*) increased significantly (P<0.05) with increasing the level of limestone supplementation up to 1% and decreased afterwards. However, the undegradable fraction (*u*) showed the opposite trend. On the other hand, the effective ADF degradability at outflow rates 2, 5 and 8%/hour increased significantly (P<0.05) with increasing the level of limestone supplementation up to 1% and decreased afterwards. These results are in agreement with those obtained by Froetschel *et al.* (1991), Kinal and Pres (1995), Resende *et al.* (2003) and Wagner *et al.* (2004).

Table 6: Effect of limestone supplementation on degradation fractions and effective degradability (%) of ADF for different kinds of silages.

Items	Degradation fractions				Effective degradability		
	a	b	c	u	K=0.02	K=0.05	K0.08
Kind of silage							
WCPS	2.44^{d}	33.50^{d}	0.0238	64.06^{a}	21.01^{d}	13.80^{d}	10.74^{d}
CSS	3.66^{a}	50.21^{a}	0.0239	46.13^{d}	31.22^{a}	20.36^{a}	15.77^{a}
FCS	2.84^{c}	39.19^{c}	0.0239	57.97^{b}	24.53^{c}	16.05^{c}	12.48^{c}
FSS	3.32^{b}	45.55^{b}	0.0238	51.13^{c}	28.46^{b}	18.59^{b}	14.41^{b}
±SEM	0.06	0.86	0.0003	0.91	0.54	0.36	0.29
Limestone %							
0.0	3.18^{a}	40.28^{b}	0.0234^{c}	56.54^{a}	24.77^{b}	15.93^{b}	12.21^{b}
0.5	3.12^{ab}	42.53^{ab}	0.0238^{b}	54.35^{ab}	26.67^{ab}	17.51^{ab}	13.64^{ab}
1.0	3.07^{ab}	45.02^{a}	0.0241^{a}	51.92^{b}	28.58^{a}	19.11^{a}	15.07^{a}
1.5	3.00^{ab}	42.59^{ab}	0.0240^{ab}	54.41^{ab}	26.72^{ab}	17.53^{ab}	13.64^{ab}
2.0	2.95^{b}	40.15^{b}	0.0239^{ab}	56.90^{a}	24.80^{b}	15.93^{b}	12.20^{b}
±SEM	0.06	0.86	0.0003	0.91	0.54	0.36	0.29

a, b, c, d: Means in the same column with different superscripts differ significantly at 5% level.

Hemicellulose disappearance:

Hemicellulose disappearance of different silages are shown in Table 7. CSS recorded significantly (P<0.05) the highest values followed by FSS and FCS, while WCPS had the lowest values. The wishing loss (zero time) of hemicellulose of different silages decreased significantly (P<0.05) with increasing the level of limestone supplementation more than 1%. While, hemicellulose disappearance of different silages at the different incubation times from 6 to 72 hours increased significantly (P<0.05) with increasing the level of limestone supplementation up to 1% and decreased significantly afterwards. The disappearance of hemicellulose for the different silages increased with increasing its content, indicating a high positive correlation between them (r = 0.95). These results are in accordance with those obtained by El Tayeb et al. (1984) who found that hemicellulose digestion was higher with the 1.5 than the .6 and 3.0% limestone diets. Wagner et al. (2004) found that the limestone treatment had a significant effect on the fiber digestibility.

Table 7: Effect of limestone supplementation on in situ hemicellulose disappearance (%) of different kinds of silages.

Items	Incubation time (hours)					
	0	6	12	24	48	72
Kind of silage						
WCPS	3.28^{c}	9.91^{c}	14.97^{c}	23.15^{c}	33.91^{c}	39.99^{c}
CSS	5.63^{a}	16.50^{a}	25.22^{a}	39.42^{a}	57.88^{a}	68.33^{a}
FCS	3.90^{b}	11.63^{b}	17.63^{b}	27.34^{b}	40.12^{b}	47.34^{b}
FSS	4.14^{a}	12.33^{b}	18.73^{b}	29.07^{b}	42.65^{b}	49.75^{b}
±SEM	0.11	0.34	0.51	0.80	1.17	1.38
Limestone %						
0.0	4.30	11.59^{b}	17.93^{b}	28.37^{b}	41.80^{b}	48.82^{b}
0.5	4.29	12.86^{ab}	19.43^{ab}	30.06^{ab}	44.06^{ab}	52.00^{ab}
1.0	4.28	14.13^{a}	20.91^{a}	31.91^{a}	46.26^{a}	54.34^{a}
1.5	4.22	12.85^{ab}	19.45^{ab}	30.14^{ab}	44.15^{ab}	52.04^{ab}
2.0	4.11	11.53^{b}	17.96^{b}	28.27^{b}	41.94^{b}	49.58^{b}
±SEM	0.11	0.34	0.51	0.80	1.17	1.38

a, b, c: Means in the same column with different superscripts differ significantly at 5% level.

The degradability fractions and outflow rates of hemicellulose of different silages are presented in Table 8. CSS silage recorded significantly (P<0.05) the highest degradability fractions (*a*&*b*) and effective degradability and the lowest undegradable fraction (*u*) followed by FSS and FCS, while WCPS had the opposite trend. This may be due to the hemicellulose content of different silages (Table 2). The rapid degradability fraction (*a*) decreased significantly (P<0.05) with increasing the level of limestone supplementation. The potential degradability fraction (*b*) and degradability rate (*c*) increased significantly (P<0.05) with increasing the level of limestone supplementation up to 1% and decreased afterwards. However, the undegradable fraction (*u*) showed the opposite trend. On the other hand, the effective hemicellulose degradability at outflow rates 2, 5 and 8%/hour increased significantly (P<0.05) with increasing the level of limestone supplementation up to 1% and decreased afterwards. These results are in agreement with those obtained by Froetschel *et al*. (1991), Kinal and Pres (1995), Resende *et al.* (2003) and Wagner *et al.* (2004).

Table 8: Effect of limestone supplementation on degradation fractions and effective degradability (%) of hemicellulose for different kinds of silages.

Items	Degradation fractions				Effective degradability		
	a	b	c	u	K=0.02	K=0.05	K0.08
Kind of silage							
WCPS	4.12^{d}	56.04^{d}	0.0239	39.84^{a}	35.03^{d}	22.81^{d}	17.65^{d}
CSS	5.60^{a}	76.26^{a}	0.0239	18.14^{d}	47.48^{a}	30.79^{a}	23.77^{a}
FCS	4.70^{c}	64.07^{c}	0.0238	31.23^{b}	39.92^{c}	26.17^{c}	20.04^{c}
FSS	4.98^{b}	67.80^{b}	0.0238	27.22^{c}	42.22^{b}	27.42^{b}	21.18^{b}
±SEM	0.07	0.98	0.0002	1.05	0.61	0.41	0.32
Limestone %							
0.0	4.96^{a}	64.07^{b}	0.0236^{c}	30.97^{a}	39.60^{b}	25.46^{b}	19.52^{b}
0.5	4.91^{ab}	66.58^{ab}	0.0238^{b}	28.52^{ab}	41.55^{ab}	27.07^{ab}	20.95^{ab}
1.0	4.85^{ab}	69.04^{a}	0.0240^{ab}	26.12^{b}	43.48^{a}	28.91^{a}	22.37^{a}
1.5	4.80^{ab}	66.63^{ab}	0.0239^{ab}	28.57^{ab}	41.57^{ab}	27.07^{ab}	20.96^{ab}
2.0	4.74^{b}	63.89^{b}	0.0241^{a}	31.37^{a}	39.62^{b}	25.49^{b}	19.51^{b}
±SEM	0.07	0.98	0.0002	1.05	0.61	0.41	0.32

a, b, c, d: Means in the same column with different superscripts differ significantly at 5% level.

Cellulose disappearance:

Cellulose disappearance of different silages are shown in Table 9. CSS recorded significantly (P<0.05) the highest values followed by FSS and FCS, while WCPS had the lowest values. The wishing loss (zero time) of cellulose of different silages decreased significantly (P<0.05) with increasing the level of limestone supplementation more than 1%. While, cellulose disappearance of different silages at the different incubation times from 6 to 72 hours increased significantly (P<0.05) with increasing the level of limestone supplementation up to 1% and decreased significantly afterwards. The disappearance of cellulose for the different silages increased with increasing its content, indicating a high positive correlation between them (r = 0.95). These results are in accordance with those obtained by El Tayeb et al. (1984) who found that cellulose digestion was higher with the 1.5 than the .6 and 3.0% limestone diets. Wagner et al. (2004) found that the limestone treatment had a significant effect on the fiber digestibility.

The degradability fractions and outflow rates of cellulose of different silages are presented in Table 10. CSS recorded significantly (P<0.05) the highest degradability fractions (*a*&*b*) and effective degradability and the lowest undegradable fraction (*u*) followed by FSS and FCS, while WCPS had the opposite trend. This may be due to the cellulose content of different silages (Table 2). The rapid degradability fraction (*a*) decreased significantly (P<0.05) with increasing the level of limestone supplementation. While, the potential degradability fraction (*b*) and degradability

rate (*c*) increased significantly (P<0.05) with increasing the level of limestone supplementation up to 1% and decreased afterwards. However, the undegradable fraction (*u*) showed the opposite trend. On the other hand, the effective cellulose degradability at outflow rates 2, 5 and 8%/hour increased significantly (P<0.05) with increasing the level of limestone supplementation up to 1% and decreased afterwards. These results are in agreement with those obtained by Froetschel *et al.* (1991), Kinal and Pres (1995), Resende *et al.* (2003) and Wagner *et al.* (2004).

Table 9: Effect of limestone supplementation on in situ cellulose disappearance (%) of different kinds of silages.

Items	Incubation time (hours)					
	0	6	12	24	48	72
Kind of silage						
WCPS	3.07^{d}	9.34^{d}	14.09^{d}	21.76^{d}	31.85^{d}	37.55^{d}
CSS	4.20^{a}	12.46^{a}	18.88^{a}	29.40^{a}	43.18^{a}	50.95^{a}
FCS	3.50^{c}	10.52^{c}	15.91^{c}	24.64^{c}	36.05^{c}	42.60^{c}
FSS	3.93^{b}	11.74^{b}	17.82^{b}	27.65^{b}	40.59^{b}	47.82^{b}
±SEM	0.06	0.20	0.28	0.42	0.60	0.70
Limestone %						
0.0	3.73^{a}	10.05^{b}	15.47^{b}	24.44^{b}	36.22^{b}	42.84^{b}
0.5	3.72^{a}	11.28^{ab}	16.97^{ab}	26.19^{ab}	38.32^{ab}	45.18^{ab}
1.0	3.71^{a}	12.53^{a}	18.43^{a}	27.96^{a}	40.46^{a}	47.48^{a}
1.5	3.65^{ab}	11.26^{ab}	16.98^{ab}	26.24^{ab}	38.31^{ab}	45.22^{ab}
2.0	3.58^{b}	9.97^{b}	15.52^{b}	24.49^{b}	36.28^{b}	42.93^{b}
±SEM	0.06	0.20	0.28	0.42	0.60	0.70

a, b, c: Means in the same column with different superscripts differ significantly at 5% level.

Table 10: Effect of limestone supplementation on degradation fractions and effective degradability (%) of cellulose for different kinds of silages.

Items	Degradation fractions				Effective degradability		
	a	b	c	u	K=0.02	K=0.05	K0.08
Kind of silage							
WCPS	3.06^{d}	41.88^{d}	0.0238	55.06^{a}	26.20^{d}	17.12^{d}	13.29^{d}
CSS	4.17^{a}	56.86^{a}	0.0238	38.97^{d}	35.45^{a}	23.08^{a}	17.85^{a}
FCS	3.48^{c}	47.53^{c}	0.0239	49.00^{b}	29.70^{c}	19.37^{c}	15.03^{c}
FSS	3.91^{b}	53.44^{b}	0.0239	42.65^{c}	33.35^{b}	21.71^{b}	16.82^{b}
±SEM	0.06	0.78	0.0002	0.83	0.49	0.33	0.26
Limestone %							
0.0	3.76^{a}	48.02^{b}	0.0235^{b}	48.22^{a}	29.69^{b}	19.08^{b}	14.64^{b}
0.5	3.71^{ab}	50.39^{ab}	0.0238^{ab}	45.90^{ab}	31.53^{ab}	20.63^{ab}	16.04^{ab}
1.0	3.66^{ab}	52.81^{a}	0.0241^{a}	43.53^{b}	33.42^{a}	22.19^{a}	17.44^{a}
1.5	3.60^{ab}	50.42^{ab}	0.0240^{ab}	45.98^{ab}	31.58^{ab}	20.63^{ab}	16.02^{ab}
2.0	3.55^{b}	47.99^{b}	0.0239^{ab}	48.46^{a}	29.67^{b}	19.07^{b}	14.60^{b}
±SEM	0.06	0.78	0.0002	0.83	0.49	0.33	0.26

a, b, c, d: Means in the same column with different superscripts differ significantly at 5% level.

Conclusion

From these results it could be concluded that the addition of limestone at the level of 1% of wet weight at making of silage improved the fiber fractions disappearance.

References

[1] Abou-Slim, I.A. and M.M. Bendary (2005). Feedstuff resources in Egypt. Sources and maximization of its utilization. Animal Production Research Institute, Second Conference & Regional Symposium on Buffalo Production, 27-29 September, Sakha, Kafr El-Sheikh, Egypt. P. 269. (In Arabic).

[2] Agricultural Economics (2003). Central Administration, Agricultural Ecnomics. Summer and Nili Crops. Economic Affairs Sector, Ministry of Agric., ARE.

[3] Bendary, M.M. and M.A. Younis (1997). Evaluation of maize stalks for feeding dairy cows. Egyptian J. Appl. Sci., 12: 11.

[4] Bendary, M.M., F.E. El-Keraby and I.A. Abou-Selim (2003). Corn silage with or without ear as effictive mean for growing fodder resources in Egypt. Egyptian J. Nutr. and Feeds (Special Issue) 6: 1399 (In Arabic).

[5] Campos, O.F. and J.T. Huber (1983). Performance and digestion by calves from limestone added to milk replacers containing soy protein concentrate. J. Dairy Sci., 66: 2365.

[6] Colenbrander, V.F.; L.D. Muller; J.A. Wasson and M.D. Cunningham (1971). Effect of added urea and ammonium polyphosphate to corn stover silages on animal performance. J. Anim. Sci., 33: 1091.

[7] El Tayeb, A.E.; M.L. Galyean and H.E. Kiesling (1984). Influence of limestone level in high concentrate and high roughage diets on site and extent of digestion in lambs. J. Anim. Sci., 59: 217.

[8] Froetschel, M.A.; L.O. Ely and H.E. Amos (1991). Effects of additives and growth environment on preservation and digestibility of wheat silage fed to Holstein heifers. J. Dairy Sci., 74: 546.

[9] Ghanem, G.H.A.; E.A. Amer and F.A. El-Zeer (2000). Evaluation of using maize stover silage by sheep. J. Agric. Res. Tanta Univ., 26: 591.

[10] Ha, J.K.; R.J. Emerick and L.B. Embry (1983). In vitro effect of pH variations on rumen fermentation and in vivo effects of buffers in lambs before and after adaptation to high concentrate diets. J. Anim. Sci., 56: 698.

[11] Hemken, R.W.; N.A. Clark; H.K. Goering and J.H. Vandersall (1971). Nutritive value of corn silage as influenced by grain content. J. Dairy Sci., 54: 383.

[12] Joanning, S.W.; D.E. Johnson and B.P. Barry (1981). Nutrient digestibility depression in corn silage-corn grain mixtures fed to steers. J. Anim. Sci., 53: 1095.

[13] Kinal S. and J. Pres (1995). The effect of dolomite, limestone and zinc oxide as feed additives on utilization of organic matter and minerals by young fattening cattle. J. Anim. And Feed Sci., 4: 183.

[14] Mehrez, A.Z. and E.R. Orskov (1977). a study of the artificial fibre bag technique for determining the digestibility of feeds in the rumen. J. Agri. Sci. Camb., 88: 546.

[15] National Campaign of Corn Crop Rising (2007). Annual Report. National Program of Corn Research, Agricultural Research Center, Ministry of Agriculture & Food, Agriculture and Irrigation Conouncil, Academy of Scienntific Research and Technology, Egypt.

[16] Ørskov, E.R. and P. McDonald (1979). The estimate of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agric. Sci. Camb., 92: 499.

[17] Perry, T.W. and M.J. Cecava (1995). Beef cattle feeding and nutrition. 2nd Ed., Academic Press, INC, USA.

[18] Resende, J.A.; M.N. Pereira; R.G. Von Pinho; A.H. Fonseca and A.R. Pereira da Silva (2003). Ruminal silage degradability and productivity of forage and grain-type sorghum cultivars. J. Scientia Agricola., 60:457-363.

[19] SPSS for windows (2004). Statistical package for the social sciences, Release 13, SPSS INC, Chicago, USA.

[20] Valdez, F.R.; J.H. Harrison and S.C. Fransen (1989). Effect of feeding silage of early and late maturing corn planted at two population densities to lactating dairy cows. J. Dairy Sci., 72: 2081.
[21] Van Soest, P.J. (1963). Use of detergents in the analysis of fibrous feeds. II- a rapid method for the determination of fiber and lignin. AOAC, 46: 830.
[22] Van Soest, P.J. and W.C. Marcus (1964). a method for the determination of cell wall constituents in forages using detergents and the relationship between this fraction and voluntary intake and digestibility. J. Dairy Sci., 47: 704.
[23] Wagner, E.; I. Schwendenwein and J. Zentek (2004). Effects of a dietary chitosan and calcium supplement on Ca and P metabolism in cats. Berl. Munch. Tierarztl Wochenschr, 117: 310.
[24] Wheeler, W.E. (1980). Effect of limestone buffers on digestibility of complete diets and on performance by dairy cows. J. Dairy Sci., 63: 1848.

The Influence of Additives for Qualitative Parameters of Silage Feeds

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Introduction

Current aspects of silage quality assessment and use of grass silage additives described Richardt (2007). Essentials and principles of silage treatment described Pahlow (2007). Silage additives have an ability of improve the fermentative process in the present of nutrient losses minimalization during conservation. Addition of lactic fermentation bacteria caused on file of observed silages the increase in lactic acid and pH reduction (Hejduk and Doležal, 2004). Biological additives caused faster pH decrease compared with a control silage without additives, especially in inoculates with LAB or with an addition of saccharolytic enzymes (Woolford, 2004). It conduce to the rapid decline of coliform bacteria, higher pH decrease intensity and to the proteolysis finish. In the silages preserved by means of the biological additives, there is the fermentative process faster finished and it is possible to feeding them earlier than the silages without preserving agents and chemical additives.

Materiál and Methods

In the experimental observation there was 42 samples of clover-grass silages from the working conditions in a piedmont area. Samples were divided into three groups. There was 14 silage samples from the first cutting on every group. In the first group there were silages without preserving agents, the second group presented silages with bacterial preparation and in the third group there was used bacterial - enzymatic preparation. Nutrient analyse, acids content in silage, pH, degree of proteolysis was determined by ÚKZUZ methods.

We tested six kinds of silage supplements in clover-grass silages – bacterial - enzymatic additives GOLDZYM, BACTOZYM , FEEDTECH F 3000; bacterial additives MICROSIL, SILLA-BAC, SILL-ALL 4x4.

Nevertheless, most of the cited papers dealt with the testing of biological additives in laboratory-scale experiments.

The aim of this work was evaluate the effect of bacterial and bacterial enzymatic additives for qualitative parameters of silage from die back grass stands under farm-scale conditions. Moreover, the effect of inoculants on fibre composition was evaluated.

Results and Discussion

Table No. 1 shows the results of statistical examination of fermentative characteristics, lactic acid, acetic acid, pH values, degree of proteolysis and nutritive value characteristics NDF, NEL, CP. Statistically evidential differences in lactic acid content between the control group and the group with bacterial additive ($P<0,05$) and bacterial - enzymatic additive ($P<0,05$) was found. Other fermentative and nutritive value characteristics (NDF, NEL and CP) in silages, where the bacterial and bacterial - enzymatic additive was used are statistically nonsignificant. Woolford (2004) described, that the biological additives caused faster pH decline compared with the control silage without additives especially in inoculants with LAB alone or with saccharolytical enzymes addition. It conduce to the fast coliform bacteria decline, higher intensity of pH decline and to the proteolysis stopping.

Table 1: Mean values of fermentation characteristics (n = 14)

	Control group without additive	Bacterial additive	Bacterial - enzymatic additive
Milk acid g.kg $^{-1}$ DM	65,24 a	79,80 b	85,90 b
Acetic acic g.kg^{-1} DM	22,40 a	26,70 a	21,80 a
Butyric acid g.kg^{-1} DM	1,3 a	1,7 a	0,8 a
pH	4,51 a	4,37 a	4,30 a
Degree of proteolysis %	10,03 a	8,4 a	8,0 a
CP g.kg^{-1} DM	154,1 a	148,7 a	157,5 a
NDF g.kg^{-1} DM	462,3 a	459,2 a	452,7 a
NEL MJ.kg^{-1} DM	5,34 a	5,42 a	5,47 a
Dry matter g.kg^{-1}	344,5 a	333,1 a	329,4 a

a,b, means with the others indexes, significant difference in rows (P<0,05)

Conclusion

It was made evaluation of fermentative characteristics at choice clover-grass silages. The clover-grass silages were separated into three groups. First group was without preservation additives, second group with bacterial additive and third group with bacterial fermentative additive. It was discovered statistical significant difference at milk acid content between control group and group with bacterial fermentative additive (P<0,05) and bacterial - enzymatic additive (P<0,05) was found. The silages with additive had higher fermentative quality in all cases. It was discovered trend to reduction of NDF values with maintainance of energy content. Fermentation parameters did not differ significantly between the variants treated with LAB inoculants or with LAB + enzymatic additives.

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References

[1] Hejduk, S. and Doležal, P. (2004). Nutritive value of broad-leaved dock (Rumex obtusifolium L.) and its effect on the guality of grass silages. Czech Journal of Animal Science, 49, 144-150.
[2] Pahlow, G. (2007). Basics and principles of ensiling. Ubesichten zur Tierernahrung, 35 (1), 1-11.
[3] Richardt, W. and Hoffman, M. (2007). Zuchtwahl und Besamung. (157), 30-32.
[4] Woolford, M. K. (2004). Silage inoculants and their live. Alltech´s 18th European, Middle East and African lecture tour, 53-70.

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