16th International Symposium
FORAGE CONSERVATION

Brno, Czech Republic, June 3-6, 2014

Conference Proceedings

NutriVet Ltd., Pohořelice, CZ
Mendel University in Brno, CZ
National Agricultural and Food Centre – RIAP Nitra, SK
Institute of Animal Science, Prague - Uhřiněves, CZ
Crop Research Institute, Prague - Ruzyně, CZ

16th INTERNATIONAL SYMPOSIUM

FORAGE CONSERVATION

3 - 6th June, 2014

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ISBN ~~xx-xxxx-xxx-x~~

Published for the 16th International Symposium of Forage Conservation, 3-6th June, 2014 Brno by Mendel University at Brno, CZ

Editors: V. Jambor, S. Malá, B. Vosynková, D. Kumprechtová

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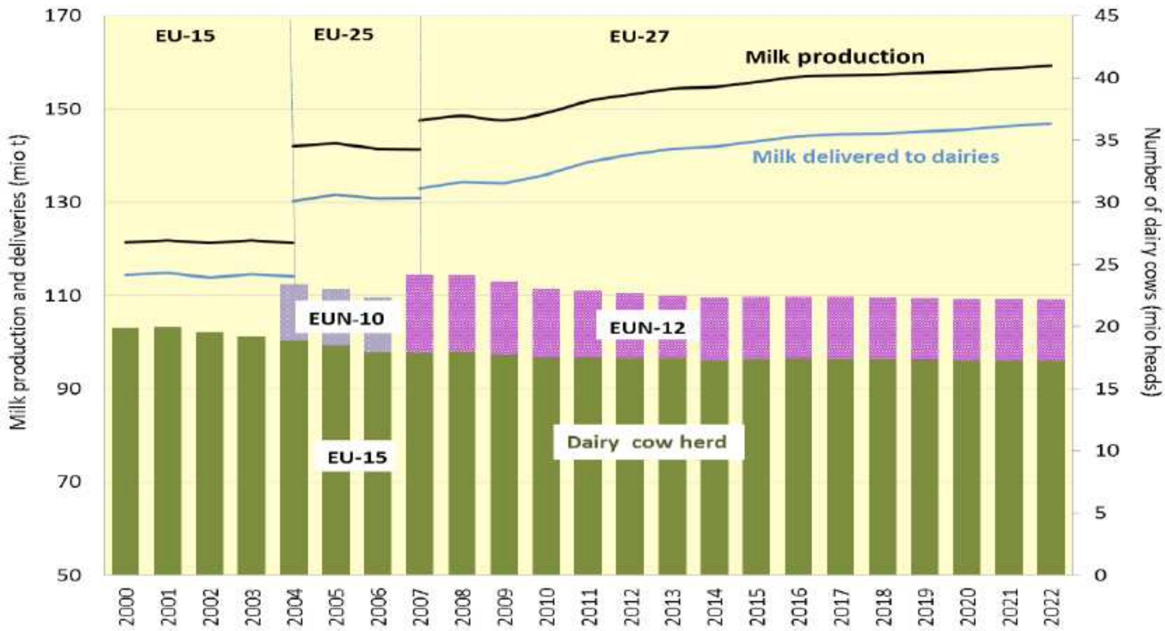
OBSAH

Production and realization of milk in the European Union

KUČERA, J.
Czech Fleckvieh Breeders Association

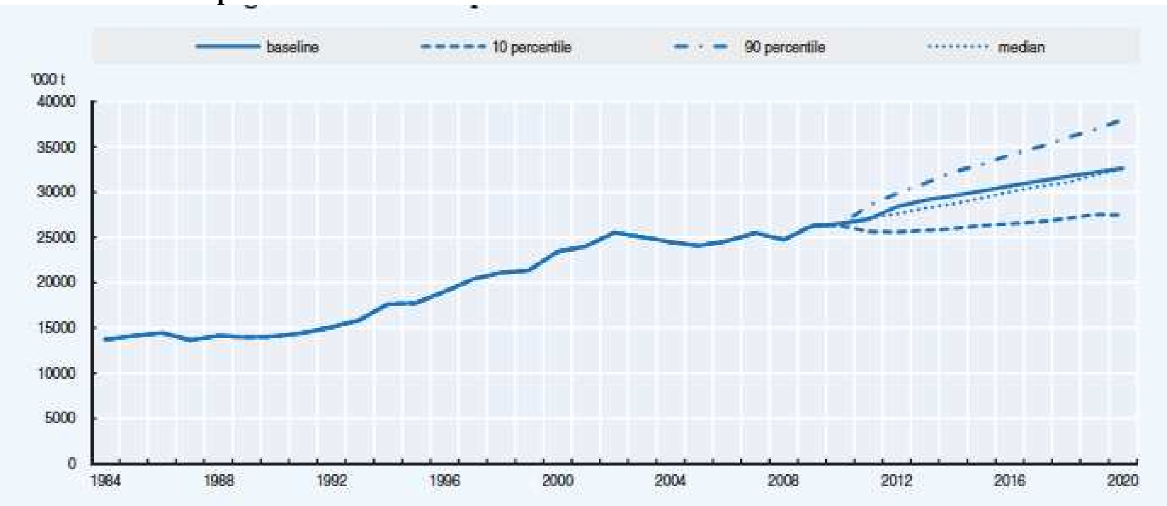
The milk market is highly globalized and responds to developments in individual areas. The European Union together with New Zealand belongs to the largest exporters of milk on the market with the same 26% share for each of the major players.

Estimation of milk production in the EU



Estimating the further development of milk production and its price is very complex task, which is confirmed by the fact that there are many studies on this topic that almost contradict each other. DG Agri estimates regarding the development of milk production in the EU till 2022 are characterized by an expected gradual increase of the total volume of production and deliveries to processing.

Estimation of milk production in New Zealand



Source: OECD and FAO Secretariats.

A trend of estimation of OECD/FAO for the second most important player on the international market - New Zealand - in the same period (till 2020) is very similar to the prediction of European production. However, if we take account of possible climate impacts, it is clear how the volume production diverge in Oceania.

The volatility of milk prices

State	Fluctuation
New Zealand	24,5
Uruguay	22,0
USA	18,9
Poland	15,0
Hungary	14,4
Australia	13,8
Czech	12,9
Germany	11,1
Brazil	11,0
Ireland	10,9
Sweden	10,9
South Africa	10,4
Netherlands	10,2
Austria	9,7
Denmark	9,3
EU average	9,1

Many studies have dealt with the volatility of milk prices recently, an analysis of the EDF among them. Within the EU only Poland and Hungary have a higher milk price fluctuations, while the large milk producers within the EU (Germany, Netherlands) are statistically behind the new EU member states.

If we look in more detail at the reasons for fluctuations respectively price stability, then it is logical that Canadian market has one of the lowest volatility of the price, thanks to a strict system of milk quotas and market management. There is the highest price fluctuation in New Zealand. However, the situation there depends mainly on the weather influence. It is also a market without any regulation in terms of quantity or price.

The examples of big and strong sales organization confirm what emerged from a study that the European Commission commissioned to the Dutch University in Wageningen: the milk price is higher by 2,5 to 4,5 Euro per 100 kg of milk in countries (regions) with a higher willingness of dairy farmers to organize themselves into strong producers organizations than in countries with lower involvement in producers organizations. Likewise, there are price fluctuations lower in states with a higher proportion of milk traded through the producers organization. Generally, the 28 EU milk sector has one of the highest participation of dairy farmers in sales organizations, which exceeds 50 %. In the long term, the most studies agree on increase of the milk production both in the EU and globally over the next ten years. A production increase by 4,5 % is expected in the old member states of the EU till 2022; only a 2% production increase is predicted in new EU member states.

The establishment of an observatory for the development of the milk price (European Milk Market Observatory) is a step, which the majority of all the participants of last year's "dairy conference" in Brussels agreed on. The site http://ec.europa.eu/agriculture/milk-market-observatory/index_en.htm keeps you informed about the development of milk prices, information on the development of the milk and milk products markets, and much more.

The history and role of feed preservation in the Czech Republic

JAKOBE, P.
SŠZP, Klatovy

Considering the way of development of agriculture in Czechoslovakia after 1950, the need for forage preservation grew very quickly as a consequence of collectivisation. The agricultural establishments founded in the country at that time were relatively large as compared with Western European farms and it was becoming increasingly difficult for them to ensure a sufficient supply of green fodder to cows. An increased field size brought about new issues connected with preservation of crops difficult to ensile, sugar beet tops and sugar refinery by-products such as molassed sugar beet pulp. All was made even worse by unfavourable climatic conditions. That is why agricultural research institutions began to address methods of forage preservation. First of all I should mention the Czech doyen of forage preservation, F. Isayev, later followed by F. Flám of the Institute of Animal Science Prague - Uhříněves. In the 1950s and 1960s these researchers investigated preservation methods of sugar beet pulp, sugar beet tops and potatoes. The quality of silages from sugar beet tops and non-pressed sugar beet pulp was very poor at that time.

Further trials were performed in the Slovak Republic, mainly at the Animal Production Research Centre in Nitra, where the research was headed by Miroslav Škultéty, or at the Grassland and Mountain Agriculture Research Institute in Bánská Bystrica.

As time went by, the leading position in research into forage preservation was gradually taken up by the Department of Biotechnology of the Research Institute of Animal Nutrition in Pohořelice that became the main coordinator of the research activities in 1964. This development mainly resulted from the work of F. Barančic at the turn of 1950 s and 1960s who built a specialised biotechnology site focused on forage preservation methods, forage treatment and preserved forage assessment. Another major research researcher A. Karkan who published the books "Silážování pokrokovými metodami" (Progressive Ensiling Methods, 1959), "Výroba a uložení kvalitní kukuřičné siláže za použití PVC plachet" (Manufacture and Storage of High-Quality Maize Silage Using PVC Silage Covers, 1963), "Silážování kukuřice s chemicky čistou močovinou a síranem amonným" (Ensiling Maize with Chemically Pure Urea and Ammonium Sulphate), and "Silážování pícnin silostanem pyrosiřičitanem sodným" (Ensiling Forage with Silostan Containing Sodium Di-Sulphite, 1969). Later, along with his colleague K. Koželuhová, he focused on storage of fodder beet and sugar beet.

Significant methodological achievements of Barančic in the 1960s included the research into and specification of the optimum dry matter level of alfalfa for haylage, within the range of 42 – 45 %. He also prepared standardized monitoring and fermentation process evaluation procedures for ensiling materials with different levels of dry matter (DM) in laboratory-scale silos and for concrete curbs used under semi-field conditions.

In 1971 these investigations were followed by trials with oat preservation aiming at producing high-energy glycide feed under less favourable conditions. At that time early varieties of maize with short vegetation times were not available in Czechoslovakia and silages that did not contain corn cobs were of poor quality due to low dry mater levels. These important studies became the basis for later development of whole crop ensiling methods. Although studies on this topic published abroad were available, the Czechoslovak agricultural sector was unaware of it. Therefore the detailed research was focused on the effects of harvesting oat at different maturity stages, from heading to milk-wax maturity, on quality of whole crop oat silages.

Silage quality of oats harvested in different maturity stages

Stage	Dry matter	pH	Acids			Lactic acid	Dry matter loss (%)
			lactic (%)	acetic (%)	butyric (%)	VFAs	
Heading	17.01	5	0.21	0.93	0.36	0.1	18
Blossoming	20.03	4.72	0.34	0.63	0.26	0.3	12
Milk maturity	24.22	4.12	0.92	0.38	0.02	1.8	11
Milk-wax maturity	33.18	4.32	1.17	0.25	0.01	3.5	8

Source: Barančic, F., Dedek, J., Jakobe, P.: "Vliv zvýšeného obsahu sušiny na kvalitu ovesných siláží a senáží" (Effect of increased dry matter levels on quality of whole crop oat silage and wilted silage), in: Živočišná výroba 20, 1975, No. 9.

In the early 1970s the quality of oat silage harvested at different maturity stages determined its further use and orientation at other cereals for the following reasons: milk-wax maturity of oats proved very appropriate, as the dry matter of about 35 % assured very good quality of silage and allowed for direct harvesting, providing that the chop length was very short, which was impossible to achieve with machinery available at that time. Therefore it was recommended to harvest oats at milk maturity, let it wilted in the field until 35% DM and then ensile. This, however, increased the dependence on favourable climatic conditions and/or need for a silage additive. Another related issue, addressed by Barančic and Přikryl, was harvesting of nurse crops, for alfalfa and clover were commonly sown under oats or barley which were left to achieve full maturity at the expense of the under-sown crop. Because of the need for quality protein from under-sown perennial crops, recommendations were published to harvest oats at heading, which meant either to feed green oats or let it wilt quickly and ensile at about 35% dry matter. This was often difficult to manage, and therefore barley was harvested at milk-wax maturity more commonly. This topic was further developed by studies of harvesting and preservation of cereals at milk-wax maturity, known as the GPS method.

In the 1970s, in addition to cereal harvesting and preservation, Jakobe studied preservation of new grass varieties in collaboration with grass improvement experts from Hladké Životice. He mainly focused on tetraploid varieties of rye grass, with higher levels of water soluble sugars, however depending on soil fertilisation. After the application of nitrogen fertilizers at higher doses, nitrogen levels grass material increased up to those measured in clover or alfalfa. Taking into account fibre and other nutrient levels, the beginning of heading was determined as the optimum maturity stage for harvest. Optimum nitrogen dose giving the highest yields was set at total 240 kg of N, given in three applications. Due to the high levels of nitrogenous substances the crop was difficult to ensile, with either 36% DM, or 25% DM plus silage additive being prerequisites for success. Newly, the methodological approach included the preservation of forage from research fields in Vatín, a crop improvement site belonging to the Forage Department of the College of Agriculture in Brno. The conditions of grass growing were accurately defined there, including fertilisation. Hartman was the first one to use liquid chromatography for laboratory measurement of water soluble sugars, pentosans and hemicellulose and their fractions. These were valuable data for crop improvers reflecting the results of their crop improvement efforts and a being an incentive for further grass growing and improvement in terms of yield and nutrient levels.

This is related to a broad issue of ensiling processes assessment which the Research Institute of Animal Nutrition paid a lot of attention to. The development of chemical methods, particularly in the 1970s and 1980s, allowed for a more accurate monitoring of fermentation processes. The micro-diffusion method for lactic acid measurement and gas chromatography for the measurement of acetic and butyric acid were introduced. At the same time the determination of free amino-acids by formol titration with calculation of the level of proteolysis was introduced. Most changes were made in analyses of lactic acid. The introduction of the micro-diffusion method was followed by gas chromatography, and since 1985 isotachophoresis has been used for lactic acid measurement. Its relevance has been confirmed by a series of silage trials aimed at monitoring of fermentation processes in difficult to ensile forage species. It was found out that parameters such as lactic acid, lactate to VFA ratio, ammonia to VFA ratio, readily soluble sugars and protein are the ones most sensitive to poor ensiling management of difficult to ensile forges. On the basis of this and other findings Hartman developed a new system of silage assessment, published in the book called "Silážování objemných krmiv" (Ensiling of Forage Crops) by Jakobe et al. (1986).

In the recent years the chromatographic methods have become the most commonly used way of measuring fermentation parameters in different silage types. The instrumentation and accuracy of computer-assisted result evaluation have been steadily improving.

In the 1970s and 1980s, due to a lack of forage, an increased attention continued to be paid to preservation of sugar beet pulp and tops by both the Institute of Animal Science in Uhříněves (Flám) and the Research Institute of Animal Nutrition in Pohořelice (Karkan). The molassed sugar beet pulp produced in sugar refineries by a new diffusion method contained very low sugar levels and only about 6 - 8 % of DM. Attempts were made to use absorbent materials or chemical additives, all without any noticeable success. The issue was resolved by the introduction of pulp pressing but other problems appeared. Also sugar beet tops were tested with absorbent material - straw chops - but a technical problem of homogeneous mixing with the tops emerged. Further efforts were made to increase dry matter content by dehydration (Karkan,1975), but the method was not established. The last stage of research into sugar beet top ensiling was inspired by the introduction of a new technology of sugar beet harvesting, which did not produce tops including the tuber heads but only cut off the leaf and petiole with a flail mechanism. Přikryl (1985) proved that in comparison to the traditionally used cutting the new method reduced the nutritional value of the forage and increased the risk of ash or soil contamination.

In the 1980s maize continued to replace oat silage in highland areas due to the of maize hybrids with earlier maturity. For the Czechoslovak agriculture maize was a new crop, and as mentioned in the introduction to this essay, Karkan described its preservation in the methodology for farmers in 1961. In the 1970s and 1980s, in the context of labour division approach applied in Czechoslovakia at that time, maize preservation was tested by Škultéty from the Animal Production Research Centre Nitra. His research results became the basis of maize preservation methods.

Assessment of maize silage harvested at milk-wax maturity (Škultéty)

Silage dry matter %	pH	Lactic acid %	Lactic acid	Nutrients on DM basis			
			Volatile fatty acids	N-substances %	Fibre %	Nitrogen free extract %	Ash %
22.25	3.90	3.59	5.70	8.58	20.00	57.12	5.69
25.64	4.10	2.75	4.40	8.12	19.42	60.50	5.38
29.67	4.30	2.15	3.42	8.00	17.23	64.78	5.33
32.63	4.40	2.67	3.44	8.02	18.11	62.87	5.08

From the very beginning of fodder preservation efforts were made to assure high-quality preserved forages for cattle even where the local environment such as low dry matter content or poor climatic conditions did not allow to achieve it. Unfavourable climatic conditions both reduced dry matter levels in fodder and did not allow for increase of dry matter level by wilting. That is why even in the Czech Republic studies were conducted to test effects of the product AIV developed by the Finnish researcher Virtanen, but the product was not widely used due to difficulties in acid handling and the necessity to neutralise the acids in feed. In the 1960s the research institutes in Pohořelice an Uhříněves worked on the application of sodium di-sulphite but practical results were not satisfactory and farmers withdrew from using it. Later on the attention was focused on the use of formic acid and a number of tests were performed, but despite very good preservation results practical applications were minimal. The widespread use was prevented by the caustic effect of acids causing skin erosions and pervasive smell. That is why attention was focused on the mixture of calcium formate and sodium nitrite in powder form called Silostan. The sodium nitrite selectively suppressed clostridia in the first stage of fermentation, thus allowing for acceleration of proliferation of lactic acid bacteria. The use of this in Czechoslovakia was described by Karkan (Animal Nutrition Research Institute) and Kozák (Institute for Scientific Management Systems). Its conservation effect on silage was said to be satisfactory then, on condition of its homogeneous introduction into the ensiled material. This was quite difficult though, and therefore the quality was variable. In addition, there were some objections against sodium nitrite from the hygienic point of view.

In those years a whole range of other silage additives was tested, such as propionic acid (for grain preservation, Barančic 1970), iso-butyric acid (for grain and hay preservation, Václavík) or benzoic acid and salts (for sugar beet conservation, Flám, Kosař). Despite the good preservation effects the widespread use was prevented by high costs.

Problems with forage quality persisted also in the 1970s and efforts were made to introduce other silage preservatives. The attention was focused on formaldehyde because of its antimicrobial action. Formaldehyde was usually used along with acetic acid. In Czechoslovakia of that time this issue was mainly analysed by Škultéty of the Nitra research centre, who prepared a methodology for use of the silage additive Chemkosil, a mixture of formaldehyde (50-56 %), acetic acid (20-25 %) and 15 % methanol, which is toxic and therefore there were objections against its use. A similar effect is produced by hexamethylenetetramine (urotropine), used abroad in mixture with sodium nitrite as Kofasil Plus. But its application was very costly and therefore Synthesia Pardubice in cooperation with Jakobe and Přikryl investigated the application of the hexamethylenetetramine based mixture with formic acid Silko M, or with acetic acid Silko A. The additive showed good effect on preservation of protein forages with a low dry matter content of 24 %. The additive was available in the liquid form and special applicators were used to inject it into the output pipeline ending of the cutter (Šeda, KUOS Žamberk). Although a correct application resulted in no health or hygiene issues, the product contained formaldehyde and potential milk contamination was not desirable.

Another trend in forage preservation was represented by probiotic silage additives. In 1979 MVDr. Míčan (State Veterinary Administration, Hustopeče) performed in-process monitoring, on the basis of which he proposed the use of a probiotic as silage inoculant. The product was manufactured by the Agricultural Cooperative of Hustopeče together with the Medipharm company. In the autumn of 1979 the Pohořelice research institute (Jakobe and Barančic) commenced laboratory, pilot and in-process testing of lactacidogenic Streptococcus faecium, strain N 74, under the commercial name of Lactisil. With 50 thousand homofermentative lactic acid bacteria per 1 g of green forage the following results were achieved:

Quality of beet top silages treated and untreated with Lactisil (Jakobe, Barančic, Přikryl)

	Dry matter %	pH	Lactic acid %	Lactic acid: Volatile fatty acids
With Lactisil	17.30	4.0	2.18	3.62
Without Lactisil	16.99	3.98	1.98	2.96

Nutritional value of beet top silages treated and untreated with Lactisil (Jakobe, Barančic, Přikryl)

	Dry matter %	N-substances %	Digestible nitrogenous substances %	Starch units
With Lactisil	17.30	3.73	3.21	10.03
Without Lactisil	16.99	3.63	2.98	9.01

The results show that quality of the preserved forage did not substantially improve, also due to the fact that preservation of beet tops was performed under optimum conditions, unlike under the field conditions. In fact, there was a big difference in the intake of forage by animals and repeated monitoring on farms showed an increase in average milk yield by 0.5 litres. Another favourable effect was an improved resistance of maize silage against secondary fermentation.

Further development of probiotics in collaboration with AB Medipharm headed towards combinations of several probiotic strains. The first one was MICROSIL that contained Streptococcus faecium, Lactobacillus plantarum, Lactobacillus casei and Pediococcus spp., with total live bacterial counts of $10x10^9$ per 1 g of product. Comparative trials confirmed that if correct technological practice of application was observed MICROSIL reduced fermentation losses and improved feed intake. Another silage inoculant tested by the research institute in the 1980 was a combination of bacteria and enzymes called BACTOZYM that consisted of lactic acid bacteria and fibrolytic enzymes. The product was designed for hard-to-ensile corps to improve fermentation, digestibility and preserve high nutritional value silage.

A retrospective look reveals that preserved fodder has played a critical role in cattle nutrition. Under the communist regime attention was paid to forage mainly because, due to the low quality of and need for animal products by the society, pressure on feed grains and concentrates, considerably limited and controlled by the regime, was increasing. In the 1970s,with the average milk yield around 3,800 litres the production efficiency of forage ranged between 6 and 8 litres of milk with about 3 kg of concentrate consumed. This was mainly due to a lack of high-standard machinery which prevented the required speed of storage and harvesting in the optimum vegetation stage. Thus silage and haylage often showed low quality and high fibre contents. Another role was performed by the fact that attention was focused on harvest of and yields of cereals as strategic commodities, and thus forage production was not among the preferences of agronomists.

A breakthrough for the Czech agriculture obviously came in 1990, when the prices and international trade were fully liberalised. Decreased prices of agricultural production, especially of milk, and the hasty privatisation disadvantaged milk production. The numbers of dairy cows have dropped from1.2 million heads in 1989 to 369 thousand these days. The figures reflecting the change are given in the table below showing percentages of concentrate in total intake of dry matter per day for the given milk yield.

Comparison of percentages of concentrate in total dry matter intake per day

Country	Milk yield kg /day	Nutrient conversion*
CSSR	10	25.0
CZ 80	17.5	34.3
CZ 390	19.5	51.3
CZ 730	20.5	46.3
NL173	19	28.9
Fr 38	17.5	31.4
UK 246	21	42.9
DE 645	19.5	56.4
AT 58	21.5	41.9

** % of concentrated nutrients in total dry matter intake in kg/day*
Source: IFCN Dairy, Braunschweig, Jakobe, 2004

The first line of the table above shows approximate values achieved in the Czechoslovak Socialist Republic in the 1970s. Average milk production was around 3,800 litres per cow and the proportion of concentrated feed dry matter was about 25 % of the whole ration dry matter. Average herd sizes are given next to CZs. The table shows that milk yield increased considerably on larger Czech, however these used a higher dietary proportion of grains (35 - 50 % on DM basis). With such diets, production efficiency of forage included in the daily ration. Nowadays such a level of grain in the ration would give about 7,433 litres of milk.

The present growth of production efficiency of forage goes hand in hand with the introduction of modern technologies and equipment to farms such as high-performance applicators of silage additives, or baled and bagged silage. Another benefit is that farmers can get modern and efficient varieties of fodder plants and maize that enable to produce high quality silage if harvested at an optimum maturity stage and good silage making practices are observed.

In conclusion it should be pointed out that Czech farmers belong in the European Union and they can make use of modern technologies and European knowledge on silage fermentation and preservation to produce high-quality foodstuffs in compliance with the EU legislation on food safety. With regard to the liberalisation of global trade the costs of milk production should be further reduced to maintain the competitiveness of the European Union on global food markets.

Fifty years progress in forage conservation and challenges for the future[1]

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Summary
Progress in the past 50 years is reviewed with reference to the major scientific disciplines involved in forage conservation. Hybrid cultivars of maize (*Zea mays* L.), the forage harvester, the large baler, polyethylene covering for silos, stretch-wrap for bales, and additives designed to improve the preservation of moist hay and the fermentation of silage all contributed to improved technological efficiency. The major biochemical pathways involved in silage fermentation have been described together with the effects on fermentation and aerobic stability during the feed-out phase of bacterial inoculation. Total mixed rations have improved efficiency of feed use by livestock. The value of covering bunker and clamp silos has been established, with recent progress in the development of co-extruded oxygen barrier film. Challenges for the future include improving the hygienic quality of silage to reduce risks to animal health, optimising crop and silage composition for biogas digesters, reducing loss of feed nitrogen to the soil and atmosphere, improving silo safety and developing edible sealants for silos.

Introduction
The five decades from the 1960s to the 2010s have seen huge changes in agriculture with increased mechanisation and larger livestock farm units. In addition, output per head of livestock increased, partly through improved animal nutrition. The development of techniques for the preservation of forage crops of good quality and sufficient quantity has allowed high levels of animal output to be sustained during periods of the year when pasture growth was inadequate to support the nutritional requirement of the grazing animal.

The process of growing, harvesting and conserving forage crops is an applied science involving five distinct disciplines: crop production, engineering, chemistry and biochemistry, microbiology, and animal nutrition. The successful production of silage and hay therefore involves an understanding of the important physical, chemical and biological factors affecting the conservation process of which the most significant are oxygen and water.

I was introduced to silage and hay whilst working on farms in the 1950s and have been involved in forage conservation research from 1965 to the present day. This review is a personal selection of some highlights of progress in forage conservation and some challenges for future improvements in technological efficiency.

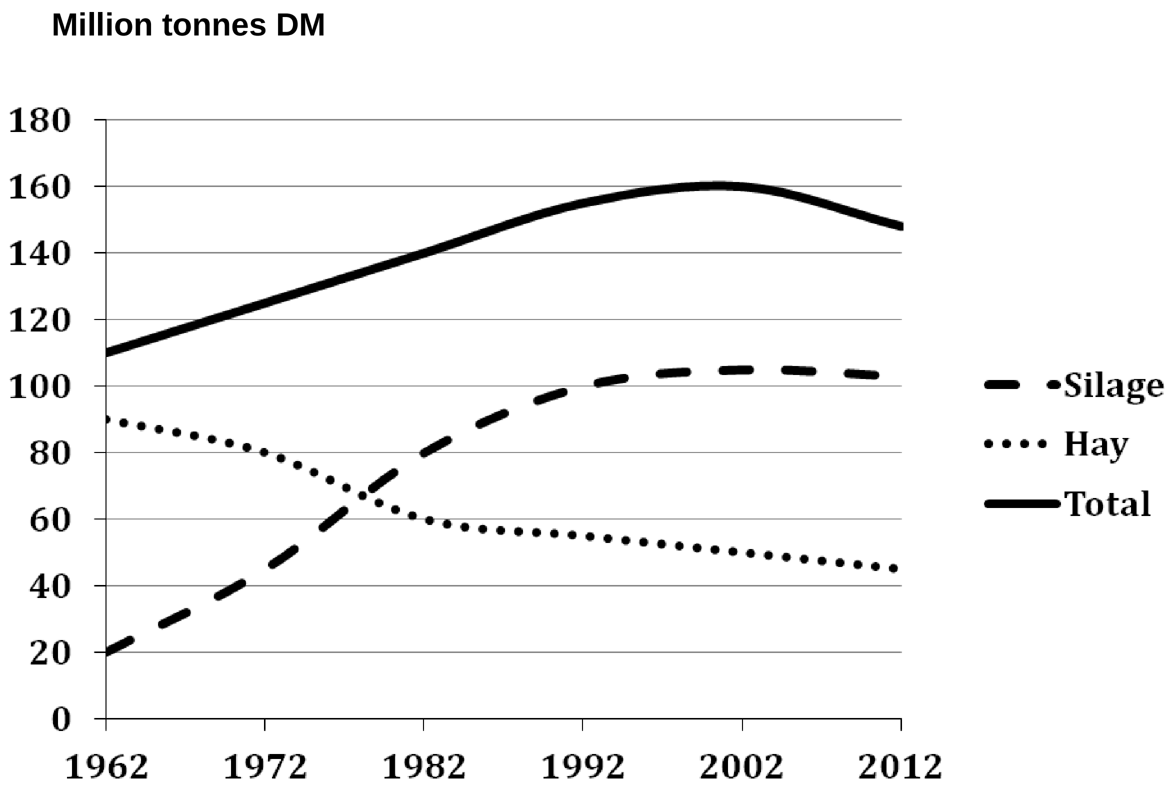
Evolution of hay and silage production

Estimates of silage and hay production in Western Europe are shown in Figure 1 for the period 1962 to 2012. There was a five-fold increase in silage DM production between 1962 and 1992, associated with the technological developments described below. Production of hay declined throughout the period so that by 2012 total output was about half that in 1962. Total production of conserved forage increased to reach a peak around 2002, with a small decrease thereafter. Maize silage increased substantially since the 1960s, especially in Western Europe so that by 2000 maize silage accounted for 48 million tonnes DM, 50% of total silage DM production (Wilkinson and Toivonen, 2003). Since then, the production of maize silage has probably increased further to around 55 million tonnes of DM in 2012.

A similar trend occurred in the USA with total silage production increasing from about 16 million tonnes DM in 1959 to 44 million tonnes DM in 2013. Maize silage currently accounts for 75% of total USA silage DM production. Of the total silage produced in the USA, it is estimated that about 85% is stored in bunkers and unwalled clamps (drive-over piles) with the remainder stored in towers, bales and bags (K.K. Bolsen, personal communication, 2014).

[1] Presented at 16th International Symposium Forage Conservation, Brno, Czech Republic, 3 to 6 June 2014.

Figure 1 Evolution of silage and hay production in Western Europe: 1962 to 2012 . (Wilkinson and Toivonen, 2003 and author's estimates).



The changes in forage conservation in Europe between 1962 and 2012 were accompanied by large reductions in the population of ruminant livestock during the period; from 201 million cattle in 1962 to 122 million cattle in 2012, and from 272 million sheep in 1962 to 129 million in 2012 (FAO, 2014). In Great Britain, inputs of fertiliser N input to grassland increased between 1962 and 1982 but thereafter the average annual quantity of fertiliser N applied to grassland decreased from 126 kg N ha^{-1} in 1983 to 55 kg N ha $^{-1}$ in 2012 (British Survey of Fertiliser Practice, 2003, 2014; Professional Nutrient Management Group, 2013). It is likely therefore that grassland yields increased in the period 1962 to 1982 and then decreased. There was also an increase in the production of concentrate feed for cattle by animal feed mills in the European Union from 32 million tonnes in 1989 to 41 million tonnes in 2012 (European Feed Manufacturers' Federation, 2013). There has been a trend in recent decades away from grazing towards more intensive feeding of cattle on diets comprised of silage and concentrates to support higher levels of animal output which, in the case of average milk production per cow, increased in Europe from 2295 kg annum^{-1} in 1962 to 5580 kg annum^{-1} in 2012 (FAO, 2014).

Much of the progress in forage conservation in the past 50 years, described in the following sections, has been made with silage and not with hay. An important disadvantage of haymaking is the need for up to seven days of dry weather during which time the crop is dried by turning and tedding (shaking) the crop once or twice daily, with consequential loss of valuable leaf tissue. More energy is needed from solar radiation and convection towards the end of the field-drying period to remove water from within flowering stem tissue than water from leaves and the outer layers of the stem that is lost rapidly in the initial phase of the field-drying process. Consequently the rate at which the crop dries decreases as drying proceeds (Jones, 1979; Jones and Harris, 1980; Wilkinson and Wilkins, 1980). The vulnerability of haymaking to loss of nutrients due to poor weather has been a major factor in the move from hay to silage in the past 50 years.

Silage in the 1950s and early 1960s was made without the use of forage harvesters. Unwalled clamps were made at the edge of the field by raking unchopped material into piles. Although plastic sheeting was first introduced as a method of covering clamps in the 1950s (Shukking, 1976), lack of covering resulted in oxygen ingress during storage with loss of extensive nutrients due to aerobic spoilage (composting or rotting). Nevertheless the probability of success was much greater with silage making than with haymaking. The risk of wet weather after cutting the crop meant that in the 1960s high quality hay was likely to be made in one out of four years in northwest Europe. Today, with only a 24 to 36-hour interval between cutting and harvesting, high quality silage is likely to be made in three out of four years.

In the following sections, progress in the major scientific disciplines involved in forage conservation is highlighted and some challenges for the future are discussed.

Crop production

The most important development in crop production was the development of high-yielding hybrid cultivars of maize (*Zea mays* L), some specifically destined for conservation as ensiled whole-crop forage, with emphasis in northern regions on earliness of maturity. In areas where summer drought and early frosts are uncommon the risk of crop failure is relatively low. Research on maize in the 1960s was mainly concerned with establishing the factors affecting the composition and nutritional value of the plant at harvest (Demarquilly, 1969; Bunting and Gunn, 1973) and after ensiling (Harris, 1965; Cummins, 1970; Danley and Vetter, 1973; Andrieu and Demarquilly, 1974ab; Deinum, 1976). This work laid the foundation for work on the role of maize silage in the nutrition of beef and dairy cattle (Thomas et al., 1975; Wilkinson and Penning, 1976; Wangsness and Muller, 1981; Phipps, 1990; Phipps et al., 1995; Bal et al., 1997; Cherney et al., 2004). Recent work has focussed on evaluating new low ferulate *sfe* and *bm3* mutants of potentially higher intake and digestibility (Jung et al., 2011).

The ability of the maize plant to yield relatively high quantities of low-cost starch per hectare, coupled with a relatively high metabolisable energy (ME) concentration (Table 1) have made the crop a popular choice for farmers in areas where the land is suitable for its cultivation. In terms of composition, maize silage is complementary to grass and grass/clover silages (Phipps, 1990). In marginal regions, whole-crop wheat forage, of similar starch concentration but lower in ME than either maize silage or good quality grass silage, is often the preferred annual forage crop to forage maize.

Table 1 Typical composition of perennial ryegrass, whole-crop maize and whole-crop wheat silages (Chamberlain and Wilkinson, 1996; Thomas, 2004).

	Grass	**Maize**	**Wheat**
DM (g kg^{-1} fresh weight)	250	300	400
pH	4.2	4.0	4.2
g kg^{-1} DM			
Ash	70	50	55
Neutral detergent fibre	520	440	460
Crude Protein	155	85	90
Total fermentation acids	120	100	80
Water soluble carbohydrates	44	15	70
Starch	trace	280	230
Metabolisable energy (MJ kg^{-1} DM)	11.5	11.3	10.6

Other notable highlights of progress in crop production for forage conservation include the development of cultivars of perennial ryegrass (*Lolium perenne* L.) with higher concentrations of water-soluble carbohydrates (Wilkins and Lovatt, 2004; Davies and Merry, 2004; Moorby et al., 2005; Marley et al., 2007) and hybrids of perennial ryegrass and tall fescue (*Festuca arundinaceae* L., Humphreys, et al., 2012) which are more resistant to summer drought than ryegrass. Tall fescue is particularly valuable as a perennial grass species for the production of high DM silage and hay because of its ability to lose water more rapidly during wilting than other grasses (Jones and Prickett, 1981).

Engineering

The most important engineering development in forage conservation in the 20th century was the development of the forage harvester. Early flail forage harvesters cut and lifted the crop into a trailer in a single operation – direct cutting – with little or no chopping to reduce particle length. Current recommendations are to chop drier crops relatively short (25 to 50 mm) to aid consolidation and to chop wetter crops to a longer average particle length (80 to 100 mm) to preserve particle length to stimulate rumination and also to reduce liquid effluent production (English Beef and Lamb Executive, 2011).

The introduction of more complex chopping cylinders on forage harvesters resulted in the separation of the cutting and chopping operation for grass crops, but not for maize and whole-crop wheat for which specialist pick-up attachments were developed. Grain-processing rollers were incorporated into the chopping equipment of forage harvesters in the 1990s to ensure more complete digestion of grain by the animal (Shinners, 2003). The benefits to grain processing are most likely to be seen with mature crops of maize and wheat (Allen et al., 2003).

The invention of the big baler in the 1970s was an important milestone in both hay and silage making. The baler allowed silage to be made for the first time in smaller, transportable packages that, like hay, could be traded easily between farms. Big bale silage gained popularity on smaller farms with limited financial resources to invest in silos and also in upland areas where the terrain was unsuitable for larger machinery. Balers could be used for harvesting straw after grain harvest in arable areas and at other times of the year for silage or haymaking. The design of both balers and self-loading forage wagons meant that they were well-suited to harvesting grass crops that had been field-wilted to 450 and 550 g DM kg^{-1} fresh weight (Shinners, 2003). In the 1980s automatic wrapping equipment followed the introduction of the large baler. Bales are wrapped in stretch-film of 25μm thickness with a 50% overlap either in-line as they are formed, or subsequently in the field or at the place of storage (Savoie and Jofriet, 2003). Baled silage probably accounts for about 25% of total silage production in Europe (Wilkinson, 2005).

Tower silos, the predominant way of storing silage in Europe and North America since the end of the 19^{th} century, represented the ultimate in efficiency in terms of low losses and mechanisation of filling, removal and delivery of silage to livestock (Wilkinson et al., 2003; Savoie and Jofriet, 2003). But towers were expensive and had restricted capacity. As livestock units increased in size, bunkers and large unwalled clamps increased in popularity along with mechanical loading and unloading equipment.

The introduction of total mixed rations in the mid 1960s was the result of adding mixing augers to a mobile forage wagon or truck so that silage and other feeds could be mixed together before the mixture was transported to the livestock building and discharged into a feed trough. Colman et al. (2011) demonstrated an improvement in feed efficiency and animal health in 273 dairy herds in France and the United Kingdom following the adoption of a total mixed ration feeding system linked to an internet-based nutrition support service.

Chemistry and biochemistry

In the past 50 years the main biochemical pathways in the silage fermentation have been described and the processes involved have been reviewed elsewhere (McDonald et al., 1991; Rooke and Hatfield, 2003). Research in the early years of the 20^{th} century was aimed at preventing undesirable fermentations that had adverse effects on the quality of cheese made from milk from cows given poorly fermented silage. The emphasis was on direct acidification (reviewed by Watson and Nash, 1960). The preservation of moist hay by acidification with mould inhibitors such as propionic acid, added at 10 g kg^{-1} fresh weight was a significant development in the 1970s (Knapp et al., 1976; Benham and Redman, 1980) since the traditional method of preserving moist hay by the addition of sodium chloride had proved to be of variable efficacy (Stuart and James, 1931, Watson and Nash, 1960). Uneven application and loss of additive can result in zones where tolerant moulds are able to develop and degrade the preservative allowing subsequent growth of other species and deterioration of the hay (Lacey et al., 1978). Despite these drawbacks, organic acids such as propionic or acetic and their salts are still in use as hay preservatives (Bagg, 2012).

Sulphuric acid, the main acid additive for silage for several decades, was superseded by the introduction in the 1960s of formic acid, a by-product of the refining of crude oil. Concern over adverse effects of sulphuric aid on the animal led to considerable research in the 1960s and 1970s into formic acid, but its relatively high cost limited its initial use (Shukking, 1976). The development of the gravity-feed applicator that allowed the acid to be added uniformly at a relatively low level (20 to 30 kg $tonne^{-1}$ fresh crop weight) accelerated the adoption of formic acid (Saue and Brierem, 1969). However, it was not until the late 1970s that the scientific basis of its use was established by the important work of Woolford (1975, 1978) who showed that whilst the mineral acids hydrochloric, sulphuric and orthophosphoric had no specific antimicrobial properties against a range of silage bacteria yeasts and moulds, other than via acidification, some straight-chain organic acids (formic, propionic and acrylic) had the dual function of both acidification and inhibitory activity against undesirable spore-bearing bacteria such as clostridia.

Animal feeding experiments confirmed the efficacy of formic acid, especially with wetter crops (Wilson and Wilkins, 1973; Waldo, 1977; Dulphy and Demarquilly, 1977) and by the early 1980s there was sufficient evidence to recommend its use by farmers as the most efficacious silage additive (Wilkinson, 1984) and also to justify its use as the positive control chemical against which other potential additives were assessed (Steen, 1991; Pflaum et al., 1996; European Food Safety Authority, 2006, 2012).

Bunkers are today the preferred way of storing silage, but in the 1960s and for several decades thereafter there was debate about the cost-effectiveness of covering them. Early research to demonstrate the effects of covering on losses showed clear advantages to covering bunkers with neoprene-nylon sheeting (synthetic rubber) over no covering (Gordon et al., 1961). Surprisingly, some farm silos in the USA are not covered today (K. K. Bolsen, personal communication, 2014) in the mistaken belief that the value of the material lost is less than the cost of the covering film, despite detailed research in the early 1990s confirming significantly higher losses in uncovered farm-scale silos than from covered silos (Bolsen et al., 1993). Loss of DM from uncovered silos was very high in the uppermost 0.5m. This finding was substantiated by a study of 127 commercial farm silos that revealed average losses of organic matter (OM) in the uppermost 0.5 m of 470 g kg^{-1} of crop ensiled for uncovered silos compared to 203 g kg^{-1} for covered silos (Bolsen, 1997).

The development of covering materials for bunkers and clamps was most rapid in northwest Europe where these types of storage structures predominated. By the mid-1970s the conventional method of covering bunkers and clamps was with a double layer of polyethylene film, each of 125 or 150µm thickness. This technology remained essentially unchanged for 30 years until the development of co-extruded oxygen barrier film (Degano, 1999).

The introduction of oxygen barrier (OB) film for covering silos was a step-change in technology that was probably as large as the initial introduction of polyethylene film itself. Silage under OB film showed less development of moulds and undesirable bacteria, including butyric acid bacterial spores, in the peripheral areas of the silo or bale during the storage period (Borreani and Tabacco, 2008; Orosz et al.,2012). The results of a meta-analysis of 51 comparisons (41 with bunker and clamp silos, 10 with baled silage) between standard polyethylene film and OB film are in Table 2. The OB film reduced losses from the outer layers of the silo during the storage period and increased the aerobic stability of maize silage. The practical implications of these findings are that less labour is needed to discard inedible material and the risk of accidentally including spoiled silage in the animals' diet is reduced. With bales, fewer layers of wrapping and less weight of film may be needed with OB stretch-wrap than with standard wrap and the process of wrapping bales may be speeded up.

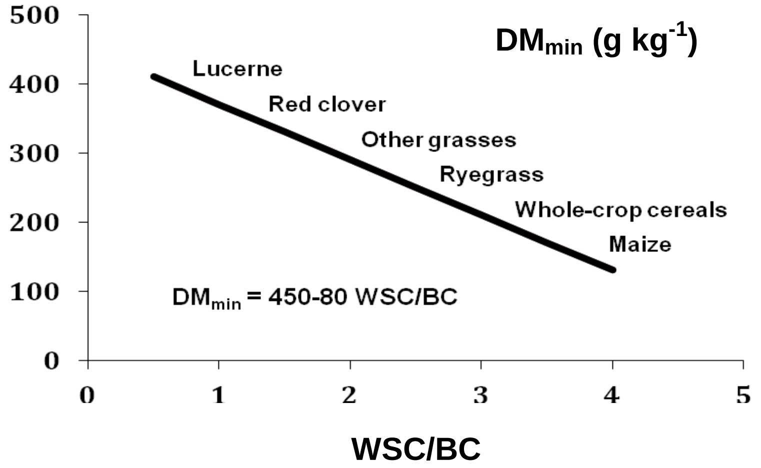
Table 2: Losses, inedible silage and aerobic stability of silage in the top surface layer stored under standard or oxygen barrier (OB) films (Wilkinson and Fenlon, 2013).

		n[1]	Standard film	OB film	Sig.
Bunker and clamp silos[2]	Loss of DM or OM (g kg^{-1})	41	195	114	<0.001
	Inedible DM (g kg^{-1})	5	107	29.6	0.022
	Aerobic stability (h)	11[3]	75.3	134.5	0.001
Baled silage	Total loss of DM (g kg^{-1})	10	7.68	4.56	<0.001

[1] Number of comparisons. [2] Includes drive-over piles and laboratory silos.
[3] All comparisons with maize silage

A significant development in the early 1970s was a better understanding of the key chemical components of the crop that affect the extent and pattern of the silage fermentation process. Weissbach and colleagues in Germany demonstrated the significance of DM, WSC and buffering capacity (BC) in determining the pattern of fermentation and the minimum DM required to achieve a stable lactic acid-dominant fermentation (Figure 2). At the same time, McDonald and co-workers were describing the energy changes in ensiling and showing that losses of energy were lower than those of DM, especially in heterolactic and yeast fermentations of glucose and in the clostridial fermentation of glucose and lactate. As a result the gross energy of extensively fermented silage is about 10% higher than that of the original crop at harvest because the end products are reduced chemically compared to the substrates (McDonald et al., 1973).

Figure 2: Minimum recommended dry matter concentrations for stable silage fermentation (Weissbach et al., 1977).



Microbiology

The significance of the growth of undesirable microorganisms such as clostridia in wet silage had been known since the early part of the 20th century (Watson and Nash, 1960) and to this day the production of silage is either prohibited or discouraged in some regions of Europe because of the risk of "late-blowing" of cheese made from milk contaminated with clostridial spores (Wilkinson and Toivonen, 2003). Research in the 1960s into the factors affecting the pattern of silage fermentation demonstrated the important loss in nutritional value associated with clostridial growth in silage, reflected in reduced proportions of total fermentation acids as lactic acid and increased proportions of acetic acid and ammonia-N in total N (Wilkins et al., 1971). Suppression of clostridial growth in crops of low DM and low WSC may be achieved by a range of technological interventions including wilting, chopping and addition of either acid (see above) or homofermentative lactic acid bacteria (reviewed by Woolford, 1984; McDonald et al., 1991; Pahlow et al., 2003 and Kung et al., 2003).

The probability of effectiveness of microbial inoculation depends on the acid tolerance of the species or strain and the number of bacterial colony forming units added per gram of crop (Pitt and Leibensperger, 1987). Heron et al., (1988) found that addition of 10^4 organisms g^{-1} was insufficient to improve the fermentation quality of ryegrass whereas 10^6 g^{-1} was adequate. Strain of lactic acid bacteria is also likely to influence the probability of a beneficial effect on the fermentation (Woolford and Sawczyc, 1984; Weinberg and Muck, 1996), especially if the bacterial strains have the ability to produce cell-wall degrading enzymes such as ferulate esterase (Dupon et al., 2012).

Work in Germany established that silages with concentrations of undissociated acetic acid of more than 8 g kg^{-1} FW were stable in air while silages with concentrations lower than 3 g kg^{-1} FW were unstable in air (Wolthusen et al., 1989). Thus, enhancement of acetic acid concentration should reduce problems of heating and moulding of silage in the feed-out period. Driehuis et al. (1999) and Driehuis and Oude Elferink (1999) reported improved aerobic stability following inoculation of maize silage with *L. buchneri*, which produces acetic acid and 1,2 propandiol from water-soluble carbohydrates and lactic acid. The same team later identified a new strain, *L. diolivorans,* which degraded 1,2 propandiol to 1-propanol and propionic acid, potentially further enhancing silage aerobic stability if present in the silage (Krooneman et al., 2002). *Acetobacter pasteurianus* may also have the potential to improve the aerobic stability of silage (Nishino et al., 1999) although the same species was implicated in the initiation of aerobic deterioration in later work (Dolci et al., 2011). Recent developments have centred on identifying microbial strains and species, some hitherto unknown, qualitatively by analysis of DNA (reviewed by Muck, 2012), which promises further elucidation of relationships between strains, species, communities, chemical composition and nutritional value of conserved forages.

Inoculants have also been developed for hay, some based on *Lactobacillus buchneri* (Baah et al., 2005) and others based on *Bacillus pumilus*, an organism that is capable of growing in relatively low concentrations of available water (a_w) and is able to compete with spoilage micro-organisms (Mahanna, 1994). However, there is relatively little independent research information to support the efficacy of inoculant products when used on hay baled below 800g DM kg^{-1} fresh weight (Department of Environment and Primary Industries, 2009; Kung, 2014).

Mould development is a particular hazard because airborne spores can cause allergies and respiratory distress in both livestock, especially horses, and also humans (Robinson, et al., 1996). Mycotoxins can adversely affect the performance, susceptibility to infectious diseases and fertility of dairy cows (Fink-Gremmels, 2008; Hofve, 2014), and some, especially aflatoxin B1, pose a risk to food safety (Driehuis, 2012). Adverse effects of mycotoxins in rations on rumen function, immune status and milk yield may be mitigated by the inclusion of a mycotoxin deactivator product in the diet of dairy cows (Kiothong et al., 2012).

Animal nutrition

The ultimate test of successful preservation of nutrients in hay or silage is the animal. Demarquilly and Dulphy (1977) comprehensively reviewed earlier work involving comparisons between silage and the corresponding fresh forage or between silage and field-dried hay made from the same original crop. They stated that both the intensity (extent) of fermentation and its pattern influenced the intake and utilisation of silage; for silage to be consumed in similar amounts as the corresponding fresh forage it must have the following characteristics: NH_3-N ≤ 50 g kg^{-1} total N, acetic acid ≤ 25 g kg^{-1} DM and other volatile acids approx. zero. They concluded that the degradation of protein during ensilage could limit the performance of animals with high protein requirements, as Clancy et al. (1977) also found in comparison of different conservation methods.

Thomas et al. (1968) reported although intake of DM as hay was higher than that of silage made from the same crops, the digestible energy concentration of silage was 1.24 times that of hay made from the same cop which explained higher efficiency of conversion of conserved forage to weight gain or milk production. Wilkinson (1980) showed that the relative yield of ME per unit of fresh crop was higher for silage than that of hay as a result of lower loss of digestible energy during conservation. The substitution (or concentrate sparing) effect of silage increases with silage intake potential (Wilkins, 1974; Huhtanen et al., 2008) reflecting increased digestibility and ME concentration (Flynn and Wilson; 1978; Steen and Agnew, 1995; Keady and Hanrahan, 2013) and improved fermentation quality (Demarquilly and Dulphy, 1977; Steen, 1991; Moran and Owen, 1994; Patterson et al., 1996) enhanced by good silage making technique (Aston et al., 1994).

Degradation of protein to water-soluble nitrogenous compounds including amines and ammonia during the silage fermentation is considered to be a factor responsible for reduced efficiency of nitrogen utilisation in animals given silage diets compared to the fresh crop or dried material (Wilkins, 1974), often attributed to lower rumen microbial protein synthesis (reviewed by McDonald et al., 1991). Some possible reasons, including asynchrony of release of energy and N solubilisation of N, were suggested by Huhtanen et al. (2012). Supplementation of silage with additional protein has given production responses and there is evidence to support histidine as a limiting amino acid for milk production, along with lysine and methionine (Shingfield, et al., 2003; Hristov, et al., 2012). Davies et al. (1997) found that high initial WSC concentration in herbage and inoculation resulted in silage with improved protein quality, indicated by a higher proportion of the leaf protein ribulose-1, 5-biphosphate carboxylase (RUBISCO) than in silage made from herbage of low WSC, without additive or with formic acid. Herbage with low WSC underwent a heterofermentative fermentation, implicating this pathway in enhanced proteolysis.

Inoculation of crops with homofermentative strains of lactic acid bacteria at the time of harvest has been the predominant type of additive for ensilage since the early 1980s in North America (Bolsen and Heidker, 1985) and since the late 1990s in Europe (Wilkinson and Toivonen, 2003). But meta-analyses of animal responses to silage inoculation have produced equivocal conclusions (Kung and Muck, 1997). Possible reasons for failures include competition from epiphytic microflora, insufficient fermentable substrate, low water activity and excessive oxygen (Kung et al., 2003). It is also important to add a greater number of homofermentative lactic acid bacteria than the natural (epiphytic) population of bacteria to increase the probability that the inoculation will dominate the fermentation. Modelling work indicated that a 10-fold increase was necessary (Pitt and Liebensperger, 1987). The target level of addition should be 10^6 colony-forming units per gram of fresh crop (Heron, 1996; Wilkinson, 2005).

Some challenges for the future

Reduced gaseous emissions from silage

The need to reduce greenhouse gas (GHG) emissions from livestock and ancillary operations is well established (Gill et al., 2009). Although the principal source of GHG emissions is methane from enteric fermentations (Opio et al., 2013), inputs of primary energy to livestock production systems that rely heavily on mechanisation (e.g. silage) should be reduced wherever possible. The energy balance of silage making is shown in Table 3 for three different crops. Primary energy, mainly non-renewable energy used in the manufacture of fertiliser, accounts for almost 60% of the total energy consumed in grass silage. In this analysis maize silage grown with reduced fertiliser and animal manure gave the highest output of ME per unit of energy input with lucerne and grass having similar ratios of ME output to energy input.. GHG emissions may be reduced by reducing the quantity of fertiliser N applied to grass, by growing alternative forages such as lucerne, and also by reducing the global warming potential of other inputs such as polyethylene film. For example, Wheelton et al. (2014) showed that use of OB film was associated with an 82% reduction in global warming potential compared to standard polyethylene film.

Table 3: Energy input and output in silage making (based on Wilkinson, 1980)

	Grass[1]	**Lucerne**	**Maize**[2]
Energy inputs (GJ ha^{-1})			
Artificial fertiliser	22.0	2.8	9.2
Field operations	13.0	13.0	12.6
Additive (formic acid)	-	7.3	-
Storage	3.4	3.0	2.7
TOTAL	38.4	26.1	24.5
Output			
Crop yield at cutting (t DM ha^{-1})	11.5	10.0	12.0
Less losses of DM during conservation (g/kg)	190	210	170
Output of silage DM (t ha^{-1})	9.3	7.9	10.0
ME (MJ kg DM^{-1})	11.0	9.0	11.2
Output of silage ME (GJ ha^{-1})	102.3	71.1	112
Silage ME Output: Total energy Input	2.7	2.7	4.6

1 Three cuts per year, 250 kg N ha-1. 2 Plus manure.

The issue of the low nitrogen use efficiency (NUE, N in animal product as a proportion of total N intake) in livestock given forage-based diets is receiving attention worldwide. N excretion is directly related to total N intake (Dewhurst, 2006; Mills, et al., 2008) and work to reduce excretion of N in manure is concentrated on the extent to which total N intake maybe reduced without at the same time reducing animal performance. A detailed study with dairy cows into the scope for reducing both methane and N excretion by manipulating forage source and diet CP concentration showed that methane emission per kg milk was lower for a maize silage-based diet than for a grass silage-based diet. Reducing diet CP concentration from 180 to 140 g kg DM-1 increased NUE, though milk yield was also slightly reduced (Reynolds et al., 2010). Greater capture of N from animal manures and residual crop N in soil will be a focus for future research into crops grown in association with maize and whole-crop cereals, either as winter cover crops or as second crops on double-cropped land in warmer regions.

Improved hygienic quality of conserved forages

Some silage is very unstable when exposed to air and can deteriorate in less than 24 hours of exposure to the atmosphere (Danner et al., 2003). Inoculation of crops with homofermentative lactic acid bacteria can reduce aerobic stability (Weinberg et al., 1993; Danner et al., 2003). Wilkinson and Davies (2012) highlighted the significance of the aerobic deterioration of silage in terms of hazards to animal health through, for example, the development of mycotoxins and bacterial endotoxins in spoiled silage. Muller (2012) stressed the importance of good hygienic quality in the production of silage for horses and highlighted the challenge of describing hygienic quality with improved accuracy.

The significance of aerobic deterioration of conserved forages in terms of the effects on the animal is now being established though the specific anti-nutritional factors remain to be fully described. Research in Germany showed an average 57% reduction in DM intake of eight maize silages differing in DM, chop length and density and exposed to air for 8 days prior to being offered to goats in a preference trial (Gerlach et al., 2013). In this trial the temperature of the silages was stable for the first 48 hours exposure to air. The mean composition and intakes of the silages exposed to air for 0, 4 and 8 days are shown in Table 4.

Table 4: Composition and intake by goats of maize silage after 0, 4 or 8 days exposure to air (Gerlach et al., 2013).

	Days exposure to air		
	0	**4**	**8**
DM (g kg^{-1} fresh weight)	360	371	395
pH	3.9	4.2	5.8
Lactic acid (g kg^{-1} DM)	58	49	8
Acetic acid (g kg^{-1} DM)	13	9	3
Ethanol (g kg^{-1} DM)	6.2	4.3	0.1
Yeasts (log_{10} cfu g^{-1})	4.6	7.2	7.3
Moulds (log_{10} cfu g^{-1})	2.4	2.8	4.2
Aerobic mesophilic bacteria (log_{10} cfu g^{-1})	4.7	5.7	6.7
Accumulated temperature (^oC above ambient)	-0.6	8.4	28.7
DM intake (3-hour period, g)	646	626	280

Dry matter concentration, pH, and counts of yeasts, moulds and aerobic mesophilic bacteria increased during exposure to air whilst concentrations of fermentation products decreased, with the largest changes occurring between 4 and 8 days exposure. Accumulated increase in silage temperature above ambient during exposure to air was the best predictor of intake. Comparable work with *Lolium multiflorum* silages also revealed a mean reduction in DM intake of 0.50 after 8 days exposure to air. However, in contrast to the work with ensiled maize there was little change in temperature, chemical composition or microbial composition during the 8-day aerobic exposure period, suggesting that small and hitherto undetected changes in chemical or microbial composition during exposure of silage to air affect animal preference and DM intake (Gerlach et al., 2014).

The use of *L. buchneri* as a silage inoculant to improve silage aerobic stability has been criticised on the grounds that its fermentation is inefficient compared with addition of homofermentative bacteria such as *L. plantarum* (Wilkinson and Davies, 2012). Kleinschmitt et al. (2013) reported that although increased additions of *L. buchneri* to maize silage were reflected in improved aerobic stability of maize silage, intake and milk yield were not improved (Table 5). The effects were attributed to increased concentrations of acetic acid in silage, in agreement with Eisner et al., (2006); though Gerlach et al (2014) found a positive relationship between acetic acid concentration and intake of grass silage by goats. Possibly there is a critical concentration of acetic acid in silage that determines whether or not the animal is likely to discriminate against the material on the basis of smell or taste; further work is needed on this topic.

Table 5: Effect of level of addition of L. buchneri CNCM 1-4323 to maize silage on acetic acid concentration in maize silage and performance of dairy cows (Kleinschmitt et al., 2013)

	Level of addition of *L. buchneri* (cfu g^{-1} fresh crop weight)				**Sig. of effects**	
	Zero	**1 x 10^5**	**5 x 10^5**	**1 x 10^6**	**Zero v additive**	**Linear effect of additive**
Silage DM (g kg fresh weight^{-1})	332	341	345	339	NS	NS
pH	3.87	3.85	3.87	3.89	NS	NS
Lactic acid (g kg DM^{-1})	47.0	35.4	31.8	40.0	NS	NS
Acetic acid (g kg DM^{-1})	28.5	40.0	44.4	48.0	0.01	NS
Aerobic stability (h)	21.5	25.6	67.6	93.9	<0.01	<0.01
DM intake (kg day^{-1})	25.6	25.1	25.3	24.6	NS	NS
Fat-corrected milk (kg day^{-1})	36.4	34.0	34.8	33.7	0.05	NS

Increased silo safety
The trend to more continuous housing of livestock and increased production of biogas from silage will increase the demand for year-round provision of high quality conserved forage. As the number of livestock per farm increases greater silo capacity is required. Many silos on livestock units were constructed several decades ago and are now too small. Instead of investing in new structures, old silos are over-filled. The issue of silo safety has been highlighted with particular emphasis on the risk of human injury and death from avalanche collapses of the silo feed face in silos greater than 3 metres in settled height (Bolsen and Bolsen, 2013).

Optimised yield of biogas methane from silage
Increasing quantities of silage are being used in the production of biogas. Weissbach (2009) found that gas yield was related to digestible (i.e. fermentable) organic matter (FOM), which in turn could be predicted from concentrations of ash and acid detergent fibre. For most crops potential biogas yield was 800 litres kg FOM^{-1} and methane yield was 420 litres kg FOM^{-1}. Amon et al. (2004, 2007) found that ensiling increased specific methane yield (litres of CH_4 per kg volatile solids) of whole-crop maize by 25% compared to use of unensiled (i.e. green) material, presumably because the products of the silage fermentation were chemically reduced and thus more suitable substrates for utilisation by methanogenic archaea. They also found that several phenotypic characters of the maize plant had a significant influence on methane yield; namely crude protein, crude fat, cellulose and hemicellulose. A challenge for the future is to improve methane yield via in-line analysis of feedstock composition, by making detailed assessments of the factors in the ensiling process that impact significantly on methanogenesis, and by breeding cultivars with higher concentrations of fermentable substrate.

New edible sealants for silos
The development of an effective material for covering silos that is also edible by livestock remains to be achieved. Berger and Bolsen (2006) described criteria for an edible sealant and experiments with a gelatinised starch/salt matrix. However, the material was costly to produce and required a protective waxy film to prevent water ingress through cracks in the matrix. Similar results were obtained in German work with a complex sprayable blend of potentially edible compounds (Uhl et al., 2011). Sodium acrylate may have potential as an ingredient of an edible silo sealant since it is i) antimicrobial (Woolford, 1978), probably due to its ability to absorb 300 times its weight of water and hence reduce a_w (Gelfand, 2014); ii) restricts fermentation and iii) improves the aerobic stability of maize silage (Wilson et al., 1979). When added at 2 kg active ingredient $tonne^{-1}$ fresh crop sodium acrylate produced silages of similar nutritional value to those made with addition of formic acid (Wilkinson et al., 1979). The potential of sodium acrylate as an edible sealant for silos should be evaluated.

Conclusions
Considerable technological progress has been made in improving forage conservation in the past 50 years but more work has to be done to achieve reduced environmental impact and increased efficiency of feed use by both live animal and mechanical fermenter. Research on improving the efficiency of the conservation of forages from field to faeces is expensive. Given the importance of silage worldwide, the increasing size of livestock units, the rising world population and the need to secure global food security it is disappointing that the capacity for multidisciplinary research on forage crops, which do not compete as food sources with the human population, has decreased with the closure in the past two decades of several major agricultural research establishments in Europe. Investment in forage conservation research brings long-term benefits to many sectors of society, especially when sponsored by both public and private sector organisations. Perhaps the biggest challenge for the

future is to create sufficient political will to secure sustained future investment in forage conservation research and development.

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Section 1: Forage production - yield, quality, fertilization

Impact of different drying techniques on hay quality

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Summary

In the years 2010 to 2012 a comparison of three different hay-drying-techniques was carried out at AREC Raumberg-Gumpenstein. The treatments „traditional field drying“, „cold air ventilation“ and „dehumidification drying“ were tested with forage of permanent grassland, cut four times per year.

The results of chemical and organoleptic analysis showed advantages of the two ventilation drying treatments in terms of hay quality compared with field drying. Differences occurred for crude protein, β-carotene, digestibility of organic matter, energy concentration and some microbial germ groups but also for sensory parameters like smell, colour and dust. In 5 of 11 trials the dehumidification technique significantly resulted in better hay quality than ventilation drying with cold air and in 9 cases better results could be achieved compared to field drying. Technical disfunctions of the dehumidification technique caused negative effects on hay quality in 4 cases. During storage of hay quality losses concerning β-carotene, digestibility of organic matter, energy concentration and development of spoilage indicative micro-organisms occurred in all treatments, but were highest in the case of traditional field drying.

Introduction

Hay and aftermath hay are still important forage conserves for ruminants even though the proportion of hay has decreased in Austria from 54 % in the year 1994 (Wilkinson et al., 1996) to 23 % in 2010 (Resch, 2013a). In some disadvantaged mountainous regions of Austria the production of „hay-milk“ is preferred by more than 8,000 farmers (fig. 1), because some sorts of special hard cheese are made of clostridia-free unpasteurised milk. 15 % of milk production in Austria is hay-milk, which in the meantime has become a very successful brand. Austrian hay farmers are located at an average altitude of 850 m (Resch, 2013b). The working committee of Austrian hay-milk farmers improved hay farming by a quality orientated marketing concept which resulted in increasing sales quantity of hay-milk products in Austria and foreign countries during the last years. On the other hand costs of concentrates are rising, so hay-milk farmers think more about strategies to improve forage quality. Approximately 24 % of profits in milk production depend on forage and its quality (Stockinger, 2009), therefore own conserved forage will be more important for successful farmers. Most of the forage in Austria originates from permanent grassland which is cut 1 to 4 times per year (max. of 6 times in Rheintal/Vorarlberg).

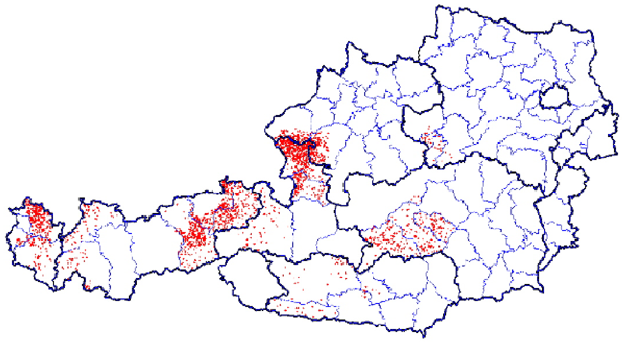

Figure 1: Designated areas with renunciation of silage production(BMLFUW, Invekos 2012)

Traditional field drying of forage is only using solar energy but this treatment takes a long field drying period with a high risk of unstable weather conditions. Dry matter contents of approx. 800 g kg^{-1} lead to increasing quality losses by spalling of leaves (Resch, 2009) and to the risk of massive propagation of epiphytic micro-organisms (Adler, 2002) in clammy grass.

Indoor drying technique basically allows to harvest forage with higher moisture contents. This reduces the field time, the risk of unstable weather conditions and the leaves spalling. But on the other hand it is a challenge for indoor drying techniques, to reduce the water content of forage within a short period as possible to avoid microbial spoilage of hay. Farmers should be familiar with the physical characteristics of forage and with the technique of the drying system, otherwise costs grow up and hay quality decreases.

The effect of a cold air drying system is limited by forage water content, air temperature and relative humidity, especially if it is cold outside in the night or air humidity is high (Nydegger et al., 2009). Ventilation with cold air reduces the efficiency of hay drying in comparison with warm air ventilation.

Treatments with air warming or dehumidification are performing better under worse environmental conditions, because they are able to remove suitable volumes of water from forage (Gindl, 2002). It is even possible to dry fresh grass by warm air, but drying costs are unecomically high in this case.

During the last decades forage science was focussed on silage conservation, whereas hay was disregarded. There is only little knowledge existing about the influence of modern drying technologies on hay quality in Austria. Experimental tests of different hay drying systems under comparable conditions were therefore necessary to provide results about mass- and quality losses during the conservation process from field to storage until feeding.

Objectives

The Agricultural Research and Education Centre (AREC) Raumberg-Gumpenstein has given a special focus on hay-quality with several projects carried out since 2007. Within the current project „Hay drying" different hay drying treatments were tested under defined conditions to provide conclusions and recommendations for farmers. The following areas were worked on:

- Technical aspects of hay drying treatments including energy consumption
- Measurement of leave spalling
- changes of hay quality during the conservation process
- changes in microbial status of hay
- feed intake, performance of dairy cows and milk quality
- analysis of costs and benefits of conservation treatments

Material and methods

A comparison of three different drying techniques for hay („traditional hay drying", „cold air ventilation", „dehumidification drying") was examined over three years (2010 to 2012) and different grassland cuts (1. to 4.) at AREC Raumberg-Gumpenstein. The experiment also included the factor storage period to determine quality changes of hay. The forage material was harvested on a permanent grassland area of in total 12 ha. The average floristic composition of the first growth was 57 % grass, 21 % legumes and 22 % herbs. In the year 2012 only three cuts were harvested because the third cut was destroyed by a flood. The harvest was managed by practice comparable machinery (Pöllinger, 2014, Resch, 2014).

Chemical and organoleptic analysis

Sampling was done by a sharpened steel drill tube (5 cm internal diameter), taking more than 20 randomised points of a forage wagon load. During the storage period hay samples were also taken from a minimum of 20 different spots to a depth of approx. 150 cm. Dynamic sampling was done at the harvest time, 7, 14, 30 and 60 days after harvest and at the beginning of feeding. The primary sample was split into subsamples for the following laboratories: AREC Raumberg-Gumpenstein, forage laboratory Rosenau (LK Niederösterreich) and AGES Linz.

Gravimetric analysis of absolute dry matter was carried out by drying at 105 °C during 24 hours (VDLUFA 3.1). For analysis of quality parameters a minimum of 300 g sample was dried during approx. 48 hours at 50 °C. Average grade of grinding was 1.0 mm. Chemical analysis of substances (Weender, structural substances, HCl-insoluble ash) and mineral macro and micro elements were carried out at AREC according to the VDLUFA book of methods. Digestibilty of organic matter was measured by the in vitro two-stage method (Tilley and Terry, 1963). Forage energy (ME and NEL) was calculated by regression coefficients on the basis of relationships between dOM and ME or NEL (DLG tables of forage for ruminants, 1997). For analysis of water soluble carbohydrates (sugar) and carotene, samples were freeze-dried and then ground to 1 mm particle size. Sugar and carotene analysis were carried out at forage laboratory Rosenau. Organoleptic evaluation of samples (smell, colour, structure and earthy contamination resp. dust) was done with the fresh material according to the ÖAG-sensoric test (Buchgraber, 1999).

Microbiological analysis

Enumeration of aerobic mesophile bacteria, yeasts, moulds and *Dematiaceae* was determined by VDLUFA-method 28.1.2 to validate microbial quality of hay samples. After a fresh sample was shred by scissors a suspension with bufferd pepton-solution (20 g of sample and 380 ml suspension) was produced in decimal dilution series. Count plates were made of suitable dilution steps with one culture medium for bacteria (Tryptose Agar with TTC) resp. with two culture media for yeasts, molds and *Dematiaceae* (Bengal Red Chloramphenicol Agar with Tergitol and Dichlorane Glycerol (DG 18) Agar). Inoculated count plates were count after appropriate time of incubation. Microbiological quality assessment was carried out on base of orientation values for microbial groups by VDLUFA-method 28.1.4 (tab. 1). Fresh harvested hay often has higher germ contents of product-typical micro-organisms as it is specified in the scheme of orientation values. If storage conditions are good, germ contents decrease during a few weeks (Bucher and Thalmann, 2006).

Table 1: Orientation values (in 10^6 cfu g^{-1} resp. 10^3 cfu g^{-1}) of VDLUFA for product-typical and spoilage-indicating micro-organism in hay, pooled in microbial groups (MG) 1 to 7 (VDLUFA 28.1.4)

Microbial Group (MG)			**orientation value**
	Mesophile aerobic bacteria	**important indicator micro-organisms**	**x 10^6 cfu g^{-1}**
MG 1	product-typical	Yellow pigmented bacteria, Pseudomonas, Enterobacteriaceae	30
MG 2	spoilage-indicating	Bacillus, Micrococcus	2
MG 3	spoilage-indicating	Streptomycetes	0,15
	Moulds and Dematiaceae		**x 10^3 cfu g^{-1}**
MG 4	product-typical	Dematiaceae, Acremonium, Fusarium, Aureobasidium, Verticillium	200
MG 5	spoilage-indicating	Aspergillus, Penicillium, Scopulariopsis, Wallemia	100
MG 6	spoilage-indicating	Mucorales	5
	Yeasts		**x 10^3 cfu g^{-1}**
MG 7	spoilage-indicating	all species	150

Orientation values of VDLUFA 28.1.4 are upper limits of microbial numbers for hay of normal condition (see tab. 1). For description of quality four quality levels (QL I to QL IV) were defined. QL I: normal status – corresponds to microbial numbers up to orientation value as a maximum. QL II: the microbial number for at least one group exceeds the orientation value up to fivefold. QL III: the microbial number for at least one group exceeds the orientation value up to tenfold. QL IV: the microbial number for at least one microbial group exceeds the orientation value more than tenfold. In this case spoilage of forage is evaluated.

Statistical analysis

All data of this project were entered into a MS-Access database and then controlled for correctness and plausibility. Descriptive analysis was done by Software SPSS (version 21), multifactorial GLM-analysis by STATGRAPHICS Centurion XV (version 15.2.14). The experimental design considered correct statistical analysis of main effects and interactions between the investigated factors drying technique, growth, storage period and year. From each quality parameter analysis of variance components was carried outto quantify the proportion of variability for each factor. Because of the comprehensive amount of results only a few selected statistical effects are presented in this paper.

Results and discussion

Concentrations of nutrients, digestibility of organic matter, sugar, vitamins and microbial status are helpful and important parameters for the evaluation of hay quality. The following results are representing the comparison of three different hay drying treatments tested in 11 exact trials and carried out in the years 2010 to 2012.

Ingredients of hay

It is of great importance in the conservation of hay to raise the dry matter content quickly above 870 g kg^{-1} FM (Nydegger et al., 2009) to ensure microbial stability for the storage period. Meisser and Wyss (1999) recommended a minimum DM of 850 g kg^{-1} FM for hay. Precious plant leaves are sensitive against spalling losses and with an increasing DM content more leaves are destroyed by mechanical work.

Multifactorial analysis showed high significant effects of the factor growth on all ingredients (p-values in tab. 2 < 0.01). Estimation of variance components indicated highest effects of growth on crude protein, natrium and copper. An increase of crude protein (121.7 to 171.9 g kg^{-1} DM) could be noticed from the first to the fourth cut whereas crude fibre dropped from 251.4 to 195.5 g kg^{-1} DM. The development of structured carbohydrates (NDF, ADF, ADL) was not non-linear in comparison with crude fibre (tab. 3). The first growth provided the highest sugar contents (154.4 g kg^{-1} DM), the lowest were measured in the third growth with 120.7 g. Hay of the fourth growth had the highest average β-carotine content of 117.6 mg kg^{-1} DM. Crude ash was increasing during the course of vegetation period with 75.1 (first cut) to 109.3 g kg^{-1} DM (fourth cut) and there was also a clear trend for the sand-content (HCl-insoluble ash, tab. 3). Sand values from the third and fourth cut exceeded 20 g kg^{-1} DM which is according to Resch et al. (2013) an indicator for earthy contamination of forage.

Significant effects on forage ingredients were observed for the factor year (tab 2) which for approx. 70 % of parameters was identified being the most influencing factor on data variability. Under comparable conditions the factor drying-treatment had a high significant effect on crude protein and β-carotine content. The average effect of air dehumidification drying was +4.9 g XP kg^{-1} DM compared with traditional field drying. Ventilation drying (cold or warm air) increased β-carotine content (+25 mg kg^{-1} DM) in opposite to field drying (tab. 3).

Table 2: Main effects and interactions of the factors year, growth, drying treatment and time of storage on different quality parameters of hay (p-values and r²)

factor	DM	XP	XF	NDF	ADF	ADL	NFC	XS	XL	XA	sand	carotene
year (y)	0,015	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
growth (g)	0,002	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
drying technique (d)	0,106	0,042	0,914	0,527	0,647	0,388	0,884	0,187	0,257	0,952	0,532	0,000
storage time (s)	0,000	0,015	0,554	0,143	0,690	0,850	0,094	0,625	0,000	0,817	0,987	0,000
d x y	0,354	0,552	0,621	0,949	0,632	0,728	0,948	0,044	0,994	0,778	0,586	0,000
d x g	0,142	0,820	0,797	0,710	0,857	0,004	0,849	0,022	0,165	0,950	0,944	0,051
d x s	0,000	0,547	0,858	0,740	0,977	0,281	0,978	0,853	0,725	0,994	0,999	0,389
s x y	0,004	0,498	0,834	0,273	0,951	0,308	0,352	0,997	0,000	0,921	0,958	0,002
s y g	0,982	0,057	0,967	0,592	0,877	0,438	0,665	0,991	0,064	0,992	0,985	0,002
R^2	0,865	0,832	0,572	0,627	0,537	0,716	0,469	0,767	0,769	0,624	0,598	0,891

p-values refered on confidenzlevel 95 % (method LSD)

Time of Storage had a statistical impact on DM-content, especially in first week after harvesting. The utilisation of ventilation techniques enabled DM-contents above 870 g kg^{-1} FM within 7 days, whereas traditionally dried hay didn´t achieve 860 g DM kg^{-1} FM during the total period of storage (fig 2). β-carotine content decreased by 40 mg kg^{-1} DM resp. 38 % from the time of harvest to the date of feeding. Crude fat (XL) remained relatively steady in the hay samples at a level of approx. 21 g kg^{-1} DM. Arrigo (2010) observed an increase of +5 to +10 g XL kg^{-1} DM from the first to the third cut of hay in Switzerland . The same trend was described in the forage value tables for alpine regions (Resch et al., 2006).

Table 3: Impact of year, growth, drying technique and storage time on different quality parameters of hay

average	count	DM	XP	XF	NDF	ADF	ADL	NFC	XS	XL	XA	sand	carotene
total	198	845,5	142,9	233,3	474,3	275,8	35,9	272,1	137,0	20,8	89,9	24,6	88,3
year													
2010	72	853,2^{b}	149,9^{c}	238,0^{b}	469,6^{b}	277,3^{b}	37,6^{b}	272,7^{b}	119,9^{a}	18,3^{a}	89,4^{b}	22,3^{a}	51,2^{a}
2011	72	837,3^{a}	142,2^{b}	240,0^{b}	497,8^{c}	292,1^{c}	43,9^{c}	256,0^{a}	129,8^{b}	20,0^{b}	84,0^{a}	21,5^{a}	133,8^{c}
2012	54	846,1^{ab}	136,7^{a}	221,8^{a}	455,3^{a}	258,1^{a}	26,3^{a}	287,6^{c}	161,4^{c}	24,2^{c}	96,2^{c}	30,1^{b}	80,0^{b}
growth													
1	54	846,9^{a}	121,1^{a}	251,4^{c}	504,1^{c}	285,4^{b}	37,2^{b}	283,4^{b}	154,4^{d}	19,9^{a}	71,5^{a}	12,8^{a}	73,5^{a}
2	54	860,5^{b}	134,0^{b}	247,1^{bc}	483,5^{b}	290,4^{b}	35,6^{b}	278,9^{b}	127,6^{b}	21,3^{b}	82,3^{b}	16,4^{a}	84,4^{b}
3	54	840,0^{a}	144,7^{c}	239,1^{b}	491,2^{b}	293,9^{b}	39,7^{c}	247,1^{a}	120,7^{a}	20,6^{b}	96,4^{c}	31,2^{b}	77,9^{ab}
4	36	834,7^{a}	171,9^{d}	195,5^{a}	418,2^{a}	233,7^{a}	31,2^{a}	279,2^{b}	145,5^{c}	21,4^{b}	109,3^{d}	38,1^{c}	117,6^{c}
drying technique													
traditional field drying	66	839,4	140,5^{a}	233,1	477,9	278,5	35,6	270,8	134,6	20,5	90,3	25,7	71,5^{a}
cold air ventilation	66	852,0	142,8^{ab}	234,2	473,4	274,3	35,4	273,4	139,4	20,8	89,5	24,6	97,1^{b}
air dehumidification	66	845,2	145,4^{b}	232,5	471,5	274,7	36,7	272,1	137,1	21,1	89,9	23,6	96,4^{b}
storage time													
0 (harvest)	33	717,9^{a}	145,1^{c}	230,2	463,7	269,3	34,7	281,5	140,8	20,6^{a}	89,1	24,4	102,2^{d}
after 7 days	33	874,5^{b}	138,8^{a}	235,7	472,8	276,0	36,5	280,1	137,0	20,4^{a}	87,9	23,8	100,6^{cd}
after 14 days	33	866,6^{b}	139,5^{ab}	237,2	486,6	281,3	35,9	264,7	137,6	20,1^{a}	89,0	24,3	96,4^{cd}
after 30 days	33	874,3^{b}	142,5^{abc}	235,0	474,4	276,7	36,5	272,8	136,7	20,4^{a}	89,8	24,8	92,1^{c}
after 60 days	33	875,4^{b}	144,3^{bc}	233,5	476,7	275,4	36,0	267,5	134,2	20,2^{a}	91,3	25,7	75,2^{b}
begin of feeding	33	864,4^{b}	147,3^{c}	228,1	471,3	276,4	35,9	266,0	135,9	23,2^{b}	92,1	24,9	63,4^{a}

units: DM [g kg^{-1} FM], carotene [mg kg^{-1} DM], other parameters [g kg^{-1} dM]

In comparison with classic crude fibre analysis, structured carbohydrates are representing nearby reality (Gruber 2009). Total content of structured carbohydrates (NDF) was similar for the first to the third cut (483.5 to 504.1 g NDF kg^{-1} DM) whereas hay of the fourth cut had significant lower NDF-contents (average 418,2 g kg^{-1} DM) which is typical for the Austrian situation in practice (Resch 2013b, 2013c). Lignin-contents (ADL) were definitely lower (35.9 g kg^{-1} DM, standard deviation +/- 9.1 g kg^{-1} DM) than the average situation in Austria without any influence of the factors drying technique or storage period.

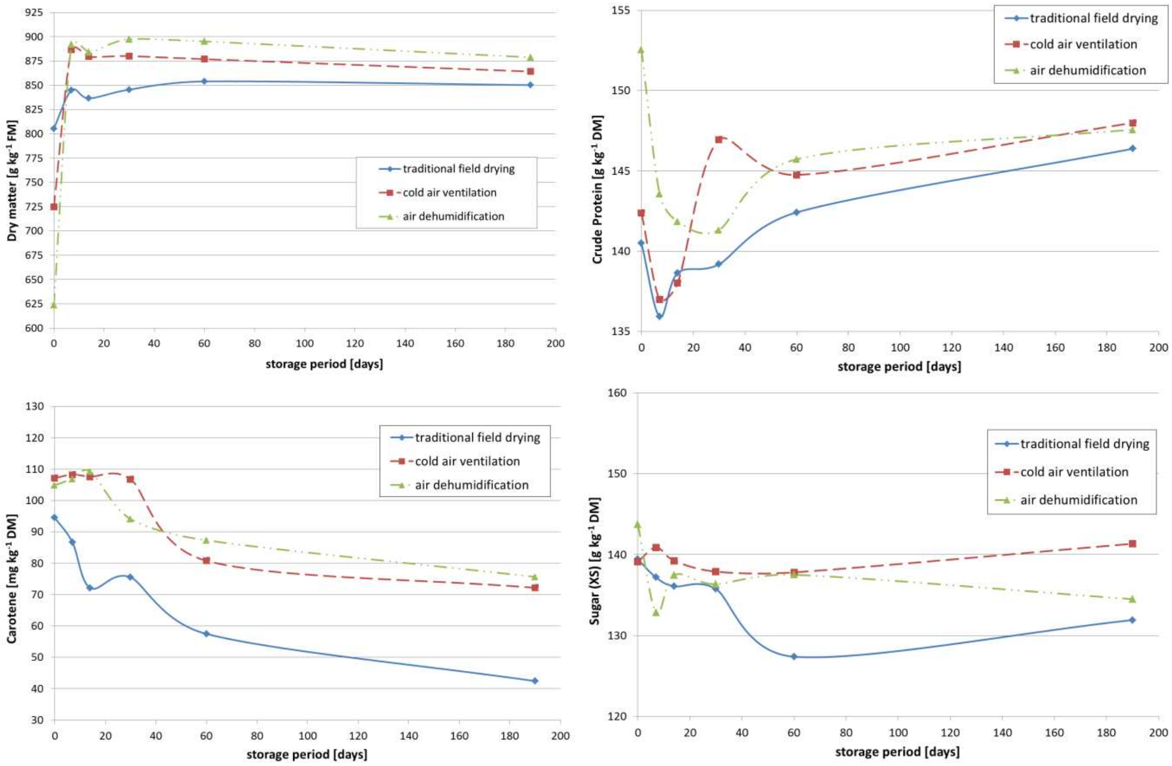

Figure 2: Influence of drying technique and storage period on DM-, XP-, carotene- and sugar-content of hay

In comparison to the average quality of hay in Austria (Resch, 2013c) much better values were found in our project for the first cut, whereas the aftermath quality was on the same level (Resch, 2014).

Digestibility of organic matter, energy content and sensory properties

Usability of forage can be classified by in vitro digestibility of organic matter (dOM). In opposite to the estimation of dOM on the basis of crude fibre (Gruber et al., 1997), the results of in vitro digestibility showed clear differences between the three drying techniques. A decreasing trend of digestibility during the storage period was observed. All tested factors significantly influenced the energy parameters ME and NEL (tab. 4).

Table 4: Main effects and interactions of the factors year, growth, drying treatment and time of storage on OM-digestibilty, energy concentration and sensory properties

factor	dOM	ME	NEL	smell	colour	structure	dust	points
year (y)	0,000	0,000	0,000	0,000	0,000	0,239	0,310	0,001
growth (g)	0,000	0,000	0,000	0,542	0,064	0,000	0,000	0,215
drying technique (d)	0,006	0,013	0,014	0,004	0,000	0,000	0,051	0,000
storage time (s)	0,149	0,020	0,041	0,008	0,380	0,055	0,011	0,003
d x y	0,143	0,169	0,178	0,075	0,004	0,021	0,617	0,009
d x g	0,184	0,265	0,271	0,011	0,053	0,017	0,264	0,021
d x s	0,985	0,996	0,996	0,583	0,935	0,759	0,942	0,842
s x y	0,924	0,966	0,965	0,806	0,596	0,160	0,104	0,710
s y g	0,130	0,202	0,160	0,702	0,706	0,234	0,035	0,399
R^2	0,666	0,668	0,669	0,454	0,509	0,595	0,490	0,502

p-values refered on confidenzlevel 95 % (method LSD)

Average hay quality in the year 2010 was significantly lower than in the following years 2011 and 2012 which might be caused by some technical problems with the air dehumidification in2010 (Fig. 4). Hay samples of the growths were significantly different in digestibility (tab. 5). Hay of the first cut had an average dOM-value of 72.6 % whereas aftermath hay of the third cut showed a low OM-digestibility of 64.2 % in all three years. Ingredients of hay like NDF, ADF, ADL or XS carried out no explanation for this dOM-depression.

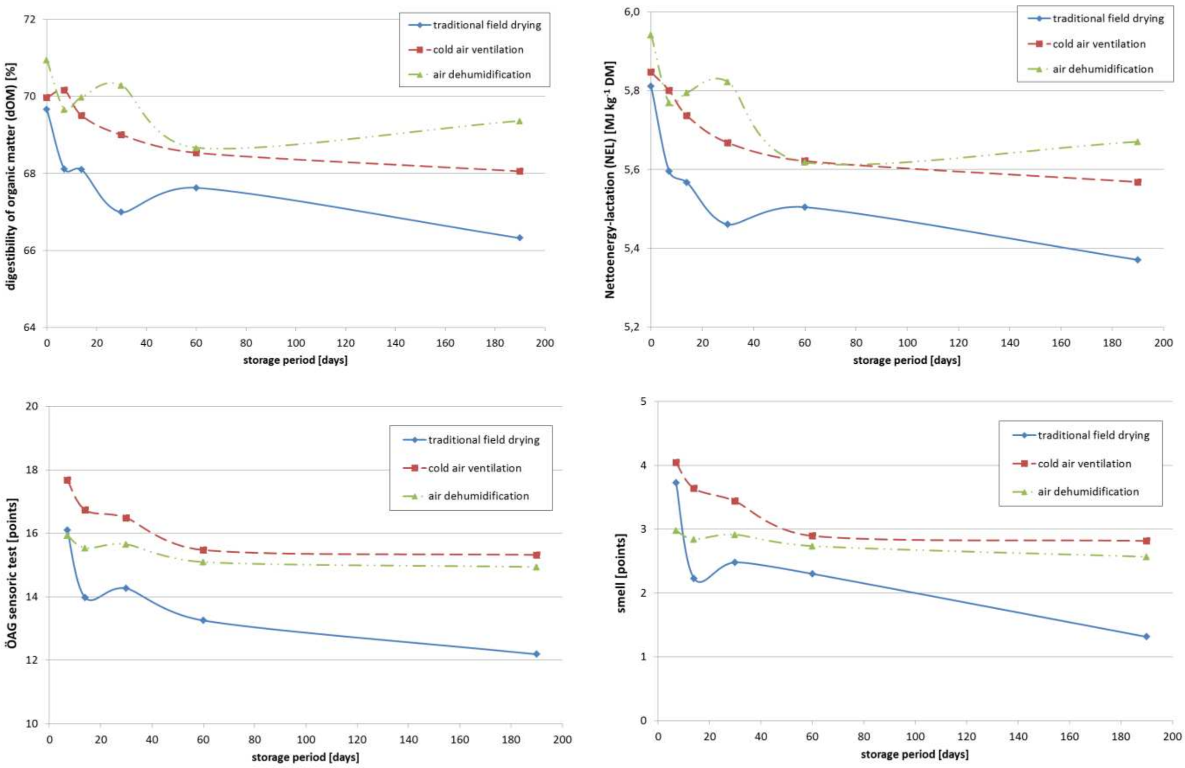

Figure 3: Influence of drying technique and storage period on OM-digestibility, net-energy lactation and parameters of the ÖAG-sensory test of hay

Hay conserved by traditional field drying had worse quality than hay processed with ventilation treatments. Quality differences between cold air ventilation and dehumidification drying were not significant (tab. 5). During the storage period a decrease of quality was observed in all treatments with an average reduction of OM-digestiblity from harvest to feeding of about 2.3 %. Quality losses of traditional field drying amounted to 3.4 %, that of cold air ventilation were 1.9 % and with dehumidification drying 1.6 % of OM-digestibiliy got lost during the storage period (Fig. 3). Loss of energy were determined with 0.4 MJ NEL kg^{-1} DM (traditional field drying) and ~0.25 MJ NEL kg^{-1} DM (ventilation treatments).

Table 5: Impact of year, growth, drying technique and storage period on OM-digestibility, energy concentration and ÖAG sensory properties of hay

factor	count	dOM	ME	NEL	smell	colour	structure	dust	points
total	198	68,9	9,62	5,68	2,9	4,0	6,7	1,7	15,2
year									
2010	72	66,6^{a}	9,25^{a}	5,42^{a}	2,2^{a}	3,7^{a}	6,6	1,7	14,2^{a}
2011	72	68,9^{b}	9,67^{b}	5,72^{b}	2,9^{b}	4,0^{a}	6,7	1,8	15,3^{b}
2012	54	71,4^{c}	9,93^{c}	5,89^{c}	3,5^{c}	4,5^{b}	6,8	1,5	16,2^{b}
growth									
1	54	72,6^{c}	10,33^{c}	6,17^{c}	3,1	4,0	6,4^{a}	2,1^{c}	15,6
2	54	69,2^{b}	9,76^{b}	5,78^{b}	2,9	4,2	6,8^{bc}	1,7^{b}	15,5
3	54	64,2^{a}	8,85^{a}	5,13^{a}	2,6	3,8	6,6^{b}	1,5^{ab}	14,6
4	36	69,8^{b}	9,54^{b}	5,62^{b}	2,8	4,3	6,9^{c}	1,3^{a}	15,4
drying technique									
traditional field drying	66	67,8^{a}	9,44^{a}	5,55^{a}	2,4^{a}	3,4^{a}	6,3^{a}	1,8	14,0^{a}
cold air ventilation	66	69,2^{b}	9,66^{b}	5,71^{b}	3,4^{b}	4,5^{b}	6,8^{b}	1,7	16,3^{b}
air dehumidification	66	69,8^{b}	9,75^{b}	5,77^{b}	2,8^{ab}	4,2^{b}	6,9^{b}	1,5	15,4^{b}
storage time									
0 (harvest)	33	70,2	9,92^{b}	5,87^{b}					
after 7 days	33	69,3	9,68^{ab}	5,72^{ab}	3,6^{b}	4,2	6,8	2,0^{b}	16,6^{c}
after 14 days	33	69,2	9,64^{ab}	5,70^{ab}	2,9^{ab}	4,1	6,8	1,6^{a}	15,4^{abc}
after 30 days	33	68,8	9,58^{a}	5,65^{a}	2,9^{ab}	4,1	6,7	1,7^{a}	15,5^{bc}
after 60 days	33	68,3	9,48^{a}	5,58^{a}	2,6^{a}	3,9	6,5	1,6^{a}	14,6^{ab}
begin of feeding	33	67,9	9,41^{a}	5,54^{a}	2,2^{a}	3,8	6,6	1,4^{a}	14,1^{a}

units: dOM [%], ME and NEL [MJ kg^{-1} DM], other parameters [points]

A comparison between the results of the hay drying-experiment and the field study „LK hay-project" (Resch, 2010, 2011, 2013b, 2013c) showed interesting effects of drying techniques and cuts on the energy concentration (fig. 4). In the first growth the differences between drying treatments was higher than for aftermath hay. Technical problems with the dehumidification drying in the year 2010 caused a significant NEL-depression (fig. 4 left side). The average energy level of hay (2011/2012) in the experiment „hay drying" was much higher than the energy level of hay from Austrian hay-milk farmers (fig. 4 right side). Disadvantages in practice caused from the later forage harvest.

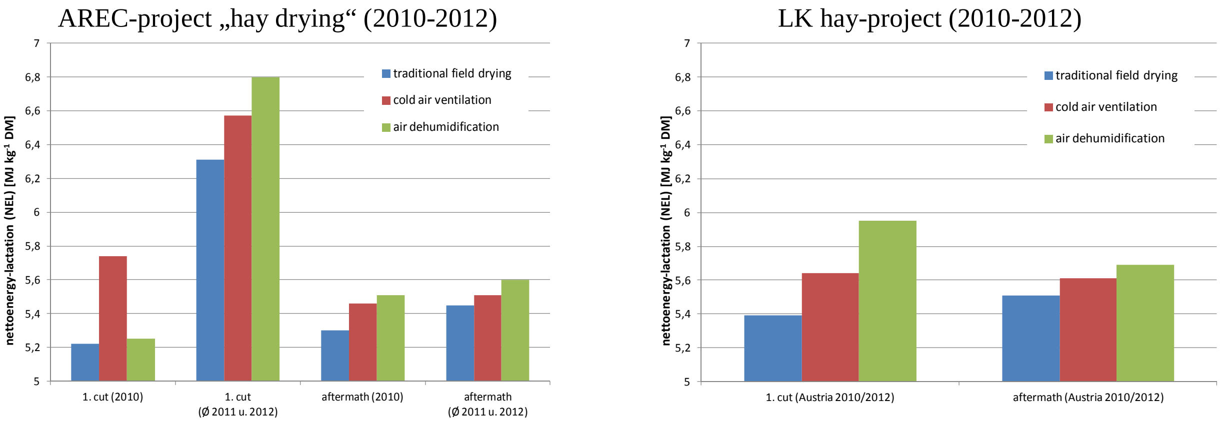

Figure 4: Influence of drying treatments on energy concentration of hay and aftermath hay (left side: AREC-Project hay drying; right side: LK hay-project)

In the AREC-experiment „hay drying" also organoleptic observations concerning smell, colour, structure and contamination (dust, earth) were carried out. Sensory properties allow to draw advanced conclusions for hay and the effects of drying techniques. For sensory properties a significant effect of the factor year was found (tab. 4), mainly caused by the bad conditions in 2010. The third cut showed significantly worse sensory quality. The drying technique had a strong influence on the conserved hay, especially on smell, colour and dust. The traditional field drying system resulted worst in terms of smell and colour but also in structure because of a lower leaf content (tab. 5).

Microbiological quality-status of hay

Product-typical microflora

In every year and for all drying techniques an almost similar field flora was determined. Bacteria flora was dominated by gram-negative germ groups like Pseudomonas and epiphytic Enterobacteriaceae. Beside yeasts there were different Hyphomycetes and Dematiaceae like Acremonium, Aureobasidium, Cladosporium, Colletotrichum or Verticillium and sometimes also toxigenic fungi like Fusarium and Alternaria occurred. Coelomycetes were detected at higher counts, especially the species Phoma and Ascochyta. The results are comparable with observations of previous AGES-experiments (Adler, 2002) and also with findings of other institutions (Brenton and Zwaenepoel, 1991; Wittenberg, 1997, Wiedner, 2008).

Table 6: Main effects and interactions of year, growth, drying treatment and time of storage on different microbial groups of hay (p-values and r²)

factor	product-typical			spoilage-indicating			
	MG 1	MG 4	MG 7	MG 2	MG 3	MG 5	MG 6
year (y)	0,098	0,784	0,018	0,000	0,000	0,075	0,001
growth (g)	0,000	0,000	0,000	0,001	0,032	0,000	0,000
drying technique (d)	0,701	0,020	0,000	0,007	0,053	0,003	0,000
storage time (s)	0,000	0,000	0,000	0,560	0,423	0,000	0,019
d x y	0,819	0,063	0,159	0,000	0,003	0,315	0,690
d x g	0,077	0,040	0,004	0,015	0,000	0,020	0,003
d x s	0,905	0,887	0,979	0,651	0,980	0,760	0,709
s x y	0,821	0,971	0,699	0,707	0,609	0,982	0,986
s y g	0,334	0,277	0,280	0,474	0,879	0,839	0,980
R^2	0,733	0,696	0,755	0,577	0,425	0,549	0,478

p-values refered on confidenzlevel 95 % (method LSD)

Numbers of product-typical bacteria (MG1) rised with every cut during the vegetation period (Adler et al., 2014). Fehrmann and Müller (1990) also found an increase of epiphytic flora on grass during the vegetation period. During the storage period composition of the microflora on hay changed because the primary flora of field-borne micro-organisms (relict flora) after harvest decreased relatively quickly. Reduction of the relict flora was higher in hay produced by traditional field drying than in hay treated with cold air ventilation resp. dehumidification drying (fig. 5). In hay samples with relevant storage flora a higher decrease of field flora was observed. Content of yeast showed an obvious reduction of germ count in every treatment. Yeast count of hay produced with the dehumidification drying system was > 0.3 log-steps higher during the total storage period than the other drying variants.

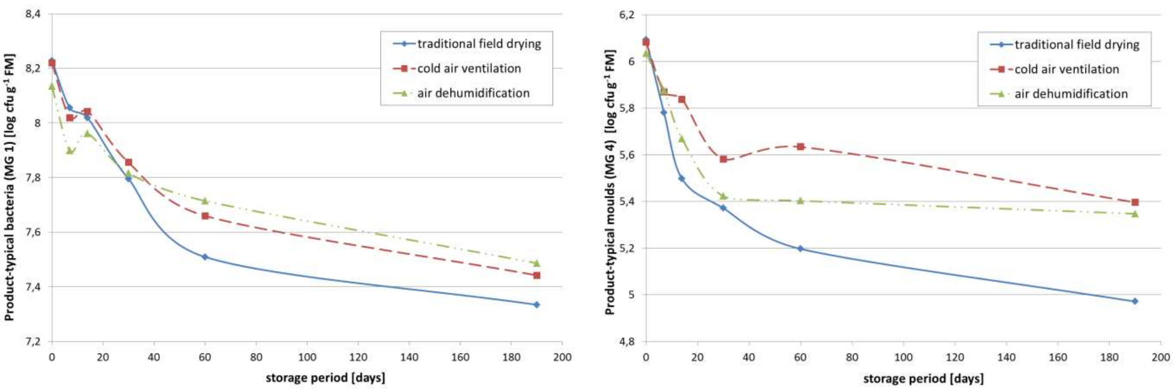

Figure 5: Influence of drying technique and storage period on product-typical bacteria (MG 1) resp. moulds (MG 4) in hay

Spoilage-indicating microflora

In the hay store the relict flora could be completed or replaced more or less quickly by a storage flora. It depends on water content and other factors (temperature, ventilation, etc.) what kind of storage flora is developing (Reiß, 1986). In hay of all treatments storage flora was characterised by little diversity of species. Spoilage indicating bacteria like Bacillus were predominant and sporadically Actinomycets occurred. Penicillium, Scopulariopsis and osmophile or xerotolerant fungi like Wallemia sebi, species of the Aspergillus glaucus-group or Mucorales dominated flora of storage-fungi. Especially in samples with high counts of fungi, Aspergillus niger and also Aspergillus fumigatus were detected. The observed flora of storage-fungi was according to other studies (Kaspersson et al., 1984; Undi et al., 1997; Reboux et al., 2006; Padamsee et al., 2012).

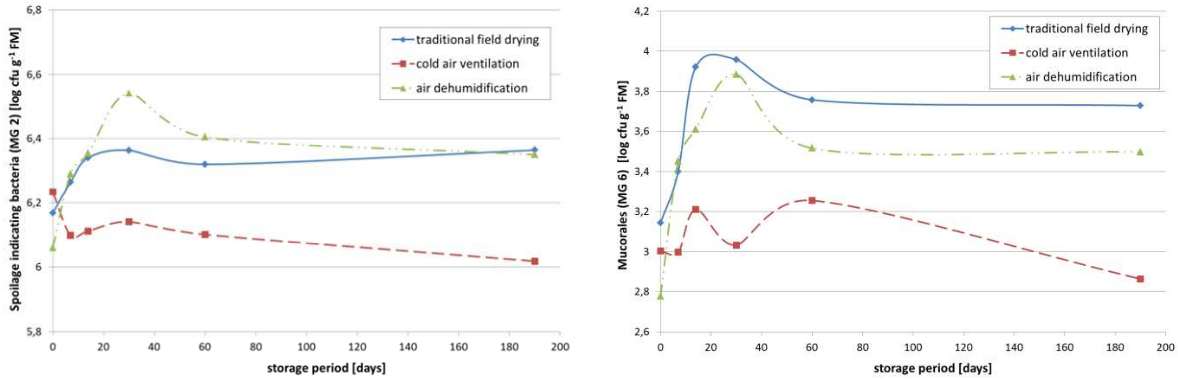

Figure 6: Influence of drying technique and storage period on spoilage-indicating bacteria (MG 2) resp. mucorales (MG 6) in hay

Hay of third or fourth cut showed consistently higher counts of storage fungi than hay of first or second cut. Reasons for more intensive development of fungi during storage could be changes in the structure of forage plants – fine or soft plant texture provide less resistance against storage fungi. High air humidity especially in late summer or autumn implies a bad drying-capability of air. Such conditions might result in an increase of fungi.

Traditional field dried hay had at least three times higher (> 0.5 log-steps) contents of important spoilage indicating fungi than hay produced by the other drying techniques.

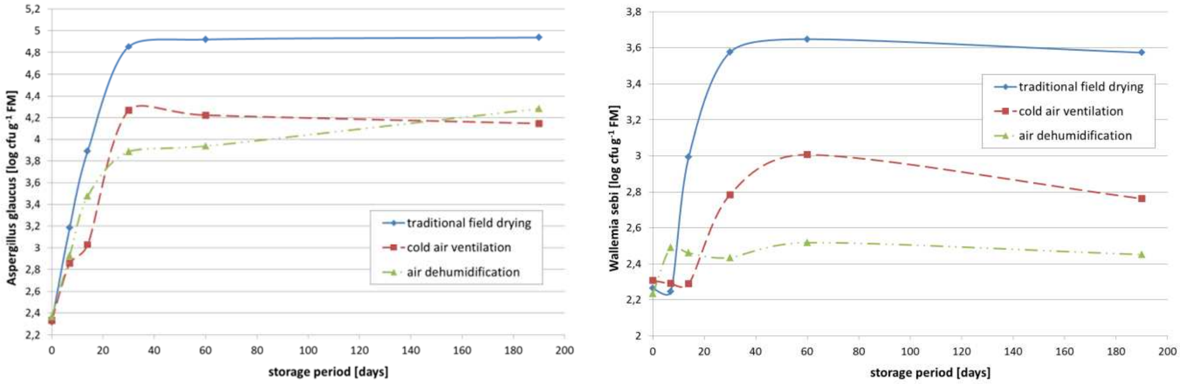

Figure 7: Influence of drying technique and storage period on Aspergillus glaucus resp. Wallemia sebi in hay

Hypothesis of microbial influence on in vitro-digestibility of OM

Results of project "hay drying" indicate a relationship between increase of numbers of spoilage indicating microbial groups and degradation of OM-digestibility during storage. GLM-model including the factors drying technique, growth, storage period, year and covariant germ group was chosen to test for the influencing effects (p-value) and to calculate regression coefficients for the correlation of different germ groups with dOM.

Table 7: Influence of different microbial groups on OM-digestibility of hay

microbial group MG	microbial species	p-value	microbial count [log cfu g^{-1} FM]	dOM [%]	regression coefficient [%]
MG 1	Yellow pigmented bacteria, Pseudomonas, Enterobacteriaceae	0,000	7,81	68,8	3,65
MG 4	Dematiaceae, Acremonium, Fusarium, Aureobasidium	0,000	5,57	68,8	2,64
MG 7	Yeast	0,466	4,68	68,9	0,37
MG 2	Bacillus, Micrococcus	0,035	6,27	68,9	-1,29
MG 3	Streptomycets	0,102	4,79	68,9	-1,64
MG 5	all species of MG 5	0,008	4,02	68,9	-0,74
MG 6	Mucorales	0,092	3,38	68,9	-0,57
MG 5-1	Penicillium	0,283	3,13	68,9	-0,32
MG 5-2	Aspergillus glaucus	0,001	3,68	68,9	-0,88
MG 5-3	Wallemia sebi	0,001	2,68	68,9	-1,07

p-values refered on confidenzlevel 95 % (method LSD)

In Tab. 7 some defined product-typical and spoilage-indicating microbial groups (VDLUFA 28.1.4) and their average effects on in vitro-digestibility of OM are displayed. For more detailed information group MG 5 was split in three sub-groups representing specific species (tab. 7). An increase of the counts of storage fungi by one log-unit caused a decrease of dOM (p-value < 0,01), in case of Aspergillus glaucus (-0,88 %) and Wallemia sebi (-1,07 %). The significant effects of product-typical groups MG 1 and MG 4 must not be overestimated, because only spoilage-indicating species could reduce easy utilisable nutrients like sugar and caused losses of digestibility of organic matter.

The results revealed that high counts of storage fungi in hay will adversely affect the digestibility of organic matter. Further experiments are necessary to confirm the causal correlation between increase of spoilage species and degradation of digestibility.

Conclusions

For more than 8.000 Austrian hay-milk farmers results of exact trials are of great importance to improve hay quality and to provide reliable decision arguments for selecting an optimal indoor drying technique. By means of the research project „hay drying“ carried out at AREC Raumberg-Gumpenstein three different hay drying techniques were tested in eleven exact trials under a four-cut-regime on permanent grassland. Under comparable conditions the ventilation techniques provided better hay quality than traditional field drying, especially concerning digestibility of organic matter, energy concentration and microbiological status. Dehumidification drying positively influenced the parameters crude protein, crude fat, and sand-content but the differences to cold air ventilation were not significant. Differences between dehumidification drying and cold air ventilation are not appearing if the year 2010 with some technical problems in the dehumidification drying treatment was eliminated from the statistical analysis. In five of eleven trials hay produced with the dehumidification drying showed better quality than that of cold air ventilation, in two cases hay quality was equal and in four cases quality was even worse, caused by technical problems. The primary problems of microbial spoilage are caused by to high water contents in the harvested forage in unfavourable combination with insufficient indoor drying. The risk of microbial spoilage is rather high for traditional field drying because of a strong development of storage microflora. The higher the water content in harvested forage the more important is a good performance and optimal management of the technical construction to produce best hay quality for dairy cows.

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Direct sowing of red clover and intergenus hybrids by three technologies - forage production and quality

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Abstract
At Jevíčko site in the Czech Republic, an accurate small plot trial was established in 2011 in two time arrangements (June and July) by three technologies consisting of directly sown seeds of red clover ´Amos´ (4n) and ´Suez´ (2n) and of two intergenus hybrids – ´Felina´ (festucoid) and ´Hostyn´ (loloid). Direct seeding was carried out by the technologies of slot seeding with a seeding drill SE-2-024, strip seeding with a prototype of PP-2 drill, with a prototype of STP 300 drill. In the trial with direct sowing technologies the DM production of red clovers was 7.43 t ha^{-1} [corrected DM (CDM) production 5.44 t ha^{-1}] for SE – 2 – 024, 6.74 t ha^{-1} (CDM production 4.20 t ha^{-1}) for STP – 300 and 7.24 t ha^{-1} (CDM production 3.21 t.ha^{-1}) for PP – 2. In the trial with direct sowing technologies the DM production of intergenus hybrids was 6.21 t ha^{-1} (CDM production 1.08 t ha^{-1}) for SE – 2 – 024, 6.11 t ha^{-1} (CDM production 0,74 t ha^{-1}) for STP – 300 and 8.09 t ha^{-1} (CDM production 2.71 t ha^{-1}) for PP – 2.

Keywords: direct sowing, red clover, intergenus hybrids, grasslands, production and quality

Introduction
Introduction of direct sowing into grasslands is influenced by a number of factors. Operational successfulness of direct sowing is still in spite of attained results insufficient and therefore the research, development and verification of new technologies continue (Kohoutek *et al*. 2002).

Materials and methods
At Jevíčko site in the Czech Republic (average annual temperature 7.4 °C, annual long-term rainfall average 545 mm, altitude 342 m, geographic coordinates: 49°37‘43“N, 16°43‘54“E), a multifactor trial with direct sowing into 30-year-old permanent grassland (PG) was established in 2011. The meadow vegetation is represented by a plant society *Arrhenatheretum*. It is well established meadow vegetation in a mezohygrophyte stand in the stream alluvium. Three direct sowing technologies were used: (1) slot seeding with SE-2-024, (2) strip seeding with PP-2 and (3) strip seeding with a prototype of STP 300 drill (discs with coulters) with spike seeding mechanism. The trials were established in two time arrangements (14th June – T1 and 19th July – T2). We sowed (L) red clover ´Amos´ (4n) and ´Suez´ (2n) at the amount of 8 million germinative seeds (MGS) per ha and (G) of two intergenus hybrids – ´Felina´ (festucoid) and ´Hostyn´ (loloid) at the amount of 12 MGS ha^{-1}. The trial plot length was 16.11 m, the length of harvested part was 11.11 m, 3 replications.

The trial plot was not fertilized in the year of direct sowing 2011. In the first harvest year 2012 the plot was fertilized with phosphorus at the rate of 35 kg ha^{-1} P and 100 kg ha^{-1} K, nitrogen was only applied on directly sown grasses at the rate of 180 kg ha^{-1} N (60-60-60) in the form of LAV by a seed drill HEGE - 80. The trial plot was harvested by three cuts. The paper evaluates dry matter production and corrected dry matter (CDM) production [(DM x % projective dominance of a directly sown species)/100)]. The forage quality was measured using the instrument NIRSystems 6500. The observed parameters were: crude protein (CP), fibre, NEL (net energy of lactation), NEF (net energy of fattening), PDIE (ingested digestive protein allowed by energy) and PDIN (ingested digestive protein allowed by nitrogen). The measured results were statistically evaluated and differences between averages were tested with the Tukey test.

Results and discussion
Dry matter (DM) production and corrected dry matter (CDM) production in the first harvest year 2012 DM production was significantly lower in the dry year 2012 in comparison with average rainfall years by about one third, especially in the growth of the 1st and 3rd cut. Average DM production in 2012 in the trial with red clovers was 7.14 t ha^{-1}, out of which 4.29 t ha^{-1} was CDM production of directly sown red clovers, in the trial with intergenus hybrids DM production was 6.81 t ha^{-1}, out of which 1.51 t ha^{-1} was CDM production of directly sown grasses. At the trial site in long-term trials with PK fertilization the original grassland produced 3.43 t ha^{-1} DM. Total DM production in 2012 (Table 1) in the trial with red clovers (zero fertilized) was 7.50 t ha^{-1} at T1 (CDM production 4.95 t ha^{-1}) at T2 it was 6.77 t ha^{-1} (CDM production 3.62 t ha^{-1}). In the trial with grasses the DM production (N fertilization: 180 kg ha^{-1}) 6.69 t ha^{-1} (CDM production 1.82 t ha^{-1}) at T1 and 6.92 t ha^{-1} (CDM production 1.19 t ha^{-1}) at T2. CDM of directly sown red clovers and grasses is conclusively higher ($P < 0.01$) at the first date of direct sowing in mid-June. There were not conclusive differences between red clover varieties in

DM and CDM production, DM production of 4n variety ´Amos´ was 7.02 t ha^{-1} (CDM production 4.20 t ha^{-1}) and of 2n variety ´Suez´ it was 7.25 t ha^{-1} (CDM production 4.36 t ha^{-1}).

There were no differences in total DM production between directly sown intergenus hybrids, the DM production of festucoid hybrid ´Felina´ was 6.75 t ha^{-1} (CDM production 0.77 t ha^{-1}) and of loloid hybrid ´Hostyn´ it was 6.86 t ha^{-1} (CDM production 2.25 t ha^{-1}, which is highly conclusive increase in comparison with ´Felina´, $P < 0.01$). Fast growing species are more suitable for direct sowing as penetrate the original sward better and resist adverse external influence.

In the trial with direct sowing technologies the DM production of red clovers was 7.43 t ha^{-1} (CDM production 5.44 t ha^{-1}) for SE – 2 – 024, 6.74 t ha^{-1} (CDM production 4.20 t ha^{-1}) for STP – 300 and 7.24 t ha^{-1} (CDM production 3.21 t.ha^{-1}) for PP – 2. Lower CDM production of strip sown red clovers was caused by a mole damaging 30 – 40 % rows after strip sowing in 2011.

In the trial with direct sowing technologies the DM production of intergenus hybrids was 6.21 t ha^{-1} (CDM production 1.08 t ha^{-1}) for SE – 2 – 024, 6.11 t ha^{-1} (CDM production 0,74 t ha^{-1}) for STP – 300 and 8.09 t ha^{-1} (CDM production 2.71 t ha^{-1}) for PP – 2. Highly conclusively higher DM and CDM production of strip sown intergenus hybrids is caused by a high proportion of loloid hybrid ´Hostyn´ on yield when CDM production was 4.15 t ha^{-1}, that is 2 – 3 times more than with other technologies and it reached the yield level of directly sown red clovers.

In the trial with red clovers average CP concentration in 2012 was 167.2 g kg^{-1} DM, fiber 201.3 g kg^{-1}, NEL 5.84 MJ kg^{-1} DM. In the trial with grasses average CP concentration in 2012 was 132.5 g kg^{-1} DM, fiber 252.9 g kg^{-1} DM, NEL 5.55 MJ kg^{-1} DM.

Conclusion

In the trial with direct sowing technologies the DM production of red clovers was 7.43 t ha^{-1} (CDM production 5.44 t ha^{-1}) for SE – 2 – 024, 6.74 t ha^{-1} (CDM production 4.20 t ha^{-1}) for STP – 300 and 7.24 t ha^{-1} (CDM production 3.21 t.ha^{-1}) for PP – 2. In the trial with direct sowing technologies the DM production of intergenus hybrids was 6.21 t ha^{-1} (CDM production 1.08 t ha^{-1}) for SE – 2 – 024, 6.11 t ha^{-1} (CDM production 0,74 t ha^{-1}) for STP – 300 and 8.09 t ha^{-1} (CDM production 2.71 t ha^{-1}) for PP – 2.

Acknowledgements

The contribution was made as a part of the research of the NAZV project QI101C199.

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Table 1: Dry matter production and forage quality from directly sown permanent grassland with legumes and grasses (Jevíčko, 2012)

Factor	(L) Legumes								Factor	(G) Grasses							
	DM	CDM	Forage quality							DM	CDM	Forage quality					
			CP	Fiber	NEL	NEV	PDIE	PDIN				CP	Fiber	NEL	NEV	PDIE	PDIN
	(t.ha^{-1})		(g.kg^{-1})	(g.kg^{-1})	(MJ.kg^{-1})	(MJ.kg^{-1})	(g.kg^{-1})	(g.kg^{-1})		(t.ha^{-1})		(g.kg^{-1})	(g.kg^{-1})	(MJ.kg^{-1})	(MJ.kg^{-1})	(g.kg^{-1})	(g.kg^{-1})
T1	7.50	4.95	169.8	202.0	5.81	5.72	86.2	99.5	T1	6.69	1.82	131.5	252.7	5.53	5.35	81.4	75.9
T2	6.77	3.62	164.5	200.5	5.86	5.78	85.3	97.2	T2	6.92	1.19	133.5	253.0	5.56	5.39	81.2	76.8
$D_{T0.05}$	0.76	1.14	5.5	6.4	0.08	0.10	0.8	3.4	$D_{T0.05}$	0.58	0.34	5.1	5.7	0.09	0.11	0.8	3.5
$D_{T0.01}$	1.03	1.55	7.5	8.7	0.11	0.13	1.1	4.6	$D_{T0.01}$	0.79	0.46	7.0	7.8	0.13	0.15	1.1	4.7
Amos	7.02	4.20	167.5	198.6	5.89	5.82	86.1	99.0	Felina	6.75	0.77	133.6	252.2	5.56	5.39	81.6	77.2
Suez	7.25	4.36	166.4	203.9	5.78	5.68	85.3	97.7	Hostyn	6.86	2.25	131.3	253.6	5.52	5.35	81.0	75.5
$D_{T0.05}$	0.76	1.14	5.5	6.4	0.08	0.10	0.8	3.4	$D_{T0.05}$	0.58	0.34	5.1	5.7	0.09	0.11	0.8	3.5
$D_{T0.01}$	1.03	1.55	7.5	8.7	0.11	0.13	1.1	4.6	$D_{T0.01}$	0.79	0.46	7.0	7.8	0.13	0.15	1.1	4.7
SE-2-024	7.43	5.44	168.7	200.1	5.85	5.77	86.0	99.5	SE-2-024	6.21	1.08	131.6	252.4	5.47	5.29	81.3	76.1
STP-300	6.74	4.20	169.7	199.2	5.91	5.83	86.5	100.7	STP-300	6.11	0.74	134.1	251.3	5.59	5.43	81.6	77.4
PP- 2	7.24	3.21	162.5	204.4	5.75	5.65	84.7	94.8	PP - 2	8.09	2.71	131.6	254.9	5.57	5.40	81.0	75.5
$D_{T0.05}$	1.12	1.69	8.2	9.5	0.12	0.15	1.2	5.1	$D_{T0.05}$	0.86	0.50	7.6	8.5	0.14	0.16	1.2	5.1
$D_{T0.01}$	1.45	2.18	10.6	12.3	0.15	0.19	1.6	6.5	$D_{T0.01}$	1.11	0.65	9.9	11.0	0.18	0.21	1.6	6.6

Crude protein fraction of lucerne in legume-grass mixture

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Abstract
The aim of the study was to determine the effect of the cut order and presence of grass component on crude protein (CP) and CP fractions in the forage of lucerne growing in monoculture or in the simple binary lucerne-grass mixture. In 2011, sampling was realized in field plot experiment over three cuts and samples were analyzed for CP content and CP fractions according to CNCPS. The CP and the soluble CP fractions were the significantly highest in the first cut whilst the insoluble CP fractions were the lowest. Direct effect of mixture's variants was lower; however it seems that increase of the grass component yield was related to increase of fraction A and B_3 in lucerne forage. Results also supported idea that effect of cut on CP fractions could be in connection with observed stand parameters such as stem length or density.

Keyword: forage, alfalfa, quality, nitrogen

Introduction
Forage legumes such as lucerne or red clover represent a major protein source for ruminant nutrition in Europe (Krawutschke *et al*., 2013). Is well known that these crops produce protein-rich forage however the proteins are extensively degraded into amino acids and ammonia in the rumen (Julier *et al*., 2003). Increasing protein escape from the rumen is a global issue and would provide an economic benefit to the dairy industry (Chen *et al*., 2009). The main objective of this study was to investigate the effect of presence of grass component in the lucerne-grass mixture on CP fraction in the lucerne forage over vegetation period.

Materisls and method
To test our hypothesis, we used a running experiment aimed at comparing four lucerne varieties (Zuzana, Jarka, Oslava, Tereza) in the monoculture (variant 100) and in lucerne grass mixture with grass hybrid Achilles (*Festulolium*) in the ratio of 10% (variant 90) and 25% (variant 75). Field plot experiment was established in the spring of 2008 at the experimental field station in the Červený Újezd (405 m above sea, temperature 7.7°C, and precipitation 493 mm). The plot experiment was arranged in completely randomized blocks with four replicates with plot size 2.5 x 7.2 m. In 2009 – 2011, three-cut regime was used and the total yield was determined from the area of 10 m^2 for each variant. CP fractions in lucerne forage were evaluated only at Jarka variety with three replicates in 2011. In each cut and plot, biomass was clipped in 4 cm height above the ground in the area 12.5 x 50 cm. The lucerne stem length of the longest stem (MSL), stem density (SD), and lucerne and grass dry matter yield (DMY) were assessed in each sample. These samples were oven-dried at 60 ºC, homogenised to a particle size of 1 mm, and analysed for CP fractions according to Licitra *et al*. (1996) into A, B_1 (soluble fractions), B_2, and insoluble fractions B_3 and C. Trichloroacetic acid was used to determine the non-protein nitrogen (NPN). The content of total nitrogen in the sample was determined by the Dumas method on Lecco analyser with calculation of crude protein (N*6.25). Two-way (cut, variant) ANOVA and principal component analysis were used for investigating of relationships among CP fractions, stand parameters, and tested variants.

Results and discussion
The result of CP concentration, CP fractions, and forage yield over three cuts and three variants are shown in Table 1. In regard to obtained values of CP fraction in our experiment, they were different from values reported by Kirchhof *et al*. (2010) which could be influenced by sample drying technique (oven-drying *vs.* freeze-drying). In the first cut, the CP content and the soluble CP fractions were the significantly highest in the first cut in contrast to second and third. Reversibly, the insoluble CP fractions were the lowest in the first cut, the highest in the third cut. This effect could be given by reached maturity stages where lucerne was in the bud stage in the first cut and in the full bloom stage in the second and third cut. According to Kirchhof *et al*. (2010) maturity stage did not affect the soluble CP fractions of the legumes but increased fraction C of lucerne. Krawutschke *et al*. (2013) also reported that the variation of CP fraction A (high soluble) was mostly positively related to N yield which is also in accordance with our results. The content of B_2 fraction was not affected either cut order or tested variant. In accordance with Kirchhof *et al*. (2010), this fraction represented the largest proportion of CP. The relationships among CP fractions, stand traits, and tested variants are shown in the Figure 1. The first axe represents the influence of cut (29 % of variability) where first cut labels are on the left side whilst second are in the figure center

and third on the right side. The second axe represents mainly effect of grass and lucerne yield of the samples (19 % of variability) where lucerne yield was related to MSL, SD and B_2 whilst grass yield in the sample was related to increasing ratio of fractions A and B_3.

Conclusion

With limited data from one year, it is possible to conclude that CP fractions in our experiment was more influenced by cut order than presence of grass component in the mixture, however it seems that increase of the yield of grass component was related to increase of fraction A and B_3 in the lucerne. Results also supported idea that cut effect on CP fractions could be in connection with observed stand parameters.

Table 1: Effect of cut and ratio of lucerne in the mixture (variant) on the total dry matter yield, content of crude protein (CP), soluble (A + B1) and insoluble crude protein (B3 + C) in forage of lucerne.

		Date	Lucerne stage	Yield (t/ha)	CP (g/kg)	Soluble CP (g/kg CP)	B_2 (g/kg CP)	Insoluble CP (g/kg CP)
Cut	1.	26. May	Late bud	6.42[a]	18.9 [a]	40.9[a]	48.5	10.6[a]
	2.	13. July	Late bloom	4.69[b]	18.3 [ab]	34.1[b]	53.0	12.9[b]
	3.	6. October	Late bloom	3.76[c]	16.8 [b]	32.7[b]	53.6	13.7[b]
	P			<0.001	0.014	0.006	0.105	0.005
Variant	100			4.83[a]	17.6	34.8	52.9	12.3[ab]
	90			4.95[ab]	18.4	35.8	53.0	11.2[a]
	75			5.10[b]	18.0	37.1	49.1	13.8[b]
	P			0.025	0.499	0.610	0.212	0.021

P = probability of F test, different letters document statistical differences in each column (Tukey HSD, α = 0.05)

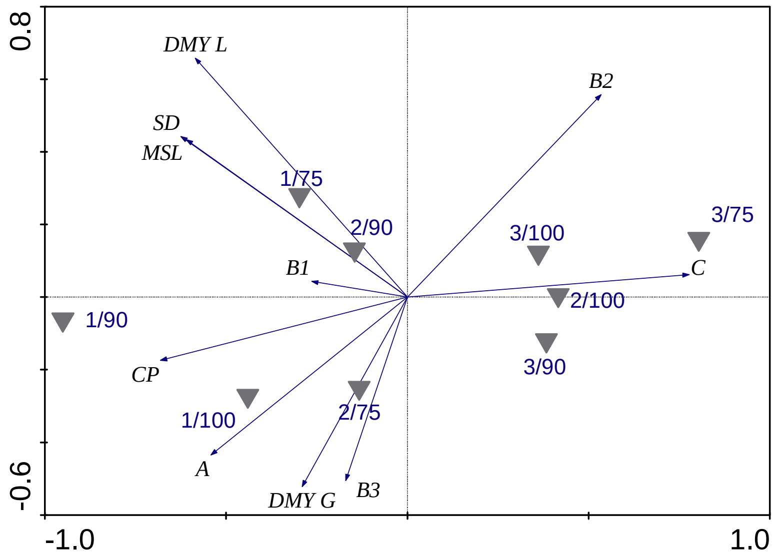

Figure 1: Biplot of Principal Component Analysis showing relation among investigated variables (presented by arrows; CP = crude protein; A, B1, B2, B3, C = fractions of CP; DMY = yield, MSL = maximal stem length, SD = stem density, G = grass, L = lucerne) and supplementary variables (presented by triangle labels; 1,2,3 = order of cut; 100, 90, 75 = lucerne sowing ratio in the mixture).

Acknowledgement

Research was supported by “S” grant of Ministry of Education, Youth and Sport of the Czech Republic.

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Section 2: Fermentation process of forage - harvest, preservation and storage

Managing the aerobic stability of silages

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Introduction

Woolford (1990) stated that "the single most important factor which influences the efficiency with which forage crops are conserved as silage is the degree of anaerobiosis achieved in the completed silo". Rapid removal of air from the forage mass and the ability to prevent air from infiltrating the silage mass during storage and feed out can have profound effects on feed quality. Excessive exposure to air at the start of fermentation prolongs the metabolism of unwanted microbes that thrive in air and delays the growth of beneficial bacteria that produce lactic acid. This can lead to undesirable fermentations and a loss in nutritive value. However, we should also remember that anaerobiosis is also important for maintaining the quality of silage during storage and feed out. Prolonged infiltration of air during storage or feed out into the silage mass can lead to aerobic spoilage. Silage that is unstable when exposed to air heats rapidly and spoils, leading to a loss of DM and nutrients with the potential for production of undesirable compounds. Aerobic spoilage during storage often is responsible for a large portion of the total DM lost in forage conserved as silage and may be as high as 15 to 25% or higher in areas of poor compaction. This review will focus on the processes involved during aerobic spoilage of silages, why aerobic spoilage is undesirable and ways to improve aerobic stability.

Aerobic spoilage of silages

When the active stage of ensiling is complete the remaining microorganisms in the mass are relatively dormant because of the low pH and absence of oxygen. However, if silage is exposed to air, the result can be a chain reaction resulting in aerobic spoilage (Figure 1). Specifically, yeasts that are able to degrade lactic acid in the presence of air usually initiate this process. Examples of these organisms include *Candida krusei (Issatchenkia orientalis)* and *Pichia membranifaciens (C. valida)* (Woolford, 1990, Inglis et al., 1999). Yeasts able to metabolize sugars (e.g. *Saccharomyces*) are also active and can add to the spoilage process. Aerobic microbial activity causes oxidation of nutrients resulting in the production of heat. Degradation of lactic acid specifically causes an increase in pH of the silage to a level that allows opportunistic bacteria (e.g. *Bacilli)* and molds (e.g. *Aspergillus*, *Fusarium*, and *Pencillium*) to then become active, furthering the spoilage process (McDonald et al., 1991). Of particular concern is the development of pathogenic bacteria and molds in aerobic spoiling silages because of their potential for producing mycotoxins and causing other detrimental effects. For example, silages sampled from the top layers of silos (where pack density was poor) and silage that was loose on the bunker floor had higher levels of *A. fumigatus* than silage sampled from the intact silo face (Prince Agri, 2007). This is of particular concern because this organism has been linked with hemorraghic bowel syndrome in ruminants (Forsberg and Wang, 2006). In some cases, bacteria from the genus, *Acetobacter* may initiate aerobic spoilage in maize silages (Spoelstra et al., 1988).

Grain crops (e.g. whole crop barley silages, maize silages, high moisture maize-grain silage) are very prone to aerobic spoilage. Sugarcane silage is also extremely prone to aerobic deterioration because it has high numbers of epiphytic yeasts and highly fermentable substrate (sucrose) (Pedroso et al., 2005). Epiphytic populations of yeasts are found on all forage crops in the field. However, their numbers are not well correlated to aerobic stability because there is a mix of non-fermentative and fermentative species present. The ensuing fermentation and level of silage management ultimately determine the number of lactate-assimilating yeasts that may survive ensiling. A list of some factors affecting the aerobic stability of silages is shown in Table 1. Porous silage masses, breaks in integrity of plastic and the amount of antifungal compounds added at ensiling, or produced during fermentation have profound effects the number of yeasts in silages. High concentrations of lactic acid and/or a low pH have minor affects on the numbers of yeasts in silages as these organisms are relatively acid tolerant and lactic acid has poor antifungal characteristics. In contrast, relatively high concentrations of acetic and/or propionic acids usually reduces the numbers of yeasts in silages because of their antifungal properties (Woolford, 1975). The concentration of acetic acid in silages can be especially high in silages with high moisture contents because the microorganisms that can produce this acid in silages (e.g. enterobacteria and heterolactic acid bacteria) thrive in wet conditions (more than 70% moisture). Thus, wet silages with high concentrations of antifungal organic acids tend to have low numbers of yeasts and are relatively stable when exposed to air. Ironically, one of the most antifungal acids sometimes produced in high moisture silages is butyric acid. This end product of clostridial fermentation is very active in inhibiting the growth of yeasts but is certainly undesirable because of the other detrimental factors associated with this type of fermentation (i.e. large dry matter loss and degradation of protein). Conversely, low moisture silages (less than 60% moisture) undergo restricted fermentations and thus produce low concentrations of organic acids and also pack poorly, often resulting in high numbers of yeasts. Ammonia also has good antifungal activity but it is doubtful that natural concentrations of this compound effects populations of yeasts in silages (see later discussion on ammonia additives). Numbers of yeasts can be reduced in silages via the direct addition of antifungal compounds (e.g., blends of buffered organic acids) or from microbial inoculation (to be discussed later).

The ambient temperature around the silage mass affects the rate of aerobic spoilage. When temperatures are very cold, microbial activity is slowed or even stopped (e.g. in freezing weather). Warm temperatures stimulate microbial activity and thus, aerobic spoilage and it is the primary reason that more spoilage typically occurs in the summer than in the winter. High concentrations of residual sugars in silage can also lead to a higher probability of aerobic spoilage.

Impact of feeding aerobically spoiled silages to ruminants

Few studies have been conducted evaluating the effects of feeding aerobically spoiled silages to ruminants. Whitlock et al. (2000) reported that feeding spoiled corn silage from the surface of a bunker silo depressed DM intake as the level of spoiled feed in the diet increased from 0 to 16% of the ration dry matter. Recently, Windle and Kung (2013) reported that heifers fed a spoiling TMR consumed less dry matter than those fed a fresh total mixed ration (TMR). In contrast the intake of cows that were fed a TMR containing aerobically spoiling high moisture corn was unaffected when compared to those cows fed fresh corn but the former produced 3.2 kg less milk per cow (Hoffman and Ocker, 1998). When animals consume spoiled silages, the exact causes of reduced intake and/or performance are not fully understood. In the study of Whitlock et al. (2000) reduced DMI probably occurred because of lower nutrient digestibility of the silage. However, in the study of Windle and Kung (2013), the nutrient composition of the diets was very similar and could not obviously explain the differences in observed intake. One major difference between diets was that the fresh TMR contained 5.0 log yeasts/g whereas the spoiled diet contained 7.8 log cfu of yeasts/g. Santos et al. (2011) added various levels of a pure culture of *I. orientalis* to *in vitro* ruminal fermentations and reported lower NDF digestibility as the amount of yeast in the culture increased suggesting that undesirable spoilage yeasts may have direct effects on ruminal fermentation. Gerlach et al. (2012) reported negative correlations between ethyl lactate and ethanol with dry matter intake in goats but the strongest negative relationship with intake was from silage temperature (as difference to ambient). Other variables that may contribute to depressions in intake include the growth of molds in spoiled silage that may result in the production of mycotoxins and effects of microbes or compounds on immune functions. Organoleptic properties (e.g., taste and smell) of spoiled feeds on intake have not been well studied. In addition to negative of effects on animal performance, spoiled silages also potentially present a contaminant to the environment if the feed is spoiled to the extent that it must be discarded.

Improving aerobic stability via silo management

Filling silos quickly with sufficient pack weight to maximize silage density and minimize porosity can minimize oxygen in a silo. Even distribution of forage in the storage structure, chopping to a correct length and ensiling at recommended dry matters (DM) for specific storage structures aids in this process. After filling, silage should be covered with plastic as soon as possible and weighted down with tires (tires should be touching) or gravel bags to exclude air. Split tires are good alternative because they are easier to handle, do not accumulate water (thus less breeding grounds for mosquitoes that could carry the West Nile Virus), and are undesirable for animals to nest in. The return on investment (labor and plastic) is extremely high for covering bunk and pile silos (Bolsen et al., 1993). Oxygen barrier plastics with low transmission rates for oxygen appear to be useful in minimizing the loss of nutrients at the silage/plastic interface (Borreani et al., 2007). This practice can also reduce the number of yeasts in silages and improve aerobic stability.

Proper management for removal of silage from silos at the feed bunk with the use of mechanical equipment (e.g., block cutters and silo facers) can help producers to maximize profits and production. Enough silage should be removed between facing to minimize aerobic spoilage. Lesser amounts may be removed in areas where ambient temperatures remain cool during the winter months. Removal of silage should be such to minimize disruption of the silage face and loose silage on the ground between feedings. Extreme care should be taken to prevent air from penetrating between the plastic and reaching the silage mass during feed out and storage and this can be accomplished by stacking tires, or lining gravel bags on the plastic at the leading edge of the feeding face.

Improving aerobic stability with additives

Chemical additives. Various chemical additives with antifungal properties have been used to enhance the aerobic stability of silages. The most common are the organic acids. For example, buffered propionic acid-ased products are commonly used in North America because of they are less corrosive and safer to handle than the straight acid. It is the undissociated (protonated) form of organic acids that is responsible for their antifungal properties and its prevalence is dependent on pH. This fact unfortunately means that more acid is needed to be effective in crops that are naturally limiting in acids from silage fermentation (e.g. crops with more than 40% DM). At the pH of a standing crop of lucerne (about 6) only about 1% of propionic acid is in the undissociated form whereas, at a pH of 4.8, about 50% of the acid is undissociated. The undissociated acid functions both by being able to penetrate into microbial cells and disrupt cytosolic functions because of the release of H^+. Undissociated acids also remain active on the surface of microorganisms and compete with amino acids for space on active sites of enzymes and by altering the cell permeability of microbes. Application of buffered propionic acid-based products in North America ranges from about 0.5 – 2 kg/t of wet forage depending on the specific situation. In previous studies, we have found that, as expected, the effectiveness of propionic acid based additives increases with higher application rates (Kung et al., 1998; Kung et al., 2000). In the past, anhydrous ammonia (about 3 kg/t of forage) was used in North America as an inexpensive way to increase the N content of maize with the added benefit that it had very good antifungal properties. However, because of safety concerns and cost, this practice is now uncommon. Potassium sorbate and sodium benzoate have also been used to improve the aerobic stability of maize silages. For example, Kleinschmit et al. (2005) reported that 0.1% potassium sorbate-EDTA and 0.1% sodium benzoate were as effective in increasing the aerobic stability of maize silage as treatment with Lactobacillus buchneri 40788 applied at 400,000 cfu/g of forage. Treatment with 0.1% potassium sorbate also improved dry matter recovery and aerobic stability and lowered the final concentration of ethanol in maize silage (Teller et al., 2012). Kincky and Sporndly (2011) reported that an additive containing sodium benzoate, potassium sorbate, and sodium nitrate improved the aerobic stability of a variety of crops with DM > 35%. We recently have shown that the same additive was effective in reducing the concentration of ethanol and improving the aerobic stability of maize silage and high moisture maize grain under North American conditions (research at the University of Delaware).

Microbial inoculants. Bacterial inoculants, based on homofermentative lactic acid bacteria are commonly added to silages to improve fermentation and increase DM and energy recovery. However, most of these inoculants are not very effective in inhibiting the growth of yeasts because they tend to maximize the production of lactic acid (poor antifungal activity) and decrease the accumulation of other organic acids that have good antifungal activity. Muck and Kung (1997) summarized the literature and found that treatment with classical homolactic acid-based inoculants improved aerobic stability about one third of the time, had no effect about one third of the time but made aerobic stability worse about one third of the time.

Lactobacillus buchneri, an obligate heterolactic acid bacterium, has been used as a silage inoculant to specifically enhance the aerobic stability by converting moderates amount of lactic acid to acetic acid in a ariety of silages (e.g. maize silage, sorghum silage, barley silage, lucerne silage, ryegrass silage, orchard grass silage, etc.) (Muck, 1996, Dreihuis et al., 1999a, Kung and Ranjit, 2001, Kleinschmit et al., 2005). Kleinschmit and Kung (2006) conducted a meta-analysis on studies evaluating the effects of *L. buchneri* on the aerobic stability of maize silage. The effects of *L. buchneri* were divided into a low level of addition (≤ 100,000 cfu/g addition, LB1) or a high level of addition (>100,000 cfu/g addition, LB2). The increase in acetic acid due to inoculation with *L. buchneri* was greater as the application rate increased in both types of crops. However, the increase was of a moderate nature as the concentration of acetic acid was only 3.89% even with the highest level of application in maize silages. (Note that this increase was the equivalent of adding about 6 kg of acetic acid/t of forage.) Treatment with LB1 also resulted in a 10-fold decrease in numbers of yeasts (3.10 log cfu/g of silage) compared to the untreated silage (4.18 log cfu/g of silage) and treatment with a high level of application (LB2) decreased the numbers of yeasts by more than 100-fold (1.88 og cfu/g of silage). Associated with these lower numbers of yeasts was in an improvement in aerobic stability but the effect was markedly greater in silage treated with the higher application rate (25, 35, and 503 h of aerobic stability for untreated, LB1, and LB2, respectively). In maize silage, the changes in lactic and acetic acids resulted in a decrease in the ratios of lactic to acetic acid in untreated maize silage from approximately 3.0:1 to 2.3:1 and 1.3:1, for the low and high dose of *L. buchneri,* respectively. Practical recommendations in the field have suggested a desirable lactic:acetic ratio of more than 3:1 (Kung and Stokes, 2001), which would be an indication of a more dominant homolactic fermentation. However, it is now evident that silages treated with *L. buchneri* should not be held to this standard.

Concerns relative to the potential of large losses of DM from silages treated with *L. buchneri* because of its heterolactic nature have not been substantiated (Kleinschmit and Kung, 2006). The loss of DM in maize silage by the higher application of *L. buchneri* was 1 percentage point more than for untreated silage. Relative to the potential beneficial effects of improved aerobic stability during storage and feeding, this loss is small. Although some have suggested that high levels of acetic acid in silages may depress intake, research studies have shown that ruminants fed silages treated with *L. buchneri* consume the same amount of DM when compared to counterparts fed untreated silages (Dreihuis et al., 1999b, Kung et al., 2003, Ranjit et al., 2002, Taylor et al., 2002). Most research on improving the aerobic stability of silages has dealt with the stability of the silages alone. However, there is good evidence that if silages are stable this benefit is transferred to the TMR. In two studies, TMR that were made with silages treated with *L. buchneri* were more stable than TMR made from untreated silages (Kung et al., 2003, Taylor et al., 2002).

When Should Additives Be Used? A question that is often asked is when should products that specifically provide enhanced aerobic stability be used in silages? Such additives may be used to treat historic problems of silages heating in the silos (over sizing, slow feed out rate, poor packing and filling). Maize silages or high moisture maize that will be stored for prolonged periods of time (more than 6-9 months) or be fed during warm weather are other good candidates for treatment. Drier silages that may be challenged because of restricted fermentations and poor packing density may be candidates for additives that improve aerobic stability. Consider treating specific silos or parts of a silo relative to summer feeding. Although some may argue that treating an entire silo may not be justifiable if the problem occurs for only a few weeks out of the year, it is extremely difficult to predict in advance the feeding challenges from a specific silo.

Improving Aerobic Stability in Moved Silage. In certain areas of North America, silages are moved between storage structures because of the need to mix silage in a feeding center. In addition, on many large dairies it is now common to find several days worth of silage fed from temporary piles (brought in from other farms or silos and stored at a staging area). In both of these instances, the chance of aerobic spoilage is increased especially in warm weather. Moving silages quickly and in cool weather minimizes the potential for aerobic spoilage. For moved silage and feeding piles, addition of chemical preservatives that contain antifungal compounds (e.g., buffered propionic acid, sorbates, benzoates, acetic acid, etc.) can be added at the time of moving to enhance stability (0.1 to 0.2%). A better practice would be to consider treating these silages at the time of ensiling with an additive to enhance aerobic stability (e.g. chemical additives or *L. buchneri*). Microbial-based additives and ammonia are ineffective on forages that have already fermented.

Improving Aerobic Stability in TMR. Because silages are often incorporated into TMR, their stability is also an issue on many farms. In a small survey of TMR sampled in DE, PA and MD over two years, more than 50% of 30 TMR (primary silages of maize and Lucerne) that were sampled within 1 hour of being made, spoiled in less than 12 h when incubated at a controlled laboratory temperature of about 22°C (Kung, Mulrooney and Morges, unpublished data Univ. of Delaware). These TMR would have spoiled even quicker if they were incubated at the ambient temperatures encountered during an average summer day (high 27 to 32 °C). Thus these TMR had the potential to spoil in the feed bunk even if the farms were feeding twice daily. If nothing can be done to alleviate the primary causes(s), additives (commonly referred to as “TMR-savers”) containing antifungal compounds can be added directly to the TMR to improve aerobic stability. The degree that silages have spoiled in the silo and ambient temperatures will determine the doses required to stop further spoilage in the feed bunk (0.2 to 0.4% of additive per t of TMR may be required to prevent further spoilage in challenging conditions). When using TMR-savers, it is best to start with a high dose for several days. If stability in the bunk has been achieved, a lower level can be used that keeps the TMR from heating in the bunk. TMR-savers can be helpful but they are not economical for long term use because the rates of addition are very high. For example, even added only at 2 kg/t of TMR, the equivalent would be adding 4 kg/t of the product per ton of forage. In addition, stopping further heating and spoilage in the feed bunk does nothing to stop the initial heating and loss of nutrients that may have occurred in the silo. Data from our lab suggests that it is better to control yeasts at that time of ensiling rather than after the fact in a TMR. The more yeasts that are present in the silage and TMR, the higher the dose of a TMR-saver will be needed to keep the feed from spoiling.

Diagnosing Problems with Aerobic Stability. Because silages heat during aerobic spoilage, the length of time that silage remains cool when it is exposed to air is often used as a measurement of aerobic stability. Many research studies determine aerobic stability by assessing the time it takes for a silage mass to increase about 2 °C above ambient or baseline temperatures after it is exposed to air. On the farm, warm silage is not always an absolute indicator of spoilage because large silos often retain relatively high core temperatures even in the winter. In a recent survey, we noted core silage temperatures as high as 32-33 °C in some silos for as long as three months. Thus, steam coming from the silage mass during silo removal is not necessarily a sign of aerobic spoilage. In a recent survey, we have noted core silage temperatures as high as +32-33 °C in some silage for as long as 90 d. In contrast, aerobically spoiled silage can often reach temperatures as high as 48-50 °C for short periods of time. Signs that silage is aerobically spoiling include measuring temperatures in excess of about 42-43 °C 10 to 20 cm in back of the silo face at feed out, reheating in the bunk, visible mold, lack of a sharp or sweet smell to the silage and/or a flat or moldy/musty smell. If a pH meter is available, a moldy smell coupled with a high pH may also be a good indicator that a feed has undergone aerobic deterioration. Relatively inexpensive temperature probes can be used to monitor temperatures in silage piles.

The number of yeasts and molds in a silage sample can sometimes be used to assess whether a silage sample has spoiled or has the potential for rapid spoilage. Care should be taken to ensure that the sample is representative of that being fed. In addition, samples for microbial analyses should be kept refrigerated (not frozen) and sent to the laboratory as quickly as possible (preferably stored with ice packs). This will minimize the growth of yeasts and molds that could grow during transit. Unspoiled silages usually contain about 1,000 to 250,000 yeasts per gram of wet silage. Samples containing more than 500,000 yeasts per gram have a high probably or spoiling rapidly in warm weather and/or have already started to aerobically deteriorate. It is not uncommon to find spoiled silages with more than 100,000,000 yeasts/g of silage.

Visible signs of molds, lack of a sharp or sweet smell to the silage and/or a flat or moldy/musty smell are also indicators of aerobic spoilage. If a pH meter is available, a moldy smell coupled with a high pH may also be a good indicator that a feed has undergone aerobic deterioration.

Measuring the production of CO2 and the time it takes for silage pH to rise have also been used to assess aerobic stability but these techniques are not well suited for use on the farm.

Conclusions

Aerobically spoiled silage is undesirable because of losses in nutrients and potential negative effects on animal performance and health. Good silo management and the use of various additives can help to minimize the incidence of aerobically spoiled silage.

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Figure 1. The process of aerobic spoilage in silages.

Silage is exposed to air
↓
Lactate assimilating yeasts are revived
↓
Silage begins to heat
↓
Degradation of lactic acid results in an increase in silage pH
↓
Readily digestible nutrients are destroyed
↓
Molds and bacteria continue the spoilage process
↓
Massive nutrient losses

Table 1. Some factors that may make silages more prone to aerobic spoilage.

Factor	Effects	Examples
High sugar content or high natural population of yeasts	Yeast use sugars as energy sources during fermentation	a) sugarcane
High DM content	High DM restricts fermentation and reduces acids that could minimize the numbers of yeasts High DM crops are more difficult to pack and allow infiltration of air into the mass	Lucerne ensiled > 45 to 50% DM Maize silage ensiled > 40% DM
Poor pack density/porosity	Allows penetration of air into the silage mass	Fill rate too fast Insufficient tractor weight
Poor feeding management	Allows penetration of air into the silage mass	Slow silage removal Loose silage Uneven silage face Intermediate feeding piles Moved silage
Poor management of plastic and weights	Allows penetration of air into the silage mass	Torn bag silos Torn silo covers Insufficient weight on plastic Plastic pulled back too far in advance
High ambient temperatures	Spoilage organisms grow faster in warmer weather	More spoilage in the summer than winter months
Addition of spoiled feeds to a TMR	Spoiled feeds bring spoilage organisms to the TMR	Spoiled wet distillers grains
Overly dominant homolactic acid fermentation	Limited production of organic acids that have antifungal properties	An extremely dominant homolactic acid fermentation caused by microbial inoculation

Silage additives – assessment of their efficiency to improve aerobic stability of silages

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Abstract
Precision chopped whole crop maize was used in a study to compare the effect of silage additive based on Na-nitrite, Na-benzoate and K-benzoate on silage quality and aerobic stability of silages ensiled in two ensiling conditions; regular and difficult. Ensiling conditions differed in packing density of forage and in air tightness of silos. Additive treated silages were found to have reduced formation of ammonia-N, acetic acid and ethanol compared with untreated control silages in both ensiling systems. Significantly eliminated yeast growth was reflected in considerably increased stability of additive treated silages only in difficult ensiling condition. Testing of silage additive for aerobic stability under difficult ensiling condition provided a better answer about a potential of the product in the agricultural practice.

Introduction
Aerobic instability is still a common problem of many types of silage, particularly of these well fermented. Experimental testing of silage additives is commonly conducted under routine ensiling condition with properly consolidated forages and silos properly sealed. Consequently, it is not surprising that results of these trials do often not display a potential of a product in the agricultural practice. Punctures and other damages of silo cover as well as uneven forage consolidation in a silo are common. These defects cause that ensiling condition becomes more difficult. Such ensiling condition challenges a silage additive to fulfil a purpose of being applied. It has been suggested that silos which were not tight under fermentation process are more prone to be aerobically unstable (Jonsson & Pahlow, 1984). Using this suggestion, German system for evaluation effects of silage additives (DLG, 2009) applies a design where silage additives are tested under difficult ensiling condition. This condition challenged by a low packing density and limited air leakage into silo during storage is used to test silage additive on improvement of aerobic stability of silages. The objective of the study was to compare the impact of regular ensiling condition with such challenged condition on silage quality and particularly aerobic stability during silage additive testing.

Materials and Methods
Whole crop maize harvested by Class Jaguar 690 at dough stage of maturity was divided into 2 fractions; one forage fraction was left untreated and was used as control and another treated with silage additive (Na-nitrite, Na-benzoate and K-benzoate) at the rate of 3 ml per kg FM. Crops were ensiled in two ensiling conditions following DLG designs (DLG, 2009): Regular condition with packing density of 160 kg DM/m^3, silos tightly sealed with a fermentation lock. Difficult ensiling condition comprised crops packet to density of 114 kg DM/m^3, silos and lids were obtained with inlets with the rubber stoppers to allow air ingression into silos, which 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
The formation of ammonia-N, lactic acid and ethanol was significantly higher but acetic acid a lower in regular ensiling condition than in difficult condition (Table 1). Additive treatments in both ensiling systems contained less ammonia-N, acetic acid, ethanol and number of yeasts compared with untreated control treatments. Moreover, additive treatments significantly reduced weight losses during whole ensiling period in both ensiling conditions. As expected, microbiological analyses revealed remarkably a higher count of lactate assimilating yeasts in untreated control silages in difficult ensiling condition than in regular one. As a consequence of that, aerobic stability of silages showed that it took significantly less time for untreated control silages to increase about 3 ˚C than additive treated silages only in difficult ensiling condition. Results give the presumption that testing of silage additive for aerobic stability under difficult ensiling condition provided a better answer about a potential of the product in the agricultural practice.

Table 1: Chemical and microbiological compositions of silages at different ensiling conditions (n=3).

Treatment	DM	pH	NH_3-N	Lactic acid	Acetic acid	Butyric acid	2.3-butan diol	Ethanol	Weight losses	Hours until temp. aerated silages increased by 3˚C (max. temp increase)	Lactate yeasts
			% of TN	% of DM							lg cfu/g FM
Regular ensiling condition										Ambient temp.: 19.9 ˚C	
Control	38.6	3.9	9.3	5.4	1.5	<0.05	0.11	0.6	2.3	148 (2.4)	2.3
Additive	39.2	3.9	4.0	5.5	1.2	<0.05	0.10	0.4	1.8	164 (1.0)	<1.7
Difficult ensiling condition											
Control	38.9	3.9	8.4	5.1	1.6	<0.05	0.12	0.5	2.6	22 (20.2)	5.7
Additive	38.3	3.9	2.9	5.3	1.3	<0.05	0.09	0.3	1.8	164 (1.4)	<1.7
$LSD_{0.05}$		**0.03**	**0.58**	**0.43**	**0.23**	-	-	**0.08**	**0.72**	**76.9**	**1.58**
P-condition		***	***	***	***	N.S.	N.S.	***	N.S.	***	***
P-additive		*	***	*	*	N.S.	N.S.	***	***	***	***
P-inter.		***	N.S.	N.S.	N.S.	N.S.	N.S.	***	N.S.	***	***

*, ** and *** at P<0.05, P<0.01 and P<0.001, respectively;
N.S. – Not significant

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Fermentation Characteristics and Aerobic Stability of Whole Crop Maize Inoculated with Homo-and heterofermentative LAB and Ensiled in Hermetic Plastic Big Tubes

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Abstract
Homo and hetero LAB (*Lactobacillus plantarum* DSM16568 and *Entrococcus faecium* NCIMB 11181/ DSM 22502 and heterofermentative LAB strain *Lactobacillus buchneri* (CCM 1819/ DSM 22501)-based inoculant improved significantly the fermentation quality of the whole crop maize silage ensiled in a big tube by significantly reduced pH value 2 days (4.00 *vs* 3.65) and 156 days (3.73 *vs* 3.60) after ensiling, by significantly increased concentrations of lactate (33.4 *vs* 36.5 g/kg DM) and acetate (17.1 *vs* 20.3 g/kg DM) and by significantly decreased butyrate (0.5 *vs* 0.3 g/kg DM). The moulds count was reduced ($P < 0.05$) in the inoculated silage with 1.15 log10 cfu/g compared with the uninoculated silage with 1.21 log10 cfu/g. The aerobic stability of the inoculated silages (laboratory scale measurement) has improved by 68 hours (2.8 days) compared with the untreated silage. The surface area covered with moulds in control big tubes was 4 -5 times greater than that in the inoculated big tubes.

Introduction
Bacterial inoculants were topical during the last two decades and their potential to improve silage fermentation (Filya at al., 2007), and animal productivity was widely demonstrated (McAllister et al., 1998; Contreras-Govea et al., 2009). However, even when satisfactory preservation under anaerobic conditions has been attained, exposure to air, particularly during feed-out, may result in aerobic growth of yeasts and fungi which results in DM and nutritional value losses. Heterolactic fermentation is less efficient in the conservation of nutrients than homolactic fermentation, but it produces acetic acid that is a more potent antimycotic agent than lactic acid (Danner et al., 2003).

The current study was designed to examine the effect of silage additive containing homofermentative LAB strains *Lactobacillus plantarum* DSM16568and *Entrococcus faecium* NCIMB 11181/ DSM 22502 and heterofermentative LAB strain *Lactobacillus buchneri* (CCM 1819/ DSM 22501) on fermentation end-products, DM loss, aerobic stability and mould developement of whole crop maize silage ensiled into an hermetic plastic big tube.

Materials and Methods
Whole crop maize, cultivar Baxxos FAO 200 was harvested and chopped in the dough stage of maturity (34 % DM) with a maize harvester “CLASS JAGUAR 840” to a length of <2-5> cm. Immediately after harvesting, the chopped forage was carted from the field to the silaging and storage area and was ensiled into an hermetic plastic big tube, using Murska tube bagging equipment with a loading tunnel can and additive applicator. During the filling of the plastic big tube, one control bag (made from four layers cheesecloth) filled with 1 kg ensiling mass was inserted into each big tube for determining DM losses. Hermetic plastic big tube silages were prepared without inoculants (control - T1) or with blend of the bacterial strains SiloSolve AS200 (T2). The inoculant was dissolved in tap water (2 g inoculant/tonne of fresh forage) and applied at rate of 4 liter suspension per tonne forage according to the label instructions for application targeting a dosage of $1.5x10^5$ CFU/g of fresh forage. The same volume (4 l/tone forage) of tap water was used instead of the suspension in the control treatment (for spontaneous fermentation). The inoculant was applied during the big tube filling process. Ten hermetic plastic big tube silages for each treatment were prepared.

Representative samples of the whole crop maize were taken (5 samples 500 g each) at the big tube filling time. Big tube silages (10 from each treatment) were sampled after 156 days of storage for chemical and microbial analyses and for laboratory aerobic stability test.

Results and discussion
Whole crop maize had a DM content 339.6 g/kg, crude protein concentration of 98.7 g/kg DM and water soluble carbohydrates concentration of 88.3 g/kg DM. Buffer capacity of herbage was law (20.6 mEq 100g DM). Based on the WSC content of the herbage prior to ensiling, the forage was considered to be moderately easy to ensile (35.0 g/kg fresh matter of WSC) according to the EFSA opinion on silage additives guidelines (European Food

Safety Authority 2008). The pH of the whole crop maize was 5.4 and the nitrate concentration averaged 253.2 mg/kg DM.

The inoculant treatment resulted in significant (P < 0.05) pH drop after 2 days and after 156 days of ensiling compared with the untreated control (Table 1). The inoculated silages had higher (P < 0.05) concentration of lactate and higher (P < 0.05) concentration of acetate than the untreated silage. The lactate:acetate ratio was decreased in the inoculated silages (1.80:1) if compared with the untreated silage (1.95:1). Moulds were reduced (P < 0.05) in the inoculated silage (1.15 log10 cfu/g) compared with the untreated silage (1.21 log10 cfu/g). The aerobic stability of the inoculated silages has improved by 68 hours (2.8 days) compared with the untreated silage.

Table 1: Effect of inoculant treatment on the fermentation variables of ensiled in a big tube whole crop maize

Variables	Control silage	Inoculated silage	Average
DM, % desiled	32.0	32.3	32.1
DM corrected, % desiled	32.9	33.4*	33.18
DM loss, %	8.7	7.4*	8.07
WSC, % DM	0.83	0.82	0.83
pH 2 days after ensiling	4.00	3.65*	3.83
pH 156 days after ensiling	3.73	3.60*	3.67
N-NH_3 fraction, % total N	3.27	3.1	3.19
Lactic acid, % DM	3.34	3.65*	3.50
Acetic acid, % DM	1.71	2.03*	1.87
Butyric acid, % DM	0.05	0.03*	0.04
Propionic acid, % DM	0.01	0.01	0.01
Alcohols, % DM	0.58	0.53	0.55
Moulds, log CFU/g fresh silage	1.21	1.15*	1.17
Aerobic stability, hours	100	168*	134

**statistically significant difference vs Control p<0.05*

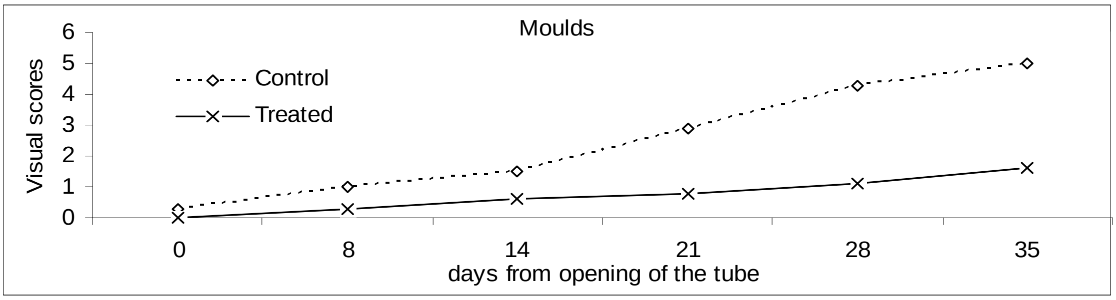

Figure 1: Dynamic of visible signs of moulds on the surface of undisturbed big tube maize silage during 35 day exposure to air

The surface area covered with moulds in control big tubes was 4 -5 times greater than that in the inoculated big tubes (Figure 1). The decreased number of molds compared with the control supports the finding that the aerobic stability has improved in inoculated silage compared with the untreated one. These findings are in agreement with those of Jaakkola et al. (2010).

Conclusions

Homo and hetero LAB-based inoculant improved significantly the fermentation variables of the whole crop maize ensiled in a big tube by reducing pH value, by increasing lactate and acetate concentrations and by decreasing lacate:acetate ratio and butyrate formation and aerobic deterioration. Mould count and visible signs of moulds on the surface of undisturbed big tube were significantly lower after removing of the plastic cover and during 35 day exposure to air.

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Acknowledgement
The authors would like to acknowledge the support from Chr. Hansen A/S

The effect of Maize-All+ on the fermentation and aerobic stability of sorghum silage in Slovakia

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Keywords
maize, yeast, mould, aerobic stability, Maize-All+, *Propionibacterium acidipropionici*

Introduction
The role of silage additives has long been to enhance the fermentation of silage by outcompeting epiphytic microflora and preserving maximal levels of the ensiled feed value and dry matter content (Ohyama). Aerobic stability of silage is detrementally affected by yeast and aerobic exposure (Woolford). Historically, if improvement in the aerobic stabillity of silage was required through the use of inoculant then *Lactibacillus buchneriI* was the only realistic option. Filya and Sucu (2007) demonstrated the ability of *Propionibacterium acidipropionici* to convert lactic acid and glucose to the more antifungal propionic and acetic acids, thus enhancing aerobic stability, and with advancements in bacterial production methods *P acidiproponici* is now a realistic commerical option for aiding the stability of ensiled corn & sorghum. Their findings have been further verified through the EU approval of *Propionibacterium acidipropionici* MA 26/4U to enhance the aerobic stability of all forage types. Work by Foltanova and Marley (2014) has shown the improved dry matter recovery and aerobic stability of corn silage treated with Maize-All+ across Europe. This trial was designed to assess the effect of Maize-All+ on sorghum silage

Material and methods
Sorghum was directly harvested using a Claas Jaguar 890 to a theoretical chop length of 10mm at a 26% dry natter. Harvested sorghum was untreated and ensiled in a concrete walled bunker. A 150Kg aliquot of untreated sorghum was homogenised and split into 2 piles. Maize-All+ (*Propionibacterium acidipropionici* MA26/4U 1.5 x 10^5 cfu/g + *Pediococcus acidilactici* 1.0 x 10^5 cfu/g, total application rate 2,5 x 10^5 cfu/g + 9,000 BAU/g α-amyslase) was applied at 10mls/Kg using a hand held sprayer in 3 x 250ml applications, Sorghum was mixed between applications. 8500g of treated sorghum was packed into each of 5 red polyvinyl net bags which were then tied with a silk tail. 8500g of untreated sorghum was was packed into each of 5 green polyvinyl net bags which were then tied with a silk tail. Bags were placed in the bunker during filling (as a Dorset wedge). Bags were sequenced Treated, Untreated, Treated, Untreated etc and were sat on 1m of untreated ensiled sorghum, The tails of the bags were drawn out toward the face and the wall of the bunker was indelibly marked to indicate the bag positions. A further 1m of untreated sorghum was added on top with standard compaction. The bunker was covered with a single plastic sheet. Bags were reached 9 weeks post ensiling, were dug out, weighed and then sampled for fermentation profile and aerobic stability. Aerobic stability was determined classically with all analysis being undertaken independently by NutriVet, Pohorelice as blind samples.

Results and discussion
Both silages were well fermented and were free of butyric acid (Table 1). Small variations in the nutritional profile were observed between treated and untreated silage (ME of treated being 9.66 compared to 9.54 MJ/Kg on untreated). Nitrate levels in both treated and untreated silage was below 1% indicating no residual store of nitrate within the ensiled sorghum. Differences in ME and nitrate can be directly attributed to variance in sampling (Table 1). The fermentation profile shows a treatment effect. Treated sorghum had a 7% increase in the level of lactic acid (1.02% compared to 0.95%). The increase in the level of lactic acid being produced from the inclusion of the homolactic *P acidilactici.* Acetic acid levels are 14% higher in the treated sorghum (1.71% compared to 1,5%). The fermentation pathway of *P. acidipropionici* converts sugar and lactic acid to propionic acid and acetic acid leading to elevated levels of both acetic acid and propionic acid. Ethanol levels in the sorghum silage increased by 22% from 0.22% to 0,27% through treatment with Maize-All+. This is consistent with the results observed by Szucs et al, but is contradictory to the results of Filya (Fylia *et al*, 2004; Fylia and Sucu, 2006). The pH of the treated sorghum is mathematically lower than that of the untreated sorghum (4.14 versus 4.19) which is in accordance with the higher level of lactic acid in the treated silage. The easily soluble sugar in the treated silage was 123% of the untreated sorghum silage. Maize-All+ contains 9,000BAU/g amylase which catalyses the breakdown of complex carbohydrate into simple sugars that are then available for silage fermentation or are available to promote the product of rumen microbial protein.

Table 2 presents the aerobic data. Both silages had a good fermentation as described, but fermentation losses were statistically reduced through treatment with Maize-All+ (from 7.0% to 4.1%). On a 1000T bunker this equates to an additional 29T of forage to feed. This supports the improved fermentation observed with treatment with Maize-All+.

Silage that was produced was classically aerobically unstable with the untreated silage heating after 20.5hrs. Maize-All+ improved the aerobic stability of the sorghum silage by 150%, increasing the time for a 2ºC rise above ambient to 34.75 hours. Propionic acid and acetic acid produced by *Propionibacterium acidipropionici* are both antifungal , leading to a reduced level of yeast and mould within the silage and leading to a parallel increase in the time taken for heating to commence.

Table 1: Comparative fermentation profile of Sorghum

	Untreated	Maize-All+
DM (%)	26.25	26.31
ME (MJ/Kg)	9.54	9.66
NO_3 (%DM)	0.9	0.8
NH_3 (% DM)	7.48	6.93
Easily Soluble Sugar (% DM)	0.47	0.58
Lactic Acid (%)	0.95	1.02
Acetic Acid (%)	1.50	1.71
Butyric Acid (%)	0	0
Propionic Acid (%)	0.14	0.16
Ethanol	0.22	0.27
pH	4.19	4.14

Table 2: Aerobic Parameters

	Untreated	Maize-All+
Fermentation Losses (%)	7.0^a	4.1^b
Aerobic stability (hrs)	20.5^a	30.75^b

Superscripts with different prefixes are statistically significantly different at P<0.05

Conclusion

Treatment of sorghum with Maize-All+ led to an improved fermentation, statistical reduction in dry matter losses and a statistical improvement in the aerobic stability of good quality sorghum silage. The Maize-All+ formulation is appropriate for use on both maize and sorghum silage.

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Section 3: Nutrition value of preserved forage (determination of forage quality by NIRs) and their effect on production and health of animals

Rapid methods of analysis of silages to improve feeding management in Dairy Farms

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Abstract
In modern dairy farms, optimization of feeding is critical to maintain animal health, lower environmental impact and maximise profitability. However, large feed and forage variability reduce consistency of composition of diet delivered to dairy cows. A feeding management plan must implement a program of feed sampling and analysis (St-Pierre and Cobanov, 2007), that ensure small variation of nutrients in diets. Traditional reference wet chemistry methods are accurate, but expensive, they can be implemented at specialized labs with relative long time of response. As an alternative to the traditional methods, near infrared spectroscopy (NIR) has received an increasing attention, with a large use in many commercial and private labs. Proficiency programs (like the National Forage Testing Association) have proven that NIR can be accurate in respect to reference method in the quantification organic feed nutrients. The paper will highlight the advantages of the use of NIR in feeding program in term of precision feeding and animal performance. It will also evaluate le challenges of on-site analysis for dairy farm as well as for biogas plants. The paper will also briefly cover the alternative methods for the determination of mineral nutrients with rapid and non-destructive methods, like x-ray fluorescence (XRF).

Introduction
As milk productivity keep increasing, feeding quality and feeding practises becomes more and more important in ensuring productivity, animal health and reduce the impact on the environment. High producing dairy cows are particularly vulnerable as they are subject to intense nutritional stress and any inconsistency in feed quality and feed delivery will affect their productivity and health (Schwartzkopf-Genswein, 2004).

Silages may represent a large portion of dairy cow diets and their quality can affect animal performance. Their composition may vary greatly not only because of the original composition of the forage, but also for the changes during ensiling. Also, silages are generally stored not protected from the environment and variation in composition during feeding can be affected by precipitation.

Silage variability and effects on cows
Silages are often stored in large single structures, made with forage that differs in composition due to the timing of harvest and location of production. In the filling of bunker silos the different sources of forage may results in layers with different composition like the one represented in Figure 1. The different sampling points of this corn silage bunker shows a map of forage layers with different DM content in the same silo. It is clear that the bottom layer is more humid with an average DM of 31%, the middle layer is drier (average 37% DM) and the top layer has an intermediate composition with an average DM of 33.9%. In this situation, there are different problems related to sampling for analysis and silage unloading that can affect the final product, which is the diet for our cows. If samples were taken at a person height or at the bottom we would obtain a composition which is not the average composition of that silo and the composition of the diet would not match formulation. During unloading, if silage is not removed from top to bottom, the composition of the loaded silage would change from day to day, making the diet inconsistent over time.

Stone et al. (2003) reported the results of analysis of 9 hay-crop and 11 corn silages sampled on top middle and bottom part of the silos. Dry Matter had the largest variation for both silages (Table 1) and hay-crop had larger variation in CP, ADF and NDF than corn silage.

Composition of alfalfa and corn silages were measured over a period of 68d during a precision feeding trial (Mertens and Berzaghi, 2009). Also in this trial corn silage was less variable than alfalfa for DM, CP and aNDF. In particular it was recorded that during precipitation events, DM content of both forages could vary more than 10 % units from one day to another one.

Variations within the silo at any given time or variations in composition over time will have an effect in the consistency of composition of the diet delivered to the animals. Mertens and Berzaghi (2009) evaluated the effects of daily changes of DM of silages on dairy cows performances. Single day variations in the composition of the diet was created artificially by adding water to either alfalfa or corn silages or both, reducing the DM between 8 to 16% units. Milk production and milk quality was monitored 3 days before (Baseline) and 3 days after (Recovery) the day of the variation. The changes in DM of the silages, on average, reduced the DM intake of 2 kg/d, which caused a loss of milk production for the following two days (Figure 2). The decrease of DMI of 1 kg for one day have caused the loss of 0.8kg of milk in each of the next two days. This short term study could only recorded short term production effects, but this sudden diet changes may also affect rumen and animal health.

Managing silage variability

Silage variability may be reduced through good agronomic, harvesting and storage practices, but it is unavoidable and we need to learn how to manage the variability in order to minimize the effects on the animals. The main tool in our hand is the implementation of a feed and forage quality control program. Such program must :

- Implement accurate and frequent sampling
- Adopt accurate analytical methods
- Have rapid return of analysis
- Be simple to implement
- Be affordable

It has being calculated that in a dairy farm with 1000 cows, improper feed sampling and analysis may cost 0.53$ per cow per day (St-Pierre e Cobanov, 2007). These researcher have developed a software to calculate optimal sampling and analysis, which would half the cost of improper feeding. For a dairy farm with 1000 cows, this plan would require sampling every 4 days and analysis run in duplicates.

If sampling and analysis protocols can be determined for optimal result, it becomes difficult to achieve the other goals of a feeding program with the traditional wet chemistry methods. Reference analytical methods are those recognize as official methods, which by definition are the most accurate that anyone can use. However, normally the reference methods for the most important nutrients are time consuming and quite expensive, which would greatly reduce the economic advantages of a feed quality control program.

In term of analytical methods, new technologies introduced in the last 25 years, have helped to make feed and forage analysis more affordable and easier to implement. In particular Near Infrared Spectroscopy (NIR) have established a key role in many research and commercial labs for the determination of organic nutrients, while very recently X-Ray Fluorescence (XRF) has being used for the rapid determination of minerals (Berzaghi e coll., 2011). Both of these techniques are rapid, non-destructive and they don’t use any type of chemical reagent.

Near Infrared Spectroscopy (NIR)

In 1976 Karl Norris (Norris et al., 1976) published the first paper on forage analysis using NIR. Since that first study, NIR has increased in popularity and its use is now widespread in a numerous forage testing labs. The advantage of NIR over the reference chemistry methods is mainly related to the rapidity of analysis. Modern NIRS instrument associated with a personal computer can collect a NIR spectrum and compute prediction in about one minute. That means that an operator working with a NIRS instrument can generate prediction of over 200 samples a day with great reduction of cost per analysis, but it also allows to return the results of analysis in less than 24 hours. The combination of accurate prediction, low costs and rapid return of results has been the key to the success of this technique. Although NIR has already establish a reputation as rapid and reliable analytical technique, the development from the publication of the first forage application in 1976 to these days has not been without pitfalls and drawbacks, which may remain in the memories of many that nowadays are still reluctant using NIR in forage analysis.

Near infrared analysis is a predictive technique based on the relationship between organic (not minerals) nutrients and the reflectance/absorbance properties of a sample in the NIR region. Before being able to make any predictions, it is necessary to develop a regression equation (model) that relates the information contained in the spectrum of a sample to the chemical composition obtained with reference methods. In practice, it is necessary to collect the spectra of several different samples, obtain their chemical composition with reference methods and develop the regression equations.

Although in the past few years there has been a great development and changes in NIRS, the general steps involved in the development of an application has not changed (Windham et al., 1989) in the past 25 years and it involves:

- Selection of several different samples with known characteristics (physical, chemical and biological) measured with reference methods;
- Collection of NIR spectra of the samples in the calibration data set;
- Computation of mathematical relationships (equation) between NIR absorption bands in the spectra and the chemical composition using chemometric techniques;
- Validation of the predicting models.

Near infrared analysis is therefore a secondary method, which predicts the chemical composition and/or biological properties (e.g. digestibility) measured with primary (reference) methods using the information contained in the NIR spectrum of the sample. The understanding of this techniques requires at least some basic knowledge of properties of the NIR spectrum and characteristics of modern chemometric methods applied in NIR analysis.

The advantage of NIR over the wet chemistry methods, is its rapidity (few minutes to few seconds), the minimal or no sample preparation, the analysis is multipara metric meaning, that with the collection of one spectrum the information of multiple parameters is obtained.

X-Ray Fluorescence (XRF)

X-Ray Fluorescence is a technique, which operatively resembles NIR, because it requires a minimal sample preparation, the quantification is based on the relationship between emission spectra and composition of calibration standards, the analysis is multipara metric and it is performed in a few minutes. However, the technique itself is completely different as it relies on emission specific for each element after the samples is irradiated with X-Ray. Because the emission of each element is highly specific and identifiable the number of samples required in a calibration is usually very small (less than 10 sample) and the calibration works across a wide range of sample types.

Accuracy and Application of Rapid Methods for the analysis of silages

NIR is used in many commercial lab replacing the traditional wet chemistry. Laboratories serving the feed industry and the feed industry itself are heavy user of this technology for its reliability and rapidity of analysis. NIR is normally implemented in the acceptance of incoming ingredients arriving at a feed mill, as well as in the quality control of the finished mixed feed.

Beside feed, forages are the next most common product analysed by NIR, silages included. Commercial laboratories offers a large array analyses by NIR (Dairyland lab, 2014), not only including the major nutrients, but also digestibility of NDF, rate of digestion and VFAs, all important traits that wouldn't be possible to quantify with wet chemistry methods for their excessive costs. There is always the question about the accuracy of NIR compared to the reference method, that often keeps people off NIR. As it was explained, NIR is a secondary method and with the reference wet chemistry methods used for calibration being the primary methods. Simply by this definition, the error of analysis using NIR will be always greater than the analytical error of wet chemistry. It is in fact generally accepted that the error of NIR prediction (Standardard Error of Prediction – SEP) should be less than 1.5 times the Laboratory Error (SEL) (Marten et al., 1989). This may scary some of the potential users regarding NIR as being inaccurate. If NIR is by definition less accurate, it is in practice accurate for the pourpose of the analysis. A proof comes from the proficiency program operated by the National Forage Testing Association (2014), which test the accuracy for wet chemistry and NIR methods in the determination of the chemical composition of forage samples. In the list of "approved" lab, meaning those that have passed the ring test, giving grades on the lab and techniques, we can find many labs that have obtained certification for NIR with also "A" grades, the maximum obtainable in this proficiency program.

The accuracy that is normally discussed is the so called laboratory accuracy. The advantage of NIR is that is very rapid and economical at in practice, can be implemented for repeated sampling and analysis, which allows for a real implementation of a quality control program for animal feeding. The frequent sampling is necessary to monitor the changes in feed composition, which are expected because of the natural variability of forages, which is not predictable. NIR then can be the analytical tool of choice to improve accuracy and precision of feeding.

Application of NIR analysis of silages normally implies drying the sample, grinding and then performing NIR analysis. This process requires the shipment of silage samples to a lab and require times for drying and grinding. However, variation within day(s) can be large and we have seen, it may have a large impact on cow's performance even if the change in silage composition is altered for a single day. For the implementation of precision feeding is then necessary to move the analytical process from the laboratory to the farm.

On-farm NIR analysis is nowadays possible thanks to new technologies that allows manufacturing of rugged instruments. In particular, NIR diode array are sensors that don't have any moving part, very small and can be used to produce portable instruments that are now available on the market (AgriNir, Dinamica Generale, Italy; MicroNir, JDSU, USA, Polispec, IOR3, Italy)). There are several challenges for these portable instruments. First, NIR spectra is dominated by water absorption (see Figure 3), which covers some of the information related to carbohydrates and proteins. Figure 3 shows spectra within the range (1100-2500nm) covered normally by laboratory instruments. Diode arrays used in portables of the NIR usually terminate at 1700nm reducing the information collected. A third technical problem that may arise is the fact that the sample presentation and scanning is performed by an unskilled analyst, which may increase the risk of mistakes and errors.

In the precision feeding trial by Mertens and Berzaghi (2009), a lab bench instrument (Foss NirSystem 6500, Foss, DK) was compared to a diode array systems (AgriNIR, Dinamica Generale, Italy) for the determination of DM in silages and the error of predictions of the diode array was about 50% greater than the laboratory instrument, but the overall error for moisture was lower than 2%. Considering that day to day variation of moisture in silage can be over 10% units (Mertens and Berzaghi, 2009), the error of 2% units of a portable instruments would still allow a significant diet correction, with a large improvement in the precision of nutrients delivered to animals. On-farm NIR analysis can then express its value, through precision feeding, improving animal performances but also potentially improving animal health.

Nevertheless, we have to remember that on-farm NIR analysis can encounter greater difficulties in correctly quantifying important parameters like forage digestibility and the implementation of precision feeding with portable instrument cannot replace periodic check-ups with more accurate laboratory methods.

On-farm NIR analysis of forages are not only limited to animal feeding, but is the past few years there has being an increase usage of silage crop such corn and grass, in the biogas production. Co-digestion of manure with energy crops or the sole use of energy crops is very popular in Germany and Italy and the potential of biogas production based on the biomass availability in may play an important role in the renewable energy supply in Europe. Biomasses are evaluated on their of bio-methane potential (BMP) or in other word in their potential of producing methane, which is the essential component of biogas of biomass. Methane can only be produced by the conversion of carbon skeleton of organic component such carbohydrates (sugars, starch, cellulose, hemicellulose), protein and fat. Water and ashes are diluting elements, which will depress the BMP potential of biomass. Therefore the determination of the composition of energy crops is very important to value such crop in the transformation into biogas.

Biogas plants may benefit on the use of NIR when they have to purchases energy crops. Corn and grass just harvested have large variation in moisture and then on BMP. The determination of moisture at ensiling will assist biogas plant in determining the value of such crops and also help them in the decisions for treatments to optimize ensiling. In the past few years John Deere, has introduce the Harvest Lab, an NIR instrument applied directly on the forage harvester that can determine Moisture content on the go, with an accuracy of 2%. (John Deere, 2014). The use of the same sensor or other portable NIR instrument during normal operation may aid the plant manager in optimizing feeding of the plant and efficiency of transformation.

Conclusions

Near Infrared is a mature techniques that has established a reputation of reliable methods of analysis and widely used in many feed and forage laboratories. As feed becomes more expensive, animal performances keep increasing the errors during feeding may have a larger economical effect. Farmer should start implementing feed quality control program and adopt practices that reduce variability of nutrient and increase feeding precision. NIR is the analytical technique of choice for such quality control program.

The introduction of portable rugged NIR instrument will help with further implementation of precision feeding, being able to adopt correction of diet formulation in real time. On-farm NIR analysis can also be very useful in the biogas industry, by improving the estimation of methane potential of biomasses that are being ensiled as well as in the evaluation of the silage being fed to the biogas plant.

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Figure 1: Dry matter content (%) of different sampling position in a bunker of corn silage

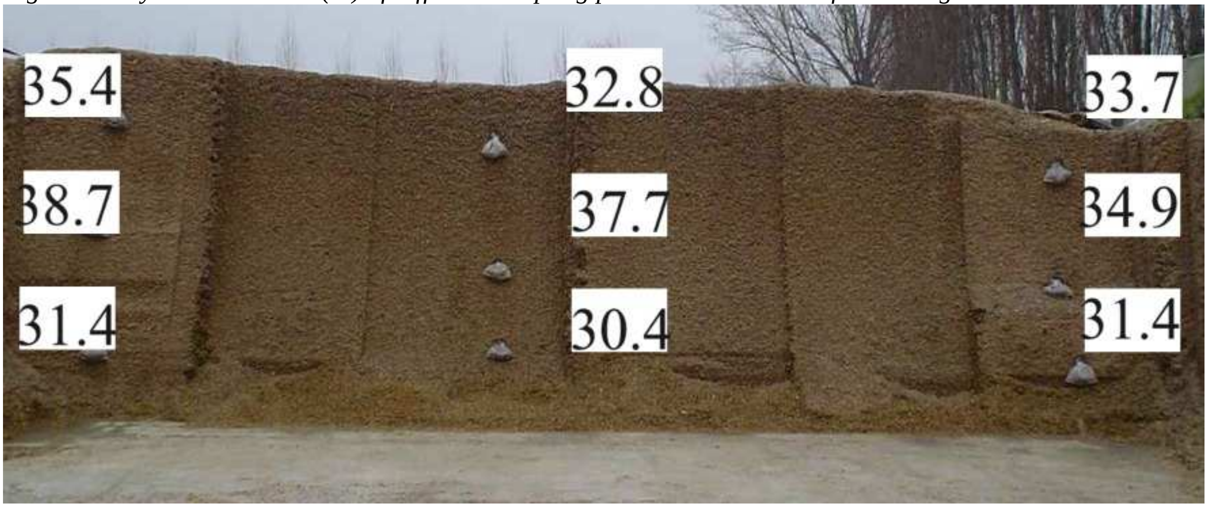

Figure 2: Changes in Milk production after one day variation of DM of the silage (Mertens and Berzaghi, 2009)

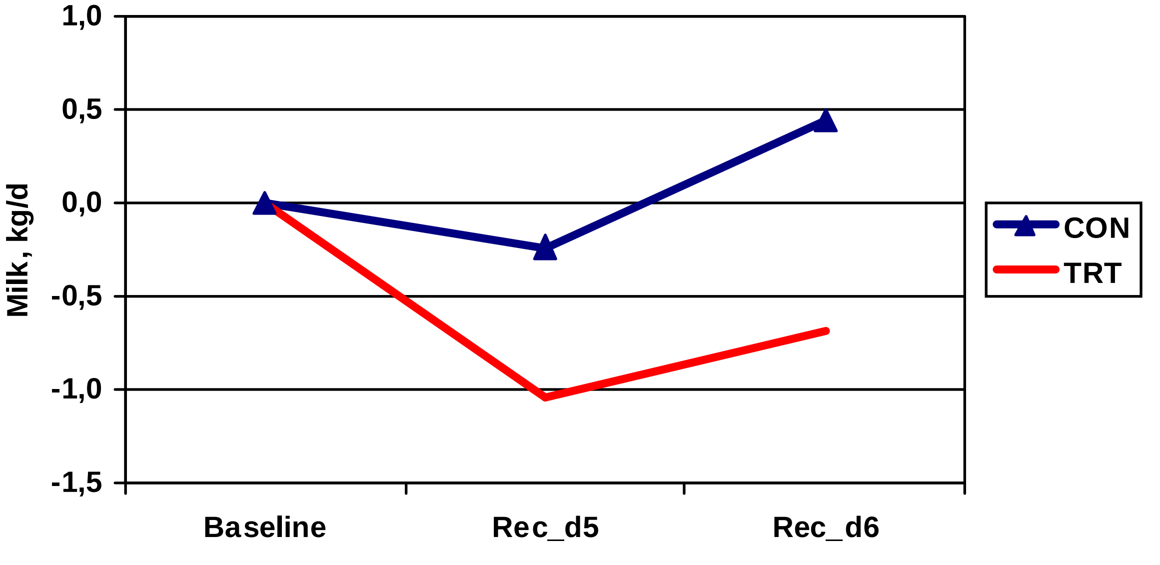

Figure 3: Spectra of total mixed ration with different moisture content and different particle size.

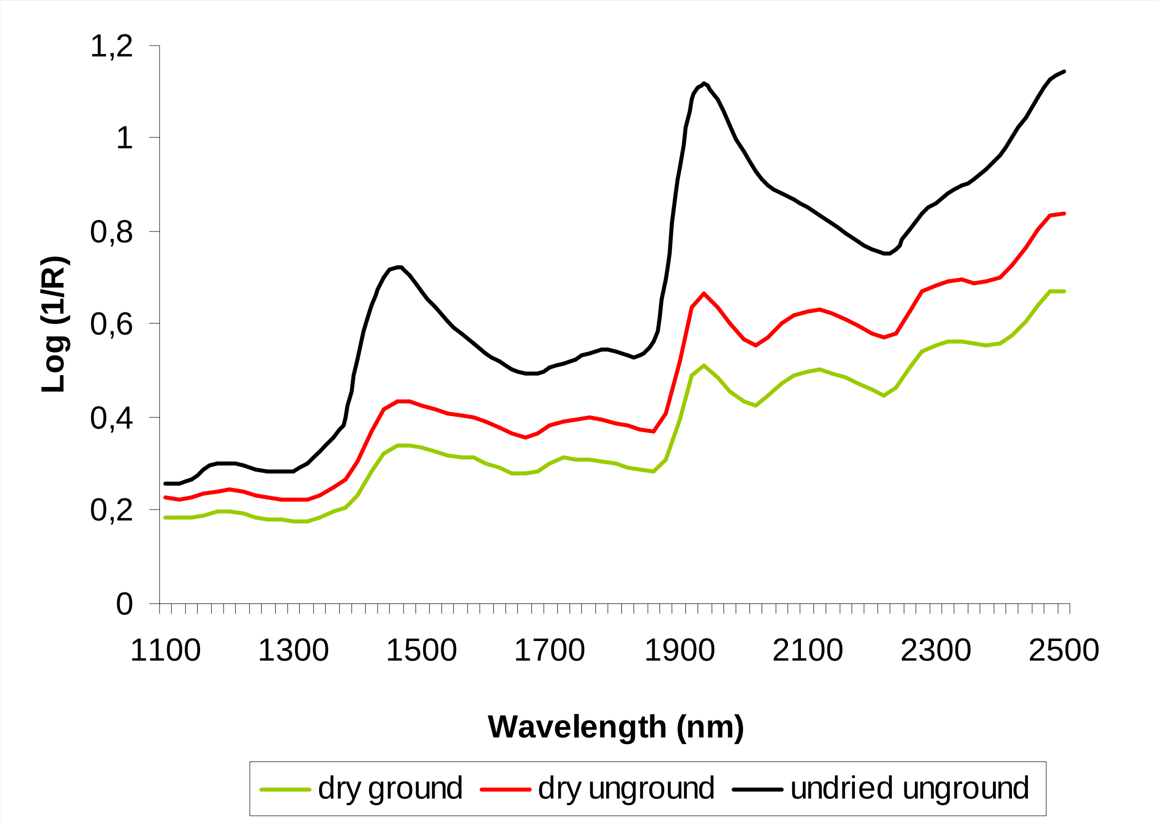

Table 1. Corn and hay-crop silage variability within bunker silos (Stone et al., 2003).

Silage	Deviation	DM	CP	ADF	NDF
		Percentage deviation			
Hay-crop	Minimum	5.2	3.3	1.1	5.4
Hay-crop	Maximum	44.7	52.1	20.0	24.8
Hay-crop	Average	21.0	17.6	10.7	14.7
Corn	Minimum	1.3	2.5	2.3	0.5
Corn	Maximum	55.0	29.5	18.3	18.6
Corn	Average	12.3	11.0	8.4	8.6

The silage fermentation – effects on rumen fermentation and implications for fertility

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Introduction
There are huge challenges facing the livestock industry, especially dairying, in the coming decades particularly the challenges of increasing production whilst reducing pollutantsfrom nitrogen and carbon containing compounds and all this with increasing pressures on the supply of quality protein and energy into the ration. Tamminga (1992) in his forward thinking review article on 'Nutrition Management of Dairy Cows as a Contribution to Pollution Control' highlighted the critical links between environmental pollution and dairy cow feeding. His review focussed on many aspects of balancing nutrient supply to the rumen and the practical ways in which we can reduce these pollutants. Whilst many steps forward have been made in these areas, especially with better supply and balance of nutrients into the diets of ruminants, most, but not all, of these improvements have been made using non forage components of the ration. With the ever present moral question of the practice of feeding food grade products in the feed of ruminants, forages must and will make a greater proportion of the diet of dairy cows. As the industry shifts back to a more forage based rationing system it is likely that the health status of the dairy herd will also improve, in turn improving profitability and sustainability of our livestock farms. In order to achieve these goals first we need to understand better what we already have, but may be don't realise it and secondly resolve some of the problems of putting these benefits into practice in the farm situation.
Some would argue that silages are already well defined, because we assess the nutrient content in terms of protein and energy, and this is more than adequate to be able to satisfactorily develop a balanced ration for the ruminant. The purpose of this review is to question this assumption and to challenge current thinking and most importantly to highlight the different languages spoken by silage biochemists and ruminant nutritionists in the hope that we can improve silage utilisation on dairy farms globally.

The review is focussed solely on one aspect of silage production, namely the silage fermentation and how that can impact on the rumen fermentation and subsequently on just one 'dairy cow production disease' namely fertility. With this in mind we should remember that *Silage is produced as a result of a fermentation process in the silo BUT is the feed for a second fermentation process in the rumen – The quality of the first fermentation can have major effects on the second fermentation!*

The silage fermentation
Firstly it is necessary to define the nutrients/chemical components that are predominantly affected by the fermentation process. These should be split into the two key components of the ration namely Carbon/Energy and Protein/Nitrogen. With respect to the carbon compounds the focus will be on Water Soluble Carbohydrate (WSC), and its fermentation products, predominantly Lactic and Acetic acids but will also consider other carbon containing volatiles. In regards to the protein/nitrogen the story is more complex so consideration will be given to the breakdown products of crude protein commonly considered by the silage biochemist, namely, soluble protein, true protein, amino acids, amines and ammonia, but we also need to put this into the context of the components used by the ruminant nutritionist such as rumen degradable protein (RDP), rumen undegradable protein (RUP) and by-pass protein and here ask the question are we speaking the same language.

Over recent years the silage microbiologist and silage additive industry has been focused predominantly on the aerobic stability of silage and have possibly lost the importance of silage fermentation quality on subsequent animal performance. The target of this review is the rumen fermentation and dairy cow fertility and so we have taken the liberty of ignoring all aspects of aerobic stability (which is the subject of another review at this conference) and are focused entirely on maximizing silage fermentation quality and the resultant silage fermentation product profile, and in turn how that affects factors such as feed intake and subsequent rumen nutrient use efficiency.

Carbon/Energy
The silage fermentation relies on the WSC to ferment to lactic acid, volatile fatty acids, ethanol and other volatile carbon containing components. The relative concentrations of these different components can have a ajor effect on intake and rumen fermentation profile. The traditional view of a good silage fermentation process was to improve the rate of fermentation in the first hours during storage and to maximize lactic acid production at the expense of all other end-products. It is well accepted that a rapid fermentation improves silage quality (McDonald *et al* 1991). Silage fermentations that produce end products other than lactic acid increase

the DM losses and Wilkinson and Davies (2013) in reviewing literature on the effect of inoculating with homofermentative lactic acid bacteria (HoLAB) compared to heterofermentative lactic acid bacteria (HeLAB) showed a greater than 2 fold difference in DM losses during fermentation with the latter approach. These losses result in an inefficient use of WSC and the resulting silages have lower levels of residual WSC. Navarro-Villa *et al* (2013) and Davies *et al* (2005) clearly showed that a silage fermentation dominated by a lactic acid fermentation increased residual sugar concentrations. The results of Davies *et al.* (1995) (Table 1) show the relationship between grass DM content, and end-point acetic acid, lactic acid and WSC concentrations.

Table 1 Composition of silages harvested at different DM concentrations and ensiled either with HoLAB or a mixture of Ho LAB and HeLAB inoculant

Target %DM	20% DM		25% DM		33% DM	
Inoculant*	1	2	1	2	1	2
Lactic acid	119.7	27.7	97.5	40.1	78.1	23.7
Acetic acid	5.7	72.0	4.9	66.0	4.6	52.5
WSC	70.2	15.4	160.5	37.8	174.9	31.5

*All values are in g/kg DM. * Inoculant 1 = HoLAB. Inoculant 2 = HoLAB+HeLAB*

The HoLAB inoculated silages had significantly higher lactic acid and WSC and significantly lower acetic acid concentrations than the silages treated with the mixed LAB inoculant at the $P<0.001$ level. It is important to consider these shifts in silage fermentation on the rumen fermentation processes.

So the first consideration is the efficiency of rumen microbial protein synthesis this will be discussed in more detail later. However WSC supply to the rumen microflora has an important role in improving efficiency of microbial protein synthesis. Merry *et al* (2002) using the *in vitro* RUSITEC system fed two silages differing in WSC concentration. The high sugar silage contained 85.7 g/kg DM WSC compared to the low WSC silage with 30.0 g/kg DM. The results showed a significant ($P<0.001$) improvement in efficiency of microbial protein synthesis from 21.4 in the low sugar silage to 23.6 (g microbial-N kg^{-1} organic matter apparently digested), or in terms of efficiency of N use 0.65 (low sugar silage) to 0.85 (high sugar silage) (g microbial-N g^{-1} Total-N fed).

An additional assessment of the energy status of the different carbon fractions to the rumen and the cow should be considered. Firstly WSC when compared to the lactic acid and other VFA products of the silage fermentation is often considered to be of greater value is this correct? Secondly should the energy content of lactic acid be considered in its own right? Harrison *et al* 2003 in their review proposed a model where an extensively fermented (inoculated) silage promoted a propionate producing rumen fermentation where as a estricted fermentation (acid treated) promoted an acetate producing rumen fermentation. Martin *et al* (1990) showed lactic acid is metabolised by rumen bacteria via the acrylate pathway to form propionate. They found a trong positive correlation between the concentration of lactic acid in silage and the molar proportion of propionic acid in the rumen. This has the potential to improve glucose supply, and spares glucogenic amino acids from catabolism. The acrylate pathway consumes hydrogen, a by-product of acetate and butyrate production, which would reduce methane emissions, and result in the improvements in energy utilisation reported with HoLAB inoculant treated silage. This was supported by Cushanan *et al* (1995) who showed that restrictedly fermented silage gave a higher acetate to propionate ration in the subsequent rumen fermentation compared to an extensively fermented silage. The results of Navarro-Villa *et al* (2013) indicated that where a combination HoLAB +HeLAB inoculant has been used that a higher level of acetic acid is produced in the silage which again promoted a lower propionate:acetate ratio in the rumen and thus reducing the energy available to the host animal.

One key factor in improving dairy cow fertility is to reduce the effects of negative energy balance NEB) in the first few weeks postpartum. One practical solution to this has been the addition to the diet of 500g of propylene glycol (Trabue *et al.* 2007). Each molecule of propylene glycol is fermented to one molecule of propionic acid and absorbed from the rumen. The energy value accorded to propylene glycol is 28 MJ/kg. This approach has a very positive effect on improving dairy cow fertility. Should we now give silage lactic acid the same level of importance? Following through with the argument, on theoretical grounds, then each lactic acid molecule fermented in the rumen produces one propionate, in addition 0.5 molecule of methane is saved. After correcting for the relative molecular weights then the equivalent energy value of lactic acid compared to propylene glycol is 23.6MJ/kg. Thus silage containing 100 g/kg DM lactic acid could be providing in excess of 2 J energy, and this does not take into consideration the energy sparing effect of reduced methane. With respect to methane and the silage fermentation process again the theory holds true that the greater the ratio of lactic:acetic acid in the silo, the lower the methanogenic potential. The study of Navarro-Villa *et al* (2013) showed that when rumen methane production is expressed in terms of Methane produced/total VFA produced that the HoLAB Inoculated silages had lower values than that of the combined HoLAB + HeLAB inoculated silages.

Protein/Nitrogen

The importance of a rapid pH decline with respect to protein degradation and the resulting nitrogen fractions present in the silage is even better established than that for WSC story. Research (Heron *et al* 1986; Kemble 1956; Charmley and Veira, 1991; McKersie 1983) has clearly shown that the breakdown of protein in the silo is a two stage process, firstly the plant's own proteases attack the protein converting it into peptides and amino acids and these breakdown products are subsequently converted into amines and ammonia by the microflora present within the silage. Thus the best controlling method is a rapid pH decline that inhibits both plant protease and microbial activity. These two facts taken together mean that ammonia-N is not always a good measure of protein breakdown as protein can undergo the first stage of the process to peptides and amino acids but not necessarily be converted further to amines and ammonia-N. This can have important implications for the efficiency of rumen microbial protein synthesis and efficiency of nitrogen use, which then influence levels of blood urea-N in the ruminant animal.

The constituents present in the nitrogen fractions can influence the efficiency of rumen microbial protein synthesis. However hereby lies a problem, and it is a language barrier between the silage biochemist and the rumen nutritionist. Often the silage biochemist examines protein breakdown products such as free amino acids, true protein, amines and ammonia. Whereas the nutritionist talks in terms of RDP, RUP Bi-pass protein and sometimes splits the protein into fast and slow degradation pools. We must begin to understand each other's *modus operandi* and find a way forward that enables us to truly represent the benefits we see from a rapid fermentation, reduced ensiling protein degradation and animal nutritional models.

There have been many studies showing a benefit from inoculation with HoLAB on silage fermentation, silage intake and animal performance, possibly more so with single species *L. plantarum* than multi species inoculation (Gordon, 1989; Mayne,1990; Merry *et al.*1995; Muck and Kung, 1997; Rooke and Kafilzadeh, 1994; Winters *et al.,* 2001). However, often the explanation of improved performance is difficult to explain by standard chemical compositional analysis.

At this point understanding how the changes occurring in the nitrogen fractions during the silage fermentation affect the rumen fermentation are of great importance. The faster the decline in pH over the first 24 – 48 h, has a significant impact on the Fraction 1 leaf protein (Cussen *et al,* 1995; Davies *et al,*1998). Fraction 1 leaf protein is the major protein involved in photosynthesis and is the most abundant protein in the plant leaf. These studies showed that inoculation with HoLAB preserved up to 35% more of this protein compared to no treatment. Further studies (Winters *et al.* 2001) used a different approach and measured total bound and free amino acids to give a gauge of true protein breakdown. These studies again showed that inoculation with *L. plantarum* increased the concentration of true protein and when fed to beef steers improved live-weight gain by 34% compared to no treatment. In the rumen the concept of balance of supply of protein and energy is used to describe the way in which rumen microbial protein synthesis can be improved (Dewhurst *et al.,*2000; Tamminga, 1992). The concept states that the supply of utilizable protein and energy needs to be synchronous for maximum efficiency of microbial protein synthesis, the concept needs to be thought of not only in terms of time, but also space, so the nutrients need to be present at the same time in the same place in the rumen for improved efficiency. So taking this balance principle further, the improvements in silage true protein content will reduce its speed of rumen degradation resulting in improved balance of supply with the energy available from cell wall degradation. Bringing the results together on silage true protein and rumen microbial protein synthesis Davies *et al.* (1999), indicated that by inoculating silage with *L. plantarum* the true protein content of silage was improved and a corresponding improvement in rumen microbial protein synthesis was also observed. Further research (Contreras-Govea *et al.* 2013) comparing silage inoculated with the MTD/1 strain of *L. plantarum* also found improvements in efficiency of rumen microbial protein synthesis and concluded that the result must be due to improvements in the true protein content of the inoculated compared to untreated silage. In terms of the changes occurring during the silage fermentation and their effects on the rumen fermentation and subsequently the animal it is important to assess the fate of the nitrogen compounds not captured by the rumen microbial flora into microbial protein. So we now need to consider the rumen nutritionists view.

Rumen degradable protein supply and reproductive performance of dairy cattle

Dietary protein can be divided into two fractions: rumen degradable protein (RDP) and rumen undegradable protein (RUP) during pre-gastric fermentation of ruminants. Rumen degradable protein provides a source of amino acids, ammonia and nucleic acid for rumen microbial protein synthesis. The efficiency of microbial protein synthesis (microbial protein synthesized divided by fermented energy) is determined by both dietary RDP and rumen carbohydrate availability. A high portion of soluble or rumen degradable protein (RDP) could increase the amount of ammonia that escapes into blood, thus increasing blood urea and lowering microbial protein synthesis.

Some of the ammonia (which is toxic to both the animal and the rumen) can permeate through the rumen wall. The blood stream carries ammonia to the liver to be detoxified by converting it to urea. Urea can easily diffuse from blood to milk; therefore milk urea nitrogen (MUN) is highly correlated with blood urea nitrogen

(BUN) and plasma urea nitrogen (PUN) (Jonker *et al.*, 1998; Broderick and Clayton, 1997). Milk urea may be used as an indication of high ruminal ammonia loss. Dietary protein nutrition or utilization and the associated effects on ovarian or uterine physiology have been monitored with urea nitrogen in plasma or milk; concentrations above 19 mg/dl have been associated with altered uterine pH and reduced fertility in dairy cows (Butler, 1998).

Meta-analysis conducted by Ferguson and Chalupa (1989) indicated that excess protein in-take may affect dairy cow reproduction through the toxic effect of ammonia and its metabolites on gametes and early embryos, and also by exacerbation of NEB. Increasing MUN levels above 18 mg dL^{-1} appears to be negatively related to dairy cow fertility, especially in the first parity. It is also suggested that the levels of MUN that are adversely associated with fertility, might be <12 and >18 mg dL^{-1}. Melendez *et al* (2000) studied a total of 1073 cows. They discovered that cows with high MUN (17-25 mg dl^{-1}) bred during the summer were 18 times less likely to get pregnant than cows with low MUN (6-16 mg/dl^{-1}) that were bred during the winter.

Jordan *et al.* (1983) discovered that uterine urea concentration was positively associated with blood ammonia and urea concentration when animals were fed high crude protein diets. Studies also reported that excessive protein intake decreases uterine pH (Elrod *et al.,* 1993; Elrod and Butler, 1993). Elrod and Butler (1993) examined the uterine pH value of dairy cows feeding different levels of dietary protein. They found that high PUN concentration was a result of cows with a high protein intake and also these animals developed a lower uterine pH. The results suggested that high PUN, which may be associated with a decline in uterine pH, could render the environment within the uterus unsuitable for early embryo development. Larson et al (1997) found that non-pregnant cows with high MUN, which had a long inter-oestrous interval, may also be associated with a low-progesterone concentration in the blood. Bovine endometrial cells in culture respond directly to increasing urea concentrations with alteration in pH gradient but respond most notably with increased secretion of prostaglandin $F_{2\alpha}$ (PGF_{α}). Increased uterine luminal PGF2a interferes with embryo development and survival in cows, thus providing a plausible link between elevated PUN concentrations and decreased fertility (Butler, 1998). Jorritsma *et al.* (2003) review also suggested that exposure of oocytes in antral follicles to elevated ammonia or urea hampers cleavage and blastocyst formation, thus affects dairy cow reproduction. These results may suggest that high dietary protein (including RDP and RUP) may affect fertility by altering the normal uterine environment and plasma progesterone concentration, which could be detrimental for embryo development.

The detrimental effect of excessive protein intake in early lactation could also be energy related. Negative energy balance (NEB), which is common in early lactation, was reported to suppress progesterone concentration (Spicer *et al,* 1990; Villa-Godoy *et al,* 1988). The NEB could be exacerbated by feeding excess protein because of the extra energy cost to detoxify and excrete urea, especially for RDP. Every gram of excess nitrogen from over feeding CP can increase the energy requirements by 13.3 kcal of digestible energy (Butler, 1998; Tyrrell *et al*., 1970; NRC, 1989).Tyrrell *et al.* (1970) demonstrated that the requirement of energy for the animal to excrete excess nitrogen as urea through urine. Therefore, if dietary intake is low in energy or high in protein to energy ratio, rumen bacteria will have reduced efficiency in utilizing free ammonia to synthesize protein, which can result in increased BUN or MUN (Broderick and Clayton, 1997; Hof *et al.* 1997; Rajala-Schultz and Saville, 2003).

Silage fermentation and reduced blood urea

Rooke *et al.* (1988) observed a marked difference in both nitrogen digestibility and nitrogen retention in sheep between treated and untreated silages. Decreases in urinary nitrogen excretion were largely responsible for the improvement in nitrogen retention with the HoLAB inoculated silages, and it is probable that this resulted from improved microbial-N synthesis within the rumen and/or reduced silage –N degradability within the rumen. Similar results have been found in the UK (Fraser *et al.* unpublished results). These studies indicate that due to the improvement in N-balance brought about by reduced urine production that the blood urea nitrogen content in these animals would by association be lower and thus reducing the toxic effect on all aspects of the cycle of the ovum. Data of O'Kiely *et al.* 2002 supports this, where in a study with beef cattle they found a better rumen microbial-N production of 94 g d^{-1} for silages inoculated with *L.plantarum* compared to 79 g d^{-1} for untreated silages and this corresponded with a significantly lower BUN of 3.96 mMol L^{-1} in steers fed the inoculated silage compared to 4.39 mMol L^{-1} for those fed untreated silages.

Silage fermentation and intake

The review thus far has indicated that the use of HoLAB inoculants can improve the lactic acid:acetic acid ratio and reduce the protein breakdown products. These factors can have an impact on dry matter intake. There are many papers examining the factors affecting intake and they are not in agreement. However if we examine the silage fermentation factors that affect intake it is fair to say that there is strong evidence that silage fermentations with elevated levels of acetic, butyric, 1-propanol, ammonia or other protein break down products have all been implicated in reducing silage intake (Gill *et al.* 1988; Huhtanen *et al.*, 2002; Kleinschmitt *et al.* 2013; Rooke *et* *al.*, 1991; Steen *et al.*,1995;) All of these compounds are reduced in silages prepared using an inoculant containing HoLAB and in particular *L.plantarum*.

So can silage fermentation really affect dairy cow fertility?

Davies *et al.* (2012) published data from a survey of fertility on 103 dairy herds in the UK. Within the study 49 herds (11621animals) had been fed silage inoculated with a specific strain of *L. plantarum* (Aber F1), whilst the remaining 54 herds (13415 animals) were fed silages either remaining untreated or treated with a range of other additives including some with other *L. plantarum* strains. The results indicated that the *L. plantarum* Aber F1 treated group had a significantly ($P<0.05$) shorter mean calving to conception interval of 125 days compared to 135 days for the other group. The data analysis took into consideration all other variables and indicates that silage quality can have a significant effect on aspects of the dairy cow nutrition and health that we may not at first expect. Whilst we could debate this study ad nausea, it does show that even with the huge range of probable nutritional status across the farms improving silage fermentation quality is important.

So in resume, the effect on fertility is likely to be multi-factorial and the transition cow is at a very finely balanced nutritional state, but by pulling together the factors that we know can affect silage fermentation quality we can begin to build up a body of evidence.

There are two key factors that affect the fertility of the cow:-

1. Negative energy balance
2. Blood Urea-N

Taking these in turn we can see how silage fermentation quality can reduce the impacts of these in early lactation

Negative Energy Balance

- Improved intake from HoLAB inoculated silages will improve energy intake and overall energy balance it is well established that if a cow can achieve a feed intake level of over 4% of its body weight by 110 days post calving it will improve conception rate.
- Improved lactic acid concentrations, improving the readily available energy to the cow and propionate uptake in to the blood. Also reducing energy losses via methane.
- Reduced urea excretion so reducing the energy required for this process and thus providing more energy for reproductive process.

Blood Urea Nitrogen

- Reduced protein breakdown in the silo results in improved microbial protein synthesis in the rumen, reducing ammonia losses from the rumen and thus reducing the formation of BUN in the liver, which then reduces the impacts on the ovum enabling a better uterine pH, and progesterone concentrations to be maintained, and reducing the direct toxic effects on blastocyst formation and survival.

However, having conducted this review there is still a need for further studies that take a holistoic approach to the subject and within a well defined experiment measure the variables discussed above. It is also important that we better understand of how we can better formulate rations based on a good understanding of dairy cow nutrition and silage biochemistry. Thus additional research is needed to detemine reliable data for rumen degradable protein (RDP), the non-degradable protein (RUP), soluble protein (SP), soluble protein to rumen degradable protein ratio of the HoLAB inoculated silages (for a range of crops) in order to calculate the modified metabolisable protein value (MP, PDIN-PDIE, DVE-OEB, MFE-MFN) for the different European evaluation systems. The knowledge of degradation rate of nitrogen fractions from a HoLAB inoculated silage is important in order to adjust this protein fraction to the correct carbohydrate source with a similar degradation rate in the rumen to maintain the synchrony of the ingredients for a more efficient microbial protein synthesis and lower plasma urea concentration in dairy cow diet. It has an important link not only with dairy reproductive performance, but also economic and environmental sustainabilty.

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Evolution of forage quality of selected grass species during the 1st harvest regrowth

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Abstract
The contribution evaluates dry matter increase and changes in concentrations of nutrients and energy of 7 grass species and varieties from 7 sequential samplings in weekly intervals from the end of April up to mid-June at the site in Jevíčko. Therefore a stationary trial was established in 2010 at the Jevíčko site on gleyic fluvisol (345 m above sea level, 7.4 °C mean annual temperature, long-date rainfall average 545 mm) with seven grass species (*Dactylis glomerata* L. ´Vega´, *Festuca arundinacea* L. ´Kora´, Festulolium ´Hykor´ and ´Hostyn´, *Festuca pratensis* L. ´Kolumbus´, *Lolium perenne* L. ´Kentaur´ (4n) and *Bromus inermis* Leysser cv. ´Tabrom´). The trial was fertilized in 2011 with N 120 kg ha^{-1} in the form of ammonium nitrate with lime applied in two doses per 60 kg ha^{-1} (in spring, after the first cut), 35 kg ha^{-1} P and 100 kg ha^{-1} K; seven sampling dates during 1st cut growth, first cut on 28th April 2011, next samplings in weekly intervals until mid-June. The dry matter (DM) production at the time of the 1st harvest was in the average of species 2.20 t ha^{-1} (1.60 – 2.76 t ha^{-1} DM). The average yield increase reached 0.96 t DM ha^{-1} and within species it ranged from 0.80 – 1.09 t DM ha^{-1}. The concentration of crude proteins (CP) in the forage in the 1st harvest was 181.8 g kg^{-1} DM, CF 176.3 g kg^{-1} DM, NEL 6.10 MJ kg^{-1} DM and NEF 6.08 MJ kg^{-1} DM. During the accrual period the CP concentration decreased by 16.6 g kg^{-1} DM (11.7 – 20.2), the fibre increased by 23.7 g kg^{-1} DM (21.3 – 26.9), NEL concentration decreased by 0.23 MJ kg^{-1} DM (0.18 – 0.27), NEF concentration by 0.29 MJ kg^{-1} DM (0.23 – 0.34) for each week of growth.

Keywords
renovation; forage quality; time of harvest; Dactylis glomerata; Festuca arundinacea; Festulolium; Festuca pratensis; Lolium perenne; Bromus inermis.

Introduction
The recent increase of individual milk production of dairy cattle in the Czech Republic (over 7000 kg FCM) lays higher demands on voluminous fodder quality. The quality of forage changes a lot during the first harvest regrowth Individual species within forage mixture of grasses and legumes demonstrated significant differences in earliness (1 – 3 weeks), and even higher differences in forage quality (3 – 4 weeks) - Pozdíšek *et al.* (2002). In order to determine an optimum date for harvest for each individual species, sequential samplings were analysed during the first harvest regrowth.

Materials and methods
A stationary trial was established in 2010 at the Jevíčko site on gleyic fluvisol (345 m above sea level, 7.4 °C mean annual temperature, long-date rainfall average 545 mm) by a method of quick renovation with seven grass species (*Dactylis glomerata* L. ´Vega´ (DG), *Festuca arundinacea* L. ´Kora´ (FA), Festulolium ´Hykor´ (Fl-Hy) and ´Hostyn´ (Fl-Ho), *Festuca pratensis* L. ´Kolumbus´ (FP), *Lolium perenne* L. ´Kentaur´ (4n) (LP) and *Bromus inermis* Leysser cv. ´Tabrom´ (BI). Species were sown in a randomised block design in pure-stand and four replications. The trial was fertilized in 2011 with N 120 kg ha^{-1} as ammonium nitrate, with lime applied in two doses per 60 kg ha^{-1} (in spring, after the first cut), 35 kg ha^{-1} P (superphosphate) and 100 kg ha^{-1} K (potassium salt). Seven sampling dates were performed during the 1st growth, with the first cut on 28th April 2011 and the next sampling in weekly intervals until mid-June. The quality of forage dry matter in 2011 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, with duplicate samples being scanned twice. The measured parameters were crude protein (CP), fibre (CF), NEL (net energy of lactation), NEF (net energy of fattening), using software WinISI II, vers. 1.50 with the aim to determine yields, nutrient content and energy concentrations evolution during the first harvest growth. Characteristics were statistically evaluated using an analysis of variance and a method of linear regression; correlation coefficients were calculated and critical values r_α (n=7) of correlation coefficient for $\alpha_{0,05}$ and $\alpha_{0,01}$ evaluated.

Results and discussion
The year 2011 was characterized by a quick arrival of spring with above normal temperatures (+2.6 °C), bellow normal rainfall (-16 mm) in April and early May which clearly accelerated vegetation development by 1 to 2 weeks. The average dry matter production of studied species at the first sampling date (28th April 2011) was 2.20 t ha^{-1}, with the highest DM production for Fl-Ho (2.76 t ha^{-1} DM) and the lowest for the, BI (1.60 t ha^{-1}).

The average weekly DM growth rate, evaluated with linear regression (Table 1a) reached 0.96 t DM ha^{-1} and ranged within the evaluated species from 0.80 (FP) to 1.09 (Fl-Ho) t DM ha^{-1}. The differences between species are statistically high significant ($P_{0,01}$) – Table 1b.

The average CP content of forage at the first sampling was 181.8 g kg^{-1} DM, CF content was 176.3 g kg^{-1} DM, NEL content was 6.10 MJ kg^{-1} DM and NEF content was 6.08 MJ kg^{-1} DM. During accrual period the concentration of CP decreased by 16.6 g kg^{-1} DM (within the range 11.7 in LP – 20.2 in BI), the fibre increased by 23.7 g kg^{-1} DM (within the range 21.3 in Fl-Hy – 26.9 in BI), NEL concentration decreased by 0.23 MJ kg^{-1} DM (within the range 0.18 in Fl-Hy – 0.27 in BI), that was slightly lower than the value 0.26 MJ kg^{-1}DM observed by Pozdíšek *et al.* (2002) in grass forage NEF concentration decreased by 0.29 MJ kg^{-1} DM (within the range 0.23 in Fl-Hy – 0.34 in BI) per week ; correlation with time of all equations of linear regression are highly conclusive ($P_{0,01}$).

Low NEL concentration in *Lolium perenne* L. was caused in 2011 by a considerable drought in the beginning of vegetation, when this species with shallow root system has difficultes with nutrient uptake, so drought lowered its growth rate and yield (Garwood, Sinclair, 1979, Thomas, 1986) unlike *Dactylis glomerata, Festuca arundinacea* and Festuloliums, which tolerated better drought, thanks to their deeper root system. Prospective grass species for high productive dairy cows are Fl-Hy ´Hostyn´ and FP ´Kolumbus´, harvested in mid-May.

Suitable grass species for suckler cows are FA ´Kora´ and BI ´Tabrom´ (BI), harvested in the second half of May.

Acknowledgements

The contribution was made as a part of the research of the NAZV project with registration number QI101C199.

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Table 1a: Parameters of linear equation y = a + bx characterizing the evolution of dry matter production and concentration of CP, CF (g kg-1) net energy of lactation (NEL) and net energy of feeding (NEF) (MJ kg-1 DM) related to time, in the period of first harvest accrual in 2011 from sequential grass sampling (n = 7) – the first sampling in the last week of April

Species	Variety	Parameter														
		DM (t ha^{-1} DM)			CP (g kg^{-1} DM)			Fibre (g kg^{-1} DM)			NEL (MJ kg^{-1} DM)			NEF (MJ kg^{-1} DM)		
		R.p.		r	R.p.		r	R.p.		r	R.p.		r	R.p.		r
		a	b		a	b		a	b		a	b		a	b	
DG	Vega	0.88	0.98	0.95**	196.2	-18.8	0.94**	145.6	22.3	0.99**	6.57	-0.27	0.99**	6.67	-0.33	0.99**
FA	Kora	1.54	0.91	0.95**	196.1	-18.0	0.94**	151.9	22.6	0.98**	6.63	-0.22	0.98**	6.37	-0.28	0.98**
Fl-Hy	Hykor	1.56	0.86	0.96**	175.2	-15.6	0.93**	162.7	21.3	0.96**	6.13	-0.18	0.97**	6.13	-0.23	0.97**
Fl-Ho	Hostyn	1.65	1.09	0.98**	169.2	-14.0	0.90**	134.1	24.7	0.97**	6.38	-0.22	0.96**	6.47	-0.28	0.96**
FP	Kolumbus	1.27	0.80	0.96**	195.1	-18.1	0.92**	127.5	26.5	0.98**	6.57	-0.27	0.98**	6.66	-0.33	0.98**
LP	Kentaur	0.58	1.08	0.98**	162.0	-11.7	0.92**	118.4	21.8	0.96**	6.40	-0.19	0.98**	6.51	-0.24	0.97**
BI	Tabrom	0.50	1.00	0.98**	219.8	-20.2	0.93**	132.5	26.9	0.99**	6.53	-0.27	0.99**	6.62	-0.34	0.99**
Species average		1.14	0.96	0.98**	187.7	-16.6	0.94**	139.0	23.7	0.98**	6.42	-0.23	0.99**	6.49	-0.29	0.99**

Table 1b. Analysis of variance

Source of variability	*df*	*SS*	F_{test}	*Sig.*	*SS*	F_{test}	*Sig.*	*SS*	F_{test}	*Sig.*	*SS*	F_{test}	*Sig.*	*SS*	F_{test}	*Sig.*
A (species)	6	32	11	**	138	21	**	273	28	**	1	5	**	2	7	**
B (cuts)	6	720	247	**	2311	259	**	4478	464	**	39	252	**	60	263	**
Total	108	923			2860			5143			46			72		

*R.p. = regression parameters, r = correlation coefficient, df = degree of freedom, SS = sum of squares, Ftest = Fisher test, Sig. = statistically significant (P0,05 = *; P0,01 = **), NS = statistically no significant.*

The nutritive quality of maize hybrids in experiments at Troubsko and Uhříněves

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Introduction

When distribution of maize hybrids by maturity is based on the FAO numbers, in practice, we often get erroneous output data. This is because the earliness expression is dependent on the genetic predisposition of hybrids and the cultivation conditions. The generalization of the specific hybrid groups leads to uncontrolled errors of the estimation of an appropriate harvest time along with losses due to both digestible nutrients and fermentation of silage. Ensiling of maize at various stages of maturity has been subject of extensive research e.g.. Andrae et al. (2001), Bal et al. (1997), Cone et al. (2008), Darby a Lauer (2002), Di Marco et al. (2002), Hetta et al. (2012), Jensen et al (2005), Johnson et al. (1999), Philippeau a Michalet-Doreau (1997). Since 2012, the research project QJ1210128 is supported by the National Agency for Agricultural Research, entitled "Innovate systems of assessment of quality of feeds with emphasis on introduction of a new national evaluation system". The main goal is a creation of an independent and unified system of assessment of maize hybrids intended for production of more valuable silage for animal nutrition during agricultural practices. The project is set so that companies offering seed maize on the Czech market can enter the research with the goal to assess the broadest range of hybrids.

Materials and methods

In 2013, eight seed companies have provided the seed of maize hybrids for our experiments. The hybrids were grown in the specific form of small-plot field. In Prague locality sowing was 26th April, into 8 rows with a length of 20 meters by sowing machine Multicorn. In Troubsko sowing was 29th April, into 4 lines with a length of 10 m by a special one-row seeder. Hybrids were sown in triplicate by random selection. The hybrids of plants were assessed from the perspective of the physiological and health condition. The harvest was planned so that the individual hybrids were harvested approximately at the same level of maturity, with average dry matter of 32%, in a range of ± 2 %. In the locality of Troubsko hybrids were sown 29th April. The harvest took place in three terms compiled by the earliness of hybrids according to FAO methodology 220-250 of 26th August, the FAO 250-280 of 4th August and FAO 290-330 of September 20, 2013. In Prague hybrids were sown 26th April. The harvest took place from the 2nd September to the 18th September. gradually by ripening hybrids. The total time lasted vegetation at both locations consistently 134 days.

Samples were taken from the chaff and dried at 55°C. The samples were then homogenized in a laboratory mill (sieve size of 1 mm) and used for determination of Dry matter amount, contents of Ash, Protein, Sugar, Rough Fibre, Starch, Neutral Detergent Fibre (NDF), and NDF digestibility (DNDF) and organic matter digestibility (DOM). The digestibility was measured with 24 hours incubation of samples in rumen liquid of dairy cow. Standard methods according to AOAC (1995) were used for chemical analysis of the samples. The statistic evaluation was performed with StatSoft, Inc. (2011). The method of analysis of variance ANOVA ($\alpha = 0.05$) Tukey HSD test was used to compare various localities.

Results and discussion

Table 1 shows the results of nutrition quality of all tested maize hybrids based firstly on the locality Prague (P) and Troubsko (T) and secondly on the groups divided by the degree of ripeness (very early, early and later early). When the dry matter content of the fresh maize plant was 31.3 ± 1.6 %, the hybrids with the same number of FAO 220-330 were harvested in two similar locations (Prague, Troubsko), but with 200 km of distance. The effect of number of FAO had no impact to the content of the Ash, Starch, Fibre, NDF in whole plant dry matter forage. The correlations were low, trend lines had R^2 lower than 0.3. Significant differences ($P<0.05$) between localities were found only in Protein (7.3 vs 8.58 % DM in Uhříněves and Troubsko, resp.) and in Starch (26.68 vs 30.75 % DM) between FAO groups of very early and later early, resp.

Conclusions

Although the hybrids with different FAO numbers (230-330) were gradually harvested in both localities approximately at the same maturity level (31.07 ± 1.05 % in Uhříněves, 31.60 ± 1.95 % in Troubsko), the results of the analyses of the nutritional and fermentation values were significantly different between the localities ($P<0.05$) in Protein, and between FAO groups of very early and of later early in Starch.

Results of 2013 were confirmed by the results of 2012. References are available from author of the article.

Table 1: Nutrition quality of maize hybrids from the locality Prague (P) and Troubsko (T)

Seed company	Fao	Hybrid	Locality	DM	Protein	Starch	NDF	DNDF	DOM
Sempol	220	Almansa	P	31.26	8.16	28.97	37.75	57,36	78,36
			T	29.09	9.09	24.91	44.67	53,90	71,36
Caussade Osiva	230	Rubben	P	30.83	8.14	27.23	42.17	54,59	72,54
			T	29.44	8.21	26.81	44.01	53,95	72,55
Saaten Union	240	Sudor	P	32.79	7.02	31.15	40.08	61,71	77,90
			T	27.81	7.48	20.99	43.79	53,79	72,14
Monsanto	260	DKC 3507	P	31.48	6.48	30.20	44.82	47,00	60,85
			T	32.57	8.90	31.82	42.03	63,29	79,93
Monsanto	260	DKC 3795 Z	P	30.14	7.26	31.59	48.55	42,97	62,43
			T	31.36	8.80	28.93	45.15	63,50	77,56
Soufflet Group	270	Dynamite	P	30.82	6.99	27.76	47.78	58,13	74,02
			T	31.37	8.87	30.54	43.04	61,40	76,11
Monsanto	270	DKC 3523	P	32.38	7.74	26.49	41.49	51,01	73,00
			T	31.00	9.17	25.51	45.57	57,97	75,44
Saaten Union	280	Susann	P	30.44	6.67	26.38	41.93	48,13	64,02
			T	31.80	8.53	24.83	45.48	44,29	64,93
Limagrain	280	LG2 32.64	P	33.09	7.50	24.82	40.48	56,33	75,82
			T	30.30	9.20	26.62	44.17	55,01	73,87
Syngenta	280	NK Silotop	P	30.02	7.06	31.76	44.19	53,48	73,22
			T	31.66	8.67	30.38	44.50	54,65	73,45
Syngenta	290	NK Octet	P	29.78	6.83	29.51	43.56	47,12	70,03
			T	32.90	7.97	32.21	40.05	53,11	75,63
Seed Service	290	GKT 288	P	30.26	8.09	29.06	41.50	47,38	70,81
			T	36.20	8.10	34.87	42.44	52,91	73,25
Monsanto	300	DKC 4014 Z	P	29.80	6.97	34.42	44.58	64,96	79,30
			T	33.62	8.47	36.36	37.63	56,38	79,94
Limagrain	320	LG1 30.311	P	30.76	6.98	24.52	42.20	55,95	75,35
			T	32.84	8.47	28.86	42.72	61,03	77,23
Sempol	330	Helena	P	32.17	7.56	32.17	38.87	54,93	77,26
			T	32.02	8.83	25.95	44.80	56,90	75,77
			Average	31.33	7.94	28.85	43.00	54,77	73,47
			SD	1.59	0.81	3.45	2.55	5,52	4,85
Location:									
		Praque	P	31.07	7.30a	29.07	42.66	53,40	72,33
		Troubsko	T	31.60	8.58b	28.64	43.34	56,14	74,61
Maturity:		**Vegetation**	**days:**						
FAO < 250		Very early	116	30.20	7.90	26.68a	42.08	54,04	71,63
FAO 250-280		Early	121	31.29	7.94	28.25ab	42.08	55,03	74,14
FAO > 280-330		Later early	131	32.00	8.02	30.75b	44.21	55,88	75,27

Different letters document statistical differences in each column (Tukey HSD, α = 0.05) for location and maturity; Unit of DM, DNDF and DOM = %, Protein, Starch and NDF = % DM; P = locality Prague, T = locality Troubsko

Dedication: MZe QJ1210128

Chemical composition, fermentation quality and aerobic stability of high moisture corn grain ensiled with different additives

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Introduction
It is assumed that the high moisture corn grain (HMCG) is easy to ensile, and for this reason it does not need any silage preservative to be well ensiled. On the other hand, HMCG is characterized by a low content of WSC and water, and for this reason, the intensity of fermentation of HMCG is much smaller than in green herbages. A slow fermentation rate in HMCG results in a slow rate of pH decreasing in ensiled biomass and finally in a low content of fermentative acids. Such conditions are favorable to the development of undesirable bacteria degrading protein and lactic acid. Furthermore, the silages made from HMCG (HMCGS) are susceptible to aerobic deterioration when the clamp or silo is opened.

We hypothesized that by using the silage preservatives, such as bacterial inoculants (to increase number of LAB and to stimulate the desired fermentation) and/or chemical additives (to inhibit the growth of undesired bacteria) we could improve the quality of fermentation, decrease fermentative losses and increase aerobic stability (AS). The aim of a study was to determine the effect of selected silage preservatives on the quality of fermentation, losses and AS of HMCGS. The preservatives were homo- or heterofermentative LAB, with or without potassium sorbate, as well as organic acids and their salts.

Materials and methods
Chemical composition of HMCG (PR38A79 hybrid; FAO 250), either crushed or ground (CG *vs.* GG), is presented in table 1. The treatments were: C or G with no additives, C or G with inoculant 1 (*L. buchneri* LN4637 ATCC PTA-2494 – 5.0×10^6 CFU·g^{-1} of grain) – CG/I1 or GG/I1; with inoculant 2 (*L. buchneri* LN4637 PTA-2494, *L. plantarum* LP285 DSM 4784 ATCC 53187, *L. plantarum* LP329 DSM 5258 ATCC 55942, *Enterococcus faecium*

Table 1: Chemical composition of high moisture corn grain

Item	Crushed grain (CG)	Ground grain (GG)
Dry matter (g·kg^{-1})	674.5	681.6
	(g·kg^{-1} of DM)	
Crude ash	10.5	12.6
Organic matter	989.5	987.4
Crude protein	93.6	88.9
NDF	104.1	104.5
ADF	28.9	29.5
Starch	795.0	783.5
WSC	13.8	16.4

SF301 DSM 4789 ATCC 55593 – 5.0×106 CFU·g-1 of grain) – CG/I2 or GG/I2; inoculant 1 + potassium sorbate (4 g·g-1 of grain) – CG/I1/PS or GG/I1/PS; inoculant 2 + potassium sorbate (as above) – CG/I2/PS or GG/I2/PS; with chemical additive 1 (formic acid - 59%, propionic acid - 20%, ammonium formate - 4,3%, potassium sorbate - 2,5%; 4 ml·g-1 of grain) – CG/CA1 or GG/CA1; with chemical additive 2 (formic acid - 55%, propionic acid - 5%, ammonium formate - 24,0%; 4 ml·g-1 of grain) – CG/CA2 or GG/CA2.

Each treatment was ensiled in triplicate, in polyethylene silo of 15 L, closed by snap lids, allowing an escape of fermentative gasses. The fermentation lasted 60 days at 15±2°C. Chemical composition and fermentation quality were determined by standard methods. AS was assessed during the 7-day-test, according to Honig [1985, 1990]. A measure of AS was the number of hours with temperature of silages not higher than 2°C above the room temperature. The results for each physical form of grain

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were subjected to the one-way analysis of variance using MIXED procedure of SAS (9.2). When the model was significant, the means were compared by PDIFF procedure with the correction for the Tukey test.

Results

There were no treatment effects on chemical composition of silages (table 2), except lower protein content in CG and GG silages (no additives), and the highest content of WSC in grain ensiled with chemical additives.

Ensiling of HMCG without any additive, irrespective of the physical form of grain, resulted in the least aerobically stable silages (table 3), with high level of protein degradation. Irrespective of the physical form of grain, all additives improved silage quality and AS. The most stable were silages made with chemical additives 1 and 2. However, the inoculants with potassium sorbate were also promising.

Conclusions

Aerobic stability of high moisture corn grain silages can be very much improved by using silage preservatives, especially chemical additives or microbial inoculants with potassium sorbate. Higher aerobic stability of HMCGS will be particularly acknowledged in the summer months.

Table 2. Chemical composition of high moisture corn grain silages

Silage	Dry matter ($g \cdot kg^{-1}$)	Crude ash ($g \cdot kg^{-1}$ of DM)	Organic matter	Crude protein	WSC	Starch	NDF	ADF
CG	664.3	14.0	986.0	86.9 b	10.8 a	794.1	101.6	273
CG/I1	661.2	13.3	986.7	91.2 a	8.6 b	790.0	96.6	28.6
CG/I2	660.0	13,5	986,5	91,6 a	7.3 b	791,9	97.0	27.5
CG/I1/PS	660.1	13.6	986.4	92.0 a	8.1 b	792.0	97.8	28.5
CG/I2/PS	659.8	13.2	986.8	92.1 a	7.1 b	792.2	97.5	27.6
CG/CA1	672.4	15.3	984.7	93.0 a	12.5 a	796.7	103.9	28.5
CG/CA2	671.8	15.8	984.2	91.3 a	12.9 a	795.6	104.0	27,8
GG	673.3	13.8	986.2	80.0 b	8,8 b	783.7	102.7	29.0
GG/I1	669.0	13.6	986.4	85.8 a	7,0 b	780.1	100.8	28.7
GG/I2	668.5	14.0	986.0	85.9 a	6.8 b	779.1	100.9	28.2
GG/I1/PS	667.5	14.2	985.8	86.5 a	6.4 b	779.3	101.3	28.4
GG/I2/PS	666.5	14.3	985.7	86.9 a	6.2 b	780.4	101.9	28,6
GG/CA1	677.9	15.9	984,9	87.7 a	13.6 a	785.5	104.3	29.3
GG/CA2	678.3	16.4	984.6	88.3 a	14.2 a	785.8	103.9	29.2

Values marked with a, b, c, d within CG or GG differ at P<0.05

Table 3. Fermentation parameters and aerobic stability of high moisture corn grain silages

Silage	pH	Ethanol ($g \cdot kg^{-1}$ of DM)	Organic acids: lactic	acetic	propionic	butyric	NH_3-N ($g \cdot kg^{-1}$ of total-N)	Aerobic stability (h)
CG	4.80 a	4.4 a	11.9 b	9.8 b	0.0 b	0.0	12.3 a	35 d
CG/I1	4.41 c	3.0 b	10.6 b	19.5 a	0.7 a	0.0	7.7 b	79 c
CG/I2	4.34 c	2.2 b	17.3 a	13.1 b	0.5 a	0.0	7.0 b	82 c
CG/I1/PS	4.44 c	2.7 b	10.4 b	22.1 a	0.6 a	0.0	6.1 b	113 b
CG/I2/PS	4.42 c	2.4 b	18.0 a	14.0 b	0.4 a	0.0	6.3 b	117 b
CG/CA1	4.63 b	0.5 c	6.6 c	5.6 c	0.0 b	0.0	3.6 c	130 a
CG/CA2	4.65 b	0.6 c	7.0 c	6.7 c	0.0 b	0.0	3.2 c	132 a
GG	4.61 a	3.2 a	12.9 b	10.6 b	0.0 c	0.0	10.9 a	46 d
GG/I1	4.31 b	2.0 b	13.0 b	27.1 a	1.0 a	0.0	6.0 b	89 b
GG/I2	4.22 b	1.8 b	19.6 a	14.2 b	0.7 ab	0.0	5.8 b	92 b
GG/I1/PS	4.20 b	1.7 b	13.7 b	28.9 a	0.5 b	0.0	5.7 b	134 a
GG/I2/PS	4.19 b	1.6 b	20.2 a	15.2 b	0.6 ab	0.0	5.5 b	136 a
GG/CA1	4.60 a	0.3 c	4.4 c	9.8 c	0.0 c	0.0	3.0 c	141 a
GG/CA2	4.61 a	0.4 c	4.1 c	7.6 c	0.0 c	0.0	3.1 c	142 a

Values marked with a, b, c within CG or GG differ at P<0.05

Silage health for animal Welfare

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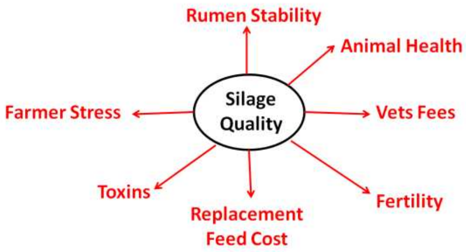
Farms across Europe are recognizing the 'all round' benefit of producing quality silage on the direct profit of their farms – the greater the silage quality is, the less the replacement feed costs are and generally the greater the animal performance. These **direct** benefits are well recognized, but what of the **indirect** benefits?

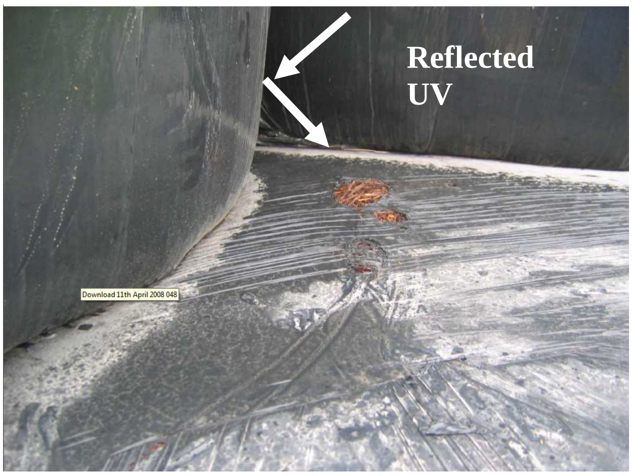
The quality of farm produced silage impacts many aspects of the dairy animal, not only nutrition. The 'health' of the silage directly impacts the health of the animal, and therefore the profitability of the farm. Recognizing this fact immediately allows the farmer to mediate a solution. Equally, recognizing which animal issues **are not** associated with silage will allow the farm to appropriately respond to animal issues. The impact of mould and mycotoxins is of enormous importance and is well recognized on farms, and has been discussed in many articles and will not be detailed here.



One issue that all farms suffer from is SARA (Sub Acute Rumen Acidosis). SARA is generally not associated with the lactic acid level of silage – it is in fact caused by excessive production of propionic, acetic and butyric acid in the rumen (the main volatile fatty acids), and an inability of the rumen to adsorb these acids quickly enough, driving the rumen pH down. This is often associated with low physically effective fibre and / or high cereal content in the diet. It is a mistake to blame a good homolactic fermentation for SARA.

Biogenic amines can be formed within silage through protein decomposition (and can also be produced by rumen microflora). Biogenic amines (histadine, putrescine, cadaverine, spermidine, tyramine etc) can be formed in 'high protein forages' such as alfalfa and clover through protein decomposition but also in maize through decarboxylation of amino acids by various bacteria. Biogenic amines have been linked to various health issues inclusive of ketonemia, systemic histaminosis and reduced nitrogen degradability. A positive correlation exists between increasing dry matter and reduced biogenic amine concentration, and between a faster fermentation and reducing biogenic amine. Use of heterolactic inoculant to increase the speed of fermentation can therefore benefit silage health and animal health with regard to biogenic amines (generally rumen microflora degrade low level ingested biogenic amines [Van Os *et al*, J Agroc Sci 1995; 125: 299-305), but this is only after the negative impact on intake].

Enterobacteria are generally rapidly killed during the early stages of ensiling by the initial decline in pH, but when the silage is aerobically challenged either through the fermentation or through damage to the sheeting, the pH rises and Enterobacteria numbers can increase to the region of ~10^8 cfu/g. The initial Enterobacteria population at ensiling is often dominated by *Erwinia herbicola* and *Rahnella aquitilis*. These are rapidly replaced by other species, inclusive of *Escherichia coli*, *Hafnia alvei* and *Serratia fonticola* (Heron *et al*, J Appl Bacteriol 1993; 75: 13 – 7), with *E coli* being the most significant threat to health as (air penetration can come from a variety of causes from inadequate sealing, animal damage and even reflected UV).



The presence of air leads to compounding problems. Air penetration slows and even stops fermentation, leading to a non stable, elevated pH, or to the elevation of the pH in localized areas, which in turn allows the outgrowth of other organisms / spores naturally present. *Clostridia sp* lead to the production of butyric acid. *C tyrobutyricum* is relatively acid tolerant and converts lactic acid to butyric acid and H_2, which in turn raises the pH of the silage and allows the outgrowth of other undesirable microbes. *Clostridia botulinum* is relatively rare in silage as it is not acid tolerant, but, as a sporolating organism it is capable of outgrowth between pH 5.3 and 6.5 (which is typical of deteriorating silage) – generally the presence of *C botulinum* is associated with an ensiled cadaver or cadaver close to point of air entry. 1g of botulium toxin is theoretically sufficient to kill 10 million people (Silage Science and Technology), and with the preferred method of feeding being TMR a small area of contaminated silage can affect an entire herd (in Israel 1,000 cows had to be slaughtered because of botulism contamination).

The aerobic conditions described above also afford for the outgrowth of *Listeria* spp. *Listeria* is generally inhibited by an anaerobic environment and low pH, but when aerobically exposed it can survive at pHs as low as 4.2 and can lead to abortion, silage eye and other welfare issues as well as leading to the possible contamination of produced milk. With aerobic exposure of silage, generally there is a visible proliferation on the surface of the silage – but this is only true if the aerobic exposure is at the surface of the silage. If air penetrates through side walls, the 'presence' can be invisible. *Bacillus cereus* grows in silage under the conditions discussed and is one of the causative agents of mastitis

Speed of fermentation brings many visible benefits – improved DM and nutrient recovery, but it also delivers equally important 'invisible benefits'. Consider your bacteria. Consider your plastic. Consider your cows.

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Quality of preserved feeds and the risk of clostridial infections in cattle

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Nutrition is regarded as the most important external factor in dairy cow nutrition that determines milk production, fertility, health and enables to fulfil the genetic potential of both the individual cow and the whole herd.

Silages make up the basic component of ruminant diets. The quality of silage determines nutritional value and palatability of the diet and total dry matter intake. Silage quality is influenced by many factors during each phase of manufacturing process, storage and inclusion in total mixed ration (TMR) and feeding. Ensiled feed may pose a certain threat to animal health, too. Even properly managed silages that meet quality requirements are prone to spoilage and may become unsafe from the hygiene point of view very quickly. Every year mainly in the spring and summer on many farms silages are exposed to aerobic spoilage. The aerobic fermentation is performed by yeasts, clostridia and fungi. Yeasts are the ones most involved in the aerobic spoilage processes. Due to the degradation of lactate silage pH is changed in favour of the growth of bacteria and fungi. In the silage products of secondary metabolism are accumulated such as mycotoxines, biogenous amines (cadaverine, tyramine, putrescine, spermidine, tryptamine and histamine). The counts of E. coli, Clostridia, Listeria and other bacterial species are growing. The nutritional value of silage is reduced and its hygiene status is unsatisfactory. Such spoilt silages have a negative impact on health, performance and fertility of cows.

The cow´s health may be harmed due to increased counts of clostridia in the silage. As a matter of fact, every silage contains some Clostridia, and they are present in the digestive tract as well. In the forage is contaminated by earth, slurry and manure, Clostridial counts are very high, leading to alterations of silage fermentation processes. Large amounts of ketogenic butyric acid, free ammonia and biogenous amines may be formed in silage. Counts of Clostridia and clostridial spores are increasing. Such silage may pose a great hygiene threat and health risk to cows. Clostridial spores may contaminate milk, deteriorate its quality, thus preventing the production of good cheese. In the digestive tract the high counts of clostridial spores may induce mucosal inflammatory reactions, producing enterotoxins that are absorbed and may damage the liver and kidneys. Clostridia may penetrate across the gut wall damaged by the inflammation into the circulation and induce inflammation in the peritoneum, liver kidneys and muscle. Clostridia produce gas that accumulates in the subcutaneous tissue, and produce toxins that impair the general health status of the animals. A massive infection may lead to fever, followed by sepsis and death of the animal. Diseases induced by Clostridia from preserved forage occur in cow dairy cow herds every year.

In the herd A clostridial infection was diagnosed in dairy cow fed wilted alfalfa silage from the pit contaminated with slurry. In the herd B the source of infection was wilted grass silages harvested under bad weather and therefore contaminated with soil. In both the herd a massive outbreak of clostridial disease occurred, manifested as diarrhoea in all the cows, drop in daily milk yield by 3-5 litres /cow/day, significant increase of somatic cell counts. In the herd A 6 cows died, in the herd B 5 cows died. In 40 % of cows subcutaneous emphysema was found. Dietary dry matter intake dropped markedly. Metabolic examination of some cows (10 and 6 cows from the herds A and B, respectively) showed dehydration and impaired liver function. Rumen fluid analyses showed rumen alkalosis. In faeces, high counts of spores of *Cl. perfringens* and *Cl. chauvoei* were found. After the exclusion of spoilt silages and introduction of dietary changes the health status was slowly improving, however, some cows showed diarrhoea for over 10 days. Milk yield increased very slowly and even after one month it did not achieve the original level. Increased SCCs in bulk tank samples persisted for 3 months.

The unsafe preserved feedstuffs are very risky for all cattle production stages. Clostridial infections cause a serious disease in cattle. They may occur at every production stage and cause considerable direct and indirect financial losses. In the two herd, a source of the infection was the conserved forage which was contaminated by soil or waste water. In cows originated disease characterized by multiple total disruption of health, a significant reduction in milk production and mortality cows. For these reasons, it is necessary to prevent these infections adherence to the principles of technology in harvesting, preservation and storage of silages. In the event that the indication of the disease in animals is the source of infection. Basic measures for suspected disease of cattle caused by Clostridia, the examination of representative samples of feaces of cattle in the herd. In case of problems containing spores in milk may just these results show that the cause of the poor feed hygiene or poor milking technology. Desirable content dispute in the feaces is < 10,000 / g respectively $< 10^4$ CFU / g of feaces. If the content of the dispute in the feaces of cattle repeatedly high (> 100,000 CFU / g , respectively > 10^5 CFU / g), it is necessary to examine the conserved fodder (silage) and the quality of the fermentation process, including aerobic stability. When proof of butiric acid fermentation (sum of butyric acid > 1.0 % of dry matter) is that there has been a multiplication or formation of spores in silage. This should not be feeding silage to dairy cows. Conversely, if the content of the butyric acid or is only traces (<0.1 % of dry matter) is a cause of severe contamination during harvest. Desirable content of clostridia spores in silage is < 1,000 CFU / g of feed content is acceptable outcome to the dispute 10,000 CFU / g respectively 10^4 CFU / g of feed in the higher incidence of 100,000 CFU / g, respectively 10^5 is not this feeding silage to dairy cows . If the content is low in dispute must be examined technique and quality of work for the collection bucket . There may be fed boundary layers (decayed or secondarily fermented silage) or too large area for the collection of silage from trough.

Observance of technological processes at harvest, silage own using suitable preservatives are essential for the production of high quality hygienic fodder. Made preserved forage quality must be well stored properly collected, and fed . Contamination by the soil is always a big risk for the development of clostridial infections.

This work was part of the research project of the Ministry of Agriculture and Food CZ

Quality Of Whole Plant Of Pea With Lucerne And Possibilities Of Ensilaging

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The whole crop of pea (*Pisum sativum*) is considered to be a source of high quality proteins and amino acids. It can offer high yields, good quality feed for ruminants and it can improve self-sufficiency of milk farms to provide crude protein in feeds from own resources (Borreani et al., 2007). One way of using is the production of silage from whole plants. Pea can be grown as monoculture as well as in mixture, namely in legume-cereal mixture or in combination with lucerne (*Medicago sativa*). Mustafa et al. (2000) reported that whole pea silages, harvested at full pod stage, can successfully replace barley or lucerne silage as a forage source for dairy cows at the beginning of lactation. They consider them to be a good alternative feed. A number of researches showed that silage from whole pea plants is very palatable for cows and it can be fed at large amounts also thanks to the low content of NDF combined with high passage of rumen. More over, in this silage is a good balance between crude protein content and energy and it seems to help save concentrates used in feed rations (Rondahl, 2004).

From the cultivation point of view pea can offer farmers a great deal of flexibility in their cropping rotations due to their quick growth in a short period providing forage of a high nutritive value and palatability (Koivisto et al., 2003). It is a very good forecrop. Growing pea for silage with underseeding of lucerne as subsequent crop seems to be very favourable. Rhizobiums are on roots of pea as well as on roots of lucerne. Thanks to them creates pea climate in the soil that is very good for growth of lucerne and development of its root system, which creates good basis for its further cultivation. If pea is sown in the field together with lucerne, it is possible to harvest 1 crop of pea for silage and 1 – 2 cuts of lucerne during one vegetation period thanks to fast growing pea.

Varieties of semi-leafless pea were developed by breeding. They are suitable mainly for regions with higher precipitation depth and for soils well supplied with easily received nutrients. High resistance to lodging is their merit (Uzun et al., 2005). Breeding such pea simplified the harvest, compared with the past; it influenced directly also the purity of feed and content of clostridia.

The objective of our work was to study the quality of semi-leafless pea grown with underseeding of lucerne during ripening and to test the quality of fermentation process and the influence of biological inoculant at low and high dry matter content in the ensilaged matter.

Material a Methods

Semi-leafless pea was grown together with lucerne at the elevation 275 m above sea level. The stand was harvested in the phase of milk ripeness in pea. The portion of lucerne represented only 10 %. One half of the cut matter wilted 4 hours, the second one 24 hours. The wilted matter was chopped, homogenized and ensilaged into laboratory silos with the content 1.7 l.

In each variant was produced control silage without ensilaging preparation and inocculated silage treated with biological preparation, which contained *Lactobacillus plantarum* DSM 3676 and 3677, *Propionic bacterium* DSM 9576 and 9577 (application dose 2 ml.kg^{-1} feed). The produced silages were stored in standard conditions for 110 days. During the storage was observed the course of weight losses by weighing in regular 21 day intervals. Parameters of the fermentation process were assessed in silage samples after the end of the experiment. The obtained results were statistically evaluated and compared by one way variance analysis.

Results and Discussion

We harvested pea with underseeding of lucerne at 3 different stages of ripening. Decrease in concentration of crude protein, water-soluble saccharides, fat as well as crude fibre took place during the ripening. It was reflected also in decrease of NEL and PDI. Increase in concentration was noticed only with starch. NDF level was generally low.

In table 2 are results of fermentation process in silages made of whole pea plants with lucerne. Addition of lucerne to pea did not deteriorate the ensilability of this feed. We found out that the fermentation process passed through more intensively in silages made of matter with low dry matter content than in silages with high dry matter content. Total content of fermentation products, which was markedly higher in silages with lower content of dry matter, gives evidence of it. Rondahl (2004) came to the same results; he confirmed that the amount of moisture present in the ensiled crop affects the total bacterial count and rate of fermentation. In wet crops with very high soluble carbohydrate levels the LAB are extremely active, and the result will be low pH silage of high lactic acid content.

Application of *Lactobacillus plantarum* at ensilaging positively influences pH decrease and creation of ammonium N, it increases lactic acid content and water-soluble saccharides in silage compared with non-inoculated feed (Fraser et al., 2000). Also in our experiment influenced the additive of biological inoculant positively the fermentation process, decreased pH, VFA, proteolysis as well as dry matter losses that occur during the fermentation. The detected differences were not statistically significant.

Table 1: Nutrition value in whole crop pea with lucerne in different phases of ripeness (fresh mass)

	Term of cutting		
	3rd July Pod swell	13th July Full pods - milk ripeness	25th July Full pods - start of wax ripeness
Dry matter in g FM	179	234	412
Organic matter in $g.kg^{-1}$ DM	933	979	855
Crude protein in $g.kg^{-1}$ DM	182	156	113
Crude fibre $g.kg^{-1}$ DM	286	283	218
NDF in $g.kg^{-1}$ DM	388	440	372
Sugars total in $g.kg^{-1}$ DM	77	51	22
Starch in $g.kg^{-1}$ DM	66	120	186
Fat in $g.kg^{-1}$ DM	29	26	25
Ash in $g.kg^{-1}$ DM	67	21	145
ME in $MJ.kg^{-1}$ DM	10.5	10.5	9.0
NEL in $MJ.kg^{-1}$ DM	5.97	5.82	5.24
PDI in $g.kg^{-1}$ DM	109.38	94.83	68.47

Table 2: Parameters of fermentation process in whole crop pea silages with lucerne

	Low content of DM (246 g)				High content of DM (424 g)			
Parameters n = 6	Control silage		Inoculated silage		Control silage		Inoculated silage	
	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
Dry matter in g^{-1} FM	238.49	1.29	238.75	0.44	397.68	5.95	398.46	3.63
Losses of DM in %	0.92	0.27	0.84	0.17	4.09	1.37	3.78	0.87
pH	3.97	0.06	3.91	0.02	4.18	0.03	4.16	0.02
Acids in $g.kg^{-1}$ DM								
- lactic	105.19	6.53	102.36	4.81	82.47	5.90	95.76	10.63
- acetic	21.61	2.96	19.27	2.51	11.47	1.23	10.77	1.20
- propionic	2.93	1.24	1.54	0.78	0.62	0.22	0.60	0.32
- butyric + i.b.	1.66	0.49	0.61*	0.14	0.58	0.28	0.44	0.23
- valeric + i.v.	0.83	0.21	0.50	0.29	0.31	0.14	0.18	0.12
- capronic + i.c.	0.64	0.24	0.48	0.33	0.07	0.02	0.09	0.07
VFA total in $g.kg^{-1}$ DM	27.67	5.12	22.40	4.43	13.05	1.34	12.08	1.72
Acids total in $g.kg^{-1}$ DM	132.86	6.70	124.76	5.79	95.52	6.60	107.84	10.70
Alcohol in $g.kg^{-1}$ DM	2.18	0.31	2.39	0.13	3.54	0.62	3.05	0.44
NH_3-N of total N in %	10.07	1.82	8.81	0.45	7.67	0.52	7.51	0.67

$* P < 0.01$

Conclusion

The following complex requirements must be met to obtain high quality silage from whole pea plants: wilting to 25 – 30 % dry matter, short cut of the matter, addition of effective additives, good pressing down and anaerobic covering. Whole pea plants are feed, which is noted for good ensilability, namely at lower as well as higher amount of dry matter compared with recommended values. The lucerne supplement in pea stand, which represented 10 % of weight, had no negative effect on ensilability. Inoculation of the ensilaged matter with biological ensilaging preparation on the basis of homofermentative LAB improved the fermentation process.

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Acknowledgement

This article was written during realization of the project „BELNUZ č. 26220120052" supported by the Operational Programme Research and Development funded from the European Regional Development Fund.

AgriNIR^{TM} - Real Time, on farm analysis for managing products variability in animal nutrition

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The technology used in feeding dairy cows continues to advance.
A primary reason is the need to improve the efficiency of nutrient used on the dairy farm, decrease nutrient excretion to the environment and improve dairy farm profitability. This is driven by a combination of animal and farm factors. There have been significant improvements in both genetics and management of the dairy cow.

In 2009, a Dairy Farm Business Summary for 93 herds in western New York revealed a total purchased feed cost and crop expense per cow equal $1,539, equal to 47% of the milk income. This indicates the importance of the feed component of dairy farm management on costs and potential profitability.

The primary feeding system used in US herds is total mixed rations (TMR's) with the scope to obtain the best ruminal environment for microbial, combining specific proportions of nutrients in the diet, mixing all the feed together and making it available 20-22 hours per day.

Since many dairy rations are based on home produced silage, a key question is how to monitor potential changes in silage dry matter and then use this information to adjust rations to maintain constant kgs of dry matter fed from the various silages.

Differences between theoretical, prepared and effectively consumed TMR by the cows were widely reported (Leonardi e Armentano, 2003)[i]. The differences, are due to errors in loading feeds (wrong weight, ecc…) and change in water content that alter the amounts of dry matter of feed loaded into the wagon (Buckmaster 1998[ii]; Ishler 2001[iii]). The value of keeping rations constant by adjusting for dry matter changes was reported in a research trial conducted by Dr. Dave Mertens at the US Dairy Forage Research Center. This indicated about a 2 kg shift in milk production under the conditions of that trial.

Due to the current situation, it is a real basic requirement for all dairy farmers to optimize the diets, providing a more consistent TMR, controlling the accuracy of the mixer operator, measuring on farm "in real time" the actual Dry Matter Content of feedstuffs and monitoring the inventory of ingredients in terms of quantity and quality.

The AgriNIR^{TM} is a portable NIR lab, able to perform real time analysis on fresh samples in order to take under control the quality of feed stuffs in your inventory measuring: Dry Matter, Crude Protein, Starch, ADF, NDF, Crude Fat and Ash.

Moreover, thanks to a specific software studied and produced by Dinamica Generale (DTM^{TM} AgriNIR), it is possible to import predictions from the AgriNIR^{TM} save them in a Data base for statistical analysis based on graph and reports. On top, DTM^{TM} AgriNIR is completely integrated in a Feeding Management Software (DTM^{TM} IC) and is possible to adjust immediately nutrients on feed ingredients and, consequently, the target quantities of ingredients in final recipes based on actual values of Dry Matter, allowing the following:

Move Rations management from As Fed to Dry Matter basis: definitely a "Great step ahead" in terms of TMR consistency.

The AgriNIR^{TM} is a tool able to help animal producers to optimize their feeding processes managing variability of feedstuffs. It is able to control wide changes in nutrients contents of feedstuffs providing necessary info to responsible in order to take actions for keeping the final TMR as consistent as possible during the time, saving costs on feeding, improving the process efficiency and reducing the environmental impact produced by farms.

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Section 4: Production of biogass by conserved forages

Biogas Production From Agricultural Raw Materials Including New Energy Crops In Germany

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Introduction

Biogas can be created form a wide range of organic material, so called biomass. It is an important fact that the process can be supplied by a multi feedstock.

The development of the biogas sector in Germany within the last 10 years was quite strong until today. In figure 1 the development of biogas installations are diagrammed. At the end of 2013 around 7,700 plant operating, the majority at the agriculture. A total power capacity of circa 3,500 MW generated by biogas plants in Germany substitute about 5 coal-fired power plant or 3 nuclear power plants. The average electrical capacity of German biogas plants are at approx. 420 kW. Beside the sector agriculture biogas installations can be found at anaerobic bio waste treatment plants, at use of landfill and sewage gas and waste water treatment plants mostly at the food industry.

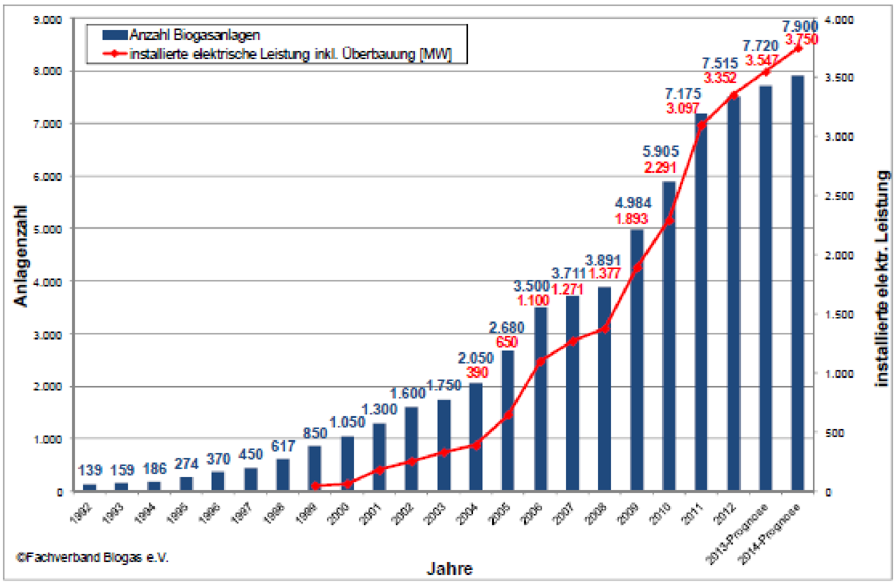

Figure 1: Development of the number of biogas plants and the total installed electric output [in MW] (as of 3/ 2014) including the forecast for 2014 [Fachverband Biogas 2014].

The strong growth of the biogas sector especially since the year 2000 was based on the German renewable energy sources act (EEG) for electrical energy from renewable (biomass) energy. The amendment of this law in 2014 with a reduction of tariffs is intended. The draft is causing strong critique by the German biogas association and the biogas industry. The use of biomass as a feedstock will be significantly limited to an electricity production of max. 100 megawatts. To meet the expansion path, the support rates for new biogas plants are drastically reduced when the biogas extension of a year exceeds 100 megawatts. Only a limited number of small scale biogas projects up to 75 kW electric and plants based on biowaste feedstock can be realized under this frame conditions at the coming years [Fachverband Biogas, 2014]. The German biogas industry will continue focusing on projects abroad and repowering of existing plants. The biogas companies will have to reduce personnel capacities under that future framework.

By biogas approximately 4% of all electricity is generated in Germany in 2013 and it supplies 7 million households with electricity. Overall, about 23% of electricity and 10% of the heat generation produced from renewable sources in Germany in 2014. In the year 2011 a number of 1,270 new biogas plants were taken during operation whereas in 2013 only 205Anlagen were built.

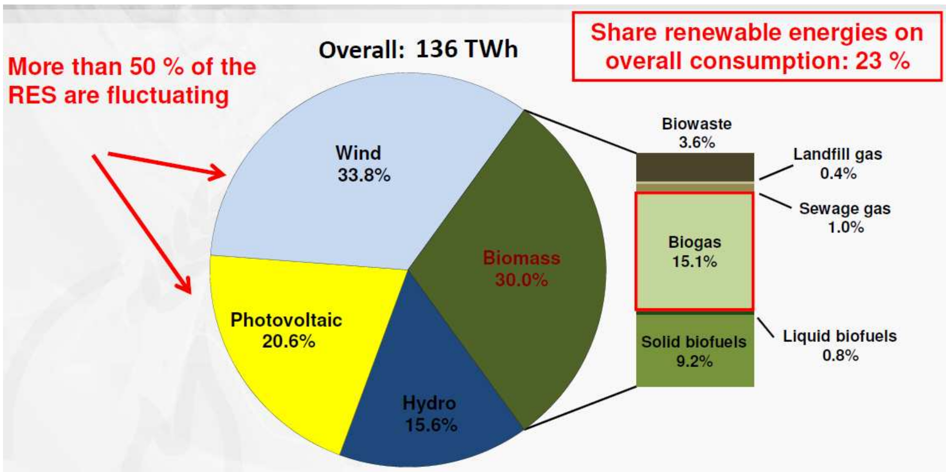

Figure 2: Structure of German electricity production from renewable energy sources in 2012 [Fachverband Biogas, 2013]

In all an electrical capacity of 3,500 MW from Biogas in 2013 are producing continuously renewable electricity and heat. In general, power creation from biomass has some advantages when comparing with power origins from other renewable resources like wind and photovoltaic, with dependency on the weather conditions. The increasing portion of renewable energy at the grid leads to a more volatile electricity production. Biogas can easily be stored at the place and can create electricity on a flexible and demand orientated basis. Some biogas plants in Germany secure by a flexible operation, the stability in the power grid. The transition to the flexible power generation is increasing and has realized especially by biogas plants. Additional capacity for gas storage (and heat) and power generation need to be created as structurally. The market bonus and flexibility bonus offer opportunities for additional income in the framework of the EEG 2012.

Members of the Biomass Association Freiberg have come together to form a group of operators of biogas plants, “Freiberger Land” and sell the electricity generated through a trading house directly. In the first stage 5 MW of electrical capacity (target 10 MW) from biogas are marketed directly together. The first plant will nowadays be upgraded to a flexible driving.

An ingenious concept to use the created heat is essential for an economic operation of a biogas project. The distribution of the heat via district heating networks or via extra gas networks and satellite combined heat and power station (CHP) located at a heat sink are some options. The portion of 60% of the generated heat must be reasonable used. That is the obligation due to the actual legal framework (EEG 2012) whereby 25% overall are counted for the process heat of the anaerobic digestion.

There are a rising number of biogas plants with upgrading of the created biogas and injection into the natural gas grid (124 plants [Fachverband Biogas, 2014]). Overall capacity of upgrading of raw biogas to biomethane is 132,000 $Nm^3 h^{-1}$. For the political aim of 6 billion m^3 biomethane at the gas grid (Integrated Energy and Climate Program of German Federal Government) in 2020 a number of around 1,500 biogas plants (average of 5 MW thermal capacity) and 1.2 million hectare fields with energy crops are needed. With the development rate till today this aim will not be realized. The application of biogas as an engine fuel for transportation in vehicles is rising and mainly based on the supply via the natural gas grid.

Feedstock in German Biogas Plants

In 2013 1.15 million hectare, from total 2.39 million hectare for energy and technical crops, where cultivated with energy crops for biogas formation, see figure 3 [FNR, 2013]. There are a strong rising to register over the last 10 years to 14.4% of all German farmland today from around 0.8 million hectare for renewable resources in 2003.

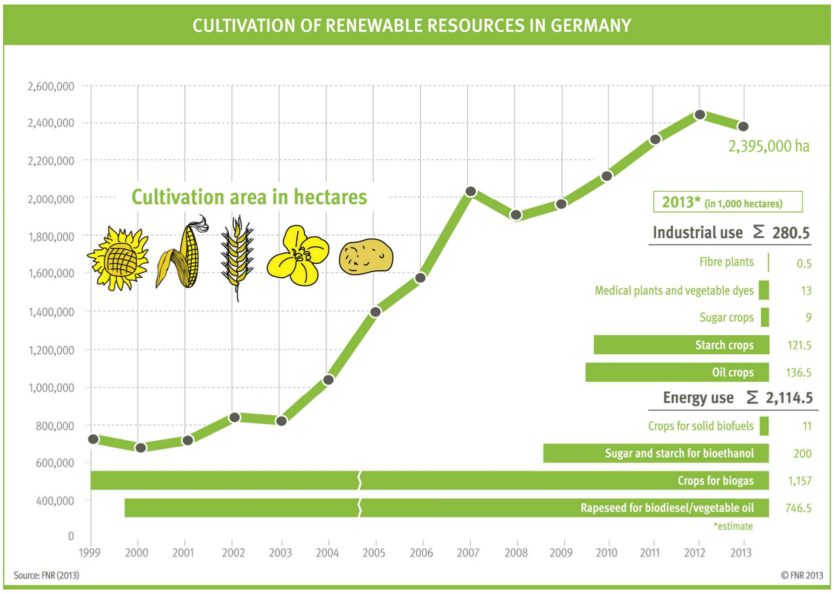

Figure 3: Cultivation of renewable resources in Germany from 1999 to 2013 [FNR 2013]

Figure 4 shows the proportions of the feedstock for German biogas plants in 2012. According to the assessment of the energy content energy crops with 81.5% has the largest share. By weight, it is 52.8%. The amount of manure is 43 weight percent. The potential of manure for biogas production is exploited only at about 20 to 30%. The use of bio-waste and industrial and agricultural waste as a feedstock previously played a minor role.

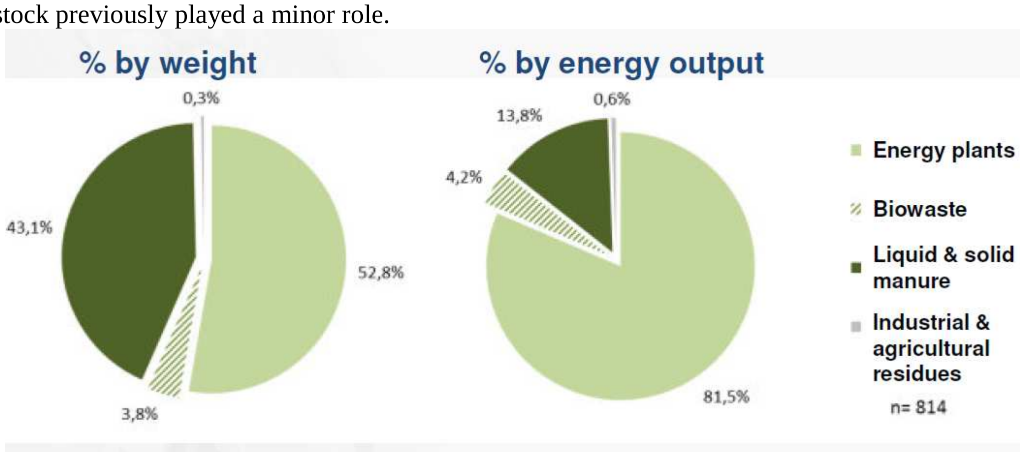

Figure 4: Feedstock in German biogas plants in 2012 [Fachverband Biogas, 2013].

The uses of silage maize as a substrate for biogas plants are well established and highly economic. The potential of one hectare silage maize is about 5,000 to 7,000 Nm³ methane gas ha^{-1}. There are 0.9 million hectares cultivated with silage maize for biogas formation. In some areas of Germany (e.g. Lower Saxony) the cultivation of maize reaches relative high portions (between 45 and 70%). Opponents in relation to the high cultivation area of silage maize point out ecological arguments (erosion, monoculture), the competition to other agriculture crops (fodder and food) and rising leasing cost for land. [Emmann et al., 2012] Figure 5 the development of the cultivation in Germany of maize by the use is shown. In recent years, there was a slight reduction of the cultivation area of maize silage for biogas due to the chance of feedstock and less new constructions of biogas plants.

In organic farming clover grass with 21% has importance as a feedstock for biogas. Up to 4,000 m³ methane gas ha^{-1} can be achieved at anaerobic digestion. Around 180 biogas plants operating on organic farms based mainly on 55% manure feedstock. Only 17% use maize silage whereas conventional biogas plants utilize approx. 39% maize as an input material. [Renews Kompakt, 2014]

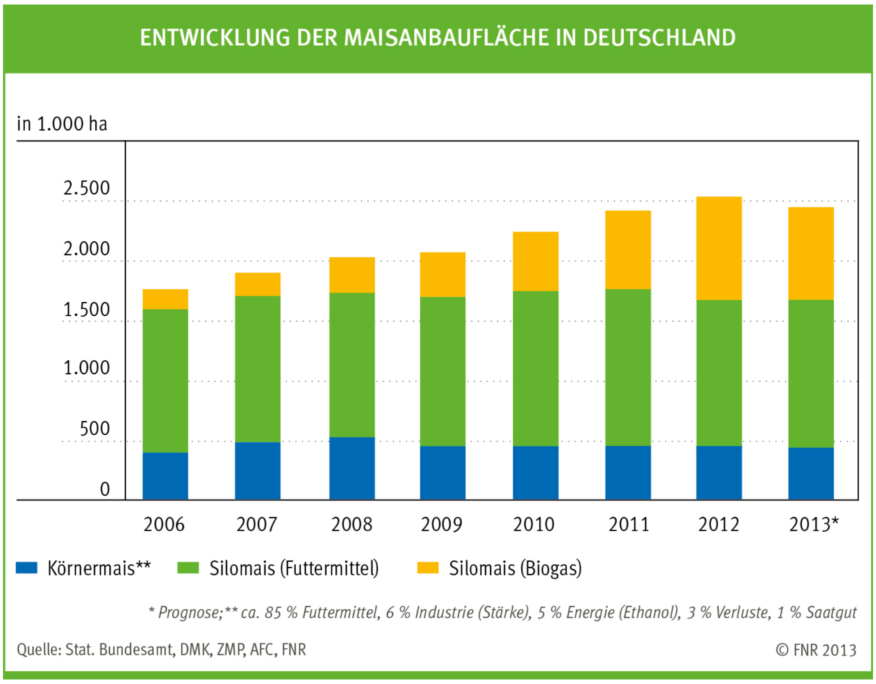

Figure 5: Development of the cultivation area for maize (blue; low part: grain maize, green; middle part: silage maize for fodder, yellow; upper part: silage maize for biogas) in Germany [FNR, 2013].

In Saxony for instance the portion of maize cultivation are 75 thousand hectare that are 11% of all Saxon fields (average in Germany: approx. 22%). [Brückner, 2013] Agricultural biogas plants in Saxony (about 205; average 420 kW electric capacity) using > 70 percent by weight manure as a feedstock. The members of the Biomass Association Freiberg operating biogas plants with total 5 MW electric capacity and using about 80 percent manure as a biogas substrate. The rest is covered by organic leftovers materials (e.g. cereal husks, bad quality silage, fodder leftovers, inter alia).

New energy crops for biogas

New energy crops offer many opportunities for a higher species diversity and acceptance of bioenergy.

Large Cultivation investigations in Germany are currently in progress in the composite project called EVA [Eckner, 2013]. The goal of the EVA project is carried out since 2005 to examine extensive energy crops (economic & ecological assessments, determination of biogas yields, irrigation experiments, two-crop use and experiments with the organic production method). Eleven typical locations plot trials were established with five crop rotations. The result showed in the project EVA in the years 2009 and 2010, crop rotation No. 1 (Winter barley-sorghum (SZF) maize winter triticale Phacelia (SZF) over the entire German locations across the highest yield with an average of 47.5 t dry matter (DM) ha^{-1}. The same level of income has crop rotation 3 (corn-winter rye sorghum triticale-one-year ryegrass) considered the average of all experimental plants with 47.4 t DM ha^{-1}. The most profitable single crop on the field with an average of 19 t DM ha^{-1} and was maize on the methane yield in the biogas plant as well of economic valuation. The following energy plants are examined within EVA project in Germany: silage or energy maize, sorghum, whole-crop cereal silage, fodder mixtures, cup plant (Silphie), sugar and fodder beets, winter turnip rape, sunflowers, rape, phacelia, legumes, cereal mixtures, blooming mixtures) [EVA, 2014]. Beets while having a very high potential on the agricultural crop land of average 5,500 m^3 ha^{-1}.

Research and Development (R&D) - projects are running with the crops buckwheat and quinoa in southern Germany (Bavaria). Buckwheat has a yield potential of 4.5 to 6 t dry matter (DM) per hectare and a gas yield of 240 to 280 Nl methane gas kg^{-1} volatile solids (VS). On average 1,200 Nm^3 methane gas per hectare can be achieved. With the cultivation of Quinoa a biogas yield of 1,600 Nm^3 methane gas ha^{-1} is possible. The advantages of both crops are short cultivation times (summer crop), at the crop rotation and ecological aspects, e.g. that it blooms [Stockmann et al., 2014].

A mixed cropping of vetch (vicia villosa) and winter rye (secale cerale) relaxes on crop rotation and would be an alternative energy plant. It could be achieved an average yield of 11 t ha^{-1} in field trials. This mixed cropping could generate 3,400 m^3 ha^{-1} of methane gas in the middle [Fritz et al., 2011].

Mixtures of indigenous wild perennial crops can achieve 50 to 60% of the biogas yield of silage maize. Advantages are ecological aspects, extensive cultivation and moderate costs. Mixture consists of 18 to 36 species at the seed. The harvesting yield varies at the second year of cultivation between 8 to

15 tons DM ha^{-1}. Relative high yields from 268 to 320 Nl methane gas kg^{-1} VS where reported (10-15 per cent lower than that from silage maize) [Vollrath, B., 2012]. The development is still in its early stages and offers potential. Figure 6 indicate a field of wild perennial crops in front of a biogas installation.

Figure 6: Mixture of wild perennial energy crops for a major diversity [Agentur für Erneuerbare Energien, 2014].

The most hopeful energy crop for biogas is the Cup plant (*Silphium perfoliatum*), a perennial composite plant from North America. Around 400 hectare of cup plant in Germany is under cultivation. Yields of up to 22 t ha^{-1} on good sites and thus methane productivity from 5,000 to 6,400 m³ ha^{-1} are achievable what is comparable to silage maize. Disadvantage is the high cost for planting. Experiments are carried out to produce seed [Biertümpfel et al., 2012]. It will help to reduce the initial costs. More details of the investigations had done near of Freiberg will be found at the passage RekultA.

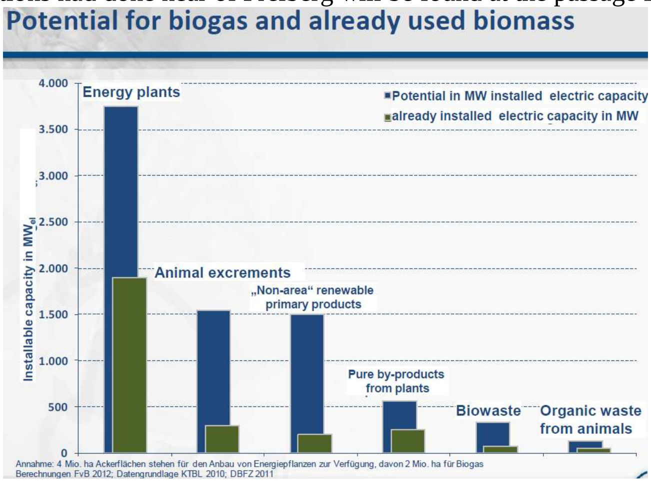

Figure 7: Potenzial for feedstock for biogas [Fachverband Biogas, 2012]

Energy plants have the highest potential in addition to manure as feedstock for biogas plants. Here, an assumption of 2 million hectares was made as a growing area for energy crops for biogas. Figure 7 displays the potential of various feedstocks and the already installed electric capacity based on that biomass.

Project rekultA

RekultA stands for: Reclamation of heavy metal polluted areas and mining landscapes of the euro region Ore Mountains by site-adapted growing of perennial energy crops; in Czech: Rekultivace těžkými kovy zatíženýcha reálů a krajin po důlní činnosti v Euroregiónu Krušné hory díky místně přizpůsobenému pěstování víceletých energetických plodin.

In the area of Freiberg/ Germany due to permanent mining and metallurgy activities heavy metal accumulation in the topsoil are present due to 800 years. On these heavy metal contaminated surfaces with feed and food are being grown, which partially limits are exceeded. If exceeding the limits in the harvested food and feed crops determine so farmers must declare their products generally as waste. High economic losses and the threat of set-aside are the consequences.

A solution for the heavy metal contaminated areas could be the cultivation of energy crops for energy. The positive aspect is that there is no competition for food or feed production on those fields.

In Ziel3 project "RekultA" (duration 2010-2014, funded by the European Union) the goal is to grow perennial innovative energy crops on heavy metal contaminated areas in the Ore Mountains to the test and to generate regional and cross-border biomass supply chains with relevant stakeholders. Here, an intensive German-Czech exchange between the project partners, the Association for the Promotion of Biomass and Renewable Resources Freiberg e.V. (short: Biomass Association Freiberg) and the Institute for Ökotoxiologie from Chomutov takes place, with the aim for a cross-border biomass network. The project partner in Chomutov performed on post-mining landscapes with marginal land cultivation experiments with new energy crops. The results of these studies can be found on German and Czech on the project page www.rekulta.org.

RekultA - growing trials in Saxony

The selected power plants should meet several criteria: Climatic and soil suitability in the area of Ore Mountains, perennial nature (erosion control, environmental friendliness), no invasive power plant and opportunities for energy recovery in biogas plants or combustion facilities. Another important criterion by farmers was that the energy crops stabilizing the heavy metals in the soil (Phyto-stabilisation). A phyto-extraction would have taken on the heavy metal polluted sites hundreds of years and would therefore not feasible in the short term. In the field tests are investigations under practical conditions, i.e. the areas are managed under production conditions (practice test) and have an area of approximately 3,000 m² (except the szarvasi grass-area in Clausnitz with approximately 2 ha). At the Biomass Association Freiberg are predominantly regional agricultural cooperatives members. These farms are interested in field trials to elicit well-founded statements under large-scale field conditions, as they are practiced in practice, and then implement the growing recommendations locally and with realistic growth and earnings expectations.

- The following energy plants were selected in Saxony:
- Cup Plant, Silphie (*Silphium perfoliatum*)
- Reed Canary Grass (*Phalaris arundinacae*)
- Miscanthus (*Miscanthus giganteus*)
- Giant Wheatgrass „Szarvasi“ (*Elymus elongatus*)
- Willow and Poplar (7 poplar varieties: Max 1,3,4; Hybride 275, Beaupre, Muhle-Larson, Androsoggin; 6 willow varieties: Tordis, Tora, Inger, Sven, Torhild, Jorr)

The objectives were to determine the crop development and income levels of the innovative energy plants in the climatic conditions of the Ore Mountains in comparison to the established energy plant maize and to analyze the heavy metal uptake.

The four experimental plots are located in the area of Freiberg (Weissenborn, Niederbobritzsch, Colmnitz, Clausnitz) in the eastern Saxon Ore Mountains. The areas are located on the edge of the Ore Mountains, i.e. in 400 to 900 m above sea level with a partially steep slope. Due to the height the growing season starts a little later than in the Saxon lowland sites and thus has a slower warming of the soil, which reduces the earning capacity. The soils are residual soils with a high stone content. Due to the slope there is a high risk of erosion.

Results

The cup plant (Silphie), that reed canary grass variety "Palaton", giant wheat grass "Szarvasi", poplar variety "Hybrid 275" and willow variety "Torah" is committed to results of RekultA growing trials for cultivation in the Eastern Ore Mountains.

On the fields with Miscanthus a high mechanical and chemical costs in the year of planting was necessary to minimize the weeds. After the first winter 2011/2012 the failure rate of Miscanthus plants was up to 87%. This meant that the field was wrapped.

The issue of cup plant (Silphie) in the first year of cultivation was in the low competitive ability against weeds. Despite mechanical and chemical plant protection measures of the weed pressure was present (see picture 1 for development details). The cup plant (Silphie) optimally developed from the second year of cultivation on the experimental plots and had winterkill in the years 2012 and 2013 by only 2% (by, inter alia, mice damage to the roots). The long flowering period of about 8 weeks in August and September is an ideal bee and insect grazing in late summer, when there is no other agricultural crops more bloom. The fostering of the cup plant shall be kept with conventional agricultural technology such as for maize. The harvest can be done with a chopper with corn head. At the RekultA - cultivation experiments the harvest went smoothly with the conventional technology. The only problem lay in the crop management operation coordinated so that the energy crop is not harvested at the optimum time and later because the same time held the corn harvest. The ensiling of cup plant (Silphie) takes place as in maize silage, but with the advantage that, even with dry matter content of about 25% no seepage formation occurs [KTBL, 2012].

July 2011

September 2011

March 2012

July 2012

August 2012

September 2012

Picture 1: Development of perennial cup plant (Silphie) on the RekultA-trial areas from 2011 to 2012

The reed canary grass variety "Palaton" and the giant wheat grass "Szarvasi" in the year of sowing had a low germination rate of only about 30% because of slow early growth, but the following year a dense plant population was present without weeds. The grasses were harvested twice a year as a feedstock for biogas production (see picture 2).

Reed Canary Grass „Palaton“ May 2011

Giant Wheat Grass „Szarvasi“ June 2012

Giant Wheat Grass „Szarvasi“ September 2013

Picture 2: Development of Red Carnary Grass “Palaton” and Giant Wheat Grass “Szarvasi” on the RekultA-trial areas

In particular, 2012, the first year of harvest cup plants (Silphie) and reed canary grass "Palaton" both cultures remained well below the corn values. When checking cup plant (Silphie) this must be explained primarily by the fact, since the plant population was still in the development phase as well as by the partial strong weed pressure, during the spring development. For some reed canary grass also the reasoning of the first harvest year are valid, but especially the culture was harvested only once this year. In Szarvasi - grass population that was planted in 2012, shows the significant influence on a two-time harvest on the total dry mass harvest. This culture comes close almost to the low maize yields in 2013. In crop year 2013 achieved both the cup plant (Silphie) as well as the reed-grass "Palaton" significantly higher dry matter yields at all trial sites. The cup plant (Silphie) had at this season about a third less income compared to the average corn yields. Also reed canary grass was found in the experiment in 2013 significantly more profitable than in the first year, especially since made two cuts. Income of the level of low corn yields were achieved, while an income was obtained from average corn yields on another trial site. See figure 8 for an overview of the yields of each single crop.

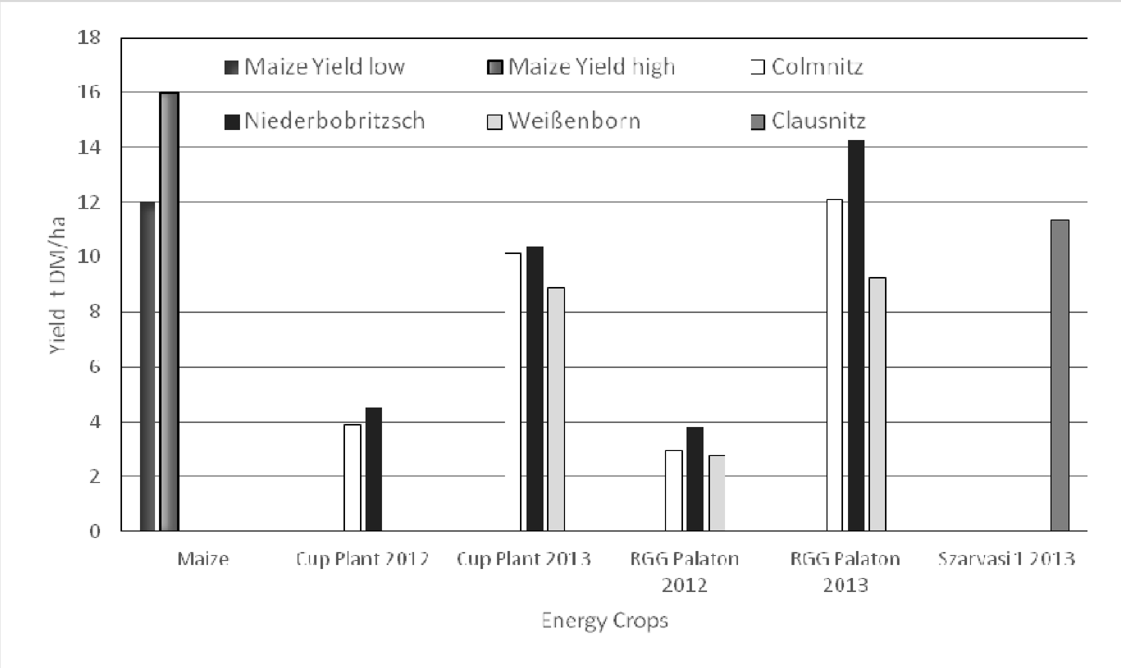

Figure 8: Overview of the dry matter yield in t DM ha-1 compared to regional maize yields (RGG Palaton is the grown reed canary grass variety "Palaton")

For an investigation of heavy metal uptake, a soil sample to vegetation beginning of the year and soil and plant samples at the harvest of the respective energy crop were analyzed by aqua regia extract dissolution and ammonium nitrate dissolution. In the soil samples, the limits for arable land from the Federal Soil Protection and Contaminated Sites Ordinance were undercut in all samples. In the energy crops, in particular through the cup plant (Silphie) and reed canary grass, low heavy metal contents were found. Only a small number of samples exceeded the feed limit for cadmium. Looking at the differences between the sites, there is no evidence of a transparent relation between the contents in the soil and the contents in the plants.

This has also been observed in other studies. A major part of the total content of a heavy metal in agricultural products can also be caused by contamination with adhering soil.

Conclusions

Energy plants offer the highest potential for biogas production in Germany. To address the partially justified criticism regarding silage maize in the public and professionals are required alternative energy crops for cultivation. Advantages of the variety of new energy plants are extensive farming and ecological aspects (bloom especially in the summer months). Biomass for biogas must be effective and environmentally friendly produced. Ensure that the climate balance, especially the greenhouse gas emissions of biogas production can be significantly improved.

Through a detailed study of the potential of biomass and the relevant actors in Saxony and in the Czech Republic possible project and approaches for different biomass value chains (e.g. power plant => biogas; Woody Biomass => combustion) are identified. In the coming years, an implementation is the plan.

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Ruminant feed production from cellulose biomass applied ammonia stripping from digested slurry of thermophilic biogas plant

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So far, livestock manures have been recycled as compost or liquid fertilizer which is digested slurry of anaerobic biogas fermentation (Ellegaard, 2002). In recent years, however, the surface or underground water has been polluted by the excess nitrogen in the compost or liquid fertilizer. In consequence, high nitrate in the hydrosphere and nitrous oxide in the atmosphere are current important environmental issues to be solved (Shoun, et al., 1989; Goolsby, et al., 1997) . Therefore, the retrieval of excess nitrogen as ammonia from livestock wastes is presumable to be one of the solution (O'Farrrell, et al.,1972; Alitalia, et al., 2012; Xia, et al., 2012). In the thermophilic (55°C) biogas plant, the greater portion of nitrogenous malodor constituents might be degraded to ammonia, whereas sulfur compounds will be discomposed to hydrogen sulfide. Thus, thermophilic biogas system has a great advantage to retrieve ammonia gas as a preprocessing procedure of livestock wastes (Takahashi, 2007; 2010). Ammonia retrieved with ammonia stripping from effluent (digested slurry) of thermophilic biogas plant was treated to wheat straw after organic desulfurization to improve feeding value of wheat straw. The nutritive value of TMR composed ammonia treated wheat straw compared favorably with control TMR with hay as forage.

Ammonolysis and ammonia stripping apparatus

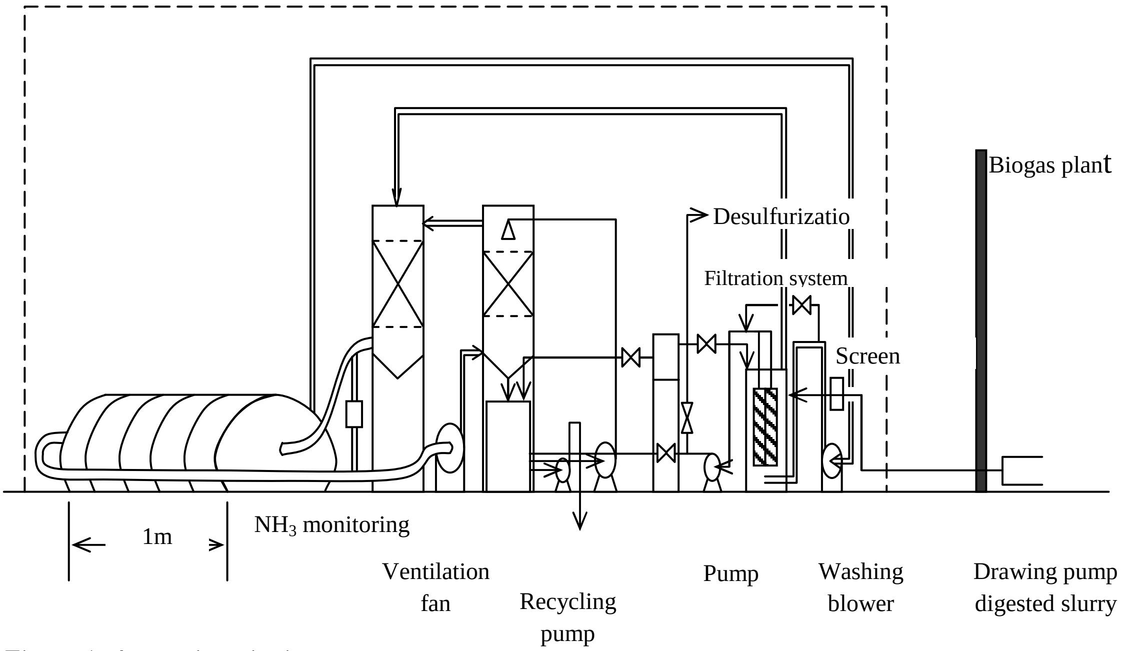

Figure 1: Ammonia stripping apparatus

Fig. 1 shows the complexed facility of the bench scale plant for ammonia stripping apparatus. Mechanism of ammonolysis reaction to soft-cellulose biomass with ammonia is presumed to degrade β1,6-glycoside bond and amide bond in the site of degradation or chemical substitution of hydroxyl group in the second portion of carbon in glucose to amide. Nitrogen content (crude protein content) hereby can be increased in the cellulose biomass. Especially, the former reaction will assist to soften the fiber fraction of cellulose biomass due to inducing partial degradation of cellulose. In consequence, feed value of cellulose biomass will be improved by ammonia treatment with ammonia stripping.

Chemical composition of digested slurry from thermophilic biogas plant and characteristics of ammonia stripping

Table 1 shows chemical composition of the digested slurry. NH_3-N content in the digested slurry was 1100-1600 mg/l. Ammonia amount collected with ammonia stripping decrease in summer season due to declining the excreta amount put into biogas plant for the summer grazing. Fig. 2-1 and 2-2 shows the characteristics of ammonia stripping. Thermophilic biogas plant has advantage in ammonia stripping, because ammonia stripping depends on pH and temperature.

Table 1: The composition of digested slurry in the thermophilic biogasplant

Item	1st	2nd	3rd	4th	Analytical method
pH	7.8	7.8	8.1	8.0	Glass electrode method
TS (%)	6.5	5.7	8.8	7.1	JIS-K-0102-14-2
VTS (%)	5.3	4.5	6.0	5.8	600±25°C Ignition loss method
CODcr (mg/l)	18,700	59,000	58,000	62,000	JIS-K-0102-20
BOD (mg/l)	210	7,300	7,500	7,100	JIS-K-0102-21
T-N (mg/l)	2,700	2,600	3,100	3,000	JIS-K-0102-45
NH_4^+-N (mg/l)	1,100	1,600	1,400	1,600	JIS-K-0102-42
P_2O_5 (mg/l)	1,100	1,400	1,400	1,500	JIS-K-0102-46
K_2O (mg/l)	3,300	3,700	3,600	3,900	Atomic absorption spectrophotometry

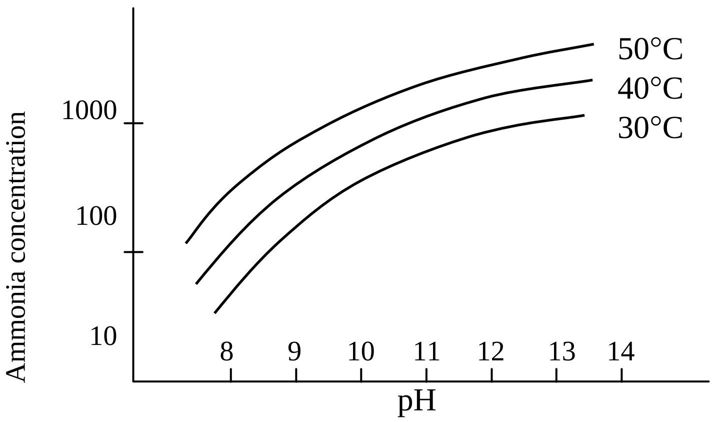

Figure 2-1: Ammonia stripping depending on temperature

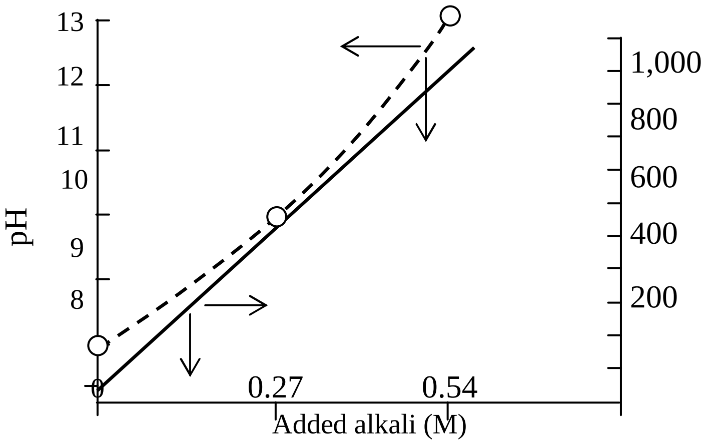

Figure 2-2: Relationship between ammonia concentration and pH in ammonia stripping

Removal effect of ammonia stripping on odorous components in the influent and the ammonia treatment of wheat straw

Table 2 shows characteristics of odorous compound removal, and Table 3 shows the results of ammonia treatment of wheat straw. There were no contaminations of sulfurous odorous compounds detected in the stripped ammonia gas after passage through deodorized tower. It was not necessary to deodorize for ammonia stripping in the digested slurry of thermophilic biogas plant.

Table 2: Removal effect of ammonia stripping on odorous components in the influent with alkaline biological deodorizing method (ppm)

Constituents	Before deodorizing tower	After deodorizing tower
NH3	<500	<450
H2S	<50	-
MeSH	<5	-
DSH	-	-
DMDS	-	-
Skatole	-	-

Table 3 Ammonia treated wheat straw with ammonia stripping

Constituents	Control	Treatment 1	Treatment 2	Analytical method
Crude protein	4.8	5.2	6.3	Kjeldahl method
Crude fat	0.8	0.8	0.8	Soxhlet method
Crude fiber	37.6	41.9	42.8	Gravimetric method
Lignin	9.3	8.5	8.7	P.J.Van Soest method
Cellulose	49.8	47.2	45.1	P.J.Van Soest method
Hemicellulose	12.4	18.1	19.1	P.J.Van Soest method
Sugar content	13.7	10.5	11.9	Saccharimeter

Treatment 1: wheat straw 15g: ammonia 0.65g; Treatment 2: wheat straw 15g: ammonia 1.10g

Feeding value of ammonia treated wheat straw with ammonia stripping

Feed value was accessed according to 4×4 Latin square designed digestion. nitrogen balance and energy metabolism trials with rumen fisturated wethers as experimental animals. Ammonia treated wheat straw was examined as TMR mixed with concentrate mixtures for dairy cow at in addition to sole feed as follows,

1) Crain grass hay
2) Ammonia treated wheat straw
3) TMR-1: crain grass hay + concentrate mixture (mixing ratio, 6:4 in ADM basis)
4) TMR-2: ammonia treated wheat straw + concentrate mixture (mixing ratio, 6:4 in air dry matter (ADM) basis)

Table 4 shows DM intake and digestibility in each experimental feed. In the aspect of feed quality, examined ammonia treated wheat straw with ammonia stripping was a mixture of wheat straw with ammonia stripping in summer grazing and wheat straw with winter feeding in barn. The efficacy of ammonia stripping was relatively low due to insufficiency of wastes collected for anaerobic fermentation, *i. e.*, lack of ammonia stripping. Consequently, dry matter (DM) and organic matter (OM) intake and OM digestibility in ammonia treated wheat straw was significantly lower than those in crain grass hay, compared to sole feed. However, there were no significant differences between TMR-1 and TMR-2.

Table 4: DM intake and digestibility of experimental feeds

	Crain grass hay	Ammonia treated wheat straw	TMR-1	TMR-2
DM intake (g/d)	910a	474b	1002a	929a
Digestibility (%)				
DM	54.8b	38.6c	63.7a	60.7ab
OM	55.4b	39.3c	64.8a	62.5a
NDF	56.0a	42.4b	54.1a	44.4b
ADF	49.9	42.1	50.7	40.3
CP	68.8ab	50.7b	79.7a	74.6b

Different superscripts in the same low indicate significant difference ($P<0.05$)

Conclusion

The system configuration of present study consists of producing renewable energy from anaerobic fermentation of livestock wastes and recycling of ammonia in the digested slurry with ammonia stripping. Consequently, nitrogen content in the slurry as a liquid fertilizer can be optimized. The retrieved ammonia with ammonia stripping will be applicable to improve feeding value of cellulose biomass.

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Biogas Production Potential of Selected Grass Species Used to Restore Grasslands

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Abstract
The most important factor for the successful development of biogas production from grassland is the knowledge of the specific yield of biogas (methane) and the potential of the biomass and methane yields per hectare for different species and varieties of grass. On the whole, it has been tested 23 varieties and 10 species of grasses in an exact small-plot field trial with four replications. The contribution of this paper is to evaluate the yield potential of biomass and the corresponding production potential of biogas and methane in the three-year average. The specific yield of biogas and methane from the grasses has been determined by a batch process in experiments with the following process parameters: a temperature of 37 °C: a stirring mode of 15 minutes every 2 hours; a total residence time of 49 days; a reactor volume of 3 l. The obtained results of biogas and methane yields vary within a relatively wide range from 370-480 Nm^3 of biogas per tonne of dry matter, respectively from 200 to 250 Nm^3 of methane per tonne of dry matter. The methane concentration ranged from 50-56 %. The average annual yields of dry matter at analysed grass in an average of three-year period ranged from 9.3 t per ha for perennial ryegrass to 12.8 t per ha for tall oatgrass. The corresponding average annual yields per hectare of methane ranged from 2054 Nm^3 per ha for perennial ryegrass to 3152 Nm^3 per ha for tall oatgrass. Based on the results, it is possible to confirm the significant influence of each grass species on the specific biogas yield and the methane yield per hectare.

Keywords: grass; biomass yield; biogas; specific methane yield; potential methane yield.

Introduction

In recent years in developed countries, the interest for alternative use of grasslands grows, particularly as a source of raw materials for the production of energy from renewable sources. The grass demand for biogas production can have positive environmental benefits due the maintenance and preservation of perennial grassland, either in terms of carbon storage in the environment, their landscaping features, the preservation of quality at groundwater and surface water, etc. (Rosch et al., 2009; Prochnow et al., 2009). The perennial grassland can bind about 0.6 t C per ha per year. The inclusion of this effect to the benefits of the used biomethane produced from grass as fuel results in a reduction of emissions from internal combustion engines by 75 % (Korres et al., 2010). Therefore, it is possible biogas production to consider as an important technology for the sustainable use of grasslands. In addition, biogas production from grasses such as non-food raw materials and its use as a transport fuel, is considered a second generation biofuel that is suitable according to the Guidelines of Renewable Energy sources in EU (EC, 2009, 2012).

Material and methods

Field trials. Exact small-plot trials were founded after the recovery of perennial grassland (below PGL) using an assortment of selected grasses at the station Jevicko on Fluvisol gleyic soil with neutral soil reaction (pH/KCl 6.7) in 2008. Preparing for a rapid restoration of PGL was made after the first mowing the application of the herbicide Touchdown Quatro (glyphosate) in a dose of 8 l per ha. Subsequently, there were seeded the selected grasses. The direct sowing was done by HEGE 80 seeder with sowing device Øyjord early in August 2008. The size of the testing area was about 10 m^2 and the trial was organized in four replications. The species and variety (in quotes) assortment of tested grasses was as follows: 1) Italian ryegrass „Lubin"; 2) perennial ryegrass „Algol", „Mustang", „Jaran", „Korok" and „Jaspis"; 3) Meadow fescue „Columbus" and „Pronela"; 4) Cocksfoot „Niva" and „Vega"; 5) Tall fescue „Kora", „Proba" and „Protahly"; 6) hybrids of Festucololiums (festucoid) „Felina", „Rebab" and „Fojtan "; 7) hybrids of Festucololiums (loloid) „Caves", „Lofa" and „Perseus"; 8) Timothy „Bobr" and „Sobol"; 9) Tall oat grass „Median"; 10) Golden oat grass „Roznovský". Overall the tested collection is composed of 23 varieties of 10 species of grasses.

The trial with an assortment of grasses was carried out in four mowings. The 1st cut took place at the end of May, the 2nd, 3rd and 4th cut every 45 days from the previous one. The total dose of Nitrogen fertilizer was 180 kg per hectare, the nitrogen application was running at a system of partial doses 60-60-60 (spring, after the first and second cut), a single spring fertilization of P and K was at a dose of 35 kg per hectare and respectively 100 kg per hectare. The measured results were statistically analyzed and differences between means were tested using ANOVA (Fisher‘s LSD – the least significant difference at a significance level of $\alpha_{0, 95}$).

Laboratory tests. Laboratory experiments were conducted on a biogasification unit of 48 per 3-liter glass anaerobic fermenters (reactors) hot up at mesophilic temperature of 37 ± 1 °C, stirred for 15 minutes every two hours. The testing of potential biogas production and methane was carried out in accordance with the methodology VDI 4630 (Anonymous, 2006). The input ratio of sample organic dry matter to the inoculum was about 3:10. The inoculum was digestate from operating biogas plant, which handles animal manure, corn silage and silage forage at ratio of about 40:40:20. Data from experimental measurement of biogas production were recorded usually once a day, at the time of highest intensity of biogas production several times a day. Qualitative biogas analysis was performed with the specialized biogas analyzer „Biogas Check Analyser” from the renowned manufacturer „Geotechnical Instruments” (UK), the measurement accuracy was checked using a gas chromatograph with a TCD detector. The total duration of the experimental fermentation was uniformly set at 49 days (7 weeks). This is sufficient time to provide the intensive phase of biogas production at all tested substrates. In many cases, the production of biogas was not completely stopped after a specified time, which is associated with a progressive fermentation of hardly degradable biomass components, such as cellulose and hemicellulose. The intensive phase of biogas usually lasted 2-4 weeks after the delay phase (lag – phase), which usually took place in about 1-5 days.

Results and discussion

The results of the production potential of selected grass species used for restoration of grassland in terms of aboveground phytomass dry matter yield and the corresponding production of methane as the main component of biogas in an average three-year period are summarized in Table 1, and are divided for the first cut and for the total annual production of four cuts.

Table 1: Phytomass dry matter yields, methane yield and the corresponding values of the potential yield of methane according to the particular tested grass species on three-year period average (2009-2011)

Grass species	Number of varieties	Annual average[1] yield of biomass dry matter in t per ha				Specific methane yield (Nm^3 per t of dry matter)	Potential annual yield of methane (Nm^3 per ha)
		2009	2010	2011	Average		
Tall oat grass	1	15,4	11,7	11,20	12,8	247	3152
Italian ryegrass	1	17,4	8,82	--	13,1[2]	237	3108
Tall fescue	1	17,5	11,1	9,60	12,7	239	3045
Timothy	2	17,1	12,1	9,40	12,9	227	2924
Cocksfoot	2	16,4	11,1	10,00	12,5	230	2873
Festucololium (festucoid)	4	16,1	10,8	8,70	11,8	228	2700
Festucololium (loloid)	3	18,3	10,6	5,90	11,6	222	2577
Meadow fescue	3	15,6	9,59	7,00	10,7	214	2293
Perennial ryegrass	5	15,2	8,47	4,20	9,3	233	2162
Golden oat grass	1	14,1	10,7	6,00	10,3	200	2054
[3] $LSD_{0,05}$		*3,82*	*2,74*	*1,97*	*2,16*	*19*	*376*

Notes: [1]in the case of variety number greater than 1 this is an average of all varieties;[2]two-year average (dropped in the third year of growth);[3]Fisher's LSD - least significant difference at a significance level of $\alpha^{0, 95}$

The production potential is given in dry matter of biomass. The yield fluctuations in particular grasses was influenced by grass species and weather patterns. For example, Italian ryegrass dropped out after the second harvest year, so there are listed averages for the two-year period. Also, the production of perennial ryegrass decreased significantly during the three-year period. Its yield on average of 5 varieties in 2011 was already the half in 2010 and 1/3rd - 1/4th compared to the first production year, when it reached a respectable 15.2 t per ha of biomass dry matter. The average annual yields of dry matter in tested grass in an average three-year period ranged from 9.3 t per ha for perennial ryegrass to 12.8 t per ha for tall oatgrass. The average range of individual years was even greater - from 4.2 t per ha of biomass dry matter in perennial ryegrass in the third production year to 18.3 t per ha of biomass dry matter in hybrids Festucololium (loloid) in the first production year.

The obtained results of specific methane yield as the main component of biogas is of relatively wide range from 370-480 Nm^3 of biogas per tonne of dry matter, respectively 200 to 250 Nm^3 of methane per tonne of dry matter. The methane concentration ranged from 50-56%. The corresponding average annual yields per hectare of methane ranged from 2054 Nm^3 per ha in perennial ryegrass to 3152 in tall oatgrass.

The second highest potential methane yield per hectare had Italian ryegrass (3108 Nm^3 per ha), although in this case it is only a two-year average with regard to the fact that this plant is biennial. In third place is tall fescue with a value of 3045 Nm^3 of methane per 1 ha. This crop with regard to its legendary endurance (up to 30 years) is considered one of the most promising grasses for biogas production needs. Least significant difference at a significance level $\alpha_{0,05}$ from a three-year annual production average of dry biomass was 2.16 t of dry matter per 1 ha and the corresponding annual production average of methane about 456 Nm^3 of methane per ha. It follows that in many cases the yields of biomass dry matter and the corresponding methane production at tested set of grass species and varieties are statistically significant at the level of probability $P_{0,\ 95}$.

From the results is evident that surrender the highest yields of biomass dry matter correlate with the highest values of specific methane yield in biogas. For example, Timothy had the highest three-year average yield of biomass dry matter (when not counting the two-year average of Italian ryegrass), but also one of the lowest biogas and methane yields (seventh place with a value of 227 Nm^3 of methane yield per t of dry matter). The grass species in the table are sorted in a decreasing sequence of a potential three-year annual yield average of methane per 1 ha (i.e., according to the last column).

Finally, it is possible to submit that the tested grasses represent not only the basis of clover-grass mixtures for grassland restoration and supplementary sowing of PGL for forage utilization with high phytomass yields, but as the results show, there are good opportunities for biogas plants because of their potential biogas (methane) production are able in certain soil-climatic conditions closer to silage corn, the potential methane yield reached in the Czech Republic had an average of about 3000-5000 Nm^3 per ha. The main advantage of growing grass instead of corn for biogas is the soil reclamation and the effective protection against erosion.

Based on the results, it is confirmed the significant influence of particular grass species on the specific yield and hectare yield of biogas and methane, which makes the research on the differences at grass species and variety in terms of the potential of biogas production and methane, a very current problem.

Dedication: This paper is based on the results of research projects MZE RO0414 (Ministry of Agriculture) and TA02021257 (Technology Agency of the Czech Republic).

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Non-traditional Crops Prospective for Biogas Production

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Introduction

In the Czech Republic (CzR) was according to the statistical data to 1.1.2013 in operation about 481 biogas plants (BGP) with a total installed capacity of 363.24 MW and 1,406 GWh of electricity production. The proportion of biogas in the renewable energy sources reached about 16 %. Of the total number of BGP, the overwhelming majority (about 65 %) of agricultural biogas plants use primarily as raw material livestock manure and crop biomass. Therefore, the current issue for Czech agriculture is the selection and verification for cultivation and use of conventional and non-traditional crops suitable for biogas production. The largest expansion to achieve these purposes has been the cultivation and use of maize, whose advantage is its high productivity and outstanding quality in terms of silage, which makes it most suitable conventional crop for biogasification. On the other hand, the intensive maize cultivation as a crop is expensive and represents a significant soil erosion risk, especially on sloping soils. For this reason, the maize in the CzR with regard to the usually high broken country has significant limitations in cultivation. For an effective protection against soil erosion and degradation, the most appropriate choice seems to be growing perennial crops, which not only reduces the risk of soil erosion, but also reduces the total financial costs of cultivation. Therefore, it is recently a growing interest in testing and practical application for biogas production of new, mainly non-traditional perennial crops, to which is directed the presented solutions as well.

Objects and developing methods

The crop selection for testing was oriented to the assortment of contrasting botanical species (grasses, legumes, herbage), which have high-production and create perennial vegetation. There was selected the following range of plants:

1.	Tree Lavatera	*Lavatera thuringiaca* L.
2.	Jerusalem artichoke	*Helianthus tuberosus* L.
3.	Topisunflower	*Helianthus tuberosus* L.*x Helianthus annuus* L.
4.	Fodder hybrid dock	*Rumex patientia* L. *x Rumex tianschanicus* A. Los.
5.	Cup-plant	*Cup-plant perfoliatum* L.
6.	Czech giant knotweed	*Reynoutria × bohemica* Chrtek & Chrtková
7.	Eastern goat's rue	*Galega orientalis* Lamb.
8.	Alfalfa (Lucerne)	*Medicago sativa* L.
9.	Reed canary grass	*Phalaris arundinacea* L.
10.	Tall fescue	*Festuca arundinacea* Schreb.
11.	Miscanthus sinensis „Giganteus“	*Miscanthus × giganteus* J. M. Greef & Deuter ex Hodk. & Renvoize
12.	Maize	*Zea mays* L.

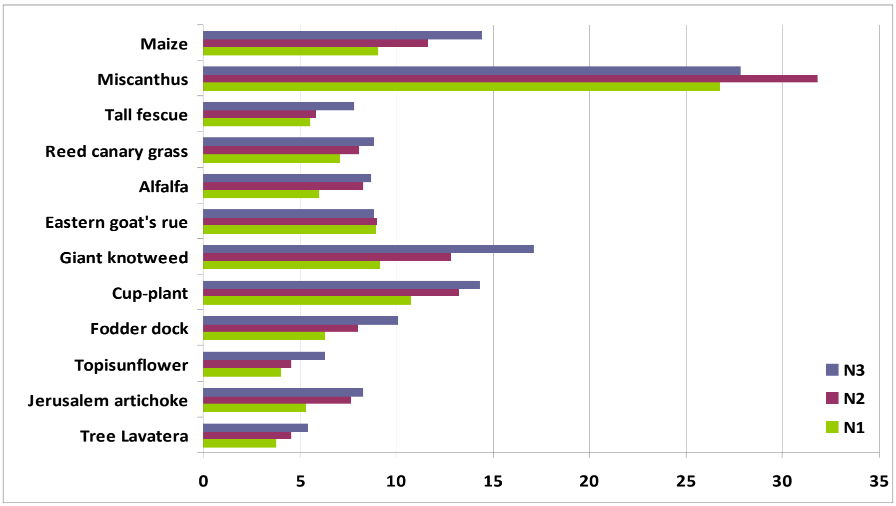
All of these crops (with the exception of maize) generate perennial vegetation. Although Jerusalem artichoke and topisunflower are not perennial crops, but in the case of tuber retention in the soil at an appropriate agricultural technology can be easily achieved perennial stands of about 8-10 years. The longest life stands were demonstrated in cup-plant, fodder dock and knotweed (15-20 years and more), lavatera life reaches from 10 to 12 years and the others, Goat's rue, reed canary grass, tall fescue and Miscanthus state about 6-8 years or more. For comparison was examined maize as a reference crop for cultivation to biogas and alfalfa as type of poorly ensilable crop. The total set of crops examined in detail, including control maize was 12 plants. For all these crops were obtained at least four years dates about yields within parcel field trials in four variants of nitrogen fertilization (50, 100 and 150 kg of N per 1 ha) and at a single dose of P and K fertilization (at 50 kg per ha of P_2O_5 and K_2O). For fertilizing of alfalfa crop and goat's rue as legumes crops was used a half of nitrogen dose.

Yield tests, chemical analyses and fermentation tests of plants were performed according to the plant different crops and fertilization variants. All analyzes and experiments were carried out in at least four repetitions. In the case that dry matter during harvest was less than 25 %, such crops were ensilaged in the fresh state and after 24 hours of withering. Before ensilaging, crops were all uniformly (same length of cut) cutting with a forage harvester TORON 690. The Dry Matter ranged from 12 to 34 %, while wilted dry matter in the range of 18-40 %. Fermentation tests for silage were largely carried out by laboratory minisilo with a capacity of 10 liters. Ensiling time was according to the normal procedures uniformly set at 90 days. The basic parameters characterizing the quality of silages were determined by standard laboratory procedures and in accordance with CNS (Czech National Standard) 46 7092.

The determination of biogasification ability closely followed the experiments with silage, starting with sample taking and preparing, and ending with the use of ensiling products for subsequent biogasification tests. Testing was performed in the phytomass of individual crops and without silage, particularly in research of biogasification differences in the various stages of plant growth. The experiments with biogasification were performed on a device 48 - nesting kit gallon glass anaerobic fermenter (reactors) heated at mesophilic temperature of 36-38 °C and agitated every hour for 15 min. The total duration of bio-fermentation test was uniformly set at 49 days (7 weeks). It is a sufficient time period, during which occurs the termination of biogas production intensive phase at all tested substrates. To completely stop of biogas production, in many cases have not yet occurred, apparently associated with the gradual fermentation of hardly biomass degradable components: cellulose and hemicellulose type. During the experiments the intensive stage in the biogas production was deducted since the end of start-up period (the lag phase) lasted usually about 2-4 weeks, then the start-up phase was usually about 1-5 days. The testing of potential biogas production and methane was carried out in accordance with the methodology VDI 4630 (Anonymous, 2006).

Results and discussion

The following graph 1 below characterizes the yield potential of the above listed non-traditional crops presenting a four-year average of dry matter yield in aboveground biomass at harvest in a single stage of mature or maturing seeds (usually in the fall, before the end of the vegetation), at segmentation of different fertilizer doses. In addition the cup-plant yields are also listed in the stage of flowering, when was the highest. For comparison, here is also shown as a reference maize crop from the point of view of biogas production, and that at harvesting for silage (approx. 32-34% of dry matter).

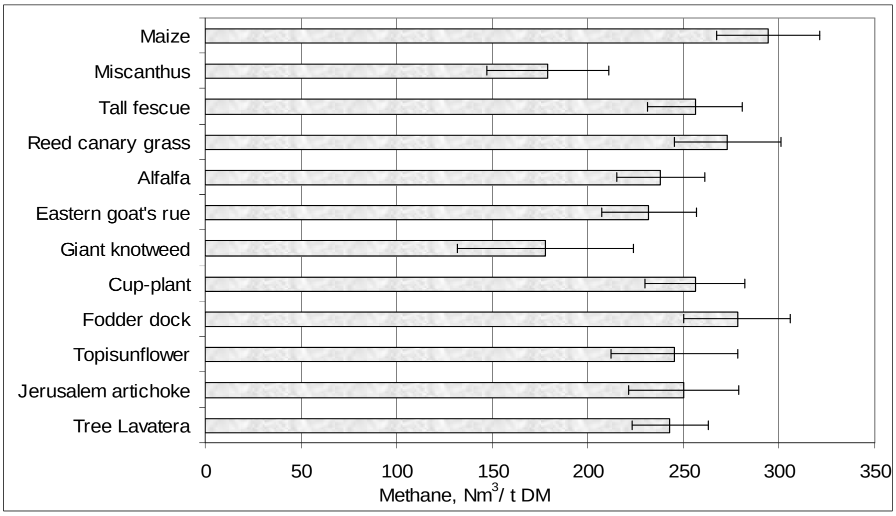


Graph 1: Comparison of aboveground biomass dry matter yield of crops in t per ha at different doses of fertilization, four-year average 2009 – 2012.

The results show that the yield of some tested plants exceeds maize. Above all, these are cup-plant and knotweed. Earlier research has shown that knotweed appears to be as the most productive plant and related to the plant protection appears as the least demanding plants suitable for the conditions of the temperate climate. Of course, when considering the knotweed cropping must first take into account its current role as a weedy invasive plant. Significant differences in yields between the different variants of fertilization at most crops is due to the large difference between nitrogen doses, which is virtually a multiple of the lowest dose (N 50 - 100 - 150 for all crops except legumes, in which the nitorgen dose is 25 - 50 - 75). The lowest yields in average of four-year period has been noted in Lavatera. In ascendant line follow topisunflower, Jerusalem artichoke, Goat's rue, reed canary grass, tall fescue, alfalfa and fodder dock - all these are crops with an average yield at four-year period less than 8 tonnes of dry matter per hectare, and that in the variant with the highest fertilization and thus yield (N3). Other crops – Miscanthus, knotweed, maize and cup-plant with an average yield for four-year period in variant N3 placed above the threshold of 14 t per ha of dry matter. Yields of Miscanthus exceeded this limit for all variants of fertilization, including N1 and in N3 achieved a remarkable result in range of 26-32 t per ha of dry matter. Since the experiments were carried out in Chomutov (except Miscanthus from our Prague trials), it is in a less favorable soil - climatic conditions, therefore maize and alfalfa yields are quite lower than indicated by the statistics and results of other research centres (e.g. Research Institute for Fodder Crops in Troubsko, see www.vupt.cz). Likewise, it can be assumed too, that other crops have lower yields here and in better soil - climatic conditions achieve higher yields.

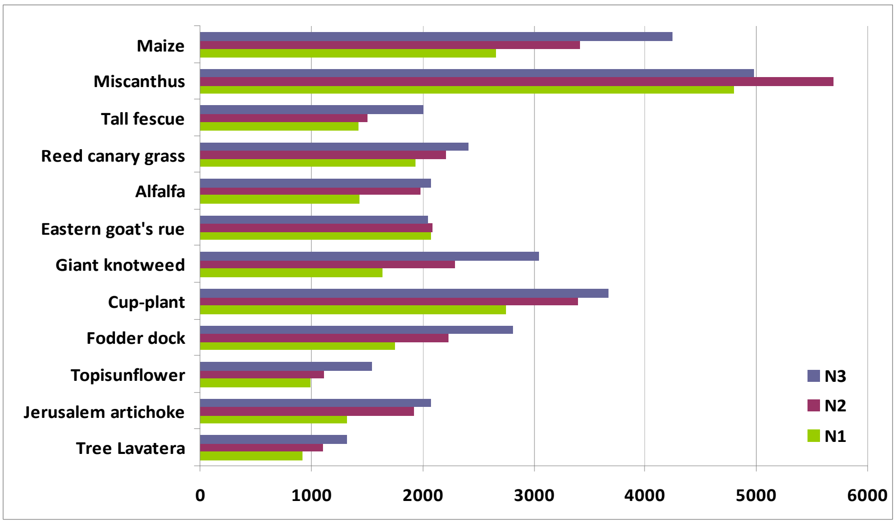
Generally, it is stated that for biogas production are preferable younger stands, on the other hand younger vegetation contain little dry matter and are therefore more difficult to harvest and transport, and are thus less suitable for silage. In the case of dry matter less than 25 %, there are necessary additional agro-technical operations in order to reduce the water content (withering, turning, collection in lines, etc.). It is favorable in terms of minimizing production costs, to harvest and collect in an "upright position" in time of maximum allowable dry matter for further processing into biogas. When the dry matter content is above 40 % (in certain crops and over 30 %) the ensiling process is worsened together with the subsequent decreased biogas production. Therefore, it must be chosen a suitable compromise between stand age and, therefore higher phytomass dry matter yields on one side and its quality for silage, and the subsequent biogasification on the other.

The following Graph 2 shows the statistically elaboration of the methane (CH_4) yield average values as the main component of biogas and the confidence interval about 95 % of probability from the summary set of results at the fresh mass of all 12 tested crops including the reference maize. From these results, it can be seen that statistically significant lower values of methane specific yield compared with maize plant have knotweed, Miscanthus and legumes. The other crops are statistically significantly different, although their average values of CH_4 yields are lower compared with maize. These results are in good relation with German database of methane yields of different traditional and non-traditional crops (Anonymous, 2009).



*Graph 2: Average values and average confidence interval (0.05) of methane yield in * Nm^3 per t of dry matter phytomass at the tested crops (year average 2009-2013)* * Nm^3 - here and further - normalized volume of methane, it is calculated on a dry basis and the so called normal conditions (0 °C and a pressure of 101 325 Pa).

The next Graph 3 shows the methane yield from silage of tested crops, corresponding to the four-year average yields achieved in the experiment divided according to the basic doses of fertilization in Nm^3 of CH_4 per 1 ha.



Graph 3: Methane yield from selected crops calculated in Nm^3 per ha.

As it is seen from the graphs, the specific biogas production and specially methane namely particular crops with exception of knotweed and Miscanthus lagging only slightly behind maize (with an average of -14 %, a range from -5 to - 21 %, and only knotweed and Miscanthus have -39 %), but with regard to the lower yields of most crops compared with maize, these differences in terms of yield from 1 ha were deepened. Without Miscanthus which has 2.5 times higher yields of biomass than maize it is an average of -30 % and the minimum value is -61 % versus maize. On the other hand Miscanthus and cup-plant, especially at the stage of flowering due to more higher phytomass yields surpasses the methane yield from 1 ha of maize as well.

Another situation appears at the evaluation of production costs during the cultivation of particular crops to biogas, both in terms of biomass and in the corresponding potential production of methane in the biogas (Table 1). For the calculation were used the average values in terms of a standard technology acquired during the implementation. It was found that virtually all the tested crops, with the exception of topisunflower, have lower production costs per unit of phytomass dry matter and to 1 Nm^3 of methane than maize. With regard to the lower yield of methane practically in all crops, the results of the comparative evaluation at monitored crops slightly improved the maize position in terms of methane yield compared with the yield of dry matter, but the overall position of particular crops remained the same. To the costs of methane production from conserved biomass is included a correction for dry matter losses during ensiling.

Table 1: Economic and production comparison of the cultivation, harvesting and ensiling of individual tested plants as the raw material for biogas production.

Evaluated crops	Phytomass average yield from 1 ha		Total production costs of phytomass in CZK per ha		Specific costs per methane yield (CZK per Nm^3 CH_4)	
	Original wet matter (t)	Dry matter (t)	Sampling in the field	Including conservation and storage	Sampling in the field	Including conservation and storage
Maize	**40**	**13**	**21 380**	**23 797**	**5.83**	**6.49**
Reed canary grass	26	9	9 139	11 588	3.97	5.03
Eastern goat's rue	38	9	6 814	9 235	3.49	4.73
Czech giant knotweed	84	20	11 214	13 607	3.05	3.70
Tree Lavatera	22	7	6 749	9 224	3.92	5.36
Cup-plant	76	18	11 651	14 044	2.64	3.18
Fooder dock	32	8	8 915	11 239	4.40	5.55
Jerusalem artichoke	24	8	10 352	12 795	5.00	6.18
Topisunflower	18	6	9 131	11 619	**6.06**	**7.72**
Alfalfa	35	8,5	6 704	9 125	3.43	4.67

Conclusion

In view of the perennial character of non-traditional crops and thus the lower costs of cultivation can be concluded, that tested crops and especially Miscanthus, cup-plant and knotweed may be a suitable replacement of maize for biogas production, especially in areas with unfavourable soil - climatic and habitat conditions, and in soils with the risk of erosion. Most of these crops can be grown for biogas production only in the so called extensive mode, it is at lower unit costs per production but also at lower overall yields per 1 hectare, because the tested crops with the exception of Miscanthus, cup-plant and knotweed, do not reach the maize productivity. On the other hand, the perennial stand character ensures soil conservation and erosion control effect of cultivation. Considering the fact that knotweed has a very low specific methane production and is considered a weed plant, it can be recommended only Miscanthus and cup-plant as a suitable replacement of maize for biogas production at intensive cultivation.

Keywords: non-traditional crops; biomass yield; biogas; specific methane yield; potential methane yield.

Dedication: This paper is based on the results of research projects MZE RO0414 (Czech Ministry of Agriculture) and NAZV QH91170 (National Agency for Agricultural Research).

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Biogas from Feeding Sorrel

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Introduction

The assurance of biomass for biogas plants (below BGP) is our mainly based on the cultivation of maize. The results are good, but recently its cultivation has been restricted, especially on the sloping land to be not threatened by soil erosion. Therefore, it is looking for other crops such as fodder mallow, sorghum or industrial hemp. A good experience are especially with feeding sorrel variety "Schavnat" (the original name of the variety before registration was "Rumex OK-2"), which is mainly known in our country as an energy crop for the production of dry biomass (Petříková et al., 2006; Usťak, 2007), but it has recently been successfully used for feeding livestock and biogas, as well (Usťak, 2012).

Results and experiences

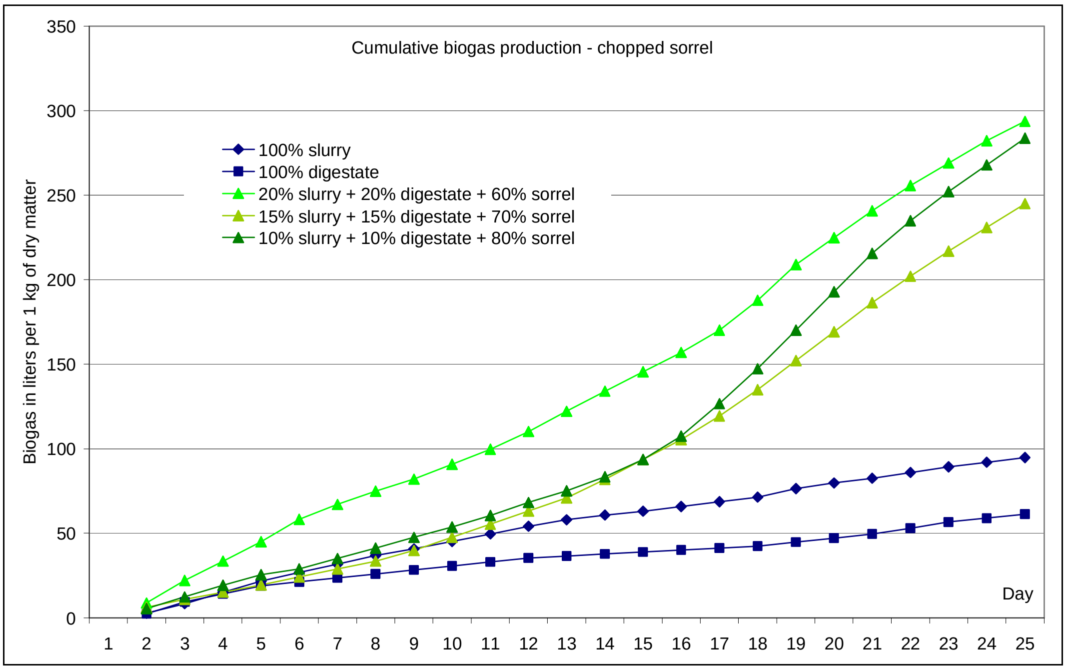
The feeding sorrel has been cultivated as a high quality forage for feeding livestock, which correspond to the data in Table 1.

Table 1: The basic compounds in the sorrel plant by several sampling dates

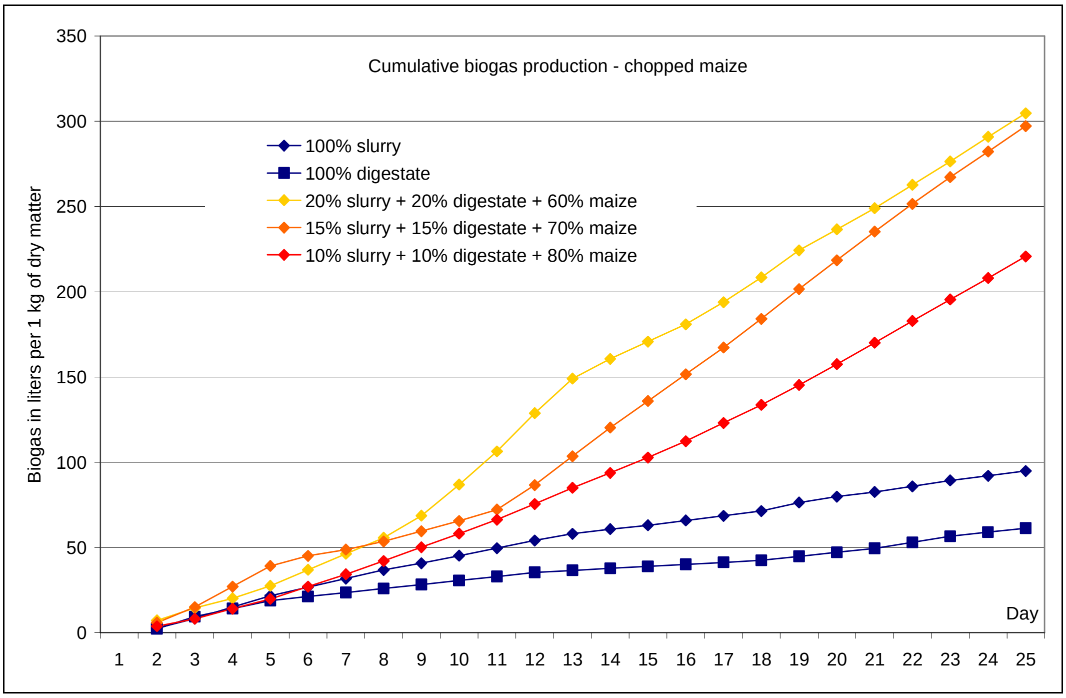
Item	**25.4.**	**5.5.**	**12.5.**	**20.5.**	**26.5.**
Dry matter (DM) content, %	11.5	8.89	11.3	13.0	12.4
Nitrogenous substances, in % of DM	31.4	23.9	19.8	13.4	12.0
Fats, in % of DM	1.47	1.87	1.73	1.13	1.18
Fibres, in % of DM	9.52	13.4	17.9	24.5	26.7
Ashes, in % of DM	11.7	11.2	10.0	8.28	8.68
Sugars, in % of DM	nd	nd	11.4	13.2	11.2

Feeding sorrel contains both high nitrogenous substances and sugar content. Therefore, we have begun to test it for use in BGP (Kára, Petříková, 2008). We have compared the biogas production from sorrel with maize, as it is evident from the results of the cumulative development based on these two cited crops. From the graphs below, it follows that sorrel (Graph 1) has a similar effect on the biogas production as maize (Graph 2).

In the case of maize in one of the variants - with addition of 80% maize to 10% slurry and 10% digestate respectively, the biogas production was even lower than the sorrel. Therefore, there are not concerns that the addition of sorrel to other biomass would hamper the course of fermentation.



Graph 1: Biogas production from chopped feeding sorrel



Graph 2: Biogas production from chopped maize

The results of model tests have been directly validated in the BGP and at the very beginning, where the correct starting of fermentation is particularly important. The basic substrate for BGP was made up of three components, each accounting with the third part: manure, corn silage and haylage from permanent grassland (below PGL). Adding a third part of sorrel to the basic substrate was started from the 9th day after the launch of BGP so that haylage of PGL was replaced. The results from monitoring the biogas production and BGP power output are given in Table 2.

Table 2: Biogas production in a new biogas plant at starting the fermentation process.

Fermentation period (days)	Power output (kWh)	Biogas production (m^3/h)		Average substrate dosage (t/day)	
		Average	Range from - to	Total	of which sorrel
7th to 8th	110	52	50 - 54	9	-
9th to 10th	150	69.5	69 - 70	9	3
11th to 23rd	200	94	80 - 105	11	3.5
24th to 28th	250	118	108 - 136	12	1.8

The results suggest that the biogas production and the BGP power output continuously increased from the beginning of sorrel addition (instead of haylage). It follows that the sorrel does not disturb the fermentation processes, and therefore it can be used for these purposes.

The date assessment thus confirmed the possibility to use feeding sorrel in other processing BGP. An example may be the large-scale cultivation for about 75 ha, where this year (2014) it is the fourth year of cultivation. To use sorrel in BGP is the most important the first mowing, which is therefore of utmost importance. The harvesting is usually in May (exceptionally early June), at the stage of full flowering when the plant reaches the highest weight, as well. The assessment to determine the correct harvest time for the first mowing is very important because it determines the overall yield of the crop. In 2013, the vegetation was at the first mowing nice, balanced, healthy growth, reaching a height of over 2 m, see Figure 1. The feeding sorrel at that time begins to age, reducing the water content and nitrogenous substances (NS) and increasing the fiber content, which is appropriate for the use in BGP. The yield of ensiled biomass of feeding sorrel was at the first mowing on average around 18 t/ha. The harvesting is provided by the same way and with the same mechanization of maize harvesting (Figure 2), which is another advantage of feeding sorrel, as it is not necessary to harvest a special technique.

As well, the way of conserving sorrel biomass in silage is similar to that of corn. The small cut biomass (Figure 3) is placed in the silos, or even "on the packed pile", as is apparent also in the Figure 4. It creates quality silage even without preservative agents, because sorrel has a high sugar content (see Table 1), that proper ensiling reliably ensures. The good quality of the sorrel silage has been demonstrated by the fact that after its inclusion on the other substrates, it was possible to reduce the total daily "ration" needed for the BGP.

Figure 1 and 2: Principal harvest of feeding sorrel for the use in BGP

Figure 3: Detail of chopped sorrel at harvesting

Figure 4: Sorrel ensiling for BGP

After the first (main) harvesting sorrel again envelopes and creates usually for at least 2 additional cuts. Its rapid regrowth contributes in particular slurry or digestate fertilization. Although it produces only basal leaves, but they are also still significant benefit to the overall harvest. For example in 2013 were harvested 2 additional cuts, with a yield of 11.7 t/ha of silage biomass, so that the total yield of sorrel in all three cuts was nearly 30 t/ha. Meanwhile, it is far below the yields of corn, but its advantage lies mainly in the fact that it is a long-term perennial crop that requires minimal treatment method compared to corn, so would significantly reduce the production biomass costs for BGP. Also, sorrel seed price is significantly lower compared to corn: usually costs 4,000 CZK/ha , as a one-time investment, up to 10 years. This means that the cost of the seed are only 400 CZK/ha/year, whereas the price of corn is about 2,000 to 3,000 CZK/ha (or more) on an annual basis.

Of course, feeding sorrel can not fully replace the BGP proven corn, but it can usefully complement it, especially on sloping land and in colder regions, where the maize is not very successful. Therefore, it can be assumed that farmers understand these benefits over time and will be more use also for these purposes.

Summary and conclusions

The perennial feeding sorrel Schavnat (Rumex OK-2) was approved in the cultivation of biomass for biogas plants. It was successfully tested in model tests and in the BGP. After the addition of sorrel silage in BPS occurred a continuous biogas production, the fermentation was reliably carried out. Therefore, it begins to be cultivated a large area and its inclusion in the recipe allows to reduce the total substrate dose. Feeding sorrel gives a yield of around 30 tons/ha, with 3 cuts per year. The importance of the first cut, after which sorrel envelops again, especially when are used slurry or digestate as fertilizers. Feeding sorrel reaches lower yields, but it is cheaper than corn production and can be grown on sloping land, which protects the soil against erosion even in colder regions, where maize is not very successful. The harvesting and conservation of feeding sorrel is provided using standard agricultural equipments.

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Dedikace: MZE RO0414

Posters

Differential of production potential of pasture stands in the protected area Moravian Karst

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Introduction

This paper aims to contribute to the evaluation of the nutritional value and production potential of pasture vegetation in protected landscape areas of the Czech Republic.

Material and Methods

Samples of vegetation were carried out on the pasture near the village Šošůvka. It is located in the Moravian Karst, in which the can operate draggy agriculture. Choice surface were grazed by a herd of dairy goats that at the separate surfaces are rotated throughout the year. Selected areas represented:

- extensive area in the former orchard that is not fertilized (site A),
- grasslands managed with medium intensity (dose approx. 90 kg ha^{-1} N - site B),
- alfalfa-grass stand (site C).

For yields of pasture stand were built at these sites fenced areas of 4 m^2. Of these the stands were removed after reaching ripeness pasture and used to determine its nutritional value. After drying of the samples at 60 ° C was determined in the laboratory content of fibre, NDF (neutral detergent fibre), ADF (acid detergent fibre), crude protein, fat and ash (ANONYMOUS 2001). Nonstructural carbohydrates (NFC) were determined by calculation (NFC = 100 - (CP % + NDF % + Fat % + Ash %). GE (gross energy), ME (metabolizable energy), NEL (net energy of lactation), NEF (net energy for fattening), PDIN (actually digestible crude protein in the small intestine) and PDIE (truly digestible crude protein in the small intestine) were calculated using regression equations (VESELY and ZEMAN, 1995, 1997).

Results and discussion

Yield aboveground phytomass integrates a number of factors - such as habitat conditions , climatic conditions , the intensity of management of the site and use a form of grassland. These should reflect the intensity of management of the area , while respecting all integrating factors. About the level of intensity in the first place decides whether in specific areas of grassland prevails productive or non-productive functions. Consequently, there is also a requirement for the quantity of aboveground phytomass yield. Consequently, there is also a requirement for the quantity of aboveground phytomass yield. The requirement to yield aboveground phytomass cannot be fixed, but varies in relation to the amount of inputs and expected outputs (production and non-production), (VESELÝ, SKLÁDANKA, HAVLÍČEK, 2011). Therefore, we focused on the comparison and the subsequent assessment of the acceptable variability said inputs and outputs on the areas of grassland with different forms and intensity of use. It will be followed the evaluation of stability of income, which is directly related to the habitat conditions, and with the form of the use of grasslands. From the data collected in the spring of 2007 shows that the dry matter yield with the intensification inputs increased to 357 and 532%. Quality phytomass does not always have to integrate with a yield of phytomass. For example, in preference to non-productive role of grasslands where grazing does not in optimal growing phase can be increased aboveground biomass production, but that it is usually accompanied by a sharp decline its quality.

One of the most important factors indicating quality of pastoral communities is the fibre content in dry matter. In the spring months, the dry matter content significantly influences their species composition. Therefore, on the extensive site (A) with a high proportion of herbs has seen the lowest content of fibre, NDF and ADF. The actual increase of the fibre content on the pasture stand during grazing goats is not the primary problem. Detected fibre content, although fluctuated the required nanny goat of 50 kg live weight (281 g kg^{-1} DM) (SOMMER et al., 1994) and when milk production is significantly lower, but the dairy herd is grazing only used as supplementary feed. The problem may be that with increasing fibre content leads to adverse developments affecting the nutritional value of these stands, the decline in digestibility of nutrients and thus the decrease the concentration of energy (CE). This trend was confirmed in the collected vegetation. Developments in the content of CP, PDIE and PDIN was not so clear.

However, despite all the differences in the nutritional value of stands, nutrient and energy yield per unit area most significantly influenced dry matter yield per unit area. Production of nitrogenous nutrients (CP, and PDIN, PDIE) with increasing intensity farming stands increased to 283-357% or 547-607%.

Production of non-structural carbohydrate (NFC) is increased to 324 and 433%, and the energy (NEL and NEV) to 335-339% and 496-504%.

Conclusion

When evaluating the nutritional value and production potential of pasture stands in protected landscape areas of the Czech Republic it is necessary to know and respect the specifics of these sites. This should be assessed when implementing grazing management in these habitats and / or the calculation of financial compensation for farmers who operate in these areas.

The content of nutrients and energy in pasture stands and yield of biomass per ha (Šošůvka 7.6.2007)

Factor		Unit	Site		
			A	B	C
Dry matter	green forage	g/kg	310,9	361,8	295,1
	yield	kg/ha	655	2336	3487
	yield – index	%	***100***	***357***	***532***
Crude fibre	content in dry matter	g/kg	254,3	281,3	294,2
	yield	kg/ha	166,57	657,12	1025,88
NDF	content in dry matter	g/kg	547,4	610,6	584,0
	yield	kg/ha	358,55	1426,36	1910,88
ADF	content in dry matter	g/kg	319,7	327,1	336,6
	yield	kg/ha	209,40	764,10	1173,72
Crude protein	content in dry matter	g/kg	115,8	91,8	132,1
	yield	kg/ha	75,85	214,44	460,63
	yield – index	%	***100***	***283***	***607***
PDIN	content in dry matter	g/kg	74,80	59,29	85,33
	yield	kg/ha	48,99	174,73	297,55
	yield – index	%	***100***	***357***	***607***
PDIE	content in dry matter	g/kg	91,34	77,37	93,86
	yield	kg/ha	59,83	180,74	327,29
	yield – index	%	***100***	***302***	***547***
NFC	content in dry matter	g/kg	223,5	203,0	181,7
	yield	kg/ha	146,39	474,21	633,59
	yield – index	%	***100***	***324***	***433***
NEL	content in dry matter	MJ/kg	6,13	5,84	5,81
	yield	KJ/ha	4,02	13,64	20,26
	yield – index	%	***100***	***339***	***504***
NEF	content in dry matter	MJ/kg	6,07	5,70	5,66
	yield	KJ/ha	3,98	13,32	19,74
	yield – index	%	***100***	***335***	***496***

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Yields and quality of silage maize hybrids grown under different climatic conditions

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Introduction

The group of C4 plants (that involves also maize) is characterised by a temperature optimum necessary for the course of their photosynthesis that rangesg from 28 °C to 32 °C. The group of C3 plants (that occur predominantly in the temperate climatic zone) requires a temperature optimum that ranges from 18 °C to 23 °C. C4 plants are considered to be more productive because, under favourable conditions, both their rate of photosynthesis and production of biomass are higher by 20 to 50 percent than those of C3 plants. In plants, biomass production is influenced (and limited) not only by the temperature but also by many other pedo-climatic factors. The availability of water is one of these important factors. Plants respond to the water deficit by a decrease in the transpiration rate and this decrease is usually associated with a decrease in the rate of biomass production and, thus, in lower yields. Just in some of those parts of the Czech Republic where maize is one of important crops, there is a lack of water because of too high temperatures and too low precipitations occurring in the course of the growing season. This paper deals with the effect of water deficits on yields and quality of silage of some selected maize hybrids.

Materials and methods

In years 2012 and 2013, small-plot experiments with eight maize hybrids were established in the locality Troubsko, Czech Republic (latitude 49.165519 °N and longitude 16.517477 °E). The aim was to to evaluate factors influencing their growth. Six and two of these hybrids were grown for silage and for grain, respectively (Tab. 2). Experiments were established in three replications and the inter row spacing was 70 cm while the intra row spacing was 15 cm. In the course of the growing season, altogether 150 kg of N was applied in two doses. Evaluated were parameters of physiological condition and health status of plants. Individual hybrids were harvested with a forage harvester in the same stage of maturity. Their dry matter content was 32 ± 2 %. In the course of harvesting, samples of chopped forage were collected and dried at the temperature of 55 °C. Dry samples were homogenised in a laboratory grinding mill (sieve mesh size 1 mm) and used for the estimation of contents of dry matter, N-substances, sugars, crude fibre, neutral detergent fibre (NDF), and NDF digestibility. Samples were incubated in rumen liquid of dairy cows for a period of 24 hours. Evaluation of temperatures and precipitations (Tab. 1) was performed using the WMO World Meteorological Organisation) recommendations and guidelines for the evaluation of climatological conditions (Kožnarová, Klabzuba 2002). Statistical analysis was performed using the software package Statistica 10, the method of variance analysis (ANOVA P>0.05), and the Tukey's test.

Table 1: Climatologic evaluation of individual months in years 2012 and 2013

Month	2012				2013			
	Temperature		Precipitations		Temperature		Precipitations	
	Mean	Evaluation	Sum	Evaluation	Mean	Evaluation	Sum	Evaluation
	0.7	1	27.5	0	-1.3	0	21.3	0
February	-3.8	-1	5.6	-2	0.4	0	47.5	1
March	6.1	1	1.8	-3	1.0	-1	42.1	1
April	9.5	0	12.1	-2	9.5	0	18.0	-1
May	16.0	1	25.4	-2	13.8	0	105.6	1
June	18.2	1	60.6	0	16.9	0	116.2	1
July	20.4	2	60.0	0	20.8	2	4.8	-3
August	19.7	2	72.4	0	19.3	1	68.8	0
September	14.5	0	32.1	0	12.5	-1	48.4	0
October	10.2	1	35.1	0	9.5	0	33.3	0
November	7	2	20.1	-1	5.1	2	21.5	-1
December	-1.8	0	29.9	0	1.7	1	4.1	-3

Point evaluation of monthly temperatures: -1= cold; 0 = normal; 1 = warm; 2 = very warm
Point evaluation of monthly precipitations: -3 = extra dry; -2 = very dry; -1 = dry; 0 = normal; 1 = wet

Results and discussion

In 2012, the weather in the locality Troubsko was characterised by very low precipitations on the one hand and very high temperatures on the other. In 2013, above all precipitations were higher. The analysis involved comparisons of mean yields and forage quality of the whole set of hybrids in both experimental years as well as comparisons of individual hybrids.

As compared with the year 2013, a lower dry matter yield of tested hybrids was recorded in 2012, obviously due to the precipitation deficit. As far as the yield of dry matter was concerned, there was no statistically significant differences among hybrids Rubben, DKC 3795, and Dynamite. In this year, the content of N-substances was significantly higher; at the level of hybrids, only Rubben showed an increased content of N-substances. In 2012, a higher content of sugars was found out in the tested set of hybrids; in hybrids DKC 3795 and Dynamite no statistically significant differences were found out. There were no statistically significant differences also in the content of fibre. In 2012, the content of NDF was generally higher but no significant differences were found out at the level of individual hybrids. In 2012, the average content of starch was higher and, when comparing individual hybrids, only values of the hybrid Rubben were different. In 2012, the digestibility of organic matter of hybrids was reduced. In this year, a significant decrease in the organic matter digestibility was observed in grain hybrids DKC 3795 and DKC 4014 and in silage hybrids NK Octet, and LG 30.311. In this year, also the digestibility of NDF of all hybrids was reduced and the response of the grain hybrid DKC 3795 to the precipitation deficit was significant.

During the growing season, the course of weather showed an essential effect on yields and quality of forage and of produced silages. These results corresponded with conclusions published by Rani et al. (2013) who studied effects of weather on the growth and yields of maize as well as with data published by Cone et al. (2008), Di Marco et al. (2002), Hetta et al. (2012), and Jensen et al (2005) who investigated the quality of maize hybrids.

Table 2: Yields and silage quality of individual hybrids (years 2012 and 2013)

Hybrid	Dry maater yield [t.ha^{-1}]		N-substances [% in dry matter]		Sugars [% in dry matter]		Fibre [% in dry matter]	
	Year		Year		Year		Year	
	2012	2013	2012	2013	2012	2013	2012	2013
Rubben	12.84	16.73	10.39	8.21	12.13	4.64	19.06	20.30
DKC 3795	10.96	15.83	10.16	8.80	7.50	5.08	23.06	21.24
Dynamite	14.89	17.01	8.57	8.87	7.04	4.51	23.84	20.21
LG 32.64	16.72	18.31	9.13	9.20	15.40	5.46	19.32	19.47
Susann	13.04	18.24	8.80	8.53	11.38	4.48	19.07	20.70
DKC 4014	15.42	21.33	8.66	8.47	8.82	4.05	19.02	20.15
NK Octet	13.03	20.71	8.76	7.97	9.73	4.20	19.38	19.14
LG 30.311	12.54	22.90	9.26	8.47	10.63	4.26	20.70	19.07
Mean	13.67 ^{a}	18.88 ^{b}	9.22 ^{b}	8.56 ^{a}	10.33 ^{b}	4.59 ^{a}	20.43 ^{a}	20.03 ^{a}
SD	0.65	0.88	0.25	0.14	0.96	0.17	0.69	0.27

Hybrid	NDF [% in dry matter]		Starch [% in dry matter]		SOH [% in dry matter]		SNDF [% in dry matter	
	Year		Year		Year		Year	
	2012	2013	2012	2013	2012	2013	2012	2013
Rubben	42.90	44.01	39.48	26.23	73.61	72.55	57.10	53.95
DKC 3795	49.24	45.15	27.19	27.93	60.74	77.56	42.82	63.50
Dynamite	47.35	43.04	30.42	26.97	71.28	76.11	53.16	61.40
LG 32.64	43.83	44.17	34.76	32.04	68.43	60.46	51.77	55.01
Susann	46.72	45.48	30.64	26.53	57.84	64.93	39.75	44.29
DKC 4014	43.92	37.63	36.25	37.87	67.54	79.94	48.30	56.38
NK Octet	43.53	40.05	32.64	33.66	70.15	75.63	50.41	53.11
LG 30.311	45.45	42.72	29.08	28.82	67.46	77.23	51.74	61.03
Mean	45.37 ^{b}	42.79 ^{a}	32.56 ^{b}	30.01 ^{a}	67.13 ^{a}	73.0 ^{b}	49.38 ^{a}	56.08 ^{b}
SD	0.79	0.95	1.44	1.47	1.88	2.41	2.00	2.16

Different letters indicate statistical differences (Tukey HSD, α = 0.05).

Conclusion

A deficit in precipitations caused above all a decrease in yields of forage. It also influenced contents of nutritive substances. However, it was not possible to evaluate explicitly the content of nutrients because such an evaluation was dependent above all on quality expectations of farmers. A lack of precipitations showed a negative effect on the digestibility of organic matter and neutral detergent fibre. However, the degree of its effects on yields and quality of forage was very individual.

Presented results were obtained within the framework of a project dealing with the evaluation of yields and quality of forage produced by several maize hybrids. The objective of this research was to develop a uniform methodology of evaluation of hybrids and to create guidelines that could enable to consultants, nutritionists, agronomists and farmers to evaluate and select individual maize hybrids.

References are available from authors of the article.

Acknowledgement

This paper was written within the framework of the research project NAZV QJ1210128.

The impact of grassland management on plant species diversity at a mesotrophic site in an eight-year-long experiment

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Abstract
This paper is aimed at evaluating the botanical composition which changed under different grassland managements. At Jevíčko site in the Czech Republic, a long-term small-plot trial with tall oatgrass stand type (Arrhenatherion) was established on permanent grassland in 2003, consisting of 16 treatments in 4 replications. The intensity of utilisation was: I1=(1st cut until May 15th, 4 cuts per year – cuts at 45-day interval), I2=(1st cut between 16th and 31st May, 3 cuts per year at 60-day interval), I3=(1st cut between 1st and 15th June, 2 cuts per year at 90-day interval) and I4=(1st cut between 16th and 30th June, 1 or 2 cuts per year, second cut after 90 days). Four levels of fertilizer application were used: F0=no fertilization, FPK=P30K60N0; FPKN90= P30K60N90, FPKN180=P30K60N180. In total 87 plant species were identified between 2003 and 2011. Botanical composition was above all influenced by nitrogen fertilization, which supports grass species and reduces legumes and other forbs. Higher grass proportion was found in two-cut regimes (I3, I4), too. A higher diversity of plant species and a more balanced proportion of functional groups (grasses, legumes, forbs) were found especially in grassland without nitrogen fertilization.
Keywords: grasslands, cutting, fertilization, biodiversity, botanical composition

Introduction
Agriculture in the Czech Republic has been considerably transformed after the reforms from the early 1990s which has brought a livestock decrease by 50 % and more, which deteriorates management and utilisation of permanent grasslands (Kohoutek *et al*., 2009). The proportion of extensively utilised permanent grasslands has recently increased in the CR up to 60 – 80 % due to agroenvironmental measures, leading to a surplus of unfeedable forage (data from Ministry of Agriculture, Green report 2009). In Switzerland, the law requires that a minimum of 7 % of total area consists of species-rich meadows and pastures (ecological compensation areas) with postponed first cut until the 15th of June in lowlands and the 15th of July in mountainous regions (Gujer, 2005). The goal is to reach about 10 % of interconnected ecological compensation areas in Switzerland. For utilisation of every further 1 % of the extensively managed permanent grasslands in the CR, it would be necessary to increase the livestock units (LU) numbers by 4000 heads which is not feasible presently and so a 'vicious circle' arises (Kohoutek and Pozdíšek, 2006).

Materials and methods
The long-term small-plot trial was performed on permanent grassland at Jevíčko site between 2003 and 2011. The vegetation on the study site was classified as *Arrhenatherion.* The experimental site is located in a moderately warm and moderately wet region - B (Tolasz *et al.*, 2007) with altitude 343 m above sea level, annual average air temperature 7.4 °C and annual average precipitation 545 mm (Tolasz *et al.*, 2007) given as the average for the years between 1966 and 1995. Soil type was classified as a fluvisol. In 2003 soil conditions were as follows: pH_{KCl} 6.5, phosphorus (by Egner method) 37 mg kg^{-1}, potassium (by Schachtschabel method) 68 mg kg^{-1} and magnesium 130 mg kg^{-1}. Before experiment establishment, grassland had been utilized by a three-cut regime without fertilization over decades. Two factorial design with four levels of each factor cutting and fertilization with all possible combination, the total number of treatments being 16, were applied. The intensity of utilisation: I_1=(1st cut until May 15th, 4 cuts per year – cuts at 45 day interval), I_2=(1st cut between 16th and 31st May, 3 cuts per year at 60 day interval), I_3=(1st cut between 1st and 15th June, 2 cuts per year at 90 day interval) and I_4=(1st cut between 16th and 30th June, 1 or 2 cuts per year, second cut after 90 days). Four levels of fertilizer application: F_0=no fertilization, FPK=$P_{30}K_{60}N_0$; $FPKN_{90}$= $P_{30}K_{60}+N_{90}$, $FPKN_{180}$ = $P_{30}K_{60}+N_{180}$. Phosphorus was applied as superphosphate and potassium as potash salt, and nitrogen as calcium ammonium nitrate. The trial was set up in small plots with an area of 10 m^2 arranged as a randomized block design with 4 replications. Botanical composition of vegetation was assessed as projektive dominance (cover) in every year and up to four times (because of up to four cuttings) per year. The botanical composition of vegetation was recorded as proportion of plant functional groups (grasses, legumes, forbs) and number of vascular plant species. The contribution evaluates the number of detected plant species per treatment (annual value contains all of the plant species detected up to four cuts per year and 10 m^2) and the effect of the treatments on the proportion of the plant functional groups averaged of over the years. The results were statistically analysed with a two-factor analysis of variance (ANOVA).

Results and discussion

The results of variance analysis (Tab. 1) show that intensity of utilisation and rate of fertilization highly significantly influence the proportion of grasses and legumes as well as the number of plant species in the grassland. The proportion of forbs is highly significantly influenced only by the rate of fertilization as well as legumes by interaction A x B.

Table 1: Analysis of variance of evaluated features (proportion of grasses, legumes and forbs in % and plant species number as no/10m²).

Source variability	*df*	Evaluated feature Grasses		Legumes		Forbs		Plant species number	
		SS	F_{test}	*SS*	F_{test}	*SS*	F_{test}	*SS*	F_{test}
A (cuts)	3	1187	**	272	**	239	*NS*	1002	**
B (fertilization)	3	13268	**	748	**	7383	**	412	**
A x B	9	419	*NS*	208	**	141	*NS*	30	*NS*
Total	63	20185		1550		11972		1697	

*Ftest – Fisher test; SS – sum of squares; df – degree of freedom; ** - statistically highly significant (P0,01); NS - statistically non-significant*

The effect of utilisation intensity and fertilization on the composition of functional groups and number of plant species is presented in Figure 1. In the average of years 2003 – 2011 grass proportion highly significantly increased from 4-cut utilisation (I1=64 %) towards two-cut utilisation (I4=75 %), also progressive N fertilization increased grass proportion from 55 % in the unfertilized control (F0) to 87 % at the nitrogen rate of 180 kg ha-1 (FPKN180). With increasing grasses proportion in the sward, the proportion of legumes and forbs decreased from 7 % resp. 26 % in 4-cut utilisation (I1) to 2 % (highly significantly) resp. 22 % in extensive two-cut utilisation (I4) (Figure 1). Increasing N fertilization also decreased the share of legumes and forbs highly significantly from 6 % resp. 37 % (F0) to 0.3 % resp. 11 % (FPKN180).

Between 2003 and 2011 altogether 87 plant species were identified. In the extensive two-cut utilisation lower number of species (18) was determined in comparison with the intensive 4-cut utilisation (28). The highest number of species was recorded on plots without (24) or with low (FPK) fertilization (24). A higher rate of N fertilization (180 kg ha-1) decreased the number of plant species down to 18.

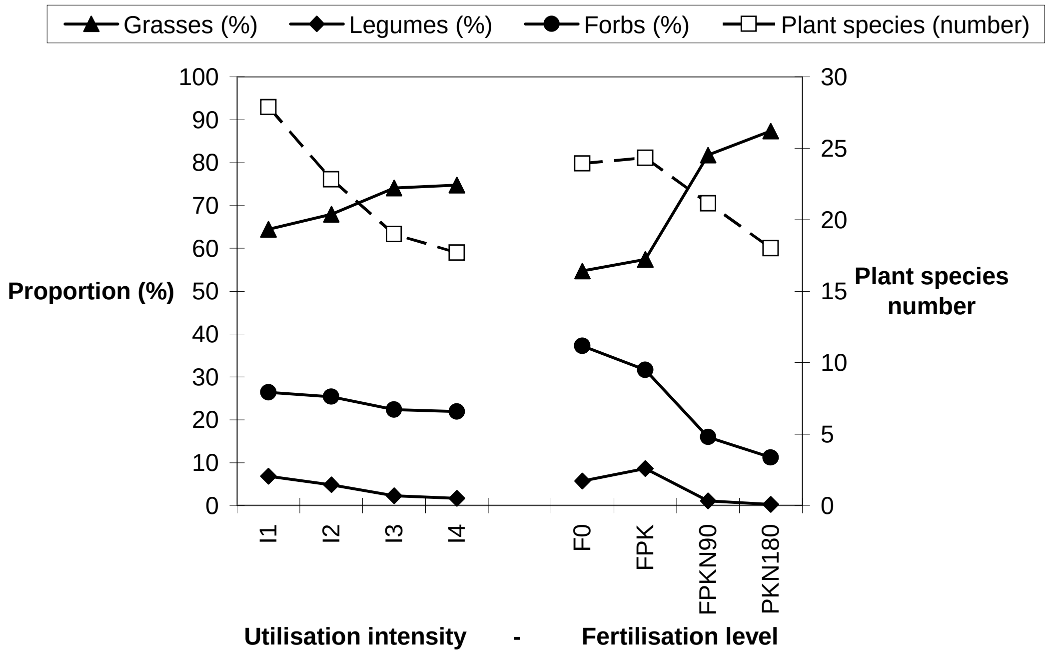

Figure 1: The effect of utilisation intensity and fertilization level on the proportion of grasses, legumes, forbs and number of plant species in permanent grassland

Two cut utilisation and increasing N fertilization promoted development and competitive ability of grasses, especially Arrhenatherum elatius and Dactylis glomerata, which reduced proportion and number of the other plants. These findings break the myth that an extensive two cut utilisation of grasslands is a way towards higher diversity of grasslands. Recent research in the CR shows that the optimum extent of extensively managed permanent grasslands, cut in mid-June and utilised for cattle (dairy and beef cows) during interlactation period, should not exceed 15 % of a managed area (Kohoutek and Pozdíšek, 2006).

Conclusion

The attained results demonstrate that more frequent cutting systems (3-4 cuts) and extensive cattle management (decrease density LU per ha agricultural land by 50 % and more) are optimum from an agricultural and ecological point of view and also from the viewpoint of grassland diversity.

Acknowledgements

The contribution was made as a part of the research of the Ministry of Agriculture of the Czech Republic, Project No. MZE 00027006.

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The effect of biological inoculant and chemical additives on fermentation characteristics and nutritive value of high moisture mechanically treated corn silage

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Introduction

Technology of conserving crimpled or coarsely ground high-moisture corn has found a number of applications. Many authors studied the issue of ground corn conservation e.g. KUNG et al., 2004; TAYLOR and KUNG, 2002; SCHAEFER et al., 1989; MLYNÁR and RAJČÁKOVÁ, 2004; HOFFMAN and MUCK, 2004; FELLNER et al. 2001; BÍRO et al., 2009 and many others. For this conservation technology, the use is recommended of chemical conservation preparations based on organic acids, esp. formic, propionic, benzoic acids and their ammonium salts, applied in dependence on dry matter content within a range from 4–5 $l.t^{-1}$ of the ensiled matter, or the use of biological inoculants (MIKYSKA, 2001). The principle of the effect of these chemical preparations consists in the reduction of pH value as well as in the reduction of undesirable microflora of epiphytes and development of their products. PETTERSON (1998) claims that an addition of formic acid can decrease starch hydrolysis in the rumen of animals. Apart from the recommended content of moisture (usually 35-54%), also a full dose of conservation agent is important for high-quality fermentation. Suitable microbial inoculants can be used for the conservation of mechanically treated high-moisture ground corn grain, too (BÍRO et al., 2009). Those usually contain – in addition to homofermentative strains of the bacteria of lactic fermentation – also some heterofermentative strains such as *L. buchneri, Propionibacterium freudenreichii* spp. *shermani* and other necessary to enhance the aerobic stability (DOLEŽAL et al., 2002; HARRISON et al., 1999; FILYA et al., 1999; MAYRHUBER et al., 1999 and others). The effect of the inoculation of ensiled high-moisture corn grain on the fermentation characteristics was studied also by RUST and YOKOYAMA (1992), WARDYNSKI et al. (1993), KUNG et al. (2004) and others.

The aim of this work was to evaluate the effect of the addition of microbial inoculants and chemical preparations on the quality of the fermentation process in high-moisture crushed maize grain ensiled in model conditions.

Materials and Methods

In our model experiment, we used coarsely ground high-moisture corn grain, which was treated in the cylindrical crusher Romill CP1 Simple with an output of up to 20 t/hour. The corn was harvested by the combine harvester at a stage of dough ripeness with an average dry matter content of 691.4 $g.kg^{-1}$. Three groups were included in the experiment as follows: untreated control-K as a negative control, inoculated silage-A and chemically treated variants-B, C and D. The treatment of the ground corn was homogeneous in all experimental variants. The chemical preparation (B) was based on organic acids (formic acid, propionic acid and ammonium formate) and the chemical preparation (C) contained ammonium propionate in addition. Both conserving preparations were applied at 4.5 l/t of grain. The last chemical additive (D) was urea applied at 4 kg/t of grain. To inoculate the crimpled corn grain, we used the bacterial inoculant (A), the active substance of which included the following bacteria strains: *Lactobacillus plantarum, Lactobacillus casei, Entrococcus faecium, Pediococcus acidilactici* and *Lactobacillus buchneri.* The converted application dose of water-soluble inoculant was 2 g/t. The treated corn was ensiled in the laboratories of Mendel University in Brno into special experimental containers, each of 9 l in volume at an average weight of 777.8 kg/m^3, which were then anaerobically sealed and stored at a room temperature of 20–25 °C. The silages were opened after six months of storage and samples were taken from each treatment (variant) for an analysis. Each silage treatment (variant) was evaluated in three repetitions. The obtained data were statistically processed in MS Office Excel and STATISTICA CZ programmes. In the evaluation, we used one-factor analysis, and the significance of differences was tested by using Scheffe test at a significance level of $P<0.05$.

Results and Discussion

Aggregative indicators of the effect of ensiling additives added to the mechanically treated high-moisture corn are presented in Tab. 1. It follows from the results that the individual preservatives have a different effect on the studied indicators of the fermentation process, namely on pH value, lactic acid content (LA), titrable acidity (TA), ratio of fermentation acids (LA:VFA) and ethanol content. The evaluation of the effect of added urea showed that the ammonized silage exhibited statistically significantly higher (P<0.05) not only the content of dry matter (728.6±5.53 g/kg) and a higher pH (4.74±0.054) but at the same time the highest content of total fermentation acids in the silage dry matter (18.92±3.884 g/kg DM). In this particular silage, we also detected the highest contents of ammonium (1.62 ±0.821 g/kg DM) and ethanol (2.27±0.821 g|/kg DM). The higher pH value in the ammonized silage is in line with the higher level of ammonium in this silage. The level of ammonia in the model control silage of mechanically treated corn grain is in agreement with the results of other authors, too (PAJTÁŠ, BÍRO et al., 2004; BÍRO, JURÁČEK et al., 2004). The lowest content of ammonia was detected in the inoculated silage (0.53±0.076 g/kg DM), which is again in line with the results of other authors (BÍRO et al., 2009). The theoretical assumption of higher ethanol formation in the untreated control silage (K) was corroborated. By contrast, the content of alcohol in the inoculated silage (A) does not correspond with the finding of WARDYNSKI et al. (1993), who detected in their experiment a statistically significantly higher (P < 0.05) content of alcohol in experimental inoculated silages as compared with the untreated control variant. The development of alcohol as a minor fermentation product in silages is connected with the factor of higher dry matter content in the ensiled matter, signalling at the same time that the proper lactic fermentation could be limited (DRIEHUIS et al., 1999). In the case of ammonized silage it appears that the correlation actually exists because this silage exhibited a significantly (P<0.05) higher dry matter content (728.6±0.553 g/kg DM). The statistically lowest (P<0.05) pH value was recorded in the inoculated silage (3.99±0.005), which is in line with the findings of other authors (BÍRO et al., 2009), while higher values are presented by SEBASTIAN et al., 1996.

Table 1: Fermentation parameters of HMGC silages

Parameters		Variants of silages				
		K	A	B	C	D
DM (g/kg)	average	687.5±0.50	695.4±0.57	695.5±0.21	744.3±0.233	728.6±0.553
	index	b	a	a	d	c
pH	average	4.47±0.041	3.99±0.005	4.21±0.019	4.51±0.019	4.74±0.054
	index	a	b	c	a	d
TA (mg KOH/100g)	average	1253.76±162.54	1258.82±182.75	1444.76±195.37	830.13±35.75	932.72±105.19
	index	a	a	a	b	b
LA (g/kg DM)	average	103.5±22.62	112.1±27.62	83.1±10.19	44.1±4.37	149.6±36.52
	index	a	ac	ab	b	c
AA (g/kg DM)	average	72.5±9.12	41.0±3,84	45.3±3.39	14.1±1.43	39.6±3.42
	index	c	a	a	b	a
PA (g/kg DM)	average	0.00	0.00	18.33±10.85	2.91±1.02	0.00
	index	a	a	b	a	a
Σ acids (g/kg DM)	average	176.0±26.10	153.1±31.27	146.7±23.73	61.1±6.06	189.2±38.84
	index	a	a	a	b	a
LA:VFA	average	1.44±0.353	2.71±0.420	1.34±0,181	2.60±0.216	3.76±0.790
	index	a	b	a	b	c
NH_3 (g/kg DM)	average	0.80±0.080	0.53±0.076	0.67±0.117	0.54±0.002	1.62±0.102
	index	b	a	ab	a	c
Ethanol (g/kg DM)	average	2.55±0.352	0.94±0.354	1.17±0.209	0.33±0.325	2.27±0.821
	index	b	a	a	a	b

Conclusions

In our experiment with the mechanically modified high moisture corn grain, the conservative effect of chemical and biological additives on the fermentation process quality of model silages was examined and compared with the control untreated silage. The conservation with the chemical preservatives resulted in a statistically significant (P<0.05) decrease of lactic acid formation from 10.35±2.292 g/kg DM in the control silage to 4.4.±0.437 g/kg DM and in the reduction of the total fermentation acid content in silages. In the inoculated silages, a positive effect on the fermentation was recorded, which was demonstrated by a significant (P<0.05) increase of the lactic acid content, and at the same time by a significant acidification pH value (3.99±0.005) in comparison with the other silages.

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Occurrence of Mycotoxins in Alfalfa Silage

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Introduction

Mycotoxins are toxic secondary metabolites produced by fungi (molds) that cause an undesirable effect (mycotoxicosis) when animals are exposed. Exposure is usually by consumption of contaminated feeds but may also be by contact or inhalation. Biological effects include liver and kidney toxicity, central nervous system effects, and estrogenic effects, to name a few. Only some molds produce mycotoxins, and they are referred to as toxigenic. The fungal toxins are chemically diverse representing a variety of chemical families and range in molecular weight from about 200 to 500. There are hundreds of mycotoxins known, but few have been extensively researched, and even fewer have good methods of analysis available. The primary classes of mycotoxins are aflatoxins, zearalenone, trichothecenes, fumonisins, ochratoxin A, and the ergot alkaloids (Whitlow and Hagler, 2010). Because of structural similarity to the steroidal estrogens, zearalenone and several of its derivatives possess estrogenic activity. Although cattle are not as sensitive to zearalenone as swine, infertility, reduced milk production and hyperestrogenism have been reported (Diekman and Green 1992). T-2 toxin is associated with gastrointestinal edema, and hematopoesis leading to death (Hussein and Brasel, 2001). Deoxynivalenol reduce feed intake, milk production (Whitlow and Hagler, 2010) and affect ovarian functions (Kolesárová 2011/12). Fumonisins have hepatotoxic and carcinogenic effects (Bondy et al., 2012). Ochratoxin A is an important nephrotoxic and nephrocarcinogenic mycotoxin (Milicevic et al., 2010). Feeds not only with high nutritional but also with high hygienic quality are needed for the animal nutrition (Kačániová, 2003; Suchý et al., 2010; Skládanka et al., 2012; Dorszewski, et al. 2013; Zachariasova et al. 2014).

The aim of the study was to determine the occurrence of mycotoxins and effect of biological additive on the concentration of mycotoxins in alfalfa silages with high dry matter content after 12 months of storage.

Materials and Methods

Alfalfa (*Medicago sativa* L.) was harvested in bud stage and wilted 48 hrs. The experiment consisted of 2 variants: alfalfa silages were ensiled without additive (control variant C) and alfalfa silages with biological additive (*Lactobacillus plantarum* $2x10^8$ $CFU.g^{-1}$) (variant LAB). Additive was applied in granular form at the rate 0.5 $kg.t^{-1}$. Biological additive was applied homogeneously. All variants were ensiled in 3 repetitions. Wilted alfalfa was filled into mini laboratory silos (4 dm^3 of volume) and stored in an air conditioned laboratory at temperature of 22°C. All silos were opened after 12 months of storage. Alfalfa silages were sampled for mycotoxins content: total fumonisins, zearalenone, T-2 toxin, deoxynivalenol and total ochratoxins. Mycotoxin content was determined by direct competitive enzyme-linked immunosorbent assay (ELISA) using Veratox quantitative test kits (Neogen, USA). Significance between individual means was identified using the Tukey's multiple range test of the software package SAS 9.1 (SAS Institute Inc., 2004). Mean differences were considered significant at $p<0.05$.

Results and Discussion

We detected the average dry matter content in alfalfa silages 581,6 $g.kg^{-1}$ (variant C) and 544,3 $g.kg^{-1}$ (variant LAB). Samples of alfalfa silages of both variants were contaminated with all determined mycotoxins. The most prevalent mycotoxin was zearalenone, followed by deoxynivalenol and T-2 toxin after 12 months of storage. Zearalenone was detected at concentrations 456.3±2.33 (C) and 427.3±16.69 $\mu g.kg^{-1}$ (LAB). Differences in zearalenone concentration between variants of alfalfa silages were not significant ($p>0.05$). Gálik et al. (2007) reported higher concentrations of zearalenone in comparison with our results (from 635.8 to 703.1 $\mu g.kg^{-1}$). Alfalfa silages without additive (C) had no significantly ($p>0.05$) higher content of deoxynivalenol (390.8±4.45 $\mu g.kg^{-1}$). In our study, data showed that concentration of T-2 toxin in alfalfa silages treated with biological additive (LAB) was significantly ($p<0.05$) higher in comparison with control (143.5±2.33 vs. 73.3±1.62 $\mu g.kg^{-1}$). Higher T-2 toxin average value (176 $\mu g.kg^{-1}$) was observed in alfalfa silages from Czech Republic (Nedělník and Moravcová, 2006). Our results not confirm previous findings, that selected strains of lactic acid bacteria are able to reduce of T-2 toxin (El-Nezami et al., 2002). Content of total ochratoxins ranged from 13.3±0.64 (C) to 13.8±1.20 $\mu g.kg^{-1}$ (LAB). The lowest contamination of alfalfa silages was by total fumonisins. No significantly lower content ($p>0.05$) of total fumonisins was found in treated silages.

Acknowledgement
This work was supported by Grant Agency of the Slovak Ministry of Education, Science, Research and Sport and Slovak Academy of Science (project n. 1/0662/11).

Table 1: Concentrations of mycotoxins in alfalfa silages

$\mu g.kg^{-1}$		FUM	ZEA	T-2	DON	OTA
C	$\overline{x}$	6.27	456.3	73.3*	390.8	13.3
	S.D.	0.01	2.33	4.60	4.45	0.64
	V	0.22	0.51	6.27	1.14	4.8
LAB	$\overline{x}$	5.4	427.3	143.5*	375.3	13.8
	S.D.	0.45	16.69	2.33	13.36	1.20
	V	8.29	3.9	1.62	3.56	8.73

*C - control variant (without additive), LAB - Lactobacillus plantarum, FUM - total fumonisins, ZEA - zearalenone, T-2 – T-2 toxin, DON- deoxynivalenol, OTA- total ochratoxins, *the values with identical superscripts in a column are significantly different at p<0.05*

Conclusions
Occurrence of observed mycotoxins was detected in all alfalfa silages after 12 months of storage. ZEA was the secondary metabolite with the highest concentration, followed by DON and T-2 toxin. Our study confirmed that application of biological additive (*Lactobacillus plantarum*) was ineffective in reduce the concentration of mycotoxins in alfalfa silages with high dry matter content.

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Effect Of Chemical Additive On Fermentation Quality Of Brewer's Grains Silage With Malt Sprouts

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Brewer's grains are characterized by high nutritive value also thanks to the fact that there remain almost 75 % out of the content of crude protein from barley. They are used in animal nutrition either as dried in feed mixtures or as fresh they are fed directly or they are ensilaged (Nishino et al., 2003; Doležal et al., 2005, etc.).

Fresh brewer's grains are characterized by very low dry matter content (200 - 220 g.kg-1). This results in very high effluence of juices inclusive of nutrients, quick microbial changes and quality deterioration during the storage. It is recommended to press fresh brewer's grains to dry matter 350-400 g.kg-1 or to use a sorbent at ensilaging. Vyskočil et al. (2009) consider malt sprouts as very convenient; they state that its additive to brewer's grains prevented effluent of silage juices.

Stability is a problem in silages from brewer's grains. The deterioration process originates through the activities of aerobic microorganisms, mainly yeasts and moulds, therefore, factors that affect these microorganisms may affect aerobic stability of the silages. The principal factors that influence aerobic stability of silages are air, substrate availability and temperature. Organic acids and their salts are one of the most efficient feed additives for aerobic stability and mould prevention.

The objective of this study was to examine the influence of chemical additive on fermentation process and stability of brewer's grains ensilaged together with the malt sprouts.

Material and Methods

We produced a mixture of brewer's grains and malt sprouts in a ratio 80 : 20. The matter was ensilaged into 1.7 l laboratory silos after homogenization. We produced one control variant (C), in which the ensilaged matter was not treated, and one trial variant (T), in which was the ensilaged matter treated by chemical ensilaging additive (22 % sodium benzoate, 8.3 % sodium propionate). Its application dose was 3.0 ml.kg^{-1} fresh matter. Silages were stored in a dark room, at temperature 20 – 22^{o} C, during the fermentation. The experiment finished 120 days after ensilaging and parameters of the fermentation process were determined in silage samples. Samples of fresh matter as well as silage samples were chemically analysed. The results were statistically evaluated by one-factorial analysis of variance.

Results and discussion

In Table 1 are nutritive and energy values of the ensilaged matter and its individual components. Malt sprouts appeared to be not only a good sorbent of the high moisture in the brewer's grains but also a very good source of sugars necessary for favourable course of the fermentation process. Mixture of malt sprouts and brewer's grains, which was prepared for ensilaging, seemed to be a valuable feed of good quality with quite a high content of energy and PDI in spite of higher NDF level thanks to high content of crude protein and low content of crude fibre.

Table 1: Nutrition value and energy of malt sprouts, brewer's grains and their mixture

Parameters	**Malt sprouts (MS)**	**Brewer's grains (BG)**	**Mixture 80 % BG + 20 % MS**
Dry matter in g^{-1} FM	901.89	188.28	338.98
Organic matter in g.kg^{-1} DM	934.06	959.70	948.68
Crude protein in g.kg^{-1} DM	239.75	250.44	235.51
Crude fibre in g.kg^{-1} DM	150.38	139.72	149.74
ADF in g.kg^{-1} DM	153.81	236.70	222.60
NDF in g.kg^{-1} DM	481.11	600.51	574.61
Hemicelluloses in g.kg^{-1} DM	327.30	363.81	352.01
Nitrogen free extracts in g.kg^{-1} DM	528.33	501.67	500.81
Sugars total in g.kg^{-1} DM	148.54	10.88	39.97
Reducing sugars in g.kg^{-1} DM	98.55	3.47	27.80
Fat in g.kg^{-1} DM	15.60	67.87	64.62
Ash in g.kg^{-1} DM	65.94	40.30	49.32
NEL in MJ.kg^{-1} DM	6.14	5.98	6.02
PDI in g.kg^{-1} DM	105.28	118.36	115.43

Results of the fermentation process (tab. 2) showed that its quality was very good also in the silage produced without any ensilaging preparation. Application of the chemical preparation slightly improved the fermentation process, which was manifested in decrease of dry matter losses occurring during fermentation, increase of lactic acid concentration and decrease of volatile fatty acids concentration, mainly acetic acid. Levels of alcohol and proteolysis were also lower in the treated silages than in the non-treated ones.

Table 2: Fermentation profile of silages

Parameters n = 5	Control (C)		Treatment (T)	
	$\bar{x}$	SD	$\bar{x}$	SD
Dry matter in g^{-1} FM	329.26	3.78	332.34	1.59
Losses of DM in %	5.22	1.62	1.64**	0.43
pH	4.07	0.06	3.93*	0.02
pH after 3 days exposure to air	4.20	0.07	3.94**	0.02
Acids in $g.kg^{-1}$ DM				
- lactic	100.54	4.75	109.76	5.49
- acetic	24.61	0.79	21.31**	1.34
- propionic	0.66	0.11	0.61	0.12
- butyric + i.b	0.25	0.10	0.21	0.09
- valeric + i.v	0.25	0.07	0.15	0.09
- capronic + i.c	0.12	0.09	0.08	0.04
VFA total in $g.kg^{-1}$ DM	25.88	0.53	22.36	1.64
Acids total in $g.kg^{-1}$ DM	126.42	5.28	132.11	4.05
Alcohol in $g.kg^{-1}$ DM	11.95	0.24	8.94**	0.25
NH_3-N of total N in %	6.06	0.26	5.37*	0.19

** *P < 0,01 , * P < 0,05*

Evaluation of the fermentation process quality from the viewpoint of acid ratio showed that the better fermentation process was observed in the treated variant because the ratio of lactic acid and volatile fatty acids was higher and better (4.90) compared with the control variant (3.88).

Although were the differences in individual fermentation parameters between treated and untreated silage low, we found statistical significance of differences in losses of weight, pH, in content of acetic acid, alcohol and ammonia N.

Similar results obtained also Vyskočil et al. (2009) in fermentation process of silage prepared from mixture of brewer's grains and malt sprouts without the additive of silage preparation. However, their experiments did not confirm the positive influence of silage additives on the fermentation process.

We measured pH after 3 days of exposure to air and we found out that the level of pH in non-treated silages increased by the value 0.13 during this period, whereas it increased only by 0.01 in the treated silages. This indicates greater stability in silages treated with chemical ensilaging additive.

Conclusion

Mixing brewer's grains with malt sprouts in the ratio 80 : 20 provided feed with high content of crude protein, low content of crude fibre and good energy potential. The course of the fermentation process was very good also in silage produced without the ensilaging additive. Application of chemical additive on the basis of salts positively influenced the fermentation. It manifested itself in decrease of losses in fermentation, pH, volatile fatty acids, alcohol as well as proteolysis. Stability in silages treated with chemical ensilaging additive was higher than in the non treated silages.

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Acknowledgement

This article was written during realization of the project „BELNUZ č. 26220120052" supported by the Operational Programme Research and Development funded from the European Regional Development Fund.

The effect of Maize-All+ on the fermentation and aerobic stability of Maize silage in Croatia

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Keywords: maize, yeast, mould, aerobic stability, Maize-All+, Propionibacterium acidipropionici

Introduction

Silage additives have been developed to enhance the fermentation of silage by outcompeting epiphytic microflora and preserving maximal levels of the ensiled feed value and dry matter content (Ohyama). Aerobic stability of silage is detrementally affected by yeast and aerobic exposure (Woolford). Historically, if improvement in the aerobic stabillity of silage was required through the use of inoculant then *Lactibacillus buchneri* was the only practical option. Filya and Sucu (2007) demonstrated the ability of *Propionibacterium acidipropionici* to convert lactic acid and glucose to the more antifungal propionic and acetic acids, thus enhancing aerobic stability, and with advancements in bacterial production methods *P acidiproponici* is now a realistic commerical option for aiding the stability of ensiled corn. Their findings have been further supported through the EU approval of *Propionibacterium acidipropionici* MA 26/4U to enhance the aerobic stability of all forage types. Work by Foltanova and Marley (2014) has shown the improved dry matter recovery and aerobic stability of corn & sorghum silage treated with Maize-All+ across Europe. This trial was designed to assess the effect of Maize-All+ on maize silage in Croatia.

Material and methods

Maize was directly harvested using a Claas Jaguar 850 to a theoretical chop length of 15mm at 30% dry matter. Harvested maize was untreated and ensiled in a concrete walled bunker. A 150Kg aliquot of untreated maize was homogenised and split into 2 piles. Maize-All+ (*Propionibacterium acidipropionici* MA26/4U 1.5 x 10^5 cfu/g + *Pediococcus acidilactici* 1.0 x 10^5 cfu/g, total application rate 2,5 x 10^5 cfu/g + 9,000 BAU/g α-amyslase) was applied at 10mls/Kg using a hand held sprayer in 3 x 250ml applications, maize was mixed between applications. 9000g of treated maize was packed into each of 5 red polyvinyl net bags which were then tied with a silk tail. 9000g of untreated maize was was packed into each of 5 green polyvinyl net bags which were then tied with a silk tail. Bags were placed in the bunker during filling (as a Dorset wedge). Bags were sequenced Treated, Untreated, Treated, Untreated etc and were sat on 1m of untreated ensiled maize, The tails of the bags were drawn out toward the face and the wall of the bunker was indelibly marked to indicate the bag positions. A further 1m of untreated maize was added on top with standard compaction. The bunker was covered with a single plastic sheet. Bags were reached 8 weeks post ensiling, were dug out, weighed and then sampled for fermentation profile and aerobic stability. Aerobic stability was determined classically with all analysis being undertaken independently by Nutrivet, Pohorelice as blind samples.

Results and discussion

Both silages were well fermented and were free of butyric acid (Table 1). Small variations in the nutritional profile were observed between treated and untreated silage (ME of treated being 10.83 compared to 10.84 MJ/Kg on untreated). Nitrate levels in both treated and untreated silage was below 1% indicating no residual store of nitrate within the ensiled maize. Differences in ME can be directly attributed to variance in sampling (Table 1). The fermentation profile shows a treatment effect. Treated maize has a 10% increase in the level of lactic acid (2.15% compared to 1.96%). The increase in the level of lactic acid being produced from the inclusion of the homolactic P acidilactici in the Maize-All+ formulation. Acetic acid levels are also 10% higher in the treated maize (0.85% compared to 0.77%). The fermentation pathway of P. acidipropionici converts sugar and lactic acid to propionic acid and acetic acid leading to elevated levels of both acetic acid and propionic acid. Ethanol levels in the maize silage were reduced by 27% from 0.15% to 0,11% through treatment with Maize-All+. This is consistent with the results observed by Filya (Fylia et al, 2004; Fylia and Sucu, 2006) is contradictory to the results of Szucs et al,and of Foltanova and Marley 2014). The pH of the treated maize is mathematically lower than that of the untreated maize (3.76 versus 3.79) which is in accordance with the higher level of lactic acid in the treated silage. The easily soluble sugar in the treated silage was 67% of the untreated maize silage. The actual difference observed was 0.01% which can be classed as irrelevant and easily explained by variance in sampling.

Table 2 presents the aerobic data. Both silages had a good fermentation as described, but fermentation losses were statistically reduced through treatment with Maize-All+ (from 7.5% to 6.1%). On a 1000T bunker this equates to an additional 14T of forage to feed. This supports the improved fermentation observed with treatment with Maize-All+.

Silage that was produced was classically aerobically unstable with the untreated silage heating after 26.6hrs. Maize-All+ improved the aerobic stability of the sorghum silage by 380%, increasing the time for a 2ºC rise above ambient to 101.25 hours. Propionic acid and acetic acid produced by Propionibacterium acidipropionici are both antifungal , leading to a reduced level of yeast and mould within the silage and leading to a parallel increase in the time taken for heating to commence. Results obtained through this system are indicative of what would be actually achieved on farm as material is ensiled under 'on-farm' conditions.

Table 1: Comparative fermentation profile of Maize

	Untreated	Maize-All+
DM (%)	30.4	28.6
ME (MJ/Kg)	10.84	10.83
NO_3 (%DM)	0.04	0.04
Easily Soluble Sugar (% DM)	0.03	0.02
Lactic Acid (%)	1.96	2.15
Acetic Acid (%)	0.77	0.85
Butyric Acid (%)	0	0
Propionic Acid (%)	0	0
Ethanol	0.15	0.11
pH	3.79	3.76

Table 2: Aerobic Parameters

	Untreated	Maize-All+
Fermentation Losses (%)	7.5^{a}	6.1^{b}
Aerobic stability (hrs)	26.6^{a}	101.25^{b}

Supercripts with different prefixes are statistically significantly different at P<0.05

Conclusion

Treatment of maize with Maize-All+ led to an improved fermentation, statistical reduction in dry matter losses and a statistical improvement in the aerobic stability of good quality maize silage. The Maize-All+ formulation is appropriate for use on both maize and sorghum silage.

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The effect of a novel combination of anti mycotic chemicals on the microbiology and nutritional value of hay

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Keywords: yeast, mould, hay, losses, Sil-All Hay,

Introduction

Hay has been used across much of Europe as a stable way of storing forage in order to provide nutrition and fibre to animals. Various forages have been used (oats, various grasses ,alfalfa etc), all of which are subject to deterioration through storage by the action of yeast and mould. Growth of yeast and mould has been well documented to reduce dry matter, reduce feed value and lead to heat damage (in the form of lost energy as generated heat but also in the linking of protein and increase in the level of ADIN) in hay and silage (Woolford). Propionic acid has historically been used to try and control microbial deterioration of hay but increasing cost of propionic acid coupled with a reluctance of contractors to use the product and a change in global climate making an adequate window of cutting, wilting and baling hay less likely to adequately dry forage. This has resulted in more hay being baled at a moisture content above the optimal 15%. Hay baled at moisture content above 15% is prone to microbial deterioration and is in need of treatment to control microbial activity. Ohyama documented the changes in nutritional value in forage through microbial action and aerobic exposue. Acid salts have been effectively used in food and forage to control fungal outgrowth and this trial assessed the proposed Sil-All Hay formulas ability to effect microbial and nutriotional parameters of hay under field circumstances

Material and methods

Third cut grass containing some leguminous species was mechanically harvested, wilted over a 48 hr period (tedded at 24hrs post cutting) and baled at a dry matter (DM) content of 79 % .

A single treatment was applied in direct comparison to untreated control (Sil-All Hay at 250g/T active application and 1l/T). The negative control received the same amount of water as the Sil-All Hay treatment. For each treatment 6 bales were produced (average weight 485Kg ±20Kg). The headlands of the field were baled in order to purge the baler of any carry over treatment and then central windrows were baled, initially untreated and then treated. 2 purge bales were made and discarded from the trial to ensure a true application rate of Sil-All Hay . The bales were barn stored at ambient temperature, unstacked and with a 0.5m space between each bale. Samples were drawn at T = 3 days and T = 3months. Samples were cored using Forage Master Probe with alcohol disinfection in between cores. Samples were refrigerated prior to analysing and were analysed within 5 hours of coring,

The laboratory examination focused on the fermentation products and the microbiological analysis. Fermentation parameters were assessed through NIR. Microbiological analysis (for lactic acid bacteria (LAB), yeasts and moulds) were performed using traditional plate count methods.

Data was compared using Minitab 16, ANOVA. Significance was declared for $P<0.05$

Results and discussion

The density of bales was measured as between 350-375 kg/m^3 .No significant difference in the feed value of the treated and untreated bales existed at baling. Application of Sil-All Hay had no impact on the initial T = 3 day nutrition of the bales, but aided with a more effective curing of the hay (average moisture content of the treated hay fell to 17.9% compared to 18.9% at 3 months) and a resultant mathematical improvement in the protein retention at 3 months (28.9% versus 27.0%). More effective curing of the hay results in greater moisture loss and concentration (w/w) of nutrient value

Retention of all energy parameters was mathematically improved through the use of Sil-All Hay. Concentration of feed values was achieved through the enhanced curing gained through treatment.

A statistically significant reduction in the Day 3 yeast count was achieved by treatment with Sil-All Hay. Average untreated hay yeast counts were at 5.9×10^7 cfu/g, whereas treated counts had been reduced by 97% to 1.5×10^6 cfu/g. A similar reduction in the Day 3 mould counts was observed through treatment with a 90% reduction from 6.3×10^5 to 6.5×10^4. The reduction in the yeast and mould counts can be attributed to the antifungal activity of the chemicals within the Sil-All Hay formulation and their natural equilibriation through the bale over the first days post baling.

Month 3 yeast and mould counts showed a mathematical difference between Sil-All Hay and control bales. Both control bales and Sil-All Hay treated bales yeast counts had reduced through the curing process as the water activity of the bales further decreased, The curing of the bales led to natural reduction in yeast counts. Mould counts for both treated and untreated bales increased between Day 3 and Month 3 analysis with the decreasing water activity of the bale favouring the growth of mould over the growth of yeast. Visual inspection of both treated and untreated bales did not show any visual growth of mould and it is possible that the mould counts are aberrantly high from mycelial growth.

Table 1: Summary Bale Analysis

	Time = 3 day		Time = 3 month	
	Control	Sil-All Hay	Control	Sil-All Hay
Moisture Content %	21.3	21.5	18.9	17.9
Protein (% w/w)	24.2	26.5	27.0	28.9
NEl (MJ/Kg)	6.0	5.7	5.7	5.8
NEg (MJ/Kg)	2.4	2.3	2.2	2.3
Relative Feed Value	118.1	120.4	123.9	133.1
Yeast (cfu/g)	$5.9 \times 10^{7\,a}$	$1.5 \times 10^{6\,b}$	1.5×10^{4}	1×10^{4}
Mould (cfu/g)	$6.3 \times 10^{5\,a}$	$6.5 \times 10^{4\,b}$	8.5×10^{6}	5×10^{6}

Supercripts with different prefixes are statistically significantly different at $P<0.05$

Conclusion

Treatment of highly wilted forage (circa 80%) with Sil-All Hay aids the curing process of hay once baled, resulting in improved nutrient and feed value protection. Sil-All Hay rapidly reduced the yeast and mould counts of the fresh forage once baled and could help to mitigate the risks associated with the baling sub optimal dry matter forage.

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Fermentation Quality Of Lucerne Silages On Agricultural Farms In West Slovakia In The Years 2010-2013

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Data on feeds that are analysed in the chemical laboratory of the Department of Nutrition at the Research Institute of Animal Production, which belongs into the National Agricultural and Food Centre, are being processed and collected in the Data Basis of Feeds. Data from this data basis serve as basis for up-dating of the legislation and innovation of systems for evaluation of nutrient requirements and nutritive value of feeds.

Lucerne is the most important perennial fodder crop in our country; it is an important source of proteins. It is appreciated in the nutrition of ruminants for its high palatability, quick passage and overall beneficial influence on the feed rations' structure. Ensilaging is the most effective way how to utilize the potential of lucerne. It is possible to produce high quality and nutritionally balanced silages only if it has high content of digestible nutrients and energy in time of harvest. Weissbach (2003) reported that the process of biomass wilting before ensilaging is the most important measure that influences the quality of fermentation process in lucerne silages. The increasing of DM content of significantly decreases the risk of undesirable course of fermentation process and also legumes with low acidification potential can be good ensilaged. Although this is well known, it often happens that little attention is paid to this knowledge in practical conditions of the agricultural farms.

The aim of our study was to compare the quality of fermentation process in lucerne silages produced on agricultural farms in west Slovakia during the years 2010 - 2013.

Material and Methods

We selected lucerne silages (analyzed in the years 2010, 2011, 2012 and 2013) from the National Data Basis of Feeds of the Slovak Republic into the statistical set. There were selected totally 362 silages. We divided the silages according to the dry matter content into five groups within individual years. First group contained silages with dry matter content up to 300 $g.kg^{-1}$, second group from 301 to 350 $g.kg^{-1}$, third group from 351 to 400 $g.kg^{-1}$, fourth group from 401 to 450 $g.kg^{-1}$ and the fifth one over the 451 $g.kg^{-1}$ FM.

For the statistical evaluation of the fermentation process we selected the following parameters: dry matter content, pH, content of ammonia nitrogen of total N, concentration of lactic acid, butyric, acetic and propionic acids. Chemical analyses of feed were performed by means of methods under the regulation of the EU commission (ES) No 152/2009.

Results and Discussion

The average parameter values of fermentation process in lucerne silages produced during the years 2010-2013 were arranged into a table according to individual groups. The group with the lowest dry matter level up to 300 $g.kg^{-1}$ was participated by 11.63 – 19.56 % of the all produced silages in individual years. The highest part (19.56 %) was noticed in silages produced in 2013. In this year was also the quality of fermentation process in this group the worst of all years. High pH (5.22), 21 % proteolysis, high concentration of butyric acid (16.97 $g.kg^{-1}$ DM) give evidence of the fact that the ensilaging was not managed well at all; the quality of these silages was very bad and its feeding to animals was a great risk for their health. On the whole we can say that the quality of lucerne silages was the lowest in the groups with dry matter content up to 300 $g.kg^{-1}$of all studied years.

Wiessbach (2003) also considers the esilaging of lucerne with low content of dry matter to be a great problem. In his opinion the increase of dry matter content from 200 to 400 $g.kg^{-1}$ FM improves significantly the ensilability of lucerne mainly because of increase in concentration of water soluble carbohydrates, which are necessary for fermentation. However, the lactic fermentation, which occurs during the ensilaging process, does not depend only on the concentration of sugars in feed. It is markedly influenced by buffer capacity, which decreases with the increase of dry matter content. This is the reason why it is necessary to decrease pH to the level 4.2 g if the dry matter content in lucerne is 200 g; if the content is 400 g it is sufficient to reduce acidification only to pH 4.75.

Our statistical survey is in agreement with this knowledge. Parameters of fermentation process improve with rising dry matter content in all studied years. The content of ammonia N and of undesirable butyric acid decreases. The highest content of lactic acid was noticed in the group of silages with dry matter level from 300 to 350 $g.kg^{-1}$ FM. Comparison of total average values of the fermentation process in silages in individual years demonstrates that the best quality was in silages produced in 2011 and the worst one was in 2013.

Table Division of lucerne silages according to dry matter content in west Slovakia in the years 2010-2013

Year, Parameters	Group with level of DM content (g.kg^{-1} FM)					Average
	to 300	300 – 350	350 – 400	400 – 450	over 450	
2010 n=91						
Ratio in %	16.48	23.08	23.08	15.38	21.98	100
Dry matter in g^{-1} FM	275.78	327.61	378.95	423.08	509.65	384.71
NH_3-N of total N in %	14.64	9.60	6.89	8.45	7.02	9.27
pH	4.71	4.63	4.45	4.61	4.66	4.61
Lactic acid in g.kg^{-1} DM	66.29	81.66	66.47	48.80	43.65	63.61
Butyric acid in g.kg^{-1} DM	5.10	1.66	0.91	2.32	0.47	2.05
Acetic acid in g.kg^{-1} DM	33.02	30.18	15.94	16.40	11.94	22.45
Propionic acid in g.kg^{-1} DM	2.05	0.81	0.39	0.63	0.56	0.88
2011 n=86						
Ratio in %	11.63	26.74	30.23	15.12	16.23	100
Dry matter in g^{-1} FM	251.32	328.80	374.87	423.89	512.59	378.01
NH_3-N of total N in %	7.14	8.08	5.75	8.55	10.69	7.68
pH	4.48	4.62	4.58	4.55	4.65	4.59
Lactic acid in g.kg^{-1} DM	65.85	66.10	61.73	55.78	64.50	63.72
Butyric acid in g.kg^{-1} DM	1.11	1.40	0.55	0.44	0.92	0.97
Acetic acid in g.kg^{-1} DM	19.23	19.85	17.05	26.21	22.24	20.05
Propionic acid in g.kg^{-1} DM	0.72	0.58	0.84	0.49	0.63	0.66
2012 n=93						
Ratio in %	15.15	24.24	33.33	21.21	6.06	100
Dry matter in g^{-1} FM	274.28	322.98	370.57	433.11	491.64	365.05
NH_3-N of total N in %	12.41	8.90	7.17	3.89	5.13	7.67
pH	5.08	4.64	4.54	4.64	4.65	4.69
Lactic acid in g.kg^{-1} DM	56.46	62.55	55.73	58.73	44.71	57.20
Butyric acid in g.kg^{-1} DM	6.64	0.65	1.34	0.56	0.21	1.85
Acetic acid in g.kg^{-1} DM	39.10	22.91	19.45	10.42	9.49	21.33
Propionic acid in g.kg^{-1} DM	1.73	1.13	1.55	0.25	0.30	1.14
2013 n=92						
Ratio in %	19.56	30.43	21.74	9.78	18.48	100
Dry matter in g^{-1} FM	256.64	327.14	374.58	425.83	495.62	364.44
NH_3-N of total N in %	21.07	11.02	9.80	7.82	7.32	11.99
pH	5.22	4.69	4.90	4.54	4.86	4.86
Lactic acid in g.kg^{-1} DM	49.97	68.23	62.68	62.77	40.07	57.74
Butyric acid in g.kg^{-1} DM	16.97	2.14	1.63	1.03	0.28	5.07
Acetic acid in g.kg^{-1} DM	42.97	30.47	21.93	18.36	9.72	27.99
Propionic acid in g.kg^{-1} DM	4.02	1.13	0.58	0.70	0.23	1.52

Conclusion

Statistical evaluation of lucerne silages produced in 2010 – 2013 shows high ratio of silages with low dry matter content (up to 300 g.kg^{-1} FM). This ratio varied from 11.6 to 19.6 % of the totally produced silages and the quality of these silages was very poor. According to the Slovak standard were these silages not suitable for feeding to animals without testing if they are not detrimental to health, except for silages of the year 2011.

We found the best quality of fermentation process in silages produced in 2011 and the worst quality was in 2013. A survey in practical conditions on agricultural farms confirmed that increasing dry matter content improves the ensilability of *Medicago sativa* and the quality of fermentation process in lucerne silages.

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Acknowledgement

This article was written during realization of the project „BELNUZ č. 26220120052" supported by the Operational Programme Research and Development funded from the European Regional Development Fund and by the ÚOP 95 112 „Determination of quality and usability of alternative crops and feeds in relation to the production of food of animal origin, updating of the national database of feeds".

Improved Production of Big Bale Lucerne Silage Using Lactic Acid Bacteria Blend Inoculant

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Abstract
The effect of lactic acid bacteria blend containing *Entrococcus faecium* (DSM 22502/NCIMB 11181), *Lactococcus lactis* (NCIMB 30117) and *Lactobacillus plantarum* (DSM16568) as an inoculant on baled lucerne silage production was evaluated. Compared with the untreated silage the inoculant treatment decreased the pH value by 0.54 units ($P < 0{\cdot}05$), alcohols by 31.2 %, ($P < 0.05$), butyric acid 4.3 times ($P < 0.05$), ammonia N by 26.8 % ($P < 0.05$) and DM losses by 46.2 % ($P < 0.05$). Inoculant treatment resulted in by 3.6 % higher ($P < 0.05$) DM content (corrected for volatiles) and higher residual WSC content (by 67.2 %, $P < 0.05$). Appreciable decreased numbers of yeast (2.07 *vs* 1.18 log cfu/g) and molds (2.66 *vs* 1.87 log cfu/g) were detected in the inoculated silage when compared with the untreated silage. At the time of plastic film removal (90 days after ensiling) the inoculated bales were scored as 0 using a scale from 0 to 5 and the untreated big bales were scored as 0.4. At the end of the aerobic stability testing (18 days after removing plastic) the untreated big bales were scored as 4.2, whereas the inoculated big bales were scored as 1.8, what was 2.3 time lower ($P < 0.05$) compared with the untreated big bales. The inoculated lucerne silage was more aerobically stable.

Introduction
The selection of the best strains is essential and based on their ability to rapidly reduce the numbers of contaminant bacteria and fungi and preventing secondary fermentation (increasing aerobic stability). Many recently developed inoculants contain multiple species, building on evidence that the growth of one bacterial species may facilitate the growth of another (Fitzsimons et al., 1992). Several studies have shown that fermentation responses differ widely among strains (Saarisalo et al., 2007) and all that could exert different effects on silage fermentation end products and, consequently, animal performance (Muck, 2002). However, information regarding the effects of inoculants on the quality of big-bale silage is limited. The aim of this work was to evaluate the efficacy of a blend of bacterial strains containing *Entrococcus faecium* (DSM 22502/NCIMB 11181; *Lactococcus lactis* (NCIMB 30117 and *Lactobacillus plantarum* DSM16568 as a microbial inoculant on the chemical composition, fermentation end-products, DM loss, aerobic stability and mold development in lucerne big bale silage.

Materials and Methods
A homogenous plot of the primary growth of lucerne *(Medicago sativa L.*) at budding stage of maturity, was mown with disk mower-conditioner, set to place wide windrows. Lucerne was wilted for 24 h without tedding to a dry matter concentration of about 35 %. The crop was picked up with a round baler (LELY WELGER RP 245 and baled into a 1.2 m wide and 1.2 m diametre cilindrical bales. The following additive treatments were applied to forage in the windrows: (1) Control – no additive and (2) homofermentative lactic acid bacterial strains *Entrococcus faecium* (DSM 22502/NCIMB 11181), *Lactococcus lactis* (NCIMB 30117 and *Lactobacillus plantarum* DSM16568. The application rate of the inoculant was 150 000 cfu/1 g herbare and applied at 4 L suspensijon per 1 tonne lucerne. The same volume (4 L/t) of tap water was used instead of the suspension in the control treatment for spontaneous fermentation. Each additive treatment was applied to a randomly selected individual windrow within a group of two adjacent windrows and four bales were made from within each windrow. Additives were applied across the full width of the top of grass windrows through a perforated dribble bar, and baling was within 15 min of additive application. Immediately after baling, the bales were carted from the field to the storage area (to dairy farm) and individually wrapped and then labelled. Six layers of plastic film (POLICROP RAPID, 750x0.025x1500 5 layer blown film) were used for wrapping a big bale to ensure anaerobic storage until opening. Five big bales from each treatment were chosen at random, weighed when prepared and again after 90 days of storage measured for dry matter (DM) losses. Prior to removing the plastic film surrounding each bale, it was examined carefully for visible damage. On removal of the plastic film all visible mould colonies on the bale surface were located numbered and scored. Each bale was core sampled for chemical and microbial analyses and for aerobic stability test.

Results and Discussion

Mean DM content of wilted lucerne reached 342.2 g/kg herbage. Lucerne had a crude protein and WSC content of 229.1 and 48.8 g/kg DM respectively. The buffer capacity of herbage was 56.4 mEq /100g DM. Based on the WSC content of the herbage prior to ensiling, the forage was considered to be moderately difficult to ensile (16.7 g/kg fresh matter of WSC) according to the EFSA opinion on silage additives guidelines (European Food Safety Authority 2008). The number of epiphytic LAB, moulds and yeasts were detected at 10^5, 10^4 and 10^4 cfu/g respectively in the pre-ensiled fresh lucerne. Dry matter content (corrected for volatiles) and WSC concentration of the inoculated silage were higher by 3.6 % ($P<0.05$) and by 67.2 % compared with thecontrol silage. Herewith, inoculation resulted in lower by 46.2 % ($P<0.05$) DM loss. Compared with the untreated the inoculant treatment decreased the pH value by 0.54 units ($P < 0{\cdot}05$), concentrations of ammonia-N and alcohols by 26.8 % ($P < 0{\cdot}05$) and 31.2 % ($P < 0{\cdot}05$), respectively. Lactic acid concentration was more than two times higher ($P<0.05$) and acetic acid concentration was numericaly (by 4.0 %) higher in the inoculated silage. Only 0.9 g/kg DM butyric acid was detected in the inoculated silage, while 3.9 g/kg DM was found in the untreated silage. The inoculated silage contained also more by 67.2 % ($P<0.05$) residual WSC (Table 1).

Table 1: Effect of inoculant treatment on the fermentation variables of ensiled in big bales lucerne

Variables	Control silage	Inoculated silage	Average
DM, g/kg	312.4	324.3	318.3
DM corrected for volatiles, g/kg	323.4	335.1*	329.3
DM loss, g/kg	87.6	47.1*	67.4
WSC, g/kg DM	6.1	10.2*	8.2
pH after 90 days	4.95	4.41*	4.68
N-NH_3 fraction, g/kg total N	58.6	42.9*	50.7
Lactic acid, g/kg DM	32.2	71.7*	51.9
Acetic acid, g/kg DM	19.8	20.6	20.2
Butyric acid, g/kg DM	3.9	0.9*	2.4
Propionic acid, g/kg DM	0.2	0.2	0.2
Alcohols, g/kg DM	6.4	4.4*	5.4
OMD, % DM	66.96	67.70	67.3
Clostridia, log cfu/g	1.0	<1.0	0.99
LAB (log CFU/g)	5.00	5.36*	5.18
Yeast (log CFU/g)	2.07	1.18*	1.62
Moulds (log CFU/g)	2.66	1.87*	2.26
Visible surface area of mould growth at the time removal of the plastic film, scores	0.40	0	0.20
Visible surface mould growth after aerobic stability test, scores	4.20	1.80*	3.00

**statistically significant difference vs Control p<0.05*

Lactobacilli numbers of the inoculated silages increased significantly ($P < 0.05$) compared with control fermentation. Appreciable decreased number of yeast (2.07 log cfu/g and molds (2.66 log cfu/g) were detected in the inoculated silage when compared with the untreated silage (1.18 log cfu/g and 1.87 log cfu/g), respectively. There was a significant interaction effect of silage additive on visible surface area of mould growth at the time of removal of the plastic film as well at the time after aerobic stability test. At the time of plastic film removal all five inoculated bales had no visible surface fungal contamination and were scored as 0. The untreated bales were contaminated with one visible colony each and were scored as 0.4. At the end of the aerobic stability testing (18 days after removing plastic) the untreated big bales were scored as 4.2 and the inoculated big bales were scored as 1.8, what was 2.3 time lower ($P < 0.05$) compared with the untreated big bales. The aerobic stability of the inoculated lucerne silages has improved by 180 hours (7 days), compared with the control silages.

Conclusions

The baled inoculated lucerne silage was superior in the onset of fermentation and in the decline of pH compared with the baled lucerne silages without additives. The result was higher concentration of fermentation acids and lactic acid, and lower butyric acid, alcohols and ammonia N fraction in the inoculated silage compared with the untreated silage. Inoculation inhibited yeast and mould count in the silage and reduced visible mould growth on the big bales surface. Higher aerobic stability of the inoculated baled silage is reflective of the higher microbiologically inhibitory environment.

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Acknowledgement

The authors would like to acknowledge the support from Chr. Hansen A/S.

Effect of seed number at sowing on yield and nutritive value of different oat-pea and oat-vetch combinations

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Summary
The authors investigated the effect of different seed number (per ha) and seed-rations on yield, nutrient content and digestibility of three summer annual mixtures (three oat-pea and three oat-vetch combinations, respectively). Mixture of oat (1.5 x 10^6 seed/ha) and vetch (2.0 x 10^6 seed/ha) had the highest green – and dry matter yield (6,3 ton DM/ha), but higher NDF content (p≤0,05), lower OM digestibility (p≤0,05) and NDF degradability (p≤0,05) compared to the other combinations (oat 2.5 x 10^6 and vetch 2.5 x 10^6 seed/ha: 3,9 ton DM/ha; oat 2.0 x 10^6 and vetch 3.0 x 10^6 seed/ha: 3,9 ton DM/ha). Mixture of oat (2.5 x 10^6 seed/ha) and pea (0.65 x 10^6 seed/ha) had the highest green – and dry matter yield (4.3 ton DM/ha) compared to the other combinations (oat 1.5 x 10^6 and pea 0.65 x 106 seed/ha: 4.2 ton DM/ha; oat 3.5 x 10^6 and pea 0.8 x 10^6 seed/ha: 3,9 ton DM/ha). Significant differences were found in nutrient content and digestibility of the different oat- pea and oat-vetch combinations.

Introduction
The authors investigated the effect of different seed number (per ha) and seed-rations on yield, nutrient content and digestibility of three summer annual mixtures (three oat-pea and three oat-vetch combinations, respectively). Nutrient content, organic matter digestibility (OMd) and NDF rumen degradability of the six different mixtures were determined by Near-InfraRed Spectroscopy.

Materials and Methods
Three different oat-pea and oat-vetch mixtures were sowed on 28th March 2013. Nitrogen fertilization was carried out before sowing in March 2013 (200 kg/ha NPK and 54 kg N/ha). Experimental plot size was 1,5m x 10m = 15nm per repetition (treatments= 6, n= 4). Treatments were the following (Table 1).

Table 1:Whole crop oat-vetch and oat-pea mixtures(28th March-19th June 2013 Kaposvár University, Hungary)

Treatments	Cultivars		Seed number/ha	
Oat and pea	MV Pehely	Rubin	1,500,000	650,000
Oat and pea	MV Pehely	Rubin	2,500,000	650,000
Oat and pea	MV Pehely	Rubin	3,500,000	850,000
Oat and vetch	MV Pehely	Maxivesa	1,500,000	2,000,000
Oat and vetch	MV Pehely	Maxivesa	2,500,000	2,500,000
Oat and vetch	MV Pehely	Maxivesa	2,000,000	3,000,000

Sampling was carried out 19th June (oat and the legume in flowering) by hand (randomized sampling with standard frame: 1 nm, n=4) on randomized experimental plots (determination of the yield n=16, samples for laboratory analyses n=4, respectively). Green- and dry matter yield, crude nutrient content, fiber fractions, organic matter digestibility and NDF degradability were determined.

Samples were dried and grinded according to the Guidelines of Samplinq® system (BLGG AgroXpertus, Wageningen), equivalently with the Hungarian National Standard. Crude nutrient content, fiber fractions, organic matter digestibility and NDF rumen degradability were determined by Near-InfraRed Spectroscopy. The spectroscopic method (dataset developed by the BLGG AgroXpertus, Wageningen, the Netherlands) uses the near-infrared region of the electromagnetic spectrum (range: 1000 - 2500 nm). A NIRS-analysis is an indirect method and has been calibrated on primary classical wet-chemical analysis, as reference methods. List of the reference methods and determination coefficients are available at the corresponding author. Spectra were determined according to the guidelines of NEN-EN-ISO 12099 (Q-Interline Quant FT-NIR analyser, ISO 12099:2010 guidelines for the application of near infrared spectrometry). BLGG AgroXpertus uses local calibration model, as special mathematical model to calculate the desired parameters based on the NIR spectra. This approach increases reliability when the relationship between the NIR spectra and an analysis feature is not linear. BLGG AgroXpertus organizes ring-test (inter-laboratory calibrations), and it validates its satellite labs to maintain reliability.

Applied statistical models were the following: Levene test for homogeneity and one-way ANOVA (comparison of the different mixtures).

Results and Discussion

Mixture of oat (2.5 x 10^6 seed/ha) and pea (0.65 x 10^6 seed/ha) had the highest green – and dry matter yield (4.3 ton DM/ha) compared to the other combinations (oat 1.5 x 10^6 and pea 0.65 x 10^6 seed/ha: 4.2 ton DM/ha; oat 3.5 x 10^6 and pea 0.8 x 10^6 seed/ha: 3,9 ton DM/ha). Mixture of oat (1.5 x 10^6 seed/ha) and vetch (2.0 x 10^6 seed/ha) had the highest green – and dry matter yield (6,3 ton DM/ha), but higher NDF content, lower OM digestibility and NDF degradability ($p \leq 0.05$) compared to the other combinations (oat 2.5 x 10^6 and vetch 2.5 x 10^6 seed/ha: 3,9 ton DM/ha; oat 2.0 x 10^6 and vetch 3.0 x 10^6 seed/ha: 3,9 ton DM/ha), showed in Table 2. Significant differences were rarely found in nutrient content and digestibility of the different oat- pea and oat-vetch combinations (Table 3).

Table 2: Changes in green- and dry matter yield and nutrient content of whole crop oat-vetch and oat-pea mixtures derived from first cut (28 March- 19th June 2013, Kaposvár University, Hungary)

Seed/ha		Green yield t/ha	DM yield t/ha	DM g/kg	Crude protein g/kg DM	Sugar g/kg DM	Starch g/kg DM	OMd %	DOM g/kg DM	NEl-VC MJ/kg DM
Oat	Pea									
1,500,000	650,000	24.0a	4.2a	176a	166a	64.5a	34.8a	70.4a	629a	5.8a
		4.5	4.5	1.0	0.1	3.9	13.1	1.1	11.0	0.2
2,500,000	650,000	24.4a	4.3a	174a	164a	61.0a	51.3a	69.6a	628a	5.8a
		3.0	3.0	0.5	0.1	8.2	21.4	1.9	20.2	0.2
3,500,000	850,000	20.6a	3.9a	191b	164a	73.3b	43.8a	68.9a	624a	5.8a
		1.0	3.0	0.5	0.1	3.0	2.8	1.1	9.7	0.1
Oat	Vetch									
1,500,000	2,000,000	34.9a	6.3a	182a	157a	57.8a	28.8a	67.5a	605a	5.5a
		4.5	4.5	0.8	0.1	13.1	11.5	1.4	18.9	0.2
2,500,000	2,500,000	22.2b	3.9b	177b	157a	56.0a	39.0a	69.0a	617a	5.7a
		2.5	2.5	0.5	0.1	8.8	20.9	1.8	21.6	0.2
2,000,000	3,000,000	22.0b	3.9b	176b	178b	44.3a	36.3a	70.9a	635a	5.9a
		3.7	3.7	0.6	0.1	8.5	15.9	0.5	6.9	0.1

abcd Means in the same column with different letters differ compared the impact of different seed /ha within the same type of mixture ($p \leq 0.05$), OMd organic matter digestibiltiy, DOM digestible organic matter,

Table 3: Changes in fibre composition and NDF rumen degradability of whole crop oat-vetch and oat-pea mixtures derived from first cut (28th March-19th June 2013,Kaposvár University, Hungary)

Seed/ha		Crude fibre g/kg DM	NDF g/kg DM	ADF g/kg DM	ADL g/kg DM	Cellulose g/kg DM	Hemicellulose g/kg DM	NDFd %	dNDF g/kg DM
Oat	Pea								
1,500,000	650,000	255a	512b	302a	30.5a	210a	271a	64.9a	333a
		12.7	41.6	10.5	3.7	33.5	11.4	2.9	39.8
2,500,000	650,000	253a	492a	299a	32.0a	194a	267a	62.5a	308a
		17.9	20.3	18.6	2.4	13.0	16.9	0.8	13.3
3,500,000	850,000	258a	488a	311a	32.8a	178a	278a	60.8b	297a
		8.0	12.2	3.2	1.3	10.7	4.1	0.6	9.3
Oat	Vetch								
1,500,000	2,000,000	266a	528a	310a	28.0a	218a	282b	64.4a	340a
		9.0	12.4	8.4	3.2	13.0	5.4	1.1	12.1
2,500,000	2,500,000	254a	511a	293a	25.3a	217a	268ab	64.7a	330a
		13.0	9.0	18.6	2.6	10.0	16.6	1.4	12.0
2,000,000	3,000,000	261a	522a	296a	26.8a	226a	269a	65.6a	342a
		9.5	18.4	12.4	2.6	10.2	11.9	0.5	11.6

abcd Means in the same column with different letters differ compared the impact of different seed /ha within the same type of mixture ($p \leq 0.05$), NDFd NDF rumen degradability, dNDF rumen degradable NDF

Conclusions

The highest oat-pea seed number/ha had positive impact on nutrient content: increasing of seed number/ha had significant effect on crude protein- and NDF-content of the mixtures. However, there were a non-significant increase in the ADL concentration, reducing the NDF rumen degradability ($p \leq 0.05$) in oat-pea mixture with higher pea and oat ration. Combination of oat and vetch, with the lowest seed number of the vetch and oat (oat: 1.5 x 106 seed/ha; vetch: 2.0 x 106 seed/ha) had the highest green – and dry matter yield (6,3 ton DM/ha), but lower nutritive value as the higher oat-vetch seed number combinations ($p \leq 0.05$). It was found that the increasing seed number/ha of vetch in combination with decreasing oat ration improved the crude protein content ($p \leq 0.05$), moreover the digestibility and rumen degradability in the mixture.

Effect of 7-14 days delay in cut on yield and nutritive value of whole crop rye during the April-May harvest period

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Summary
Authors aim was to determine the effect of the cultivars and the different phenological stages on green and dry matter yield, nutrient content, organic matter digestibility (OMd) and NDF rumen degradability of whole crop rye with 7 and 14 days delay, respectively. Crude nutrient content, fibre fractions, organic matter digestibility and NDF degradability of two different varieties and three hybrids were determined by Near-InfraRed Spectroscopy (NIR) before earing (29th April), in earing (7th May) and in flowering (13th May) in Hungary (randomized experimental plots, n=4). Crude protein content decreased with 8-20% during 7 days, while there was 39-42% reduction ($p \leq 0,05$) comparing of flowering stage to the initial phenological phase (ear in stem). The ADL concentration elevated by 27-48% during earing and 49-71% after 14 days ($p \leq 0,05$). The NDF rumen degradability decreased with 8-16% during 7 days and 18-32% during 14 days after the optimal harvest time ($p \leq 0,05$). Organic matter digestibility dropped by 9-13% (7 days), and 18-24% (14 days) compared to the early stage ($p \leq 0,05$).

Introduction
Authors aim was to determine the effect of the cultivars and the different phenological stages on the green and dry matter yield, additionally nutrient content, organic matter digestibility (OMd) and NDF rumen degradability of whole crop rye plant by Near-InfraRed Spectroscopy during the first cut period with 7-14 days delay, respectively (April-May 2013, Hungary).

Materials and Methods
Two different varieties (Diament and Protector) and three hybrids (SU Phönix, SU Santini, SU Mephisto) were sowed on 10th October 2012 (130 kg seed/ha). Farm manure (40 ton/ha) was applied on 24th September before sowing. Nitrogen fertilization was carried out in 12th March 2013 (81 kg N/ha). Experimental field was 1024 nm (applied plot size W 4m x L 16m = 64nm per repetition, treatments= 4, n= 4). Sampling was carried out before earing (29th April), in earing (7th May) and in flowering (13th May) by hand (randomized sampling with standard frame: 1 nm, n=4) on Latin square designed experimental plots (determination of the yield n=16, samples for laboratory analyses n=4, respectively). Green- and dry matter yield, crude nutrient content, fiber fractions, organic matter digestibility and NDF degradability were determined.

Samples were dried and grinded according to the Guidelines of Samplinq® system (BLGG AgroXpertus, Wageningen), equivalently with the Hungarian National Standard. Crude nutrient content, fiber fractions, organic matter digestibility and NDF rumen degradability were determined by Near-InfraRed Spectroscopy. The spectroscopic method (dataset developed by the BLGG AgroXpertus, Wageningen, the Netherlands) uses the near-infrared region of the electromagnetic spectrum (range: 1000 - 2500 nm). A NIRS-analysis is an indirect method and has been calibrated on primary classical wet-chemical analysis, as reference methods. List of the reference methods and determination coefficients are available at the corresponding author. Spectra were determined according to the guidelines of NEN-EN-ISO 12099 (Q-Interline Quant FT-NIR analyser, ISO 12099:2010 guidelines for the application of near infrared spectrometry). BLGG AgroXpertus uses local calibration model, as special mathematical model to calculate the desired parameters based on the NIR spectra. This approach increases reliability when the relationship between the NIR spectra and an analysis feature is not linear. BLGG AgroXpertus organizes ring-test (inter-laboratory calibrations), and it validates its satellite labs to maintain reliability.

Applied statistical models were the following: Kolmogorov-Smirnov test, Shapiro-Wilk test, one-way ANOVA (comparison of the cultivars), two-way ANOVA (effect of cultivars *vs.* pheonological stages), correlation and regression analyses between parameters of the different cultivars and phenological stages.

Results and Discussion
Crude protein content decreased with 8-20% during 7 days, while there was 39-42% reduction ($p \leq 0,05$) comparing of flowering stage to the initial phenological phase (ear in stem). The ADL concentration elevated by 27-48% during earing and 49-71% after 14 days compared to the initial stage ($p \leq 0,05$).

Related to the lignification, NDF rumen degradability decreased with 8-16% during 7 days and 18-32% during 14 days after the optimal harvest time (p≤0,05). Based on the cell-wall effect, the organic matter digestibility dropped by 9-13% (7 days), and 18-24% (14 days) compared to the early stage (p≤0,05).

Table 1:Changes in green- and dry matter yield of whole crop rye derived from first cut (between 29th April-13th May 2013 Hungary, n=16)

	29th April 2013 (before earing)		7th May 2013 (in earing)		13th May 2013 (in flowering)			
	Green	DM	Green	DM	Green	DM	Green	DM
	t/ha	t/ha	t/ha	t/ha	t/ha	t/ha	std error	std error
Diament	39.6ab	6.2a	45.5ac	7.5abc	43.4a	9.5a	0.118	3.268
Protector	37.5a	6.0a	42.6b	8.2ac	-		0.153	4.003
SU Phönix	42.2bc	5.2a	44.1ab	7.5ab	42.3a	9.0bc	0.123	3.268
SU Santini	40.1c	5.1a	43.2ab	6.9b	45.4b	9.1b	0.121	3.268
SU Mephisto	39.2bc	5.2a	47.4c	8.1c	43.6a	8.6c	0.120	3.268
Std error (columns)	0.541	0.95	0.531	0.93	0.592	0.104		

abcd Means in the same column with different letters differ (p≤ 0.05)

Table 2: Changes in nutrient content, organic matter digestibility and NDF rumen degradability of whole crop rye derived from first cut (29th Apri l- 13th May 2013 Hungary, n=4)

		DM g/kg	Crude protein g/kg DM	Crude fiber g/kg DM	Sugar g/kg DM	NDF g/kg DM	ADF g/kg DM	ADL g/kg DM	OMd %	NDFd %	dNDF g/kg DM
29th April 2013 (before earing)											
Diament	Mean	157a	213a	283a	103a	619a	285a	31a	76a	62a	381a
	St.dev	19.1	19.3	7.0	16.7	10.5	13.0	2.6	1.0	0.7	3.2
Protector	Mean	158a	191a	291a	110a	634a	302a	34a	74a	61a	384a
	St.dev	8.3	23.9	8.4	15.5	9.6	11.1	1.4	1.1	0.9	5.5
SU Phönix	Mean	124a	208a	283a	102a	618a	290a	33a	75a	62a	385a
	St.dev	2.1	14.5	9.5	4.2	8.7	13.7	2.1	0.9	0.8	6.6
SU Santini	Mean	128a	215a	290a	87a	548a	288a	31a	76a	60a	328a
	St.dev	9.3	10.2	6.9	13.7	165.1	13.1	1.3	1.2	2.1	92.8
SU Mephisto	Mean	131a	199a	284a	109a	613a	293a	30a	76a	64a	394a
	St.dev	12.3	12.0	5.1	16.6	7.9	8.8	2.9	1.0	1.0	9.2
7th May 2013 (in earing)											
Diament	Mean	166a	169b	354b	38b	689b	358b	46b	67b	52b	359b
	St.dev	7.9	12.0	13.3	8.3	9.8	12.0	2.4	0.9	2.1	14.7
Protector	Mean	193b	157b	353b	55b	691b	366b	47b	64b	51b	350b
	St.dev	7.4	12.4	17.1	3.3	14.3	16.1	2.9	1.9	2.7	14.7
SU Phönix	Mean	170b	182a	336b	51b	672b	345a	43b	67b	53b	356b
	St.dev	11.9	20.3	16.0	6.4	19.1	17.8	2.9	1.9	1.3	11.4
SU Santini	Mean	160b	185b	331b	42b	667b	346b	44b	68b	55b	369b
	St.dev	4.6	8.0	12.9	9.0	7.5	9.7	3.5	1.1	1.2	4.8
SU Mephisto	Mean	171b	183a	329b	47b	663b	339b	38b	69b	56b	373b
	St.dev	24.7	15.9	2.4	6.1	14.7	7.8	3.4	0.9	3.4	17.5
13th May 2013 (in flowering)											
Diament	Mean	219b	122c	372b	69ab	683b	390c	53c	62c	48b	328c
	St.dev	3.9	12.0	7.3	2.0	4.9	9.2	1.0	0.6	1.4	7.8
Protector	Mean	219c	114c	371b	85c	686b	383b	51b	61b	46c	312c
	St.dev	61.5	19.4	14.2	10.1	12.9	17.9	1.7	1.4	1.4	9.3
SU Phönix	Mean	214c	128b	356b	77c	680b	377c	50c	62c	49b	334c
	St.dev	13.0	14.2	15.3	13.7	8.4	12.8	3.3	1.5	2.6	16.0
SU Santini	Mean	200c	137c	362b	52	683c	381c	51c	61c	50b	338c
	St.dev	4.3	9.9	7.9	10.4c	3.3	5.7	2.2	1.1	1.1	6.7
SU Mephisto	Mean	197b	116b	410c	42b	703c	415c	51c	57c	44c	308c
	St.dev	4.8	3.2	19.5	12.9	11.0	13.0	2.1	1.8	1.6	6.3

abcd Means in the same column with different letters differ comparing the effect of the phonological stages (p≤ 0.05), OMd organic matter digestibility, DOM digestible organic matter, NDFd NDF rumen degradability, dNDF rumen degradable NDF

Conclusions

It was found that the effect of the three different phenological stages (7 and 14 days delay compared to the optimal harvest time) was significant on green and dry matter yield of the whole crop rye cut in very early stage (April-May 2013). However, the delayed harvest had significant detrimental effect on nutrient content, OMd and NDFd. According to our results, it is strongly recommended to cut the whole crop rye before earing, in order to maintain the nutritive value of the crop. The impact of 'lignification model' was more considerable as compared to the effect of the different cultivars/hybrids on the same parameters on the same stage.

Changes in nutritional parameters of maize when harvested at different times

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Keywords: Maize, hybrids, yields, nutrients

Introduction
Maize is an important crop in Czech Republic. Especially as a feed for cattle. Today maize is utilized for biogas production too. High yields and energy per hectare are the most important reasons for its application as animal food. Maize silage constitutes an important part of the winter feeding programme at numerous agricultural enterprises. Each year different maize hybrids are evaluated at the Institute of Animal Science to determine the nutritional parameters and yields at the individual plant components.

Choice of harvest time and the physiological maturity of the crop at harvest are the most important factors that can influence the quality of crop. Farmers often underestimate the maturity stage of plants during the harvest. Changes in nutrient contents and plant yields of a maize hybrid with a FAO number of 300 were monitored during four harvests in three weeks.

Material and methods
The maize hybrid was grown on the experimental field of the Institute of Animal Science in Prague (sugar beet growing region, 280 m above sea level). Temperature in this area averaged 9.7°C in the last years with an average total precipitation of 601.3 mm. Maize was grown under conventional agricultural practices. Ripped planting lines was 75 cm apart and the distance between seeds in each line was 15 cm. Eight lines each 150 m long were used. Number of plants was counted in June. Dry matter (DM) of maize silage should fall within an optimum range, ideally from 28 % to 33 %. Maize was harvested at four different DM contents a three week-period. In each harvest time 3x10 plants were cutting. Yield of whole plants, cobs and plants without cobs (residual plants) were investigated. Some chemical analyses were determined as quality indicators of maize. Digestibility of organic matter was determined by an in vitro method (48 h by Tilley and Terry 1963, modified by Resch 2004).

Results

		Harvest				
		1	2	3	4	SEM
Whole plant						
DM	%	27.6^{a}	29.8^{b}	34.5^{c}	40.0^{d}	2.15
Cob						
DM	%	43.1^{a}	48.0^{b}	52.2^{c}	54.01^{c}	2.42
Crude protein	g/kg DM	83.25^{a}	93.68^{b}	94.41b^{c}	97.36^{c}	1.75
Crude fiber	g/kg DM	84.90^{a}	78.21^{b}	77.30^{b}	74.46^{c}	1.99
Starch	g/kg DM	456.89^{a}	482.88^{a}	516.52^{b}	575.69^{c}	4.23
Ash	g/kg DM	23.03	21.67^{a}	21.55^{a}	24.45^{b}	0.30
Fat	g/kg DM	57.24^{a}	68.59^{b}	63.27^{b}	68.89^{b}	0.92
Digestibility	%	78.94	80.43	81.52	-	0.32
Yield	t/ha	8.8^{a}	12.1^{b}	13.3^{b}	14.0^{b}	0.49
Residual plant						
DM	%	20.5	22.5	24.4	28.65	1.78
Crude protein	g/kg DM	87.91^{a}	78.07^{b}	69.83^{c}	48.01^{d}	1.82
Crude fiber	g/kg DM	314.03^{a}	332.16^{b}	332.28^{b}	358.53^{c}	3.98
Ash	g/kg DM	66.39	68.26	69.78	69.85	1.22
Fat	g/kg DM	15.47^{a}	18.28^{b}	14.41^{a}	7.68^{c}	0.84
Digestibility	%	60.24	59.71	59.57	-	0.28
Yield	t/ha	9.0	8.1	9.1	9.1	0.23
Cob proportion	%	49.5^{a}	59.7^{b}	59.4^{b}	60.5^{b}	0.25

^{a,b,c}*Values in the same row with the different letters are significantly different (P < 0.05).*

With advancing maturity stages and increasing DM content, contents of crude protein in the residual plant was decreased (P<0.05) and crude fiber increased (P<0.05). This is in accordance to Ettle et al. (2003), who dealt in detail with the effect of maize variety harvested at different maturity stages on feeding value. Values of organic matter digestibility in the residual plant were decreased but these changes were not statistically significant. In parameters, elated to cobs crude fiber was decreased (P<0.05) and crude protein increased (P<0.05). These changes were most evident in maize harvested between 27.6 % and 29.8 % of DM contents. Similarly starch contents increased (P<0.05) when DM contents became higher. Value of organic matter digestibility of cobs were increased but the changes were not statistically significant. Yields were increased only in cobs over the DM range of 27.6 to 40.0 % with no significant changes found in residual plants.

Conclusion

In order to produce high quality maize silage, DM contents of maize have to be monitored. Most of the nutritional parameters depend on the maturity stage of the harvest. This study evaluated the changes of basic nutritional parameters of a maize hybrid at four different DM contents. With quality of the residual plant deteriorating as time proceed harvest time is critical. Most changes occured when the DM contents of whole plants were between 34.5 and 40.0 %.

Acknowledgements Supported by project MZERO0714

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The variability of the results of the nutritional value of maize silage obtained by the apparatus AgriNIR

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Introduction

The aim of one part of our project was to find the possibility of using methods of the near-infrared reflectance spectroscopy (NIRs) to analyse nutritional values of the fresh material (plats or silages) in practice. Near-infrared reflectance spectroscopy (NIRS) is a method of analysing composition of forages in a fast and repeatable way by exposing a sample to near infrared light and recording which wavelengths are absorbed and which are not. All of the major components of maize silage have known absorption rates of near infrared light. By calculating which wavelengths of light are absorbed and which ones are reflected back, a value can be assigned for how much of each component a feed has. AgriNIR™ is a portable NIR analyser for forages and grains that quantifies, in seconds, the percentage of Humidity or Moisture (dry matter), Starch, Crude Protein, Acid detergent fibre (ADF), neutral detergent fibre (NDF), Ash and Crude Fat of the plant material being analysed. The technical data for accuracy are: Humidity or Moisture 2%, all nutrients 3%. The complete analysis can be ready in 60 seconds. The use of screening instruments for detecting AgriNIR nutritional value of the food is even greater interest by laboratories, consultants and representatives of agricultural services (seed companies).

However, there are not many articles (comparative studies) in the scientific literature that would provide information about the variability of the results obtained for comparison with the results of chemical analyses. If results in an article on this topic in the scientific literature were found, obtained results are from NIRs device that handles dry matter, not fresh green matter. Review of the near-infrared spectroscopy has been written by Roggo et al. (2007).

The main goal was to find out how to alter the nutritional value of maize silage stored in a large tube bag.

Material and methods

We tested maize silage stored in a large plastic bag. The first part (the control) of the bag (K) was filled with chop maize in 10 mm long, additives were not used, the second part, experimental (P) was filled with the same chopped material, but into which we applied the additive based on salts of benzoic acid, sorbic acid and sodium nitrite, 3 litres per tonne. The experiment lasted 90 days. On weekdays the samples of silage (K and P) and samples of feed residues from the feeding experiment with 22 bulls (Kzb and Pzb) Holstein breed (black and white), were collected into small bags, weight at the beginning was 212 kg. The samples were stored in a freezer set on minus 18^{o}C till the end of the collecting process. Afterwards they were unfrozen in the room ambient temperature (20^{o}C). The infrared spectra were measured by AgriNIRs equipment of 4 x 62 samples of maize silage. From the same large plastic bags we took samples three times (5 + 2 + 5). We analysed the samples of silage with use of chemical methods (2 x 12 samples), also of control (Kc) and of experimental (Pc) silages. Dry matter (DM), Protein, Starch, ADF, NDF, Ash and Fat was determined in the samples both by AgriNIRs equipment and chemical analyses, the AOAC (2005) methods we used.

Results and discussion

The results are in table 1. Coefficients of variation ranged from 2.5 to 6.7% were higher than in residues in fresh silage. The residues in comparison with fresh silages were found higher solids starch ADF and NDF.

The correlation between the dry matter content and the consumption of silage were low in both the control (0.01) and the experimental (-0.5) silage. Consumption the experimental silage (P) was significantly higher (P> 0.05) than control silage (K = 17.55, P = 17.48 grams per head per day, respectively).

There were significant differences (P<0.05) between the analysis of maize silage AgriNIRS apparatus and laboratory methods. The variance values of the analyzed by apparatus AgriNIRS within a silage was low. Differences between control and experimental silages which have been preserved with the addition of additives was not significant (P>0.05).

Lundström (2013) measured 12 samples of maize silage with AgriNIRs apparatus (A) and 6 samples chemically (R). The content of DM was insignificant (P> 0.05) between A (43 ± 7%) and R (45 ± 7%), similar results were insignificant for the protein (A = 7 ± 0.5%, B = 8 ± 0.4%). Significant differences (P <0.05) were found in NDF (A = 32 ± 3%, B = 51 ± 4%).

Schaaf (2013) compared the results of the measurement repeatability of the results recorded on two apparatus AgriNIR. There were a total of 79 maize silage samples taken from 36 dairies. All samples were tested in the AgriNIR Forage Analyzer first. The same sample was then taken and analyzed by Dairy One Forage Analyzing Laboratory. There was a large difference in the results between the two machines. They had disagreement in their test results and the disagreement varied by component. Several components had a low correlation between the two machines, so the disagreement was not linear. Other components had a high correlation, but they had a large difference in actual values.

According of Zimmer et al. (1990) which measured maize stover, there are positive and significant correlations between LAB and NIRS analyses.

Conclusion

There were significant differences (P<0.05) between the analysis of maize silage AgriNIRS apparatus and laboratory methods, but the variance of values analyzed by apparatus AgriNIRS within the silage was very low. Differences between control and experimental silages which have been preserved with the addition of additives was not significant (P>0.05).

The study showed the NIRS analyses should be useful for maize breeding programs and for evaluating of maize silage for feeding ruminants, nevertheless a large number of samples needs to be evaluated, but it neers more research and calibration of equations.

Table 1: Nutrition values measured by the apparatus AgriNIR and analysed chemically

	n	DM %	Starch % DM	Protein % DM	NDF % DM	Ash % DM	Fat % DM
K	62	32.48a	28,8a	8,07b	41,61b	4,45b	2,94b
P	62	32.01a	29,8a	8,45c	41,61b	4,59b	2,91b
Kz	62	35.40b	31,5b	7,80ab	46,73c	4,10a	2,44a
Pz	62	35.41b	31,4b	8,43c	41,31b	4,18a	2,66a
Ka	12	35.19b	32.23b	7.39a	38.40a	4.43b	3.14c
Pa	12	36.01b	29.91a	7.34a	38.19a	4.20a	3.14c

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Dedication: QI91A240 a MZERO0714

OMD determination by ELOS (enzymatic soluble organic matter) method in grasses

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Abstract
The paper is aimed at possibility to specify prediction of NEL and NEV concentrations using OMD determined by the laboratory method ELOS (enzymatic soluble organic matter) in forage with the use of a very cheap domestic preparation from Trichoderma reesei with defined main enzyme activities, convenient for routine practice. This should improve current situation when determination coefficients are mainly taken from feeding tables. The accuracy of OMD prediction in vitro by ELOS method provides in the range of values common for grasses at technical maturity (grazing, conservation), that is 60 – 80, results practically identical (±2.43 units of OMD) to the method of Tilley and Terry (1963). The difference is caused by 'inaccuracies' of both methods.
Keywords: grasses; OMD; forage quality; Dactylis glomerata; Festuca arundinacea; Festulolium; Festuca pratensis; Lolium perenne; Bromus inermis.

Introduction
The recent increase of individual milk production of dairy cattle in the Czech Republic (over 7000 kg FCM) lays higher demands on voluminous fodder quality. The quality of forage changes a lot during the first harvest regrowth. Individual species within forage mixture of grasses and legumes demonstrated significant differences in earliness (1 – 3 weeks), and even higher differences in forage quality (representing about 3 – 4 weeks) - Pozdíšek *et al.* (2002). In order to determine an optimum date for harvest for each individual species, sequential samplings were analysed during the first harvest regrowth.

Materials and methods
A stationary trial was established in 2010 at the Jevíčko and Hladké Životice site by a method of quick renovation with seven grass species (*Dactylis glomerata* L. ´Vega´ (DG), *Festuca arundinacea* L. ´Kora´ (FA), Festulolium ´Hykor´ (Fl-Hy) and ´Hostyn´ (Fl-Ho), *Festuca pratensis* L. ´Kolumbus´ (FP), *Lolium perenne* L. ´Kentaur´ (4n) (LP) and *Bromus inermis* Leysser cv. ´Tabrom´ (BI). Species were sown in a randomised block design in pure-stand and four replications. The trial was fertilized in 2011 and 2012 with N 120 kg ha^{-1} as ammonium nitrate, with lime applied in two doses per 60 kg ha^{-1} (in spring, after the first cut), 35 kg ha^{-1} P (superphosphate) and 100 kg ha^{-1} K (potassium salt). Seven sampling dates were performed during the 1^{st} growth, with the first cut on 28^{th} April 2011 and the next sampling in weekly intervals until mid-June. OMD of 196 samples was determined by (a) ELOS method, using domestic cellulase preparation from *Trichoderma reesei* (Míka *et al*. 2009) on instrument Ankom DaisyII, with original packets F57 and (b) reference method of Tilley and Terry (1963), modified by Štýbnarová – Pozdíšek (2009). Characteristics were statistically evaluated using method of linear regression; correlation coefficients were calculated and critical values r_α (n=196) of correlation coefficient for $\alpha_{0.05}$ and $\alpha_{0.01}$ evaluated.

Results and discussion
The acquired results are presented in Fig. 1. In two years' time and from two sites 196 samples of grass forage were collected and analysed with an overall range of OMD *in vitro* by Tilley and Terry 53.22 – 85.80, respectively by ELOS method 51.40 – 88.16. There is regularly a close relation between the values from both methods while samples by ELOS method with lower OMD in three out of four collections were undervalued whereas the samples with higher OMD were overvalued in all four collections. If we consider the whole range of values (samples from the earliest growth stages with the highest OMD values up to late stages with the lowest OMD), the total average within the assessed collection (n=196) is 71.48 by Tilley and Terry method and 71.38 by ELOS method.

The intersection of the calculated slope of line for the given collection (n=196) with a diagonal (at an angle 45°) is at the value of 70.73 OMD (Fig. 1); in this area both methods provide without additional correction identical numerical results with tolerance ±2.43 unots of OMD within the range from 60.73 – 80.73. It must be added that within this OMD range are most samples collected at the stage of optimum technological maturity. The equation of line $y = 1.2427\ x – 17.384$ displays high value of determination coefficient (0.7915), which explains 79.15 % cases. Correlation coefficient r = 0.89** is statistically highly significant (P0.01).

The accuracy of OMD determination *in vitro* by ELOS method provides in the range of values common for grasses at technical maturity (grazing, conservation), that is 60 – 80, results practically identical (±2.43 units of OMD) to the method of Tilley and Terry (1963). In this respect the ELOS method is acceptable and according to the correlation coefficient r provides useful information about factual OMD. Our last paper (Míka et al., 2009) already showed remarkable coincidence of individual parallel replications (n = 3). With error tolerance of 1.5 % (relatively) the minimum number of replications was 1.7 for the ELOS method, whereas it was 2.5 replications for nowadays 'classic' method *in vitro* with rumen fluid by Tilley and Terry (1963).

Conclusion

The ELOS method uses a very cheap domestic cellulose preparation with balanced cellulotic and other activities for hydrolysis of lygnocellulosic materials. The accuracy of OMD prediction *in vitro* by ELOS method provides in the range of values common for grasses at technical maturity (grazing, conservation), that is 60 – 80, results practically identical (±2.43 units of OMD) to the method of Tilley and Terry (1963). The difference is caused by 'inaccuracies' of both methods.

The ELOS method can be favourably used for accurate determination of the optimum date of harvest of grasslands with respect to anticipated livestock yield and also for more accurate calculation of NEL and NEV concentrations using actually determined OMD values in comparison to digestibility values taken from feeding tables.

Acknowledgements

The contribution was made as a part of the research of the NAZV project with registration number QI101C199 and MZE00027006.

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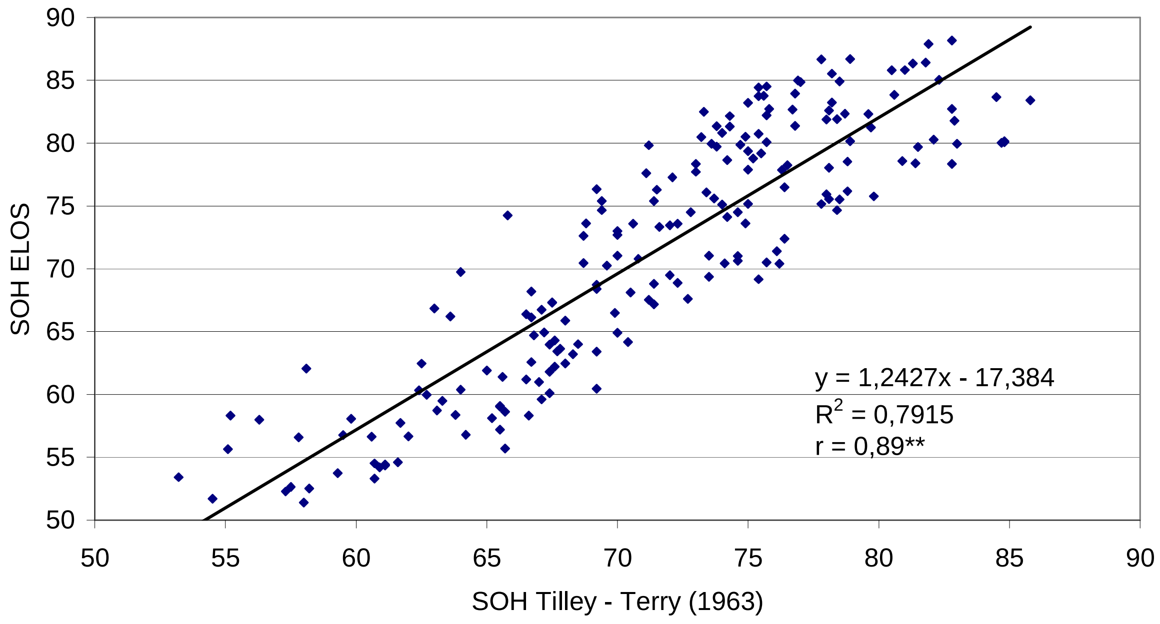
Figure 1: OMD determined by ELOS method vs. Tilley – Terry (1963), n = 196



The effect of feeding mycotoxin-contaminated silage on milk performance and parameters of rumen milieu of dairy cows.

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Abstract
The aim of the study was to reveal the effect of feeding the maize silage contaminated with mycotoxins deoxynivalenol (DON), nivalenol (NIV) and zearalenone (ZEA) on milk performance and rumen characteristics of dairy cows. The trial was carried out on 3 ruminally cannulated lactating dairy cows and was divided into 2 periods of 7 d (4 d preliminary period and a 3 d experimental period). In the first period, two cows received the control diet (C) based on control maize silage, lucerne hay and a supplemental mixture, and one cow received the experimental diet (M), in which part of control silage was replaced with mycotoxin contaminated silage. In the subsequent period, the cows were switched to the other treatment. Dry matter intake was not affected (P>0.05). Intake of DON and NIV was higher in M (9941 and 2714 mg/d) than in C (2965 and 2253 mg/d, respectively, P<0.01). Milk yield and protein content differed significantly between groups (P<0.05). Mean concentration of metabolite DOM-1 in the rumen fluid in M was higher (15.1 ng/ml) than in C (1.6 ng/ml, P<0.01).

Introduction
Silage of whole plant maize represents a high-energy feed component of dairy cow rations. Under central European cultivation conditions maize is often infested by different moulds of the genus *Fusarium*, whereby *F. graminearum* and *F. verticillioides* are the most important (Miedaner et al., 2010) contaminating silage especially by deoxynivalenol (DON), but also zearalenone (ZON) and fumonisins (FUM, Logrieco et al., 2002). Mould contamination in silage is associated with reduced palatability, reduced nutritional value and feed intake, animal health problems, decreased productivity and fertility and increased disease susceptibility (Fink-Gremmels, 2008; Scudamore and Livesey, 1998).

The aim of the study was to reveal the effect of feeding the maize silage contaminated with mycotoxins deoxynivalenol (DON), nivalenol (NIV) and zearalenone (ZEA) on milk performance and rumen characteristics of dairy cows.

Material and Methods
The experiment was carried out on 3 ruminally cannulated lactating Holstein cows with a similar milk production of 18.9 ± 1.1 kg/d. The experiment was carried out in the form of a cross-over design and was divided into 2 periods of 7 days (a 4-d preliminary period and a 3-d experimental period). Cows were divided into 2 groups. In the first period, two cows received the control diet (C) based on control maize silage, lucerne hay and a supplemental mixture, and one cow received the experimental diet (M), in which part of control silage was replaced with mycotoxin contaminated silage. In the subsequent period, the cows were switched to the other treatment. Cows were fed individually twice daily (6.30 and 16.30 h) *ad libitum* the above mentioned diets (Table 1).

Table 1: Composition of diets g/kg (on DM basis)

	C	M
Maize silage – control	485	263
Maize silage – contaminated	-	233
Lucerne hay	96	95
Supplemental mixture	419	418
Total	1000	1000

Feed intake and respective refusals were monitored daily and samples of them were taken in each period and analysed for the basic nutrients (according to AOAC, 1984) and for the mycotoxins. Cows were milked twice a day (7.00 and 17.00 h). Milk yield was recorded at each milking. During the experimental period, samples of milk were taken at each milking; they were conserved by Bronopol (D&F Control Systems, Inc. USA), cooled to the 6 °C and analysed by infrared analyser (Bentley Instruments 2000, Bentley Instruments Inc., USA) on basic milk constituents.

In each experimental period five samples of ruminal fluid were taken daily at 6:30, 8:30, 10:30, 13:30 and 16:30 h to determine pH, and the content of volatile fatty acids, ammonia and mycotoxins and their metabolites.

Mycotoxins and their metabolites were determined using ultra performance liquid chromatograph ACQUITY (Waters) coupled with tandem mass spectrometer QTRAP MS/MS (AB Sciex).

Results and discussion

Intake of DM and mycotoxins is given in Table 2. Although DM intake did not differ significantly between groups (P>0.05), refusals were noted in M. This is in accordance with Coppock et al. (1990) or Trenholm et al. (1985) but in contrast to other studies, e.g. Dänicke et al. (2005) or Korosteleva et al. (2007) where no feed refusals were observed. Intake of DON and NIV was higher in M than in C (P<0.01), while intake of ZON was not affected by the treatment (P>0.05).

Table 2: Intake of dry matter (DM) and mycotoxins

Intake of	Unit	C	M	SEM	P
dry matter	kg	16.1	15.0	0.61	0.249
DON	µg/d	2964.7	9940.8	341.90	<0.001
NIV	µg/d	2253.0	2714.4	114.30	0.014
ZON	µg/d	948.5	871.1	41.10	0.208

Table 3: Yield and composition of milk

	Unit	C	M	SEM	P
Milk yield	kg/d	17.8	19.9	0.43	0.006
Fat	g/kg	43.8	45.7	1.39	0.342
Protein	g/kg	34.2	31.9	0.30	<0.001
Casein	g/kg	26.5	24.9	0.47	0.033
Laktose	g/kg	46.4	46.9	1.07	0.743
Urea	mg/100 ml	15.1	14.4	1.72	0.751

Yield and composition of milk is presented in Table 3. Milk yield in M was higher than in C (P<0.01). While concentration of protein and casein in M was lower than in C (P<0.05), content of other milk compounds was not affected by the treatment (P>0.05). Results concerning the DON and ZON effect on milk yield and composition are inconsistent. Although some negative effects were observed with regard to milk yield and composition (Charmley et al., 1993; Coppock et al., 1990; Whitlow et al., 1986; Trenholm et al., 1985) no effect of mycotoxins was observed in the other studies (Winkler et al., 2014; Korosteleva et al., 2007; Seeling et al., 2006; Dänicke et al., 2005).

The average ruminal pH in C and M was 6.42 and 6.53, respectively. Total VFA concentrations were on average 2.55 ml/l in C and 3.72 ml/l in S. Mean concentration of metabolite DOM-1 in the rumen fluid in M was higher (15.1 ng/ml) than in C (1.6 ng/ml, P<0.01). Changes in postprandial concentration of DOM-1 in the rumen fluid are presented in the Figure 1. The highest concentration of DOM-1 was observed 4 h after feeding.

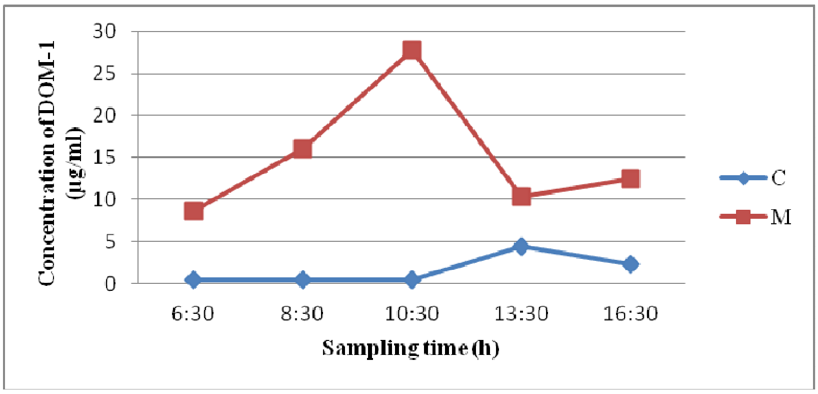

Figure 1: Concentration of DOM-1 in rumen fluid of cows fed either control (C) or mycotoxin contaminated silage (M) in dependence on time of sampling

Conclusion

After feeding maize silage contaminated with mycotoxins DON, NIV a ZON to lactating dairy cows feed intake and composition of milk was negatively affected. No mycotoxins except of metabolite DOM-1 was found in the rumen fluid. The highest concentration of DOM-1 in the rumen was determined 4 h after feeding.

Acknowledgement

This study was supported by the Ministry of Agriculture of the Czech Republic, project No. QI111B044 and by the institutional support by decision RO0313 from February 28th 2013.

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Determination of protein digestible in intestine in grass silage by the NIRS method

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The aim of this work was to evaluate the measured spectra of samples of grass silage by the dispersive NIR spectrometer 6500 with NIRS reflectance detection. The samples were evaluated in the wavelength range 400 - 2500 nm. The file comprised 89 samples of grass silage. Samples were analysed by reference methods in accordance with the Decree of the Ministry of Agriculture No. 2145/2004-100 and protein digestible in intestine (PDI) was calculated. Correlation between results of reference methods and measured spectra was used to develop an equation "SGR.EQA". Chemometric methods of the NIRS software were used to validate the calibration equation. According to reference methods, PDI minimum value, PDI maximum value, MEAN, SEC, SECV and RSQ were 47.78 g.kg-1, 81.55 g.kg-1, 68.17 g.kg -1, 4.78 g.kg-1, 7.48 g.kg-1 and 0.85, respectively. Mahalanobis distance was used to determine outliers. After outlier selection, 61 samples were used for development of new equation SGR.EQA. According to new equation SGR.EQA, PDI minimum value, PDI maximum value, MEAN, SEC, SECV and RSQ was 57.51 g.kg-1, 81.55 g.kg-1, 67.55 g.kg -1, 0.93 g.kg-1, 2.12 g.kg-1 and 0.92, respectively. Parameters of new equation SGR.EQA were optimal and correlation coefficient RSQ showed a strong dependence. Therefore calibration could be identified as very reliable.

Introduction

Animal nutrition is one of the most important factors and influences animal health and performance. Feed-stuffs fulfil animal requirements for nutrients and also enter the food chain. For this reason, the feed-stuffs have to be classified. It is necessary to determine the best feeding ration with considering the animal health and performance by using such diagnostic methods which allow to provide information concerning forage quality in a short term. Near infrared spectroscopy (NIRS) eliminates time consuming sample preparation, reduces costs of chemicals, energy, laboratory equipment and protects the environment, as well.

Material and methods

Grass silage samples were taken from mountain and submontane regions of Slovakia. The silage samples were assembled from agricultural firms directly and were expedited to laboratories for analysis. Samples were prepared and analysed in agreement with valid legislation of the Slovak Republic according to the Decree of the Ministry of Agriculture No. 2145/2004-100 on legal sample assembling and on laboratory feed-stuffs analysis and evaluation. The content of Dry Mater (Directive No.1971/393/EC), total N (Kjeldahl, Directive No. 1993/28/EC), fat (Soxhlet No. 1998/64/ES)**,** and products of the fermentation process in silage samples, carboxylic acid and alcohol (according to the Decree of the Ministry of Agriculture No. 2145/2004-100 part L). Following the analysis the samples that did not comply with the analytical tolerance for laboratory results of nutrient contents were selected (as defined by "Analytical tolerance" - Decree No.39/1/2002-100 issued by the Ministry of Agriculture of the Slovak Republic). The content PDI (protein digestible in intestine) was calculated by Petrikovič et al. (2000). We carried out diagnostics, sc. control of the applience's parametres: 1. Measure NIR repeatability (NIR repeatability is a measure of the deviations in optical (Log/R) data at each wavelength), 2. Measure wavelength accuracy (It is measured with polystyrene and didymium inside the instrument), 3. Measure instrument response (It is a measure of the absolute reflectance from the ceramic) .We scanned the check cell and then the silage samples on the NIRS 6500 device. Prior to the scanning, the silage samples had been adjusted by drying at 60 °C and disintegrated to the 1 mm fraction. Following the scanning – measuring of the silage samples adjusted in this manner on the NIRS 6500 we acquired absorption spectra composed of 89 samples.

Results and Discussion

The objective of this study was to develop calibrate equation SGR.EQA. The software of NIRS 6500 performs different mathematical procedures: PCR, PLS, MPLS. The linear regression method was based on modified partial least squares (MPLS). The first file contained 89 samples (Tab.1.). From the total of 89 samples after selection of outliers, outliers were determined by Mahalanobis distance a set of 61 for PDI. The most commonly used distance measures are the Euclidean distance (ED) and the Mahalanobis distance. Both distances can be calculated in the original variable space and in the principal component (PC) space (De Maesschalck et al., 2000). Obtained spectra were matched to calculated values for and used to develop a calibration file SGR.EQA. To develop the best calibration equation (Tab. 2.), mathematical matrix of linear dependence was controlled using statistical methods. Partial least squares (PLS) regression is similar to principal component regression (PCR), but uses both reference data (chemical, physical, etc.) and spectral information to form the factors useful for fitting purposes (Martens, H., Naes, T. 2001). MPLS is often more stable and accurate than the standard been calculated, are standardised (dividing by the standard deviations of the residuals at each wavelength) before calculating the next factor. When developing MPLS equations, cross-validation is recommended in order to select the optimal number of factors and avoid overfitting (Shenk, J.S., Westerhaus, M.O. 1995).

In order to verify the research we have used a validating set VSGR.CAL of 20 randomly selected silage samples which had been used for the laboratory analysis. The set of the samples was scanned on the NIRS 6500 and was evaluated by the use of SGR.EQA equation. We compared the calculated results PDI marked as LAB and results ANL acquired following the scanning and evaluating of samples on NIRS 6500. We evaluated the parametres (Tab 3): standard error of prediction (SEP), standard error of cross validation (SECV), and proportion of reference method variation explained by cross validation with predicted values (1-VR), coefficient of determination of cross validation (RSQ). Davies and Williams 1996 it has been reported that the SECV is the best single estimate of the prediction capability of the equation, and that this statistic is similar to the average standard error of prediction (SEP) from 10 randomly chosen prediction sets. Since near infrared spectroscopy (NIRS) was first developed, thousands of papers have demonstrated the ability of this technology to predict traditional chemical values and other parameters of nutritional interest for different feeds and forages (Bertrand, D. et. al, 2000). Forage quality is affected by many factors, including the characteristics of the environments (soil, temperature, precipitation, light conditions), grassland management (fertilization, cutting, grazing) and preservation techniques (Juráček et al., 2001; Gallo et al., 2006; Bíro et al., 2002, Gálik, 2007). The use of NIRS in evaluation of quality of agricultural production reported Dardenne *et al.* (2006) and Kalinin et al. (2008).

Conclusion

It has been demonstrated throughout the study that near-infrared spectroscopy provides fast and accurate information about feed nutritive value. Based on this it is possible to correct and balance the feeding ration and to avoid overfeeding as well as insufficient nutrition. In our study the grass silage samples were used to develop a calibration file SGR.EQA. This new model provides a possibility to evaluate grass silage samples brought to the laboratory by a client. Near infrared spectroscopy allows reduction of costs by avoiding traditional analysis and protects the environment. From this point of view, near infrared spectroscopy is a fast, effective and perspective method.

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Table 1: Calibration file SGR.CAL

Variable	N	Min. value	Max. value	Mean	SEC	SECV	RSQ
PDI ($g.kg^{-1}$)	89	47.78	81.55	68.17	4.78	7.48	0.85

Table 2: SGR.EQA

Variable	N	Min. value	Max. value	Mean	SEC	SECV	RSQ
PDI ($g.kg^{-1}$)	61	57.51	81.55	67.65	0.93	2.12	0.92

Table 3: Validation file VSGR. CAL

Variable	N	Mean (ANL NIRS)	SEP	SECV	1-VR	RSQ
PDI ($g.kg^{-1}$)	20	65.67	1.93	2.62	0.88	0.84

Effects of a homofermentative inoculant on dry matter losses, fermentation pattern, aerobic stability, protein quality and biogenic amines in grass silage

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Introduction

Grass silage plays an important role in the feeding of dairy cows and can significantly contribute to meeting the dietary demand for protein, thereby enabling the partial replacement of expensive protein concentrates. However, fermentation and protein quality often do not meet the required standards, and biogenic amines as end-products of amino acid degradation by undesired microorganisms may impair feed intake and health of animals (Krizsan und Randby, 2007 and cited references there). The benefits of homofermentative lactic acid bacteria products (inoculants) were summarized by Kung *et al*. (2003). However, comprehensive information on their effects on protein quality and formation on biogenic amines in particular is still lacking. Therefore, this trial aimed at gaining more knowledge about it.

Materials and Methods

Smooth meadow grass (*Poa pratense*) was harvested May 14, 2012 with a plot harvester, slightly wilted and chopped to a theoretical particle size of 20 mm. The crop contained 220 g/kg dry matter (DM). Concentrations (g/kg DM) of crude protein, crude ash, water-soluble carbohydrates (WSC) and nitrate were 154, 93, 99 and 0, respectively. Buffering capacity (BC) was 4.9 g lactic acid/100 g DM, giving a SC/BC ratio of 2.0 and a fermentability coefficient (FC) of 39. Thus, the material, having an epiphytic lactic acid bacteria (LAB) count of log 6.5 cfu/g, was considered moderately-difficult-to-ensile. Prior to filling the material into glass jars (1.5 L), the homofermentative inoculant KOFASIL® LAC (ADDCON EUROPE GmbH, Bitterfeld-Wolfen, containing *L. plantarum* DSM 3676, 1k20731 and *L. plantarum* DSM 3677, 1k20732, at 50/50 ratio, inoculation rate: 100,000 LAB/g fresh forage) was applied at 10 l/kg forage, whereas the control treatment received tap water at 10 ml/kg. The three replicate silos per treatment were stored at 25 °C for 121 days. The losses of DM, which was corrected for the loss of volatiles during drying, and the fermentation pattern were analyzed by routine analytical procedures. Aerobic stability (ASTA) was determined for 10 days by employing the temperature method, and aerobic DM losses were calculated based on the accumulated temperature (Honig, 1990). Protein quality was evaluated by the Cornell Feed Protein Evaluation Scheme, and biogenic amines be detected by GC-MS-S (Richardt *et al*., 2011). Data were subjected to statistical analysis by PROC GLM of SAS, version 9.3.

Results and Discussion

Data given in table 1 demonstrate the positive effect of inoculation with KOFASIL LAC on many of the tested parameters resulting in superior quality to that of untreated silages. Despite good fermentability and high epiphytic LAB count of the forage was untreated silage subjected to undesired fermentations, as reflected by high butyric acid. This is likely to have been caused by the lack of nitrate in the fresh crop and too less efficient epiphytic LAB resulting in a slow acidification rate. This, in turn, enabled saccharolytic clostridia to form butyric acid in the early stages of fermentation only (Weissbach und Honig, 1996) since the pH after 121 days of anaerobic storage was still on an acceptable level.

By stimulating homolactic fermentation, treatment with KOFASIL LAC decreased anaerobic DM losses by 32 %, but simultaneously reduced aerobic stability and increased associated aerobic DM losses. This is in line with earlier observations by Honig and Thaysen (2002) who evaluated the effects of homofermentative inoculants based on a total of 309 comparisons with untreated silages made from moderately-difficult- and easy-to-ensile forages. They found a mean decrease in DM losses by 31 and 9%, respectively, and aerobic stability was reduced by 1 day.

Additive treatment positively affected protein quality characteristics by increasing the concentrations of true protein, fractions B1 and B2 as well as by lowering the NPN content (fraction A), which supports findings by Kramer *et al.* (2012). Nadeau *et al*. (2012) who used a different homofermentative inoculant and a chemical additive in their study also observed an increase in rumen undegradable protein, which was shown in this study for KOFASIL LAC.

The use of the inoculant decreased the total biogenic amine concentration as well as that of most individually detected amines. The observed amine levels are in line with those found by Richardt *et al.*

(2011) and Krizsan and Randby (2007), who reported mean values of 2.86 g/kg DM (n=99) and 4.82 g/kg DM (n=24) ranging from 0-20.7 g/kg DM and 0.97-13.8 g/kg DM, respectively. As found in this trial, putrescine and tyramine were shown to occur at the highest concentrations. High correlations were found between the contents of biogenic amines and butyric acid and ammonia by Richardt *et al*. (2011) and Krizsan and Randby (2007). These findings are confirmed for butyric acid whose formation was completely prevented by the additive, but the effect on ammonia-N level was less pronounced. It was shown previously that the activity of saccharolytic clostridia in the early stages of fermentation is associated with only limited protein breakdown (Kaiser *et al*., 1997) compared with the activity of proteolytic butyric acid bacteria. The strong relationship between fermentation quality and biogenic amine concentration, as reported by Richardt *et al*. (2011), was substantiated by the results presented in this study.

Table 1: Effects of KOFASIL LAC on DM losses, fermentation pattern, aerobic stability, protein quality and biogenic amines

Parameter	Control[1]	KOFASIL LAC[1]	SEM	*P value*
Fermentation pattern				
pH	4.3	3.9	0.003	*<0.001*
Ammonia-N (% total N)	15.5	12.2	0.127	*<0.001*
Lactic acid (g/kg DM)	57.0	82.7	3.085	*<0.01*
Acetic acid (g/kg DM)	1.7	8.5	0.690	*<0.01*
Butyric acid (g/kg DM)	26.1	0	0.520	*<0.001*
Ethanol (g/kg DM)	13.4	3.9	0.471	*<0.001*
DM losses during fermentation (%)	7.1	4.8	0.024	*<0.001*
Aerobic stability (days)	10.0	7.3	0.177	*<0.001*
Aerobic DM losses (%)	0	7.9	0.822	*<0.01*
Protein quality				
True protein (g/kg DM)	35.4	42.9	0.781	*<0.001*
Fraction A (% CP)	76.0	70.5	0.504	*<0.01*
Fraction B1 (% CP)	1.1	4.0	0.656	*<0.05*
Fraction B2 (% CP)	16.6	19.7	0.187	*<0.001*
Fraction B3 (% CP)	3.9	3.1	0.232	*ns*
Fraction C (% CP)	2.4	2.6	0.167	*ns*
UDP5[2] (% CP)	13.2	14.3	0.247	*<0.05*
Biogenic amines (g/kg DM)				
Putrescine	1.7	0	0.158	*0.01*
Cadaverine	0.8	0	0.297	*ns*
Histamine	0	0	-	-
Phenylethylamine	0.3	0.3	0.029	*ns*
Tryptamine	0.8	0	0.031	*<0.001*
Tyramine	2.0	0.6	0.134	*<0.01*
Total	5.6	0.9	0.571	*<0.01*
γ-amino butyric acid (GABA, g/kg DM)	10.4	7.7	0.369	*<0.01*

[1]values given as LSMEANS, [2]undegradable protein at passage rate of 5% h^{-1}, ns not significant

Conclusions

Homofermentative inoculants exerted a positive effect on the course of fermentation and associated DM losses even in forages which were not expected to be poorly fermented. As inoculation resulted in less stable silages upon exposure to air, higher feed-out rates are required to avoid excessive losses. By enhanced protein quality, treated silages may partially replace supplemented protein concentrates, thereby contributing to a more profitable milk production. The reduction of biogenic amine concentrations is likely to have beneficial effects on feed intake and animal health.

Acknowledgements

The authors are deeply indebted to Kirsten Weiß, Humboldt University Berlin, Germany and Wolfram Richardt, LKS mbH, Lichtenwalde, Germany for analyzing fermentation pattern as well as protein quality and biogenic amines, respectively. The help of Elisabet Nadeau, SLU Skara, Sweden, with running statistics is greatly appreciated.

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FARM-SCALE COMPARISONS OF OXYGEN BARRIER FILM WITH STANDARD POLYETHYLENE FILM AS TOP SURFACE COVERINGS FOR ENSILED GRASS CROPS

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Abstract
There is little data on the effect of oxygen barrier (OB) film on losses from the peripheral areas of bunker silos filled with grass crops. Primary growth ryegrass was ensiled in two farm-scale bunkers divided longitudinally and covered either with OB film (45 μm thickness) or with two layers of PE film (250 μm total thickness). Net bags were sampled, buried in the upper 30 cm of each silo, recovered after 235 days (Silo 1) or 223 days (Silo 2) and sampled again for analysis. Mean estimated loss of organic matter (OM) from the upper 30 cm was 188 g kg^{-1} for silage stored under OB film and 268 g kg^{-1} for silage stored under PE film (P=0.02). Silage pH, ammonia N and ash tended to be lower and lactic acid tended to be higher for silage under OB than under PE film. It is concluded that loss of OM from the upper layers of ensiled ryegrass in farm-scale bunker silos is reduced by the use of OB film, and that silage quality may also be improved.

Introduction
Losses of crop dry matter (DM) from the peripheral layers of silos can be considerable if the covering film is either absent or is permeable to oxygen (Bolsen et al., 1993). Oxygen barrier (OB) film ('Silostop', B Rimini Ltd, London, UK) has substantially reduced permeability to oxygen compared to standard polyethylene film (Degano, 1999; Borreani and Tabacco, 2012). In a meta-analysis Wilkinson and Fenlon (2013) found that mean losses of DM or organic matter (OM) from the top surface layers of bunker and clamp silos were reduced from 195 g kg^{-1} for standard film to 114 g kg^{-1} for OB film (P<0.001). However, 32 of the 41 comparisons were with whole-crop maize (*Zea mays*), and only 3 comparisons were with grass or grass/clover. In this paper previously unpublished data was analysed from farm-scale comparisons of OB with standard film to test the hypothesis that the findings of the meta-analysis were valid for grass crops ensiled under farm conditions.

Material and methods
Two comparisons were undertaken on dairy farms in the UK. Primary growth grass crops (predominantly *Lolium perenne*) were wilted for 2 to 3 days in May 2003 and ensiled in good weather after treatment with an inoculant additive. Bunker silos were divided longitudinally into two halves. One half of the top layer of each silo was covered with either two layers of black polyethylene (PE) film of 125μm thickness (250μm total thickness) or a single layer of oxygen barrier (OB) film ('Silostop') of 45μm thickness. Corresponding side walls were lined with single layers of the same type of film used on the top surfaces. On the top surface of Silo 1, PE film was protected by used car tyres and OB film by woven polypropylene netting with gravel bags around the edges. On Silo 2 both films were protected by polypropylene netting with gravel bags around the edges. Net bags, 6 per treatment containing approx. 7 kg of herbage fresh weight, were placed at random in the upper surfaces of each silo to 30 cm depth. Losses of organic matter (OM) were estimated using the equation of Bolsen (1997) from the concentration of ash in samples of herbage taken from the net bags at the time of ensiling and in samples of silage taken from the net bags when recovered after 235 (Silo1) and 223 days storage (Silo 2). Statistical analysis was performed using Genstat 16 (Lawes Agricultural Trust, Rothamsted, UK). Data were examined using a linear mixed model with type of film as a fixed effect and silo as a random effect.

Results and discussion
Composition and estimated losses of OM from the upper 30 cm of the silos are in Table 1. Values for pH, ammonia N and ash tended to be lower and concentrations of lactic acid tended to be higher in silage stored under OB film than in silage stored under PE film but the differences were not significant. There were no significant interactions between silo and type of film. The percentage reductions in loss of OM due to use of OB film were similar for both silos at 32% for Silo 1 and 28% for Silo 2. The mean reduction in loss of OM by use of OB film was 80.3 g kg^{-1} (P=0.02).

Table 1: Composition of herbage at harvest and of silage and estimated losses of OM from the top 30 cm of the silos during storage.

	Silo 1		**Silo 2**		s.e.d.	Significance of effects		
Type of film	OB	PE	OB	PE		Silo	Film	Silo x Film
Herbage at harvest								
Dry matter (DM, g kg^{-1} fresh weight, FW)	289.3	292.5	210.3	207.7	10.54	<0.001	NS	NS
Ash (g kg^{-1} DM)	76.7	73.8	83.0	82.2	2.88	0.001	NS	NS
Silage								
DM (g kg^{-1} FW)	279.0	280.0	222.0	202.5	14.35	<0.001	NS	NS
Ash (g kg^{-1} DM)	89.7	94.3	104.0	115.0	6.66	<0.001	NS	NS
pH	4.07	4.32	4.27	4.53	0.282	NS	NS	NS
Ammonia-N (g kg^{-1} total N)	30.0	32.2	66.7	87.3	14.53	<0.001	NS	NS
Lactic acid (g kg^{-1} DM)	130.0	64.2	78.7	57.2	35.29	NS	NS	NS
Estimated loss of OM (g kg^{-1})	157.0	231.8	219.0	304.8	42.79	0.03	0.02	NS

Conclusions

The mean percentage reduction in top surface loss of OM from ensiled grass crops for OB compared to PE film of 30% was similar to the mean reduction in loss of 42% reported for 41 comparisons of OB with PE film by Wilkinson and Fenlon (2013). The results reported here demonstrate under commercial ensiling conditions the superiority of a single layer of OB film over a double layer of standard PE film in terms of lower losses and potentially better fermentation quality of grass silage in the upper layer of the silo.

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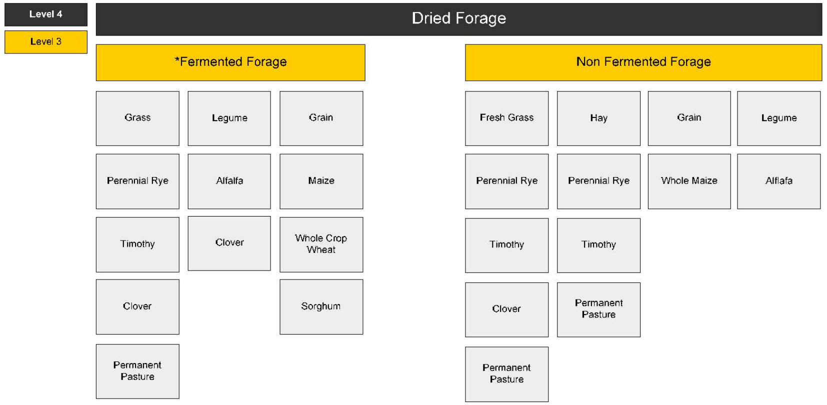

Available Calibrations: Maize

Parameter	Min%	Max%	Correlation
Moisture	3.00	7.33	0.8400
Protein	6.00	8.00	0.9700
Crude Fibre	13.50	22.00	0.9500
Ash	1.50	5.33	0.8600
Starch	6.81	38.67	0.9700
NDF	36.00	41.33	0.9400
ADF	15.00	24.67	0.9500
ADL	0.35	3.33	0.7900
DigDM ◊	56.67	76.50	0.8300
DigOM ◊	58.00	72.00	0.8400
Sugar (Total Reducing)	4.20	12.67	0.9300
Sugar (Total Soluble)	5.60	16.00	0.9800

Available Calibrations: Alfalfa

Parameter	Min%	Max%	Correlation
Moisture	1.20	12.60	0.9300
Fat Ether Extract	0.90	22.47	0.9800
Fat Ether Extract + Acid Hydrolysis	3.45	21.93	0.9900
Protein	10.20	41.73	0.9900
Crude Fibre	0.15	19.47	0.9500
Ash	1.20	25.00	0.8900
Starch	2.85	42.47	0.9800
Sugar	0.75	24.00	0.9700
NCGD	61.20	66.00	0.9700
NDF	0.45	45.93	0.9700

Available Calibrations: Grass

Parameter	Min%	Max%	Correlation
Moisture	1.50	8.67	0.8500
Protein	3.65	20.67	0.9700
Crude Fibre	12.00	27.33	0.9600
Ash	4.00	12.00	0.8700
NDF	26.00	46.67	0.9400
ADF	13.00	28.67	0.9400
ADL	0.50	6.00	0.9200
DigOM ◊	37.00	53.33	0.9400
DigDM ◊	40.00	54.67	0.9400
Sugar (Total Soluble)	0.68	25.33	0.9700

* Clamp, Big Bale and Tower are the methods of ensiling used fermentation on the Forage

◊ denotes calibration parameter does not use % units, please contact Aunir for further details

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