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Mycotoxins and forage crops
Problems of the occurrence of mycotoxins in animal feeds

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In recent years there has been a growing concern of professionals and wider public about the origin and quality of food products. For this reason high-quality animal feed has become a matter of great importance. Now, we are facing problems of recurrent incidence of mycotoxins in all types of feeds. Mycotoxins are secondary metabolites of fungal pathogens with different levels of toxicity to warm-blooded animals (including humans), which may cause health problems and consequently economic losses in farm animal breeding.

At present nearly 400 mycotoxins produced by a wide spectrum of fungal pathogens are described but the occurrence of only the most frequent and harmful ones is monitored on a regular basis. Among the frequent producers of these substances are, for example, species of the genera *Alternaria, Aspergillus, Ceratocystis, Fusicoccum, Fusarium, Helminthosporium, Penicillium, Rhynchosporium, Stachybotrys,* and others (Lew *et al.,* 2001). Some of the above mentioned fungal species may be found in a very large range of host plants in different developmental stages. It is, therefore, evident that the presence of their metabolites is inevitable to some extent (Salomonsson *et al*., 2002, Palermo, 2002).

Toxicogenic fungi are found practically worldwide. Since the outbreak of the so-called „turkey-X" disease in Great Britain (Blount, 1961) it has become clear that the international trade with feed raw materials facilitates transmission of mycotoxins from endemic regions to areas with intensive farming. Nowadays, contamination of feed commodities with moulds and mycotoxins is considered to be one of the most significant negative factors in crop production and quality of animal feeds (Steyn 1998, Scudamore 1998).

The effects of mycotoxins on the animal organism are very adverse, depending on the type of toxin, rate and length of the effect, species, age, sex and animal health. The symptoms may be reduced immunity, allergic reactions, reproductive disorders, nervous system disorders, respiration disorders, and reduced conversion and utilization of feeds or higher mortality in animal farming. Mycotoxins also damage mucous membrane and thus reduce nutrient absorption and also impair the function of the liver, kidneys, reproductive organs and the immune system. Gastrointestinal absorption means that toxins penetrate into the blood and bodily tissues. After being consumed and absorbed in the digestive tract, mycotoxins penetrate into the liver where their biological transformation takes place. Under normal conditions, biotransformation reduces toxicity of harmful substances but as for mycotoxins their contents are increasing as well as their ability to penetrate into edible bodily tissues. In comparison with monogastric animals, ruminants are more resistant to some mycotoxins because their rumen microflora breaks down the substances and disposes of them. Rumen microorganisms are able to transform some mycotoxins. For example, B aflatoxins are transformed to M type (Bertuzzi *et al*., 2003, Gimeno, Martins, 2002). However, the biggest effect of mycotoxin-contaminated feed on animal production is subclinical. The primary impact of mycotoxins on animal and human health is immune suppression and reduced efficiency of a number of metabolic processes resulting in increased sensitivity to negative factors of the environment (Danicke, 2001). Mycotoxins may cause acute

intoxication but predominantly they may negatively affect animal performance chiefly by reducing weight gains, feed conversion and resistance to infectious diseases. Studies carried out with purified toxins pinpointed major target organs, and detailed studies summarizing experimental data on all major toxins were published. They include aflatoxins (Miller, Wilson 1994), trichothecenes (Eriksen, Alexander 1998), deoxynivalenol (Rotter at al. 1996), zearalenone (Kuiper-Goodman et al. 1987) and fumonisins (Marasas 1995).

A search study of the effect of low-quality silages on animal health was published by Wilkinson (1999). The risks connected with silages are divided into three categories: (1) contaminating microorganisms – *Listeria*, enterobacteria, clostridia, moulds, (2) their chemical products, i.e. mycotoxins and (3) high acidity and other reverse metabolic disorders. Other extensive studies on this subject were published in the year 1998 (Scudamore, Livesey 1998) and in the year 2000 (Driehuis, Elferink 2000).

In feeds of plant origin the most frequent mycotoxins are aflatoxins, fumonisins, ochratoxin A, patulin, roquefortin C**,** zearalenone and mycotoxins belonging to the group of trichothecenes.

Aflatoxins

These are substances produced by the fungi *Aspergillus flavus* and *A. parasiticus*. These fungal organisms are sometimes labelled as storage moulds. We distinguish between major aflatoxins – B_1, B_2, G_1, G_2 and derived aflatoxins – M_1, M_2, which are produced by conversion in the process of digestion of feeds contaminated with major aflatoxins. Aflatoxin B_1 is recognized as one of the strongest natural carcinogens ever described.

Aflatoxin producers need a relatively high temperature to stay alive – their life optimum is 28°C – and high relative moisture content of the substrate. It is known that if the moisture content of the substrate is below 12 %, the life cycle of the pathogen stops.

Aflatoxins have a toxic effect on the liver and kidneys. All species of farm animals are sensitive, especially poultry, young animals and pregnant females. The most obvious symptoms of intoxication are loss of appetite, gastroenteritic troubles, subcutaneous haemorrhage, bleeding from body openings and mortality. The liver of dead animals is enlarged and exhibits signs of necrotic changes.

Fumonisins

Fumonisins are a group of mycotoxins produced by species of the genus *Fusarium*, specifically *F. moniliforme* and partly *F. proliferatum* (Bezuidenhost et al., 1988, Gelderblom et al., 1988, Vesonder et al., 2000). These pathogens are isolated most often on corn and corn products. Fumonisins are relatively most dangerous for horses, donkeys, sows and sheep, which as a result of consuming contaminated feed suffer from such diseases as leukoencephalomalacia (fatal disease of the brain, liver and kidney, characterized by muscular tremors, incoordination, aimless wandering, leg and neck extending to total paralysis) and lung oedema. In poultry fumonisins cause the so-called syndrome of feed toxicity. The available literary data indicate that cattle are relatively little sensitive to mycotoxins of this group (Marasas, 2001). According to the World Health Organization – International Agency for Research on Cancer (IARC-WHO) fumonisins are classified as potential carcinogens for humans (class 2B). Fumonisins are structurally similar to sfingosines and can be biologically active in blocking key enzymes of biosynthesis of sphingolipids.

Ochratoxins

Production of ochratoxins A, B and C is described predominantly in the species *Aspergillus ochraceus, Penicillium viridicatum* and *P. verrucosum.* Ochratoxins occur quite often under our conditions, optimum living conditions of their producers are at a temperature of 3 – 5°C and humidity of about 20%. These mycotoxins are most often found in barley, rye, oats, wheat, rise and corn (Palermo *et al*., 2002). The most toxic ochratoxin A displays immunotoxic, teratogenic and carcinogenic properties, it causes damage to kidneys, immune system and digestive problems. Sensitive are all species of farm animals, especially pigs. Ochratoxin may cause neuropathy of pigs. It is a disease affecting the kidneys and the liver which are enlarged, pale and have a rough surface. Ochratoxin A is detected in meat.

Ochratoxins are also very toxic to poultry. Ruminants are resistant to these substances because detoxication takes place in the rumen.

Patulin

Patulin is produced by the genera *Aspergillus* and *Penicillium* and very often it is present in fruit and fruit products and also in silages. Intoxications are reported very often in poultry where they cause damage to the central nervous system, spleen, liver, stomach, kidneys and the respiratory system.

Roquefortin C

In connection with silages toxins produced by *Penicillium roqueforti* are often discussed. This fungus is able to adapt to conditions with low pH (Muller, Amend 1997). Irrespective of toxicity proved during experimental studies, their importance as feed contaminants is limited because of their low biological accessibility to oral administration, which prevents clinical intoxication. However, they may cause unfavourable effects due to their well-known antimicrobical activity resulting in disbacteriosis. Rumen flora is highly sensitive and disturbed synthesis of fatty acids may cause ketosis. Corn silages from farm silos from northern Germany were analyzed for the fermentation process, mould content and presence of mycotoxins. The authors reported that with increasing dry matter content of silages the content of free fatty acids decreased and the proportion of samples with a high proportion of mould propagules increased. Increased secondary contamination, predominantly with *Penicillium roqueforti* together with *Aspergillus* spp., *Mucor* spp., *Monascus* spp. resulted in silage of poor quality (Auerbach et al. 1998).

Trichothecenes

This largest group of mycotoxins produced by fungi of the genus *Fusarium* comprises more than 140 described substances. The most important substances are deoxynivalenol, nivalenol, T-2 toxin, HT-2 toxin, diacetoxyscirpenol, etc. (Danicke 2002, Bottalico et al., 2002, Desjardins et al. 2001). All groups of animals are endangered (Eriksen, Pettersson, 2004).

Deoxynivalenol (vomitoxin-DON) – is apparently the most frequent trichothecene. The most sensitive animals are pigs. Diseased animals refuse feed, vomit and suffer from diarrhoea. Other symptoms of intoxication are incoordination, haemorrhage of mucous membranes, abortions in pregnant females or sudden death. Ruminants are less sensitive. DON action is manifested, for example, by reduced milk production, reduced feed conversion and diarrhoea. The experiments conducted suggested that DON penetrates into milk only sporadically.

T-2 toxin (T2) – causes chiefly skin problems, there are often bleeding sites in the area of the head and sexual organs of animals. In pigs, it causes most often reproductive problems. In cattle it causes reduced immunity in calves, blood coagulation problems and haemorrhage. T-2 toxin is known for its high acute toxicity (Salomonsson *et al*., 2002).

Zearalenone

Zearalenone is another metabolite of many *Fusarium* species in our conditions. It can very often be found in different agricultural products of plant origin. It causes serious problems in farm animals by its estrogenic effects. It is often referred to as a „non-steroid hormone". A lot of reproductive problems in animal breeding are caused just by this toxin (Minervini et al., 2001). It blocks functions of natural hormones, it causes enlargement of vulva, uterus and ovaries, vaginal and rectal prolapse, formation of follicular cysts, oestrus and fertility problems, foetus development problems, etc. Significant is transmission of this mycotoxin into milk. The most sensitive animals are pigs. Cattle are less sensitive.

With the aim of ensuring good health and performance in animals it is important to produce silage with high nutritional value and good hygienic quality. Besides silage contamination with undesirable or pathogenic microorganisms such as, for example, *Clostridium tyrobutyricum, Clostridium botulinum, Listeria monocytogenes* or *Escherichia coli*, the occurrence of filamentous fungi and their secondary toxic metabolites (mykotoxins) is another important factor causing poor performance and health problems of cattle in many cases. It is also necessary to point out that in comparison with cereals or protein feed materials, the knowledge about these microorganisms, their metabolites in silages and their effect on animal health and quality of animal products is still very poor.

Filamentous fungi and mycotoxins in silages

Primary contamination

A number of mycotoxins analyzed in silages are produced during vegetation well before harvest and storage. Pathogenic microorganisms, predominantly representatives of the genus *Fusarium*, were isolated from all plant parts. Fungi invade host tissues through the root, stem, leaves, and also by means of vectors, for example, nematodes. In the course of the phytopathogenic process host plants are contaminated with mycotoxins which are detectable in this period. Mycotoxin contents increase in the last weeks before silage maturation but infection is more frequent on dead tissues. Maximum levels of toxin content are recorded at the time of harvest. Afterwards they do not change much.

Some trichothecenes (e.g. DON and ZEA) were detected in grasses at a concentration of about 2 mg/kg. Silage corn contained also DON and ZEA in different amounts in the range of 0.005 to 13.75 mg/kg. The occurrence of these mycotoxins and their concentrations were markedly affected by the analyzed part of the plant. It was interesting that corncobs contained less ZEA and in lower concentrations than stems. A statistically significant correlation was found between the level of dry matter at harvest and ZEA content. In silage corn the presence of OTA was also recorded. Gotlieb (1997) summarized results of many studies in the United States and indicated a high occurrence of DON in silages (up to 3 mg/kg). In Germany ZEA, DON and OTA were most often isolated from silages produced from the entire corn plants. The concentration of ZEA in silages ranged from 33 to 51 ppb, the concentrations of DON and OTA were 673-4297 ppb and 17-37 ppb, respectively. Grass and corn silages might be

contaminated with trichothecenes and zearalenone. Mycotoxin contents were recorded in the lower variance.

Whitlow and Hagler (2002) analyzed corn silages from farms in North Carolina and detected a number of toxic fungal metabolites. Medium concentrations of AFL, DON, ZEA and T-2 toxin were 28 ppb, 525 ppb, 1991 ppb, and 569 ppb, respectively. Fumonisins were detected quite often.

Changes in the content of mycotoxins coming from primary contamination during fermentation

Changes in mycotoxins induced by the phytopathogenic process during silage making have not been well explained yet. Nevertheless, it has been proved that most of these metabolites exhibit high stability in a strongly acid environment. In the course of fermentation of corn silage no decrease in ZEA and DON concentrations was observed (Lepom *et al*. 1990). In a series of laboratory experiments with grass and corn silage making, no significant effect of fermentation on DON content was found. The lower limit of concentration of this mycotoxin was 570 ppb, after a prolonged time of fermentation the average concentration of DON was 620 ppb. Lindenfelser and Ciegler (1970) studied changes in the content of aflatoxins during silage making and found only a small or practically no decrease in the concentration of these substances after 26 days of storage. Ineffectiveness of aflatoxins described by some authors may be explained by the action of some organic, predominantly, lactic acids. The effect is highly dependent on the concentration and the period of use.

Dynamics of fungus growth during fermentation and after silage opening

The growth of filamentous fungi (moulds) is determined by a number of factors influencing the final composition of mycoflora of silages. The most important factors are temperature, composition of the atmosphere, substrate properties involving moisture content, water activity, pH and chemical composition as well as biotic factors (presence of insects, vertebrates and other microorganisms).

Populations of filamentous fungi continuously undergo important changes between the period of vegetation before silage making and the opening the finished silage. A crucial role in the changes of mycoflora during the first stages of silage making is played by oxygen availability. Once an anaerobic environment has been created during the initial stage of fermentation, *Fusarium spp.* cannot survive long. A*lternaria spp.* and *Cladosporium spp*. are dying as well. It is evident that any delay in silage closing may result in increased numbers of fungal propagules in silages in the initial stages of storage.

According to the classification of filamentous fungi in silages on the basis of their tolerance to oxygen deficit, species of the genus *Fusarium* are strictly aerobic. Among tolerant moulds are *Aspergillus fumigatus*, some of *Mucorales* and *Penicillium,* as well as *Monascus ruber*. Some other species of the genera *Mucor* or *Penicillium varioti* and *P. roqueforti* are regarded as indifferent to the presence of oxygen.

In a series of experiments on corn plants Auerbach *et al*. (2000) revealed continuous reductions in the number of native fungal propagules when the material was stored in anaerobic conditions from the very beginning. The presence of oxygen in the initial stages of fermentation caused growth of some species of moulds before their number started to decrease. The only species of filamentous fungi detected viable even after 60 days of storage was *Penicillium. roqueforti.*. On the other hand, permanent presence of oxygen did not reduce the number of fungi during the entire test period.

Another factor influencing the succession of microorganisms during fermentation are changes in pH caused by natural production of organic acids (lactic, propionic, etc.). Although the level of pH does not markedly affect filamentous fungi which may grow or remain in the latent stage with pH in the range of 3 to 8, fluctuations between these values may have an effect on their sensitivity to other environmental factors. Resistance of fungal organisms to organic acids varies with genera and species. Lactic acid does not usually have any negative effect, unlike propionic acid, which is a potential inhibitor of moulds.

The growth of filamentous fungi is also possible during feed withdrawal when silage is oxygenated again. Oxygen promotes the growth of filamentous fungi if other factors such as temperature, organic acid contents, substrate composition and competing organisms do not restrict their development. All micro-aerophilic species have the advantage that they can start to propagate dramatically at low concentrations of oxygen and relatively high concentrations of carbon dioxide. Experimentally, it has been proved that e.g. P. *roqueforti* requires a minimum concentration of oxygen of 4.2 % for growth provided the concentration of CO2 does not reach 80%.

Mycoflora of finished silages

During the analysis of microflora from 1230 samples of finished corn silages from French and Italian farms almost 70 fungal species were isolated. *Penicillium roqueforti* was a dominant species detected in 76 % of the samples. The occurrence of the genera *Monascus, Aspergillus*, *Byssochlamys* and *Paecilomyces* was 31%, 21%, 41% and 27%, respectively. A high proportion of *Mucoracea*e was also reported. Mycological analyses of samples from 98 grass silages and 135 corn silages collected during the years 1997 and 1998 in southern Germany also revealed the dominance of three main species. *P. roqueforti* was detected in 30% of samples and *M. ruber* and *A. fumigatus* were present in 19% and 9% of silages, respectively. In Austria in the samples of 455 grass and corn silages a spectrum of filamentous fungi was recorded. *P. roqueforti, B. nivea, A. glaucus* and *M. ruber* prevailed in 53.6 % (Adler 1993).

Variable results from research into mycoflora in silages, with respect to the presence, abundance and dominance of certain fungal species, may be attributed to many factors. Some effects may be exerted by methods used for laboratory analyses of filamentous fungi. Incubation in the anaerobic and aerobic environment influences the range of cultivated species. An important role is played by temperature and composition of growth substrate. Results of some studies also indicated that the population of fungi may differ with the type of silage. More diverse mycoflora was described in grass silages than in corn silages.

Formation of mycotoxins in silages

The detection of filamentous fungi in silages is not convincing evidence of the presence of mycotoxins. Like in the above mentioned growth of fungi, formation of mycotoxins is also affected by a number of environmental factors. Not all species of the genera of moulds are able to produce mycotoxins, but the development of toxicogenic species in silages is a prerequisite of the production of toxic metabolites in these feeds. Although production of mycotoxins formed *in vitro* under artificial conditions appears to be the common characteristic of species isolated from silages, not always do these data correlate with reality *in situ.*

When air gets in after opening the silage, the growth of moulds and mycotoxin production may be initiated. It was proved that *P. roqueforti* and *A. fumigatus* in grass and corn silage may form roquefortin C in high concentrations as well as verruculogen

and fumitremorgen B. It is very likely that the type of accessible carbon sources and their utilization may better promote the growth of *P. roqueforti* and formation of roquefortin C in corn silages, compared with grass silage. These silages have often a surplus of sugar, and moulds grow twice as fast on sugars as on other fermentation products

Sample collection

Determination of mycotoxins in feeds or other commodities is a relatively expensive process. It is important to ensure that such determinations are as accurate and reliable as possible. Close attention is therefore given not only to the analytical determination itself but also to sampling and sample preparation for analysis. Results of analyses may be useless if the sample is not representative enough of the whole batch and if the portion taken for the test is not representative enough of the sample. According to many authors, erroneous sampling of material and the collection of an aliquot from the collected sample may account for more than 90 % of the total error of the mycotoxin analytical process. The importance of correct sampling of the test batch for producing accurate results is dependent on two typical properties of contamination with mycotoxins: low concentration of these substances in the commodity and their uneven distribution. The probability of detecting contamination can be increased only by increasing the content of particular samples and increasing their numbers – samples should be taken from as many places of the silage pit as possible. Some comparative examples are given just to illustrate the occurrence of mycotoxins in low concentrations. The average occurrence of mycotoxins is given in mg/kg i.e. ppm (parts per million) or μg/kg i.e. ppb (parts per billion). Now, the illustration of how very small the concentration is:

1 mg/kg = 1 ppm – 1 kg of wheat has 30000 grains, and 1 grain in 30 kg represents 1 ppm.

1 μg/kg = 1 ppb – 1 grain of corn in 3.5 wagons

Sample collection is defined as taking a certain amount of material from the whole for testing in such a way that the occurrence and amount of tested substances in the sample correspond to their occurrence and amount in the whole. Collecting samples for mycotoxin analysis from silage pits must include taking some amounts of silage and mixing them to make a composite sample. After opening the silage pit with corn silage it is recommended to take three samples from the front part of the silage wall, each at three horizontal levels. Each sample should weigh 1.5 kg. The total weight of samples will be 4.5 kg. This average sample must be well homogenized or the size of particles must be mechanically prepared. After that, a proportional analytical sample of 1 kg in weight will be taken and from this sample a 100g sample as well as a reserve sample of the same weight will be collected. Immediately after collection, the samples will be put in the freezer and stored at a temperature of – 20°C until they are later processed. Freezing samples is important because it prevents further production of toxic substances by metabolizing microorganisms during storage. Certainly, it is ideal to analyze the content of mycotoxins immediately after sampling, but it is not always possible.

Analytical determination of mycotoxin contents

For the determination of mycotoxins different analytical procedures are used. One group are chromatographic analyses (TLC, GC, HPLC), the second group which is suitable for screening determination is the immuno-enzymatic analysis using ELISA assays. At present ELISA kits produced by two foreign manufacturers are available on

the Czech market. Essentially, they do not differ, but still there are some differences in the spectrum of analytes offered for particular toxins and in sensitivity (ppm, ppb). Test kits contain a basic set of reagents including serial dilute standards. More accurate results are provided by kits where the results are taken on the spectrophotometer at a certain wavelength of the transmitted light (quantitative determination). Interim results can be obtained by using the so-called FAST tests where the resulting colour reaction is determined visually by the laboratory staff. These tests give semi-quantitative results - less, the same as or more than the standard. These tests last for ca 20 minutes without sample preparation.

Even though ELISA determination of mycotoxin contents requires less laboratory apparatus than most chromatographic procedures, it is necessary to have some important instruments, aids and chemicals in place.

Mycotoxin contents in silages – Czech Republic

Since the year 2002 the mycological and mycotoxicological laboratory of the Fodder Crop Research Institute Ltd. Troubsko has been conducting the study of the hygienic quality of different feeds of plant origin for farm animals. Besides other evaluations (nutrient content, sensory evaluations, total number of native colonies of yeasts and moulds), tests for the presence and concentration of mycotoxins are also performed.

In the years 2002 and 2003, a total of 65 samples of different preserved materials were tested for the presence and concentration of five of the most frequent and serious mycotoxins. These were aflatoxins, deoxynivalenol, fumonisins, T-2 toxin and zearalenone. The samples were supplied by companies located in northern and southern Moravia. For analyses the ELISA assay was used.

The following table gives average values of individual mycotoxins in samples of the feeds tested.

Table: Average concentrations of mycotoxins in DM of different feeds ***/ ppm***

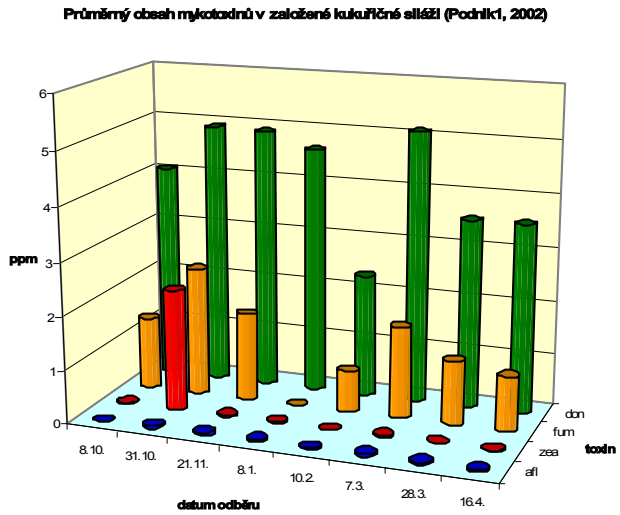
	AFL	T2	FUM	DON	ZEA	Percentage of positive samples
alfalfa silage	0.0035	0.176	0.050	0.500	0.577	100
corn silage	0.0014	0.260	1.870	0.960	1.377	96
clover-grass silage	0.0028	0.242	0.470	0.630	0.179	100
grass silage	0.0024	0.207	1.110	0.550	1.197	93
GPS barley	0.0024	0.163	1.130	1.370	0.500	100

It is evident that the presence of mycotoxins in all types of feeds of plant origin is reality. From the results of analyses it follows that as for mycotoxin contents corn silages are the most serious problem. On the other hand, clover-grass and alfalfa silages were contaminated with these harmful substances least of all, compared with the other feeds. The primary source of contamination is infection of host plants with pathogenic organisms during the process of vegetation. During the harvest and the conservation process the content of mycotoxins does not change markedly except for the substances produced by *Aspergillus* spp. or *Penicillium* spp. when in the course of storage secondary infection and successive production of these substances may occur.

Interesting is the detection of fumonisins in grass silages. This type of mycotoxins has so far been described predominantly in corn but it is possible that there is a link between *Fusarium* spp. producers and other monocotyledonous hosts.

A frequently asked question is about changes in mycotoxin contents, e.g. during silage making. The following graph shows the content of deoxynivalenol and other mycotoxins during the process of silage making in corn started in the year 2003. The samples for analysis were taken from the upper part of the silage feeding trough at 3 to 4-week intervals. It is evident from the graph that if mycotoxins are already present in the material supplied their content does not change much in the course of the process. And what is even more important is the fact that there are no reductions. But the opposite is true, especially when silage is badly established and there will be secondary contamination with fungal microorganisms.

Graph: *Changes in the content of selected mycotoxins in corn silage in the course of silage making as illustrated by one of the companies studied.*



Besides the Fodder Crop Research Institute (VÚP) Troubsko, analyses of mycotoxins are also made by the State Veterinary Institute (Státní veterinární ústav) in Jihlava, which is an accredited workplace for analysis of these mycotoxins. The results of monitoring summarized for the years 2000-2002 were presented at the workshop organized by SZÚ Brno „Mycotoxins and toxinogenic micromycetes in foodstuffs (Honzlová, 2003) in Proceedings of SZÚ Brno, October 2003). In the laboratory of the SVÚ Jihlava a wide spectrum of feed samples were analyzed for DON, ZEA, T-2 and FUM. The monitoring of feeds for DON content indicated that, for example, in the year 2000 out of 48 samples of dry forage crop 48 were positive with an average occurrence of 1.61 mg/kg. Of three samples of hay two were positive with an average of 0.35 mg/kg. In the year 2002 seven samples were analyzed for DON content in corn silage. All of the seven samples were contaminated with this toxin with an average of 0.93 mg/kg. During the analysis for ZEA content of 48 samples of dry forage crops 44 were positive with an average of 0.19 mg/kg. All seven corn silage samples were positive again, the average occurrence was 1.01 mg/kg. As for T-2 toxin, the average contamination in corn silage was 0.32 mg/kg. Besides roughages, sugar-beet pulp, wheat, rape and alfalfa meal, feed mixtures, seed oil cakes, etc. were analyzed for the

content of these three mycotoxins, The colleagues from the SVU pointed out that as for DON content, besides feed mixtures for pigs, predominantly silages, green fodders are a great hazard. In feed mixtures, the highest concentrations are recorded in wheat and corn.

Hygienic limits

Unlike foodstuffs, in most European countries there are no maximum permissible concentrations of individual mycotoxins in individual categories of animals and feeds. For this reason comparisons of detected results with recommended limits published in the United States are used. The limit of zearalenone in all feeds and categories of animals is 0.5 mg/kg. The limit of T-2 toxin is similar. The maximum permissible limit of fumonisin is 5 mg/kg. As for deoxynivalenol there are several categories specified, for example, in cattle the limit is 10 ppm in less than 50 % of feed, in pigs the limit is 5 ppm.

Measures

Essential for the production of high-quality roughages free of mycotoxins is the **integrated system of plant cultivation with appropriate elements of plant protection and well-respected principles of good agricultural practice.** These are predominantly an optimum site for the crop, selection of a suitable variety for a particular growing region, well balanced nutrition and appropriate pesticide applications to control fungal diseases and insect pests, which by their activity create the gateway to pathogens producing toxic substances (some mycotoxins even in small amounts are more toxic than residua of common pesticides). Very important is also scheduled harvest of the crop and immediate and correct ensiling. In some enterprises there are still problems, such as poor preparation of fresh silage material (cutting to optimum particle size) and also imperfect trampling and air removal from the silage. Another quite frequent problem impairing the quality of silages is their insufficient and imperfect cover which allows air penetration and secondary contamination with fungal pathogens. Important is also well-organized withdrawal of finished silage. Even after improper opening and non-compact withdrawal, secondary contamination may occur. The risk of excessive growth of fungal microorganisms and subsequent formation of mycotoxins in stored feeds, for example, might be reduced by application of the so-called „mould-preventing" agents. Major ingredients of these preparations are acids and substances reducing corrosiveness of the preparation and improving mechanical properties of feeds. Most often these preparations contain a combination of organic acids and essential oils or other substances which are effective against a broad spectrum of fungal organisms, make the feeds looser and have reduced corrosiveness. If mycotoxins are present in the commodity (feeds, silage, etc.), the effectiveness of these mould-preventing preparations is very low to zero. In this stage we have to start discussing possibilities of binding mycotoxins which is, however, a much more complicated procedure. The elimination of mycotoxins, predominantly under our conditions characterized by most widespread fusariotoxins, is complicated by low polarity of their molecules and consequently by limited potential of adsorption which is less stable. For mycotoxin binding clay-based preparations were used until recently. These preparations selectively adsorb polar mycotoxins (aflatoxins, partly ochratoxin). The adsorption component is specially-treated activated aluminosilicates with crystal texture. The size of pores in the crystal texture ensures selectivity in the effect only for molecules of particular sizes and the distribution of polar groups. Adsorption is possible only in molecules that have functional polar groups. Adsorbed mycotoxins cannot penetrate through the intestinal

wall into the blood, but they pass through the digestive tract of the animal and leave the body with excrements. Now, these preparations incorporate inactivated biomass of *Sacharomyces cerevisiae* with preserved enzymatic activity of esterases and epoxidases. These enzymes break down molecules of trichothecenes and zearalenone into non-toxic metabolites which are naturally excreted from the animal. These preparations are added to the feed. Adding to the ensiled material has not been practised yet (Visconti et al., 2000, Dvorska, Surai, 2001, Smith et al., 2001).

Following the above mentioned recommendations, it will be possible to keep the level of undesirable mycotoxins in feeds at the acceptable level and to minimize economic losses in animal breeding caused by the effects of these substances.

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Strategies for Minimizing Clostridia Spores in Silages

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Introduction

Improved quality control and quality management has become essential in agriculture and food industry. Since the beginning of this year the EU regulation No 183/2005 on feed hygiene has been in effect. This regulation places the farmers under the obligation to ensure that all used feeds are suitable for animal feeding in accordance with the current EU legislation. As a method to control quality problems, the use of the HACCP (Hazard Analysis Critical Control Points) concept will become compulsory. This method has proved to be very worthwhile in many fields of the food industry.

Using the HACCP concept means 1) to determine the critical points within the production process which are decisive for the quality of the final product, 2) to set special quality standards for these critical points and 3) to control them regularly. In most cases of its use it became obvious that securing and improving the quality of a certain final product could reliably be achieved only if the preceding steps of production were included in these controls. The aim of this paper is to demonstrate the necessity to include the entire production chain from crop production to milking, if good milk hygiene standards with special regard to clostridia are to be achieved.

Problem

Spores of clostridia in milk cause off-flavours and texture defects of various cheese types. The source of udder and milk contamination with clostridia spores is the faeces of cows from which the milk is taken. Even with best hygiene during the milking process, at least a few spores are transmitted into milk (Stadhouders and Spoelstra, 1990). But the risk of contamination increases significantly with increasing the spore content of the cow's faeces. In order to restrict disturbances of the cheese manufacturing process, the dairies have to use different measures for compensation of this quality failure of the milk. These measures are costly (e.g. centrifugation) or undesirable with regard to consumer safety (e.g. chemical additives). Because silages are the main source of clostridia spores in the diet and consequently in the cow's faeces, silage feeding is not allowed in some regions where special cheese types are produced. In other regions, the clostridial spore content of the milk is checked by the dairy industry more and more strictly.

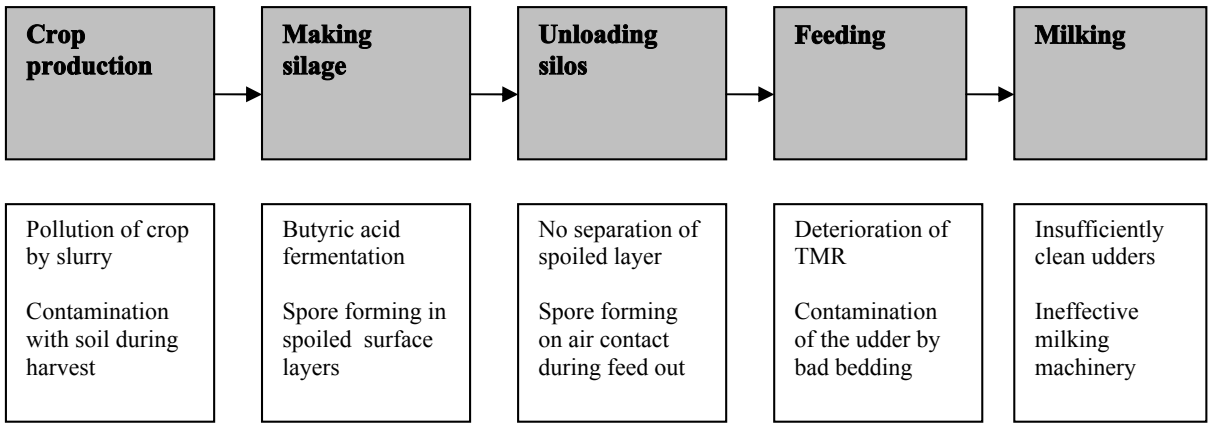
In addition, serious health disorders in dairy herds have been associated with a high clostridia load in the feed. A newly emerged disease has been observed, and the name "visceral botulism" has been suggested (Böhnel, 1999, 2004; Böhnel et al., 2001; Schwagerick and Böhnel, 2001, 2003; Schwagerick, 2004). Although some elements of the pathogenesis have to be further investigated, the occurrence of this disease has been regularly related to feeding silages with bad hygienic quality to dairy cattle. This coincides with the old experience that bad silages can make cows sick. It concerned mostly individual animals in the past. But with the strong increase in milk yield on big dairy farms and the use of TMR (Total Mixed Ration) feeding procedure, it seems to have developed into a serious problem of dairy herds currently.

Both reasons require to control and to improve feed hygiene in general and the quality of silages in particular.

Production chain

Figure 1 shows the individual links of the production chain and the main risk factors which endanger the hygienic quality of milk. Indeed, the contamination risks start already in crop production, namely whether the meadows to be used for silage production are fertilized with slurry and how this is done. Slurry from cattle regularly contains very high numbers of clostridia spores. The contamination of grass with the residues of slurry but also with dead plant material and with soil particles can generate high spore numbers in the silage even without proliferation of clostridia during the fermentation process.

Figure 1: Production chain of a dairy farm with silage based feeding
- Main risk factors for clostridia spore contamination -



However, butyric acid fermentation in the silo is the major cause for high spore numbers in silage (Weissbach and Köller, 1998; Stadhouders and Spoelstra, 1990). During butyric acid fermentation, clostridia cell numbers and mostly also the numbers of clostridia spores increase. Therefore, butyric fermentation is the most important risk for feed hygiene and has to be strictly prevented. Pre-wilting of the herbage is the main measure to inhibit clostridial growth when making grass or legume silages. But this measure should be regularly combined with the use of a suitable silage additive in order to compensate an insufficient or varying degree of wilting of the crop. Particularly in case of very low nitrate content and of soil contamination of the crop, silage additives with special inhibitory effect against clostridia are always necessary (Weissbach et al., 1993; Weissbach and Honig, 1996; Kaiser and Weiss, 1997; Weiss, 2000; Kaiser, 2001; Polip, 2001).

Although clostridia are obligate anaerobic bacteria, high spore numbers are often found near the surface of the silage stack and also in the silage after extended air contact during unloading the silo (Kwella and Weissbach, 1991). Subsequent air exposure may cause spore formation by living clostridia cells when they had found conditions to proliferate before. Conditions which enable clostridial growth may not be given everywhere in a silo. But nevertheless they can exist in several ecological niches within the silage stack, particularly near its surface. For instance, diffusion of air into the silage during storage enables the growth of yeasts and moulds which metabolize lactic acid. As a consequence, pH increases, thereby creating favourable conditions for clostridia to grow. In this way, even maize silage can become a source of high spore numbers, as was recently found (Driehuis and te Giffel, 2005).

The fate and behaviour of clostridia spores in the intestinal tract of cattle is not sufficiently understood yet. However, it is well proven that spores not only pass the rumen and the gut undamaged, but can also germinate and cells can proliferate in this habitat (Bani et al., 1991). Evidence is provided that botulinum toxin formation in the gut may occur (Böhnel, 1999, 2004; Böhnel et al., 2001; Schwagerick and Böhnel, 2001, 2003; Schwagerick, 2004).

Finally, the technological systems of livestock housing and milking can substantially influence the risk of contamination of milk with clostridia spores. However, all efforts to minimize the contamination of the udder and of cleaning it before milking cannot prevent the transmission of spores into milk as long as silages with very high clostridial load are fed.

Critical control points

Generally, silages containing high clostridia spore numbers have to be excluded from feeding to dairy cows. As silage is a very inhomogeneous material for microbiological analyses, it has therefore been tested if the spore content of cow faeces can be a more reliable parameter to assess feed hygiene (Weissbach and Köller, 1989).

Comprehensive methodical investigations into repeatability and into sampling techniques for determination of clostridia spores in faeces have been carried out. The laboratory method used was the determination of the MPN (Most Probable number) of the total gas-producing anaerobic spore-forming bacteria. This group of micro-organisms includes all bacteria belonging to the genus Clostridium, besides spores of some Bacillus species. The use of this method proved to be more effective for monitoring purposes than other, more specific methods (Bergere et al., 1990).

It was shown that the analysis of a pooled sample taken from the faeces of at least 10 randomly selected cows provide representative and repeatable results for the evaluation of feed hygiene in a given herd (Weissbach et al., 1993). Data from samples, which were taken on several farms, revealed big variations in the spore content between farms and between feeding periods. The expected relationships between spore numbers in faeces and milk have been confirmed (Kalzendorf, 1994, 1996). Some results of a dairy farm monitoring are shown in Tables 1 and 2.

Table 1: Farm monitoring results on clostridia spore content in cow's faeces

Farm	number of samples	Numer of Clostridia spores (MPN/g)	
		Mean	Maximum
Winter Frediny			
A	35	3 000	250 000
B	38	14 200	250 000
C	33	16 000	450 000
D	68	20 700	520 000
E	10	37 000	450 000
F	52	194 000	2 500 000
G	18	230 000	450.000
Summer feeding including silages			
D	18	66 000	250 000
Summer feeding without silages			
B	26	150	4 500
A	36	220	2 500

This results demonstrate level and variation of spore content in the faeces, which are currently to be expected in practical conditions when this method is used. The great difference between feeding periods - with versus without silage feeding - confirm that

the major clostridia load always originates from silages (Table 1). However, the level of clostridia spores and the risk of milk contamination clearly depend on silage quality (Table 2).

Table 2: Farm monitoring results on grass silage quality and clostridia spores

Farm	Monitoring period	Butyric acid in silage % of DM	Clostridia spores		Frequency (%) of milk samples with a high clostridial spore load*
			in faeces MPN/g	in milk MPN/ml	
I	02.01. – 16.04.	0.2	5 000	1.2	8
II	18.12. – 18.03.	0.7	6 800	1.7	19
III	06.01. – 22.03.	0.7	23 000	3.0	26
IV	21.01. – 18.05.	1.2	163 000	4.6	32
V	08.01. – 25.03.	1.5	224 000	14.0	63

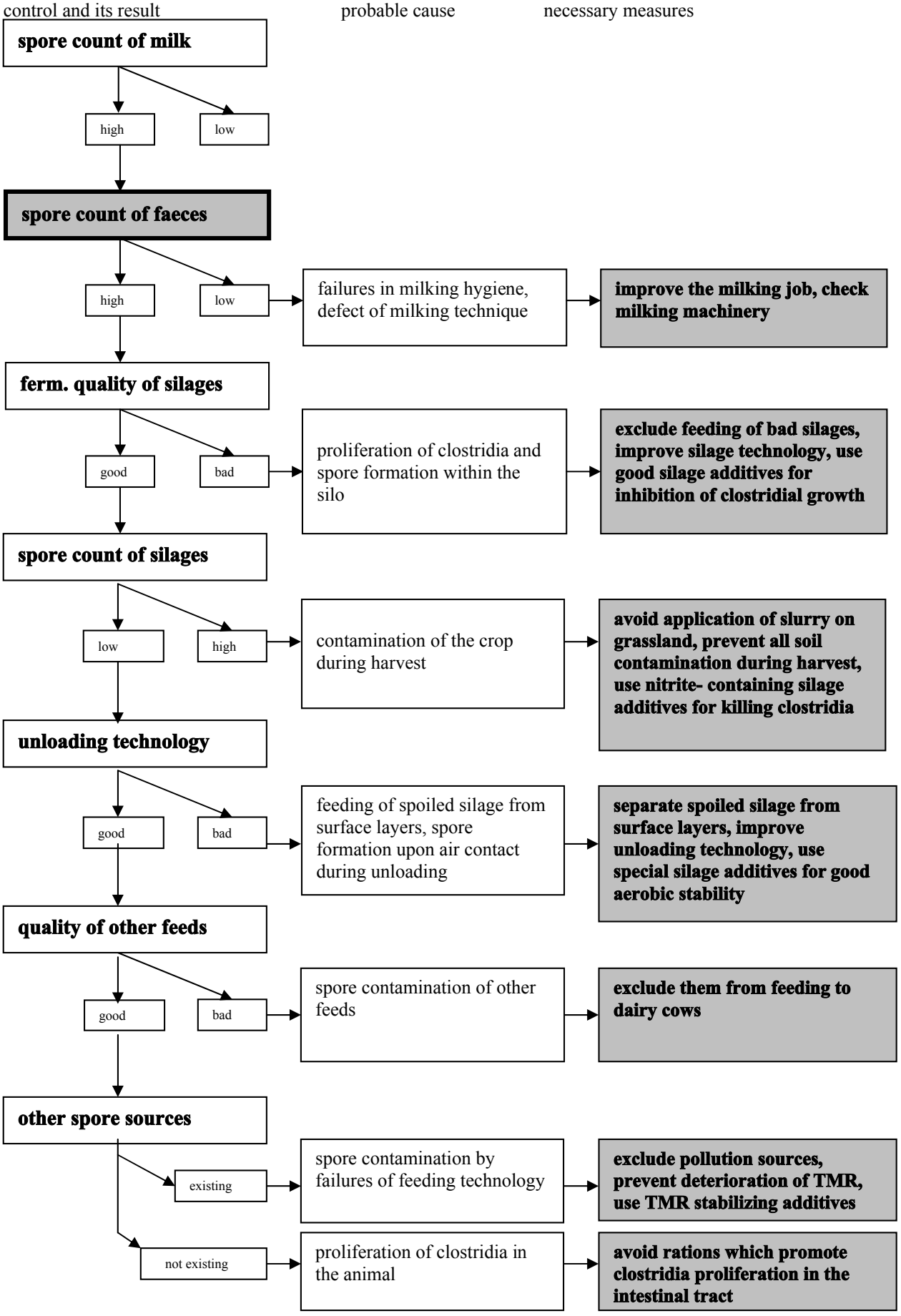
* MPN >6 spores/ml

Hazard analysis

In conclusion, the clostridia spore content in a pooled sample from cow faeces may be a suitable indicator to evaluate the quality of feed hygiene on a dairy farm, e.g. such a farm which was identified to deliver milk with too a high spore content or which had serious animal health problems. Less than 10 000 spores/g faeces are the desirable level, more than 100 000 spores/g faeces are a signal for great risks in terms of milk quality and animal health (Weissbach, 1997).

Faecal clostridia spore number enables to characterize the hygienic level of the total feed ration, including feeding technology. It can be used as the basis of a causal analysis of the entire production chain. A scheme of such a causal analysis has been designed and is demonstrated in Figure 2. Adherence to this scheme not only allows to evaluate the hygienic level but also to analyse the causes of the given disturbance and to deduce recommendations to solve the problem.

Figure 2: Causal analysis of high clostridia spore load



Improvement of silage technology and the use of more effective silage additives proved to be the decisive points suitable to change the situation in most farms which have hygienic problems now. The general experience obtained during the last years was that the high quality level needed today can be not ensured without the use of silage additives. These additives are needed to compensate the naturally changing biological properties of the crop, the variation of weather conditions and also the unavoidable weaknesses of technology in constantly ensuring optimal conditions. Therefore, application of silage additives should be a regular component of each ensiling procedure. Chemical silage additives proved to be much more effective for this purpose than inoculants. Regarding chemical silage additives, aqueous neutrally-reacting solutions of salts of preservatives are much easier to handle than acid mixtures.

Abstract

Improving and securing feed hygiene in dairy operations mainly mean decreasing clostridia spore load in silages. A quality management system based on a HACCP concept is proposed. Clostridial spore content of dairy cow faeces was identified as the main critical control point in such a system. Results of a farm monitoring on clostridia spores levels were shown.

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Mycotoxins in the forage and health problems in ruminants

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Mycotoxins are toxigenic metabolites of fungi, which occur worldwide and are a potential risk for humans and animals as well when feeds and cereals are contaminated. Their importance and occurrence was recognized in the last decades. By oral uptake of mycotoxin contaminated feeds so called mycotoxicoses in animals are observed. In cattle the course of mycotoxicoses is acute or chronical, but dependent on the amount of these toxic substances fed to the animals. Cows are predisposed to infectious diseases by decrease of the natural resistance mechanisms and the immunogenesis. Furthermore mycotoxin contaminated feed-stuffs are a potential risk for the consumer because of aflatoxin residues in meat- and milk-products. Reports about aflatoxin residues in milk were seen in Italy 2003. Specific mycotoxins affect certain organs or tissues like liver, kidney, brain, mucous membranes of the gastrointestinal- and genital-system as well.

In this paper a general view of the most important moulds and mycotoxins regarding their effects on ruminants are shown. In forage we can observe more and other mycotoxins than in cereals. Based on the climatic conditions the most important mycotoxins in Austrian food-stuffs are as follows: Fusarium toxins (deoxynivalenol, DON = vomitoxin and other trichothecenes), alone or in combination affecting the mucous membranes of the digestive tract and zearalenone, ZON causing infertility.

Trichothecenes cause irritations of the mucous membranes of the digestive tract. They comprise approximately 100 different toxins with various toxicity. In farm animals especially deoxynivalenol (=DON or vomitoxin), nivalenol, T-2-toxin, diacetoxyscirpenol (DAS) and the macrocyclic trichothecenes as roridin A and verrucarin A cause intoxications. These secondary metabolites of fungi are developed by Fusarium species, and as well known main producers are Fusarium roseum (Gibberella zeae), F. graminearum and F. culmorum.

In Austrian farms DON and the estrogenic active zearalenone have caused important economic losses. Humid and cold weather-conditions as well as abrupt changes of temperature between day and night enhance the severity of fusariumtoxin contamination of different cereals and maize species. T-2-toxin, DAS and the macrocyclic trichothecenes are more toxic than DON. Thereof following clinical symptoms of incriminated animals may occur: decreased feed intake, general weakness and severe bleeding, cardiovascular shock and apathy. As local cytotoxic effects inflammation of muzzle, lips, tongue and pharynx are described. T-2-toxin causes in calves after 6 weeks of feeding (4 mg/kg feed) atrophy of thymus.

Concerning the effects of DON in cattle only a few informations are available. Experiments using dairy cattle, which were fed for five days with 43 and 83 mg total DON did not exhibit any clinicopathological symptoms. Milk yield as well as minerals were in the normal range. DON was excreted via feces and urine in a very short period of time but not via milk. Therefore it can be concluded, that DON is metabolized by rumen flora. A trichothecene toxicosis can only be diagnosed by positive chemicoanalytical detection of the incriminated toxin in the feed. Because of the rapid

metabolism in the rumen T-2-toxin, DON and DAS could neither be found in tissues nor in serum, urine and liver respectively.

Zearalenone (ZON) effects particularly the genital system and is elaborated by Fusarium spp. on the field. Frequently this estrogenic effective fungal metabolite occurs with DON and sometimes its active alcohol zearalenol. The temperature optimum range of the enzyme activity which is essential for the elaboration of ZON should be 12 - 24 °C and changes of temperature are the basis for toxin production. Chemically ZON shows a similar configuration as estradiol, is connected at cytoreceptors and causes estrogenic effects as well as abnormal estrus. Heifers display a prolonged estrus as well as decreased conception- and non-return-rates. Dairy cows, which were fed with fusarium-contaminated grain (25 mg ZON/kg) showed vaginitis, extended estrus, decrease of feed intake and milk yield as well. Corn-silage, maize kernels and wheat were frequently contaminated and exhibited the highest zearalenone levels.

Mycotoxins in growing forage grasses

Various endophytic fungi of the genus *Neotyphodium* (formerly denoted *Acremonium*) may infect growing pasture grass. Endophytes are often considered to be mutualists, as they confer a variety of benefits to the host plants. However, endophytes produce a wide range of alkaloids, such as ergot and indole diterpene-type alkaloids, inducing staggers, gangrene of extremities, reduced conception rates and heat intolerance in livestock.

In Europe, *Neotyphodium* infected grass, particularly *Lolium perenne*, has only recently been described to occur in natural grassland, depending on season and climate. Ergot and clavine alkaloids are the toxins formed by *Neotyphodium* species, but these toxins are less toxic to mammalians. **Lolitrem B** is the most prominent toxin involved in livestock intoxications following ingestion of infected perennial ryegrass (*Lolium perenne*).

Perennial ryegrass (*Lolium perenne*) habituates a wide range of climatic zones. *Neotyphodium lolii* infects all varieties of ryegrass and the formed toxins are lolitrems. It is an indole terpenoid. The typical clinical symptoms are staggers of grass grazing animals. Early symptoms are head tremors and muscle fasciculation of the neck and shoulders and later of the legs. Young cattle tend to lay down, have difficulty in rising and show abnormal position. Horses show muscle fasciculation, tremor and ataxia. Sheep sway while standing. If the animals are forced to move or encounter other stress, the severity of symptoms suddenly increases, progressing into tetanic convulsions. Deaths rarely occur. Lambs showed reduced live weight gains and health conditions and organ functions were not affected by the intake of lolitrem. A carry over of lolitrem into the blood could not be verified. In Europe the concentrations of lolitrem are lower than e.g. in Australia, therefore no “ryegrass staggers” are observed.

The mechanism of action of lolitrems is still not fully elucidated and an interaction with GABAergic pathways and an increase in excitatory amino acids in the central nervous system have been described. Animals may recover completely when withdrawn from the infected grass. In sheep and cattle maximum tolerable toxin concentration is close to 2 mg/kg dry matter and for horses half the concentration is enough.

Cattle consuming endophyte infected tall fescue (*Festuca arundinacea*) in the USA showed reduced weight gain, lower conception rates, reduced milk production and intolerance to high temperatures. The responsible toxin was found to be the **ergovaline**. It acts as a dopamine-receptor agonist and is associated with the “fescue foot”, referring to gangrenous lesions at the extremities caused by the strong vasoconstrictive effect.

Loss of appetite may be related to an acidemia, as increased tryptophan and serotonin levels have been found in peripheral blood and central nervous system. A decreased level of prolactin has been found in affected cattle, which explains the reduced milk production and retarded growth rates in calves. The carcass quality of ergovaline exposed animals remains altered in fat necrosis and watery, soft muscle tissue. In horses agalactia and increased mortality in foals have been reported. A decreased immunocompetence in affected animals was observed. Ergovaline is held responsible for prolonged luteal function, prolonged gestation, retained placentas and decreased conception rates in mares. Therapeutic approaches are based on counteracting the dopaminergic activity of ergovaline.

Mycotoxins in hay and straw

Due to fermentation activity lolitrem B declined over a period of three months to residual non-toxic values. Other toxins such as slaframine in legumes has been found to persist. Typical storage moulds are *Alternaria* and *Aspergillus*. Spores are involved in the pathogenesis of recurrent airway disease (heaves or RAO, or COPD) in horses, cattle and even humans. In straw various *Fusarium* toxins have been found, especially T-2 toxin and satratoxin. Both toxins are dermatotoxins and produce skin lesions.

Mycotoxins in silage

Penicillium roqueforti is the prevailing mould species, followed by *Aspergillus fumigatus* and *Monascus ruber* in grass silages. All moulds modify the sensory quality of the substrate, which affects palatability and reduced feed intake, particularly in dairy cows fed mouldy silage is observed. The reduction in feed intake may result in considerable production losses and even adverse health effects in young, high producing dairy cows, as these animals easily develop a negative energy balance with the onset of milk production. The rumen fermentation is modified resulting in an increase in pH, reduction of methane and short chain fatty acids. **Mycophenolic acid**, **roquefortin C**, **PR toxin**, **penicillic acid** and **patulin** are the most prominent toxins. They are formed at different stages in the ageing of a fungal colony and that hinders routine toxin monitoring and evaluation of actual adverse health effects. The feeding of silage contaminated with roquefortin C in practice relevant concentations proved to be of low toxicity. Mycophenolic acid is a well known immunosuppressive agent, but no acute toxicity could be observed by feeding experiments in sheep, however, an influence on parameters of the immune system could be demonstrated at the highest dosage. Monacolin K is a well known inhibitor of the 3-OH-Me-glutaryl-CoA-reductase and inhibits the rumen flora and thereof the digestion of the crude fiber. Citrinin is very low in silages and has no relevant toxicity for ruminants.

Aspergillus fumigatus is a very common mould species and produced a wide range of **tremorgenic mycotoxins**, e.g. verrucologen, fumitremorgen and penitrem A. They induce tremor and other signs of neurotoxicity, however, the oral bioavailability is low and the neurological symptoms are rarely seen under practical conditions. **Gliotoxin** is found in cattle suffering from therapy-resistant mastitis and induces as an immunotoxic agent apoptotic cell death in numerous cell types.

Aspergillus clavatus is another silage spoiling mould which produces patulin that impairs the barrier function of the intestinal tract due to its ability to bind glutathione. Other symptoms are neurotoxic but not seen under practical conditions. Patulin was formerly used as an effective antibiotic agent but recently was recognized as a carcinogenic substance.

Fusarium species and their toxins are of high prevalence in corn silages originating from the preharvest period. Deoxynivalenol, zearalenone and fumonisins are the most common and important mycotoxins. Deoxynivalenol (vomitoxin) affects the gastrointestinal tract, resulting in reduced nutrient absorption and growth retardation and induced proinflammatory and immunosuppressive effects. Zearalenone is known to bind to estrogen receptors, thereby causing hyperestrogenism and impaired fertility. The forestomach flora of ruminants will degrade the toxins, but this process is saturable. Fumonisins are potent inhibitors of the ceramide synthetase and disrupt sphingolipid metabolism and thus many cell-cell interactions including the gastrointestinal barrier. Fumonisins have been related to a variety of diseases in horses (equine encephalomalacia), pigs (pulmonary edema) and cattle and sheep (reduced performance and nephrotoxicity).

Effects of rumen flora on the metabolism of mycotoxins

The fusariotoxins ZON, T-2-toxin, Diacetoxyscirpenol (DAS) and DON are potential risk factors for cattle. In cattle with completely developed forestomach-system the rumen fluid content represents however for certain mycotoxins as ZON, T-2-toxin, DAS and DON a detoxyfying barrier. Examinations have shown, that mycotoxins mentioned above are metabolized to significant lesser toxic substances. ZON is metabolized into α-zearalenol and β-zearalenol, DAS and T-2-toxin into Monacetoxyscirpenol (MAS) and HT-2-toxin deacetylated. Such metabolic changes in the rumen are natural defense mechanisms of ruminants against toxigenic feed-components. DON is metabolized into deepoxymetabolite 1 (DOM 1) and excreted via urine.

Diagnosis

It is really difficult to diagnose mycotoxicoses in cattle. First of all the case history containing clinical symptoms (indigestion, hemorrhagic diathesis, central nervous disturbances) and the feeding regimens are of utmost importance. Both concentrate and roughage can be spoiled. Frequently an antibiotic therapy is ineffective, a seasonal occurence of such intoxications (spring, fall) is observed, storage conditions of the different feed components should be checked and visible mold contaminated feed-stuffs are suspicious for mycotoxin elaboration. Other pathogens like bacteria, virus and parasites must be excluded. By chemicoanalytical detection of the mycotoxins in the feed or possible residues in tissue samples (liver, kidney) and blood serum samples respectively the diagnosis "mycotoxicosis" is confirmed.

Prevention

For preventive measures of mycotoxin contamination of feed-stuffs, high moisture grain should be dried immediately after harvest, proper attendance during harvest, transportation and storage can decrease mold growth and subsequent mycotoxin elaboration. To preserve high moisture grains for mold invasion organic acids (e.g. propionic acid) are used.

Many methods of detoxification of mycotoxin contaminated feed-stuffs have been investigated, but they are expensive and in general not applicable under practical conditions. The experienced mycotoxicologist together with the veterinarian and nutritionist should make the final decision about use or destruction of fungal or mycotoxin contaminated feeds.

Botulism and silage
Part I. Botulism

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Clostridium (C.) botulinum is a soil bacterium. There are many different species and types which are characterized by the production of so-called botulinum toxins (BT). They have in common:

- anaerobe metabolism
- spore formers.
- toxin producers.

With rare exceptions spores and/or toxins enter the body of man and animals by the oral route and may cause a disease complex which is known as "botulism".

Bacteria

C. botulinum lives and multiplies (and dies) in the soil. Some strains are adapted to the intestines of animals. In modern agriculture additional substrates for multiplication are especially bio-compost and substrate of biogas production. Poultry and pig manure as well as feline faeces may contain the pathogen also in a high number. The different types of C. botulinum have demands on different nutrients, on different soil types, and may withstand different microbial/protozoal competitors and predators. Hence it is not possible with our actual knowledge to predict what may happen to the different types of C. botulinum spread in the environment. However, experience shows that once the disease appears for the first time, it may be present in the following years.

The bacteria multiply in an atmosphere with reduced oxygen pressure (anaerobiosis). If the survival is not secured the vegetative forms convert to survival forms, the spores. These may resist adverse environmental conditions for many decades in the soil, in feed, and in other contaminated material. They may withstand temperatures like those existing during silage conservation, hay drying, feed pelleting, so called "hygienisation" in composting or biogas production processes. Once the spores realize a favourable environment for propagation they germinate and become vegetative forms again. The biochemical pathways remain obscure.

Toxins

Many clostridial species are characterized by the production of metabolites which have a negative effect on the metabolism of many animals, including man. There are different types of toxins

- neurotoxins (A-G), mixed forms, unknown types
- cytotoxic substances (C2, C3)
- haemolysins
- others.

We really do not know why and when the bacteria produce toxins and when they stop to do so, although many genetic factors have been described.

Toxin production may take place in animal or plant protein. Besides other nutrient and physiological factors a certain water activity (water available for microbial metabolism, not identical with water content) and temperatures normally higher than 20 °C are necessary. Almost ideal conditions may prevail during ensiling processes. This problem will be dealt with in a separate session. Inappropriate storage of hay, concentrate, or even mineral mixtures may cause propagation of C. botulinum and toxin production as well.

Toxins are thermolabile, i.e., they are destroyed at temperatures above 60-70 °C. The pharmacological action is on muscles and internal organs, as it was described already by Kerner, 1820. Unfortunately nowadays botulism is immediately taken as muscular paralysis, only. The term "bulbar paralysis" indicates that all target organs of those nerves leaving the medulla may be affected.

It seems worthwhile to list examples of those organs which may be touched by botulinum toxins. (The pharmaceutical research normally operates with toxin concentrations which are much higher than those in natural cases. There are no known studies on long-time actions of minor doses occurring under natural conditions.)

musculature	striated muscles	smooth mm.	heart mm.
nerve systems	sympathetic nerves	parasympathetic nn.	autonomous nn.
	brain	medulla	hippocampus
	spinal cord		
endocrine organs	pituitary	pancreas	adrenal glands
	liver		
urogenital system	uterus	urinary bladder	kidney
	spermatozoa	mammary gland	
blood	erythrocytes	leucocytes	blood vessels

Diseases

Although botulinum toxins are those toxins with the highest biological activity, the clinical picture may vary by

- type, amount and duration of resorption of the toxin
- animal, breed and type
- age
- simultaneous action of mycotoxins, other bacterial or vegetal toxins
- intestinal homoeostasis (equilibrium of virus, bacteria, protozoa, parasites)
- feed and water uptake
- other underlying diseases.

Many symptoms may not be seen or not be attributed to botulism, like milk reduction, untreatable milk fever, displacement of abomasum, placental retention, movement disorders

The diseases may be characterized in different ways:

- uptake of toxin
  - intoxication
  - intestinal infection (toxico-infection)
  - wound infection (rare)
- clinical aspect
  - muscular botulism

- visceral/systemic botulism
- general weakness

- onset and duration
  - hyperacute
  - acute
  - subacute
  - chronic
- outcome
  - lethal
  - recovery
  - chronic weakness
  - reduced production.

Diagnosis

Diagnosis normally needs a presumptive diagnosis, possibly with exception of classical cases of muscular paralysis. Laboratory diagnosis, pathological signs, epidemiological evaluation may lead to the final diagnosis, hence excluding any differential diagnosis. Chronic cases may remain non conclusive.

In any case the help of a laboratory specialized in botulinum diagnosis is recommended, especially in new cases.

Treatment

In acute cases there is no specific treatment. In farms with preceding history of botulism the use of antitoxins may be helpful if they are available and of the specific toxin type.

The (enteral) use of antibiotics is of no great help, it may aggravate the clinical picture due to release of toxin by affected bacteria.

Symptomatic treatment may be helpful, especially in animals which are not affected too much and in chronic cases.

Restoration of the intestinal equilibrium by feed management will be dealt with in another session.

Prophylaxis

As with many clostridial diseases the vaccination with toxoid vaccines may prevent disease. This holds true especially for muscular botulism. For the visceral form there is still a lack of experience.

With so many different types of botulinum toxins the vaccine must contain the specific toxin type. There are almost no cross-protections between the different types. On the world market there are vaccines against types B, C, and D for animals. Their quality is not always secured.

As a general management tool, production, harvesting, and storing conditions should reduce the additional contamination of the soil by C. botulinum. Hence, contact of animals with bacterial forms and toxins may be reduced. European legislation, by supporting use of bio-fertilizers and bio-gas production, causes unintentionally the amendment of new types of C. botulinum and in higher quantity in the environment. Due to the survival in nature C. botulinum may pose a risk for future agriculture and consumer's health.

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Botulism and silage
Part II. Diagnosis and discussion

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Diagnosis

Since March 1999 at first a previously unknown disease of dairy cows has been observed in about 20 dairy herds of Mecklenburg (North Eastern Germany). The diagnosis turned out to be difficult due to unspecific symptoms at first sight:

- increased cow losses after calving without clear reasons
- problems in calving performance
- high rate of recumbancy
- individual cows with body mass reduction and appearing tucked-up
- individual cows of the same feeding groups with diarrhoea or constipation, increased incidence of abomasal displacement
- lameness
- ataxic gait
- unable to rise voluntarily
- prostration
- afebrile
- reduced milk yield
- reduced milk yield compared to feed intake.

Mainly cows after calving and recently acquired animals suffer from these disturbances. Late in lactation and in the dry period problems are rarely observed. In farms affected for a long time, there were young calves with somnolence, unable to suckle and to stand up.

After exact investigations with no obvious indications of other diseases there remained clinical signs which might be due to the effects of botulinum toxins:

1. Striated musculature

    - flaccid paralysis of the skeletal muscles, particularly of the extremities: ataxia, crossed forelegs, swaying while standing,
    - drooping tail
    - difficulty in swallowing
    - paresis of the tongue

2. Smooth musculature

    - reduced intestinal motility
    - decreased ruminal activity
    - abomasal dislocation
    - uterine inertia

3. Autonomous nervous functions

    - profuse salivation
    - decreased pupillary light reflex
    - reduced sensory perception of the eyes
    - drooping ears
    - somnolence
    - reduced reflexes.

In general these symptoms could be taken for bulbar paralysis, a typical sign of botulism.

4. Circulatory system

    - respiratory distress, high or low heart rate
    - engorged veins on the legs and the head
    - oedema on the abdomen and the udder
    - cold and dry skin
    - sudden death.

5. Immune system

    - general immune suppression
    - swollen accessory lymph nodes on the neck
    - inflamations of skin: e.g., eczema in the inguinal region.

After the clinical investigation – it represents the most important part of botulism diagnosis –, with the help of laboratory investigations the differential diagnosis has to be considered.

Except for a tendency for acidosis, no other metabolic disturbances like mineral deficiency or ketosis were identified. On the other side tests confirmed an intestinal flora comprising spore forming bacteria and botulinum toxins. Botulinum toxins were found as well in the rumen content, the liver and the small intestine. But not only Clostridium botulinum was detected. Other clostridia species like C. perfringens or C. septicum were found as well. The general term "clostridiosis" was to be discussed. A decreased level of blood cholesterin and vitamin B12 resulted from the ruminal and intestinal dysbacteria. Infectious diseases were to be excluded. Very different levels of botulinum toxin antibodies were found. Mainly in sick cows often a low titre of antibodies were determined in contrast to the rest of affected cows. A break down of immune response could be possible.

Discussion

In all affected farms one common cause could be observed: grass silage of poor hygienic quality or decreased stability was fed in a total mixed ration (TMR; one exception: water contaminated by a carcass). After changing the silage respectively conservation of the TMR cow health situation was improved.

Typical features of these silages are:

- low content of conservation acids like acetic acid, propionic acid, and lactic acid

- higher rate of several butyric acids and more then 10 000 spores per gram (MPN)
- soil content > 100 g/kg
- rather high content of proteins (>17 g/kg)
- normally the first grass cut in the year
- originating from extensive managed grassland
- overgrowth of weeds
- necrotic material in the cut grass (especially after flood)
- manured with sewage or digestate of biogas production
- fast running harvesting vehicles, low cut above the ground.

In addition farmers often tried to compensate lower milk yield by higher feeding of concentrates and maize silage, thus making the situation even worse. Maize silage with a high starch content enhances the risk of mass clostridia proliferation within the intestine.

Conclusions

The described disease proved to be a chronic form of botulism. Already in the early 20th century several authors described exactly these symptoms as of botulism. The recently well known "food borne" botulism is an acute intoxication. Presumably we must accept the existence of a protracted intoxication after clostridia intake and intestinal formed toxin (Toxico-infection). Poor hygienic quality of grass silage seems to be an important factor of chronic botulism outbreak in dairy cows. Grass silage is a well known cause of horse botulism. We have to consider the natural circle of clostridia concentration from soil to cut grass, silage, and from the intestine back to the soil again.

It is absolutely necessary to break the circle in avoiding the described management mistakes in feed production and feeding.

References

For references the reader is referred to the other contributions of this session.

Capillary electrophoresis in feed analysis – use of IONOSEP analyser

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Methods of capillary electrophoresis (CE) for determination of substances important in farm animal nutrition are summarized in this contribution. The substances are divided into three groups (natural ones, additives and contaminants). The principle of CE method is briefly described and examples of use of analyser IONOSEP in this field are presented.

Basic features of CE

Capillary electrophoresis encompasses a family of related separation techniques that use narrow-bore fused silica capillaries to separate a complex variety of large and small molecules. High electric field strengths are used to separate molecules based on differences in charge, size and hydrophobicity. Sample introduction is accomplished by immersing the end of the capillary into a sample vial and applying pressure, vacuum or voltage. Depending on the types of capillary and electrolytes used, the technology of CE can be segmented into several separation techniques. Examples of these include:

Capillary Zone Electrophoresis, also known as free-solution CE (FSCE), is the simplest form of CE. The separation mechanism is based on differences in the charge-to-mass ratio of the analytes. Fundamental to CZE are homogeneity of the buffer solution and constant field strength throughout the length of the capillary. The separation relies principally on the pH controlled dissociation of acidic groups on the solute or the protonation of basic functions on the solute.

Capillary Gel Electrophoresis (CGE) is the adaptation of traditional gel electrophoresis into the capillary using polymers in solution to create a molecular sieve also known as a replaceable physical gel. This allows analytes having similar charge-to-mass ratios to be resolved by size. This technique is commonly employed in SDS-Gel molecular weight analysis of proteins and the sizing applications of DNA sequencing and genotyping.

Capillary Isoelectric Focusing (CIEF) allows amphoteric molecules, such as proteins, to be separated by electrophoresis in a pH gradient generated between the cathode and anode. A solute will migrate to a point where its net charge is zero. At the solutes isoelectric point, migration stops and the sample is focused into a tight zone. In CIEF, once a solute has focused at its pI, the zone is mobilized past the detector by either pressure or chemical means. This technique is commonly employed in protein characterization as a mechanism to determine a protein's isoelectric point (pI).

Capillary Isotachophoresis (CITP) is a focusing technique based on the migration of the sample components between leading and terminating electrolytes. Solutes having mobilities intermediate to those of the leading and terminating electrolytes stack into sharp, focused zones. Although it is used as a mode of separation, transient ITP has been used primarily as a sample concentration technique.

Electrokinetic Chromatography (EKC) is a family of electrophoresis techniques named after electrokinetic phenomena, which include electroosmosis, electrophoresis and chromatography. A key example of this is seen with cyclodextrin-mediated EKC. Here the differential interaction of enantiomers with the cyclodextrins,

allows for the separation of chiral compounds. This approach to enantiomer analysis has made significant impact on the pharmaceutical industry's approach to assessing drugs containing enantiomers.

Micellar Electrokinetic Capillary Chromatography (MECC or MEKC) is a mode of electrokinetic chromatography in which surfactants are added to the buffer solution at concentrations that form micelles. The separation principle of MEKC is based on a differential partition between the micelle and the solvent. This principle can be employed with charged or neutral solutes and may involve stationary or mobile micelles. MEKC has great utility in separating mixtures that contain both ionic and neutral species, and has become valuable in the separation of very hydrophobic pharmaceuticals from their very polar metabolites.

Micro Emulsion Electrokinetic Chromatography (MEEKC) is a CE technique in which solutes partition with moving oil droplets in buffer. The microemulsion droplets are usually formed by sonicating immiscible heptane or octane with water. SDS is added at relatively high concentrations to stabilize the emulsion. This allows the separation of both aqueous and water-insoluble compounds, and is used effectively by the pharmaceutical industry as generic methodology to analyze a broad spectrum of pharmaceuticals.

Non-Aqueous Capillary Electrophoresis (NACE) involves the separation of analytes in a medium composed of organic solvents. The viscosity and dielectric constants of organic solvents affect both sample ion mobility and the level of electroosmotic flow. The use of a non-aqueous medium allows additional selectivity options in methods development and is also valuable for the separation of water-insoluble compounds.

Capillary Electrochromatography (CEC) is a hybrid separation method that couples the high separation efficiency of CZE with HPLC and uses an electric field rather than hydraulic pressure to propel the mobile phase through a packed bed. Because there is minimal backpressure, it is possible to use small-diameter packings and achieve very high efficiencies. Its most useful application appears to be in the form of on-line analyte concentration that can be used to concentrate a given sample prior to separation by CZE.

On-line combination of capillary isotachophoresis with capillary zone electrophoresis (CITP-CZE) offers the separation of analytes from sample matrix without the use of discrete spacers. It has been shown that this combination of ITP and ZE is suitable to analysis of trace ionogenic constituents present in a large excess of matrix ions. The CITP–CZE mode utilizes advantages of both methods. The ITP step enables injection of large amounts of a sample (up to several hundred microliters) and thus permits analysis of ionogenic constituents below mmol/liter. The sample constituents are separated into a stack of the zones with minor constituents focused into narrow bands. Bulk ionogenic components are forced to migrate out of the separation compartment at the end of the preseparation column. The minor analytes concentrated and cleaned up from the bulk component in the ITP step are transferred into the analytical column as a narrow sample pulse in the ZE step. The removal of bulk component is well defined and reproducible when it is based on the signal from the conductivity detector of the preseparation column. The ZE step offers high resolution and aids in the identification of minor components using migration times.

Application of CE in feed analysis

From the point of view of analytical chemist feed samples are very complex and heterogeneous which brings many difficulties determining compounds of interest. Advantages of CE applied to such analysis are:

1. Non-ionic compounds (carbohydrates, starch, fibre, fat, etc.), which are frequently bulk components of the sample in question, do not move electrophoretically at selected conditions, and thus do not interfere with the analysis of ionic compounds
2. CE separations are advantageously carried out in free solutions so that no interaction between analytes and separation media (e.g. sorbent in HPLC column).
3. Minimal sample treatment (usually extraction of solid sample followed by filtration and/or dilution in the case of liquid samples).
4. Low running cost (two order of magnitude compare with HPLC).

Compounds, which are usually determined in feedstuffs, can be divided in to three groups:

- ✓ **Naturally occurring feed constituents** *(alkali and alkaline earth cations, inorganic anions, organic acids, amino acids, amines, proteins, anti-nutrients and toxins*)
- ✓ **Feed additives** *(essential amino acids, water soluble vitamins, antioxidants, preserves, acidificators, synthetic growth stimulators*)
- ✓ **Feed contaminants** *(pesticides, heavy metals, some inorganic anions)*

CE was also applied to evaluate the homogeneity of feed mixtures through the determination of minor constituent of feed additive such as lysine, choline, halofuginone, etc.

Ionosep is analyser designed for analysis ionogenic compounds, working on principle of CITP (single capillary analyser Ionosep 2003), two-dimensional CITP and/or CITP-CZE (coupling capillary analyser Ionosep 2002, 2004). It enables analysis a few or more compounds within one analysis in a short time at mM and sub mM levels. Reachable detection limit is up to 10^{-8} mol/l. It is equipped with 10-position automatic changer of samples. This changer of samples can be used separately from analyser what brings big advantage for outdoor work. Using UPS – reserve source and superior notebook - it is able to run analyser for 40 hours = for 150 analysis out of the basis laboratory. IONOSEP 2002, 2003 and 2004 are constructed as fully automatic analysers. All works, including dosing, analysis run, and data collecting and evaluating, everything is run by computer. Ionosep 2003 is equipped with contactless high frequency conductivity detector which is comparable with its parameters to contact conductimeter. Ionosep analyser 2002 and/or 2004 are equipped with two conductimeters and UV-VIS detector and Ionosep 2004 is equipped with three conductivity detectors – two of them for ITP mode and the third one for CZE mode. Analyser Ionosep are controlled by software package running on personal computer under WINDOWS 95/98/2000/NT/XP. List of applications of Ionosep analyser in feedstuffs is given in Table.

Conclusions

Capillary electrophoresis (especially isotachophoresis or its combination with zone electrophoresis) is powerful electrophoretic method especially for the

determination of non-UV-absorbing small ions, i.e., alkali and alkaline earth metals, inorganic and organic acids, and other. Due to the concentrating effect of CITP the excellent detection limits as low as 10^{-8} mol/l can be reached. Very promising is its on-line combination with CZE where CITP step serves as preconcentrating and/or sample clean-up step for analyses of sample with complex matrices such as feedstuffs are. With the help of Ionosep analysers one can determine most of ionogenic nutritionally relevant components of feedstuffs.

For further reading about the application of CE in feed/food analysis see review [1, 2, 3] or visit websites [4, 5].

Abbreviations

BALA = beta-alanine; BTP = 1,3-bis[tris(hydroxymethyl)methylamino]propane, bis-tris-propane; EACA = 6-aminohexanoic acid; GLYGLY = glycylglycine; HEC = hydroxyethylcellulose; HIS = DL-histidine; HPMC = hydroxypropylmethylcellulose; MHEC = methylhydroxyethylcellulose; TRIS = 1,1,1- Tris (hydroxymethyl)-aminomethane

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http://www.recman.cz

http://www.vscht.cz/ktk/www_324/staff/FK/cz/default.htm

Application of Ionosep analyser in feedstuffs analysis

Analyte	Electrophoretic mode/detection	Electrolyte composition	Sample	Sample treatment
NH4, K, Na, Ca, Mg	CITP/conductivity	LE: 7.5 mM H_2SO_4 + 7 mM 18-crown-6 + 0.05 % HPMC TE: 5 mM BTP + 10 mM caproic acid	Silage, feed supplements, various feedstuffs	Extraction with water or 0.1 M HCl, dilution, filtration
Fe	CITP-CZE/UV at 254 nm	LE: 10 mM HCl + 14 mM BALA + 0.05% HPMC TE: 10 mM acetic acid BGE: 100 mM acetic acid + 20 mM BALA + 0.05% HPMC	Drinking water, various feedstuffs	Addition of EDTA
Heavy metals (Cd, Cu, Pb, Zn) + Al	CITP/conductivity	LE: 30 mM NH_4Ac + 10 mM glycolic acid TE: 5 mM acetic acid	Drinking water, various feedstuffs	Extraction on selective sorbent (Spheron OXIN 1000)
Chloride, bromide, iodide, sulphate	CITP/conductivity	LE: 6 mM $Cd(NO_3)_2$ TE: 10 mM tartaric acid	Drinking water	None
Nitrate, nitrite, sulphate, sulphite, phosphate	CITP/conductivity	LE: 10 mM HCl + 10 mM BALA + 3 mM BTP + 0.05% HPMC TE: 10 mM citric acid	Drinking water, various feedstuffs	Dissolution, dilution, filtration
Bromate, chlorate, chlorite	CITP-CZE/conductivity	LE: 10 mM HCl + 20 mM BALA + 0.05% HPMC TE: 10 mM succinic acid BGE: 10 mM succinic acid + 5 mM BALA + 0.05% HPMC	Drinking water	None
Phosphate (and some organic acid)	CITP/conductivity	LE: 5 mM HCl + GLYGLY, pH 2.8 TE: 10 mM caproic acid	various feedstuffs	Extraction with hot water, filtration
Fluoride	CITP/conductivity	LE: 2 mM HCl + 5 mM EACA + 0.05% HPMC TE: 2 mM tartaric acid	Feed mixtures, phosphate, various feedstuffs	Microdiffusion from 25% perchloric acid and trapped in 0.5 M NaOH
Iodide	CITP/UV at 254 nm	LE: 10 mM HCl + HIS + 0.2% HEC + 6% PVP, pH 6 TE: 10 mM MES + HIS, pH 6	Drinking water	Addition of sulphate and fluoride as discrete spacers
Organic acids (lactate, acetate, succinate, propionate, butyrate)	CITP/conductivity	LE: 10 mM HCl + 22 mM EACA + 0.05% HPMC TE: 10 mM caproic acid	Silage, various feedstuffs	Extraction, dilution and filtration

Analyte	Electrophoretic mode/detection	Electrolyte composition	Sample	Sample treatment
Organic acids (citric, benzoic, formic, fumaric, lactic, malic, phosphoric, tartaric)	2D-CITP/conductivity and UV at 254 nm	LE1: 10 mM HCl + 5.6 mM BTP + 0.05% MHEC LE2 : 20 mM HCl + 30 mM glycine + 20 mM beta-cyclodextrin + 0.05% MHEC TE :5 mM caproic acid	Feed mixtures, feed supplements	Extraction, dilution, filtration
Organic acids (lactic, succinic, beta-hydroxybutyric)	2D-CITP/conductivity	LE1: 10 mM HCl + 20 mM BALA + 0.05% MHEC LE2 : 5 mM HCl + 20 mM BALA + 0.05% MHEC TE :5 mM caproic acid	eggs	Extraction, dilution, centrifugation, filtration
Phytic acid and phosphate	CITP/conductivity	LE: 10 mM HCl + 5.6 mM BTP + 0.05% HPMC, pH 6.1 TE: 10 mM MES	Cereal grains, legumes, various feedstuffs	Extraction with 3.5% HCl, filtration, dilution
Methionine (free)	CITP/conductivity	LE: 10 mM HCl + 20 mM arginine + 0.1% PVP TE: 10 mM valine + $Ba(OH)_2$ to pH 10	Feed mixtures and supplements	Extraction, dilution, filtration
Lysine, arginine, ornithine (free and/or bounded)	CITP/conductivity	LE: 10 mM KOH + 20 mM valine + 0.05% HPMC TE: 10 mM Tris + HCl to pH 8.3	Feed mixtures and supplements	Extraction or alkaline hydrolysis with barium hydroxide, dilution, filtration
Histamine	CITP/conductivity	LE: 10 mM KOH + 20 mM valine, pH 9.9 TE: 20 mM Tris + HCl, pH 8.3	Fish meal, various feedstuffs	Extraction with 0.01 M HCl
Glucosinolates (sinalbin, sinigrin)	CITP/conductivity and UV at 254 nm	LE: 10 mM HCl + 20 mM GLYGLY + 0.05% HPMC TE: 10 mM acetic acid	Mustard seeds, various feedstuffs	Extraction with hot water
Glycoalkaloids (chaconine, solanine)	CITP/conductivity	LE: 2 mM HCl in methanol TE: 5 mM $Zn(NO_3)_2$ in methanol	Potato, various feedstuffs	Extraction with methanol, SPE treatment
Biofactors (choline, lysine, kurasan, vitamin B1 and B6	CITP/conductivity	LE: 10 mM NH4OH + 20 mM acetic acid + 0.05% HPMC TE: 10 mM acetic acid	Feed supplements	Extraction, dilution, filtration

New Technologies for Bunker Silo Management in North America

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Why Density is Important

As forage compacts, air entrapped within the pore space is forced out. Thus there is less oxygen within the forage to support aerobic microbial activity. Aerobic microbes break down the sugars to carbon dioxide and water and release heat. This appears as forage heating and dry matter loss.

The volume of pore space relative to the total volume occupied by the crop is called porosity. Highly porous forage allows for easy gas exchange with the air around the forage. Gas exchange allows oxygen to penetrate the forage mass during fermentation and throughout the storage and feed out periods. Silo walls and plastic covers restrict exposure of the forage to the air. However, the materials used are not 100% effective at excluding oxygen. Cracks and holes and permeability of materials allow oxygen to enter the storage. Open feed out surfaces are always exposed to the air. When forage is packed densely and the feed out surface is kept smooth, oxygen penetration through the porous material is limited. Densely packed forage helps to limit the penetration, which limits dry matter losses and increases bunk life of feed.

When aerobic organisms thrive for extended periods, the populations of bacteria, fungi, molds and yeasts explode. During this process, sugars, starches and acids are decomposed. This increases fiber levels, crude protein, ammonia levels, and silage pH in the remaining silage. Accompanying heating decreases the levels of available protein.

Pitt (1983), as reported in Pitt (1990), showed temperature rise in tightly sealed silos increased with high dry matter content and low bulk density (Table 1).

Denser material at higher moisture content is less permeable and resists oxygen penetration better than low-density, low-moisture material.

Table 1: Temperature rise (°C) after a silo is tightly sealed at various compaction levels (bulk densities) and dry matter contents (Pitt, 1983)

	DM content (%)					
Bulk density (kg/AF m^3)	**20**	**30**	**40**	**50**	**60**	**70**
	Temperature Rise (°C)					
320.4	2.7	2.9	3.3	3.8	4.3	5.0
380.6	1.4	1.6	1.8	2.1	2.4	2.8
640.8	0.8	0.9	1.0	1.2	1.4	1.7
801.0	0.4	0.4	0.6	0.7	0.8	1.0
961.2	0.1	0.2	0.3	0.3	0.4	0.6

When studying 19 bunker silos after an average 96-day storage period, Ruppel (1992) found a relationship for dry matter loss as a function of density. His average rate of dry matter loss was 2.5% per month. This author has converted the Ruppel dry matter loss values to a loss per day value and then multiplied by 180 days to generate a relationship of dry matter loss for a 180-day period based on dry matter density. The expression is,

$$DM\ loss\ (\%) = 29.1 - 0.058 \times DM\ density\ (kg/DM\ m^3)$$

From Ruppel and the work of others, dry matter loss with resultant effect on feed quality is inversely related to dry matter density.

Factors Affecting Density

There are many factors affecting the density of dry matter in a silo. This makes it very difficult to develop a mathematical equation which precisely describes density as a function of these factors. Table 2 lists some of the research and demonstration projects conducted to investigate factors affecting silage density in tower and bunker silos. A **+** symbol indicates the study found a direct relationship between a factor and silage density. A **–** symbol indicates silage density decreased as this factor increased. The **o** symbol indicates the researchers considered this factor but found no impact on silage density.

Early studies, using little packing effort, found increasing forage moisture content caused forage bulk density to increase, yet dry matter density remained unaffected. The wet density increase can be explained largely by the increase in forage weight due to added water. Messer and Hawkins (1977b) found wet bulk density increased as moisture increased, but dry matter density was unaffected and was in the range of 125-152.2 (kg/DM m^3). They also found corn silage had a lower wet density than hay for the same moisture after 18 days of fermentation.

The maximum average value of 273.9 (kg/DM m^3) obtained at depth of 21.3 m of silage is not as high as is measured in some bunker silos that are actively packed with heavy tractors. This suggests that active packing at shallow stack depths can actually achieve higher densities than just allowing gravity to work on silage.

Table 2: Factors influencing dry matter density

Reference	Messer & Hawkins, 1977a	Messer & Hawkins, 1977b	Pitt, 1983	Negi et al., 1984	Jofreit & Zhao, 1990	McGechan, 1990	Darby & Jofriet 1993	Ruppel et al., 1995	Muck & Holmes, 2000	Bernier-Roy et al, 2001	Johnson et al., 2002	Vokey, 2002	Muck et al, 2004b	Savoie et al, 2004	D'Amours & Savoie, 2004	Visser, 2005	Craig & Roth, 2005
Factors influencing DM Density																	
Depth in storage		o	+			+	o		+			+			+	+	+
Distance from storage wall/edge			+												+	+	+
Distance from feedout face															+		
DM kontent	o	o	–	+		+			+	+			+	o			
Packing time/ frequency						o/+		+	+	+			o	o			
Surface area								+						+			
Tractor weight	+	+			+		+	+	+								
Pressure				+						+			+	+			
Layer thickness									–	–			o	–			
Grain percentage															+		
Corn maturity	o					–					–						
Particle size	o	o				–			o		–		–				
Crop type	+	+							o	+			+				
Processing											+			o			
Storage type																+	
Surface cover												+					
Dual Wheel									o								
Overfilling storage																	–

+ Positive impact; **–** Negative impact; **o** Considered but no impact observed

The unit density "at depth" of a column in Table 3 is the expected density at the bottom of a given column height of silage. It is increasing with depth and is larger than the average density for the column. It is also in the range of values measured at the base of some bunker silos. However, some actively packed bunker silos will have densities higher than the 312.4 (kg/DM m^3).

Table 3: Silage density and equivalent tractor weight in a tower silo based on ASAE Standard D252.1

Silage Depth (m)	**Aver. Bulk D** (kg/AF m^3)	**Aver. DM Den** [30% DM] (km^3)t^3)	**Unit Bulk Density at Depth** (kg/AF m^3)	**Unit DM Density at Depth** [30% DM] (kg/DM m^3)	**Pressure Exerted by C** (kg/m^2)	**Equivalent* Tractor Weight** (kg)
3.1	608.8	182.6	785.0	235.5	267,178	2,086.5
6.1	720.9	216.3	881.1	264.3	632,790	4,898.8
9.1	785.0	235.5	929.2	278.7	1,033,557	7,883.2
15.2	865.1	259.5	992.3	298.0	1,898,370	14,696.4
21.7	913.1	273.9	1041.3	312.4	2,791,307	21,591.0

***** Four-wheeled tractor, 0.46 m × 0.61 m contact area per wheel

To get a sense of the pressure exerted by an equivalent weight of a packing tractor "at depth", some calculations were made and the results entered in Table 3. The pressure was determined by multiplying average bulk density for a given depth times the depth. For example,

$$6.1\text{-m depth} \times 720.9 \text{ (kg/AF } m^3\text{)} = 4397.5 \text{ kg/}m^2$$

The tractor was assumed to have a wheel contact area of 0.46 m × 0.61 m and four wheels. The equivalent tractor weight is entered into Table 3 for a *6.1-m*. depth is calculated as:

$$\text{Tractor weight (kg)} = 4{,}397.5 \text{ kg/}m^2 \times 0.28 \text{ } m^2\text{/wheel} \times 4 \text{ wheels}$$
$$= 4{,}925.2 \text{ kg}$$

Since modern packing tractors are heavier than 7,883.2 kg, one would expect average densities to exceed the average density of 235.5 (kg/DM m^3) obtained by gravitational packing of 9.1 m of silage depth.

Jofriet and Zhao (1990) proposed an equation that related dry matter density to packing tractor weight (m = tonnes) as:

$$\text{DM density (kg/DM } m^3\text{)} = 200 + 4 \text{ x } m$$

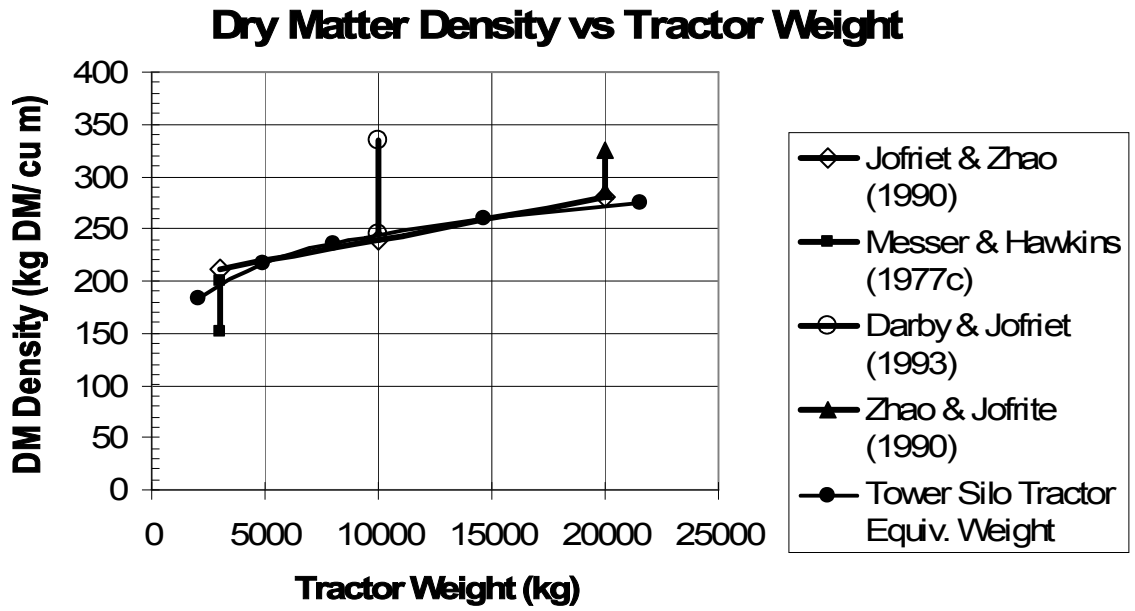
This curve is plotted in Figure 1 where tractor weights are selected based on weights used in research over the years. Messer and Hawkins (1977c) used a 2,994-kg tractor, Darby and Jofriet (1993) used a 9,979-kg tractor, and Zhao and Jofriet (1990) used a 19,958-kg tractor. The densities achieved in the corresponding studies and the equivalent tractor weight densities of Table 3 are plotted in Figure 1 as well. The Jofriet and Zhao equation seems to under-predict dry matter density for two of the three tractor weights used. It appears to quite closely reflect the equivalent tractor weight achieved by gravity in tower silos.

Muck and Holmes (2000) studied bunker silo density by probing at four locations across the bottom portion of the face of 168 bunker silos storing hay crop and whole plant corn silages. They found the most important characteristics influencing density were: silage depth, tractor weight, prepacked forage layer thickness, and time packing per ton. The relationships developed from this work were incorporated into a spreadsheet. The spreadsheet can be downloaded from the UW-Extension Team Forage web site located at

URL http://www.uwex.edu/ces/crops/uwforage/storage.htm

This spreadsheet has been used by many farm advisors as a teaching tool to help explain options for bunker silo packing to increase silage density.

Figure 1: Density as affected by packing tractor weight.



Using a tractor weighing 6,577 kg, Muck, et al. (2004b) packed alfalfa on one side of a bunker silo with one tractor pass (4.52 min/t DM) and the other side of the bunker with two tractor passes (5.92 min/t DM). The packing layer thickness averaged about 0.26 m while the top surface layers were about 0.2 m. The forage dry matter content varied widely between 24.8% and 76.6% DM, averaging about 42.7% DM. The first twelve loads on each side were particularly dry (>50% DM). Based on weight of forage placed into a measured volume of storage, they found the density following placement was 301.2 (kg/DM m^3) for the single pass packing and 291.6 (kg/DM m^3) for the double pass packing. The dry matter densities obtained by core sampling are shown in Table 4.

Table 4: Alfalfa silage dry matter density by height above floor for 1x and 2x tractor passes (Muck, et al., 2004b)

Sampling Date Dec. 18, 2003			**Sampling Date Feb. 3, 2004**		
Height above Floor (m)	**1x**	**2x**	**Height above Floor** (m)	**1x**	**2x**
	Density (kg/DM m^3)				
0.49	370.1	362.1	0.49	298.0	301.2
1.19	282.0	293.2	1.10	357.2	324.4
1.89	269.1	246.7	1.58	225.9	270.7
2.59	169.8	225.9	2.19	253.1	304.4
3.29*****	168.2	185.1	2.71*****	163.4	195.4
Average	253.1	262.7	Average	259.5	278.7

***** 0.49 m below top surface of silage

The average density for two-pass packing was higher on both dates compared to one pass. The bottom layers were packed to similar densities, but the 2x packing improved the density over 1x packing in the two sampling locations closest to the top surface of the silage on both sampling dates. Silage sampled on February 3 was close to the back wall of the bunker silo. Access by the packing tractor to the back wall was limited by the bucket. Forage placed near the back wall was much lower in moisture content than the rest of the storage. This may explain the inconsistent trends in density with height of silage above the floor. These results suggest that extra packing time should be devoted to the top of the silage storage where 2x packing gained an average of 40 (kg/DM m^3) over 1x packing. It should be noted, the average dry matter density obtained in this study was well above the minimum recommended of 224.3 (kg/DM m^3). This can probably be attributed to the large amount of time available for packing, even though the packing tractor weight was relatively low.

Muck, et al. (2004b) studied the effect of tractor weight on packing density in whole crop corn. They packed one bunker silo with a tractor weighing 6,577 kg while the other tractor was 9,662 kg. Spreading and packing time was about 1.42 min/t DM for the light tractor and 1.63 min/t DM for the heavy tractor at the beginning of packing, and 3.56 min/t DM for the light tractor and 4.08 min/t DM for the heavy tractor at the end of the packing process. The packing layer thickness was 0.4 m for the lighter tractor and 0.33 m for the heavier. The corn dry matter content averaged about 33.9%.

At the time of filling, average density for both silos was very close at 236.3 (kg/DM m^3) (heavy tractor) and 235.5 (kg/DM m^3) (lighter tractor). The unexpected lower average dry matter density for the heavier tractor may be explained by the heavier tractor having: a) axles extending beyond the wheels, preventing packing next to the walls, and b) front wheels located farther back from the front of the tractor, preventing packing near the back wall.

The feedout face of each bunker was sampled for density twice during emptying (Table 5). The average density for the heavier tractor is about 24 (kg/DM m^3) denser (12%) than for the lighter tractor. This higher density manifests itself at depths below the top layers (below 0.46 m from top surface) of the silage. The top layers are all quite low density and consistent across the four samplings. This further supports the argument for doing a good job of packing the top surface but highlights the limitations of accomplishing high density in the top zone by improved practices.

Table 5: Corn silage core densities collected at different depth during bunker feedout. (Muck, et al., 2004b)

Tractor Weight (kg)	**6,577**		**6,577**	**7,662**		**7,662**	
Height Above Floor (m)	**DM Density** (kg/DM m^3)	**Height Above Floor**(m)	**DM Density** (kg/DM m^3)	**Height Above Floor** (m)	**DM Density** (kg/DM m^3)	**Height Above Floor**(m)	**DM Density** (kg/DM m^3)
0.46	233.9	0.46	235.5	0.46	312.4	0.46	320.4
0.98	227.5	1.13	249.9	1.04	257.9	1.13	245.1
1.46	235.5	1.80	245.1	1.65	264.3	1.77	246.7
1.98	211.5	2.47	195.4	2.26	241.9	2.44	219.5
2.5*	195.4	3.11*	184.2	2.83*	182.6	3.11*	190.6
Average	221.1		221.1		251.5		243.5

* 0.46 m below silage top surface

Average dry matter density with the lighter tractor was just below the minimum recommended of 224.3 (kg/DM m^3). In the hay silage study (Muck, et al., 2004b), much higher than minimum recommended dry matter densities were achieved with the same tractor. The different results between the two studies might be explained by smaller layer thickness (0.26 m *vs*. 0.4 m), higher average dry matter content (42.7% *vs*. 33.9%), and longer packing time (4.5 min/t DM *vs*. 3.2 min/t DM) for the hay silage *vs*. the corn silage. Each of these differences in packing would be expected to reduce density in the corn silage *vs*. the hay silage. Using the 50% heavier tractor did overcome some of the expected density-reducing factors of the corn silage packing process.

Vokey (2002) studied 14 bunker silos considering silage density based on distance from the top surface and the quality of that forage. Vokey found (Table 6) density and silage quality increased with depth down to 1.83 m from the silage top surface.

Covered silage values are similar to those found by Craig and Roth (2005) for their 2004 data (Table 9), and the deeper samples for the uncovered case are similar to Craig and Roth 2005 data. The one exception is the low density at the 0.3 m depth in the uncovered storages. Here, it appears density is appreciably reduced by decomposition due to exposure to air.

Table 6: Effect of depth from top and covering of bunker silos (Vokey, 2002)

Silage Depth (m)	**Uncovered**	**Covered**
	- - - - **Density** (kg/DM m^3) - - -	
0.30	141.0	176.2
0.91	221.1	197.0
1.83	241.9	227.5

Visser (2005) sampled 48 hay crop and 69 whole plant corn silage bunkers and piles. He reported dry matter density in corn silage was lower than hay silage (Table 7). Silage density in piles was generally lower than in bunkers and combination bunker/pile storage systems for both hay and corn silage. The lower density for corn silage may be attributable to the higher harvest rate for corn compared to hay crop and operators not changing packing practices to compensate for the reduced time available for packing. Visser found dry matter density increased with depth (Table 8) and at distances greater than 1.22 m from the sides of the storage. Visser concluded there were storages not achieving adequate dry matter density, leaving opportunities to significantly improve density. Some of the corn silage storages were as low as 78.5 (kg/DM m^3) which is typical of that found in forage wagons (Holmes, 1995), indicating that perhaps no mechanical packing was being conducted.

Table 7: Dry matter density by storage type for hay crop and whole plant corn silage (Visser, 2005)

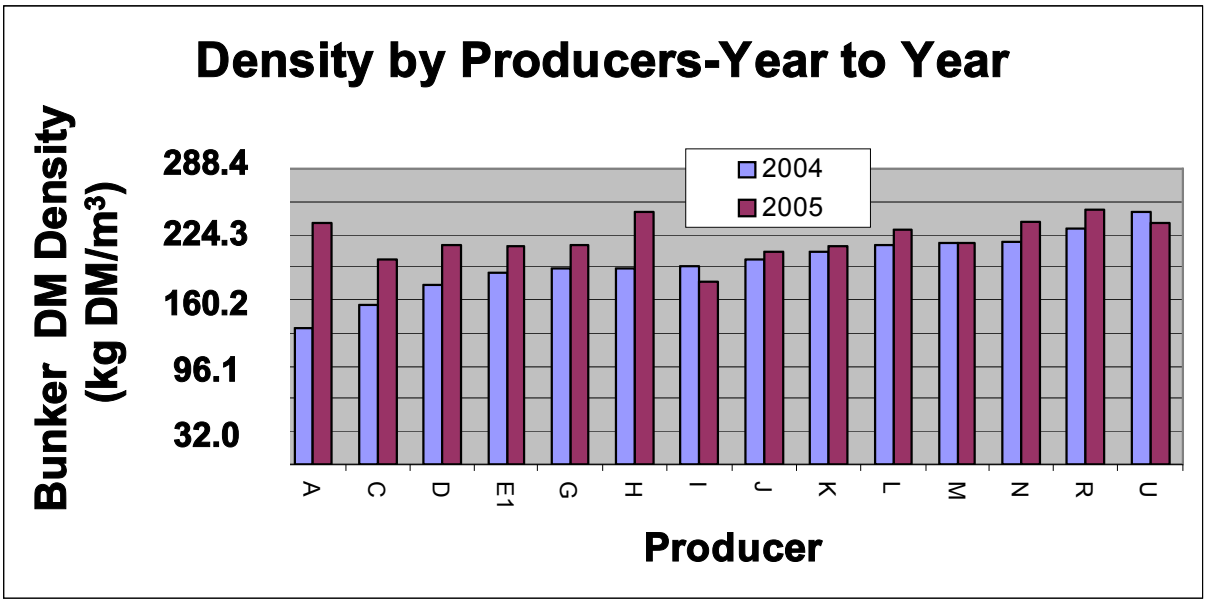
Storage Type	**Hay Silage**		**Corn Silage**	
	Average	**Range**	**Average**	**Range**
	- - - - - - - - - - **DM Density** (kg/DM m^3) - - - - - - - - -			
Bunker	254.7	158.6-435.7	193.8	102.5-378.1
Pile	219.5	131.4-366.9	176.2	78.5-299.6
Bunker/Pile	357.2	235.5-581.5	195.4	78.5-298.0

Craig and Roth (2005) used a probe to collect samples for density measurements on 22 bunker silos in 2004 and 21 bunker silos in 2005. They sampled at four locations across the feedout face at three elevations. They found six of seven producers who had achieved an average density less than or equal to 192.2 (kg/DM m^3) in 2004 were able to increase the forage density to above 192.2 (kg/DM m^3) in 2005 (Fig. 4). This improvement was the result of an educational effort explaining the factors affecting bunker density and the operators changing practices to achieve higher density.

Table 8: Dry matter density as affected by location and crop type. (Visser, 2005)

Location on Feedout Face	**0.9-1.2 m from Top**	**Center of Mass**
Crop	- - **DM Density** (kg/DM m^3) -	
Hay	238.7	275.5
Corn	169.8	214.7

Figure 2: Average dry matter density achieved by producers studied in 2004 and 2005. (Craig and Roth, 2005)



Craig and Roth (2005) found density to be a function of location within the silo. Density increased with depth, and improvements in packing practices affected the middle and bottom slightly more than the top (Table 9). Overfilling and inability to pack the upper volumes adequately contributed to lower density in the top portions of some bunker silos.

Table 9: Average bunker silo silage dry matter density by depth. (Craig and Roth, 2005)

Level within Bunker	**Average Density** (kg/DM m^3)	
	2004	**2005**
Top	179.4	190.6
Middle	206.7	222.7
Bottom	224.3	241.9

Samples collected within 2.44-3.05 ft of the bunker silo walls were consistently lower than those collected at greater distance from the walls (Table 10). Two practices can contribute to this difference, namely a shallower depth near the wall *vs*. the interior and the frequency of packing tractor wheel passage over silage near the wall. The latter is the most likely explanation. This is also corroborated by Muck, et al. (2004b) as shown in Table 4. As a packing tractor moves across the forage surface, both wheels pass over a given point in the interior of the silo, but only one wheel passes the point within one tractor width of the wall. Thus, the interior is packed twice as often as an exterior area per pass of the tractor. Table 10 shows operators increased density at both the interior as well as near the walls from one year to the next. This suggests that operators may need to spend more effort packing next to the wall to improve density in this area exposed to oxygen through wall materials and cracks. Figure 3 shows density is affected more by depth than position within the silo (Craig and Roth, 2005).

D'Amours and Savoie (2004) monitored six bunker silos as they were filled and packed with corn silage. They measured density with a probe at four heights near the center of the feedout face and near one wall. Samples taken at the center of the bunker were 7% denser than those near the wall (Table 11), corroborating the results of Craig and Roth (2005). From Table 12, it is apparent there is an increasing density with distance below the top surface. Intermediate 1 sample is closer to the floor than Intermediate 2.

Table 10: Average bunker silo silage dry matter density based on proximity to walls. (Craig and Roth, 2005)

Location within Bunker	**Average Density** (kg/DM m^3)	
	2004	**2005**
Near wall	197.8	212.3
Interior	209.1	225.1

Figure 3. Dry matter density as affected by position and depth within a bunker silo. (Craig and Roth, 2005)

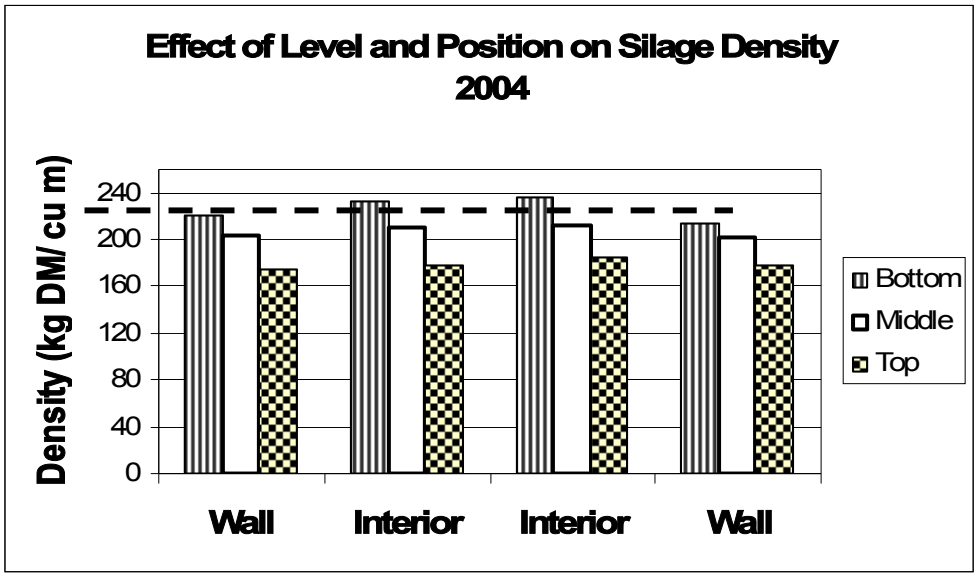


Table 11: Dry matter density by location within the bunker silo (average of two dates). (D'Amours and Savoie, 2004)

	- - **DM Density** (kg/DM m^3) - -	
Farm Site	**Wall**	**Center**
1	283.6	298.0
2	217.9	235.5
3	216.3	240.3
4	200.3	205.1
5	246.7	278.7
6	253.1	275.5

Table 12: Silage dry matter density as influenced by height of silage. (D'Amours and Savoie, 2004)

Farm Site	**1**	**2**	**3**	**4**	**5**	**6**
Height	- - - - - - - - - **DM Density** (kg/DM m^3)- - - - - - - - -					
0.5 m above floor	312.4	259.5	249.9	221.1	290.0	- - -
Intermediate 1	291.6	233.9	232.3	206.7	272.3	272.3
Intermediate 2	296.4	216.3	222.7	193.8	257.9	264.3
0.5 m below top surface	264.3	200.3	208.3	185.8	229.1	241.9
Average	291.6	227.5	229.1	201.9	262.7	264.3

Layer Thickness

The density of forage before it has been mechanically packed is of importance at the top of tower silos, in forage wagons, and when estimating layer thickness prior to packing a bunker silo or pile. Holmes (1995) reported the dry matter density of forage in a wagon at about 80.1(kg/DM m^3). Because most people find it difficult to estimate or measure layer thickness, one can use an estimate of density to establish the area over which to spread forage to achieve a desired layer thickness. For example, using the capacity of trucks (about 2,495 kg DM) used by Muck, et al. (2004b), a layer density of 80.1 (kg/DM m^3), and a desired layer thickness of 0.15 m, the area over which the forage must be placed before packing is 207.7 m^2.

$$2{,}495 \text{ kg DM} \div 80.1 \text{ (kg/DM m}^3\text{)} \div 0.15 \text{ m} = 207.7 \text{ m}^2$$

If the silo has an average width of 9.14 m, the length of slope upon which the forage must be placed is 22.7 m (207.7 m^2 ÷ 9.14 m). If the wall height is 3.05 m and one uses a progressive wedge technique from the floor to the top of the wall, the horizontal distance from the floor edge of the filling slope to the top of the wall needs to be about 22.49 m which is close to the slope length of the filling surface. To facilitate these calculations, a spreadsheet has been developed (Holmes, 2005a) which suggests a horizontal distance of packing to achieve a specified thickness when the following variables are specified.

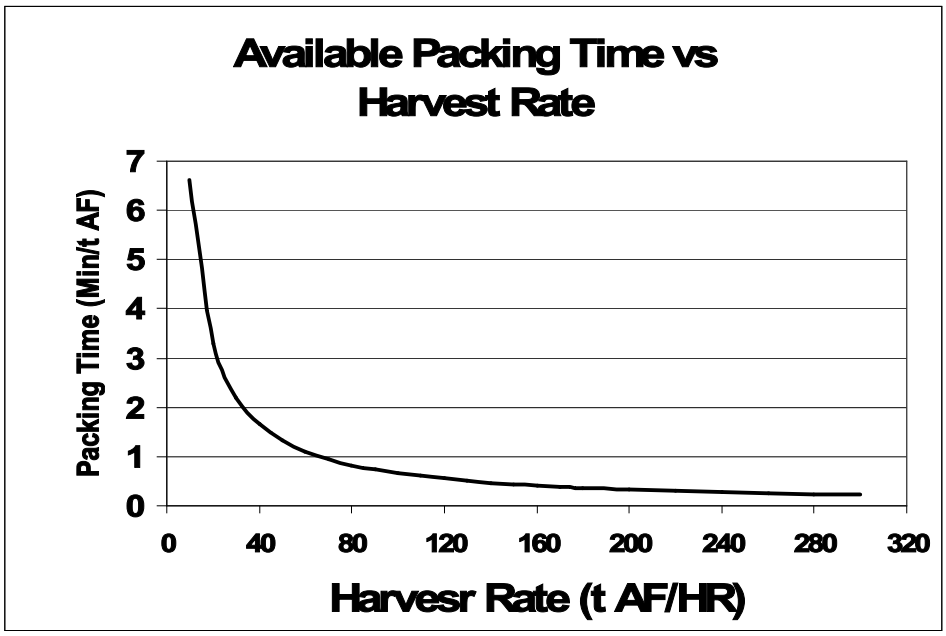
1. Dimensions of the transport vehicle.
2. Height of filling slope.
3. Width of filling slope.
4. Estimate of dry matter density of forage in the transport vehicle.

One way to use a shorter filling slope to achieve a lower layer thickness is to push in one-half of a load, pack it, and then push in the other half of the.

Harvest Rate

Harvest rate influences time available to pack a bunker silo and/or silage pile (Figure 4). The harvest rate (t AF/hr) is generally faster for corn silage than it is for grasses. This is largely due to the ability to keep the throat of the forage harvester full. Self-propelled forage harvesters will generally have much higher harvest rates than tractor-powered units. Some of the higher capacity machines on the market can harvest at the rate of 136.1-181.4 t AF/hr (available packing time of 0.33-0.44 min./t AF). Lower weight tractors can achieve adequate packing density (> 224.3 (kg/DM m^3)) when there are several minutes per ton of dry matter available to pack the forage. However, as harvest rate increases, measures must be taken to assure good packing. Adding weight to packing tractors or using heavier packing tractors are options available that appear to increase forage dry matter density. At some point, harvest rate can be so high that one packing tractor will be insufficient to achieve the desired density. Using tractors of similar weight is more advantageous than one heavy tractor and one light weight tractor. Care must be taken when more than one tractor is packing to avoid collisions.

Figure 4: Time available to pack forage as influenced by harvest rate.



Determining On-farm Average Density

Many research and demonstration projects report results of density measurements using probes similar to that described by Muck and Holmes (2000). The on-the-farm limitations of the probing methods include:

- No access to the probing equipment.
- Number of probe holes to obtain a representative sample.
- Variability of density across the feedout face (depth, wall *vs*. center, disturbance of feedout face, cover *vs*. not, variability of packing procedures, etc.)
- Hazard of injury/death from feedout face avalanche during sampling.

To resolve some of these concerns, Holmes and Muck (2004) have suggested a method for calculating average density. The procedure involves weighing silage as it is

fed with the feed wagon scales and dividing by the calculated volume removed. A spreadsheet available on the UW-Extension Team Forage: Harvest and Storage web site will perform the average density calculation, Holmes(2005b).

Silage Facer

Bunker silo facers are a form of silage unloader. They are frequently constructed as teeth attached to a drum. The drum is mounted on a boom attached to a power unit (skid steer, tractor, telehandler, etc.) The rotating drum is drawn down the feed out face of the silage in a bunker or pile. The teeth remove the silage and generally drop it onto the floor of the storage. The silage remaining in the bunker has a smooth face with a relatively low exposure to oxygen. The facer is an alternative to a front-end loader which is the most common piece of equipment for removing silage from a bunker face. It is difficult to leave a relatively smooth face with a front-end loader. Frequently, the loader operator leaves a rough face with deep fissures in the silage. This exposure to air causes major losses (up to 10%) on these faces.

The difference in loss between that obtained by the front-end loader and the facer is influenced by many factors, from how the forage was ensiled to how it is removed. An estimate of dry matter loss differences is listed in Table 13. These estimates presume the dry matter loss differences are higher as fewer recommended practices for silage management are followed.

Table 13: Dry matter loss improvement by using a silage facer versus a front-end loader.

Dry Matter Loss Improvement (%)	**Storage Management Characteristics**
1	Harvest forage in the 60-70% moisture range Short chop length Pack forage densely (> 256.3 kg/DM m^3) Remove 0.31 m per day from silo face Good face management with front-end loader
3	Harvest forage in the 55-65% moisture range Long chop length Pack forage to average density (224.3-240.3 kg/DM m^3) Remove 0.15 m per day from silo face Moderate face management with front-end loader
5	Harvest forage in the 50-60% moisture range Long chop length Pack forage to below average density (< 224.3 (kg/DM m^3) Remove less than 0.075 m per day from silo face Poor face management with front-end loader

A spreadsheet has been developed to establish the break-even cost of a facer. This spreadsheet was used to develop Table 14. A producer can afford to spend less than the break-even cost and maintain profitability. The break-even cost of the facer when converted to an annual cost equals the sum of improvement in dry matter loss value, additional labor, additional equipment, and additional fuel use costs. The labor, equipment and fuel use could actually be savings if the facer operates at a faster rate than the front-end loader.

In Table 14, the front-end loader and facer are assumed to remove silage from the bunker at the same rate. The forage is valued at $129/t DM. There is no additional cost or savings for labor, equipment or fuel use. A smaller facer may cost between $3,500 and $5,000. From Table 14, a producer with a small amount of forage and using good

management (1% DM loss difference) will break-even with the cost of a smaller facer. Larger operations or those with less good management will have significant profits by investing $4,500 for a facer. For example, a producer with 1,860 t DM stored and improving dry matter loss by 3% would have a $29,667 ($34,167 – $4,500) profit over a 10-year period or $2,967/year.

Table 14: Break-even cost with no additional time required by the facer for forage removal compared to a front-end loader.

Increased DM Loss Using Front-end Loader (%)	**Quantity Stored** (t DM)	744	1,860	3,719	5579	7439
	No. of Cows with Heifers	100	250	500	750	1000
		Break-even Investment ($)				
0.5		2,278	5,694	11,389	17,083	22,778
1		4,556	11,389	22,778	34,167	45,556
2		9,111	22,778	45,556	68,333	91,111
3		13,667	34,167	68,333	102,500	136,667
4		18,222	45,556	91,111	136,667	182,222
5		22,778	56,944	113,889	170,833	227,778

Other Benefits

Benefits of a bunk facer which may be difficult to quantify a monetary value for include:

- Elimination of silage chunks which are difficult to meter into a feed mixer from a loader bucket and sometimes don't blend in the mixer;
- Blending of the forage before placing into feed mixer;
- Particle size is not reduced.

To access the spreadsheet referenced above and a more complete discussion of this topic (Holmes, 2003) can be downloaded from the Team Forage, Harvest and Storage website at URL: http://www.uwex.edu/ces/crops/uwforage/storage.htm

Take Home Messages

- Dry matter loss is inversely related to silage density.
- Actively packing bunker/pile silos increases silage density.
- Many producers are not achieving the recommended minimum silage density. Producers should check silage density and adjust packing procedures if needed.
- The rate of forage push-up and packing must be increased as harvest rate increases to assure adequate packing.
- Increasing packing tractor weight increases silage density.
- Increasing the number of packing tractor passes over the forage increases silage density. More tractor passes requires more packing time per ton.
- Increasing packing passes near bunker silo walls may be needed to increase density in that area of the silo.
- Extra packing effort (more tractor weight, packing time per ton etc.) may be needed on top layers of the forage to increase density in those layers most exposed to oxygen.
- Silage facers may pay for themselves very quickly in midsized to larger operations if

dry matter loss can be reduced compared to front end loader methods of removing silage.

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A Trouble-shooter for Common Silage Problems in Mexico and the USA

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Introduction

Regardless of the size of an operation, beef and dairy producers know problems occur in every silage program. This paper describes possible causes and solutions for 10 common problems, which include:

- Safety concerns
- Effluent
- Large variation in nutritional quality of the ensiled forage
- Missing the optimum harvest window for whole-plant maize
- Clostridial, butyric acid-containing field-wilted silage
- High acetic acid levels in wet maize silage
- Heat damaged silage
- Aerobically unstable maize silage during feedout
- Excessive surface-spoilage in sealed bunkers and piles
- High 'forage in' versus 'silage out' losses in bunkers, piles, and bags

Producers (and their nutritionist) should discuss these problems and solutions with everyone on their silage team as a reminder to implement the best possible silage management practices.

Safety Concerns for Bunker Silos and Drive-over Piles

Consistently protecting workers, livestock, equipment, and property at harvest, filling, and feeding does not occur without thought, preparation, and training. We have nothing to lose by practicing safety; we have everything to lose by not practicing safety (Murphy and Harshman, 2006)

Major hazards and preventive measures).

- Tractor rollover.
  - ✓ Rollover protective structures (**ROPS**) create a zone of protection around the tractor operator.
  - ✓ When used with a seat belt, ROPS prevent operators from being thrown from the protective zone and crushed by the tractor or equipment drawn by the tractor.
  - ✓ A straight drop off a retaining wall is a serious risk so never fill higher than the top of a wall.
  - ✓ Sighting rails should be fitted to the walls. These rails indicate the location of the wall to the pack tractor operator but are not intended to hold an over-turning tractor.
  - ✓ Consider adding lights to the rail if filling will occur at night.
  - ✓ Form a progressive wedge of forage when filling bunkers or drive-over piles. The wedge provides a slope for packing, and a maximum 3 to 1 slope minimizes the risk of pack tractor roll-overs.
  - ✓ Backing up the slope can prevent roll backs on steep slopes.
  - ✓ Use low-clearance, wide front end tractors and add weights to the front and back of the tractors to improve stability.
  - ✓ When front end loaders are used to carry feed into the silo, do not carry bucket any

higher than necessary to help keep the center of gravity low.
  - ✓ Front-wheel and front wheel-assist drive tractors provide extra traction and stability.
  - ✓ When two or more pack tractors are used, establish a driving procedure to prevent collisions.
  - ✓ Dump trucks, which are used to transport chopped forage in large scale operations, can roll-over on steep forage slopes, particularly if the forage is not loaded and packed uniformly.
  - ✓ Raise the dump body only while the truck is on a rigid floor of the storage to prevent turn overs.
- Entangled in machinery.
  - ✓ Keep machine guards and shields in place to protect the operator from an assortment of rotating shafts, chain and v-belt drives, gears and pulley wheels, and rotating knives, which are on tractors, pull-type forage harvesters, self-propelled harvesters, unloading wagons, and feeding equipment.
- Run-over by machinery.
  - ✓ People on foot (especially children) should never be allowed in or near a bunker or pile during the filling operation.
  - ✓ Rear view mirrors should be properly adjusted on all tractors and trucks.
- Fall from height.
  - ✓ It is easy to slip on plastic when covering a bunker, especially in wet weather, so install guardrails on all above ground level walls.
  - ✓ Use caution when removing plastic and tires, especially near the edge of the feeding face.
  - ✓ Never stand on top of a silage overhang, as a person's weight can cause it to collapse.
- Crushed by an avalanche/collapsing silage (Schoonmaker, 2000).
  - ✓ The number one factor contributing to injuries or deaths from silage avalanches is overfilled bunkers and drive-over piles!
  - ✓ Do not fill higher than the unloading equipment can reach safely, and typically, an unloader can reach a height of 3.5 to 4.5 meters.
  - ✓ Use proper unloading technique that includes 'shaving' silage down the feeding face and never 'dig' the bucket into the bottom of the silage. Undercutting, a situation that is quite common when the unloader bucket cannot reach the top of an over-filled bunker or pile, creates an overhang of silage that can loosen and tumble to the floor.
  - ✓ Do not allow people to stand or gather near the feeding face, and a common rule-of-thumb is never being closer to the feeding face than three times its height.
  - ✓ The perimeter of bunkers and piles should be fenced and a sign posted, '*Danger: Do Not Enter. Authorized Personnel Only'*.
- Complacency.
  - ✓ Think safety first! Even the best employee can become frustrated with malfunctioning equipment and poor weather conditions and take a hazardous shortcut, or misjudge a situation and take a risky action (Murphy, 1994).
  - ✓ It is always best to take steps to eliminate or control hazards in advance of an event rather than to rely upon yourself or others to make the correct decision or execute the perfect action each time they encounter a hazard.

Effluent

Effluent has a very high biochemical oxygen demand. It should always be contained near the silo of origin and never allowed to enter groundwater and/or a nearby pond or

watercourse. When seepage occurs, the plant materials that threaten water quality are also nutrients that are lost from the silage.

Causes

- Forage was ensiled too wet (low DM content) for the type and size of silo.
- Forage was not conditioned.
- Forage was in a windrow that was too bulky for the time allowed for field-wilting.
- Weather did not allow the forage to be field-wilted properly before chopping.
- Person(s) responsible for determining the DM content of the forage made a mistake.
- Whole-plant corn or sorghum harvested at an immature stage of growth.
  - ✓ Silage contractor arrived earlier than expected.
  - ✓ Chopping began too early because of a large number of hectares to harvest.

Solutions

- Use weather forecasts to make forage management decisions.
- Take advantage of new mowing, cutting, and conditioning machines and equipment technologies.
- Coordinate the merging of windows with the time of chopping.
- Monitor the dry-down rate and whole-plant moisture content of each field of maize so harvest can begin on time.
- Select a range of maize hybrids with differing maturities to widen the effective harvest window.

Large Variation in the DM Content and Nutritional Quality of the Ensiled Forage

Causes

- Interseeded crops of different maturity.
- Multiple cuttings or multiple forages ensiled in the same silo.
- Delay in harvest activities because of a breakdown or shortage of machinery and equipment or a shortage of personnel.
- Seasonal or daily weather affects crop maturing and field-wilting rates.
- Differences exist among maize hybrids. Hybrids with the stay-green trait tend to be wetter at a given kernel maturity than non stay-green hybrids.

Solutions

- Use multiple silos and smaller silos, which improve forage inventory control.
- Ensile only one cutting and/or variety of 'hay-crop', field-wilted forage per silo.
- Minimize the number of maize and/or sorghum hybrids per silo.
- Shorten the filling-time without compromising packing density.

Missing the Optimum Harvest Window for Whole-plant Maize

Causes

- Harvest equipment capacity is inadequate.
- The crop matures in a small harvest window.
- Warm, dry weather can speed the maturing process (and dry-down rate) of the grain and forage components of the plant.
- Wet weather can keep harvesting equipment out of the field.
- Silage contractor does not arrive at the scheduled time.

Solutions

- Plant multiple maize or sorghum hybrids with different season lengths.

• Improve the communication between the beef or dairy producer, crop grower, and silage contractor.
• Change harvest strategy, which might include kernel processing, shorter theoretical length of cut
(**TLC**), or adding a packing tractor(s).

Clostridial, Butyric Acid-containing Field-wilted Silage

Causes

• The forage is ensiled too wet and undergoes a fermentation dominated by clostridia.
• Lucerne and other legumes, which experience a rain event in the field after mowing, are at a higher risk because rain leaches soluble sugars from the forage.
• The forage is harvested too wet for the type and size of storage.

Solutions

• Chop and ensile all forages at the correct DM content for the type and size of silo.
• Proper packing to achieve a minimum density of 240 kg of DM per m^3 excludes oxygen and limits the loss of plant sugars during the aerobic phase (Visser, 2005; Holmes, 2006).
• Apply a homolactic bacterial inoculant (**HomoLAB**) to all forages to ensure an efficient conversion of plant sugars to lactic acid.
• Do not contaminate the forage with soil or manure at harvest.
• When it is not possible to control the DM content by field-wilting, the addition of soluble sugars can reduce the chance of clostridial fermentation and problems associated with butyric acid silage.

High Levels of Acetic Acid, particularly in wet Maize Silage

Causes and symptoms

• When the whole-plant has a low DM content at harvest, it is predisposed to undergo a prolonged, heterolactic fermentation.
• This silage has a strong 'vinegar' smell, and there will be a 0.4 to 0.8 meter layer of bright yellow, 'sour' smelling silage near the floor of a bunker silo or drive-over pile.

Solutions

• Ensile all forages at the correct DM content, and especially not too wet.
• Apply a HomoLAB silage inoculant to ensure an efficient conversion of soluble carbohydrates in the plant to lactic acid.

Heat-damaged Silage

This silage has a dark brown color and a strong, burnt caramel/tobacco smell. The concerns with heat-damaged silage are reduced digestibility of the protein and energy components.

Causes

• In well-managed silage, the temperature of the ensiled forage should not increase more than 8 to 16° C above the ambient temperature at harvest, and when the temperature of the ensiled forage exceeds 46 to 48° C during the first 1 to 2 weeks in the silo, heat-damage can occur.
• Most of the heat is from plant and microbial respiration, which continues as long as oxygen is
present in the ensiled mass.

• Chemical reactions, called Maillard or 'browning', bind plant sugars and hemicellulose with proteins and amino acids.

Solutions

• Before filling a bunker silo, seal cracks in the walls and/or line walls with polyethylene.
• Harvest at the correct stage of maturity, and especially not too mature.
• Ensile all forages at the correct DM content, and especially not too dry.
• Do not chop forages too long, which would typically be longer than 2.5-cm theoretical length of cut for field-wilted forages and 14 to 18 mm TLC for whole-plant corn or sorghum.
• Achieve anaerobic conditions as quickly as possible in the ensiled forage mass.
• Fill silos in a timely manner and distribute the forage evenly in the silo.
• Achieve a minimum packing density of 240 kg of DM per m^3.
• Cover/seal the surface as quickly as possible following filling (within 24 hours).

Aerobically Unstable Maize Silage during Feedout

Research into the processes of aerobic deterioration has not explained why maize silages differ in their susceptibility to aerobic deterioration. Microbes, primarily lactate utilizing yeast, as well as forage and silage management practices contribute to the aerobic stability of an individual maize silage (Uriarte-Archundia et al., 2002).

Solutions

• Harvest at the correct stage of kernel maturity and especially not too mature.
• Ensile at the correct DM content, and especially not too dry.
• Do not chop longer than 18 mm TLC if the crop is processed or 12 mm if not processed.
• Achieve a minimum packing density of 240 kg of DM per m^3.
• Maintain a uniform and rapid progression through the silage during the entire feedout period.
  - ✓ Remove a minimum of 15 to 25 cm daily in cold weather months.
  - ✓ Remove a minimum of 25 to 40 cm daily in warm weather months.
• Use proper silage removal procedures to maintain a smooth, dense face. Use the shave down method with a bucket or consider using a face cutter.
• Minimize the amount of time maize silage stays in the commodity area before added to the ration, and silage might need to be moved from a bunker or pile to the commodity area twice daily.
• Do not leave maize silage rations in the feed bunk too long, especially in warm, humid weather.
• Add about 1 to 2 kg of a buffered propionic acid product per tonne of TMR if heating does occur.
• Consider re-sizing a silo and subsequent feedout face for the time of year a silage will be feedout.
  - ✓ Feed from 'larger feedout face areas' in cold weather months.
  - ✓ Feed from 'smaller feedout face areas' in warm weather months.

Excessive Surface-spoilage in Sealed Bunker Silos and Drive-over Piles

Solutions

• Achieve a minimum of density of 240 kg of DM per m^3 within the top 0.75 meters of the bunker or pile surface.
• Shape all surfaces so water drains off the bunker or pile, and the back, front, and side slopes should not exceed a 3 to 1 slope.

• Seal the forage surface immediately after filling is finished (Bolsen, 1997).
• Two sheets of polyethylene or a single sheet of oxygen barrier film is preferred over a single sheet of plastic (Berger and Bolsen, 2006). (www.silostop.com).
• Arrange plastic sheets so runoff water does not contact with the silage.
• Overlap the sheets that cover the forage surface by a minimum of 1 to 1.5 meters.
• Sheets should reach 1.5 to 2 meters off the forage surface around the perimeter of a drive-over pile.
• Put uniform weight on the sheets over the entire surface of a bunker or pile, and double the weight
placed on the overlapping sheets (Ruppel, 1993).

- ✓ Bias-ply truck sidewall disks, with or without a lacework of holes, are the most common alternative to full-casing tires.
- ✓ Sandbags, filled with pea gravel, are an effective way to anchor the overlapping sheets, and sandbags provide a heavy, uniform weight at the interface of the sheets and bunker wall.
- ✓ Sidewall disks and sandbags can be stacked, and if placed on pallets, they can be moved easily and lifted to the top of a bunker wall when the silo is being sealed and lifted to the top of the feedout face when the cover is being removed.
- ✓ A 15 to 25 cm layer of sand or soil or sandbags is an effective way to anchor sheets around the perimeter of drive-over piles.

• Prevent damage to the sheet or film during the entire storage period.

- ✓ Mow the area surrounding a bunker or pile and put up temporary fencing as safeguards.
  against domesticated and wild animals.
- ✓ Develop a rodent control program for the farm.
- ✓ Use a mesh or resistant secondary cover to exclude birds.
- ✓ Store waste polyethylene and cover weighting materials so it does not harbor vermin.
- ✓ Regular inspection and repair is recommended because extensive spoilage can develop quickly if air and water penetrate the silage mass.

• Discard all surface-spoiled silage because it has a significant negative effect on DM intake and nutrient digestibility (Whitlock et al., 2000).
• Do not store waste spoilage near the storage so it will not harbor vermin.
• Full-casing discarded tires were the standard for many years to anchor polyethylene sheets on bunker silos. These waste tires are cumbersome to handle, messy, and standing water in full-casing tires can help spread the West Nile virus, which is another reason to avoid using full-casing tires on beef and dairy operations (Jones et al., 2004).

High 'Forage in vs. Silage out' Losses in Bunker Silos, Drive-over Piles, and Bags Solutions

• Harvest at the optimum stage of maturity and whole-plant DM content.
• Use the correct size of bunker or pile, and do not over-fill bunkers or piles.
• Employ well-trained, experienced people, especially those who operate the forage harvester, packing tractor, or bagging machine (Muck and Holmes, 2004). Provide training as needed.
• Apply a HomoLAB inoculant.
• Achieve a uniform packing density of at least 240 kg of DM per m^3 in bunkers and piles.
• Provide an effective seal to the surface of bunkers and piles and consider using double polyethylene sheets or oxygen barrier film (Bolsen, 2004) (www.silostop.com).
• Follow proper face management practices during the entire feedout period.

• Start a silage quality control program and schedule regular meetings with your entire silage team.

Profitability of Sealing Maize and Lucerne Silages in Bunker Silos and Drive-over Piles: A Spreadsheet

The spreadsheet was developed from research conducted at Kansas State University from 1990 to 1995 and equations published by Huck et al. (1997). The authors noted that about 75% of the total tons of maize and sorghum silage made in Kansas from 1994 to 1996 were not sealed, and the value of silage lost to surface spoilage estimated to be 6 to 10 million dollars annually.

Presented in **Table 1** are examples from the spreadsheet to show the profitability of sealing bunker silos and drive-over piles. The net benefit from effective sealing makes it very clear that beef and dairy producers should pay close attention to the details of this 'highly troublesome' practice.

Profitability of HomoLAB-treated Maize Silage for Lactating Dairy Cows: A Spreadsheet

Many beef and dairy producers and nutritionists are concerned about whether it is economical to apply a HomoLAB when making whole-plant maize silage. Presented in **Tables 2** is a spreadsheet, which can be used to calculate the profitability of inoculating maize silage with HomoLAB.

The dairy herd in this example had an average milk production 34.0 litres per cow per day and a daily ration DM intake of 23.6 kg. The increase in net income with HomoLAB-treated maize silage, calculated on a 'per cow per day' and 'per cow per year' basis, is from improvements in both forage preservation efficiency and silage utilization. The additional 'cow days' per tonne of maize ensiled because of the increased silage DM recovery (1.3 percentage units) and the increased milk production per cow per day (0.125 litres) gave an added net income of 18.5¢ per cow per day and $56.31 per cow per year. The increase in net return per tonne of maize ensiled was $8.81.

Profitability of Increasing DM Recovery of Maize Silage fed to Growing Cattle: A Spreadsheet

The spreadsheet©, which incorporates preservation and utilization efficiencies, allows producers to assess the profitability of specific silage management inputs. Three changes implemented in the example shown in **Table 3** were: 1) a HomoLAB inoculant applied at $1.00 per tonne, 2) an additional pack tractor used at $1.25 per tonne, and 3) a more effective seal/cover provided at a cost of $1.00 per tonne. These changes resulted in a $8.19 net benefit per ton of whole-plant maize ensiled, which came from a 5-percentage unit improvement in DM recovery and a 0.25 kg improvement in feed to gain ratio (DM basis).

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Table 1: Profitability of sealing maize and lucerne silages in bunker silos and drive-over piles.[1]

Inputs and calculations	Bunker 1 maize	Pile 1 maize	Bunker 2 maize	Bunker 3 lucerne
Silage value, $ per tonne	35	35	32.50	60
Silage density, kg of fresh wt per m^3	700	700	700	600
Silo width, m	12.2	12.2	30.5	12.2
Silo length, m	30.5	30.0	76.2	30.5
Silane lost in the original top1meter:				
unsealed, % of the crop ensiled	55	55	60	50
sealed, % of the crop ensiled	20	20	20	15
Cost of the covering sheet, ¢ per m^2	48.0	48.0	48.0	48.0
Silage in the original top 1 m, tonnes	260	534	1,627	223
Value of silage lost if unsealed, $ per silo	5,014	10,275	31,724	6,698
Value of silage lost if sealed, $ per silo	1,823	3,726	10,575	2,009
Sealing cost, $ per silo	579	1,186	3,615	579
Value of silage saved by sealing, $ per silo	2,612	5,353	17,535	4,110

[1]Numbers in highlighted squares and bold are user inputs.

Table 2: Profitability of HomoLAB-treated maize silage for lactating dairy cows.[1]

Maize silage, other forage, and grain or supplement inputs:

Ration ingredients	DM intake, kg	DM, %	As-fed, kg per day	$ per kg	Feed cost, $ per day
Maize silane	**6.8**	**33.3**	20.4	**0.035**	0.71
Other forage (silage)	**4.1**	**45.0**	9.1	**0.060**	0.54
Other forage (hay)	**1.8**	**88.0**	2.0	**0.150**	0.31
Grain or suplement	**10.9**	**88.0**	12.4	**0.180**	2.23
Total	23.6		43.9		3.79

Maize silage inventory and inoculant cost:

Maize silage required per cow per year, tonnes	7.22
HomoLAB cost per tonne of crop, $	**1.00**

Table 2 (cont.): Profitability of HomoLAB-treated maize silage for lactating dairy cows.[1]

Komponent	Untreated maize silage		HomoLAB maize silage
Preservation efficiency:			
Silage recovery, % of crop ensiled[2]	**85.0**	**(1.3)** [3]	86.3
Silage recovered per tonne of crop ensiled, kg	850		863
Amount of corn silage fed per cow per day, kg	20.4		20.4
Cow days per tonne of crop ensiled	41.62		42.26
Increased cow days per tonne of crop ensiled			0.64
Milk production per cow per day, kg			**34.0**
Milk gained per tonne of crop ensiled, kg			21.6
Milk price, $ per kg			**0.40**
Increased milk value per tonne of crop ensiled, $			8.66
Utilization efficiency:			
Increased milk per cow per day, kg			**0.125**
Increased milk value per tonne of crop ensiled, $			2.11
Preservation efficiency + utilization efficiency:			
Increased milk value per tonne of crop ensiled, $			10.77
Increased feed cost per extra cow day, $			3.08
Increased feed cost per tonne of crop ensiled, $			1.96
Increase net return per tonne of crop ensiled, $			8.81
Added cost of HomoLAB: per cow per day, $			0.024
per cow per year, $			7.22
Added income as milk: per cow per day, $			0.208
per cow per year, $			63.58
Net benefit from HomoLAB: per cow per day, $			0.185
per cow per year, $			56.31

[1]Numbers in highlighted squares and bold are user inputs.
[2]Shown in parenthesis is the response to HomoLAB expressed in percentage units, which is a 19-trial average across all HomoLAB products (Bolsen et al., 1992).

Table 3: Profitability of increasing DM recovery of maize silage for growing cattle.[1]

Ration ingredients	DM basis	Untreated ration	HomoLAB ration	Untreated ration	HomoLAB[2] Response	HomoLAB ration
	%	DM, %	DM, %	kg / day		kg / day
Maize silage	**87.5**	**0.333**	**0.333**	6.76		6.85
Other silage or hay	**0**	**0.90**	**0.90**	0		0
Grain or supplement	**12.5**	**0.90**	**0.90**	0.97		0.98
Total[3]	100			7.73		7.83
Avg. cattle wt, kg	**300**					
Cattle price, $ per kg	**2.60**					
Avg daily gain, kg				1.09		1.14
DMI, kg per day				**7.73**	**+ 0.10**	7.83
Ration DM per kg of gain, kg				**7.1**	**- 0.25**	6.85
Silage per kg of gain, kg of DM				6.21		5.99
DM recovery, % of the ensiled crop				**82.5**	**+ 5.0**	87.5
Silage fed per tonne of crop ensiled, kg as-fed				825		875
Gain per tonne of as-fed crop ensiled, kg				44.2		48.6
Extra gain per tonne of as-fed crop ensiled, kg						4.4
Value of the extra gain per tonne of crop ensiled, $				---		11.44
Cost of HomoLAB per tonne of crop ensiled, $				---		**3.25**
Net benefit per tonne of HomoLAB-treated crop ensiled, $				---		8.19

[1]Numbers in highlighted squares and bold are user inputs.
[2]Response is a 19-trial average across all HomoLAB products (Bolsen et al., 1992).
[3]User must adjust ration ingredients to 100 percent.

Reduction Of Fungi In Silane By Homofermentative Lactic Acid Bacteria

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Summary

Homofermentative (HOM) lactic acid bacteria (LAB) that are osmotolerant and possessing anti-fungal properties are highly desirable characteristics in bacteria-based silage additives. The objectives were to identify and commercialize HOM LAB strains that were osmotolerant and possessed anti-fungal properties when used in silage. *Lactobacillus plantarum* MiLAB 14 and MiLAB 393 were osmotolerant at a simulated dry matter (DM) concentration of 450 g/kg fresh matter (FM). Yeast counts in grass and maize were lower for *L. plantarum* MiLAB 393 compared with other LAB strains and untreated control (1.87 – 3.10 x 10^3 colony forming units (cfu)/g fresh matter (FM) vs. 0.14 – 1.70 x 10^5 cfu/g FM). Aerobic stability in grass and maize silage treated with the HOM LAB strains was variable, but similar to the untreated control in 290 g/kg DM maize, similar or better than that in grass with 396 g/kg DM and lower in grass of 437 g/kg DM. Fermentation characteristics were consistently improved by the use of HOM LAB compared with the untreated control in all trials. It was possible to identify and commercialize one HOM LAB strain that was osmotolerant and possessed antifungal properties.

Introduction

Silage is an important source of feed to cattle, sheep and horses during periods when fresh forage cannot be fed. For optimal use, silage should be of high nutritional and hygienic quality. Growth of fungi may seriously reduce both the nutritional and hygienic quality in silage and result in high DM losses, poor aerobic stability, and reduced animal performance. The LAB may protect silage from microbial spoilage, such as that of fungi, through competitive growth, production of antagonistic metabolic substances or by forming other antimicrobial compounds (Schillinger et al., 1996; Stiles, 1996). Presently, few LAB-based additives contain this combination of characteristics to effectively reduce fungal growth, particularly in high DM crops that require osmotolerant LAB.

Traditionally, heterofermentative (HET) LAB strains, such as Lactobacillus buchneri, have been used to reduce fungal growth in silage and improve aerobic stability through production of relatively high proportions of acetic acid, which cannot be used as substrate by fungi. However, most HET LAB are not as competitive in the silage environment that contains a large number of other micro-organisms, such as HOM LAB (Weinberg and Muck, 1996). In silage that does not have a significant problem with fungi, the HOM LAB reduce the pH-value more quickly, have lower DM losses and result in higher animal performance than HET LAB. Therefore, identification of HOM LAB that are highly osmotolerant and have a direct action against fungal growth are highly desirable in silage additives.

The objectives were to identify and commercialize homofermentative lactic acid bacteria strains that were osmotolerant and possessed anti-fungal properties when used in silage.

Materials and methods

This presentation focuses on presenting results on osmotolerance and antifungal properties of Lactobacillus plantarum MiLAB 393, and to some extent of L. plantarum L4, L. plantarum MiLAB 14 and L. coryniformis Si3 in ensiled grass and maize. Through

collaborative work between the Swedish University of Agricultural Sciences (SLU) and Medipharm AB, a silage additive based on MiLAB 393 was launched by Medipharm AB on the market in 2005. A patent describing the antimicrobial effects of MiLAB 393 linked to the production of cyclo(L-Phe-L-Pro), cyclo(L-Phe-trans-4-OH-L-Pro) and 3-phenyl lactic acid has been written and studies on antimicrobial resistance has shown that MiLAB 393 is safe to use. Large-scale studies on fermentation, aerobic stability and animal performance of a commercialized product containing L. plantarum MiLAB 393 have also been conducted.

Diluted LAB cultures from 13 strains were surface spread on MRS agar plates supplemented with 10% KCl simulating a crop with 450 g/kg DM and a water activity (aw) of 0.91. Strains were considered osmotolerant if 30% of the cfu remained after 48 h of anaerobic incubation at 30 C compared with the control plate containing no KCl. According to these criteria, MiLAB 393 and MiLAB 14 were osmotolerant, while L4 was partly osmotolerant and Si3 was not osmotolerant (Ström et al., 2005).

The suppression of yeast by the LAB strains with antifungal properties was tested in two grass silage experiments where the DM concentration of grass was 396 g/kg FM (A) and 437 g/kg FM (B), respectively.

Results and discussion

Suppression of yeast by the four antifungal LAB strains added to ensiled grass with DM concentrations of 396 g/kg fresh matter (FM) and 437 g/kg FM showed that yeast counts were lower for MiLAB 393 (0.87 – 3.10 x 103 cfu/g fresh crop) compared with other antifungal LAB strains and an untreated control (0.14 – 1.70 x 105 cfu/g) (Ström et al., 2005). While none of the silages had alarmingly high yeast counts (<1.7 x 105 cfu/g), an early suppression of yeast reduces the risk of future fungal growth. In the same trial, aerobic stability was determined in silage of grass and maize that was aerated for seven days. In grass silage with 396 g/kg DM it took between 0.3 and 1.2 days to increase the temperature by +2 °C. In grass silage with 437 g/kg DM the corresponding values were 1.5 to 7 days and in maize silage at 290 g/kg DM the range was 3.2 to 5.5 days. Only in grass silage with 437 g/kg DM was aerobic stability higher (more days to increase the temperature) in the untreated control compared with other treatments. Remaining treatments differed little in aerobic stability (Ström et al., 2005). Silage is generally considered aerobically stable if it takes more than two days to increase the temperature by +2 °C, indicating that treatment with antifungal LAB in grass was less successful than that in maize. However, untreated grass silage had higher concentrations of butyric acid, an acid that reduces silage quality but increases aerobic stability.

In trials conducted at Medipharm with various additives containing MiLAB 393 or commercial strains without MiLAB 393 showed that MiLAB 393-based additives were more osmotolerant and tended to have higher aerobic stability when added to silage based on grass, whole-crop cereals and maize (Bruzelius & Zander-Jakobsson, 2002). Furthermore, the benefit of using MiLAB 393-based additives was highest when ensiling high DM grass (550 g/kg DM), while differences in fermentation and aerobic stability compared with commercial strains without MiLAB 393 were smaller when crops were ensiled at lower DM concentrations.

Conclusions

All trials, including large-scale trials and on-farm trials, in which MiLAB 393 was added alone or in combination with other strains indicated that the fermentation quality of the silage was excellent and that the strain performed well when high-DM crops were ensiled. Results on suppression of fungal growth have been variable, but generally positive compared with commercial Medipharm strains without MiLAB 393 or untreated silage.

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Grass Silage In Columbia

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The system to store fodders as silos was developed in countries as a practical solution to fulfill the needs of herbivorous animals during nutritious shortage due to the winter season. This system has permeated to countries without seasons and for the last 50 years we have been using it in our country. Colombia is one of those countries that is normally considered as being tropical but with the peculiar condition of being located in the equatorial zone. In the small equatorial zone where Colombia is located there are no dramatic temperature changes throughout the year but there are periods of droughts and periods of rains. The periods of droughts are not present in all zones during the same months and there are zones in which the lack of rain during certain part of the year will end up in economic losses; there are also zones in which the problem is an excess of rain and finally there are also zones where there are no problems and in which short rain and sunny days are permanent all year long.

In Colombia the environmental temperature constancy as well as the presence of enormous mountains, along with limiting with two seas - the Pacific and the Atlantic oceans , and its large number of rivers ranks Colombia as third in the world in water resources, also possessing one fifth of its area (200.000 km2) as forests. Part of Colombian land belongs to the Amazon system and another part to the Orinoco system, with continuous 12 hours night and 12 hours day, all conditions that help cataloguing Colombia as a very particular country.

For the above reasons we have found in Colombia 293 different frog species and 1780 bird species, but neither the frogs nor the birds are found within the same ecological niche; some of them grow and reproduce themselves into the forest, others are found in the mountains, others live in the plains, others near by the sea. Due to those special environmental conditions and for the last 200 years, 24000 native plants have been classified.

But Colombia was not a place where large herbivorous animals were to be found. Our land was not the place for enormous flocks of herbivorous animals such as those found in Central Africa or as the North American buffaloes.

It was not until 1541 that the Spanish colonizers brought cows into the country being the same route used form horses, chickens, hogs, sheep, goats as well as the cereal grains such as wheat, barley and oat. While those products entered the country, some native products left to other places as it was the case for potatoes, tomatoes, cocoa, corn, cassava, very hot spices and some special vegetables known to be used as good medicines.

But the environmental constancy and relative high humidity did not help only species used by the human kind, they are also the best environment for insects, as well as for the butterflies, mosquitoes, fungus and bacteria and as consequence of their presence there will be illness in all animals that share this environment.

Therefore in our country there is a continuous attack by plagues to plantations as well as to animals. Damage is comparatively higher than that found in other parts of the world as a result use of insecticides, fungicides and antibiotics in our plantations and animal exploitations would largely surpasses the quantities utilized in other countries.

Colombia is a country where its farmers are as efficient as any other farmers within their own country limitations and conditions, our productions are lower than those obtained in seasonal countries mainly because there is no way to compare productions in two totally different natural conditions and when productions are compared without taking into

account their differences in ecosystems, it is impossible not to make mistakes when interpretating of nature.

Therefore solutions proposed to our problems in agriculture might be different to those proposed in other countries. We may not simply apply to our land and to our products all the solutions found to be very suitable for solving problems in other countries.

We also believe that our proposals should not be accepted without objections by farmers living under conditions different than ours.

Nevertheless our country possesses 24 million heads of cattle that are fed in pasturelands in which grass from various origins grows. Most of the grasses we actually used as fodder for cattle have arrived from equatorial Africa. As an example the "kikuyu grass" (Pennisetum clandestinum) that we used as main grass in our high lands is original from the Kilimanjaro mount. Kenya's altitudes are as equatorial and as high as Colombian mountains and this grass found fertile land in our plateaus located to 2600 meters above sea level. On this grass we are grassing Holstein cows that if fed with grass alone will produce per lactation between 3 - 4 tons of milk. Pastures seeds have also been imported from England, Italy and the U.S. but they are only to be seed in the plateaus of the high mountains and they will never grow in the lower zones. In the lower zones (between 0 and 1000 m of altitude) mainly African varieties are growing and our native grasses are being moved away based on the greater load capacity and dry matter production of the African varieties.

Plateaus of our mountains being very similar to those found in Kenya are subjected to conditions quite different one to each other. Clouds covering our mountains are originated in the Amazon forest and are responsible for blocking the direct sun irradiation to our high plateaus during certain months of the year (rainy season) . During this time the total available solar energy that reaches the plant is lower than the solar energy required by the kikuyu grass for its complete metabolism. The relative deficiency in solar energy (compare with kikuyu grass requirements) has an enormous effect on the distribution of nitrogen fractions within the kikuyu grass: from the total nitrogen content in the kikuyu grass only an average of 50% can be attributed to protein nitrogen being the other half accounted by peptides, amino acids, nitrates and in smaller quantities other nitrogenous substances. Later in time and after a period of relative dry season, with high brightness days, with a low availability of fodder for grazing, the rain period arrives, pastures grow quickly but under conditions of low sun brightness due to the continuous presence of clouds, they accumulate large quantities of nitrates that acts as a poison to the animals that consume the grass and some cows may die. In Colombian high lands during clear days with no clouds the nocturnal loss of heat is enormous because it flees to space without being stopped by clouds. Freezing temperatures may go as low as -1º C at 06:00 and be as high as 18º C by 10:00. This fast unfreezing of the grass leaves does not aloud the cell walls to strech at the same velocity as it happens for the internal water; this phenomenon breaks the cells apart and "burns" all pasture. In our altitudes it is not possible to forecast when these lower temperature days will be present. The most you may have is a 24 hours advice but it may not happen any thing at all.

European grasses growing in the high plateaus are very capable of withstanding these freezing changes of temperature but under normal conditions the kikuyu pasture behaves a lot better; it is the one that is the predominant in those zones, due to its root system having lower fertilization and water demands requests and those characteristics resulting in a lower productive cost for grassing, albeit suffering enormously during this freezing phenomenon. The cattle raiser then should decide if his land is so prone to suffering from "burn grassing" that he has to invest in a costly and "new" grass.

In the low zones Holstein cattle is not found. The environmental conditions, especially the average temperature (28º C), the high relative humidity, diseases transmitted by ticks and mosquitoes and a longer period of drought are simply natural conditions not compatible with selected animals from European origin. Due to these conditions Zebu cattle proliferate. Milking is an important cash product and milk is usually provided by F1 crosses between Zebu cattle and Holstein cows. Milk production is lower in the crosses cows but as the total number of cows in the country is concern its number is larger that the Holstein cows. It has been calculated that 70% of the 5 million tons of milk produced yearly in Colombia is coming from cross cattle found in low lands. The milk producer uses low technology and milk composition has higher levels of protein and fat. The remaining yearly milk: 30% - 1.5 million annual tons – is produced in high lands.

To avoid the problems of production related to the effects on grasses of a longer than normal dry season and/or the effect of freezing fodders are stored as silos.

The storage of fodders is done primarily as silos and not as hay simply because rains or sunny days cannot be predicted in advance.

In some places – usually located in low land zones – a grass is seeded mixed with a legume. This mix will increase the average protein of the outcome. Grasses in Colombia are very different in their protein content. While in high land pastures protein varies between 14% and 20% (N x 6.25) in low land pastures the best ones will go up to 9% protein. Corn production as fodder for cattle is also different depending upon zones. High zones corn requires 200 days to be ready for making silage, while in the low zones corn can be cut to store in a silo in only 85 days. Corn will produces an appreciable amount of fodder by unit of surface but it very low nutrient density, low protein, high lignin, it has also few soluble carbohydrates but is very palatable feed and can be consumed in large quantities. Corn is made as silo in low lands and it is offered to milk producers located in the high lands but corn silo water content is high (aprox 70%) and transport is expensive, then in terms of kilogram of dry matter corn silo is quite expensive if transport is required.

Under these conditions grass silo is stored as close as possible to the place where it is to be used and grass that is normally grown is the one selected to produce the grass silo.

The fermentation process into a silo requires a fodder quite high in soluble carbohydrates. Pastures of the equatorial zones do not possess high levels of soluble carbohydrates. It is necessary to add some source of these carbohydrates, usually the molasses (by product of the sugar cane industry) is used in quantities of 30 – 40 kilograms by ton of green fodder. Molasses are dissolved in approximately twice as much volume of water and applied to the layers of fodder when the silo is being formed.

Losses in a silo can be very high and it has been calculated that dry matter losses can be between the 8% and the 50%, and distributed according to the following parameters

1.- Losses during the harvesting…….	3% to 14%
2.- Losses by respiration and aerobic fermentation……	5% to 18%
3.- Losses by efluyentes……..……	0% to 8%
4,. Losses during feeding……..…….	1% to 10%.

It is clear that the largest losses are found during the initial time after the silo has been finished and that the corresponds to the residual respiration of the recently cut plants and the initial aerobic fermentation of the silage.

Losses originated by the residual respiration obey to the oxidation of fodder sugars to CO_2 and water and they are impossible to avoid. The serious losses are those caused by the aerobic respiration, mediated by micro organisms which will be present if oxygen is high in their surroundings and pH is higher than 4. During fermentation in a regular silo these two processes are carry out simultaneously. During this process of packing the silo oxygen is diminished while pH is high (pH > 6.0) during this time aerobic micro organisms

will multiply and will produce heat (aerobic bacteria, yeast and fungi). Besides during this initial period free proteasas from the grass plants will act on proteins given rise to free amino acids and degrading them to ammonia and amines, this last change in terms of time will depends on rate al which pH drops.

Once the oxygen levels inside the silo are utilized by aerobic the micro organisms the anaerobic bacteria will increase in numbers these species will produce acetic acid, lactic acid, ethanol and CO_2 originated from the fermentation of simple sugars: glucose and fructose. Once the pH has begun to drop the predominant metabolite is lactic acid which is the main responsible for pH drops. pH will drop down to near 4 units moment at which the silage will be maintained in stable conditions if it remains without being disturbed.

The changes carried into a silo are identical if we used other processes like the "Silo press" or the plastic "balls" or "cylinders" systems of storage that are mechanically packed, covered by a plastic sheet and left directly on the grassing land. Plastic used in Colombia for making "Silo press" as well as for the "balls" and basic silos has to be specially treated so as to block large amounts of ultraviolet light. All these methods are utilized in the Colombia but basic silos (pressed with tractor weight and covered with a plastic lining) are most common.

We have carried research to diminish losses and costs in basic silos.

We will refer to a method used for storage certain foods that being important in human diets are producing more than one million metric tons of by products that under certain type of storage would make products for feeding animals.

Among the by products used to feed animals are: potatoes (Solanum tuberosum), cassava (Manihot sculenta), yam (Dioscorea spp) and carrots (Daucus carota).These as well as other products can be stored to be used as feed.

We have called this method LIQUID SILO.

As it can be recognized by LIQUID SILOS it is a system that help to storage feed in a medium that contains anaerobic bacteria, water and sources of soluble carbohydrates all under anaerobic conditions. Anaerobic bacteria are just contaminants which presence is helped by human manipulation, water soluble carbohydrates are by products of the sugar industry and the anaerobic conditions are obtained by pouring a small amount of vegetable oil on top of the water. This oil layer will act as a membrane that permits the exit for the gases produced into the silo but avoids the air entrance.

Under the conditions achieved in the LIQUID SILO the micro organisms that will form the basic bacterial contaminants are those found in the same material that will be ensiled and those normally present in the surrounded air. Anaerobic bacteria will multiply enormously, they will produce lactic acid and species will be selected by the effect of their own lactic acid production. Changes in pH in the LIQUID SILO was found after 7 days of initiated to be stabilized at 3.9 + /- 0.2.

Once the pH in the silo has been stabilized the extensive number of bacterial species diminished dramatically and almost all colonies belong to Lactobacilus planctarum.

The bacterial growth after 15 days was of 30×10^{11} UFC by ml

It holds that if a LIQUID SILO drops its pH to near 4 units then any product into the acidic and anaerobic solution will be subjected to the same conditions.

Potatoes and cassava were selected as by products to be used as be ensiled in LIQUID SILO.

To produce potatoes in a LIQUID SILO it is ideal is to use good quality potatoes. Each normal potatoes should be divided into 4 portions just because a normal recipient will hold a larger weight.

Once the portions of potatoes are deposited into the selected recipient a solution of molasses in water (10:90 w/w) is poured in an amount suitable to cover the potatoes layer with an excess of about 10 cm.

Vegetable oil is added in the amount needed to cover the water molasses solution with a layer of 4 mm thick.

If LIQUID SILO is to be used as to storage carrots or cassava it is not needed to add molasses when making the system as the soluble sugars in carrots and in cassava are enough to supply the bacterial requirements.

Rain should be avoided on the LIQUID SILO as excess water would pour the oil out.

LIQUID SILOS could be used for feeding animals as soon as they are finished but after 20 days the LIQUID SILO contents are ensiled.

To remove the product that has been ensiled as LIQUID SILO oil should be withdraw before taking any of the contents out. Once the selected amount has been taken out the layer of oil initially withdraw must be poured again.

LIQUID SILO as a solution of water molasses (90:10 w/w) contaminated with micro organisms by washing a hand full of grass into the solution and covered with the oily layer of 4 mm, will show a pH drop to 4 after 10 days.

The above solution after 10 days will contain more than 20×10^{1} micro organisms per ml. Most of them Lactobacillus plantarum.

The LIQUID SILO after 10 days, prepared as stated before may be used as anaerobic bacteria inoculant's to grass silos when the silo is initially formed.

By the use of LIQUID SILO solution in grass silage it is possible to decrease losses due to aerobic fermentation.

Production of high-quality silages for horses

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Introduction

Overproduction of food within EU is a great economic and social problem. Agreements to reduce food production and convert fertile land to other use and activities will be asked for. During the latest decades the interest in horse sports has increased specifically among girls and a number of farms has changed from food production to horse raising. This keeps the landscape open and the cultural and social life running.

The Horse Industry in the European Union

The number of horses is rapidly increasing and the latest numbers close to 6 million corresponding to 12,5 inhabitants per horse. The leisure of riders 6.5 million, the number of riding schools about 20.000 and members in riding schools 2.7 million.

The social importance is also growing. By utilizing farms on the country side the villages will still be kept alive. The horse sport is mainly a girl sport – in Sweden the same number of girls are riding as boys playing soccer – around 500.000. In economic terms horse breeding is of the same economic dignity as pig production (480 million Euro) on the agricultural side and 2.7 milliard Euro totally. There is lack of information on the educational side and big differences between countries in education capacity. During the decrease period of horse breeding you lost a lot of experiences and basic knowledge. The destribution of horses also have changed from beeing part of a farm animal to horse farms only why knew conservation, feeding and logistic strategies has to be developed.

Production of horse feed

- Forage intake in relation to conservation systems – risks and possibilities
  Weather related problems
  Forage related problems
  Hay production
  Silage production

- Silage technology for horses
  Stage of maturity – nutrient concentration – DM content
  Ensiling in bales – density – costs – storage stability – stretch film quality – use of silage additives

- Feeding silage to horses
  Palatability
  Silage quality
  Future challanges

Table 1: Number of horses per 1000 inhabitants within EU member states. (Final Report, EU Equus 2001 – The Horse Industry in the EU Union).

Member state	**Tot number of horses**	**Inhabitants**	**Horses per 1000.**
Austria	**81.900**	**8.200.000**	**10**
Belgium	**200-250.000**	**10.200.000**	**22**
Denmark	**150.000**	**5.300.000**	**28.3**
Germany	**1.000.000**	**82.000.000**	**12.2**
	1.200.000 (later estimates)		
Greece	**35.000**	**10.600.000**	**3.3**
Spain	**350.000**	**39.600.000**	**8.8**
Finland	**57.400**	**5.200.000**	**11.0**
France	**452.000**	**59.100.000**	**7.7**
Ireland	**60.000**	**3.700.000**	**16.2**
Italy	**323.000**	**57.300.000**	**5.6**
Netherlands	**400.000**	**15.800.000**	**25.3**
Polen	**27.000**	**9.900.000**	**2.5**
Sweden	**280.000**	**9.000.000**	**31.1**
UK	**965.000**	**58.800.000**	**16.4**
Total	**4.676.000**	**375.331.000**	**12.5**

Table 2: The relation between feed ration and use of the agricultural area for horse production. (Final Report, EU Equus 2001 – The Horse Industry in the EU Union).

Ration A = 8 kg hay and 2 kg oats per day. B = 4 kg hay, 2 kg straw and 4 kg oats. Based on 165 grazing days and 200 days indoors. Horse live weight, 500 kg. Percent of the total agicultural area used for horses.

Member state	Ration A	Ration B
Austria	2.4	1.9
Belgium	11.8	9.8
Denmark	5.4	4.1
Germany	4.2	3.5
Greece	1.7	1.5
Spain	2.5	2.4
Finland	3.0	2.5
France	1.2	1.1
Ireland	0.9	0.7
Italy	3.5	3.2
Netherlands	14.0	11.4
Polen	1.7	1.8
Sweden	9.7	7.6 (estimates 2004)
UK	4.1	3.3
Total	3.15	2.68

Table 3: The number of leisure riders and riding schools.

Member state	Leisure riders	Number of riding schools	Members in riding schools
Austria	**200.000**	**1.100**	**80.000**
Belgium	**-**	**800**	**15.000**
Denmark	**100.000**	**500**	**70.000**
Germany	**> 2.000.000**	**5.000**	**100.000**
Greece	**-**	**-**	**-**
Spain	**100.000**	**273**	**-**
Finland	**60.000**	**500**	**26.000**
France	**600.000**	**5.939**	**426.000**
Ireland	**30.000**	**-**	**-**
Italy	**50 – 70.000**	**1.200**	**25.000**
Netherlands	**400.000**	**1.000**	**260.000**
Polen	**-**	**400**	**700**
Sweden	**500.000**	**600**	**215.000**
UK	**2.400.000**	**2.280**	**1.440.000**
Total	**6.460.000**	**19.592**	**2.657.700**

Comments:

In Sweden 500.000 boys are football players and 65.000 licenced ishockey players why 85 % of the riders are girls = 425.000. 80 % of the horse industry is owned / lead of girls). This industry is very important in keeping the landscape open and is a great part of the farmland economy. Even within EU this development has a high priority !!

Table 4: Existing possibilities to attand formal education in the horse area and the number of graduates from such programmes in year 2000.

Member state	Upper secondary	Higher education
	reported number of graduates	reported number of graduates
Austria	**yes**	**-**
Belgium	**yes – 60**	**no**
Denmark	**no**	**-**
Germany	**no**	**yes - 12**
Spain	**no**	**yes**
Finland	**yes – 230**	**yes - 12**
France	**yes – 1185**	**no**
Ireland	**yes**	**yes**
Italy	**no**	**no**
Netherlands	**yes – 60**	**yes - 10**
Polen	**yes**	**yes – 24 + 23**
Sweden	**yes – 300**	**yes – 35 + 55 (riding / trotting)**
UK	**yes – 5000**	**yes – 600 - 1000**

Comments:

After the hugh decrease in horse interest after the second world war a lot of knowledge has disappeared. The increasing interest in horse activities both as a possibility of living but also in training and competition has put a new attiontion to the lack of feeding and training the horse. In Sweden the horse buisness is increasing and need of knowledge and information great. A specific foundation for horse activities is also astablished to stimulate the youngsters interest and healthy sport activities. We look forward to collaborate in a larger program within EU.

Forage intake in relation to conservation systems – risks and possibilities

Three important points will influence the forage value and choice of conservation systems:

a) Harvest and conservation at an optimal stage of maturity / nutritive value. The value will rapidly decrease from 200 Euro per ton silage DM to 100.

b) Secure an acceptable hygienic quality. Spores of clostridium tyrobutyricum in milk reduces the milkprice / silage DM value by 45 Euro.
c) Reduce nutrive losses from harvest to feeding. A problematic weather only can cost 90 Euro per ha.

Weather related problems

Weather conditions will influence the harvesting time and the possibility to make high quality hay. Data from the last years will explain the great importance of a flexible conservation possibility.

Table 1: Forage preservation in Sweden 1990 to 2004.

System	1990	1998	1999	2000	2004
Hay	30 %	5 %*	20 %	10 %*	10 – 15 %*
Bunker silo 20	20	20	20	25	
Tower 20	20	20	20	20	
Bale silage	30	55	40	50	50
Ton x 10^3 stretch film:					
	6	9	8	10	11

* Problematic weather conditions

The new bale technology has increased because of its flexibility and heavy competion in relation to investments in permanant silos and machinery to avoid hygienic quality problems and reduce losses.

Forage related problems

A studie of 2817 horses distributed over a number of farms / riding schools around the Stockholm / Uppsala area, most of them half blood riding horses were examined by a questionare. The results are presented below. 59 % fed hay only, 14 % silage / haylage only and 27 % both hay and silage. 29 % of the managers had noticed health problems. Mouldy hay was the main problem but loosen manure when changing from hay to silage often occurd.

Table 2: Number of health disturbances on farm level.

Health problem	Number of occations
Diarrhoea	17
Fertility	0
Respiration	25
Laminites	2
Reduced intake	9
Botulin	2
Allergic symtom	4
Other	10

Data from assurance companies show the same pattern. **Respiration** problems cost a lot of money – **botulin is not** a problem.

Bale size is another promblem. A restricted amount of horses on a farm cannot consume a big bale of 300 kg DM in a reasonable time, why the silage will heat and get mouldy during a mild autumn. There is a demand of small bales – something that has to be developed. Silage additives can reduce the problem.

The horses preferens to conserved forage ??

The first harvest from a pure grassward was preserved as

a) Barn dried hay – 86 % DM
b) Bale silage – 35 % DM
c) Bale silage – 55 % DM
d) Bale silage - 70 % DM

and individually fed to four horses. All forages were fed in four separate boxes to each horse. The places of the boxes were changed before every feeding period during fourteen days experiment period after an one week adoption period.

The horses preferenses were:

a) Silage of 35 % DM
b) Silage of 55 % DM
c) Silage of 70 % DM = hay. The horses avoided the hay more than the silages

Hay production

Hay of good nutritional compositon and without hygienic quality problems will be prefered of many horse owners with one or two horses. Its easy to handle and store and the DM content does not vary why it is easy to predict the amount to be fed.

The sensiblity to weather conditions makes it difficult to harvest at an optimal stage of maturity, why the nutritive value can vary between years and harvests. The main problem is to discover its hygienic quality. During the latest years foggy autumns have caused mould growth of high quality barned dried hay during storage, which has increased the interest in using bale silage. See table 5.

Silage production

The estimated use of bale silage to horses in Sweden is 50 % of the forage ration / producers (2004). Bale silage has been excepted by the horse industry.

Many horse owners are afraid of wet silage – often because of the smell but also through advice from many vets. *Clostridium Botulinum* has been pointed out as a big risk. This cannot be prooved from statisics (table 6 and the preferens study).

The general opinion on stage of maturity / nutritive value is that forages shall be late cut / rough material of low crude protein content. High wilting of this crops is hard to dense and will easily puncture the stretch film. The silage get mouldy.

New experiments will demonstrate the possibility to feed high quality silage decreasing the amount of concentrate and leading to cheeper rations and more healthy horses. Bed weather conditions resulting in mouldy hay have resulted in a change to use of silage and convinced many horseowners.

Silage hygienic quality is of specific importance in the horse market. You cannot always regulate the weather condintions why some silages has to be baled at lover DM content than wanted. The late development of silage additives adopted to the long cut forage processed through the balers can overcome this quality problems.

- **Silage technology for horses**
  Stage of maturity – nutrient concentration – DM content
  Ensiling in bales: bale system – bale density – stretch film quality / number of layers – use of silage additives – storage stability – costs

The main bale silage is produsedon an ordinary farm and transported to the horse stables. Bale silage is the base in this production chain. As ensiling is an anaerobic process the forage density / lack of oxygen within the bale is very important. Several factors is of importance in such a system.

The crop. No baler can reduce the moisture content during baling. Crop DM has the strongest impact on bale density – R^2 = 0. 75 – 0.85. Crop maturity and crop structure cover 5 – 15 %. As early cut forage also gives higher nutritive value this is a positiv economic factor.

Table 3. Relation between crop DM, bale density, number of bales per ton DM and film requiered. Round fix and flex chamber balers – 120 x 120 cm size.

	Forage DM, g per kg fresh forage		
	200	350	500
Bale density, kg DM, m^{-3}	100 – 105	135 – 160	200 – 240
Number of bales per ton DM 7.5	4.6	3.2	
Kg stretch film per ton DM	8.3	5.1	3.5

The bale systems also influence bale density

Fix or flex chamber balers without or with knives can improve the silage process and the packing capacity.

Square bales are preferred for long transport distances. The bale corner are sensible to film puncture why the producers will use 12 – 16 layers. Small high density square bales are useful when few horses have to be fed. C. Müller, 2005 got good results with small bales (Welger AP 730 and New Holland 575) when the bale weight exeeded 35 kg. Comparing 6, 8 and 10 layers of stretch film and using Lactic Acid Bacteria and Kofasil Ultra she improved the gas tightnes by increasing the number of layers and improved the silage quality and storage stability by use of additives. Kofasil Ultra gave the most consistent results. The method needs a lot of labour !!

Moving / conditioning – wilting quality

One very sensitve factor is the DM variation in a windrow before baling. The baler cannot distribute the forage within the baler during baling. If the DM content is 350 – 400 g on average the bottom part often is 200 and the topp 500 g DM per kg fresh forage. 200 g DM of long cut material will ferment very slowly why clostridie fermentation take place and part of the bale has a bad hygienic quality. One good example is presented in table 8.

Table 4: Untreated and Kofasil Ultra treated, 4 ltr per ton crop, baled silage. Results from 10 bales per treatment.

	Control		Kofasil Ultra	LSD p< 0.05
DM, g,kg FM	242		250	
pH	4.79		4.60	0.20
NH_3 – N, g/kg total – N	154		76	15.1
G per kg DM:				
WSC	3.8		26.3	4.9
Lactic acid	30.5		43.2	8.6
Ethanol 12.7	4.9		3.3	
Butyric acid	17.1	< 0.3	3.7	
Clostridie spores per g				
Silage	1.259.000	< 100		

Stretch film quality.

Oxygen leakage into a bale will both cause nutrient losses and hygienic quality problems. The stretch film must keep a resistant tightness of CO_2 within the bale and prevent a permeation of O_2 into the bale. Film colour is important. A white film will reflect sunshine and keep the surface cold / tight while a black film will increase the permeation. As high temperature also stimulate growth of undisired bacteria and mould a black film will cause losses and hygienic quality problems. A good example is presented in table 9.

Table 5: Nutrient losses in relation to use of silage additives and film colour.

Baler: Krone fixed chamber. Wrapper: Kverneland Sila Wrap 7556.

Film: Triowrapp 750 mm x 25 µm x 65 % prestretching.

Crop: Ryegrass 460 g DM/kg FW; 107 g CP, 177 g WSC, 11 MJ per kg DM.

Treatment: with without and with 4 l Kofasil Ultra per ton FW. 30 bales per treatment.

	Nutrient losses, Kg / t silage DM.		
Film	No additive	Kofasil Ultra	Difference
Black x 6 layers	90	65	25
White 58	41	17	
Difference	32	24	
LSD p< 0.05			5.2

If the nutrient is compared with molasses one kg Ne loss costs 0.33 Euro.

Oxygen leakage in relation to use of stretch film in combination with Kofasil Ultra.

Table 6: Nutrient losses in relation to use of stretch film.

Baler: Krone fixed chamber. Wrapper: Kverneland Sila Wrap 7556.

Film: Triowrapp 750 mm x 25 µm x 65 % prestretching.

Crop: Ryegrass 610 g DM/kg FW; 107 g CP, 177 g WSC, 11 MJ per kg DM.

Treatment: with without and with 4 l Kofasil Ultra per ton FW. 30 bales per treatment.

	Nutrient losses, Kg / t silage DM.		
Film	No additive	Kofasil Ultra	Difference
White x 4 layers	63	38	25
White x 6 layers	39	21	18
White x 8 layers	38	23	15
LSD $p< 0.05$			3.5

What is the explanation ??

Table 7: Relation between round bale systems (New Holland 658 and Claas 255), crop maturity, DM content and silage density / nutrient losses. 8 layers of Rani Wrap are used. (Olsson, M., Willson, D & Lingvall, P. 2003)

Baler	DM, g	Volyme, m^3	Density, kg DM,m^3	Losses per bale, kg	Nutrient losses, kg/ton DM
Crop A. G per kg DM: CP – 149, Ash – 89, NDF – 455, MJ (ME) – 10.3					
Flex chamber	401	1.65	174	6.1	54
Fix chamber	395	1.80	167	6.8	57
Crop B. G per kg DM: CP – 174, Ash – 100, NDF – 390, MJ (ME) – 10.5					
Flex chamber	465	1.65	220	6.4	42
Fix chamber	531	1.79	218	6.3	41
Flex chamber, 8 km	578	1.66	261	5.8	31
10 km	613	1.67	241	6.1	34
Fix chamber, 8 km	523	1.81	219	7.7	49
10 km	539	1.82	220	6.5	38
$LSD^{P>0.05}$	29	0.04	14.2	1.4	9.2

Table 8: Comparison of two stretch film qualities and 6 or 8 number of layers on external damages and nutrient losses. 16 bales per treatment. (Jacobsson, F & Lingvall, P. 2002)

	Trio base		Horse wrap		
Number of layers	6	8	6	8	$LSD^{P>0.05}$
DM g per kg forage	539	564	556	565	6.5
Density, kg DM per m^3	218	219	197	217	21.8
Tightness in seconds	40	102	63	105	
Surface damage, % of the bale surface	9.2	0.6	2.9	0.4	4.4
Nutrient losses, kg per ton Silage DM	152	67	92	65	23.5
Estimated as surface Losses	93	8	29	8	13.7

Conclusions

Part 1.

- Claas 255 will expand the volyme when the chamber is filled – the density will be reduced!
- The differenses within treatments between bales in DM was not expected.
- The bale density is related to the fiber content and ranged from 167 to 261 kg DM per m^3.
- High wilted forage baled in New Holland 658 reduced the bale density when the speed increased.
- The Claas 255 never exeeded 220 kg DM per m^3
- Our hypothesis is that the losses is related to the size of the bale surface and the gas permeation through the stretch film. The permeation is related to the film quality, number of layers and the surface temperature. The variation in weight losses is very small. The nutrient losses will reflect the amount of forage in the bale. 95 % of the bales were extreemly tight when meassured before opening the bales for examination.

Part 2.

- Bale tightness is mainly related to the number of layers used.
- Surface damage is totally avoided by use of eight layers of stretch film.
- The higher stretch film quality of Horse Wrap film reduced the nutrient losses from 152 to 65 kg per ton silage DM compared to six layers of Trio base corresponding to 3.9 Euro per bale when used to a highly nutritional well shaped round bale !!

Palatability

Silage versus hay is already discussed. The relation between crop composition, DM content and feed intake of silage to horses have to be examened as problems are available in the litterature without any clear explanation. The market will not be safe until they feel secure.

Silage quality

From the field study in Sweden it is clear that silage hygienic quality is of importance. A.T. Ursin and M. Johannessen, 2004, Norway, has studied palatability and feed intake of bale silage without additive and after use of GrasAAT – Plus and Kofasil Ultra. There were small differences in chemical composition and fermentation pattern but significant differences in feed intake between the additive treatments.

Table 9: DM intake of silage fed without any concentrate to 5 Norvegian cold blood trotters – 500 – 550 kg live weight.

	Control	Grassat Plus	Kofasil Ultra
Application rate, l / ton FW	-	3 – 5	3.5
DM, g per kg FW	420	460	490
pH	4.3	4.5	4.6
NH_3-N, g per kg Tot – N	89	82	76
g per kg DM:			
Ash	6.3	6.7	6.7
Ethanol 5.6	3.2	1.6	
Lactic acid	24.2	16.1	17.9
Acetic acid	4.2	2.8	3.5
Butyric acid	-	-	-
Feed intake, kg DM per day	10.2	11.0	11.9 $p< 0.01$

The WSC – content is not published but you can expect a higher content after use of Kofasil Ultra. The very rapid effect of Kofasil Ultra when a bale is closed will reduce both respiration and bacterial losses.

Future challenges

The increasing interest in horse sports is of great value for the society. A better understanding of feeding forage to horses can reduse many of the digestion disturbences among horses fed to much starch. At the same time larger grass and grazing areas can be used to keep our traditinal landscape open and conserved in an efficient and economic way.

Most of the present information looks promising. There are alredy activities started in improving the utilization of forages to horses. Now we look forward to start a collaboration within Europé to utilize the research capacity and the knowledge in a scientific and economic way. As a consequense of the Swedish experiences more than 50 % of the total horses are fed high quality wilted and baled silages as the only feed. Until now we cannot se any problems with silage in rations for competing trotters and riding horses.

Acknowledgement

A number of foundations and companies have supported us in our work on silage for horses. Those are Addcon Agrar GmbH / Hanson & Mohring LTD, BPI / UK, DOW Chemical Switzerland, The Kverneland Group europé, Agri Pac Scandinavia, Trioplast LTD / Sweden, Welger GmbH / Germany, Medipharm LTD / Sweden, Perstorp Special Chemicals / Sweden Tellefsdal A.S. / Norway, Kungsaengens Res. Centre, Uppsala, Sweden and Dep. of Agric. Biosystems and Technology, Alnarp, Sweden.

FORAGE PRODUCTION

Quality of Meadow Forage as Related to the Level of Fertilisation, Exploitation a Their Mutual Interactions

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Introduction

The paper deals with effects of intensity of exploitation, level of fertilisation and their interactions on contents of crude protein, fibre, PDIE, PDIN and energy (NEL/MJ) in forage harvested on meadow stands ot the Cynosuretum type.

Problems associated with the effect of NPK fertilisation, exploitation and, especially, of their intensifying effects on productive and qualitative characteristics of grassland stands has been permanently discussed in many scientific papers (KOHOUTEK et al. 2003; BUCHGRABER, HRABĚ 2001). Special attention was paid to an early date of the first cut, which significantly influences the concentration of nutrients (esp. NEL > 6.0 MJ.kg $^{-1}$ DM) and qualitative yields per unit area (HRABĚ, BUCHGRABER 2004) as well as to the appealness of fodder for cattle (WEISSBACH 2001). It is also necessary to mention the importance of research concerning extensive use of grassland stands when applying the system of extended autumn grazing and/or winter rearing of cattle (OPITZ von BOBERFELD et al. 2006; HOCHBERG 1998; SKLÁDANKA, HRABĚ 2003), which takes into account not only a decrease in nutrient concentration but also hygienic aspects of fodder intake when applying this specific system of stand exploitation.

Material and methods

Experimental plots are situated in a potato-growing region in the Field Research Station Vatín, county Žďár n. Sázavou (altitude 540 m; annual sum of precipitation 736 mm – 440 mm during the growing season; average annual temeprature 6.1°C – 12.6 °C during the growing season). Soil conditions: fluvial modal loamy gley, developed on an non-carbonate alluvial sediment (Glmf), with the groundwater level 0.72 m. Stand composition in the time of stand establishment (2003) – mesophylous TTP association corresponding with the Cynosurion union.

Experimental variants:

Intensity of stand exploitation:	1 (I)	four cuttings (15 May; 30 June; 15 August; 30 Sept.)
	2 (SI)	three cuttings (30 May; 30 July; 30 Sept.)
	3 (MI)	two cuttings – early (15 June; 15 Sept.)
	4 (E)	two cuttings – late (30 June; 30 Sept.)
Intensity of fertilisation:	A	no NPK
	B	$P_{30} + K_{60}$ kg.ha^{-1}
	C	$N_{90} + P_{30} + K_{60}$ kg.ha^{-1}
	D	$N_{180} + P_{30} + K_{60}$ kg.ha^{-1}

Application of fertilisers: P+K in the form of superphosphate and potassium salt in the spring; N-fertilisation: Variant 1 (four cuttings, intensive expoitation) and Variant 2 (three cuttings, medium intensity of exploitation) - 30 (60) kg in the spring, 30 (60) kg

after the 1st cutting and 30 (60) kg after the 2nd cutting; in Variants 3 (low intensity, early) and 4 (extensive, late) – 45 (90) kg in the spring and 45 (90) kg after the 1st cutting.

Tabulated qualitative characteristics under study were estiimated using the NIRS method and represent means of four replications. Regarding the limited extent of this paper only average values of corresponding cuttings are presented as well as averages of both experimental years (2004 and 2005). Differences between averages were evaluated using the method of variance analysis.

Results and discussion

As far as the effect of intensity of exploitation was concerned (expressed by the number of cuttings and the date of cutting - Tab. 1) it is obvious that all qualitative characteristics of the system of 4 cuttings (1st cutting on 15 May) were significantly better than those of variants with three and/or two cuttings. The same is valid when evaluating differences between variants with three and two cuttings. No differences were found out between the early and the late variants of harvests on stands with two cuttings. In fodder from extensively exploited two-cut stands, the contents of crude protein (111-116 $g.kg^{-1}$ DM) and PDIN were lower by approximately 1/3 and the concentration of PDIE was decreased by rel. 10%. On the other hand, the concentration of fibre was higher (263-264 $g.kg^{-1}$ DM). Fodder harvested in systems with three and four cuttings met cattle requirements for energy content (NEL 5.4-5.6 $MJ.kg^{-1}$ DM). In extensive systems with two cuttings, the concentration of energy was relatively low (4.9 $MJ .kg^{-1}$ DM).

The effect of intensity of fertilisaton on qualitative parameters should be evaluated individually for each experimental variant. As comapred with other variants, in the variant with a high level of N-fertilisation (180 $kg.ha^{-1}$), the concentration of N-substances (crude protein, PDIN, PDIE) was significantly higher. It is surprising that there were no differences in conentrations of both nitrogen compounds and fibre and energy between variants with no fertilisation and with a lower dose of N (90 $kg.ha^{-1}$). Fodder of low quality was harvested in variants with application of P+K only.

Results of research, which demonstrated a significant effect of increasing doses of N+PK on fibre concentration and their effect (and especially of N) on the content of energy (from 5.4 MJ in the variant without dressing to 5.1 MJ in the variant with N-doses 180 $kg.ha^{-1}$) were corroborated. Minimum differences in fodder quality harvested on non-fertilised meadow stands and those with a low dose of nitrogen could be inluenced, with the exception of a higher percentage of *T. repens* in fodder harvested in the variant without dressing (Tab. 3), also by an increased proportion of *Festuca arundinacea*, i.e. a species with a lower fodder quality; its share in fodder harvested on N-fertilised plots was as much as 17,7% while in stands without fertilisaton it was only 8.2% (1st cuttings).

Table 1: Concentration of nutrients in fodder from variants with a different intensity of exploitation and with different levels of fertilisation. (Vatín 2004-2005)

Intensity of exploitation	CP	CF	PDIN	PDIE	NEL	NEV	Level of fertilisation	CP	CF	PDIN	PDIE	NEL	NEV
	($g.kg^{-1}$ DM)				($MJ.kg^{-1}$ DM)			($g.kg^{-1}$ DM)				($MJ.kg^{-1}$ DM)	
1	161,6	196,1	96,4	85,2	5,63	5,52	A	130,7	223,7	77,8	79,9	5,38	5,21
2	141,7	224,2	83,9	81,9	5,39	5,22	B	118,2	239,8	69,7	78,1	5,15	4,93
3	116,2	263,7	68,2	76,6	4,93	4,65	C	130,1	245,4	77,6	79,4	5,17	4,95
4	111,1	264,6	64,8	75,0	4,88	4,60	D	151,6	239,8	88,2	81,3	5,14	4,89
Average	132,6	237,1	78,3	79,7	5,21	5,00	prům.	132,6	237,1	78,3	79,7	5,21	5,00
$D_{T(0,05)}$	7,43	7,11	4,03	1,14	0,06	0,08	$D_{T(0,05)}$	7,43	7,11	4,03	1,14	0,06	0,08
$D_{T(0,01)}$	9,06	8,67	4,91	1,39	0,08	0,09	$D_{T(0,01)}$	9,06	8,67	4,91	1,39	0,08	0,09

The uniformity of quality of fodder harvested in the variants without fertilisation and with a low supply of N (90 $kg.ha^{-1}$) as well as with the application of P+K only should be evaluated with regard to differences in the share of clover species in harvested fodder (Tab. 2).

Table 2: The share of clover species (%) in fodder harvested in the 1st cutting in individual variants of fertilisation. (Vatín 2004-2005)

Variant of fertilisation	Share of clover spec. (%) in harvestet forage			
	2004 - total	1st cut 2004	2005 -total	1st cut 2005
A	11,0	8,9	8,8	5,9
B	6,4	4,6	6,4	4,3
C	2,1	2,0	1,6	1,0
D	2,5	2,3	1,4	1,3

However, from the practical point of view the evaluation of results concerning interactions of both factors (i.e. intensity of fertilisation and intensity of exploitation) is more favourable. As shown in Tab. 3, where means of all 16 variants are presented, an above-average quality of fodder was recorded in all stands with four cuttings (and at all levels of fertilisation); similar results were obtained also in variants with 3 cuttings (with the exception of concentration of N-substances in variants with no fertilisation and with the application of P+K only).

Table 3 Mean concentrations of nutrients in fodder harvested in all combinations of the intensity of exploitation and fertilisation – average values of all 16 variants. (Vatín 2004-2005)

Parametr	Nutrient ($g.kg^{-1}$ DM, NEL/ MJ DM)				
	CP	CF	PDIN	PDIE	NEL
Average Range	132,6 94,2-172,6	237,1 285,2-187,2	78,3 56,6-102,0	79,7 73,8-86,9	5,2 4,7-5,8
Dt 0,01	23,5	22,5	12,7	3,6	0,2

Conclusion

Results obtained within the period of 2004-2005 corroborated a significant effect of an increased intensity of exploitation (4 and 3 cuttings *vs.* 2 cuttings) on fodder quality (i.e. concentration of nutrients) harvested in variants without fertilisaton and with application of P+K only and, especially after the appliccation of 90 kg and 180 kg

N. ha^{-1} + PK. In ther variant with a high level of fertilisation (180 $kg.ha^{-1}$), concentration of nutrients was significantly higher than in all other variants of fertilisation. However, no significant differences in concentration of nutrients were found between variants with a lower supply of N (i.e. 90 $kg.ha^{-1}$) and without NPK. As far as the interaction of both factors (i.e. exploitation x fertilisation) was concerned, fodder of significantly higher quality was harvested in varinats with four and three cuttings than in extensive variants with two cuttings only. The effect of dominance (i.e. percentages) of clover species and of *Festuca arundinacea* was discussed as well.

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Literature can be provided on request

Production, persistence and quality of forage of legumes in conditions of the Czech Republic

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Summary

At the Jevíčko site in the Czech Republic (CR) we observed 6 legumes varieties in accurate trials which were sown in 1986 and we evaluated production ability and persistence of seven varieties and of a newly bred variety of *Trifolium pratense* L. (*TP*), of five varieties of *Trifolium repens* (*TR*), of two varieties of *Trifolium hybridum* L. (*TH*), of three varieties of *Medicago sativa* L. (*MS*), of one variety of *Medicago lupulina* (*ML*) and of one variety of *Lotus corniculatus* (*LC*). Legume species were sown as monoculture and cut three times. Evaluation of the system comprised determination of dry matter production, botanical composition of the stand and forage quality by Weenden analysis, OMD. Among evaluated legumes the most productive are *MS* and *TP*, medium productive are *LC* and *TH* followed by *TR*. The least productive was black medick. The most persistent is MS (8 years), *TP* was persistent for 3 years, *TR* was persistent three to five years depending on variety, *TH* three years, *LC* three years, *ML* only a year.

Keywords: legumes, yield, persistence, Weenden analysis, OMD

Introduction

The increase of milk production of cows in The Czech republic requires raising forage quality and concentration of energy in forage. Legumes, which make a stable component of feeding ration, were grown on 317 thousand ha in 1990 and consisted of (a) red clover on the area of 170 thousand ha with the yield of 10.7 t/ha of fodder and (b) lucerne on 147 thousand ha with the yield of 9.2 t/ha of fodder. In 2002 the area of legumes was 142 thousand ha, out of which (a) red clover covered 59 thousand ha with the yield of 8.6 t/ha of fodder and (b) lucerne on 83 thousand ha with the yield of 7.9 t/ha of fodder.

Materials and methods

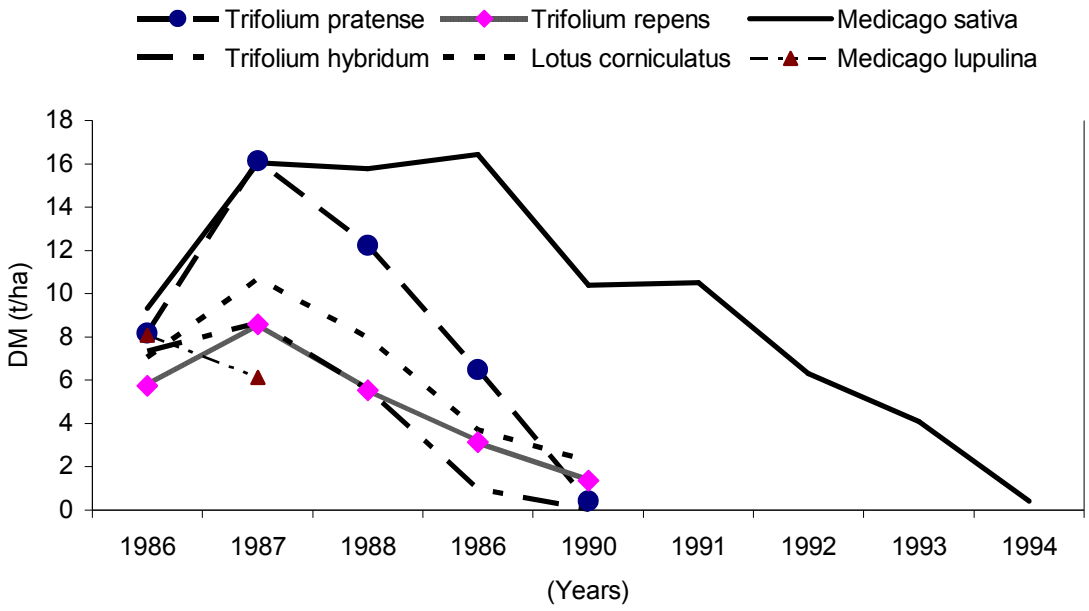
At the Jevíčko site in the Czech Republic (CR) we observed 6 legumes varieties in accurate trials which were sown in 1986 and we evaluated production ability and persistence of seven varieties and of a newly bred variety of *Trifolium pratense* L. (*TP*), of five varieties of *Trifolium repens* (*TR*), of two varieties of *Trifolium hybridum* L. (*TH*), of three varieties of *Medicago sativa* L. (*MS*), of one variety of *Medicago lupulina* (*ML*) and of one variety of *Lotus corniculatus* (*LC*). Accurate small plot trials with legumes were established by grassland sward renovation at the Jevičko site (335 m above sea level, average annual temperature 7.5 °C, average annual precipitation 629 mm) in The Czech republic. The type of soil is fluvisoil. The trial was fertilised with 50 kg/ha of nitrogen in the sowing year, in production years with 60 kg/ha of nitrogen, 35 kg/ha of phosphorus and 100 kg/ha of potassium in autumn. The legumes were cut three

times. Our contribution evaluates dry matter production of sown varieties and their persistence (observation during the occurrence of variety in the plot), concentration of nutrients by Weenden analysis and methods of wet chemistry, digestibility of organic matter according to Tilley & Terry (1963), modified by Lampeter (1970) and concentration of net energy determined by calculation (Sommer et al., 1994). The acquired results of dry matter production were evaluated by variance analysis.

Results and discussion

Among the evaluated legumes (fig. 1) was *MS* the most persistent (8 years) and productive (yield in the first three years 15.77 – 16.42 t/ha).

Figure 1: Dry matter production of leguminous varieties



The second most productive legume is *TP* with the highest yield in the first harvest year (yield 16.1 t/ha). Concentration of net energy reaches 5.75 – 6.00 MJ NEL/kg of DM in *TP*, *TR* and *TH*, in *MS* and *ML* it is cca by 0.2 – 0.4 MJ/kg of DM decreased, because during growth period they age faster and to reach the optimal quality four cuts are required. CP concentration is highest in *TR* (214.2 g/kg of DM), in other legumes it is between 180 – 190 g/kg of DM, which is optimal mainly for highly productive dairy cows. OMD in highest in *TR* (75.6 %) and is vere good in *TH*, *TP*, *LC* and *ML* (year of sowing and first harvest year). Concentration of fibre (CF) is very favourable, it is among 183.4 – 214.3 g/kg of DM, it only higher in *MS* (248.9) and *LC* (229.0).

Conclusions

In the CR legumes are used not only as pure stand on arable land, but also in legumegrass mixtures and for strip-seeding into grasslands. Main legumes are red clover, lucerne and white clover.

Acknowledgements

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Production and quality of forage from grass species intended for extended grazing season of suckler cows in conditions of the Czech Republic

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Summary

In the last decade suckler cows' breeding has been progressively developing in conditions of the Czech Republic. One of the possibilities is to extend grazing season in autumn, in case of mild winter grazing could be taken into account in winter months. Therefore in 1997 and 1999 there were at the Jevíčko site in the Czech Republic established accurate small plot trials with selected grass species (*Dactylis glomerata, Lolium perenne, Festuca arundinacea, Festuca pratensis,* genus hybrids and *Bromus marginatus*) which were intended for harvest in extended grazing season (from October till April of the following year). We observed the growth after the second harvest in three time series (2001-2003) in the middle of July and August. In our paper we evaluate these features: DM and concentration of NEV, NEL, CP and CF by infrared spectrometry.

Keywords: extended grazing season, grasses, forage quality, NIR spectrometry

Introduction

In the Czech Republic there has been a progressive development of suckler cow production in the last decade. The number of heads was 140 thousand in 2004, in 1990 there wrere almost no suckler cows. Effective management requires among other s to decrease running costs. This is possible by year-round grazing. Preparation of grazing vegetation for utilization in winter season is based on carrying out the last summer cutting in June or july, or possibly in August (Opitz von Boberfeld, 1997).

Material and Methods

In 1997 and 1999 were at the site Jevíčko in the Czech Republic (335 m above sea level, average annual temperature 7.5 °C, average annual precipitation 629 mm) established accurate small plot trials with eight selected grasses species and varieties - Dactylis glomerata (cv. Niva), Lolium perenne (cv. Sport and Mustang), Festuca arundinacea (cv. Kora), Festuca pragensia (cv. Rožnovská), genus hybrids (cv. Hykor and Bečva) and Bromus marginatus (cv. Tacit), intended for harvest in extended grazing season (from October till April next year). We observed the growth of the third cut after the second harvest in the middle of July and August in three time series. Grasses were fertilized [120 kg/ha N - 60 kg/ha N in spring, 60 kg/ha N after second cut + P35K100 applied in spring] in the form ammonia nitre with calk. We evaluated dry matter production and forage quality in parameters of NEV (net energy of fattening), NEL (net energy of lactation), crude protein (CP) and crude fibre (CF), predicted with NIRSystems 6500. Recorded data were statistically evaluated by variance analysis.

Results and Discussion

Dry matter production (Fig.1) of eight observed varieties was in the average of three harvest years influenced by the date of summer cutting, the growth from the mid-July was more productive, in the average of six samplings 1.54 t/ha DM, compared to mid-August growth with average production 0.93 t/ha DM, the acquired results are statistically highly significant (P0.01).

Concentration of NEV and NEL (Fig. 2) compared to dry matter production is higher from August than July growth, because the vegetation is younger. NEV and NEL concentrations have maximal values from samplings till the beginning of winter and they are 5.8 – 6.3 MJ/kg DM, during the winter season until beginning of March they sharply fall to 3 – 4 MJ/kg DM and they start to rise with initiating overgrowth in the beginning of spring. CP concentration has similar progress, which from autumn values of 133 g/kg DM falls to 75 g/kg DM and rise again with the beginning of vegetation and grassland overgrowth start.

Figure 1. Dry matter production (t/ha) in extended grazing season in the average of sowing years, regrown and varieties

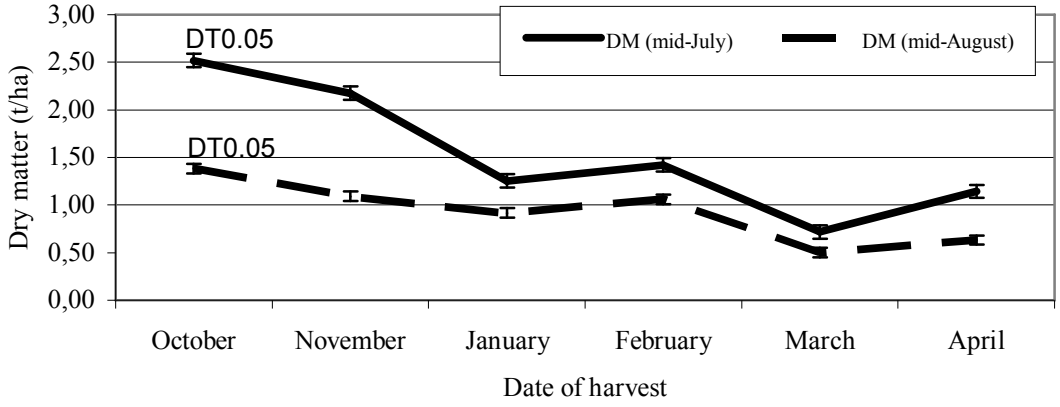
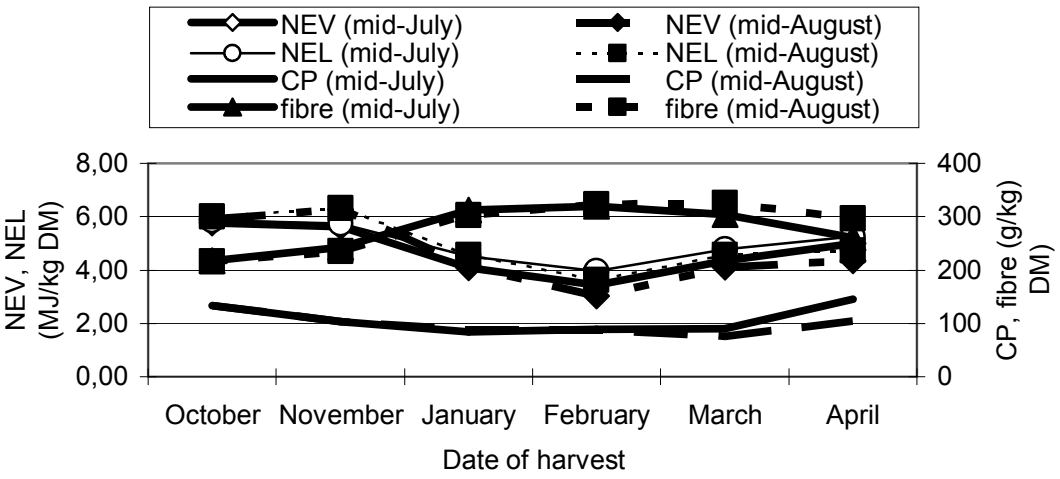


Figure 2. Quality of grass forage in extended grazing season form two growths in the average of sowing and harvest years and evaluated varieties



Fibre concentration has the opposite progress, it is the lowest in October (216.1 – 217.3 g/kg DM) and during winter it exceeds 300 g/kg DM. With beginning grassland overgrowth its concentration begins to decrease. The acquired results are in accord with the findings of Skládanka (2005) and others.

Conclusion

In the conditions of the CR it is practicable to extend the autumn grazing season for suckler cows by 2 – 3 weeks, on lower lands without snow cover even longer. The forage quality is still very good in this period, however it decreases during winter by permeability of organic matter. *Dactylis glomerata*, *Festuca arundinacea* and genus hybrid are convenient grass species. To achieve the growth of grazing vegetation it is necessary to cut or graze the grassland on the turn of July and August at the latest so that the pasture had enough time to overgrow.

Acknowledgement

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The influence of precipitation shortage on forage yield of *Dactylis glomerata* L., *Dactylis polygama* Horvat, *Festuca arundinacea* L. and genus hybrids in a long-term experiment (1986-2004)

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Summary

At the Jevíčko site in the Czech Republic (CR) we observed 19 grass varieties in accurate trials which were sown in 1986 and evaluated production ability and persistence of five varieties of *Dactylis glomerata* L. (*DG*), one variety of *Dactylis polygama* Horvat (*DP*), two varieties of *Festuca arundinacea* L. (*FA*) and two Festulolium hybrids (FlH). Evaluation of the system comprised determination of dry matter production and botanical composition of the stand. *Dactylis glomerata* L., *Festuca arundinacea* L. and Festulolium hybrids were among the most productive, *Dactylis polygama* Horvat was less productive but the most persistent grass species in the conditions of the CR. These species are the basis of permanent grass / legume mixtures for grassland renovation and strip seeding into grassland. Interannual differences in yield are markedly influenced by precipitation in growing season. The paper evaluates the influence of precipitation in vegetative season (April – September) on yield of above mentioned species and varieties of grasses.

Keywords: *Dactylis glomerata* L., *Dactylis polygama* Horvat, *Festuca arundinacea* L., Festulolium hybrid, persistence, yields

Introduction

Festuca arundinacea L. has spread significantly in developed forage countries in the last decades due to its favourable qualities, e.g., production ability, persistence, good health (Buckner and Bush, 1979). *DG*, *FA* and Festulolium hybrids are among the most persistent and productive grasses of the grasses used in the Czech Republic (Kohoutek *et al*., 2000).

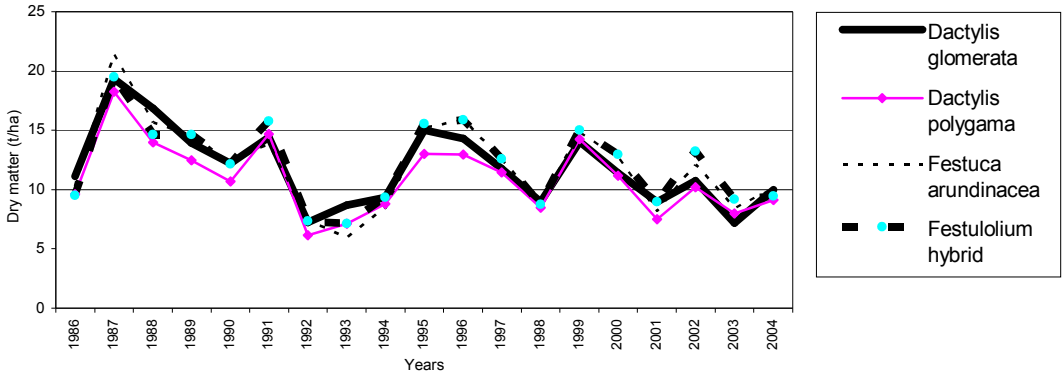
Material and methods

Nineteen grass species were sown at the Jevíčko site in 1986, in a trial to evaluate species of *Dactylis glomerata* L., *Dactylis polygama* (syn. *Dactylis Aschersoniana* Graeb.), *Festuca arundinacea* L. and Festulolium genus hybrids. They were sown at the Jevíčko site in 19 simple grass / legume mixtures (60 % grass species, 25 % *Trifolium pratense* L., 15 % *Trifolium repens* L.) with 4 replications in small plots (10 m^2). They were studied in a 3-cut system. Grass / legume mixtures and pure cultures were fertilised with nitrogen at rates of 120 kg/ha (1987), 180 kg/ha (1988 and 1989) and 240 kg/ha (1990-2004). We evaluate influence of precipitation in vegetative season (April - September) on yield.

Results

Dry matter (DM) production (Figures 1) of the evaluated grasses was similar even in the trial years, the differences between years are caused by rainfall (Figure 3).

Figure 1: Dry matter production (t ha^{-1}) of DG, DP, FA and Festulolium hybrid at the Jevíčko site in 1986-2004.



The influence of precipitation on yield is presented in Figure 2 and Table 1. Grass species (*DG*, *FA*, Festulolium hybrid) react positively to precipitation in vegetative season, the correlation coefficient of regression line is in case of *DG* and Festulolium hybrid statistically significant (P0,05), dry matter production is between 15.1 – 15.7 kg DM per 1 mm of precipitation, in case of *DP* only 2,7 kg DM. Reaction to the drought is in descending order: *DG* < *FA* < Festulolium hybrid < *DP*; *DP* reaction in a longer time is negatively influenced by its medium persistency.

Figure 2 Effect of precipitation on dry matter production of the grass species

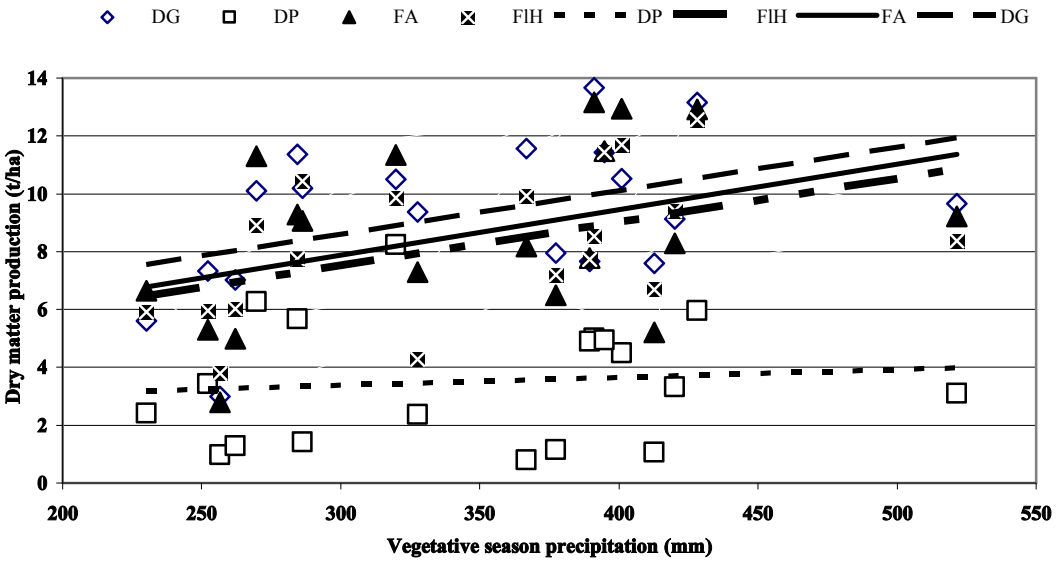


Table 1: Parameters of line equation y= a + bx defining yield of sown species in dependence on precipitation in vegetative season of evaluated years (n=19)

Species	Parameter		Coefficient	
	a	b	of determination	of correlation
Dactylis glomerata	4.0809	0.0151	0.2033	0.45 *
Dactylis polygama	2.5693	0.0027	0.0096	0.10 N.S.
Festuca arundinacea	3.1582	0.0157	0.1669	0.41 N.S.
Festulolium hybrid	3.0120	0.0151	0.2296	0.48 *

Remark.: * = statistically significant (P0,05)
N.S. = non significant

Discussion

The importance of *Festuca pratensis* Huds. and *Lolium perenne* L. among the typical grassland species in the conditions of the Czech Republic has decreased in the last 10-15 years, because they have poor persistence. *Festuca arundinacea* L. together with inter-generic hybrids of the festucoid type have started to gain importance, as was the case in the USA, France and other countries in the 1970s and 1980s (Buckner and Bush, 1979).

Acknowledgements

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Revitalization of biotope with stinging nettle - Urtica dioica L. dominance in the Veľká Fatra National Park

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Introduction

Ruderal phytocoenoses of synatropic plant species were formed by animal disturbance and extremely animal excrement overmanured (nitrogen, potassium) of some agricultural exploited pastures. These phytocoenoses are stabile and species composition is invariable many years. Livestock doesn't intake this forage (NOVÁK, 1992; NOVÁK, SLAMKA, 2003). Number of plant species decreases markedly, stability of grassland ecosystems disturbs and forage production and forage quality decreases, too (NOVÁK, 1992). Stinging nettle is expansionary, competitively robust species, which grows nearly all the world over with the exception of tropical Africa and South America (OPITZ VON BOBERFELD, 1994; NOVÁK, 1997). Stinging nettle is dioecious plant pollinated by wind mainly. Vegetative reproduction by underground rhizomes dominates, rhizomes branch out in all direction and create dense focuses that represses nearly all weed species and cultivated plants. Diasporas in the soil are potential weed source of biotope, because stinging nettle diasporas retain germination ability many years. We found out by soil samples analyses from ruderal biotopes that the nettle soil seed reserve is large (14 100 seeds per m^2) (KOSTKA, NOVÁK, 2004). Experimental research associated with a proposition for revitalization of ruderalized agricultural exploited pastures has not done in the Slovak national parks yet.

Material and methods

Examined area is localized in the West Carpathians Mts., Veľká Fatra National Park, locality Pod Ploskou. Altitude ranges 1240 m with various acclivity up to 5^0, exposition SW, average temperature during vegetation period (April - September) is 9 ^{0}C and 4 ^{0}C during all year, sum of downfall ranges 820 mm during vegetation and 1250 mm during all year. Soil substratum comprises Mesozoic calcites and marly shale and soil type is Rendzina. On the basis of floristic analyses and forage value of determined plants species we evaluated and compared grasslands quality (E_{GQ}) of ruderal vegetation (variant 1) and revitalized vegetation (variant 3) by NOVAK (2004) method in 2004 and 2005. For revitalization of ruderal vegetation we used hand sowing (the 20th of August) of seed's mixture from wild plant (table 1) after preceding chemical application of herbicide with Glyphosate active substance (6 l/ha). Wild plant seeds were collected from pasture vegetation near by examined area during vegetation period 2004. Variant 1 we cut once and variant 3 three times during vegetation period. This research allowed Ministry of the environment of the Slovak Republic.

Table 1: Seed mixture for revitalization

Species	Part in mixture [%]	Sowing rate [g/m^2]
Dactylis glomerata L.	25.00	2.50
Festuca pratensis Huds.	10.00	1.00
Phleum pratense L.	10.00	1.00
Poa pratensis L.	10.00	1.00
Festuca rubra L.	5.00	0.50
Trisetum flavescens (L.) P. Beauv.	5.00	0.50
Trifolium repens L.	15.00	1.50
Trifolium pratense L.*	3.00	0.30
Lotus corniculatus L.	3.00	0.30
Plantago lanceolata L.*	2.00	0.20
Achillea millefolium L.*	2.00	0.20
Carum carvi L.*	2.00	0.20
Taraxacum officinale Weber*	2.00	0.20
Alchemilla vulgaris L.*	2.00	0.20
Daucus carota L.	1.00	0.10
Acetosa pratensis M i l l.	1.00	0.10
Leucanthemum vulgare Lam.	1.00	0.10
Prunella vulgaris L.	1.00	0.10
Total	**100.00**	**10.00**

Results and discusion

Table 2 shows a floristic composition of ruderal biotope (variant 1) and revitalized biotope (variant 3). Ruderalized biotope with stinging nettle dominance (40 %) and high proportion of mosses (*Bryophyta*) and empty places (60 %) affected like monoculture with very low floristic diversity and quality (E_{GQ} = 5 from 100 points). Seeds of sowing species germinated well and emerging greensward closed terrene during next vegetation period. The stinging nettle's seed reserve was large (22 028 seeds/m^2), but new emerging greensward inhibited stinging nettle's seed germination and next growth and development. Therefore predominance of stinging nettle decreased from 40 % to 3 % after chemical application and sowing during next vegetation period. Ruderal biotope was characteristic with 6.65 times higher phosphorus content and 2.75 times higher potassium content in soil compares with soil from pasture vegetation near by examined area. The best development from floristic group of grasses (*Poaceae*) had meadow foxtail (*Phleum pratense* L.) with predominance 13 % and meadow fescue (*Festuca pratensis* Huds.) with 6 %. For these species are climatic conditions of examined area the most suitable. White clover (*Trifolium repens* L.) (predominance 13 %) from floristic group of leguminous (*Fabaceae*) covered gaps by over ground stolons very well. Only swine's snout (*Taraxacum officinale* Weber.) from floristic group of other families of dicotyledonous species we didn't record in revitalized vegetation. Ruderal vegetation with stinging nettle dominance and low quality was changed to typical pastures with total predominance 75 % and with better quality (E_{GQ} = 53.60). NOVÁK (1997) achieved similar results by sowing of cultural cocksfoot (*Dactylis glomerata* L.) and cultural white clover (*Trifolium repens* L.) in altitude 600 m. Sowing of wild plant's seeds was used for revitalization of ruderal biotope first time in conditions of Slovak national park (NOVÁK, KOSTKA, SLAMKA, 2005).

Table 2: Floristic analyses and evaluation of grassland quality

Species	FV	Year 2004 D (%)		Year 2005 D (%)	
		var. 1	var. 3	var. 1	var. 3
Grasses (*Poaceae*)					
Agrostis capillaris L.	5				+
Dactylis glomerata L.	7				3
Deschampsia caespitosa (L.) P. B.	3/1				+
Festuca pratensis H u d s.	8				6
Festuca rubra L. *ssp.rubra*	5/3				3
Phleum pratense L.	8				13
Poa pratensis L.	8				2
Poa trivialis L.	6/4	+	+	+	2
Trisetum flavescens (L.) P. Beauv.	6/4				4
Grasses (*Poaceae*) total		+	+	+	**33**
Legumes (*Fabaceae*)					
Lotus corniculatus L.	7/5				2
Trifolium pratense L.	7				3
Trifolium repens L.	8				13
Legumes (*Fabaceae*) total					**18**
Others herbs					
Acetosa pratensis M i l l.	2!				1
Achillea millefolium L.	5/3				3
Alchemilla vulgaris L.*	5				+
Capsella bursa-pastoris (L.) Med.	1				+
Carduus acanthoides L.	0				+
Carum carvi L. *	5/3				3
Cirsium eriophorum (L.) Scop.	0				+
Daucus carota L.	3/2				+
Galeopsis tetrahit L.	2!				2
Leucanthemum vulgare L a m.	2				+
Plantago lanceolata L. *	6/4				1
Prunella vulgaris L.	2				2
Ranunculus repens L.	-1				4
Rumex alpinus L.	2!	+	+	+	
Rumex obtusifolius L.	1!	+	+	+	3
Stellaria graminea L.	2!				1
Urtica dioica L. *	1!	40	40	40	3
Veronica chamaedrys L.	2				1
Others herbs total		**40**	**40**	**40**	**24**
D % total		**40**	**40**	**40**	**75**
***Bryophyta* + empty places**		**60**	**60**	**60**	**25**
E_{GQ}		**5**	**5**	**5**	**53.60**

D = predominance of species in % , FV = forage value of species,
E_{GQ} = evaluation of grassland quality, + = predominance to 1 %,
* = medicinal plant

Conclusion

Eutrophized biotope with ruderal vegetation and with stinging nettles dominance (40 %) and high proportion of mosses (*Bryophyta*) and empty places (60 %) affected like monoculture with very low floristic diversity and quality (E_{GQ} = 5 from). Ruderal vegetation we revitalized by seed's sowing of 6 grass species, 3 leguminous species and 9 species of other families of dicotyledonous species from wild plants. The best development from floristic group of grasses (*Poaceae*) had meadow foxtail (*Phleum pratense* L.) with predominance 13 % and meadow fescue (*Festuca pratensis* Huds.) with 6 %. For these species are climatic conditions of examined area the most suitable. White clover (*Trifolium repens* L.) (predominance 13 %) from floristic group of leguminous (*Fabaceae*) covered empty places by over ground stolons very well. Only swine's snout (*Taraxacum officinale* Weber.) from floristic group of other families of dicotyledonous species we didn't record in revitalized vegetation. Ruderal vegetation with stinging nettle dominance and low quality was changed to typical pastures with total predominance 75 % and with better quality (E_{GQ} = 53.60).

Keywords: *Urtica dioica*, floristic analysis, evaluation of grassland quality, revitalization

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Biomass Production of Different Early Maize Hybrids

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Summary

Dry mass content and morphological parameters of different early maize hybrids were studied in experimental field of Czech University of Agriculture in Prague. We tested four hybrids with FAO number 200, 250, 300 and 380. In 2005 samplings of maize plants were practiced in 14-days intervals at the beginning of vegetation and in 2-4 days intervals before harvest time to determine the formation of dry mass content. Early hybrid had the most rapid shape of formation of dry mass content. Medium early hybrid showed slowly formation of fry mass content. Medium late and late hybrids were characterized by stagnation from 5th to 7th and 8th sampling, respectively. After this period rapid enhance of dry mass content was determined. Tested hybrids reached silage maturity 120 days (FAO 200), 127 days (FAO 250), 132 days (300) and 134 days (FAO 380) after seeding. At harvest time the significant influence (α=0.05) of hybrid to measured morphological parameters (plant height, ear height, stalk diameter, ear length, ear diameter, number of leaves) was found.

Keywords: maize; FAO number; dry mass content; morphological parameters

Introduction

Silage maize is the most important annual forage crop in Czech Republic. In particular production area there is necessary to choose maize hybrid with optimal FAO number which produce high biomass yield and good quality (Šuk *et al.*, 1998). Important parameters to characterized particular hybrid contain plant height, ear height, grain weight etc. (Revilla *et al.*, 1999). These parameters could be significantly influenced by maize production conditions (Gonzalez-Ponce and Salas, 1995; Ali *et al.*, 1999). The objectives of this research were to evaluate the formation of dry mass content and the morphological parameters of different early maize hybrids.

Materials and Methods

The plot experiment with silage maize was established in the field of Czech University of Agriculture in Prague. Four different hybrids were tested in the year 2005: early hybrid Birko (FAO 200), medium early hybrid Daxxar (FAO 250), medium late hybrid Kuxxar (FAO 300), and late hybrid Lucia (FAO 380). The experiment was established 28 April in Latin square. The plot area was 20 m^2. The sowing rate was 85,000 plants per hectare. Samplings of maize plants were practiced during the vegetation to determine the formation of dry mass content. Actions were realized in 14-days intervals at the beginning of vegetation and in 2-4 days intervals before harvest time. From each plots 3 plants were taken for analysis. The samplings were practiced to optimal harvest maturity of silage maize (milk-waxy maturity). In the term of harvest (Birko: 26 August, Daxxar: 2 September, Kuxxar: 7 September, Lucia: 9 September) we measured selected morphological parameters on 10 plants from each plots: plant height, ear height, stalk diameter 0.1 m above the ground, ear length, ear diameter and number of leaves. The weight of selected plant parts (leaves, stalks, ears) was determined. The

dry mass content was fixed at 105 °C. The statistical evaluation was accomplished by Analysis of Variance (ANOVA), Tukey HSD, on 0.05 probability values.

Results and Discussion

Formation of dry mass content of tested hybrids during the vegetation is shown in Fig. 1. The difference among the hybrids is evident. Early hybrid has the most rapid shape. Medium early hybrid shows slowly formation of fry mass content. Medium late and late hybrids are characterized by stagnation from 5th to 7th and 8th sampling, respectively. After this period rapid enhance of dry mass content was determined.

Hybrid Birko reached silage maturity 120 days, Daxxar 127 days, Kuxxar 132 days and Lucia 134 days after seeding. Average weight of one plant and dry mass content in the harvest time were 236.4 g, (31.4 %) for hybrid Birko, 229.4 g (31.9 %) for Daxxar, 280.4 g (29.8 %) for Kuxxar and 330.4 g (30.1 %) for Lucia. Dry mass content of leaves and stalks ranged 25.4 - 27.3 % and 20.6 - 21.5 %, respectively. Dry mass content of ears were: Birko 42.5 %, Daxxar 44.3 %, Kuxxar 40.7 % and Lucia 42.8 %. Percentage ratio of plant parts is particularly influenced by hybrid Bosák *et al.* (2000). In harvest time was found equable ratio of leaves (13.4 - 14.8 %). Share of stalks ranged from 25.2 to 31.9 %. Ears represented 58.8 % (Birko), 61.4 % (Daxxar), 54.1 % (Kuxxar) and 55.1 % (Lucia) from total weight of plant.

Statistical analyses showed significant influence of hybrid to measured morphological parameters (Tab. 1). Significant lowest plant height was found for hybrid Daxxar. Hybrid Lucia produced the highest plants. Difference between these hybrids was 0.33 m. Hybrid Kuxxar (2.82 m) was significantly different in comparison to hybrids Daxxar and Lucia. Hybrid Daxxar is characterized by the lowest ear height. The highest level of this parameter was measured at hybrids Kuxxar and Lucia without mutual significant difference. Stalk diameter 100 mm above ground ranged from 19.3 to 20.2 mm (hybrids with FAO number 200 - 300). Hybrid Lucia produced significantly highest stalk diameter (23.1 mm). Ear length increases with FAO number. All tested hybrids significantly differed in this parameter. Ear diameter ranged from 42.5 to 46.1 mm. Significantly lowest diameter was found at hybrid Daxxar. Hybrids Birko and Daxxar had significantly lower number of leaves in comparison to hybrids Kuxxar and Lucia.

The highest levels of measured parameters were found at hybrid Lucia and the lowest once at hybrid Daxxar. Planted hybrid significantly affects characteristics of maize above ground biomass (plant height, number of leaves, yield of dry mass, etc.) (Ford and Pleasant, 1994). This assumption is in contrast to results of Berzsenyi *et al.* (1998). They found higher impact of year to plant height and other parameters than selected hybrid characteristics (e.g. earliness of hybrids).

Figure 1: Formation of dry mass content of tested hybrids in the year 2005

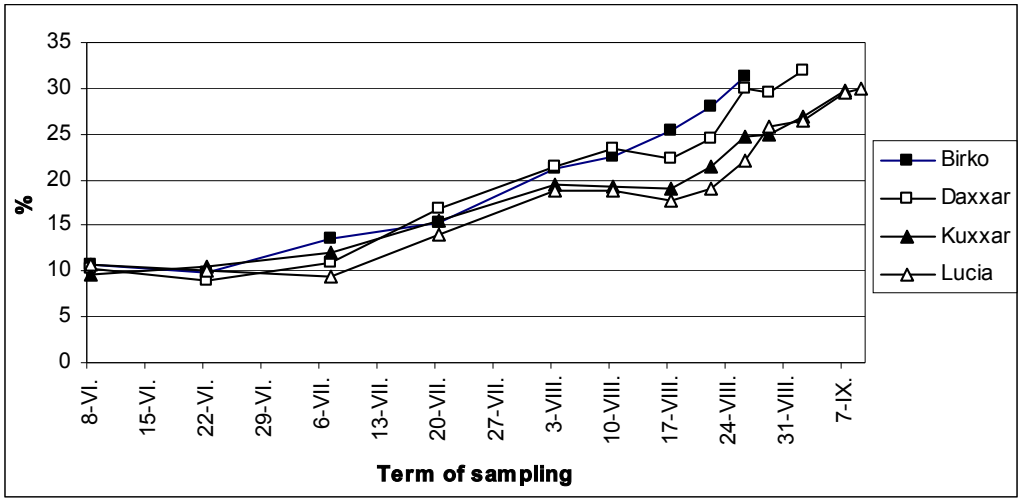

Table 1: Morphological parameters of tested hybrids in the year 2005 (ANOVA, Tukey HSD)

Hybrid	Plant height (m)	Ear height (m)	Stalk diameter (mm)	Ear length (mm)	Ear diameter (mm)	Number of leaves
Birko	2.77^{AB}	1.10^{B}	19.3^{A}	178.6^{A}	45.1^{B}	12.1^{A}
Daxxar	2.69^{A}	0.96^{A}	20.0^{A}	194.0^{B}	42.5^{A}	12.0^{A}
Kuxxar	2.82^{B}	1.36^{C}	20.2^{A}	217.4^{C}	46.1^{B}	14.4^{B}
Lucia	3.02^{C}	1.33^{C}	23.1^{B}	235.1^{D}	45.7^{B}	13.5^{C}
F-test	31.43	115.24	29.18	145.28	22.12	137.31
p-value	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

A, B, C, D - differences significant on $\alpha = 0.05$

Conclusion

Hybrids with higher FAO number show better potential to reach upper values of morphological parameters and yield. On the other side, lower ears ratio of total above ground biomass yield was found. Evident differences in formation of dry mass content among tested hybrids were determined. Earlier hybrids shown rapid increase of this characteristic compared to later hybrids. Formation of dry mass content study will continue in next years and will be evaluated in relation to agrometeorological conditions.

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Determination Of Lucerne First Cut Term By Growing Degree Day Method

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Abstract

Growing degree-days (GDD) are a temperature-derived index which is related to lucerne quality and used for prediction of harvest term in the first cut. It is necessary to access these equations for different environments, thus the goal of this study is an assessment of preliminary equations in the conditions of Czech Republic. In 2004, the samples were taken over the first cut period in 10-days interval from 12 May to 22 June in the experimental field of Czech University of Agriculture Prague. There were two replications by three lucerne germplasm in this experiment. The neutrodetergent (NDF), acidodetergent (ADF), and crude fiber (CF) content was assessed by each sample. In this experiment, the correlations between GDD and fiber content were highly significant ($P<0.0000$) and provided R^2 around 80% with residual standard deviation from 2 to 3 percentage units. According to preliminary data set, we can calculate harvest term with allowable NDF and ADF content as approximately 430 GDD in our experimental condition.

Keyword: lucerne, harvest term, GDD

Introduction

Growing degree-days (GDD) are a temperature-derived index representing the amount of heat plants are exposed to (Sulc et al, 1999). This method is well known and used successfully for prediction of maize harvest (Zea mays L.) in the Czech Republic. The similar model is used for lucerne in the USA where lucerne is one of the most important crops. The GDD for lucerne are calculated as follows. Average the maximum and minimum temperature for each day (24 h period) beginning March 1, substract the base temperature (41 ºF = 5 ºC), and sum the growing degrees for all day that have a positive number (Allen, 1996). According to Allen and Beck (1996), accumulated GDD are related to neutrodetergent fiber (NDF) content in the spring growth of lucerne. As noted Van Soest (1996), GDD relate reasonably well to forage quality in perennial forages when soil moisture is not limiting, but later in the season when moisture is typically limiting, GDD do not relate well to quality. This is presumably because forage growth is limited more by soil moisture than by heat unit. Because of this, GDD is highly related to quality only for spring harvest lucerne with adequate rainfall and not for subsequent cuttings (Allen, 1996). The using of GDD method for harvest prognosis in European conditions was described by Nykänen et al (2005). In Finland there is an Internet service for harvest prognosis of silage for grasses and red clover. These models are verified for other forage crops including lucerne. In contrast to the American studies, it is based on mathematical model which describing the relation between organic matter digestibility (OMD) development and effective temperature sum. The base temperature is 5 ºC as well as is published by Allen (1996).

Generally, GDD is considered as a good and simple model for prediction NDF content or OMD. Although GDD and lucerne NDF content are highly related within an

environment, GDD prediction equations have not been consistently accurate across environments (Sulc et al, 1999). It is necessary to access these equations for different environments, thus the goal of this study is an assessment of preliminary equations in the conditions of Czech Republic.

Materials and Method

To test the GDD method, we used a running experiment aimed at comparing 3 germplasms of lucerne (*Medicago sativa L.*) in the experimental field of Czech University of Agriculture Prague. There were two lucerne experimental candivars (ŽE XLII, ŽE XLV) and variety Jarka in completely randomised blocks. The forage sampling was performed from square 20x20 cm in two replications by each lucerne germplasm. In 2004, the sampling was repeatedly realized over the first cut period in approximately 10-day interval in following dates: 12, 21, 31 May and 9, 22 June. According to Allen (1996), GDD was calculated for ºC as 324, 405, 471, 578 and 735 GDD, respectively. The neutrodetergent fiber (NDF), acidodetergent fiber (ADF), and crude fiber (CF) content was assessed by each sample. We did not assess OMD but according to Míka (1997) ADF is related to forage digestibility. The data analyses were performed by simple linear regression and analysis of variance in Statistica 6.0.

Results and Discussion:

Based on ANOVA results, we did not record any significant differences among germplasms as well as germplasm*GDD interactions in fiber content. The GDD terms were highly significant (P = 0.0000) for NDF, ADF and CF content. The obtained statistically significant differences among GDD terms for NDF and ADF were exactly the same. For CF content, there were stronger differences in beginning of evaluated period and less strong in end of this period in comparison with ADF and NDF content.

Míka et al (1997) presented that the optimal values of fiber content in lucerne forage are up to 400 and 300 g/kg for NDF and ADF, respectively. The crude fiber content in lucerne forage for silage should be up to 290 g/kg (Bíro, Juráček, 2003). From point of view of fiber content, the optimal term of harvest was between 471 and 578, 405 and 471, 578 and 735 GDD for NDF, ADF, and CF, respectively. If we presume ADF content as a parameter related to forage digestibility, it seems that the harvest term according to this parameter is earlier in comparison with harvest term according to NDF and CF content.

The regression results of neutrodetergent, acidodetergent and crude fiber content for each germplasm and for all of them are shown in Table 2. The obtained R^2 ranged from 78.50 (ADF) to 80.91 (NDF) for all germplasms. The obtained R^2 and accuracy of estimate measured as residual standard deviation (RSD) varied among germplasms. It can indicate possible differences in accuracy among germplasms but one year data set must be taking into account. An average RSD 26.5, 25.9, and 19.5 of g/kg were recorded for NDF, ADF and CF content, respectively. According to Allen (1996), it is possible predict NDF content within ±30 g/kg 68% of the time and within 60 g/kg 90% of the time. Based on our one year equations, we can calculate last harvest time with acceptable lucerne quality (NDF, ADF = up to 400, 300g/kg) as 430 GDD. Allen and Beck (1996) conducted investigation by lucerne stands in six states of USA. Their report indicate harvest time about 700 to 750 GDD (Fº) so it means about 370 to 400 GDD in ºC.

Table 1: Relating lucerne fibre content (y; NDF, ADF or CF g/kg) and growing degree-day (x; ºC) by candivars and variety Jarka (linear regression, y=a+bx), RSD = residual standard deviation.

predicted parameter	germplasm	a	b	R^2	F-ratio	P-value	RSD	Df
NDF	XLII	215.50	0.332	79.62	31.25	0.0005	26.82	9
	XLV	189.37	0.361	88.48	61.44	0.0001	20.83	9
	Jarka	167.52	0.414	79.34	30.74	0.0005	33.74	9
	all	190.79	0.370	80.91	118.67	0.0000	26.51	28
ADF	XLII	198.20	0.263	90.45	75.80	0.0000	13.64	9
	XLV	140.74	0.375	84.30	42.95	0.0002	25.83	9
	Jarka	138.18	0.366	73.28	21.94	0.0016	35.30	9
	all	159.04	0.334	78.50	102.24	0.0000	25.89	28
CF	XLII	143.38	0.213	81.90	36.20	0.0003	16.02	9
	XLV	97.44	0.297	84.30	42.97	0.0002	20.47	9
	Jarka	101.05	0.281	79.63	31.28	0.0005	22.70	9
	all	113.96	0.264	79.96	111.72	0.0000	19.53	28

Conclusion

With limited data from one year, it is difficult to conclude that GDD model is the sole indicator needed to select the optimum lucerne harvest date. In our experiment, the correlations between GDD and fiber content were highly significant and provided R^2 around 80% with residual standard deviation from 20 to 30 g/kg. The accuracy of fiber content prediction among germplasm varied thus should be investigated. According to preliminary data set, we can calculate harvest term with allowable NDF and ADF content as approximately 430 GDD in our experimental condition. The experiment is continuing so results can not be definitive.

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The Effect of Harvest Term and Grass Species on Qualitative Parameters of Biomass Used For Direct Combustion

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Abstract

The dynamics of DM content, energy and nitrogen content in biomass of *B.inermis, B. marginatus, F. arundinacea* and *Festulolium* Felina was studied in the years 2003-2004 to determine an optimal term of harvest of grass species grown for the production of biomass for direct combustion. The experiment was established at the Czech University of Agriculture in Prague (latitude: 50°08´ N, altitude: 14°24´ E) in 2002. The selected characteristics were studied during vegetation in one month intervals from the mid-May. DM content was highest in *B. inermis* and *B. marginatus* in the last two terms of sampling. The highest gross calorific value was in *B. inermis* and *B. marginatus* biomass 18,26 kJ/g and 18,17 kJ/g respectively. Net calorific value was strongly influenced by sampling term and ranged from 2,61 kJ/g in the first sampling to 9,78 kJ/g in the fifth sampling term. The nitrogen content was significantly higher in the first sampling compared to the others ($P < 0,0001$).

Introduction

Growing of energy crops is the possible solution of arable land surplus in the Czech Republic. The simplest, cheapest and most common method of obtaining energy from biomass is direct combustion. Perennial grasses seem to be suitable plants for production of energy biomass. Many different types of biomass can be grown for the express purpose of energy production. There are two main factors which determine whether a crop is suitable for energy use. Good energy crops have a very high yield of dry material per unit of land and good quality (Rice, 2003). The main quality parameters of biomass produced for direct combustion is high dry matter content, energy content (El Bassam, 1998) and low concentration of nitrogen, sulphur, chlorine and ash (Lewandowski *et al*., 2003). Dry matter content at harvest influences the cost of transportation and handling, as well as the recoverable energy level, since moisture vaporization requires energy during the combustion process (McLaughlin, *et al*., 1996). The amount of energy which can be produced from biomass crop must be higher than the amount of energy required growing the crop (Rice, B., 2003). The low concentration of nitrogen improves the combustion quality and leads to decreased emissions of nitrous oxide during the combustion of biomass (Lewandowski *et al*., 2000). N concentration in biomass also influences the N quantity removed in the biomass. It would be important in determining fertilization needs and usefulness as a feedstock (Reynolds *et al*., 2000). The aim of this study is to compare DM, N and energy content in biomass of selected grass species during the vegetation to find an optimal term of the first harvest according to these most important qualitative parameters.

Materials and methods

Festulolium (*Festuca arundinacea* Schreb. x *Lolium multiflorum* Lam.) cv. Felina, tall fescue (*Festuca arundinacea* Schr.) cv. Kora, mountain brome (*Bromus marginatus*

Nees ex Steud.) cv. Tacit and smooth brome (*Bromus inermis* Leyss.) cv. Tabrom, were studied in a plot experiment. The experiment was carried out in 2002 at the Czech University of Agriculture in Prague (latitude: 50°08´ N, altitude: 14°24´ E), 286 m above sea level. The area is classified as having a moderate to warm and mostly dry climatic. The average growing period is 172 days and the mean annual temperature is 7.9 °C (30 year mean). The long-term average precipitation was 526 mm per year. Pure stands of *Festulolium, F. arundinacea*, *B. marginatus* and *B. inermis* were sown at a rate of 40, 45, 85 and 40 kg ha^{-1} respectively and established in two replications. Seeds were drilled with rows 125 mm apart. Samples of biomass were taken during vegetation at approximately one month intervals (six sampling terms from mid-May to mid-October) in the years 2003 and 2004. Biomass was hand cut 50 mm above the ground from each plot, weighed, dried to constant weight and reweighed to determine dry matter content. N content was analysed in Ekolab Žamberk. Energy content of biomass was expressed in gross and net calorific value. Gross calorific value of the biomass was assessed using an automatic adiabatic calorimeter LAGET MS 10 A. Net calorific value was calculated from gross calorific value and DM content according to Pastorek *et al*. (2004). The data analyses were performed by multivariate analysis of variance in Statistica 6.0.

Results and discussion

Results of statistical analysis are shown in table 1. Sampling term, grass species and their interaction influenced significantly dry matter (DM) content in biomass. *B. inermis* (471,2 g/kg) and *B. marginatus* (492,8 g/kg) provided biomass of higher DM content than *Festulolium* (433,0 g/kg) and *F. arundinacea* (421,4 g/kg). The DM content in bromes was higher particularly in the last three terms of sampling. Although delayed harvest reduced moisture contents recorded in studied grasses, these were still above the threshold of 230 g/kg fresh matter set by Lewandowski and Kicherer (1997) for safe storage of stalky biomass.

Gross calorific value ranged from 17,84 kJ/g in *Festulolium* to 18,26 kJ/g in *B. inermis* biomass and was not influenced by sampling term. It corresponds with conclusions of Lewandowski and Kicherer (1997) that energy content of dry grass biomass usually fluctuate around the value 18 kJ/g. Changes in gross calorific value in time are small because elemental composition, mainly C, O and H content, remains almost the same during the vegetation.

Average net calorific value was lowest in *F. arundinacea* 4,21 kJ/g and highest in *B.marginatus* 4,93 kJ/g. Net calorific value was strongly influenced by sampling term and ranged from 2,61 kJ/g in the first sampling to 9,78 kJ/g in the fifth sampling term. The dynamics of net calorific value in grass biomass was in all species similar. Steady increase of net calorific value was recorded from the first to the fifth sampling term which was similarly as the dynamics of DM content. The highest values of this parameter were obtained in sampling terms 5 and 6.

*Table1: Effect of sampling term, grass species and their interaction on selected qualitative parametres, * significant at $Tukey_{0,05}$ probability level, ** significant at $Tukey_{0,01}$ probability level, BI - B. inermis, BM - B. marginatus, F - Festulolium, FA - F. arundinacea*

Variable	Factor	Df	F-ratio	P-value	Multiple range test
DM content	Sampling term	5	161,57	0,0000	1 < 2 < 3 < 4 < 6, 5**
	Species	3	13,44	0,0000	BM, BI > F, FA*; BM > FA**
	Sampling term*species	15	3,30	0,0045	-
Gross calorific value	Sampling term	5	1,06	0,3879	-
	Species	3	9,29	0,0000	BI, BM > F**
	Sampling term*species	15	0,70	0,7722	-
Net calorific value	Sampling term	5	169,45	0,0000	1 < 2 < 3 < 4 < 6, 5**
	Species	3	16,46	0,0000	BM, BI > F, FA**
	Sampling term*species	15	3,50	0,0031	-
N kontent	Sampling term	5	39,43	0,0000	1 > 2, 6, 4, 3, 5**
	Species	3	0,93	0,4440	-
	Sampling term*species	15	0,84	0,6260	-

The effect of sampling term on N content in grass biomass was significant. The highest average N content was in the first sampling term (16,91 g/kg). In the biomass of all grass species was quoted a substantial decrease in N content in the period between the first and second sampling term. The decrease of N content in grass biomass during vegetation can be explained by the diluting the mineral pool due to plant growth (Marschner, 1995). No significant differences in N content were found among the sampling terms 2,3,4,5 and 6. Grass species did not influence the N content in biomass but differences in N dynamics were recorded. In *B. inermis* and *B. marginatus* the N content in the first sampling term was higher than in *Festulolium* and *F. arundinacea.* The decrease in the sampling terms 1, 2, 3 in bromes biomass was sharper and slow decrease was noted also in the terms 4, 5, 6. It can be ascribed to a process that N-containing substances, can be actively exported from the senescent parts to still active organs e. g. storage organs, but may also be leached (Marschner, 1995). In *Festulolium* and *F. arundinacea* biomass the decrease at the beginning of vegetation was slower and from the second sampling term the N content fluctuated in both species around 8,5 g/kg.

Conclusions

More favourable quality for direct combustion is obtained in delayed harvest term in all grass species. Only gross calorific value was not influenced by sampling term. Biomass of *Bromus inermis* and *Bromus marginatus* provided better values of selected qualitative parameters.

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UTILIZATION, NUTRITIVE VALUE AND HYGIENIC ASPECTS OF PRESERVED FORAGES ON PRODUCTION HEALTH OF ANIMALS

Health Risks Posed by Feeding Low Quality Silage

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Silages are a basic component of ruminant diets. Their quality implicates nutritional value and palatability of feed, and total feed intake. High quality maize silage has a high energy level, it is well accepted by animals and stabilizes fermentation processes in the rumen. In most dairy herds, maize silage is a basic component of the diet throughout the year. In combination with maize LKS or CCM silages, it is an important energy source in early lactation and peak lactation. It affects milk yield and quality, health status and fertility. In combination with preserved forage with high protein content and feed concentrate it provides optimum condition for formulating TMR with high nutrient concentrations, required by high-producing lactating cows, minimizing the development of indigestion and health disorders. High quality preserved forage is indispensable for high-producing cows. Both maize silage, LKS and CCM provide maize starch that is digested slower than wheat and barley starch and partially escapes rumen fermentation, which is a great advantage. When digested in the intestine, starch is a source of glucose in the period of negative energy balance, thus reducing the occurrence of ketosis. The quality of silage is influenced by many factors in all stages of manufacture, storage, mixing in TMR and feeding. Harvesting technologies and a range of preservatives available in this country are sufficient and comparable with those used abroad. Still, the quality of preserved roughage is not on a required level on many farms and brings about some health risks. Preserved feeds have low levels of energy, crude protein, inappropriate structure, dry matter and disputable hygiene quality. Even silage of good quality when produced, can quickly change and become of poor hygiene quality. The aerobic (secondary, or spontaneous) fermentation is caused by yeasts, bacteria and fungi. Yeasts are highly acid-tolerant and they bear a wide temperature range (from 8°C to 35°C), and they can survive in silage for a long time, like the spores of fungi and some bacteria. Under favourable conditions (enough oxygen, water, 12 – 35 °C) yeasts multiply and impair the preserved feed. Yeasts intensively metabolize lactic acid and readily fermentable sugars. They produce heat, carbon dioxide and water. Preserved forage loses energy and dry matter. It has been demonstrated that a dry matter loss can be 2% to 3 % in one day. By breaking down lactic acid, silage pH changes and conditions suitable for the growth of bacteria and fungi are created. Thus, products of rotting, mycotoxins and many biogenic amines such as cadaverine, tyramine, putrescine, spermidine, tryptamine and histamine accumulate in the silage. Counts of *Escherichia coli*, *Klebsiella* spp., *Clostridium* spp., *Listeria* spp. and other bacteria increase. In the heated silage material indigestible substances are formed. Preserved feed, affected by aerobic fermentation has a low nutritive value and poor hygiene status. It has many adverse effects on health, and production and reproduction performance of animals.

Preserved forage, impaired by aerobic processes, decreases the value of total mixed ration. Yeasts readily break down sugars added to feed ration as molasses or feed sugar, fungi grow quickly and produce large amounts of mycotoxins, decompose protein. Conditions are suitable for the growth of pathogenic bacteria. These undesirable

processes take place until feed is ingested by the animals or removed from the feeding trough.

Metabolites produced during the aerobic fermentation, decrease feed palatability and dry matter intake (30%). There are great changes in the fore stomachs in cattle. The rumen fermentation requires an optimum nutrient intake. Aerobic fermentation reduces the concentration of soluble carbohydrates, digestibility of protein, the ingested bacteria adversely influence the rumen microflora and microfauna. Mycotoxins exert very adverse effects on the rumen fermentation. For the above-mentioned reasons, digestion disorder may develop such as simple indigestion, alkalosis and rotting of ruminal content. Characteristic signs due to the ingestion of spoiled forage are reduced appetite, performance, changed quality and composition of milk, higher occurrence of mastitis, scours, increased incidence of hoof diseases and purulent endometritis. The health status of the mother is reflected in the health status of the calf. Calves born are less viable, they do not want to suckle colostrum, there are low levels of immunoglobulines in colostrum, therefore the calves have poor colostral immunity. They show a high incidence of diarrhoea and high mortality.

Feeding of spoilt roughage adversely influences fermentation processes in the rumen. An increase in rumen fluid pH, decrease in VFA concentrations, decreased propionate concentrations and increased butyrate concentrations have been observed. Ammonia concentrations have been increased, which is a sign of insufficient utilization of crude protein and low formation of microbial protein. A marked decrease in counts of infusoria in rumen fluid has been observed. Often, their counts are lower than 100 000 in 1 ml rumen fluid. Rumen fluid is of changed colour, texture and odour.

In the summer, yeasts are causative agents of mastitis. The therapy of yeast mastitis is difficult and they are often recurrent. A high concentration of T-2 toxin causes haemorrhages in the mammary gland and milk is of pink to reddish colour. A frequent complication is subsequent mastitis.

Ingestion of impaired feeds adversely affects fertility. The pathogenesis of such disorders includes nutrient deficiencies due to reduced appetite and underlying metabolic disorders, liver diseases, altered function of the thyroid, adrenals and ovaria. In cows affected by T-2 toxin we have found significantly decreased thyroid hormone concentrations. Some metabolites, mycotoxins above all, exert toxic effects on sperms, oocytes and embryos. Zearalenone – mycotoxin with pronounced estrogenic effects – delays oestrus and has a toxic effect on sperms and oocytes. It is responsible for the unsatisfactory situation in many herds. Toxic metabolites, along with subclinical hypocalcaemia and other factors (selenium and vitamin E deficiencies) participate in the development of endometritides in the early puerperium.

A seriousness of adverse consequences of feeding forage impaired by aerobic fermentation is magnified by the fact that the above-mentioned production and health problems in cows are concurrent. Therefore it is very difficult to find the causes of these problems and direct and indirect losses occur. An efficient prevention is vital for the minimization of losses. Strict adherence to technological procedures during the harvest, silage making and the use of suitable preservatives that reduce aerobic fermentation are the basis of production of high quality preserved forages. High quality preserved forage must be stored under suitable conditions and withdrawn and fed in the right way. A danger of aerobic fermentation is high particularly in silages with high contents of carbohydrates.

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Precision Chopped Grass Silage or Roundbale Silage for Dairy Cows

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Introduction

Precision chopped (PC) grass silage from bunker silo was compared with roundbale silage (RB) to dairy cows.

Materiále and Methods

A primary growth of a timothy-dominated sward was mown at heading on June 10 and June 13, in Skedsmo, Norway (60° N, 11° E) in 2002, and wilted for 1 to 5 h to reach 242-265 g/kg DM (Table 1). The herbage was applied 3.9 l/t GrasAAT silage additive (645 g/kg formic acid, 60 g/kg NH_3, Norsk Hydro, Oslo), and ensiled in a 6 m x 24 m bunker silo using Taarup 602B precision chopper with 32 knives, or in bales using Vicon RF 130 MP fixed chamber roundbaler with 14 fixed knives (Kverneland, Klepp, Norway). Bales were wrapped using 6 layers of 0.025 mm white stretch plastic. Due to 28 mm rain on 11-12 June, ensiling was delayed, during which the bunker was temporarily covered by plastic. The bunker was carefully compacted by driving a 6.5 tonne tractor continuously in the silo between each load of herbage.

The harvested crop was low in energy, 5.6 MJ NE_l/kg DM as assessed by NIRS, and contained, per kg DM: 148 g CP, 122 g WSC, 636 g NDF and 365 meq buffering capacity.

Table 1: Harvested crop

	Roundbales (RB)	Precision chopped (PC)
Kg	112061	150860
DM, g/kg (n = 14 (RB), n = 18 (PC))	246 ± 17.8	248 ± 14.4
DM, kg	27591	37484
GrasAAT, l/t	3.89	3.90
Number of bales (RB) or loads (PC)	153	50
Per bale (RB) or load (PC), kg	732	3017
Per bale (RB) or load (PC), kg DM	180	750

Both silages were chopped using Serigstad RBK 1202 bale chopper prior to feeding. Each silage was fed *ad libitum* to 16 Norwegian Red dairy cows in mid lactation (122 d.i.m.). A 4-week preliminary period preceded a 10-week experimental period. Two concentrates, both based on barley, oats, rapeseed- and soybean meal were also evaluated using a factorial design. No significant silage by concentrate interactions were found, and only silage results are presented here. The concentrates were fed separately in four daily meals, and contained, on average, per kg DM: 161 g CP, 48 g fat, 362 g starch and 207 g NDF.

Results and discussion

No mould was found in the silages. RB and PC silages were aerobically stable for one and two weeks, respectively. Both silages were well fermented (Table 2). The RB silage underwent a restricted fermentation that spared water soluble carbohydrates (WSC) in the silage, whereas PC silage was more extensively fermented.

Table 2: Composition of silages, n = 14

	Roundbales	Precision chopped	SEM	*P*
DM, g/kg	249	250	2.1	NS[#]
OM, g/kg DM	930	925	2.0	0.06
CP, g/kg DM	144	144	3.3	NS
NDF, g/kg DM	591	591	4.4	NS
ADF, g/kg DM	352	352	3.7	NS
WSC, g/kg DM	69	28	4.7	<0.001
Lactic acid, g/kg DM	46	69	1.3	<0.001
Formic acid, g/kg DM	7.3	7.1	0.2	NS
Acetic acid, g/kg DM	13	19	0.7	<0.001
Butyric acid, g/kg DM	0	0.6	0.31	NS
Ethanol, g/kg DM	7.7	8.5	0.83	NS
NH_3-N, g/kg TN*	84	75	3.4	0.09
pH	4.14	3.94	0.032	0.001
Digestible OM, g/kg DM	605	608	4.8	NS

* Corrected for NH_3-N applied with the silage additive
[#] $P \geq 0.1$

Silage intake was 10.8 and 11.9 kg DM for RB and PC silage, respectively (Table 3). This difference was attributed to the measured shorter median chop length of PC silage (20 mm) than of RB silage (100 mm) when fed. The increased intake of silage PC was reflected in a higher milk yield, with a higher fat concentration ($P = 0.11$) and a higher yield of ECM. Milk urea was lower and milk α-tocopherol higher when using PC compared with RB silage.

Table 3: Feed intake, milk production, changes in body weight and condition score (BCS), and N utilisation

	Roundbales	Precision chopped	SEM	*P*
Silage DM, kg	10.8	11.9	0.28	0.01
Concentrate DM, kg	6.06	6.06		
Total ration DM, g/kg BW	31.2	32.9	0.54	0.04
Total ration NDF, g/kg BW	14.1	15.1	0.27	0.01
Milk, kg	21.3	22.1	0.34	0.097
ECM, kg	21.7	22.9	0.31	0.01
Milk fat, g/kg	42.0	43.4	0.62	NS
Milk protein, g/kg	33.6	33.4	0.30	NS
Lactose, g/kg	46.4	46.7	0.13	0.07
Milk taste score*	4.08	3.95	0.124	NS
Milk urea, mM	5.61	5.23	0.078	0.003
α-tocopherol, mg/l	0.45	0.54	0.020	0.004
BW change, g/d	301	321	55.2	NS
BCS change, points/100 d	-0.048	0.035	0.092	NS
Milk N / feed N	0.270	0.268	0.005	NS

* Five-point scale where 1 = poor quality milk and 5 = high quality milk with no deviation from normal taste

Rumen fluid from cows fed PC silage had lower pH (6.63 vs. 6.70) and contained a lower NH_3-N concentration (12.4 vs. 14.0 mM), a lower proportion of acetic acid (66.0 vs. 67.3 molar%) and a higher proportion of propionic acid (17.7 vs. 16.9 molar%) compared with cows fed RB silage.

The roundbale technique produced silage with a more desired chemical composition than the precision chop & bunker silo technique. In spite of its slightly higher NH_3-N concentration, the RB silage was expected to give rise to approx. 3% higher silage intake than the PC silage due to its lower concentration of fermentation acids (Huhtanen et al 2002). Contrary to this, a 10% higher intake was observed using silage PC, which means that the shorter particle length of silage PC totally overrode the effect of the better fermentation quality of silage RB in terms of intake. An increased intake of 10% is also recently found in another trial where silage chop length was reduced from 67 to 22 mm, and fermentation quality was equal (Randby 2005). However, the positive effect of shorter chop length seems to require that chop length is reduced to nearly 20 mm, since no effect of chop length was found comparing grass silages with 135 and 60 mm mean chop lengths, with identical chemical composition (Toivonen and Heikkilä 2005). According to Nørgaard (2003), a reduction in eating time with reduced particle size is observed only for chop lengths below 40 mm. Ruminating time is reduced for feeds with chop lengths below 20 mm. Particle lengths below 20 mm may, however, reduce the physical structure of the ration, and give rise to rumen dysfunction, low milk fat concentration and related problems (De Brabander et al. 1999).

The increased energy intake with PC silage compared with RB silage was well utilised, and converted quantitatively to milk energy, since the cows did not increase their body weight gain or their body condition score.

Conclusions

In situations where grass silage produced at the farm is cheaper than alternative feeds, a reduction in particle size is of great practical significance. The shorter eating time needed to consume each kg of DM when it is finely chopped may be the reason for the higher intake. Further studies are needed to determine the effect of chop length on intake of highly digestible grass silage.

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Forage Drying – Cause Study on the Formation of Dioxines

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Introduction

Since the end of the1990's, elevated values of dioxines and other contaminants have been established in different feeds during official feedstuff controls in Germany. In many cases the source of pollutants could be traced back to dried feedstuff from forage drying plants. European-wide the process of direct current drying in rotating drum dryers is used for the drying of forage and other feed. The reasons for the formation of toxic substances, however, are still unknown. It is assumed that the direct firing and heating system and particularly the solid fuels applied in forage drying plants could be the source of such undesirable materials. Investigations on different drying plants have shown that also other reasons for the pollutant formation are to be considered.

Materials and methods

State of the forage drying

In 2000 in the EU about 5 Mio. tons of dried forage have been produced with the main producers to be Spain (1.95 Mio. t), France (1.43 Mio. t) and Italy (0.70 Mio. t). In Germany almost 340.000 tons of dry forage were made with additional 125.000 tons which have been imported. Hence, the degree of self-supply with crude proteins for the feeding of farm animals in Europe is about 22-25% (Kämpfe and Hentze, 2001). From economic reasons, the number of forage drying plants has decreased in the last few years. Today about 70 drying plants still operate in Germany which are organized in the German Industrial Union of Agricultural Drying Plants (Fürll et al., 2003).

European-wide the process of direct current drying in rotating drum dryers is used for the drying of forage and other feed. During this process, the forage is directly contacted with the flue gas from the combustion system which is diluted with external air before entering the drum as drying air. Mainly natural gas and heating oil but also wood (chips), lignite and black coal are utilized as fossil fuels. The temperature of the drying air at the entrance of the drum varies between 350°C and up to 1000°C (Mayer and Rutzmoser, 2001). The exit air reaches values of 80-140°C. The moisture content of the wet green forage is between 60% w.b. and 80% w.b. whereas the moisture content of the dry forage amounts to < 13% w.b. The advantage of the direct drying process is its high thermal efficiency which surpasses that of the indirect drying process by more than 30%. The disadvantage is that there is a potential risk of entry of dioxines and other pollutants into the feedstuff if the combustion is improperly carried out.

Measurements of dioxine concentrations in forage

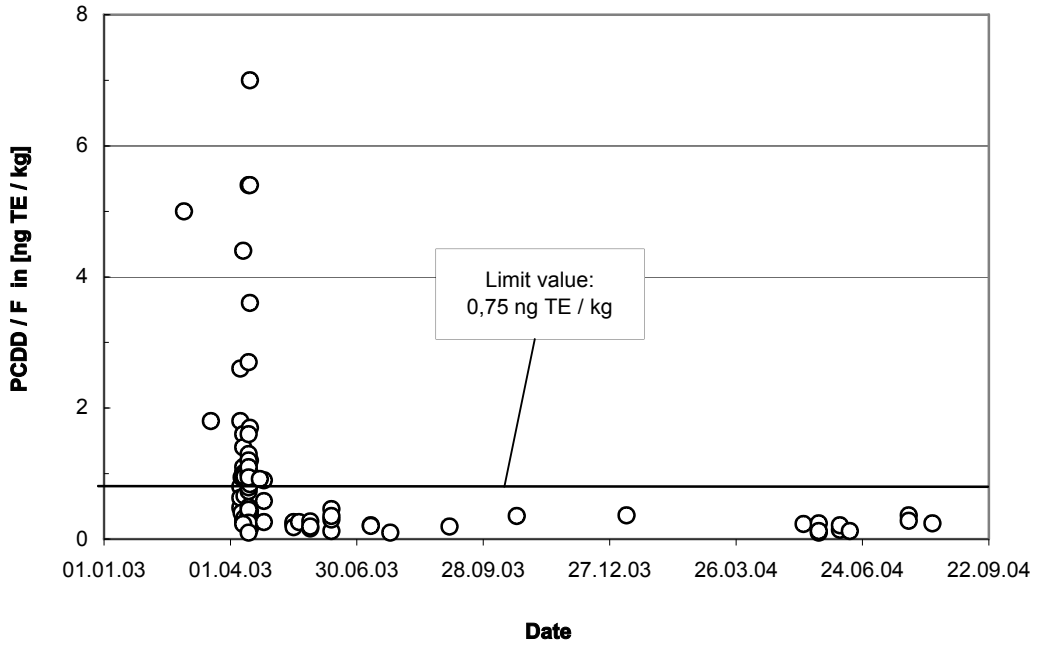
In order to find out the reasons for the dioxine formation in dried forage investigations at different drying plants accompanied with measurements of the flue gas composition and dioxine concentration in samples of farm-fresh as well as dried forage have been carried out. The limit value for dioxines in forage is fixed in German Animal Feed Order from 2002 to 0,75 ng TE/kg (TE = toxicity equivalent) based on a dry substance content of 88% w.b.

As an example, the dioxine concentrations measured in a drying plant in 2003 and 2004 are depicted in Figure 1. This plant is operating with a solid fuel mixture of black coal and briquette wear. As can be seen from the figure, there was a significant singular incident which occurred in April 2003. At that time, high concentrations of dioxines up to 7 ng TE/kg were found in samples of one forage charge. The cause study revealed that this production charge was from September 2002. Due to the long time distance between the production period and the date of ascertainment as well as the circumstances of the storage of the dried forage, the real date of production and, therefore, the effective reason for the dioxine formation could not be found out. Possibly, a disaster like a fire in the drum dryer or problems at the beginning of the drying process were the reasons.

In November 2004, at the same drying plant measurements have been conducted to investigate the start-up procedure of the drying process in detail. These investigations devoted interesting results. It could be demonstrated that two reasons for a possible dioxine formation are important:

1. the pollution of the green farm-fresh forage,
2. the dioxine formation in the start-up period of the drying plant.

Figure 1: Dioxine concentrations measured in samples of dried forage from a drying plant directly fired with a mixture of black coal and briquette wear



Results and discussion

The following potential sources of entry of dioxines into dried forage have been determined or recherched:

- the basic dioxine load of the farm-fresh forage,
- concentrated dioxines in particulate matter (cyclone dust),
- dioxine entry from the combustion, especially during the start-up procedure or disasters (drum fire),
- dioxin formation during the drying process within the drum.

The dioxine concentrations of farm-fresh forage measured in November 2004 amounted up to 0,66 ng TE/kg. The highest total values are to be found in particulate matter such as cyclone dust with up to 15 ng TE/kg (Fürll et al., 2003). During the start-

up procedure dioxine values up to 1 ng TE/kg have been measured. By now no measurements are known about the possible dioxine formation during the drying process within the drum dryer.

Conclusions

Dried forage is an important long-term storable feedstuff for healthy feeding of farm animals due to its high content of crude proteins. In comparison to the indirect drying process, the direct forage drying in rotating drum dryers should be maintained and prefered due to its high thermal efficiency and high capacity which is needed during the harvest period. To avoid dioxine formation, the drum dryers should be regularly operated. The temperature within the combustion chamber should go beyond 900°C. If this is always guaranteed the dioxine formation during the combustion of fossile fuels is suppressed on account of the element sulfur. Sulfur prevents the formation of chlorine (Cl_2) which is a source material for dioxines (Oehme, 1998). In fossile fuels the S/Cl-ratio is higher than 5:1. The supression effect of sulfur already occurs if S/Cl > 0,64. Therefore, operating instructions and the dryer control must be adapted and improved. If this will be considered the direct forage drying can develop to a state-of-the-art technique and will persist.

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Content Of Structural Sacharides And Their Influence On Degradability Of Lucerne Crude Protein In Different Stage Of Maturity

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Summary

The influence of maturity stage of lucerne was investigated on the content of crude protein (CP), NDF, ADF, N-ADF and in sacco CP degradability in the rumen of bulls.The content of CP decreased significantly ($P<0.05$) with the increasing of lucerne maturity. The part of N-ADF appears to be higher in the period of full flowering than in two previous periods. The effective CP degradability decreased about 10 % in relation to the increasing of NDF and ADF contents ($P<0.01$) in dependance of lucerne maturity. Correlation between observed parameters of lucerne was high significant.

Introduction

Lucerne (Medicago sativa, L.) represents in our conditions important roughage the nutritive quality of which is related to the stage of phenophasis. Legumes are better digestible than grasses because they contain less fibre [3]; it is due to the differences in their morphology and anatomy (Deinum, 1973). However, during maturation of lucerne the proportion of leaves to stems is decreased from 1.5 to 0.5 (Buxton and Redfearn, 1997), fiber in stems become more lignified and it decreases digestibility as well as quality of lucerne as feed. As described by Atanassova et al. (1994) the content of fibre has negative effect on degradation of crude protein in lucerne , and content of crude protein has positive effect.

Materials and methods

We studied the relation of crude protein degradability and of content of structural polysaccharides, represented by cell walls (NDF), in lucerne (variety Palava), at three growth stages – creation of flower buds (A), beginning of flowering (B) and in the late flowering (C). We analysed the lucerne samples in three repetitions (we took the samples always from the area 1 m^2). We determined dry matter, crude protein (N-6.25) by Kjeldahl's method, NDF and ADF as described by van Soest and N-ADF.

We determined the degradability in four young bulls with large rumen cannula by means of standard in sacco method. We did four repetitions for each incubation period and animal. Effective degradability was calculated as described by Orskov and McDonald (1979).

The studied parameters were evaluated by two-way analysis of variance, and the dependence of effective crude protein degradability (EdgCP) on content of observed parameters was determined by means of linear regression (Grófik a Fľak, 1990).

Results and discusion

Concentration of NDF (cell walls), ADF as well as the proportion of N-ADF from total content of N rose with maturation of lucerne. On the contrary, content of N decreased significantly (Table 1). Content of hemicelluloses changed only nonsignificantly. This fact is a consequence of the change of leaves and stems proportion in the plant.

Table 1: Content of nutrients, N-ADF (g/kg DM)

Phenophasis	Dry matter	Crude protein	NDF	ADF	N-ADF	Hemicelulosis
A	170±8,9[a]	239±8,7[a,b]	338±9,6[a]	284±8,8[a]	1,58±0,07	28,4±4,5[a]
B	187±17,8[b]	210±11,0[b]	396±17,4[a,]	332±19,0[a]	1,73±0,14	54±4,4[a]
C	229±10,2[a,b]	173±1,9[a,]	464±4,1[a]	388±12,7[a]	1,60±0,22	76±12,7[a]

Means in the same column with the same letters differ significantly (P<0,05, resp. P<0,01)

Leaves are of relative constant composition independent from the vegetation stage with very low lignin content. Content of lignin in stems is two times higher than in leaves in very young plants alrerady, and it increases significantly with maturation of lucerne (Čerešňáková et al., 2002). Nitrogen linked to ADF represents the lignified fraction of N and is not utilizable by animals. Its portion represented 5.7 % at the stage of late flowering. This fact became evident significantly also in decrease of effective degradability of crude protein from 79.6 % at the stage of flower buds creation to 69.8 % at the stageof late flowering. The markedly decreasing of soluble N fraction may significantly limit effective crude protein degradability (fig. 1). As mentioned by Lindberg (1985) it was the fibre structure which protects crude protein from degradation in rumen.

Content of crude protein is in negative correlation with the content of NDF, ADF, and the correlation of soluble N with effective crude protein degradability is strongly positive (Table 2). Degradability of non-soluble N fraction decreases significantly with the increase of NDF and ADF content

Figiure 1: Portion of effective degradable CP, soluble N and N linked on ADF from the total content of N in lucerne

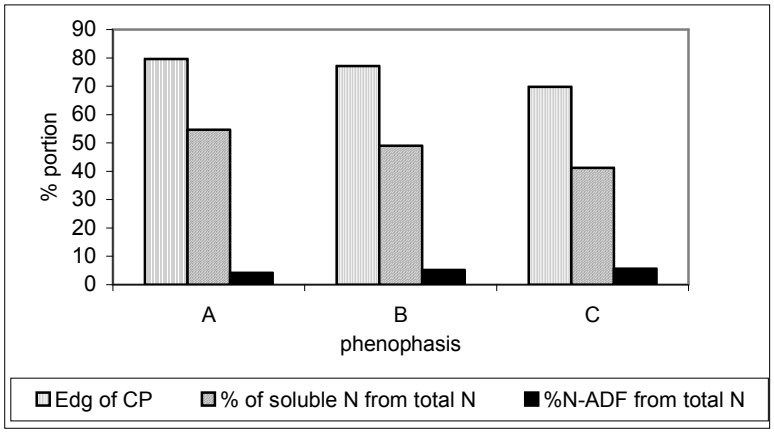

Table 2: Correlation between observed parameters of lucerne

Parameters	Soluble N	ADF	%N-ADF from N	NDF	Edg of CP
CP (Nx6,25)	0,760**	-0,765**	-0,804**	-0,833**	0,623*
Soluble N		-,0737**	-0,778**	-0,614*	-0,778**
ADF			0,721**	0,916**	-0,668**
%N-ADF from N				0,697**	-0,549*
NDF					-0,842**

** P<0.01; * P <0.05

Conclusion

The obtained results show that the increase of content of cell walls – structural polysaccharides with ageing of Lucerne, which is a natural process in plants, has negative relation to degradability and therefore also utilization of Lucerne crude protein as important roughage in nutrition of cattle.

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Early or Normal cut Grass Silage for Dairy Cows in Organic Farming

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Introduction

The sub-arctic climate of northern Norway provides challenges to organic farmers who want to be self-sufficient with feed. Early harvested grass has higher nutritional value than grass harvested at normal time and may, due to low crop yields, be chosen when acerage is not a limiting factor. Silage of early harvested clover grass may reduce the need of supplementary protein feeds and concentrates in the ration. Traditionally, many farmers in Northern Norway harvest grass at a relatively late stage of maturity, and supplement with relatively high levels of concentrates.

Materials and Methods

Early cut organic grass silage (roundbales) was compared with silage harvested 17 days (180 daydegrees) later in a continuous production experiment with 32 Norwegian Red dairy cows (24 multiparous and 8 primiparous cows) in early lactation (63 ±21 d.i.m.). The experiment was carried out in Bodø, Norway (67° 17" N, 14° 23" E). A half of the cows received a feed ration with 40% concentrates (H), and the other half 10% (L) on an annual energy basis. During the experiment, this made up, on a daily basis, 944 g DM fishmeal (587 g/kg DM CP, 48 g/kg DM fat) plus mineral and vitamin supplements to all cows, plus for cows on H 4.7 and cows on L 1.0 kg DM of cereals containing barley, oats, and some peas (dried oats-barley: 120 g/kg DM CP, 247 g/kg DM NDF, 51 g/kg DM starch; ensiled oats-pea: 126 g/kg DM CP, 226 g/kg DM NDF, 43 g/kg DM starch). Early cut silage (June 11, 2004) was harvested when timothy axes were perceptible in the stem and normal cut (June 28, 2004) when timothy axes were completely visible. The roundbale grass silage was acid treated (Ensimax, early cut 5.0 l/t, normal cut 3.0 l/t; 213 g/kg formic acid, 200 g/kg acetic acid; Borregaard Industries, Sarpsborg, Norway) and fed *ad libitum*. The harvesting time had no significant effect on the botanical composition of the clover grass (average: timothy (*Phleum pratense* L.) 478 g/kg DM, meadow fescue (*Festuca pratensis* L.) 163 g/kg DM, other grass species 230 g/kg DM, red clover (*Trifolium pratense* L.) 30 g/kg DM, white clover (*Trifolium repens* L.) 3 g/kg DM, herbs 95 g/kg DM).

Data were analyzed using analysis of variance with the GML model procedures of SAS. Results regarding milk yield and milk composition were covariance corrected for data from the preliminary period, whereas data pertaining to feed intake and BW changes were analyzed without covariance correction, since no reliable covariate existed for these parameters.

Results and Discussion

Early cut resulted in lower crop yields (2.17 t DM/ha) compared with normal cut (4.66 t DM/ha). Energy and protein concentrations were higher for early cut than for normal cut silage (6.4 vs. 5.6 MJ NE_l, as assessed by NIRS, 136 vs. 105 g/kg DM CP). Both silages were mainly well preserved, with minor amounts of butyric acid and low

NH_3-N values (Table 1). Ethanol concentrations, however, were high in both silages. The low levels of WSC in the silage were most probably due to the extensive ethanol fermentation. Driehuis and van Wikselaar (1996) reported levels up to 57 g/kg DM in silages with ethanol as the major fermentation product.

Table 1: Composition of 1st cut silages, cut early or at a normal time

	n	Early[1)]	Normal[1)]	s.e.m.	*p*
DM, g/kg	22	296	271	8.7	0.1
OM, g/kg DM	5	935	943	1.9	0.02
CP, g/kg DM	5	136	105	3.7	0.0005
NDF, g/kg DM	5	414	569	6.8	< 0.0001
ADF, g/kg DM	5	222	318	2.5	< 0.0001
ADL, g/kg DM	5	16	23	1.2	0.007
WSC, g/kg DM	6	20	8	3.0	0.02
Crude fat (EE), g/kg DM	5	37.2	29.9	0.94	0.0008
Lactic acid, g/kg DM	6	41	48	7.4	NS[2)]
Acetic, g/kg DM	6	19	20	2.3	NS
Formic acid, g/kg DM	6	2.4	4.1	0.52	0.11
Butyric acid, g/kg DM	6	0.7	0.1	0.21	0.10
Ethanol, g/kg DM	6	26	34	4.4	NS
NH_3-N, g/kg TN	5	40.5	45.0	5.37	NS
pH	6	4.31	4.08	0.072	0.02

[1)] Concentrations weighted according to the number of days each silage portion was used
[2)] $p \geq 0.2$

Cows on H refused to eat 1.9 kg of their daily concentrate allotment when fed the early cut silage. When fed the normal cut silage, however, only 0.3 kg was left over. Also Thuen (pers. comm., 2006) experienced poor palatability of rolled barley to dairy cows in organic production when cows were offered high quality grass silage *ad libitum*. Silage, total DM and total NDF intakes were in general high in the present experiment (Table 2). Intake of early cut silage was significantly higher than normal cut silage. Total ration intake was only slightly higher on H than on L, but milk yields were notably higher on H, possibly due to the higher energy concentration in ration H than L. The production level in L might also have been limited by adaptation to low concentrate levels over several lactations. Cows in L fed normal cut silage had the lowest feed energy concentration and lost body weigh and reduced body condition score during the experiment. Cows in H fed normal cut silage gained body weight and maintained body condition score. Cows fed early cut silage gained body weight and body condition score both on H and L. Although the same roundbaler and silage bale chopper was used, median chop length of silage was 94 mm for early cut and 136 mm for normal cut (measured on 3 samples), due to different physical conditions of the two crops.

Cows at H produced 27.5 kg ECM per day with early cut silage and 25.3 kg ECM with normal cut silage, and cows at L produced respectively 24.7 and 22.4 kg ECM. Cows offered early cut silage had highest milk protein concentration (H: 34.6 vs. 32.8; L: 34.4 vs. 31.4 g/kg). Also Rinne *et al*. (1999) found higher milk yields at early maturity stage but smaller differences in milk protein concentration, with the highest level at the second of four cutting times. In the present experiment the harvesting time did not influence the sensoric quality of milk, but low concentrate level reduced the milk taste slightly. Although milk free fatty acid levels were low, they were

significantly higher for cows fed normal, than early cut silage on both H and L concentrate level. This may relate to the lower energy concentration in the latest cut silage.

Table 2: Feed intake, production, body weight (BW), body condition score (BCS), and N-utilization

Concentrate level (annual):	High (40% on energy basis)				Low (10% on energy basis)			
Cutting time 1st cut:	Early	Normal	s.e.m.	*p*	Early	Normal	s.e.m.	*p*
Silage DM, kg	17.1	14.6	0.66	0.02	16.7	15.4	0.66	0.18
Concentrate DM, kg [1)]	3.72	5.31	0.367	0.008	1.94	1.98	0.017	0.09
Total ration DM, g/kg BW	35.3	34.0	1.24	NS [2)]	34.3	32.7	0.87	NS
Total ration NDF, g/kg BW	13.3	16.1	0.49	0.001	13.3	17.0	0.42	< 0.001
Milk, kg	26.3	23.9	0.84	0.07	23.3	21.4	0.79	0.11
ECM, kg	27.5	25.3	1.07	0.16	24.7	22.4	0.98	0.13
Milk fat, g/kg	43.3	44.5	0.92	NS	45.0	44.9	1.04	NS
Milk protein, g/kg	34.6	32.8	0.52	0.03	34.4	31.4	0.52	0.002
Milk lactose, g/kg	45.8	47.1	0.55	0.11	45.8	46.9	0.33	0.04
Milk urea, mM	3.37	3.36	0.090	NS	3.43	3.46	0.052	NS
Milk FFA IR, meq/l	0.38	0.60	0.032	< 0.001	0.58	0.76	0.064	0.060
Milk taste score [3)]	4.28	4.19	0.160	NS	3.80	3.93	0.172	NS
BW (initial), kg	584	587			543	539		
BW change, g/d	397	255	88.6	NS	380	-114	98.7	0.003
BCS (initial), points [4)]	3.16	3.30			3.10	2.98		
BCS change, points/100 d	0.38	0.00	0.116	0.03	0.35	-0.17	0.149	0.03
Milk N/feed N	0.272	0.290	0.0118	NS	0.266	0.282	0.0077	0.16

[1)] Differences due to concentrate left-overs: H: Early 1.90, Normal 0.30, L: Early 0.07, Normal 0.0

[2)] $p \geq 0.2$

[3)] Five point scale, where 1 = poor quality milk and 5 = high quality milk with no deviation from normal taste

[4)] Five point scale with 0.25 point intervals, where 1 = emaciated and 5 = very fat animals

Conclusions

Feeding early cut silage of an organic ley increased feed intake, milk yield and milk protein concentration compared with normal cut. Left-overs of concentrates were a problem in the experiment and ways to improve the palatability of on farm produced cereals have to be developed. Early cut crop yields were only about half of the crop yield at normal cut. Therefore early cut may only be recommended to farmers in northern Norway when acerage is not a limiting factor.

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The effect of acetic acid, caproic acid and tryptamine on voluntary intake of grass silage by growing cattle

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Introduction

On average reductions in the voluntary intake of grass silage by cattle compared to the fresh parent crop have been observed (Mayne and Cushnahan, 1994/95). This has been attributed to the end products of fermentation (Dulphy and Van Os, 1996). Fermentation is a complex process generating a vast number of compounds. High performance liquid chromatography (HPLC) and gas chromatography (GC) were used to further separate silage components. The objective of the present study was to point out substances negatively related to intake by using Partial Least Squares Regression (PLSR) analysis, and to verify the impact of these components on the voluntary intake of silage.

Materials and Methods

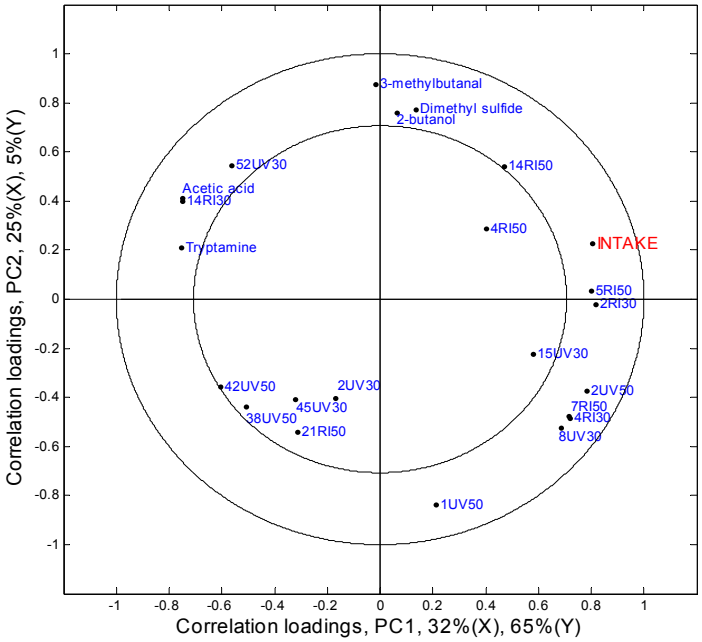
The samples subjected to HPLC and GC analyses were from 24 low DM silages. The silages were harvested from the same sward (>80% Phleum pratense) at the same time (within 60 h) using different ensilage techniques. Chemical analyses and intake registrations of the 24 silages existed from a previous feeding trial (Krizsan and Randby (submitted)). The HPLC analysis was performed with a Bio-Rad HPX-87H organic acid column at 30 and 50°C (mobile phase: 0.0075N H2SO4 at 0.8 ml min-1) with a UV spectrophotometric detector at 210 nm or a refractive index (RI) detector. Run time for each sample was 55 min. The GC analysis was performed with an automatic headspace sampler, and a flame ionization detector. A HP-Innowax fused-silica column was used. The carrier gas was helium with a flow rate of 1.0 ml min-1. The column temperature was held initially at 40°C for 1 min, increased to 60°C at 5.0°C min-1, then isothermal at 60°C for 10 minutes, increased to 200°C at 10.0°C min-1, and held for 15 minutes. Previous analysed fermentation products and compounds registered as peak area or heights from the GC and the four different HPLC chromatograms were related to intake by PLSR analysis using The Unscrambler®8.0.5. Only significant variables were kept in the model as estimated by jack-knifing, and the model was validated by full cross validation (Martens and Martens, 2001). Compounds with unknown identification and negatively related to intake were identified/quantified with LC-MS/HPLC-UV. The effect of three successfully identified components, singly or as a mixture, was examined in a feeding trial. The substances were added to untreated wilted grass silage in amounts that aimed to correspond to the highest detected level in the samples of the 24 silages. The silage was preserved as round bales and before feeding commenced the bales were processed through a chopper (Duks GS-120, Ans By, Denmark) producing a median chop length of 45 mm. Thirty steers of Norwegian Red (initially 176 kg, s.e. 28.3) were blocked by daily intake and bodyweight, and randomly assigned to treatment within block. The experiment was performed as three pairs of 5 × 5 Latin Squares balanced with regard to carry-over effects, and with experimental periods of two weeks. Five dietary treatments were studied: addition of 60 g/kg DM of acetic acid (AcA), 2.5 g/kg

DM of caproic acid (CA), 1 g/kg DM of tryptamine (TrpA), a mixture of AcA, CA and TrpA providing the same concentrations, and a control diet (C) of silage with nothing added. The animals were fed silage as sole feed during the whole trial. The amounts of added substances were based on individual measurements of bodyweight and an expected daily silage DM intake (SDMI) of 2.2 kg DM/100 kg live weight (LW). Statistical analysis was carried out using MIXED procedures in SAS.

Results and Discussion

Ranges (mean value/s.e.m.) of earlier analysed fermentation quality parameters in the 24 silages were: WSC 16.3-70.9 (33.0/15.3), AcA 11.5-64.7 (28.6/14.6), propionic acid (PA) 0-5.2 (1.0/1.6), butyric acid (BA) 0-25.1 (6.0/8.3), lactic acid (LA) 2.2-102 (49.3/23.8), ethanol 3.4-13.3 (6.8/2.4), 2-phenylethylamine 0-0.257 (0.100/0.083), histamine 0-1.43 (0.347/0.396), TrpA 0-0.643 (0.085/0.151), tyramine 0.294-2.68 (1.49/0.650), putrescine 0.174-3.73 (1.44/0.897), cadaverine 0.122-5.41 (1.36/1.34) g/kg DM, NH_3-N 89.3-255 (153/41.5), ADIN 12.2-28.9 (18.9/4.90) g/kg total N. A total of 128 peaks from the HPLC chromatograms and 26 peaks from the GC chromatograms were separated. Identified compounds by standards in the GC chromatograms were: propanal, 2-methylpropanal, methyl ethanoate, ethyl ethanoate, 3-methylbutanal, methanol, 2-butanol, 1-propanol, dimethyl sulfide and ethyl butanoate. From the two-component PLSR model AcA, CA and TrpA were picked among compounds negatively related to intake. The relation of the significant explanatory variables to intake is presented in the correlation loading plot (Figure 1). The 42nd peak with regard to retention time in the HPLC-UV chromatogram at 50ºC (42UV50 in Figure 1) was identified as CA. The highest detected level of CA in the silage samples was 2.1 g/kg DM. Other substances in the south-west quadrant in Figure 1, close to 42UV50, are also anti-correlated to intake. The majority of unknown substances negatively related to intake were separated by HPLC. Therefore, a combined LC-UV(210) and LC-MS system was prioritized for the identification. Analysis of samples containing the substances of interest suggested possible identities. However, the suggested identities could not be confirmed with standards in HPLC-UV except for CA. Moreover, substances with known identity most negatively related to intake were AcA and TrpA (Figure 1).

Figure 1: Correlation loading plot of the variables in the PLSR model.



The chemical composition of the round bale silage was (s.e.m.) (N = 10 unless otherwise stated): DM 314 (16.6) g/kg, CP 137 (4.42), NDF 577 (26.0) (N=3), ADF 349 (8.32) (N=3), ADL 46.1 (3.24) (N=3), WSC 26.6 (1.84), ethanol 7.9 (1.09), AcA 20.7 ($2.05), PA 0.2 (0.325), BA 0.0 (0.0), LA 79.9 (2.32), CA 0.0 (0.0), TrpA 2.95 (0.217) (N=5) g/kg DM, NH_3-N 136 (4.32) and ADIN 16.1 (1.79) (N=3) g/kg total N. Measuring daily SDMI indicated significant differences between treatments (P=0.002). Estimated least squares means (LSM), and differences of LSM for the treatments compared to the control are presented in Table 1. However, no significant treatment effects were observed when total daily DM intake (silage DM and added amount DM of chemical substances in the treatments) was considered (P=0.562). The average daily quantity of AcA and the mixture treatment ate during the whole trial amounted to 0.10 and 0.11 kg DM/100 kg LW, respectively. These amounts corresponded almost precise to the significant (P<0.05) differences in Table 1. The average concentration of chemical substances provided in the silage DM during the trial were: 53.5 AcA, 2.21 CA, 0.887 TrpA g/kg DM, and in the mixture 53.6 AcA + 2.24 CA + 0.927 TrpA g/kg DM.

Table 1. Daily SDMI (kg DM/100 kg LW) and estimated contrast compared to the control diet.

Treatment	SDMI	Contrast	P
Acetic acid (AcA)	1.98	-0.13	0.035
Caproic acid (CA)	2.16	0.05	0.426
Tryptamine (TrpA)	2.14	0.03	0.572
AcA + CA + TrpA	1.97	-0.14	0.021
Control	2.11	-	-

Conclusions

Dietary addition of acetic acid, caproic acid, tryptamine and a mixture of these compounds to untreated restrictively fermented grass silage did not significantly alter daily DM intake of growing steers fed silage as sole feed.

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Nutritive Value of Maize Silage with Different Nitrogen Fertilization Intensity

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Abstract

The aim of this work were characteristics of maize silage nutritive value changes influence of different rates nitrogen fertilization. We have studied three levels of nitrogen fertilization. The plants of maize we harvested at content of dry mater from 291,3 to 399,3 g.kg^{-1}. In variant N_0 maize grew in ground, which wasn´t fertilized for 6 years. In rest of variants (N_{60}, N_{180}) maize grew in conditions with different rates nitrogen fertilization (60 and 180 kg N per hectare). After analyzing we found, that with increasing fertilization intensity was the content of crude protein statistically significantly higher and content of nitrogen free extract decreased. In variant without nitrogen fertilize we found the maximal value of NEL. Value of protein digestible in intestine increased in dependencies from fertilization intensity.

Introduction

Maize is very important feed in animal nutrition, which presents elemental source of energy in nutrition of ruminant. Combinative hybridyze with heterosis effect utilization pour into different nutritional quality feed. Significant variances of amount to in structural and non-structural carbohydrate content, which determine digestibility of organic mater and fermented organic mater content (Bíro, 2001). Maize silage is the basic feed in livestock nutrition, but quality of maize silage in practical conditions is unsuitable (Sommer 1997). Essential factor quality of silage is technological discipline (Doležal et al. 2003). An area that has received passing attention from time to time is the influence of fertilizer on composition of maize (Summers 2001). Fertilizing with N, P, K resulted in increased crude protein of the maize and an increase in some of the essential amino acids (Bird, Olsen 1972).

Materials and Methods

Maize (*Zea mays, L.*) was grew in the same agroclimatic conditions with different intensity of nitrogen fertilization. In experiment was used silage hybrid MILA 400. Individual stands was in autumn 2002 fertilized by superphosphate and potash salt (tab. 1). In the spring 2003 was fertilized by nitrogen: 2/3 of rate before seeding and 1/3 of rate during vegetation. Experiment had five variants of fertilization: N_0 – maize was grew in ground, which was not fertilized by nitrogen six years (control variant). In variant N_{60} was rate of nitrogen 60 kg/ hectare and in N_{180} 180 kg N/ hectare. Maize was harvested in stage at the beginning of milk-waxy maturity at the end of august 2003. Whole plant of maize was cutted directly after harvesting on the lenght 8-12 mm. Matter of maize in all variant was silaged without additives. Silage mater was pressed by pneumatic press into the units with capacity of 15 dm^3 and hermetized. After 56 days we sampled of average samples for chemically analyses on the content of nutrients (MP SR, 1997).Energy and protein value in maize silage was caltulated according to MP SR (2002). Differences between of variants was evaluated statistically by program *Statgraphics, version 5,0.*

Table 1: Rates of applied nutrients

Variants	Rates of nutrients kg/ha		
	autumn 2002	year 2003	
		before seeding	during vegetation
N_0	-	-	-
N_{60}	20 P + 80 K	40 N	20 N
N_{180}	60 P + 240 K	120N	60 N

Results and Discussion

We found statistically significant decreased content of dry mater in comparison to control variant by 70,5 $g.kg^{-1}$ DM (N_{60}) and by 84,9 $g.kg^{-1}$ of DM (N_{180}) with intensification of nitrogen fertilization (tab.2). Nitrogen fertilization increases yield of maize first of all content of crude protein (Khandaker and Islam, 1988). In comparison to maize silage made from mater grew on soil without fertilization had maize silage of variant N_{60} by 31,4 % and silage of variant N_{180} by 49,8 % higher content of crude protein. Differences was statistically significant ($p<0,001$). Identical results confirmed Brucknerová (2004). With intensity of nitrogen rates we have not found statistically significant differences in content of fat, crude fiber, ash and organic mater in silage. Maize silage of variant N_{180} had the highest content of crude fiber and the lower content of fat. Brucknerová (2004) found higher content of aciddetergent of fiber in variants with nitrogen fertilization. Content of ash with intensity of fertilization decreased, content of nitrogen free extract too. Silages of variant N_{180} had in comparison to control variant statistically significant ($p<0,01$) lower content of NFE by 41,6 $g.kg^{-1}$ of DM. With intensity of nitrogen rates value of NEL in maize silage decreased from 6,3 (K) to 6,23 $MJ.kg^{-1}$ of DM, however differences were not statistically significant. The same trend was in value of NEG and silage N_{180} had statistically significant lower value of NEG like control silage. With intensification of nitrogen doses increased value of protein digestible in intestine (PDIN) in maize silage. This value, which is limiting fraction of PDI in maize like carbohydrate feed, was in variants with fertilization higher against variant without fertilization by 10,2 – 16,1 $g.kg^{-1}$ of DM. The application of nitrogen fertilization increased significantly value of PDIE too.

Table 2: Nutritive value of maize silage

Parameter (g/kg DM)	Control K	N_{60}	N_{180}	Statistical significance	
	$\overline{x}$			K : N_{60}	K : N_{180}
Dry matter (g/kg fresh matter)	374,2	303,7	289,3	+++	+++
Crude protein	53,2	69,9	79,7	++	+++
Fat	26,7	29,2	25,4	-	-
Crude fiber	204,9	199,9	216,7	-	-
Ash	43,1	45,6	47,7	-	-
Nitrogen free extract	672,1	655,3	630,5	-	++
Organic matter	956,9	954,4	952,3	-	-
Net energy of lactation (MJ/kg DM)	6,3	6,28	6,26	-	-
Net energy of gain (MJ/kg DM)	6,29	6,26	6,23	-	+
PDIN*	32,2	42,4	48,3	++	+++
PDIE*	66,2	68,6	68,9	+	+

DM - dry matter, * protein digestible in intestine, + P<0,05, ++ P<0,01, +++ P<0,001

Conclusions

In experiment we found influence of different rates of nitrogen fertilization on nutritive value of maize silages. From results of observation of influence nitrogen fertilization on nutritive value of maize silage originate that:

1. with increasing of intensity nitrogen fertilization decreased content of dry mater and energy value (NEL and NEG) and content of nitrogen free extract,
2. increased content of crude protein,
3. content of crude fiber was the highest in variant with rate of nitrogen 180 kg/ hectare,
4. with rate of nitrogen fertilization increased value of protein digestible in intestine (PDIN and PDIE).

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The evaluation of quality and nutritive value of sedge herbage ensilaged in big bales

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Introduction

Sedge vegetation contains more vitamins and nutritive components and some micronutrients than the grass meadows herbage know as qualitatively the best. First of all sedge herbage it characterized by higher fat content and lower content of crude fibre than grasses Almost all sedges are the richest sources of the vitamins A and C. They contain also more than grasses copper and cobalt Denisiuk (1980). But bog meadows traditionally were mown extensively for hay and used mostly for horse feeding. Because of the high silica content and structural fibre cattle are reluctant to eat it (Denisiuk, 1980; Moraczewski, 1996; Nowiński, 1966). Recently, agricultural use of bog areas has been abandoned and this has caused the disappearance of bog meadows. They become overgrown by natural generation of trees and shrubs and develop into coppice communities. This study was aimed to find the ways of improvement the nutritive values of sedge herbage by ensilaging with the use of rolling up presses and to consider the possibility of sedge silage use in agriculture production.

Materials and methods

During the years 2002-2003 the study in the Experimental Station in Biebrza in Podlaskie province was carried out. Ensilaged plants were harvested from two bog meadows situated in and near the area of Biebrza National Park. It was a vegetation of *Calthion* alliance consisting in 70 % of low sedges: *Carex panicea* (59%), *C. oederi* (6%), *C. fusca* (5%) (meadow I) and a vegetation of *Magnocaricion* alliance from flooded meadow composed in 80 % of sedges, mostly *Carex gracilis* (meadow II). In 2002 the meadows were mowed in the middle of June and in 2003 in the beginning of July. After mowing the herbage coming from both meadows was pre-wilted and harvested with the rolling up press. Half of ensilaged material was inoculated with Polmazym containing LAB and enzymes (1 l/t of herbage). For comparison the herbage from cultivated meadow composed in 80% of grasses: *Poa pratensis* (40%), *Dactylis glomerata* (14%), *Phleum pratense* (7%), *Alopecurus pratensis* (6%), *Festuca rubra* (6%), other species (7%) and in 20% of herbs and weeds was ensilaged. In the same time the herbage from the same bog meadows was dried for hay. During the winter period the forages were fed to 32 heifers in age of 18-23 months and mean starting body weight 322 kg in 2002 and 303 kg in 2003, divided into 4 feeding groups. These groups were: group I – fed with sedge silage, group II – sedge silage supplemented with Polmazym, group III – hay of sedges and group IV – silage from cultivated meadow. The feeding experiment in 2002 lasted 40 days and 63.5 days in 2003. Animals were fed as a group "ad libitum". Along with the forages the animals received 1.5 kg concentrate per head and day. The nutritive value and quality and of tested feeds was determined. In 2003 the air stability of silage samples in temperature 21°C for 12 days was evaluated.

Results

The quality of silage prepared from sedge herbage varied between years especially due to different level of DM in the ensilaged herbage. Good weather conditions during harvest process in 2002, pre-wilting of ensilaged material to the proper DM content (> 500 g/kg DM) caused that the quality of all silages was very good. No butyric acid was found and the content of lactic acid in FM of sedge silage prepared both with and without Polmazym, was similar to that in silage made from cultivated meadow sward. Because of rain falls in 2003 and resulting from this insufficient pre-wilting of some parties of the herbage on thicker swaths, some bales of silage were of low quality value. The butyric acid and greater quantities of acetic acid, especially in silage made without the Polmazym addition were stated (Table 1).

Table 1: Chemical evaluation of tested silage

Year	2002			2003		
Type of silage	sedge	sedge + Polmazym	cultivated meadow	sedge	sedge + Polmazym	cultivated meadow
DM (g kg^{-1})	553.7	523.7	661.9	439.6	496.5	511.8
pH	5.00	4.90	5.60	4.90	4.70	5.10
Lactic acid (g/kg FM)	2.70	2.67	2.79	2.03	2.62	2.93
Acetic acid (g/kg FM)	0.24	0.33	0.22	0.73	0.46	0.35
Butyric acid (g/kg FM)	0.00	0.00	0.00	0.11	0.06	0.00
Sum of acids	2.94	3.00	3.01	2.88	3.14	3.28
Points	100	100	100	74	85	100
Evaluation acc. to Flieg - Zimmer scale	very good	very good	very good	good	good	very good

Silage prepared from sedge vegetation contained significantly more crude protein than hay prepared from the same type of vegetation. Also the content of crude fat and phosphorus was higher and crude fibre lower. The nutritive value of feeds was significantly improved by ensilage process. The silage made with addition of Polmazym had higher nutritive value than control silage (without additives). In both years of study they had higher content of phosphorus and crude protein in the first year of investigation. The phosphorus content both in the hay and in sedge silage was significantly smaller than in the silage from cultivated meadow (Table 2).

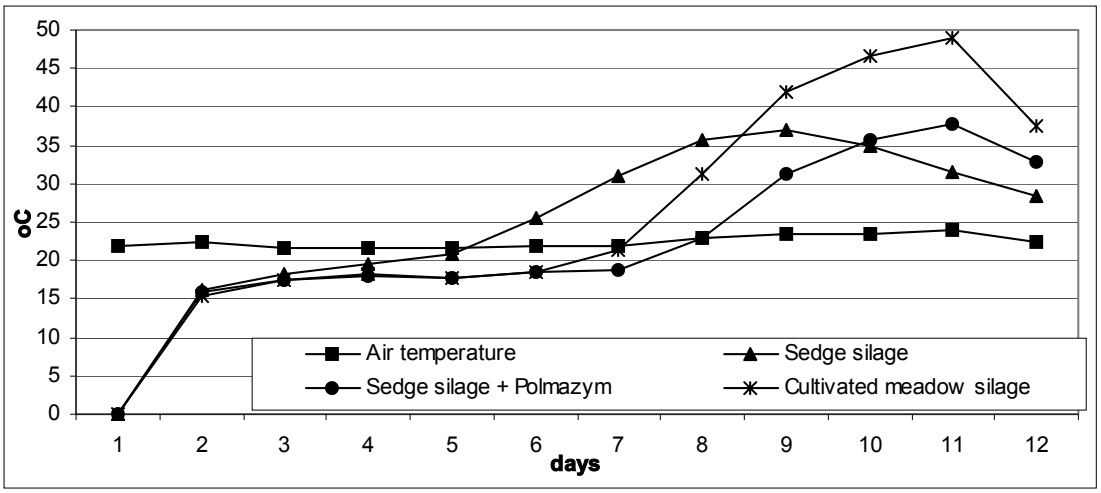
Table 2: Nutritive value of tested feeds.

Year	2002					2003				
	I	II	III	IV	LSD	I	II	III	IV	LSD
Crude protein (g/ kg DM)	151.0	186.3	134.8	190.7	10.5	150.1	146.8	131.4	199.7	23.4
Crude fibre (g/ kg DM)	229.1	257.3	323.3	286.6	8.7	343.0	332.0	399.1	321.9	51,6
Crude fat (g/ kg DM)	37.5	37.6	35.6	37.9	ns	-	-	-	-	-
Phosphorus (g/ kg DM)	2.4	2.7	2.3	3.4	0.20	3.6	4.4	2.5	6.5	1.10
Daily gains of animals (g/head)	575ab	695a	384b	717a		690a	748a	367b	813a	
Daily intake of feeds (kg DM/head)	7.47	8.30	5.94	7.81	-	7.66	8.82	6.21	9.58	-

The animals receiving the silage from sedge meadows obtained higher body gains than animals fed with the sedge hay and did not differ significantly from the body gains of animals fed with the silage from cultivated meadows, but they were a little lower. Animals receiving the sedge silage with Polmazym addition obtained higher body gains than animals fed with the control silage (without additive), but the differences were not significantly different The level of body gains was positively correlated with a nutritive value of feeds and the level of intake. The animals ate more fodder in the form of silage than hay. The consumption of the silage with additive was higher than silage without additive and was similar to the silage from cultivated meadow (Table 2).

Polmazym addition improved the air stability of evaluated silages. The stability of the sedge silage was 5 days, 6 days - silage from cultivated meadow and 6 days sedge silage supplemented with LAB+enzyme additive (Figure 1).

Figure 1: The aerobic stability of examined silage in 2003



Conclusions

The quality and nutritive value of silage and obtained body gains of animals in two years of study demonstrate that ensilage of sedge herbage was a better method of food conservation than haymaking. Well-prepared silage from sedge herbage had a value similar to that of feeds from cultivated meadow grasses and was suitable for feeding farm animals. The addition of Polmazym to ensiled sedge herbage significantly improved the quality and nutritive value of silage.

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Effect by various intensity of grassland management to forage nutritive value and forage nutrient production

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Summary

The aim of this paper was to assess the influence of different grassland management on the dry matter production and the nutritive value of the forage. The small plot trial was managed during 2003 and 2004 in four levels of the cutting intensity and in four levels of the fertilization. The model cattle load was 0.1 and 2 LU.ha^{-1}. The lowest value of the dry matter production (6.55 t.ha^{-1}) was ascertained in the intensive utilization. The fertilizer application significantly increased ($P<0.01$) dry matter production compared to nil fertilization (from 5.83 to 8.27 t.ha^{-1}). The results show decrease of concentration of energy (NEL, 5.83 to 5.07 MJ.kg^{-1} DM) and PDIN (111.0 to 77.1 g.kg^{-1} DM), PDIE in connection with decrease of the utilization intensity. With the application of the different utilization and fertilization it is possible to influence significantly the amount and the quality of the fodder from grasslands.

Key words: grasslands, cutting frequency, forage quality, fertilizer level

Introduction

In relation to the stagnation of the agricultural products consumption it could be expected other expansion of the permanent grassland areas. In this situation it is necessary to find out objectively the most suitable methods of the grassland management for the next time. The permanent grassland management by means of the cattle breeding is the most rational alternative.

Pozdíšek *et al.* (2002) point out that these parameters are depended on the species and varieties and also on the type of the management. With the suitable frequency of the utilization it is possible to achieve the energy concentration (NEL) on the value of 6.1 MJ.kg^{-1} DM. On the contrary the lower frequency of the utilization (older stand) causes the decrease of this value up to 5.1 MJ.kg^{-1} DM.

Materials and Methods

In 2003 it was founded the long-term small plot trial on the permanent grassland sites in the locality Rapotín. It consists of 16 treatments, in 4 replications, with a 10 m^2 harvest plot size. The grassland vegetation on the experimental stands was classified as *Arrhenatherion*.

It was managed with four levels of the intensity of utilization:

1 = intensive (1st cut until May 15th, 4 cuts per year – cuts at 45 day interval),
2 = medium intensive (1st cut between 16th and 31st May, 3 cuts per year at 60 day interval),
3 = low intensive (1st cut between 1st and 15th June, 2 cuts per year at 90 day interval) and
4 = extensive (1st cut between 16th and 30th June, 1 or 2 cuts per year, second cut after 90 days).

Each type of the utilization was furthermore divided in four levels of fertilization:

F_0 = no fertilization, $F_{PK}=P_{30}K_{60}N_0$; $F_{PKN90}=P_{30}K_{60}+N_{90}$, $F_{PKN180}=P_{30}K_{60}+N_{180}$.

It was measured the annual dry matter production for all plots. The samples from these plots (352 in total) collected during 2003 and 2004 were analyzed in laboratories of the Research institute for cattle breeding, Ltd., Rapotín. By means of the Weenden analysis there were estimated the values of nitrogen compounds, fat, crude fibre and ash. Furthermore, it was counted the quantity of the nitrogen free extract in dry matter of each sample. Forage quality in terms of crude protein (CP), fibre, NEL (net energy of lactation), PDIE (ingested digestive protein allowed by energy), PDIN (ingested digestive protein allowed by nitrogen) was predicted by means of the regression equations for the organic matter digestibility (Pozdíšek *et al.*, 2001) and by means of the equations that mentions Petrikovič *et al*., 2000. The results were statistically evaluated with two-factor analysis of variance with one observation in the subclass; the differences between the averages were tested by the Tuckey test ($DT_{0.05}$, $DT_{0.01}$).

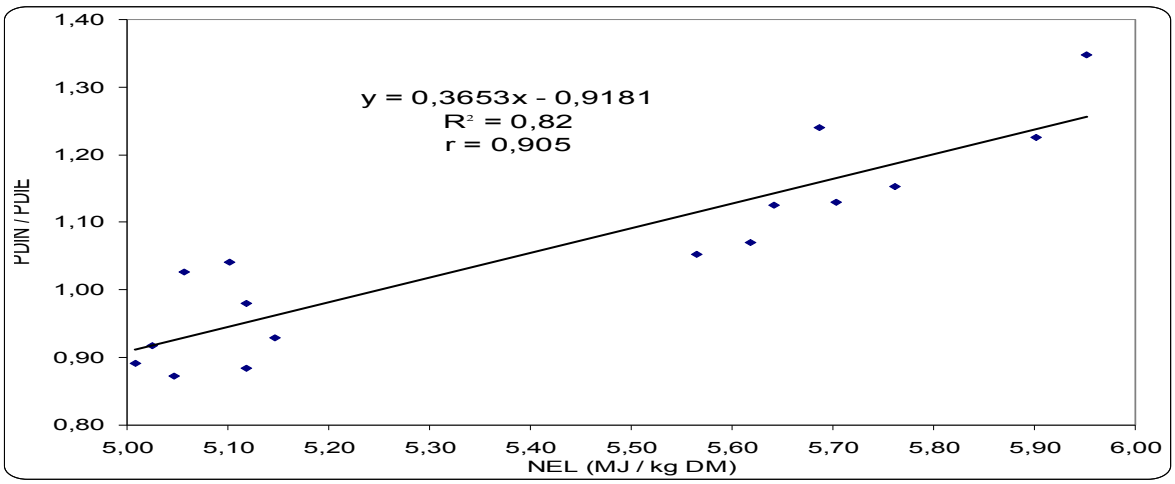
Results and Discussion

In table 1 we show average values of dry matter production during years 2003 and 2004. We have noticed the height production in sixteen treatment combinations. The dry matter production was the lowest in the intensive utilization (6.55 $t.ha^{-1}$). It we can conform to results of Gruber *et al.* (2000). They state, on the basis of the evaluation of the long-term trials at BAL Gumpenstein, that the graded cutting frequency causes the decrease of the yield from grasslands, especially four-cut utilization (8.65, 8.05, 6.51 $t.ha^{-1}$ DM, with 2, 3 and 4 cut-utilization).

The fertilizer application over two years (see table 1B) significantly increased ($P<0.01$) dry matter production compared to without fertilization, i.e. from 5.83 to 6.35 $t.ha^{-1}$ in F_{PK} fertilised treatments, or to 7.76 $t.ha^{-1}$ in N- fertilization of 90 $kg.ha^{-1}$, or to 8.27 $t.ha^{-1}$ in N-fertilization of 180 $kg.ha^{-1}$, respectively. The energy (NEL) and PDIN, PDIE concentration decreases in connection with decrease of the utilization intensity. The highest concentration of energy was acquired in 4-cut utilization. Figure 1 shows relation between energy concentration (NEL) and PDIN/PDIE ratio. PDIN/PDIE ratio with the sufficient concentration of NEL was ascertained in the medium intensity of utilization and in the F_{PK} fertilization or in the low nitrogen dose, respectively.

Treatments intensity of utilization and fertilization	DM ($t\ ha^{-1}$)	NEL ($MJ\ kg^{-1}$ DM)	CF ($g\ kg^{-1}$ DM)	CP ($g\ kg^{-1}$ DM)	PDIN ($g\ kg^{-1}$ DM)	PDIE ($g\ kg^{-1}$ DM)	PDIN/ PDIN
A							
1	6.55	5.83	239	173	111.0	91.3	1.214
2	7.54	5.63	254	156	100.3	88.7	1.122
3	7.46	5.09	297	120	78.1	82.0	0.949
4	6.66	5.07	298	118	77.1	81.1	0.936
Mean	7.05	5.40	272	142	91.6	85.8	1.055
$DT_{0,05}$	0.59	0.11	10	4	2.6	1.1	0.024
$DT_{0,01}$	0.71	0.13	12	5	3.2	1.3	0.030
B							
F_0	5.83	5.37	273	131	84.8	84.5	0.991
F_{PK}	6.35	5.37	274	133	86.2	85.0	1.005
F_{PKN90}	7.76	5.42	271	144	92.7	86.2	1.062
F_{PKN180}	8.27	5.45	272	161	102.8	87.3	1.164
Mean	7.05	5.40	272	142	91.6	85.8	1.055
$DT_{0,05}$	0.59	0.11	10	4	2.6	1.1	0.024
$DT_{0,01}$	0.71	0.13	12	5	3.2	1.3	0.033

Figure 1: Relation between concentration of nettoenergy and PDIN:PDIE ratio



Conclusions

By means of the various types of the grassland utilization and fertilization it is possible to influence significantly the amount and the quality of the fodder. These findings are important for the cattle nutrition security and for the sufficient grassland management. It is necessary the other knowledge enlargement.

Acknowledgements

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Changes in Nutrient Content of Corn Forage in Ensilage Process in Dependence on Contamination with Fusarium strains

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Introduction

While most fungi only reduce the yield or nutritive value of the feed they infest (available carbohydrates and other nutrients are converted to carbon dioxide and other fungal metabolites not readily available as animal nutrients (DiCostanzo, 1995), some fungi have the ability to produce toxic chemicals, mycotoxins. Mycotoxins are now more frequently being associated with crops like corn silage that include not just grain but a high percentage of stalks and stover. Recently, mycotoxins in corn silage have been associated with dairy herd health problems during years. Field molds in corn are mainly caused by *Fusarium*, the most common species associated with mycotoxin problems in livestock. Fusarium toxins in corn are probably not reduced by ensilement (Lepon, 1990). The general conditions needed for *Fusarium* field molds to proliferate are high humidity (>70%), oxygen, and temperatures that fluctuate between hot days and cool nights (Seglar, 2001).

The objective of the study was to determine the changes in nutrient content of corn forage and corn silage after the artificial infection with *Fusarium* strains in comparison with untreated plants.

Material and methods

In 2005 a field trial was conducted in the Research Institute of Plant Production Praha-Ruzyně. The experimental field of corn forage was divided into two areas – control (C) and inoculated (I). The inoculation was as follows: the cops in milk stage of maturity and the stalk were harmed with wire brush and subsequently all plants were infected with a prepared suspension of *Fusarium* strains. Entire corn plants were harvested at the soft dough stage of maturity. Stalks with cops were cut in a branch grinder and leaves were cut separately in a chopper in such a way that the 70% of the forage was 5-10 mm and 30% within 12 mm. Forage was than mixed by hand and packed into the silage microtubes (approximately 6,5 kg per microtube). Samples of raw forage were taken. Immediately after the filling the microtubes were closed and stored at 25 °C (±1°C). After the fermentation microtubes were opened, the contents were mixed thoroughly and samples of silage were taken for analyses. Obtained results were analysed using the Statgrafics 7.0 package.

Results

Table 1 shows the nutrient content of fresh corn forage. Control (C) plants had significantly higher content ($P<0,05$) of dry matter, organic matter and crude protein than the corn infected with *Fusarium* strains (I). The values of crude fibre and fat were similar and were not affected by the treatment.

Similar results were observed in the nutrient content of corn silage as presented in Table 2. The dry matter and the organic matter content of untreated silage (C) was significantly higher (P<0,05) than that of infected corn silage (I). Furthermore, control group tended to have higher content of crude protein, fiber and fat. These findings were not significant.

Table 1: Nutrient content of fresh corn forage

Nutriet		C (n=5)		I (n=5)	
		Průměr	SEM	Průměr	SEM
Dry matter	g/kg	362,1 [a]	0,2	350,7 [b]	0,2
Organic matter	g/kg	315,2 [a]	1,5	295,0 [b]	1,9
Crude protein	g/kg	65,9 [a]	1,5	59,3 [b]	2,0
Crude fiber	g/kg	179,6	2,1	178,1	1,5
Fat	g/kg	21,65	0,831	21,7	0,487

[a, b] means followed by the superscripts are significantly different (P<0,05)

Table 2: Nutrient content of corn silage

Nutriet		C (n=5)		I (n=5)	
		Průměr	SEM	Průměr	SEM
Dry matter	g/kg	347,1 [a]	0,3	331,2 [b]	0,5
Organic matter	g/kg	297,6 [a]	3,1	274,0 [b]	6,1
Crude protein	g/kg	67,3	1,6	63,5	1,8
Crude fiber	g/kg	166,9	5,4	156,9	6,2
Fat	g/kg	23,8	0,8	22,1	0,6

[a, b] means followed by the superscripts are significantly different (P<0,05)

Conclusion

Corn plants artificially infected with the suspension of Fusarium strains had lower nutritive value in fresh status as well as after ensilage.

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Comparison And Evaluation Of Quality Of Lucern Silage In CZ In Years 1997 – 2005 And Effect Of Dry Matter On Nutrtional Indicators And Proteolysis

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Summary

František Mikyska: COMPARISON AND EVALUATION OF QUALITY OF LUCERN SILAGE IN ĆR IN YEARS 1997 – 2005 AND EFFECT OF DRY MATTER ON NUTRITIONAL INDICATORS AND PROTEOLYSIS. AgroKonzulta Žamberk s.r.o., Klostermanova 1258, 564 01 Žamberk.

A monitoring system of the analysed feed collected in the databank of feeds at AgroKonzulta Žamberk s.r.o. provides us a comparison of quality of voluminous feed among the particular years. From 1997 to 2005 only feed from the laboratories in ČR that meet the same criterias of evaluation and calculation to enable their comparison in a certain period, were collected in the databank of feed. We were evaluating lucern silage for the mentioned period. Following the indicators that had been monitored (dry matter, crude protein, NEL, crude fibre, crude ash, pH, KVV, lactic, acetic and butyric acids, fermentation class, all-in class and NH_3) we evaluated the effect of the year on silage quality. Coming out of the analysis from years 2004 and 2005 we compared the effect of dry matter on quality indicators and mostly on proteolysis. The effect of dry matter had narrow correlation with proteolysis of lucern silage. When dry matter was low proteolysis went up. Protein, ADF, NDF, acetic and butyric acids had also effect on dry matter and when dry matter was low the values were of the highest and when dry matter increased the values decreased.

The values of other nutrients were optimal in different dry matters.

Introduction

In past 15 years the agriculture in ČR has gone through significant changes that affected manufacture and quality of voluminous feed. Changes regarding stabling technologies, from fasten to free, feeding with feeding (mixing) cars (TMR feeding) and there was also a request on increasing efficiency of diary cattle. The mentioned changes had a direct influence on purchase of high quality automatic equipment, adoption of high performance hybrides, increased nutrients concentration, earlier harvest of forage and stabilization of fermentation process with preservation products.

A choice of mechanization has significant effect on quality of voluminous feed, mainly in lucern silages but the base will always be to follow technological discipline that regards optimal time of cuts, pressing, turning, chopped of roughage food, application and a type of conservant, stamping, covering with mats, avoiding access of air etc.. The major motivation of meeting quality of feed is increasing efficiency and income for milk and meat. But the basic thing is optimal animal nutrition related with animal health that is a condition of maximal efficiency.

Materials and Methods

About 38 000 samples of feed from laboratories in the Czech Republic that met the same criteria of evaluation were collected in the databank of feed from 1997 to 2005. The analysed feed were produced in agriculture companies of the Czech

Republic. Comparison and evaluation of feed show possibilities of further development towards quality of voluminous feed.

Following 1685 analysis of lucern silages for the mentined period we made a table where the following indicators are monitored: frequency of analysis, crude protein, NEL,crude fibre, crude ash, pH, KVV, lactic, acetic and butyric acids, fermentation class, all-in class and NH_3). Of the results we evaluated the effect of the year on silage quality. Regarding 534 analysis for 2004 and 2005 we compared the effect of dry matter on quality indicators and mainly on proteolysis. We made 8 intervals in dry matter and the analysis done in this interval was averaged. (Intervals of dry matter: 20% - 24%, 24,1% - 27%, 27,1% - 33%, 33,1% - 36%, 36,1% - 40%, 40,1% - 45%, 45,1% - 50%, 50,1% - 55% and over 55%). Besides proteolysis, we also monitored the following nutrients: crude protein, NEL, crude fibre, crude ash, pH, KVV, acetic and butyric acids and NH_3).

Results

Table 1 shows that year 2002 had the best quality lucern silage, particularly value of fibre 21,59% was the lowest for the monitored period. Content of protein 21,49% was among the highest. In 2004 and 2005 the quality of lucern silages was negatively affected mostly by starting cold and rainy weather during the beginning of vegetation period and bad climate, mainly in the first cuts. In Table 1 we can see that protein decreased on 20,64% in 2005 and herewith they got under the long-term standard of 21%. NEL values significantly changed during the monitoring. The values of fibre of 24,45% and 24,42% were very high in past two years compared with other years and only 1997 had higher values. The values of fermentation process and NH3 were average in years 2004 and 2005.

Table 2 and Graph 1 show narrow correlation between dry matter and proteolysis of lucern silages. Protein, ADF, NDF, acetic and butyric acids also had relation to dry matter, the values were the highest when low dry matter and the values went down with increasing dry matter. The rest nutriants had their optimal values in different dry matters. In NEL the highest value was 5,20 MJ when dry matter was 34,69% (Graph 2).

Average of lucern silages for 1997 – 2005

Table 1

Year	Number of analysis	Dry matter %	NL %	NEL %	Fibre %	Ash %	pH	KVV	% Lactic acid	% Acetic acid	% Butyric acid	Fermentation class	All-in Class	NH_3 %
			In 100% dry matter				In initial mass							
2005	218	39,4	20,64	5,08	24,42	11,38	4,65	1488	2,76	0,81	0,05	1,51	1,92	0,150
2004	236	37,7	21,32	5,01	24,45	11,01	4,67	1511	2,86	0,83	0,053	1,40	1,86	0,158
2003	156	40,9	21,33	5,02	22,53	11,30	4,78	1459	2,62	0,82	0,1	1,80	1,82	0,149
2002	170	39,5	21,49	5,04	21,59	11,27	4,72	1445	2,6	0,87	0,07	1,87	1,6	0,148
2001	256	36,8	21,50	5,09	23,23	11,65	4,78	1488	2,68	0,95	0,09	2,09	1,90	0,150
2000	216	39,4	21,2	5,10	22,37	11,30	4,85	1467	2,43	0,88	0,13	1,98	1,87	0,150
1999	89	42,1	20,4	5,05	22,92	11,26	4,84	1682	2,85	0,84	0,07	3,50	1,65	0,160
1998	190	39,5	19,3	5,02	23,74	11,31	4,73	1606	2,47	0,69	0,07	2,24	1,56	0,160
1997	154	40,2	19,7	5,02	26,42	10,98	4,97	1424	2,30	0,72	0,07	2,16	1,58	0,240

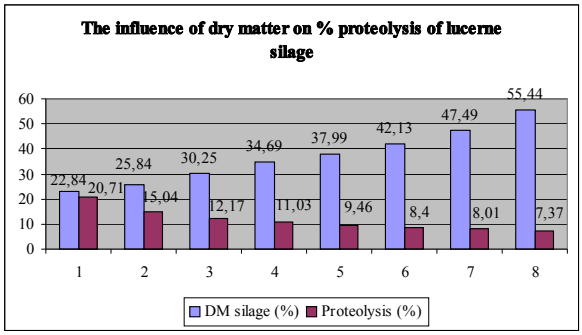
Analysis of lucern silages in 2004 and 2005

Nutrients are counted over on 100% dry matter

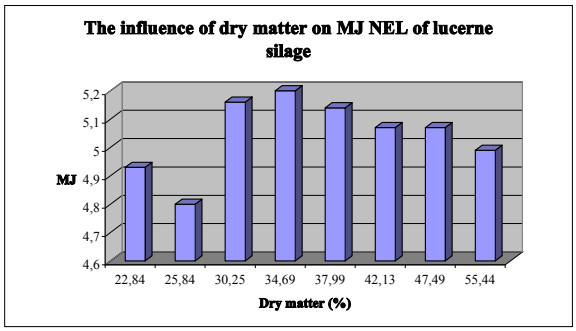
Table 2

Analysis			Dry matter Inicial weight	Crude protein	Crude fibre	NEL	Crude ash	pH	KVV	Lactic acid	Acetic acid	Butyric acid	NH_3	Proteolysis	ADF	NDF
Number	%	Range of dry matter	(%)	(%)	(%)	(MJ)	(%)			(%)	(%)	(%)	(%)	(%)	(%)	(%)
15	2,81	20% - 24%	22,84	22,86	26,80	4,93	11,68	5,05	873	5,21	5,47	1,93	0,092	20,71	371,9	447,1
22	4,12	24,1% - 27%	25,84	22,53	25,44	4,80	11,44	4,98	983	5,57	4,88	0,81	0,066	15,04	383,6	429,3
99	18,54	27,1% - 33%	30,25	22,39	24,65	5,16	11,38	4,70	1304	8,27	3,77	0,40	0,053	12,17	341,4	373,4
79	14,79	33,1% - 36%	34,69	21,53	24,12	5,20	11,55	4,66	1478	8,07	2,82	0,35	0,046	11,03	331,0	372,5
122	22,85	36,1% - 40%	37,99	21,50	23,98	5,14	11,10	4,57	1564	8,05	2,13	0,05	0,039	9,46	327,7	367,4
102	19,10	40,1% - 45%	42,13	20,38	24,39	5,07	11,07	4,57	1677	7,69	1,66	0,02	0,033	8,40	334,9	394,8
52	9,74	45,1% - 50%	47,49	20,32	24,06	5,07	11,13	4,73	1589	6,04	1,39	0,04	0,032	8,01	309,0	356,4
38	7,12	nad 50.1%	55,44	18,90	25,14	4,99	10,89	4,96	1419	4,04	0,94	0,02	0,027	7,37	295,9	348,1
534	100,00	Average	381,25	21,01	24,49	5,10	11,20	4,69	1467	7,19	2,28	0,21	0,39	9,65	332,6	379,8

Graph 1



Graph 2



Conclusion

The differences in quality among the monitored years were evaluated in lucern silages. Regarding the monitored period, in years 2004 and 2005 the quality was significantly worse and it concerned increased level of fibre and decreased level of protein. During monitoring the effect of dry matter in lucern silages on proteolysis the narrow correlation was found out. When dry matter of lucern silages was under 30% the percentage of proteolysis was higher so hereby its unstability. Decreased quality of silages has the influence on worsening animal health, decreasing efficiency and following economics of breeding of diary cattle.

Protein Degradability and Biogenic Amines Content of Silage

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Introduction

High protein content of legumes can arise several problems related to feeding and nitrogen emission. Legume protein is intensively degradable during drying, ensiling or ruminal fermentation. High protein degradability of legumes decreases their usage as an efficient protein source.

Little attention has been paid to the content of different protein end-products in silage and their effect on the animal organism. The most common biogenic amines in silage are putrescine, cadeverine, histamine and tyramine. It has been approved that biogenic amines have negative effect on dry matter intake as they inhibit ruminal contractions, reduce dry matter digestibility and the passage rate of feed particles in the digestive tract.

Objective of the study was to explain the effect of wilting and additives on silage protein hydrolysis and on the content of biogenic amines.

Materiál and Methods

The mixture (50:50) of red clover – *Trifolium pratense L.* and timothy – *Phleum pratense L.* was studied. The material was conserved in 3-litre jars with three replicates. For treatment, chemical additive (AIV 2000) and biological inoculant (*L.plantarum* + *L.fermentum*) were used.

The chemical composition of silages was determined according to the generally accepted methods and degradability was studied by the *in sacco* method with two ruminally fistulated cows. Soluble fraction (A) was determined by washing the bags in a washing machine set to a cold cycle. For determination of rapidly degradable fraction B1, the samples were incubated in the rumens of fistulated cows for 8 hours; for potentially degradable fraction B2, the incubation lasted for 64 hours. Ruminal protein degradability of silage was calculated by nutrient losses from the bags during the incubation period.

The content of biogenic amines was determined gas chromatographically, using HP 6890 Series GC System with capillary column HP-5.

Data were analysed by using GLM procedure of SAS.

Results and Discussion

Dry matter content of unwilted control silages was 140 to 171 g/kg, and that of wilted silages 256 to 295 g/kg; crude protein contents were 153 to 172 g/kg and 172 to 178 g/kg, respectively (Table 1). The content of NH_3-N in the control silages was considerably higher that in the treated silages. Also it was lower in silages treated with the biological additive, compared to these treated with the chemical additive ($p<0.0001$).

In our study, considerable amounts of biogenic amines were found only in the untreated direct cut silages (Table 1). In the wilted silages, the amount of biogenic amines was very low or they were not present. All biogenic amines under investigation were present in untreated direct cut silages. Histamine had the highest and purtrescine

somewhat lower concentration: 5.24 g/kg and 0.86 g/kg, respectively. Additives totally inhibited the formation of putrescine, histamine and tyramine, and also resulted in approximately 100-fold reduction in cadaverine formation.

Table 1: Effect of additive and wilting on chemical composition and biogenic amines content of silages

Treatment / additive	Dry matter g/kg	Crude protein g/kg DM	NH_3-N total N %	Putrescine g/kg DM	Histamine g/kg DM	Cadaverine g/kg DM	Tyramine g/kg DM
Unwilted							
kontrol	140	153	18.7	0.86	5.24	2.32	2.00
biological	171	161	1.19	0	0	0.03	0
chemical	162	172	5.8	0	0	0.03	0
Wilted for 24 hours							
kontrol	256	178	6.3	traces	0	0.05	0.08
biological	274	172	1.6	0	0	0	0.01
chemical	295	172	3.9	0	0	0.03	0

Potential ruminal degradability of silage protein was approximately 90% as indicated by fraction B2. Solubility ranged from 60 to 74.4%; after 8 h of incubation there was an additional increase of 10% in protein degradability (Table 2). In the unwilted silage, the additive decreased NH_3-N content and increased the protein degradability of fraction B2. However, no effect on protein degradability for fractions A and B1 was detected. In the wilted silage, the additive decreased protein solubility, and degradability of fraction B1. The effect of chemical additive on protein solubility and hydrolysis of fraction B1 was to some extent higher for the wilted silage than for the unwilted silage. The effect of an additive on the decrease of silage nitrogen degradability and kinetics has been shown by Flores et al. (1999), who indicated that silage nitrogen degradability of ryegrass reduced from 81.22 to 79.31%. Hristov and Sandev (1998) concluded that protein solubility of lucerne silage, prepared with chemical additive, decreased by 9.5%.

Table 2: Effect of additive and wilting on silage protein hydrolysis

Treatment / additive	Total N, g/kg	Soluble fraction, % total N A	Rapidly degradable fraction, % total N B 1	Potentially degradable fraction, % total N B 2
Unwilted				
kontrol	24.5	67.2	77.1	89.6
biological	25.8	65.5	79.8	91.1
chemical	27.5	64.2	76.1	94.3
Wilted for 24 hours				
kontrol	28.5	74.7	77.2	92.6
biological	27.5	65.2	76.0	92.6
chemical	27.5	60.0	68.0	91.8
Significant difference, P				
Unwilted				
control *vs*. biological	<0.0001	0.3150	0.0210	0.0025
control *vs*. chemical	<0.0001	0.0003	0.3677	<0.0001
biological *vs*. chemical	<0.0001	0.0928	0.0018	<0.0001
Wilted for 24 hours				
control *vs*. biological	0.0062	<0.0001	0.2852	0.9491
control *vs*. chemical	0.0027	<0.0001	<0.0001	0.1146
biological *vs*. chemical	0.8847	<0.0001	<0.0001	0.1011

Conclusions

Potential ruminal nitrogen degradation of the silages was approximately 90%. Protein solubility and ruminal degradability were lower for silages treated with the chemical additive than for those treated with the biological additive, or for untreated silage.

All biogenic amines under investigation were present in untreated direct cut (140g/kg) silages. The content of histamine was the highest, it was followed by putrescine. Wilting and treatment with additive inhibited the formation of biogenic amines in silages.

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Releasing of Calcium, Magnesium and potassium from forages in the rumen of cows

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Summary

An in sacco technique was used to measure release of Mg, Ca, K from six forages – lucerne hay from the 1st cut (LH1), and the 2nd cut (LH2), orchard grass hybrid Rela (GR) and hybrid Niva (GN), grass silage (GS), red clover silage treated with Feedtech (CSFT) and /or with Kofasil (CSKO). The forages were different in content of macrominerals and also large diferences ($P<0.01$) were in elements release in the rumen among experimental forages and the elements. The extent of disappearance of Ca and Mg was higher from LH1, LH2, CSFT, CSKO than from G and GS, except of K. The release of individual elements through all incubation time are very well expressed by cubic polynoms.

Introduction

Solubilization and release of mineral elements from the feedstuffs are important proceses for their utilisation by animals. According to Playne et al. (1978), the elements not released from feedstufs in nylon bags after 48 h incubation in the rumen are probably unavailable for absorption to the animal. The location of mineral elements in the forage structure may influence its release (Gralak et al., 1997). High proportions (> 60 %) of P, K and Mg were released during short incubation in the rumen. Ca had the lowest disappearance. Disappearance of elements from the bags in the rumen depends on mineral content and fibre source of incubated feeds (Flachowsky and Grün, 1992) .

Materials and Methods

To study the release of mineral macroelements we used following forages: lucerne hay from the 1st cut (LH_1), two hybrids of orchard grass (hybrid Rela (GR) and hybrid Niva (GN)), grass silage (GS), red clover silage treated with chemical (Kofasil) and biological (Feed Tech) conserving agent.

An in sacco method was used to study the release of mineral elements from forages. Incubation of forages was done in the rumen of 3 cows with large rumen cannula. The animals were fed a ration consisting of lucerne hay, maize silage, cereal meal and mineral and vitamine feed additive. Access to water was ad libitum. The conditions of in sacco experiment were mentioned by Čerešňáková et al. (2005).

Dry matter and crude protein conten of forages was determined by STN 46 9072, and cell wall content according to the procedure of van Soest (Lutonská a Pichl, 1983). Ca, Mg and K were determined by AAS Solar 9000 (Unicam. Cambridge UK).

The influence of forage and time of observations were evaluated by two way analysis of variance with the fixed effects with one observation per cell (*ij*- subclasses in feed x time), with determination of Tukey test of nonaditivity of feeds x times. Dependences of analysed traits on the time were evaluated, the most suitable polynom was polynom of 3rd (cubic) degree.

Results and Discussion

Solubilization and release of mineral elements from the structure of forage is very important precondition of their utilization in animals There are large differences between forages in cell wall content that affect degradability of NDF (Čerešňáková et al., 2000) and release of mineral elements (Ledoux a Martz, 1991). We found that the partial regression coefficients of mineral elements release on NDF and time are positive highly significant regression on NDF (Čerešňáková et al.,2005). Potassium was released rapidly from all tested forages and appeared to be independent of NDF content (Emanuele and Staples, 1990).

Table 1: Chemical composition of experimental forages (g/kg DM)

NUTRIENS	LUCERNE		DACTYLIS GLOMERATA		SILAGES		
	1. CUT	2. CUT	HYBRIDS		GRASS	RED CLOVER	
	13.5.1998		RELA	NIVA		FeedTech*	Kofasil*
DM (G/KG)	218.6	307.6	171.1	172.8	212.8	299.1	314.2
NX6.25	210.0	191.3	140.7	148.4	174.6	211.6	226.8
NDF	351.7	383.7	597.7	565.4	545.5	325.5	361.7
CALCIUM	15.82	1.7	3.66	3.78	4.76	15.29	15.54
MAGNESIUM	3.3	10.4	1.46	1,54	1.9	3.6	3.7
POTASSIUM	34.79	31.38	29.98	23.18	28.98	23.18	34.79

Our results document differences between grasses and legumes in content of NDF, and crude protein (Table 1). Concentration of macroelements generally was much higher in LH_1 than in GR, GN and GS. Plant species showed differences mainly in Ca and Mg concentration. We found significant differences ($P<0.01$) for minerals releasing between forages in incubation time (Table 2) also. Cubic functions of release of individual elements from feeds in dependence on incubation time have high values of R^2, however, not all of them are statistically significant (Table 3a, 3b).

Table 2: Two-way Anova of selected elements with the test of nonaditivity of group (forage) x time of incubation

Element		Groups (A)	Time (B)	Error (e)	N	R
		$f_a = 6$	$f_b = 6$	$f_e = 36$	$f_N = 1$	$f_r = 35$
Ca	MS	133.85^{++}	10.187	4.993	30.11	4.27
	F	26.81^{++}	2.04		7.41^{+}	
Mg	MS	0.5021	0.1244	0.0437	0.2071	0.039
	F	11.50^{++}	2.85^{+}		5.31^{+}	
K	K	MS	0.0394	0.0147 0.93	0.0437	0.3439
		F	2.49^{+}			53.16^{++}

$F_{0.05}(6, 36) = 2.364$; $F_{0.01}(6,36) = 3.351$; $F_{0.05}(1, 35) = 4.120$; $F_{0.01}(1, 35) = 7.42$

Table 3a: Parameter estimation of cubic polynomials $y = b_0 + b_1t + b_2t^2 + b_3t^3$ of Ca and Mg release from selected feeds in the time of ruminal incubation ($^{**}P<0.01$, $^{*}P<0.05$)

Feeds	b_0	b_1	b_2	b_3	R^2	b_0	b_1	b_2	b_3	R^2
	Ca					Mg				
CS_{FT}	16.6816	-0.4157	0.0070	-3E-05	0.852**	1.6352	-0.0412	0.0007	-3E-06	0.926**
CS_{Ko}	16.9005	-0.4450	0.0086	-5E005	0.922**	1.8639	-0.0657	0.0015	-1E-05	0.966**
LH_1	15.3549	-0.5568	0.0207	-0.0002	0.930**	1.3461	-0.0898	0.0032	-3E-05	0.985**
LH_2	12.1543	-0.5862	0.0205	-0.0002	0.903**	1.1137	-0.0718	0.0021	-2E-05	0.797*
GS	3.8092	0.0659	-0.0034	3.6E-05	0.373	0.5888	0.0074	0.0002	1.4E-06	0.311
G_{NIVA}	4.1848	0.2050	-0.0070	6.1E-05	0.269	0.9158	-0.0025	-0.0001	1.9E-06	0.344
G_{RELA}	4.0793	0.2018	-0.0071	6.3E-05	0.260	0.8413	0.0007	-0.0002	2.6E-06	0.217

*Table 3b: Parameter estimation of cubic polynomials $y = b_0 + b_1t + b_2t^2 + b_3t^3$ of potassium release from selected feeds in the time of ruminal incubation (** $P<0.01$, $^{*}P<0.05$)*

Feeds	b_0	b_1	b_2	b_3	R^2
	P				
CS_{FT}	0.1906	0.0009	-5E-05	5.9E-07	0.598*
CS_{Ko}	0.2686	0.0071	0.0002	-1E-06	0.671*
LH_1	0.1539	0.0028	2.7E-05	-7E-07	0.947**
LH_2	0.1265	0.0034	-7E-05	4.3E-07	0.689*
GS	0.4477	0.0325	0.0009	7E-06	0.587
G_{NIVA}	0.3372	0.0204	-0.0009	8.3E-06	0.500
G_{RELA}	0.3264	0.0258	-0.0011	1.0E-06	0.503

Conclusion

In sacco technique is usefull for determination of differences among forages in mineral elements releasing in the rumen. The rumen is the major site of K, Ca and Mg release from these forages.

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The effect of different urea doses on fermentation characteristics of maize silage

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Introduction

In the Czech Republic, maize is the most important crop grown for all-year feeding of silage. This crop gives high and stable yields of dry matter per hectare. however, the produced silage contains only low amounts of protein and for that reason there are efforts to improve this situation and to increase the content of nitrogen in preserved material. One of the possible ways how to increase the content of nitrogen in silage is to use urea, which at the same time shows also a good preserving effect. As early as in 1931 BRIGL and WINDHEUSER (In: KUMANOV and TODOROV, 1965) performed in Germany experiments with supplementation of 1 to 2 % of urea into the maize silage and their results very positive. Later on, a number of authors studied this problem, e.g. Mc CARTHY et al., 1970; SHIRLEY et al. 1972 etc. According to KALAČ (1975), a urea supplement results in the development of mixed bacterial microflora that is able to fix ammonia-N and increase the content of this element in silage and, at the same time, to increase the digestibility of all nutrients without any adverse effects on fermentation processes. BAITNER et al. 1985, PAHLOW 1979 and KNABE et al. 1984 corroborated this statement because in their experiments a supplement of urea in the dose of 5 kg/ton increased the content of crude protein and ammonia-N in silages and did not reduce fermentation processes and aerobic stability of preserved forage (due to a high production of acetic acid). The preserving effect of ammonia is based on the release of ammonia and carbon dioxide, which takes place in the course of its enzymatic hydrolysis by urease. This property is attributed above all to heterofermentative bacteria and only partly also to lactic acid bacteria.

$$CO(NH_2)_2 + H_2O \xrightarrow{\text{urease}} 2NH_3 + CO_2$$

Ammonia produced through the microbial hydrolysis of urea is usually converted to NH_4^+, which prevents its reverse absorption. This means from the theoretical point of view that one urea molecule can neutralise two molecules of lactic acid (BAITNER et al. 1985). Problems concerning the application of urea as a preservative were studied by many authors, e.g. ADOGLA-BESSA et al. 1999, KIRIKOV 1999, Ó-KIELY 1998, KRISTENSEN a OHLSSON 1995, WILKINSON 2005 and others.

The objective of this study was to evaluate effects of differentiated doses of urea on fermentation parameters of maize silage on the base of a comparison with untreated Control.

Material and Methods

The maize hybrid Romario FAO 250 (supplier KWS, seed materials s.r.o.) was used in a model experiment. The harvested crop was homogenised, treated with urea and ensiled. The average DM content in the harvested crop (hybrid *Romario*) was 265.2 $g.kg^{-1.}$ The model experiment was divided into three different treatments, viz. untreated Control, Variant 1 treated with 2.5kg/t and Variant 2 treated with 5kg/t. In both

experimental variants dry urea was applied homogenously to harvested crop and the treated material was ensiled in three replication into 50 l aluminium containers. In each container the amount of compacted biomass corresponded in average to the specific density of 800 $kg.m^{-3}$. Experimental fermentors were sealed and stored at room temperature of 20 – 25 °C. After eight months, the containers were opened and 6 representative samples were taken from each variant for analyses and evaluation of the fermentation process

Analytical methods

The DM content was estimated on the base of drying at the temperature of 103±2 °C to a constant weight. Analytical method used (including the preparation of water extract) were described in on of our earlier papers (DOLEŽAL, 2002). The obtained samples were analysed to estimate contents of volatile fatty acids, lactic acid, and ammonia, pH, and titrable acidity. The content of alcohol was estimated according to method described by HARTMAN (1974). Results were statistically analysed using the method of unifactorial analysis of variance (SNEDOCOR and COCHRAN, 1967).

Results and Discussion

The DM of fresh maize was 265.2 g/kg. After eight months of fermentation, the DM content changed as follows: in Controls it decreased to 249.0 $g.kg^{-1}$, in Variant 1 it slightly increased to 267.3 $g.kg^{-1}$ and in Variant 2 the corresponding DM content was 263.8 $g.kg^{-1}$. The application of urea decreased pH from 3.95 (Control variant) to 3.70 (Variant 1) and 3.71 (Variant 2). This observation was at variance with data published by WILKINSON (2005), and KUMANOV and TODOROV 1965) who observed an increase in pH from 3.80 to 4.05 after the application of urea in the dose of 5 kg/t. However, BAITNER et al. (1985, KRISTENSEN and OHLSSON (1995) on the other hand reported a decrease in pH. The lowest and the highest values of titrable acidity were found out in Control and in Variant 1 (786 ± 99.80 mg KOH/100 g and 1,067 ± 142.09 mg KOH/100 g, resp.); in Variant 2, the corresponding value was 895 ± 232.26 mg KOH/100 g. As far as the quality of fermentation processes was concerned, an increased content of total acids was observed in both experimental variants after the application of urea: in Variant 1 it increased from 5.15 ± 1.06 % of DM Control to 6.42 ± 0.89 % and in Variant 2 to 5.35±1.42 %. This increase was caused mainly by lactic acid and the differences between Variants 1 and 2 on the one hand and Control on the other were 66.6 % and 30.5 %, respectively. The effect of acetic acid was smaller and the corresponding values were only 21.1 % and 10.6 %, respectively. This means that a lower dose of urea (i.e. 2,5 kg/t) resulted in an inhibition of production of acetic acid and stimulated synthesis of lactic acid. This observation corresponds with data published by KRISTENSEN and OHLSSON (1995) who observed an increase in the content of lactic acid in silage with the supplement of urea. As compared with control (54.98 ± 8.37), a positive effect on LA/VFA ratio was observed in both experimental variants (69.53 ± 2.02 in Variant 1 and 65.02±7.16 in variant 2). The lowest content of ammonia was found out in Variant 1 (0,11±0,14); this also corresponded with the highest content of alcohol (0.55 ± 0.09). This means that these silages contained not only homofermentative but also heterofermentative bacteria strains that produce not only acids but also ethanol (and utilise ammonia). WILKINSON (2005), and KUMANOV and TODOROV (1965) observed after the application of urea (5kg/t) a decrease in ethanol content from 12.8 g/kg to 8.7g/kg DM. In our experiment this

observation was not corroborated; quite on the contrary, its contents increased in both variants (by 22.2 % and 13.3 % in Variant 1 and 2, resp.).

Conclusion

Maize silage is the most common feedstuff for cattle and for that reason its quality must be very high. In this experiment the we tried to analyse the effect of urea on the quality of fermentation processes. It was found out that both doses of urea (i.e. 2.5 kg/t and 5 kg/t) increased production of lactic acid and total acids and partly inhibited production of acetic acid so that its preservative effects were corroborated.

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The effect of yeast culture SC-47 as a in THE DIET of cows on rumen fermentation

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Introduction

Nowadays, yeast cultures are a frequent component of feeding rations of farm animals. Under specific anaerobic conditions of rumen, yeast show a positive effect not only on milk components and the performance of ruminants but also on the ruminal digestion *per se* (LYONS, 1993; SUNE et al., 1998; ALSHAIKH et al., 2002 and others). In the rumen of animals receiving yeasts, total numbers of bacteria and infusoria considerably increase (SUNE, 1998 etc.). However, an improvement of cellulolytic activities need not be always dependent only on the size of the bacterial population. In presence of yeast, the bacterial activity is usually increasing. Yeast cultures improve ruminal cellulolytic activities probably due to the fact that they increase the numbers of bacteria, improve the intensity of fibre digestion, reduce accumulation of lactates and decrease concentration of oxygen in rumen fluid. An improved utilisation of starch supplied in the diet results in a decrease in the rate of production of volatile fatty acids (VFA) and an improved stability of rumen environment; this, in the final effect, shows a positive impact on the digestion in rumen (BLAKE, 1993; BAX, 2004). A positive effect of yeasts on farm animals is manifested also through an increased intake and improved digestibility of nutrients (JOUANY, 2001). LYONS (1993), as well, mentioned that some yeast strains showed a better capability to use lactates and/or stimulate their utilisation by propionic acid bacteria. The utilisation of lactates by these bacteria is of the cardinal importance for the stabilisation of ruminal environment. DOREAU and JOUANY (1998) observed that in individual animals yeast reduced the diurnal variability of pH values and, at the same time, decreased differences among different individuals; this, therefore, resulted in an increased stability of rumen environment during the day. The available literary data also indicate that in rumen fluid of animals receiving yeast preparations not only the total content of VFA and of propionic acid increase but also that the content of ammonia goes down (ENJALBERT et al., 1999). The total numbers of bacteria (and also infusoria) is significantly increased (SUNE, 1998; KAMRA et al., 2002 etc.).

Material and Methods

The experiment involved 12 post-partum dairy cows with a similar performance level. Control (1) and experimental (2) groups (six animals each) were fed on the same diet consisting of maize silage with an increased content of DM (16 kg), wilted clover-grass silage (16 kg), meadow hay of average quality (3 kg) and feed mixture (7.5 kg). The feed mixture for the experimental group (2) contained also a yeast culture in the amount of 5 g/head/day. The experiment lasted 60 days. After 30 days, rumen fluid was sampled using a throat (oesophagus) probe to evaluate the response of animals to the inclusion of yeast culture into the diet; for details see HOFÍREK and DVOŘÁK (2002). In the rumen fluid, the total content of VFA and percentages of acetic, propionic and

butyric acids were estimated; pH value was determined by means of potentiometry, content of ammonia according to Conway (AOAC, 1980) and the total numbers of infusoria according to HOFÍREK and DVOŘÁK (2002). The obtained values were compared with reference data (VRZGULA et al., 1990).

Parameters of rumen digestion were statistically analysed by means of variance analysis and the differences between average values were analysed by means of t-test. The programme Statgraphic (Version 5.0) was used for the statistical analysis of obtained results.

Results and Discussion

In Fig. 1, pH value is near the bottom of reference range and is lower than in controls (6.3). This result corresponds with data published by KUNG et al. (1997) and PUTNAM et al. (1997). We were not able to confirm results published by other authors (LYONS, 1993; AUCLAIR, 2004; ILLEK, 2004; KAMRA et al., 2002) that observed an increase and stabilisation of pH in rumen fluid after the application of yeast cultures.

As shown in Fig. 2, production of VFA increased in the experimental group from 84 mmol/l to as much as 116 mmol/l of rumen liquid. This result is in accordance with data published by other authors (LYONS, 1993; ILLEK, 2004; KAMRA et al., 2002 etc.). On the other hand ALSHAIKH et al. (2002) and PUTNAM et al. (1997) did not find any changes in production of ruminal VFA. According to DVOŘÁK (1994), BÍREŠ (2000), and VRZGULA et al. (1990) the total amount of VFA and the percentages of individual acids in rumen are changing in dependence on both qualitative and quantitative composition of the diet and its hygienic quality.

As compared with control, the application of yeast resulted in a better utilisation of ammonia (Fig. 3). These results confirmed data published by ILLEK (2004), LYONS (1993) and other authors. On the other hand, PUTNAM et al. (1997) did not find any significant effect on the content of ammonia in rumen fluid while NEWBOLD et al. (1996) demonstrated its increase. SPANN (1993) mentioned that when feeding a diet containing 13 % of crude protein and 5.9 MJ NEL/kg DM the degradation and synthesis of bacterial protein are in equilibrium.

Results of a number of studies indicate that the microbial proteosynthesis is closely correlated with the amount of available energy. The synthesis of 7.5 – 10.5 g of protein requires 1.0 MJ ME. A lack of available energy reduces synthesis of microbial protein and results in a decrease in numbers of infusoria. It was also demonstrated that if the complete diet contained high amounts of concentrates (more than 70 %), the protein synthesis was relatively low. Increased concentrations of ammonia indicated a low percentage of roughage in the diet while its higher amounts were associated with higher percentages of easily degradable N-compounds in the rumen (SOMMER and PETRIKOVIČ, 2003).

The applied concentration of yeast can significantly stimulate metabolic activities of ruminal infusoria (Fig. 4) and, thus, increase their numbers. Similar results were published also by ILLEK (2004), LYONS (1993), YOON and STERN (1996), STRZETELSKI et al. (1996), AUCLAIR (2004) and other authors. On the other hand KAMRA et al. (2002) did not observed any positive effect on infusoria activities after the supplementation of yeast into the diet. According to BAX (2004), as much as 50 % of microbial protein resulted from infusorial synthesis. ZELENKA (1996) mentioned that in high-performance dairy cows the microbial protein was not of full value because some essential amino acids were missing. Protozoa are very sensitive to changes in pH and for that they quickly disappear from the ruminal fluid after any decrease of this

value. Our results showed that there was an explicit and significant relationship between the dose of yeasts and the number of infusoria in 1 ml of rumen liquid. This corresponds with data published by ALSHAIKH et al. (2002) who observed similar relationships between the dose of yeast culture and parameters of rumen fermentation.

Conclusions

A supplement of 5 g/head of a yeast preparation increased significantly the diurnal production of VFA and reduced the amount of ammonia. Differences in average numbers of infusoria that existed between both groups of dairy cows were statistically significant ($P < 0.05$) while those in pH values were statistically insignificant ($P > 0.05$). Significant differences (in favour of the experimental group) were found also in percentages of propionic and butyric acids in the sum of ruminal VFA.

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TECHNOLOGY OF FORAGE HARVEST, PRESERVATION AND STORAGE

Improvement of aerobic stability of pressed sugar beet pulp silage, made in large plastic silage bags, depending on ensiling period and silage additive

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Introduction

Silage making in large plastic bags has been grown rapidly in the last few years in Europe (WEBER 2005) as well as in the U.S. (MUCK and HOLMES 2005), but not much research to this technology can be found. Aerobic instability of silages, mainly caused by yeast (BECK and GROSS 1964) is a major subject in research (SPOELSTRA 1993). After methodical adaptation of the "buried bag method" for using on silage bags (WEBER et al. 2004) improvement of aerobic stability of beet pulp silage was investigated. Ensiling period of only 14 days, often in farms with feed shortage, and 183 days as well as influence of air stress behind open bag silo surface was considered in combination with a chemical silage additive.

Material and Methods

Fresh pressed sugar beet pulp from one sugar factory was ensiled by AG BAGGER G 7000 in 2,70m diameter silage bag on two days. "Buried bags" were putted into the bag by method according WEBER et al. (2004). Silage additive was applied by sprayer installed above press rotor. Each variant was stored in a single bag with about 25 tonnes pulp. After ensiling period bags were opened on both ends for statistical reasons and silage quality was measured. After 5 days open surface one meter was fed out from both ends and the "buried bags" were collected, the same was done after another 5 days. Aerobic stability was measured according HONIG (1990). Yeast was measured as whole cell count, all other parameters according VDLUFA (1997). Software SPSS was used for statistical analyses. Levene homogenity - and Tukey – or Dunnett T3 multiple test were used with $\alpha \leq 0{,}05$, significant differences marked below tables.

Results and Discussion

Table 1 is showing characteristic of fresh beet pulp and of silage while bag opening after ensiling period. Ensiling took place 4 to 6 hours after leaving the sugar extraction process.

Table 1: Characteristic fresh beet pulp before ensiling (n=12) and silage (n=4)

trial number	ensiling period	pulp	DM	pH	sugar	lactic acid	acetic acid	yeast	mold
	days		%		p. kg DM	in DM		per g FM	
					g	%		CFU	
1	14	before ensiling	24,5 *0,9*	4,9 *0,2*	89,8 *7,3*	0,3 *0,1*	0,3 *0,1*	$2,9x10^5$ *$4,7x10^5$*	0,0 *0,0*
		silage	23,6 *0,5*	3,6 *0,0*	7,6 *10,7*	4,7 *0,6*	0,9 *0,2*	$8,2x10^3$ *$3,1x10^3$*	*0,0* *0,0*
2	183	before ensiling	24,1 *0,7*	4,7 *0,1*	92,4 *5,6*	0,4 *0,1*	0,4 *0,1*	$9,0x10^4$ *$1,4x10^5$*	$2,5x10^1$ *$8,7x10^1$*
		silage	23,8 *0,6*	3,6 *0,1*	57,6 *41,0*	4,5 *1,4*	1,0 *0,1*	$6,6x10^4$ *$9,1x10^4$*	0,0 *0,0*

Beet pulp of trial one (14 days) was ensiled one day before the other trial (183 days), but no significant differences in fresh beet pulp were found. Dry matter had expected level of 24%, sugar content with 9% in DM was higher than expected. Some fermentation already took place during storage and transport as lactic and acetic acid content demonstrated. Number of yeasts with 10^5 CFU/g pulp had already a high level in fresh pulp. This shows a high microbial activity which may have influence on aerobic stability later in the silage.

In *table 2* is shown the aerobic stability and yeast content of the silage depending on air influence and ensiling period. Ensiling process reduced higher yeasts content in fresh pulp. It shows airtight system of silage bag, but stability with 2 days was not high without additive.

Table 2: Aerobic stability (ASTA, in days) and yeast content (in CFU per g FM) of beet pulp silage depending of ensiling period (14 and 183 days) and air influence to open surface

silage additive		air influence by open bag surface							
		none (n=4)				5 days (n=8)			
		ensiling days							
		14		183		14		183	
		ASTA	yeast	ASTA	yeast	ASTA	yeast	ASTA	yeast
none	x	$2,1^a$	$8,2x10^{3a}$	$2,9^a$	$6,6x10^{4a}$	$1,6^{aA}$	$1,1x10^{7a}$	$3,2^{aB}$	$6,2x10^{5a}$
	s	*1,2*	*$3,2x10^3$*	*0,6*	*$9,1x10^4$*	*1,1*	*$9,0x10^6$*	*1,5*	*$6,2x10^5$*
2,5 l/t	x	$5,1^b$	$6,0x10^{2a}$	$4,9^b$	$0,0^b$	$1,9^{aA}$	$1,2x10^{5aA}$	$6,6^{bB}$	$0,0^{bB}$
	s	*1,4*	*$9,0x10^2$*	*0,4*	*0,0*	*0,8*	*$1,2x10^5$*	*0,7*	*0,0*
5,0 l/t	x	$6,7^b$	$0,0^b$	$6,3^b$	$0,0^b$	$4,7^{bA}$	$7,3x10^{2b}$	$7,0^{bB}$	$0,0^b$
	s	*0,6*	*0,0*	*1,4*	*0,0*	*1,7*	*$5,0x10^2$*	*0,0*	*0,0*

small types: significant difference between additive treatment
large types: significant differences between ensiling days in same group of air influence

Addition of 2,5 l Mais Kofasil Liquid (Na–Benzoate and Na–Propionate) improved aerobic stability from 3 to about 5 days, 5 l/t brought another increase of 1 - 2

days. Extremely air stress by 5 day feed out period led to air influence one meter behind surface on short ensiling period. The stability was significant lower than with 183 day ensiling period, without silage additive. 2,5 l/t additive did not increase aerobic stability with short ensiling period, but with 183 ensiling days highly significant. Reduced yeast content by chemical inoculant correlated with higher aerobic stability. 5 l/t of chemical additive were able to kill yeast in any situation.

Conclusions

The ensiling period of beet pulp in a silage bag and feed out air stress had influence on aerobic stability of the silage. It could be improved from 2 up to 5 - 7 days with a chemical additive. A short ensiling period of only 14 days required 5 l/t to get an acceptable aerobic stability of 5 days while a long ensiling period of 183 days required only 2,5 l/t to get nearly 7 days of aerobic stability.

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High Moisture Grain Crimped and Bagged in a Plastic Tube Silo

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Introduction

Low profits from the sale of grains have increasingly led to cereals being used in feeding on the farm and for biogas production. The preservation of high-moisture grain is an alternative for reducing drying costs; advantages and disadvantages are given in table 1. The preservation of combine-harvested grain at a moisture content of 200 g kg^{-1} requires the application of organic acids. For ensilage, grain must have a moisture content of at least 300 g kg^{-1}, and it must be stored under anaerobic conditions with a density of 1000 kg m^{-3} (Zimmer, 1985; Ratschow, 1986).

Table 1: Advantages and disadvantages of preserving grain with a high moisture content compared with drying (cf. Buchanan-Smith et al., 2003)

Advantages	Disadvantages
higher yields resulting from earlier harvesting of the grain at its maturity;	requires considerable investments in storage facilities;
saves drying costs and reduces fuel consumption;	reduces the time frame for harvesting grain;
increases flexibility in grain harvesting;	increases storage losses slightly;
allows rapid handling of large volumes of grain during harvest;	more power required to process grain in this form;
may reduce investments in processing equipment;	loss of flexibility in selling the grain on the cash market;
feeding value is as high as or higher than the feeding value of grain ground when dry;	loss of fluidity in moving the grain;
reduces dust in rations and in livestock barns.	grain is liable to freeze in winter and to attract flies in summer;
	improper storage may result in complete loss of feed.

A new system (Crimper Bagger, AG BAG) offers the possibility of crimping and storing grain in plastic tube silos in one operation. Bagging systems do not require long-term investments for storage constructions, and bag size varies with the quantity of forage harvested. Bag silos thus filled offer the flexibility to act according to changing market situations. Especially for expanding farms planning to produce biogas, pressed bag silos might be a good option. Losses are said to be low with bag silos (Muck, 2004).

To assess the influence of the moisture content of crimped grain on fermentation in tube silos, a series of tests was carried out at the Institute of Agricultural Engineering at the University of Bonn.

Materials and Methods

Trials were conducted at the agricultural research station “Frankenforst” of the University Bonn in 2005. Winter wheat with a moisture content of 20% was harvested and crimped. Half of the wheat was moistened to a moisture content of 30%. Both variants were stored in laboratory silos (1.5 l), each with four different application rates of a chemical additive to improve aerobic stability (0 l/t FM, 2 l/t FM, 4 l/t FM, 6 l/t FM Kofagrain). A similar experiment was implemented on a large scale, using the crimper-bagger and plastic tube silos (50 t).

The fresh crop was analysed for dry matter content, concentrations of water soluble carbohydrates (WSC), buffering capacity, and microbial composition. For the laboratory experiments, the 1.5 l silos were filled with the different variants, sealed and fermented at a constant temperature of 25°C. All samples were prepared in three replications. After 90 days, the fermentation quality was analysed (pH, concentration of lactic acid, acetic acid, butyric acid and ammonia).

The aerobic stability of the silages was determined according to the Völkenrode System (Honig, 1990). This method is based on monitoring temperature increases resulting from microbial activity in samples exposed to air (on the 28^{th} and 42^{th} day, for 24 hours each). The temperature was measured automatically with Gemini 'Tinytalk' temperature loggers. Silage was deemed to be stable unless the internal temperature was more than 3°C above the ambient temperature.

Analyses of the chemical and microbial composition and the fermentation quality of the material were conducted as further indicators of quality.

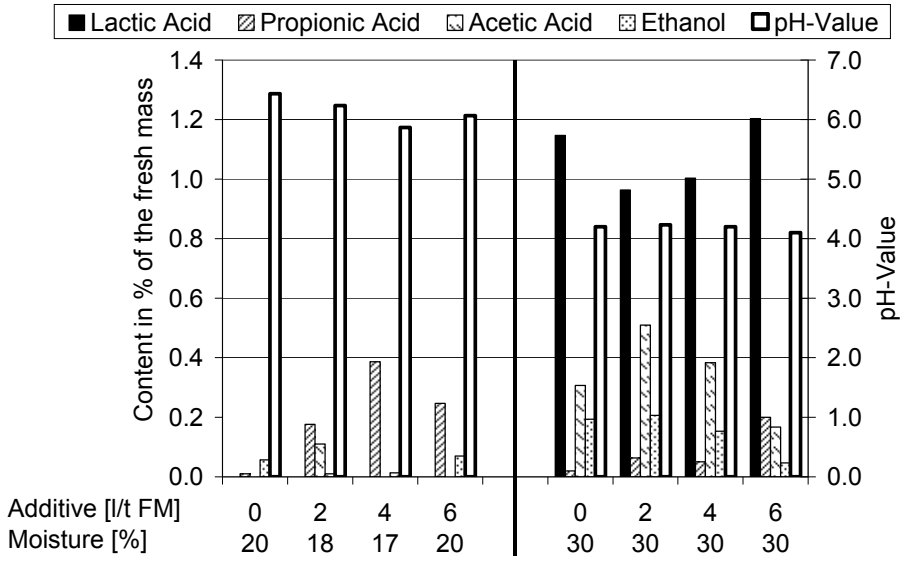
Results and discussion

The results of the experiments show that the moisture content has a considerable influence on the production of lactic acid. In Figure 1, the percentages of the different fermentation products are assigned to the different moisture degrees of winter wheat and to the different application rates of the additive. At a moisture content below 20%, lactic acid fermentation could not be shown to have taken place. Consequently, the pH values are at a level between 5.8 and 6.4.

At maximally 6 l/t FM, the low application rates of the silage additive can only lead to a minor decrease in pH. However, the analysis of the aerobic stability of the silages showed that even a small amount of the additive can significantly delay temperature increases in the wheat after exposure to air. Thus, it is clear that the additive reduces moulds and yeasts and that this property is independent of the pH of the feed.

Ensilage clearly takes place above moisture contents of approximately 30 %.This is reflected in the lactic acid and acetic acid contents. At first sight, at less than 2 % DM, they seem to be low compared to other silages. However, with the substrate consisting of grains only, it lacks buffering substances. Thus, even these low acid contents can lower the pH to around 4. None of the variants contained undesirable butyric acid. Only traces of ethanol were found, with the ethanol contents decreasing with higher silage additive application rates. This is an indicator that the silage additive inhibits ethanol-producing yeasts. The propionic acid levels are mainly a consequence of the use of the silage additive.

Figure 1: Influence of moisture content and application rate of a chemical additive on the fermentation quality of winter wheat in laboratory silos



Thus, achieving aerobic stability is easier with wet grains than with dry grains because higher moisture contents lead not only to better fermentation but also to a better compression of the material in the silage bag, which reduces air infiltration into the opened silage bag.

Conclusions and Reference

Initial results suggest it is possible to produce stable silage from crimped grains with a moisture content of 300 $g*kg^{-1}$. Moreover, it seems to be sufficient to apply silage additive at a rate of 2 to 4 $l*t^{-1}$.

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The Effect of Different Density on Fermentation Profile and Aerob Stability in Corn-sorghum Silage – Investigated in a New Model Silo System

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Introduction

Silage density is one of the major factor in anaerob fermentation and aerob stability (Woolford, 1984; McDonald et al, 1991). Some of the silage additive have advantageous effect on stability of silage exposed to air. In recent experiment authors's aim was to investigate the effect of density (150: 150 kg DM/m^3, 200: 200 kg DM/m^3 , 250 : 250 kg DM/m^3) on silage fermentation profile (pH, lactic- and volatile fatty acid content) and aerob stability in corn-sorghum silage (with original dense). Additionally, the authors investigated the effect of a biological addititive (*Streptococcus faecium, Pediococcus acidilactici, Lactobacillus plantarum and Lactobacillus salivarius*, amilase, pentosanase, cellulase, hemicellulase, germ: 10^5 /g, dose: 5 g/tonna) on quality and aerob stability of corn-sorghum silage ensiled with different density (TR 150: 150 kg DM/m^3, TR 200: 200 kg DM/m^3 , TR 250: 250 kg DM/m^3) in order to find whether inoculation is able to compensate the poor management (low density) and aerob deterioration.

Materials and method

Ensiling was carried out in a newly improved model silo system (airtight sealing, built in temperature sensors, 0,041 m^3). Sampling was carried out on the 60^{th} day of fermentation. Crude nutrients (dry matter, crude protein, crude fat, crude fiber, crude ash, nitrogen-free extract), pH, lactic- and volatile fatty acid content were measured at the end of anaerob phase according to the Hungarian National Standards. Temperature changes were detected in aerob phase after opening (0,5; 48;240 hours) with sensors of model mini silos (in 110 mm depth).

Results and discussion

Authors have found that the different densities did not have significant effect on pH in control corn-sorghum silage (Table 1) . Inoculation reduced the pH in silage ensiled with 250 kg DM/m^3compared to the control. (DM content of control and inoculated silage were 30,8% and 31,4%, respectively.)

Table 1: The pH-changes of control and treated corn sorghum silage ensiled with different density (n=3)

	150	200	250	TR 150	TR 200	TR 250
Mean	3,91	3,95	3,90	3,97	3,92	3,88
St.dev.	0,02	0,02	0,01	0,04	0,01	0,01
Rel. coeff.	0,51	0,39	0,30	0,88	0,15	0,30
T-test p-value		0,069		0,063		0,013
		NS		NS		*
	150-200	200-250	150-250	150-200	200-250	150-250
T-test p-value	0,65	0,17	0,63	0,32	0,031	0,004
	NS	NS	NS	NS	*	**

Note: NS = not significant difference; * p< 0,05; ** p< 0,01

In recent experiment different densities did not modify significantly lactic- and volatile fatty acid content in control corn-sorghum silage (Table 2). Inoculation slightly decreased the acetic acid content in treated silage. Authors have found significant differencein acetic acid concentration between inoculated and control silage ensiled with 200 kg DM/m^3density.

Temperature changes compared to environmental temperature were lower in the case of 200 and 250 kg DM/m^3 ($p< 0,05$) on the 30th minute, 48th and 240th hour of aeration (Table 3). Inoculation reduced significantly the temperature corn-sorghum silage ensiled 150,200 and 250 kg DM/m^3 in the 48th hour of aerob phase.

Table 2: The lactic- and volatile fatty acid content of control and treated corn sorghum silage ensiled with different density (n=3)

	150	TR 150	200	TR 200	250	TR 250
Lactic acid	g/100g fresh material					
Mean	1,44	1,16	1,40	1,31	1,49	1,23
St. dev.	0,25	0,32	0,04	0,13	0,28	0,17
		NS		NS		NS
	150-200	200-250	150-250	TR150-TR200	TR200-TR250	TR150-TR250
	NS	NS	NS	NS	NS	NS
Acetic acid	g/100g fresh material					
Mean	0,70	0,70	0,80	0,61	0,73	0,65
St. dev.	0,07	0,16	0,09	0,03	0,11	0,11
		NS		*		NS
	150-200	200-250	150-250	TR150-TR200	TR200-TR250	TR150-TR250
	NS	NS	NS	NS	NS	NS
Total acid	g/100g fresh material					
Mean	2,14	1,87	2,21	1,93	2,24	1,88
St. dev.	0,25	0,46	0,13	0,15	0,38	0,27
		NS		NS		NS
	150-200	200-250	150-250	TR150-TR200	TR200-TR250	TR150-TR250
	NS	NS	NS	NS	NS	NS

Note: NS = not significant difference; * $p< 0,05$; ** $p< 0,01$

Table 3: Temperature changes in control and treated corn-sorghum silage during aerob phase compared to environmental temperature (110 mm layer; n=3)

Aerob period (h)		150	TR 150	200	TR 200	250	TR 250
	Mean	4,8	5,5	3,4	4,8	3,9	4,9
	St. dev.	1,0	0,5	0,3	0,9	0,5	0,8
0,5			NS		NS		NS
		150-200		200-250		150-250	
		NS		NS		NS	
	Mean	4,5	1,0	3,1	1,4	3,6	0,0
	St. dev.	1,0	0,1	0,3	0,5	0,4	0,0
			**		*		**
48		150-200		200-250		150-250	
		NS		NS		NS	
	Mean	2,7	1,3	0,8	2,5	0,1	1,9
	St. dev.	0,2	0,4	0,2	0,5	0,0	0,2
240			NS		*		*
		150-200		200-250		150-250	
		*		NS		*	

Conclusions

Authors summarized that inoculation have significant effect just in the case of high density (250 kg DM/m^3) on pH. We concluded that good compactness is needed for anaerob homofermentative latcic acid bacteria in additive to ferment and reduce pH-value. Therefore poor management can not be compensated by inoculation. Additionally, higher density (200 and 250 kg DM/m^3) reduced the temperature changes in aerob phase, therefore improved the aerob stability compared to silage ensiled with 150 kg DM/m^3. In recent experiment inoculation (*Streptococcus faecium, Pediococcus acidilactici, Lactobacillus plantarum and Lactobacillus salivarius*, amilase, pentosanase, cellulase, hemicellulase, germ: 10^5 /g, dose: 5 g/tonna) improved the aerob stability compared to the control corn-sorghum silage ensiled 150, 200 and 250 kg DM/m^3 on the 48th hour of aeration, respectively.

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Density measurement on silage

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Introduction

Together with high-value and energy-rich green forage, low-loss and quality-oriented preservation is of outstanding importance when preparing silage. Fungi and their metabolic products, toxins, represent a special danger in preservation because they can infiltrate the human organism via the food chain and cause damage to health there. Practical experience and a large number of scientific studies document that in addition to dry matter content and cutting time, the storage density in the silo is one of the factors exerting the greatest influence on the quality of fermentation and fungi growth. There are specifications for compaction, depending on the material to be ensiled. So far, however, there has been no practical procedure for fast and continuous density measurement during feed storage intake.

Objective

The objective of this research is to develop an online procedure enabling the operator of mobile compaction units to receive a constant display of the green forage density actually achieved while driving over the feedstock. Only in this way can the operator produce the minimum compaction necessary in fermentation biology cost-effectively throughout the entire silo. The core of the process is a suitable density sensor. This must be able to capture the density in the most recently stored layer of approx. 30 cm. Suitable sensors are to be selected by investigating physical measuring principles, and a measuring procedure is to be developed.

Radiometric measurement with gamma backscattering probe

The process is based on the partial absorption and simultaneous scattering of the radiation of radioactive nuclides on passing through the material. The measuring device consists of a radioactive source of radiation and a detector uniting a transmitter and receiver that are screened against each other. The number of gamma quanta registered by the detector is a function of the density of the surrounding material. Practical embodiments are measuring probes that are inserted in the material, or block-shaped devices placed on top of the material.

Microwave Resonance Procedure

The method uses the dipolar properties of the water molecule. A harmonious electromagnetic resonance field is produced through a generator via a sensor. The water bound in the product enters into interaction with this field. By measuring the detuning of the resonance frequency and attenuation of the resonance amplitude caused by the material to be measured, and by analysing this on the basis of a patented process of Messrs. TEWS, it is possible to determine the moisture content and the density separately. The material to be measured must be in contact with the cylindrical sensor.

Georadar or reflection sensors

The georadar "Ground Penetrating Radar" works on the basis of propagating high-frequency electromagnetic waves and counts as one of the impulse radar processes. A short, energy-rich impulse is transmitted in order to obtain a distinct and clearly defined signal, the echo. It is also possible to obtain a continuous signal. The effective speed of wave propagation and/or the reflection coefficient are connected in linear fashion with the density. The process does not require any contact with the material.

Results

All three sensor types are prepared for density measurement of silage and ready for use. Gamma backscattering probes already proved their suitability for discontinuous measuring at errors of < 3% in the 1980s. Only minimum Problems result from the use of radioactive material. Test measurements with microwaves produced measuring signals with a high degree of determinateness at moisture contents of 40-70 % and densities up to 600 kg/m³. It is necessary to expand the measuring range and to separate density and moisture content better. In view of the advantage of contact-free measurement, further developments of the technically more demanding reflection sensors could be worthwhile.

Application

For online measurement it is planned to connect the sensors with the mobile compaction machines. Sensors that require contact with the material are to be arranged on the circumference of a measuring wheel suspended floating on the machine, which rolls over the surface of the compacted material during travel. The sensor transmits the measurement signal is to an evaluation unit. This calculates the density, compares it with the specified target values, can link it with location coordinates and display this appropriately to the machine operator on a monitor. The data are also to be stored for subsequent readout or can be transferred to a stationary computer.

Effectiveness of Conditioning Herbage at Harvesting

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Summary

Herbage from permanent grassland as well as from grass/clover mixtures was harvested by machinery equipped with a swath conditioner. The efficiency of conditioners was investigated. Regardless of their working tool type, positive effects of conditioning on protein digested in the intestine (PDI) were found. The energy values in herbage were well-balanced at all the research treatments.

Keywords: conditioners, forage harvesting, wilting, nutritive value

Introduction

The harvesting time at forage ensiling should be as short as possible to comply with the preservation techniques. Therefore, an effective design and construction of working tools used in harvesters is necessary. A research was carried out to investigate the mechanical treatment of swath by forage harvesters equipped with conditioners.

Materiále and Methods

At the 1st cut, permanent grassland (PG) and a grass/clover mixture (GCM) were harvested by forage harvesters equipped with the conditioners and the efficiency of machinery was investigated. A comparison was made between the herbage harvested with mechanical swath conditioners (MD-5K) using either plastic breaking fingers or metallic ones, attachable as appropriate, or cut by a standard mower (ŽTR-165). The wilting process in swath was monitored using a moisture meter (*Fortuna 2*). The moisture content was measured immediately after cutting and then in two-hour intervals. The investigation of herbage started immediately after cutting at 08.00 o` clock when fresh herbage was sampled for laboratory analyses. The times of wilted herbage sampling are given in the table, namely: a = Day 1 at 10.00 o` clock; b = Day 1 at 14.00; c = Day 2 at 10.00 and d = Day 2 at 14.00. In compliance with the approved research methods, the herbage samples were analysed to determine dry matter content (DM) and organic nutrients. The data were used to calculate the nutritive value of herbage defined as protein digested in the intestine (PDI), net energy for lactation (NEL), net energy for fattening (NEV) and metabolizable energy (ME). The data were submitted to analysis of variance (*Anova)*.

Results and Discussion

The content of DM was lower in fresh herbage from GCM (192.67 - 219.35 g/kg) than in that from PG (280.80 - 323.64 g/kg)., probably in relation to the botanical composition of swards and to the phenological stages of botanical species at the time of harvesting. During the research trial, the largest proportion of species in PG were at the stage of full earing or after flowering while the species in GCM were at the earing stage. The analyses of wilted herbage showed significant differences not only between the sward types but also between the machines and the periods of time passed after harvesting and the sampling dates, respectively. A linear increase in herbage DM content was recorded at all the samplings and treatments. The highest decrease in herbage moisture content (584.77 g/kg) was found at PG when swaths were treated by the metallic conditioner while DM content in herbage cut without conditioning was only

397.71 g/kg at the time given. The highest DM content in GCM herbage (437.31 g/kg) was recorded at swaths treated by the plastic conditioner, and similarly, the lowest DM content (353.05 g/kg) was found in herbage cut at the treatment without conditioning. Under nearly identical field and climatic conditions, Buchgraber (2002) reported that mechanically conditioned herbage reached the storable content of moisture in hay 3- to 3.5-fold faster than unconditioned swath. Gonda, Kunský and Šesták (1998) recorded 86.8 and 95.6 % of damaged plants, respectively, with the use of conditioners.

The nutritive value of herbage showed a decrease in degradable crude protein (CP) in connection with the longer time of wilting. Considering the lower CP content in PG herbage, PDI was also lower than that in GCM. The lowest PDI in PG herbage was recorded at the treatment with the plastic conditioner applied while the lowest PDI in GCM herbage was found at the treatment cut without conditioning. At PG harvesting, the highest values were recorded at treating with the metallic conditioner and also in herbage cut without conditioning while at GCM it was at the treatment with the plastic conditioner. An assessment of all the treatments and samplings showed nearly the same levels of NEL, NEV and ME, only a small decrease was found when the wilted and the fresh herbage was compared. From this point of view, PDI was a limiting factor in the potential production. An earlier research (Čunderlíková and Polák, 2003) had also shown increased nutritive value of herbage treated with mechanical conditioners.

Sward types	Machinery treatment	Herbage	Sampling	DM	PDI	NEL	NEV	ME
				G/kg DM		MJ/kg DM		
PG	Cutting only	fresh		280.80	77.60	5.25	5.00	9.035
		wilted	a	357.32	75.30	5.23	4.98	9.001
			b	384.28	68.00	5.22	4.98	8.980
			c	391.58	66.20	5.20	4.96	8.938
			d	397.71	62.00	5.19	4.93	8.914
	Cutting and plastic conditioner	fresh		323.64	70.10	5.29	5.03	9.092
		wilted	a	334.52	67.00	5.25	5.00	9.011
			b	332.96	62.30	5.20	4.95	8.940
			c	491.83	61.50	5.20	4.95	8.942
			d	543.91	56.10	5.18	4.94	8.901
	Cutting and metallic conditioner	fresh		290.69	78.90	5.27	5.01	9.022
		wilted	a	323.25	73.90	5.25	5.00	9.019
			b	339.27	68.80	5.24	4.99	9.012
			c	435.57	66.70	5.25	5.00	9.018
			d	584.77	59.20	5.24	4.94	9.024
GCM	Cutting only	fresh		192.67	89.70	5.22	4.94	8.989
		wilted	a	253.30	83.80	5.22	4.93	9.003
			b	244.25	82.30	5.20	4.92	8.981
			c	287.00	73.70	5.16	4.87	8.911
			d	353.05	65.80	5.15	4.87	8.889
	Cutting and plastic conditioner	fresh		219.35	87.90	5.22	4.94	9.003
		wilted	a	247.72	78.90	5.22	4.93	8.999
			b	277.79	78.20	5.20	4.91	8.973
			c	321.13	74.10	5.20	4.92	8.966
			d	437.31	72.30	5.20	4.92	8.963
	Cutting and metallic conditioner	fresh		205.43	89.20	5.18	4.90	8.951
		wilted	a	230.72	80.50	5.22	4.94	9.013
			b	278.34	79.30	5.22	4.93	9.000
			c	355.16	77.90	5.20	4.92	8.964
			d	417.55	70.50	5.15	4.87	8.879
Tukey (P<0.05) + (P<0.01) ++			sward	++	++	+	+	+
			machinery	+	+	+	+	+
			sampling	++	++	++	++	++

Conclusiona

The research showed positive effects of conditioning on maintaining the nutritive value of herbage. At PG, the highest PDI was found in herbage harvested without conditioning and in that treated with the metallic conditioner. At GCM, the highest PDI was recorded in herbage harvested with the plastic conditioner attached. However, a rather even level of energy value was found in herbage from all the research treatments. The mechanical conditioning of herbage at harvesting showed positive effects mainly on PDI, regardless of the working tool design used for the machinery.

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Investigations on ensilability of pea seeds (*Pisum sativum*) harvested before maturation

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Introduction

Patchy maturation in field peas grown for seed production is a known problem resulting in high post harvest costs for technical drying of the seeds. Developing a method for preserving peas harvested before maturation by lactic acid fermentation would have several advantages:

- supplying a low cost feed high in protein from local sources
- initiation of harvest independent on moisture content of pea seeds
- early clearing of pea fields for following crops
- reducing harvesting losses from cracked pods
- saving costs for technical drying of the material

Thus, the aim of the investigation was to verify ensilability of pea seeds harvested at approximately 65 % dry matter content.

Material and Methods

Bruised seeds of pea (*Pisum sativum*, variety "Lisa") harvested with an experimental harvester at approximately 65 % dry matter (DM) were used for an ensiling study. Six hundred grams of this material were filled in plastic bags (3 per treatment) either pure, with molasses addition (2% of fresh matter [FM]) or inoculated with lactic acid bacteria (LAB; commercial product containing *Lactobacillus plantarum*, inoculation with $3x10^5$ cfu/g FM) alone or in combination with molasses. Air was evacuated and bags were sealed using a vacuum sealer. Bags were stored at 20°C for 5, 15, 50 and 90 days. After incubation silage extracts were prepared (50 g silage in 200 ml *aqua dest*., stored for 15 h at 5°C) and pH-values were measured. Fermentation parameters in the silage extracts as mentioned in table 2 were determined by HPLC and GC. A T-test (Duncan) was performed to compare the results.

Nutrient contents in the harvested material were analysed according to the Weende feed analysis.

Results and Discussion

The nutrient content of the harvested material is shown in table 1. Regarding the high content of crude protein in peas in comparison with the low content of easily fermentable carbohydrates known from the literature (Bastianelli *et al*., 1998) ensilability was expected to be low. However, organoleptic evaluation of the resulting silages revealed good quality in all variants tested. This could be approved by analytical determination of the fermentation parameters: The pH was below the DM-dependent critical value in all silages. Butyric acid did not occur in any of the samples and contents of acetic and propionic acid as well as ethanol were low.

Regarding the additives molasses had no effect on fermentation except for slightly increasing the amount of lactic and acetic acid produced. On the contrary, addition of lactic acid bacteria resulted in a faster and deeper decrease of pH, significantly higher amounts of lactic acid and reduced fermentation losses. Providing molasses in

combination with LAB had no further positive impact on fermentation quality suggesting that enough fermentable carbohydrates are supplied by the pea seeds.

Conclusions

1. Preliminary results prove good ensilability of pea seeds harvested at approximately 65 % DM without and with addition of silage additives.
2. Addition of lactic acid bacteria accelerates pH-decrease and improves fermentation parameters of resulting silages.
3. As addition of molasses has no effect on silage quality, providing additional sources of easily fermentable carbohydrates is not necessary for the preparation of pea seed silage.

The preliminary results suggest ensiling as a suitable method for conserving pea seeds harvested with a moisture content not adequate for dry storage.

Summary

Patchy maturation in peas results in high post harvest costs for technical drying of the seeds. In order to verify possibilities of preserving peas with high moisture content by lactic acid fermentation, model silages from pea seeds harvested at 65 % DM content were prepared without and with addition of molasses and/or lactic acid bacteria and incubated for 5 to 90 days at 20°C. Organoleptic evaluation showed good quality of all silages, even in the variants prepared without silage additives. Addition of lactic acid bacteria improved fermentation. Molasses addition had no effect on silage quality. Thus, ensiling seems to be a suitable method for conservation of pea seeds harvested before maturation.

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Table 1: Nutrient contents of pea seeds harvested before maturation

Parameter	Dry matter	Crude ash	Crude protein	Crude fibre	Crude fat
	(% OS)	(% DM)			
Pea seeds	62,5	3,4	23,0	7,8	1,8

Table 2: Fermentation parameters of silages from pea seeds depending on additives and time of storage (means: n=3, ± s)

Incubation (d)	Variant	DM (% OS)	FL (%)	pH	Parameters in % DM				
					LA	AA	PA	BA	Ethanol
5	**Control**	**63,30^{a,AB}**	**0,69^{a,A}**	**4,88^{c,C}**	**1,86^{a,A}**	**0,35^{b,A}**	**0,01**	**0**	**0,81^{a,B}**
	± s	*2,58*	*0,26*	*0,03*	*0,15*	*0,03*	*0,00*	*0*	*0,17*
	Molasses	**64,24^{a,AB}**	**0,54^{a,A}**	**4,74^{b,D}**	**2,19^{b,A}**	**0,50^{c,A}**	**0,01**	**0**	**0,74^{a,B}**
	± s	*2,10*	*0,01*	*0,03*	*0,15*	*0,03*	*0,00*	*0*	*0,01*
	LAB	**64,73^{a,C}**	**0,39^{a,A}**	**4,24^{a,C}**	**3,48^{c,A}**	**0,26^{a,B}**	**0,01**	**0**	**0,53^{a,A}**
	± s	*0,76*	*0,10*	*0,01*	*0,05*	*0,02*	*0,00*	*0*	*0,09*
	LAB+Molasses	**64,19^{a,AB}**	**0,64^{a,AB}**	**4,26^{a,C}**	**3,57^{c,A}**	**0,34^{b,A}**	**0,01**	**0**	**0,57^{a,A}**
	± s	*2,05*	*0,29*	*0,01*	*0,23*	*0,01*	*0,00*	*0*	*0,05*
15	**Control**	**61,52^{bc,A}**	**1,15^{b,B}**	**4,58^{c,B}**	**2,99^{a,B}**	**0,47^{c,AB}**	**0,01**	**0**	**1,17^{c,B}**
	± s	*0,09*	*0,08*	*0,03*	*0,05*	*0,01*	*0,00*	*0*	*0,06*
	Molasses	**61,58^{c,A}**	**1,06^{b,B}**	**4,47^{c,C}**	**3,41^{b,B}**	**0,60^{d,B}**	**0,01**	**0**	**0,90^{b,B}**
	± s	*0,08*	*0,06*	*0,01*	*0,07*	*0,02*	*0,00*	*0*	*0,00*
	LAB	**61,39^{ab,B}**	**0,43^{ab,A}**	**4,13^{a,A}**	**4,54^{c,B}**	**0,33^{a,C}**	**0,01**	**0**	**0,70^{a,B}**
	± s	*0,08*	*0,25*	*0,00*	*0,17*	*0,03*	*0,01*	*0*	*0,05*
	LAB+Molasses	**61,25^{a,A}**	**0,56^{a,A}**	**4,16^{b,B}**	**4,69^{c,B}**	**0,41^{b,B}**	**0,01**	**0**	**0,74^{a,A}**
	± s	*0,12*	*0,01*	*0,01*	*0,06*	*0,01*	*0,00*	*0*	*0,06*
50	**Control**	**61,43^{b,A}**	**1,53^{c,C}**	**4,37^{b,A}**	**4,09^{a,C}**	**0,54^{ab,AB}**	**0,01**	**0**	**1,10^{a,B}**
	± s	*0,22*	*0,03*	*0,01*	*0,04*	*0,06*	*0,00*	*0*	*0,12*
	Molasses	**61,23^{ab,A}**	**1,39^{b,C}**	**4,35^{b,A}**	**4,16^{a,B}**	**0,64^{b,B}**	**0,01**	**0**	**0,82^{a,B}**
	± s	*0,32*	*0,01*	*0,01*	*0,16*	*0,06*	*0,01*	*0*	*0,08*
	LAB	**60,83^{a,A}**	**0,87^{a,B}**	**4,12^{a,A}**	**4,80^{b,B}**	**0,35^{a,C}**	**0,01**	**0**	**0,66^{a,B}**
	± s	*0,22*	*0,02*	*0,01*	*0,17*	*0,03*	*0,00*	*0*	*0,01*
	LAB+Molasses	**61,30^{ab,AB}**	**0,83^{a,C}**	**4,14^{a,A}**	**4,85^{b,B}**	**0,27^{ab,AB}**	**0,01**	**0**	**0,42^{a,A}**
	± s	*0,28*	*0,03*	*0,00*	*0,02*	*0,23*	*0,00*	*0*	*0,36*
90	**Control**	**61,77^{ab,B}**	**1,88^{b,D}**	**4,37^{c,A}**	**1,91^{a,AB}**	**0,30^{a,AB}**	**0,01**	**0**	**0,43^{a,A}**
	± s	*0,08*	*0,18*	*0,00*	*0,40*	*0,18*	*0,00*	*0*	*0,31*
	Molasses	**62,25^{b,B}**	**1,87^{ab,ABC}**	**4,38^{d,B}**	**3,41^{b,B}**	**0,41^{a,A}**	**0,01**	**0**	**0,41^{a,A}**
	± s	*0,30*	*0,43*	*0,01*	*0,81*	*0,08*	*0,00*	*0*	*0,24*
	LAB	**61,50^{a,B}**	**1,11^{a,B}**	**4,14^{a,B}**	**3,26^{b,A}**	**0,21^{a,A}**	**0,01**	**0**	**0,43^{a,A}**
	± s	*0,34*	*0,04*	*0,01*	*0,35*	*0,02*	*0,00*	*0*	*0,05*
	LAB+Molasses	**61,54^{a,B}**	**1,09^{a,D}**	**4,17^{b,B}**	**3,78^{b,AB}**	**0,28^{a,AB}**	**0,01**	**0**	**0,47^{a,A}**
	± s	*0,17*	*0,09*	*0,01*	*0,57*	*0,05*	*0,00*	*0*	*0,09*

OS: original substance, FL: fermentation losses, CA: crude ash, CP: crude protein,
LA: lactic acid, AA: acetic acid, PA: propionic acid, BA: butyric acid
Control: without additives
Molasses: addition of molasses
LAB: addition of lactic acid bacteria (*Lb. plantarum*)
LAB+Molasses: addition of lactic acid bacteria (*Lb. plantarum*) and molasses
^{ab}: different lower case letters in a column show significant differences between variants at a given incubation time
^{AB}: different capitals in a column show significant differences between incubation times within a variant

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Aerobic stability of sorghum-maize mixed silages

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Abstract

Aerobic stability of silages has great importance in practice. When the silo is opened, yeasts and molds can grow due to the exposition to air. The process cause significant loss of nutrients and harmful silage will be produced. There are lot of published results on the fermentability, the nutritive value and the digestibility of sorghum-maize mixed silages, but we have only limited knowledge about their aerobic stability. Here we report the results of our experiments concerning the aerobic stability of mixed silages prepared from different ratios of sorghum and maize, and the use of the inoculant *Lactobacillus buchneri*, the heterolactic bacterium.

Introduction

Silo maize is the most important forage of ruminants in Hungary, where the average annual temperature and the precipitation is 10.6 °C and 550-600 mm, respectively. Due to the climate changes caused by global warming, the number of droughty years are increasing, and that is while the cultivation of drought resistant plants become important. It is well known that sorghum (*Sorghum bicolor L.*) belongs to drought resistant plants. Its yield may be twice that of maize during drought. The carbohydrate content of sorghum is fairly high, so the aerobic stability of sorghum silage has been intensively studied **(Filya et al. 2002; Weinberg et al. 2002).** The effect of a heterofermentative bacterium *Lactobacillus buchneri* has been also investigated on the aerobic stability of sorghum silage. The bacterium decreased the growth intensity of yeasts and molds, and it was established the aerobic stability of silages were improving **(Froetschel et al. 1995;Filya et al. 2002)**. In the drought south plan region of Hungary, maize and sorghum are co-cultivated and ensilaged frequently. There are published data about the advantages and disadvantages of their co-cultivation **(Avasi 2001, Orosz 2003),** the fermentation dynamics and nutritive values of mixed silages **(Avasi 2001),** but there are no results on the aerobic stability of sorghum-maize silages. Here we report on the aerobic stability of mixed silages in accordance with the variation of sorghum-maize ratios.

Materials and Methods

Maize at dent stage and sorghum at the milk stage of maturity were harvested and chopped to approximatelly 10-12 mm and ensilaged in 120 litre capacity model-size silos. The packing density was 536-680 kg/m^3 dependent of the ratio of sorghum.

Treatments	Marks
100% maize	Maize
75% maize and 25% sorghum	75% - 25% M/S
50% maize and 50% sorghum	50% - 50% M/S
25% maize and 75% sorghum	25% - 75% M/S
100% sorghum	Sorghum

..

The treatments used in two repeated experiments are as follow:

Treatments: 1. (control) without inoculant

2. with inoculant (treated with 5g/t fresh forage)

Inoculant: *Lactobacillus buchneri* NCIMB 40788 $6x10^{10}$ cfu/g

The inoculants was suspended in 20 ml water and spread on the chopped material. Silos were opened and sampled after 60 days of ensilage. The chemical composition of the fresh plant material and the silages were subjected to Weendei-analysis. The lactic acid and volatile fatty acids (VFA) was analysed by Chrom-5 gas chromatograph device. pH was examined with digital pH-meter OP 211/1, and NH_3 from watery extract with OP 264/1 NH_3 measuring device. The water soluble carbohydrates (WSC) were determined according to Mac Donald P. and Henderson J. (1964) using Anthron reagent and sulfuric acid, applying spectrophotometry. Buffer capacity was determined by lactic acid titration. The aerobic stability was determined 60-67 days after ensiling using the standard of Honig system (1986). The temperature was measured every hours by computer.

Results and Discussion

The mature stage of maize and sorghum is characterized by lower dry matter and higher fermentable carbohydrate (WSC) content at the time of harvest **(Table 1)**. In mixed silages increasing the rate of sorghum resulted in the increase of lactic- and acetic acids, while decrease in the ammonia contents. Significantly higher amount of acetic acid was produced when the silages were treated by *L. buchneri* **(Table 2)**. The silages containing 100 to 75% sorghum were less stable (**Fig. 1)** but as a concequence of bacterial inoculation the aerobic stability was improved by 24 hours **(Fig 2).**

Table 1.: Some chemical parameters of fresh forages

Parameters		Maize	Sorghum
Dry matter	g/kg	487	237
Crude protein	g/kg DM	71,9	84,8
WSC	g/kg DM	50,6	141,4
Buffer kapacity	g/kg DM	16,2	16,0
pH		5,95	5,87

Table 2: Chemical compositions of the maize-sorghum mixed silages

Parameters		Untreated silages (control)					Treated with inoculant (*L. buchneri*)				
		Maize	75-25 M/S	50-50 M/S	25-75 M/S	Sorghum	Maize	75-25 M/S	50-50 M/S	25-75 M/S	Sorghum
DM	g/kg	486	422	333	323	261	496	455	354	308	253
CP	g/kg DM	68,1	71,5	80,4	73,5	80,2	62,1	68,3	78,7	73,7	78,7
LA	DM%	2,55	3,06	4,41	3,10	3,68	2,80	2,97	3,61	3,80	3,88
AA	DM%	1,25	1,37	1,38	0,78	1,5	1,51	1,21	1,83	1,88	1,82
BA	DM%	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
PA	DM%	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
NH_3	mg%	69,7	55,4	51,6	51,5	46,1	63,8	55,0	43,8	34,8	34,9
pH		3,86	3,82	3,76	3,74	3,73	3,91	3,91	3,91	3,86	3,83

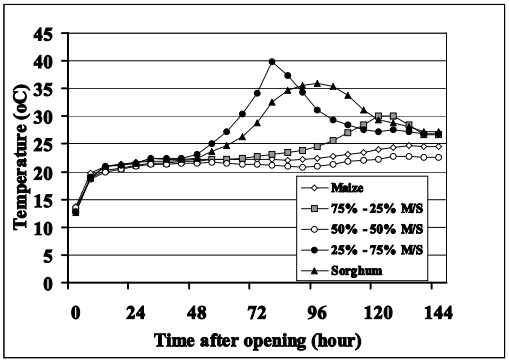

Fig 1. :Aerob stability of the control mixed silages

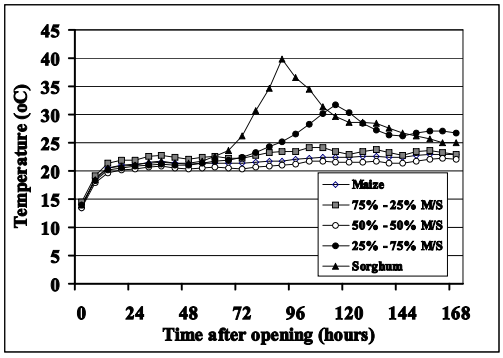

Fig 2.: Aerob stability of the treated silages

Conclusion

Sorghum has unfavourable effect on the aerobic stability of mixed silages so its use in higher than 50% can not be suggested.

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Figure 1: Aerob stability of the control mixed silages

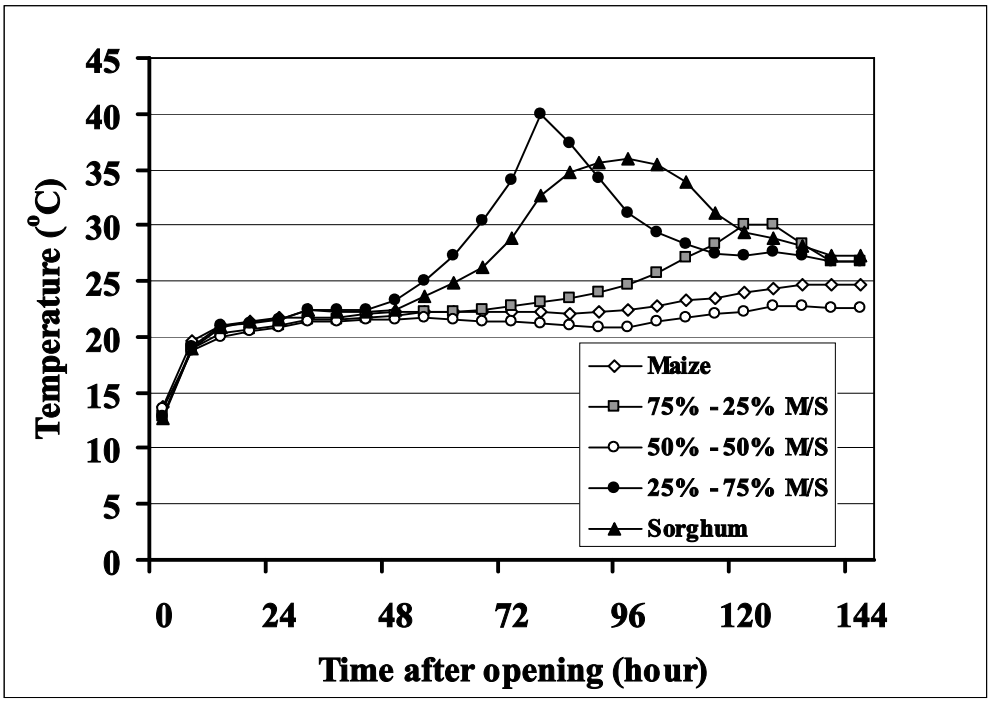
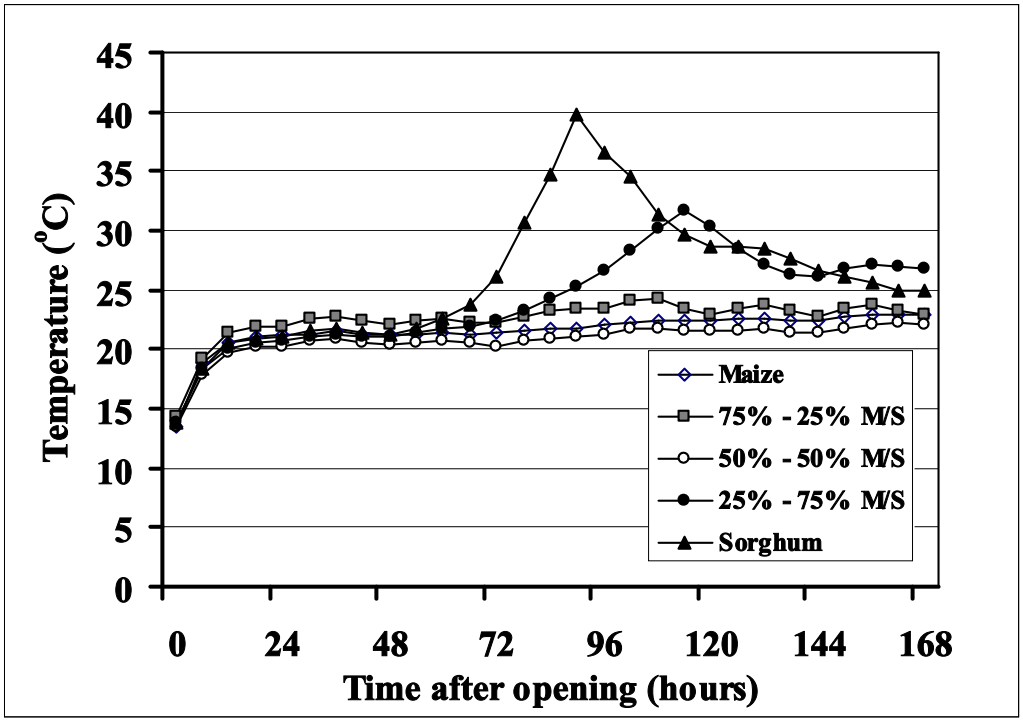


Figure 2: Aerob stability of the treated silages



MICROBIOLOGY AND CONTROL OF ERMENTATION PROCESS

The High-Through-Put Laboratory at Chr. Hansen A/S: a powerful research tool.

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Introduction

In many of the experimental tasks performed today at Chr. Hansen, including screening for new silage cultures, very large sample numbers are encountered. Clearly, traditional manual screening approaches are inadequate for the evaluation of such large sample numbers, and therefore automation and High-Through-Put (HTP) screening are required (Fox *et al*., 2002; Handen, 2002).

High-Through-Put (HTP) screening can simply be considered as using the power of robotics to carry out repetitive laboratory manipulations in a very rapid and highly reproducible fashion. This technology has experienced rapid growth and development in the last decade, with many equipment suppliers having developed dedicated robots for specific laboratory operations (Meier and Nolte, 2002).

Materials and Methods

The High-Through-Put laboratory at Chr. Hansen consists of two principal units; a colony picking robot and a liquid handling robot.

Colony picking robot

The colony picking robot (Genetix Ltd., United Kingdom) is used for automated colony picking and colony replication; the system consists of three principal components. The first component comprises, an imaging and software system, which identifies and selects the colonies for picking from the source agar plates. Colonies can be selected on the basis of size, presence/absence of clearing zones and color (white/blue screening). The second component of the colony picking robot consists of a robotic arm to which a 96-pin picking head is attached. The robotic arm and picking head move in the x, y, and z-axes, and are responsible for picking the selected colonies from the source agar plates and transferring them to either 96- or 384-well destination microtitre plates. Colonies are picked and transferred at a rate of 4000 colonies/hour, which is about 20-fold faster than that which can be performed manually. The third component of the colony picking robot consists of an automated cleaning and sterilization system. The 96-pin picking head is cleaned and sterilized by a combination of ethanol rinsing and heat inactivation. The three components of the colony picking robot are fully integrated, with both the source agar plates and destination microtitre plates automatically fed into the robot.

Liquid handling robot

The liquid handling robot (Tecan Schweiz AG., Switzerland), which is used for enzymatic assays, metabolite determination and strain characterization consists of four principal components. The first component of the robot is the pipetting unit, which consists of 8-channel and 96-channel systems. The 8-channel pipetting system is used for pipetting volumes in the range of 1-1000 µl, with each channel capable of being

controlled separately. This configuration gives great flexibility, however, the pipetting speed is moderate. In contrast, the 96-channel system pipettes volumes at very fast pipetting speeds, but the channels cannot be operated separately. Both pipetting systems can use either fixed or disposable tips. The second component of the robot consists of a centrifuge, which can handle up to four 96- or 384-well microtitre plates, and is mainly used for harvesting, washing and resuspension of cells after growth. The third component of the system consists of a controlled atmosphere incubator for microtitre plates, in which the oxygen and carbon dioxide levels can be adjusted for optimal growth. The fourth component of the robot is a microtitre plate reader, which can operate in absorbance, fluorescence or luminescence modes, and is used for cell growth and for various enzymatic assay measurements. Other minor components of the liquid handling robot include, a 96-well sonication unit for cell lysis, a 95ºC toaster for cell/enzyme inactivation, and a shaker for mixing and cell resuspension.

All the components of the liquid handling robot are linked together *via* two robotic manipulating arms, which move the microtitre plates between the various components of the robot. The entire robot is controlled by a sophisticated software program, which tracks the position of all the microtitre plates in the system. In addition, the software system maximizes the utilization of the individual components of the robot, in order to ensure the greatest possible sample throughput.

Results

The colony picking robot and the liquid handling robot are used to screen and characterize lactic acid bacteria for key technological characteristics.

Growth rate. The growth rate of the lactic acid bacteria is an important screening parameter, whereby the fastest possible growth rate is normally desirable. This assay is performed in 96- or 384-well microtitre plates and is based on kinetic measurement of optical density.

Metabolite determination. Determination of the production of metabolites such as lactate, malate, urea, ammonia, acetoin and diacetyl is performed by removal of cells from the growth medium, and measuring the level of the metabolites in the supernatant. Typically, these metabolites are determined in coupled enzymatic assays in which the oxidation/reduction of NADH/NAD+ is measured spectrophotometricly on the liquid handling robot.

Production of antimicrobial compounds. Screening for the production of antimicrobial compounds such as bacteriocins is performed by using the colony picking robot to pick the colonies and transfer them into mircotitre plates. Following growth, the culture supernatants are tested by replicate inoculation into indicator organism destination plates.

Molecular species identification of bacteria. Molecular species identification of the isolated lactic acid bacteria is performed in 96-well format. Using the liquid handling robot, cells are grown, harvested by centrifugation, and lysed. DNA is purified from the resulting lysate by anion-exchange chromatography and used as a template in a Polymearase Chain Reaction (PCR) using species-specific primers. The subsequent PCR products are purified and the nucleotide sequence determined.

Discussion

There are a number of critical issues when considering automating a laboratory process.

(i) Does the sample number justify automation, *i.e.* low throughput (100-1000) or high throughput (1000-100,000) ?

Table 1: Example of comparison of total time taken for manual versus automated laboratory processes.

Sample number	Manual process (total time taken in hours)	Automated process (total time taken in hours)
500	5	7
1000	10	10
2000	20	12.5

As illustrated in Table 1., there is a considerable time investment in the initial set-up of any particular assay on a robotic system. It is only when large sample numbers are encountered that the advantage of automating an assay becomes evident.

(ii) Can the assay be successfully scaled down and performed in microtitre plates ? This is a very important consideration as robotic systems, generally, can only handle samples in microtitre plates. Scale down of the assay increases throughput and reduces assay costs. However, there are some practical limitations to ever increasing assay scale down, and therefore 96- and 384-well microtitre plates are most frequently used in High-Through-Put (HTP) screening.

Conclusios

The High-Through-Put (HTP) laboratory at Chr. Hansen constitutes a unique system whereby new silage cultures can be isolated, identified and characterized. This can be performed with a high sample throughput, low cost per sample and high degree of reproducibility by exploiting the flexibility and power of robotics.

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Dry Matter Losses in Green Chopped and Ensiled Sugarcane (*Saccharum officinarum* L.) Treated With Chemical Additives.

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Abstract

The utilization of sugarcane in animal feeding presents some limitations. The use of additives can minimize dry matter losses whether sugar cane is daily harvested and chopped or ensiled. The objective of this trial was to evaluate dry matter losses of green chopped or ensiled sugarcane treated with calcium oxide, calcium carbonate and calcium sulfate in experimental silos, in a complete randomized experimental design. Calcium oxide was effective to decrease dry matter losses during the early ensilage period. Dry matter losses and gas production were significantly reduced by calcium oxide and calcium carbonate in ensiled sugar cane.

Introduction

Brazil is the largest sugarcane producer of the world. Last year its production was 400 million metric tons (IBGE, 2005). Approximately ten percent of the harvested sugarcane is used in animal feeding (Landell et al., 2002). Sugarcane is a feedstuff with high relative feed value due to forage yield, low dry matter cost and constant nutritive value after maturity. Fresh chopped sugarcane is frequently used in animal feeding and possesses some limitations such as daily harvesting and low fiber digestibility. In addition, its high soluble sugars content, results in sugarcane silage with high ethanol levels, which in turn increases dry matter losses and lowers its nutritive value. Silage fermentation may be changed by using chemical and biological additives. The objective of this trial was to analyze the effects of adding calcium oxide (CaO), calcium carbonate (limestone - $CaCO_3$) and calcium sulfate [gypsium - $Ca(SO_4)_2$] on dry matter losses of green chopped and ensiled sugarcane.

Material and Methods

Two trials were conducted at the Department of Animal Science, University of Sao Paulo, Piracicaba-SP, Brazil. In Trial 1 sugarcane was green chopped in a stationary chopper and mixed with finely ground CaO, at four levels (0, 5, 10 e 15 kg/t of fresh forage). The chopped forage was placed unpacked in 20 liters plastic buckets (experimental units), and stored for ten days. Buckets were weighed and sampled daily. Variables analyzed were: dry matter losses accumulated during two periods of storage (days 1 to 5 and 5 to 10), and during the entire period. Each treatment had four replicates, in a competed randomized design. In Trial 2 green chopped sugarcane was ensiled in 20 L plastic buckets mixed with CaO or $CaCO_3$ finely ground (10 and 15 kg/t of fresh forage) or $Ca(SO_4)_2$ (10 kg/t of fresh forage), both additives diluted in water solution at a rate of 40 liters/t of fresh forage and *L. buchneri* ($5x10^4$ CFU/g fresh forage). Each treatment had four replicates in a completed randomized design. Packing density in the buckets was 500 kg fresh forage/m^3. Buckets were closed and equipped with Bunsen valves. At the bottom of the buckets there was a sand layer covered with cheese cloth and a fine plastic sieve to allow effluent collection. Dry matter content, gas

losses, effluent yield, dry matter losses and recovery rate were measured after a storage period of 90 days. Losses were estimated by bucket weight difference between day 0 and day 90 of storage. Statistical analyses for both trials were performed according to PROC GLM by SAS, and means compared by the Tukey test.

Results and Discussion

Table 1 shows total dry matter losses accumulated, dry matter loss from day one through day five and dry matter loss from day five through day ten. CaO significantly reduced total dry matter losses during the entire storage period when compared to control. There were no differences among CaO levels. Dry matter losses from day 0 through day 5 showed the same trend, although increasing CaO levels showed a trend to decrease losses. The lack of response from day 5 through day 10 probably is due to the intense microbial activity favored by the high availability of soluble sugar at the beginning of the experimental period. CaO probably inhibits microbial activity and thus diminishes dry matter loss.

Table 1: Dry matter losses in fresh sugarcane treated with calcium oxide.

Variables	CaO level (kg/t FF)				C.V. (%)
	0	5	10	15	
Total loss, kg/t DM	260.25^{a}	210.01^{b}	190.87^{b}	190.30^{b}	8.01
Loss 0-5 days, kg/t DM	170.44^{a}	120.50^{b}	110.76^{bc}	100.34^{c}	9.46
Loss 5-10 days, kg/t DM	80.81	80.52	80.10	80.96	11.29

Means with different superscripts in the same row differ (P<0.01).

CaO at both concentrations (10 e 15 kg/t fresh forage) reduced gas production and total dry matter loss and increased dry matter recovered during the ensiling period as compared to control. This is in accordance with Neto et al. (2005), who showed 190 and 800 kg/t DM of gas loss and DM recovery, respectively, in sugarcane silage treated with CaO (20 kg/t fresh forage). $CaCO_3$ also reduced losses, although at a lower efficacy. $Ca(SO_4)_2$ was not effective in reducing losses.

Ensiling sugarcane results in significant dry matter loss, but on the other hand allows farm operations of harvesting, hauling and storage to be done in a short period of time, as compared to daily green chopping.

Table 2: Dry matter losses and effluent production in sugarcane silage treated with chemical and microbial additives

Treatments	Total losses (kg/t DM)	Gases losses (kg/t DM)	Effluent production (Kg/t FF)	DM recovery (kg/t DM)
Control	340.31^{c}	320.11^{b}	310.26^{ab}	650.69^{c}
L. buchneri, 5x10^{4} CFU/g FF	350.78^{c}	320.83^{b}	410.89^{b}	640.22^{c}
CaO, 10 kg/t FF	160.90^{ab}	150.12^{a}	200.55^{a}	830.11^{ab}
CaO, 15 kg/t FF	150.90^{a}	140.16^{a}	190.68^{a}	840.11^{a}
$CaCO_3$, 10 kg/t FF	200.00^{ab}	170.19^{a}	320.82^{b}	800.01^{ab}
$CaCO_3$, 15 kg/t FF	210.00^{b}	180.47^{a}	300.07^{ab}	790.00^{b}
$Ca(SO_4)_2$, 10 kg/t FF	340.10^{c}	310.82^{b}	310.78^{b}	650.90^{c}
C.V. (%)	7.98	9.18	16.13	2.67

Means with different superscripts in the same row differ (P<0.01).

Conclusions

CaO reduces dry matter loss when added in green chopped sugarcane, apparently at the optimum dose of 5 kg/ton of fresh forage. CaO and $CaCO_3$ did reduce total losses in sugarcane silage, whereas $Ca(SO_4)_2$ and *L. buchneri* were ineffective.

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The effect of absorbents and Preservatives on the fermentation characteristics of brewers‘ grains silage

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Introduction

Brewers' grains is a feedstuff with a high nutritive value and a different rumen degradation of protein (COSTA et al., 1995; COSTA et al., 1994). Brewers' grains contain approximately 1/7 of the nitrogen-free extract and nearly 75 % of crude protein of malting barley. The biological value of protein is dependent on the content of amino acids of malting barley and is further enhanced by yeasts activities. According to COSTA et al. (1994) 1 kg brewers' grains dry matter contains 16.19 % of crude protein, 38.63 % of nitrogen-free extract, 48.60 % NDF and 18.83 % ADF. Of saccharides, glucose and maltose are the most abundant sugars and the content of energy ranges from 6.1 to 6.7 MJ NEL per kg DM (LOHNERT et al., 1996; SPANN, 1993). The digestibility of fresh brewers' grains organic matter is relatively high and ranges in average from 63 – 65 % (LOHNERT et al., 1996). A characteristic but undesirable feature of brewers' grains is its relatively low stability and tendency to a quick deterioration. Its low content of dry matter causes a release and runoff of silage liquids immediately after ensiling on the one hand and makes its ensiling very difficult on the other. for that reason brewers' grains are pressed to increase its content of dry matter to 35 – 40 % (BUCHGRABER and RESCH, 1997); another possibility is to ensile it with admixtures of asome moisture-absorbing materials (RIDLA and UCHIDA, 1997; PEREIRA et al., 1998; TANAKA, AKIYAMA, YAMADA et al., 2001; De BRABANDER et al., 1999) and others.

The aim of this study was to evaluate the effect of addition of two absorbents and ensiling OPs on the quality of fermentation process when ensiling brewers' grains under exactly defined model conditions.

Material and Methods

In a model experiment, fresh brewers' grains were used with the dry matter content of 187,4 g/kg. The ensiled material was transported into the laboratory of the Department of Animal Nutrition and Forage Production, Faculty of Agrnomy (MUAF Brno) where it was homogenised with admixtures of tested preservatives (malt sprouts and barley groats) in such a way thjat the final dry matter content was higher than 30 %. Two controls (A with malt sprouts and B with barley groats) and two experimental variants with an preservatives (C_A and C_B) were used. The preservative (a biochemical preparation containing selected strains of lactic acid bacteria and natrium benzoate) was applied in the dose of 4 L/t. In variants A and B, the average dry matter contents of ensilge brewers' grains were 32.99 % and 33.43 %, respectively. This material was ensiled in three replications into special exoperimental containers and stored at 20–25 °C. After eight months the containers were opened and six representative samples were taken off to evaluate the quality of fermentation process. All experimental samples weere compared with untreated control without an addition of absorbents (D). Dry matter content was estimated by drying to a constant weight at the temperature of 103±2 °C. Analytal procedures (including the preparation of water extract) were described earlier (DOLEŽAL, 2002). Samples weree analysed for the contents of volatile fatty acids, lactic acid, ammonia, pH, and titration acidity. Alcohol content was determined

according to the method described by HARTMAN (1974). Results were statistically analysed using the method of unfactorial variance analysis (SNEDECOR and COCHRAN, 1969).

Results and Discussion

One kilogram of fresh brewers' grains contained 246.3 g crude protein, 79.4 g fat and 209.3 g crude fibre. Contents of ME (10.43 MJ/kg DM) and NEL (6.11 MJ/kg DM) corresponded with available literary data. The average fermentations characteristics are presented in Tab. 1. Results of this model experiment indicate that there are statistically significant differences between individual variants of ensiled brewers' grains. As compared with untreated brewers' grains, a supplement of both absorbents into the ensiled material resulted in a statistically significant ($P<0.01$) increase in the DM content. As compared with control samples, a higher DM content in silages containing absorbents was able to reduce both production and escape of silage liquids. An increase of DM content above 30 % showed a positive effect on fermentation of ensiled material. The addition of malt sprouts resulted in a statistically significant ($P<0.01$) increase in production of lactic acid (2.20±0.211 %) as compared with control (D) silage made of wet brewers' grains (0.30±0.177 %) and/or silage with the supplement of barley groats (0.61±0.172 %), resp. These results correspond with data published by BUCHGRABER and RESCH (1997) that found out only low amounts of lactic but high levels of acetic and butyric acids, resp. WYSS (1997) as well, mentioned very low or even zero production of lactic acid in silage made of brewers' grains; besides, its level gradually decreased during its storage. An preservative supplement resulted in a better preservation effect above all in silage containing barley groats (as compared with that containing malt sprouts). Model silages wiht malt sprouts showed better parameters of fermentation process than those with barley groats and the preservative. These silages showed not only a higher content of lacticv acid but also a better LA/VFA ratio and a lower content of AA. In our model experiment, a statistically highly significant ($P<0.01$) increase in the content of butyric and propionic acids (0.75±0.42 % and 0.14±0.04 %, resp.) was observed in untreated control variant. Experimental silages did not contain any amounts of these undesirable VFA, the presence of which indicates the degradation of protein. These observations corroborate data published by BUCHGRABER and RESCH (1997), and WYSS (1997) about silages made of pressed brewers' grains. In the experimental silage containing a supplement of malt sprouts, the level of acetic acid was significantly the lowest of all treatements(0.56±0.02 % and 0.68±0.03 %, resp.); this difference was statistically significant ($P<0.01$) as compared with other treatments. These silages also showed the lowest ($P<0.01$) values values of pH (4.29±0,01 and 4.30±0,04), resp. Experimental silale containing malt sprouts showed the significantly highest production of all ferementation acids ($P>0.01$)???. There were also differences in the content of ethanol in silages containing absorbents and in untreated control. It was found out that malt sprouts reduced production of ethanol morde intensively than barley groats, which showed to be unsuitable due to its content of starch.

Table 1: Fermentation characteristics of silages

Groups Average ±SE	A	B	CA	CB	D
DM (%)	32.76±0.412 B,CB,D	35,66±0.575 A,CA,D	32.17±0.252 B,CB,D	34.80±0.565 A,CA,D	25.87±0.853 A,B,CA,CB
pH	4.29±0.017 B,cb,D	4.56±0.053 A,CA,cb ,d	4.30±0.039 B,cb,d	4.47±0.015 a,b,ca	4.43±0.122 A,b,ca
TA (mg KOH/100 g)	1436.8±29.9 B,cb	1083.7±119.5 A,CA,CB,D	1431.3±67.0 B,cb	1547.7±94.1 a,B,ca	1457.5±125.2 B,cb
LA (%)	2.20±0.211 B.CB,D	0.61±0.172 A,CA,D	2.27±0.047 B,CB,D	0.70±0.103 A,CA,D	0.30±0.177 A,D,CA,D
AA (%)	0.56±0.015 B,ca,CB,D	0.80±0.116 A,ca,cb	0.68±0.027 a,b,CB,D	0.94±0.434 A,b,CA,D	0.81±0.219 A,CA,CB
PA (%)	0	0	0	0	0.14±0.037 A,B,CA,CB
BA (%)	0	0	0	0	0.75±0.418 A,B,CA,CB
Sum of acids in DM	8.43±0.727 B,CB,d	3.94±0.312 A,CA,CB,D	8.15±2.466 B,CB,d	5.18±0.362 A,B,CA,D	7.73±0.657 B,ca,CB
LA/VFA	3.91±0.309 B,CB,D	0.80±0.303 A,CA,D	3.36±0.200 B,CB,D	0.64±0.093 A,CA,D	0.19±0.118 A,B,CA,D
Ethanol (%)	0.02±0.008 B,D	0.08±0.031 A,CA,CB,D	0.03±0.005 B,d	0.02±0.008 B,D	0.04±0.019 A,B,CB

A- with malt sprouts, B - with barley groats, CA - malt sprouts + preservative,

CB - barley groats + preservative, D - untreated control silage of brewers´silage

Conclusions

The aim of this study was to evaluate the effect of a supplement of malt sprouts and barley groats used as absorbents and of a silage preservative on the quality of fermentation process in model silages made of wet, non-pressed brewers' grains as compared with an untreated control. The best fermentation profiles were found in silages with supplements of malt sprouts and preservative because they showed not only a better LA/VFA ratio but also significantly lower ($P<0.01$) pH and ethanol content on the one hand and significantly higher ($P<0.01$) levels of lactic acid, total fermentation aicds and significantly reduced production of acetic acid than control. This supplement of absorbents resulted in an increase in the content of DM in stored brewers' grains, reduced production and escape of silage liquids and inhibited formation of butyric and propionic acids. Statistically significant differences between iindividual groups were observed also in pH and content of VFA and lactic acid.

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The Influence Of Some Biological Additives on Fermentaion And Quality Of Whole-Crop Barley Silage

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Introduction

Whole crop cereal silage also has a place on other farm types. It can be used on beef farms to increase weight gain in pre-mating and calving cows, and to grow finishing stock.

Whole-crop cereals represent the group of forage where the relation between the stage of maturity and concentration of water-soluble carbohydrates seems to have the most pronounced impact on their ensilability. However, WSC development in forage plants might contain the ensilability of whole crop cereals since the dough stage of growth is considered as the most desirable stage to harvest cereals for silage. The optimal harvesting time of whole crop cereals considering the yield, feeding value and ensilabity is between early and late dough stage (Knický, 2005).

Westwood (2003) also suggested the most critical factor is to get the time of harvest right. Harvesting at 38 % dry matter and when grain is at the "cheesy-dough" stage will maximize grain content and digestibility, which is where all the energy and starch comes from.

The use of biological silage additives in process of preserving whole crop cereals is very important. Fermentation improvement forms conditions of minimalization the risk of undesirable components in silage.

The aim of our experiment was to study the effect of biological and biological-enzymatic preservatives on the fermentation and nutrient composition of whole-crop barley silages.

Material and Methods

Whole-crop spring barley was cut in the mid-dough stage of maturity. We ensilaged the fresh matter of forage wilted free. Table 1. shows the nutrient composition of barley.

Table 1: Nutrient composition of whole crop barley before conservation

Dry matter in $g.kg^{-1}$ FM	OM	Crude protein	Crude fibre	ADF	NDF	WSC total	Starch	Fat	Ash
	in $g.kg^{-1}$ DM								
358.11	949.43	100.18	257.12	268.02	544.75	87.39	256.43	20.48	50.57

The chop crops were homogenized and to objective results filled into 1.7 l laboratory silos. Four different groups were examined - a non treated control and three experimental variants treated as follows:

1. treated with a biological additive (Medipharm) consisting of *Enterococcus faecium* M-74, *Lactobacillus plantarum, Lactobacillus casei* and *Pediococcus spp.* 1 l of solution was applied per 1 ton of feed.

2. treated with a biological additive (Pioneer) consisting of *Enterococcus faecium* DSM 4788, 4789 and *Lactobacillus plantarum* DSM 4784, 4785, 4786 and 4787. 0.5 kg was applied per 1 ton of feed.
3. treated with a biological-enzymatic additive (Alltech) consisting of *Streptococcus faecium*, *Pediococcus acidilactici, Lactobacillus plantarum* and enzymatic component with amylase, cellulase, hemicellulase and pentosanes. 2 l of solution were applied per 1 ton feed.

The laboratory silos were placed in a dark room at 20-22 ^{o}C. After 200 days of fermentation the silages were examined for nutrition composition, fermentation parameters and losses of dry matter.

Results and Discution

The nutrient and fermentation quality of whole-crop barley silage are shown in Table 2.

Table 2: Nutrient composition and fermentation parameters in whole-crop barley silane

Parameter		Control silage		Silages with additives						Statistical significance of differences	
				1		2		3			
		$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	P < 0,05	P < 0,01
Dry matter	in g.kg^{-1} FM	311.0	3.29	341.6	3.31	342.2	3.48	343.4	2.68		C :1,2,3
Organic matter	in g.kg^{-1} DM	942.1	1.95	946.4	2.04	946.7	0.35	947.3	0.76		C :1,2,3
Crude protein	in g.kg^{-1} DM	101.6	2.47	103.3	2.09	101.1	2.95	105.6	3.03		
Crude fibre	in g.kg^{-1} DM	282.2	8.91	253.1	10.86	244.9	13.69	246.9	13.20		C :1,2,3
ADF	in g.kg^{-1} DM	297.5	17.72	266.0	11.08	263.0	12.35	262.4	12.72		C :1,2,3
NDF	in g.kg^{-1} DM	541.6	21.15	498.3	32.93	493.7	20.33	476.9	23.63	C :1	C :2,3
Sugar total	in g.kg^{-1} DM	43.9	6.37	45.6	8.68	38.7	5.73	41.1	1.87		
Starch	in g.kg^{-1} DM	146.6	9.34	190.7	30.61	216.0	16.23	179.8	8.24		C :1,2,3 2 :3
Fat	in g.kg^{-1} DM	32.2	2.14	29.3	1.54	29.6	0.91	27.6	0.73	C :1	C :3
Ash	in g.kg^{-1} DM	57.9	1.95	53.6	2.04	53.3	0.35	52.7	0.76		C :1,2,3
Dry matter losses in %		15.23	1.07	5.09	0.86	4.95	0.94	4.49	0.71		C :1,2,3
pH		4.47	0.02	3.78	0.07	3.72	0.06	3.66	0.01		C :1,2,3 1 :2
Acids in g.kg^{-1} DM											
- lactic		41.57	5.01	66.09	3.02	67.19	6.33	56.60	3.89	2 : 3	C :1,2,3 1:3
- acetic		5.82	2.43	4.30	1.03	4.86	0.60	4.95	0.58		C :1,2,3
- propionic		2.23	0.35	0.29	0.15	0.43	0.08	0.22	0.05		C :1,2,3 3 :2
- butyric + isob.		5.02	0.24	0.76	0.37	0.52	0.08	0.70	0.19		C :1,2,3
- valleric + isov.		0.83	0.38	0.04	0.03	0.03	0.00	0.04	0.03		C :1,2,3
- capronic + isoc.		0.74	0.17	0.03	0.00	0.04	0.01	0.03	0.00		C :1,2,3
Total volatile fatty acids		14.64	2.68	5.42	1.54	5.88	0.64	5.93	0.77		C :1,2,3
Total acids		56.21	6.53	71.51	3.55	73.07	6.95	62.53	3.53		C :1,2
Alcohol in g.kg^{-1} DM		5.71	0.91	2.01	0.23	1.93	0.43	2.58	0.16		C :1,2,3
NH_3-N of total N in %		11.81	0.69	7.71	0.88	6.88	0.48	6.86	0.60		C :1,2,3

All silages treated with additives improved fermentation in comparison to silage not treated. In treated silages werehigh significantly lower pH values, butyric acid, and all volatile fatty acids, alcohol, ammonia N of total N, and high significantly higher content of lactic acid than in the control silage. Differences of fermentation parameters were minimal between treated silages.

Improved fermentation process in the treated silages became obvious in decreased weight and nutrient losses. Crude fibre content, NDF, ADF, starch, and ash levels

revealed highly significant differences between the untreated and treated silages. Among the three treated silages were non-significant differences. High significantly lower NDF and starch values were only between the silage treated with biological-enzymatic additive and silages treated with biological additives.

The application of enzymes caused a decrease in the contents of crude fibre and its fractions. However, the differences of crude fibre and ADF were minimal.

Hristov and McAllister (2002), Kung et al. (2004), Nadenau (2005) also confirmed that microbial inoculants improved the fermentation process and DM recovery in whole-crop cereal silages.

Conclusion

The results suggest that application of biological and biological-enzymatic additives had a positive effect upon the quality of the fermentation process and the nutrient levels in whole crop barley silages. The positive effect of the biological-enzymatic additive on degradation of crude fibre and its fractions in comparison to biological additives was minimal.

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Application Of Probiotic Products In Ensilaging Of Pea With Share Of Cereals

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Introduction

Meanwhile in other countries technology of harvesting and conservation of cereals, possibly legumes, were developed by one-crop system (Zimmer 1987), in the agriculture of the Czech Republic these crops were mainly used as cover crops in laying out perrenial fodder crops. Oat, later barley and broad bean were commonly used as cover crops in past. Mixture of leafy varieties of pea with spring wheat started to be used 20 years ago. Technology of growing and method of conservation are described by Přikryl and coll. 1987 and comparison of digestibility of leaf peas silages with shares of cereals and other cover cereals and legumes are described by Přikryl and coll. 1989.

Application of earlier and less gyrate peas has been extended just in past few years. The probiotic products by MEDIPHARM CZ intended for conservation of the mentioned crops have been used with very good results for more than 35 years in the Czech Republic.

Material and Methods

In two agriculture corporations the areas of perrenial forage (clovergrass and lucern clovergrass) were laid out in cover crops (a mixture of semi-leaf pea and barley).

The recommended sowing mixture was 200kg peas and 70kg barley. The crop was done in lactic ripeness of peas and lactic ripeness of barley. In the first corporation the product Microsil (a mixture of lactic bacteria Lactobacillus plantarum, Lactobacillus casei, Enterococcus faecium and Pediococcus pentosaceus) was used for conservation. And Lactisil 200NB (mixture of lactic bacteria Lactococcus lactis, Pediococcus acidilactici, Enterococcus faecium and Lactobacillus plantarum in a mixture with celulolytic enzymes and natrium benzoate) was used for surface treatment of silages against moulds. The mass was ensilaged into two silo bunkers during one week period. In the second corporation the mass was ensilaged in plastic bags. In the first plastic bag the mass was ensilaged with Microsil and in the second plastic bag the mass was ensilaged with a product based on formic acid. When feeding to diary cows their intake of the silage and efficiency including composition of lactic protein were monitored.

Results and Discussion

Table 1: Real seeding rate and hectare yield

Seeding rate	corporation	
	I.	II.
pea	170 kg	200
barley	70 kg	70
perrenial forage	clover grass 17kg, grass 3kg (Bečva)	lucern 10kg, clover grass 1,5kg, grass 3kg (Felina 1,5kg, Bečva 1,5kg)
efficiency	19,9 tons/ha	21,5tons/ha

In the first corporation sowing amount of pea was not followed and efficiency was also lower comparing to the second corporation. The laid perrenial forage was very good in the first year after sowing.

In the first corporation the silage that was concluded a week earlier had better parameters of roughage proteins and fibre. Similarly, in the second corporation the silage that was concluded as first (treated with formic acid) showed better nutritional parameters. The final fermentation process was very good in all variants, higher content of acetic acid was identified only in the variant with the chemical product. The peas silages showed very good durability (low content of ethanol). Feed intake of diary cows was very good (a higher value of pH) in very good fermentation process. Higher content of fibre was found out, for instance the content was three times higher comparing to the level of fibre in corn silage. In the first breeding with 386 diary cows of red fleckfeed breed and with efficiency of 6405 litres the production of lactic acid increased by 0,2% after including 7kg pea silage. Consequently, the efficiency increased. In the second breeding with 500 diary cows of Holstain breed and efficiency of 9920 litres the tendency to higher level of lactic acid after including 6 kg the silage has been claimed.

Table 2: Final silage quality - selected indicators

Nutrients	corporation			
	I.		II.	
	Microsil Silage concluded on 13.7.	Microsil Silage concluded on 19.7.	Formic acid	Microsil
Dry matter	37,65	31,15	31,94	30,68
Roughage protein (g/kg)	15,90	14,62	15,81	13,25
Fibre (g/kg)	24,89	25,90	25,31	24,54
Soluble fibre (g/kg)	95,7	107,7	86,1	96,8
Easily soluble saccharides (g/kg)	12,0	7,6	10,1	8,3
Fermentation				
pH	4,48	4,52	4,64	4,62
Lactic acid (g/kg)	33,2	28,7	22,4	19,2
Acetic acid (g/kg)	6,8	9,8	11,9	4,1
Butyric acid (g/kg)	0	0	0	0
KVV	1330	1440	1380	1310
Ethanol	1,1	0,9	0,4	0,5

Conclusion

Application of mixtures of peas with barley as cover crop has great perspective. It is a very good cover crop that does not limit growth of perrenial forages.

Higher content of easily fermented saccharides in ensilaged mixture of peas and barleys enables to use microbial preparations, in our case Microsil. Such preserved silage shows very good durability. Any negative effects on diary cows were found out after feeding them with the silage, the intake of silage was without problems. Nutritional value of the silages was very good (sufficient share of roughage proteins with high share of soluble fibre). After incorporation of the silages with the mentioned composition in feeding dosage of diary cows the level of protein share increased in dry matter of milk.

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Effect Of Application Biological Additives on Fermentation Quality Of Red Clover Silage

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Introduction

Interest in leguminous forages is increasing as farms reduce their reliance on purchased concentrate feeds and search for improved production form homegrown forages. Red clover is a high yielding, high protein legume than can be grown successfully in submountainous and mountainous regions of Slovakia.

Red clover is high protein forage. Moore and Patterson (2005) reported that red clover silage has a high crude protein content of 16 % to 20 % and a ME content of 10 to 12 MJ/kg DM, depending on the growth stage at cutting.

The quality of clover silage is affected by poor climatic conditions in crop time. Pahlow et al. (2000) states that increase of dry matter ensilaged legumes above 25 % decreased the risk of butyric acid production in non-treated silages.

The aim of this experiment was to study the effect of application a biological and a biological-enzymatic additive on fermentation quality and digestibility of red clover silages with different wilting levels of the forage.

Material and Methods

The tetraploid red clover from the second cut was ensilaged under laboratory conditions. Different wilted cut red clover had 279 and 423 g dry matter. Next value of clover nutrient composition before conservation shows the table 1.

Table 1: Red clover - fresh matter

Level of DM content	Dry matter	Organic matter	Crude protein	Crude fibre	ADF	NDF	Hemi-cellulose	Nitrogen-free extract	Sugar total	Fat	Ash
	g	g . kg^{-1} dry matter									
Low	279.21	919.31	195.03	271.92	359.99	431.95	71.96	428.55	58.05	23.82	80.69
High	423.12	914.11	201.95	286.73	377.41	429.29	51.88	403.50	94.09	21.94	85.89

Following wilting the matter was chopped, homogenized and filled into 1.7 l laboratory silos. The fermentation was observed in untreated control (U), and in the two experimental variants treated with:

T_1 - a biological additive Kofasil Life (*Lactobacillus plantarum* 3676, 3677, and *Propionic bacterium* DSM 9576, 9577). The applied amount was 2.0 ml solution of additive per kilogram of feed.

T_2 - a biological additive Kofasil Life with enzymatic complex (cellulase, hemicellulase, and glucosooxidase). The applied amount was 2.0 ml of additive + 0.1 ml enzymes per kilogram of feed.

The silos were placed in a dark room at 22 ^{o}C. Silage losses of dry matter were determined at regular 21-days intervals. After 180 days of fermentation were the samples of silages determined on fermentation parameters, nutrient composition, and digestibility of DM and OM.

Results and Discussion

The chemical analyses of fermentation process, and of nutritive value are presented in table 2. The increase of DM content in red clover became reflected in increased pH and sugar level in the silage, decreased lactic, acetic, and butyric acid contents, and increased NH_3 - N of total N.

In comparison to untreated silages the silages treated by additives were in silages with lower level of dry matter stated to have lower pH, acetic acid, butyric acid, NH_3 - N of total N levels, and losses of dry matter, too. The differences between the pH, NH_3 - N of total N, lactic and acetic acid were highly significant, respectively. In silages treated by biological additive with enzymes were higher losses of dry matter if in silages treated only by biological additive.

The effect of application both silage additives was higher in silages with 26 % content of dry matter compared to silages with DM content on 40 % level.

The application of silage additives influenced the nutrient composition in silages minimal. Highly significant differences were found in content of crude fibre, and sugar. The enzymes effect increased level of crude fibre about 1.5 % compared to untreated silage.

The results obtained in our study coincide with those previously reported by Gallo et al. (2002, 2003), Rajčáková et al. (2005) but also by Fychan et al. (2002), Speijers et al. (2001), and Winters et al. (2002).

Table 2. Nutrient composition and parameters of the fermentation in red clover silage

Parameter n = 6	Level of DM content											
	Low						High					
	U		T_1		T_2		U		T_1		T_2	
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s
DM in g	262.99	2.47	265.34	2.24	261.54	0.82	400.30	2.40	407.44	2.82	402.48	2.42
CP in $g.kg^{-1}$ DM	192.14	0.94	188.20	2.40	187.60	1.50	191.90	1.85	191.94	0.89	194.12	2.65
CF in $g.kg^{-1}$ DM	288.92	4.86	281.71	4.59	**271.52	1.52	310.22	5.87	303.03	3.53	**295.92	7.45
ADF in $g.kg^{-1}$ DM	357.65	3.14	353.40	5.98	**343.96	2.08	384.01	4.96	382.31	8.02	377.04	5.91
NDF in $g.kg^{-1}$ DM	400.17	4.22	400.70	6.03	394.30	9.16	423.31	3.44	425.90	6.04	**416.31	1.55
Sugar total in $g.kg^{-1}$ DM	14.41	3.74	21.05	6.69	**29.07	3.63	27.77	2.48	**18.40	2.37	23.98	3.17
Fat in $g.kg^{-1}$ DM	29.46	0.91	30.07	2.41	27.74	0.89	26.99	1.88	26.02	1.15	27.06	0.62
Ash in $g.kg^{-1}$ DM	87.35	0.97	85.18	0.41	84.70	0.47	90.68	0.87	90.20	0.91	90.22	0.19
pH	4.45	0.02	**4.14	0.15	**4.13	0.08	4.94	0.11	**4.39	0.05	**4.49	0.03
DM losses in %	6.46	0.89	5.25	0.80	6.45	0.41	6.12	0.55	**4.14	0.64	5.32	0.58
Acid lactic in $g.kg^{-1}$ DM	58.12	6.32	**86.75	12.14	63.06	2.16	27.69	2.80	**48.51	10.32	**45.88	2.99
Acid acetic in $g.kg^{-1}$ DM	13.18	1.10	**7.61	1.69	**6.25	0.11	8.32	1.08	8.65	1.04	8.53	0.87
Acid butyric in $g.kg^{-1}$ DM	0.41	0.14	0.30	0.02	0.29	0.03	0.35	0.26	0.32	0.13	0.28	0.03
NH_3-N of total N in %	9.24	0.22	**6.73	0.94	**6.48	0.29	13.24	2.84	**8.77	0.20	**6.18	0.24
DM digestibility in %	60.34	0.60	60.52	0.76	60.41	1.76	57.15	0.41	57.09	0.36	55.82	1.04
OM digestibility in %	56.84	0.66	57.11	0.75	57.30	1.82	53.47	0.37	53.21	0.44	51.87	1.13

U – untreated, T_1 - Kofasil life, T_2 – Kofasil life with enzymes, CP – crude protein. CF – crude fibre

* $P < 0.05$ ** $P < 0.01$ The statistics is performed for the individual trials only.

Conclusion

The treatment of red clover with a biological additive with other without enzymes improvement of fermentation process and quality of silages in this experiment. The application of biological additive with enzymes was not signify compared to application of the biological additive.

The level of dry matter ensilaged red clover affects efficiency of biological silage additives. Treatment effect in silages with DM content on level 26 % was higher compared to silages with 40 % dry matter.

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Control of clostridia fermentation in silage using a silage additive based on a mixture of lactic acid bacteria

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KEYWORDS: butyric acid, clostridia spores, grass silage, lactic acid bacteria (LAB), silage additive

Introduction

Clostridia are undesired micro organisms in silage. Anaerobe deterioration is caused by unrestricted growth. For feeding purpose it is very important that counts of clostridia spores are kept at a low level to avoid health problems and cuts in animal performance.

In the presented study a mixture of lactic acid bacteria was tested in slightly wilted silages because of its higher risk for clostridia contamination. The results showed that the additive improved silage quality. The main effects were the increased production of lactic acid (highly significant) connected with a highly significant decrease of pH value. Additionally the activity of clostridia was reduced as indicated by lower amount of butyric acid formed and lower counts of clostridia spores.

Materials and Methods

Ensiling Material and Treatment

Three first cut grass materials (permanent grassland) from different farms with dry matter content in the range of 25 – 36% were used. Each of the three chopped grass materials were ensiled once as untreated control and once treated with a new biological silage additive (three homofermentative lactic acid bacteria: *Lactobacillus paracasei*, *Pediococcus acidilactici* and *Lactococcus lactis*). The lactic acid bacteria were sprayed onto the grass as a liquid bacteria solution with a concentration of $2.5x10^5$ cfu/g grass. Laboratory silos (6.5l) were analysed after 90 days of storage at 20°C.

Table 1: Parameters of the fermentation process after an ensilage period of 90 days

Laboratory silage day 90	Silage 1		Silage 2		Silage 3		Mean values		S. E.		significance
Treatment	Cont.	LAB	Cont.	LAB	Cont.	LAB	Cont.	LAB	Cont.	LAB	
DM (g/100 g FM)	25.5	26.1	24.1	25.6	37.6	35.9	29.1	29.2	7.4	5.8	-
pH – value	4.9	4.1	5.3	4.0	5.6	4.0	5.3	4.0	0.4	0.0	**
Weight losses (g/100 g FM)	2.0	1.4	3.2	1.5	3.4	1.3	2.9	1.4	0.7	0.1	*
Lactic Acid (g/100 g DM)	3.8	8.7	0.2	9.3	1.9	7.7	2.0	8.6	1.8	0.8	**
Butyric Acid (g/100 g DM)	2.4	0.1	4.0	0.4	1.2	0.0	2.5	0.2	1.4	0.2	*
Ammonia-N (g/100 g TKN)	10.2	6.5	13.7	6.4	6.1	5.4	10.0	6.1	3.8	0.6	-
Cl. spores (log (cfu/g FM))	6.3	4.2	7.2	4.1	4.7	3.4	6.1	3.9	1.3	0.4	*

Cont. = control; LAB = treated with lactic acid bacteria; TKN = total kjeldahl nitrogen; FM = fresh matter; DM = dry matter; * $P<0.05$, ** $P<0.01$

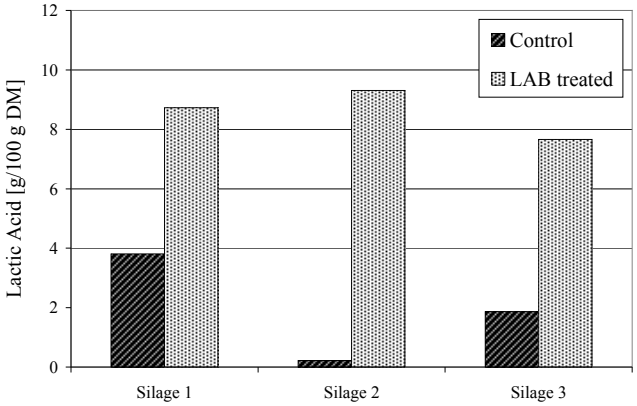
Measured Parameters

From an aqueous silage extract (50 g silage extracted with 250 g distilled water) the pH-value and the fermentation patterns (HPLC, Agilent 1100, Column: Transgenomic ICSep ICE-ION 300) were analysed. Ammonia-N was determined by distillation (Gerhardt). A MPN-method in micro titre plate scale with RCM-bouillon containing D-cycloserine and neutral red was used for the determination of the amount of clostridia spores in silage (Jonsson, 1990; Kaufmann & Weaver, 1959).

Results and Discussion

Ensiling materials of low (25%) and medium (36%) dry matter contents were used. After 90 days of fermentation in untreated controls the pH was decreased to 5.3 (Table 1). The silages treated with lactic acid bacteria reached a pH of 4.0, which is significantly lower. Furthermore, significantly reduced weight losses were measured in treated trials. High values of lactic acid were produced in the inoculated silages (7.7 – 9.3 g/100 g DM). In control samples only low levels of lactic acid (Figure 1, Table 1) but high amounts of butyric acid and ammonia-N were (Table1) detected. These values, strongly deviating from the LAB treated silage, may be caused by clostridia fermentation. The high counts of clostridia spores in untreated controls are further evidence.

Figure 1: Lactic acid content in untreated control and silages treated with lactic acid bacteria; Amounts of lactic acid in inoculated silages are significantly increased ($p<0.01$).



Conclusion and References

There is a high risk for grass material with low dry matter content to fail the conservation process as deterioration by clostridia fermentation may occur. The silage additive used in these trials improved silage quality remarkably, which was proven by several parameters. Most important for successful conservation is the production of high amounts of lactic acid with the consequence of a rapid pH decrease. Subsequent constant low levels of pH are responsible for reduced activity of clostridia. The inhibition of clostridia in inoculated silages is indicated by significant lower counts of clostridia spores ($p<0.05$), formation of significant lower amounts of butyric acid ($p< 0.05$) and a tendency towards lower contents of ammonia nitrogen.

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Microbial changes of draff during storage

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Intorduction

Draff consists of insoluble remains of endosperm, glumes, and retained flocks of substances precipitated during the process of mashing. It remains in the straining tank after straining the wort. The draff belongs therefore to the offal of the brewing industry. If the straining tank is used, 100 kg of dry brewer‘s malt yields 120 to 130 kg of wet draff containing 75 to 80 % of water. (Kosař et al. 2000). Fresh wet draff is warm and contains substances undergoing a relatively fast destruction. It also contains about 5 % of N-substances, 2 % of fats, 4 % of fibrous material and 10 % of NFES (nitrogen free extractive substances), particularly water soluble saccharides with prevailing glucose and maltose. Smaller amounts of water soluble substance are also present (Doležal et al. 2006). Fresh draff is notable by enhanced contents of the group of B vitamines.

Draff belongs not only to sought after raw material of the food processing industry, but it is also used for direct feeding of domestic animals. due to its favourable dietary value, high digestibility.of organic matter and a relatively easy availability. However, low storability, high humidity, high contents of N-substances and saccharides, as well as mildly acid pH belong to its disadvantages. Fresh draff can not be usually used for feeding longer than for 48 hours since afterwards begin undesirable sensory and biochemical changes. An important role in such undesirable processes can be played by microorganisms. Fresh warm draff is a practically sterile material after the wort has been strained. The occurrence of microorganisms participating on the deterioration of wort is due to the secondary contamination from transport means and storage facilities in the breweries and agricultural plants. Draff can be considered in general as spoiled when the concentration of yeasts and moulds is higher than 10^{5-6} and that of bacteria than 10^{8} (Doležal et al, 2005). Besides bacteria also the yeasts (*Saccharomyces, Candida, Pichia, Hansenula*) can participate on decomposition processes. They belong to the acid-tolerant microorganisms able to proliferate within a rather wide temperature zone (0 - 45°C with an optimum at about 30°C). This is why draff with its pH and nutrient contents presents for them an ideal substrate. The counts of yeasts in draff should not exceed 10^{5} per g, since otherwise they can be the cause of diarrhoeral disorders and disturbances of ruminal digestion. Moulds reproduce in draff more slowly than yeasts although it is for them also a suitable substrate. The dangerousnes of moulds rests in the first place on the possible formation of mycotoxines.

The goal of our study was to appreciate the influence of selected additiva on the dynamics of colonization of fresh brewery draff by yeasts and moulds.

Material and Methods

Four variants of fresh draff treatments with selected additives were studied: A (control) – no additives, B – preparation with formic acid, propionic acid and

ammonium formate, amount added 3 l/t, C - preparation with formic acid, propionic acid and ammonium formate, amount added 6 l/t, D – biochemical preparation containing bacteria of lactic fermentation (*Lactobacillus plantarum, Pediococcus pentosaceus, Lactobacillus lactis, Enterococcus faecium*), cellulolytic enzymes and sodium benzoate, amount added 4 l/t. Storage was taking place under aerobic conditions at 22 ^{o}C. Samples of 20 g were taken from experimental variants after 0, 24, 48 and 72 hours. Each sample with 180 ml of sterile distilled water was shaked for 10 min on a shaker. Subsequently followed the serial dilution by a factor of 10. 1 ml of respective dilutions was transferred on a Petri dish and overflown with culture medium. The counts of yeasts and moulds were estimated on Chloramphenicol Glucose Agar (Biokar Diagnostics, France) after 120 hours at 25 ^{o}C.

Results and Discussion

Fresh draff had the pH 6,50, a high humidity, and was rich in nutrients. This made it an ideal substrate for microorganisms. Graphs 1 – 4 demonstrate the changes in counts of yeasts and moulds in the course of 72 hours. From the graphs is evident the fast proliferation of yeasts whose numbers attain the limit of 10^5/g already after 48 hours. The higher increase of yields in B variant was probably due to an inadequate concentration of active substances in the preparation with additives. A rapid decrease of pH to 4,17 in the C variant, contrasting with pH 6,50 of the control, followed the application of a twice higher amount of the additive, i.e. 6 l/t. The efficiency of organic acids is under such conditions higher. The counts of yeast cells are in this variant very low and they do not exceed the value of 10^3/g after 48 hours. The development of yeasts in the D variant is related to the fact that the respective preparation is used for the preservation under anaerobic conditions. Thus, conditions for the development of lactic fermentation bacteria are not fulfilled. Also the influence of sodium benzoate present in the preparation is disputable with respect to the pH of draff. The pH 4,50 is considered according to Drdák 1989 as the limiting acidity for an effective usage of benzoic acid or benzoate in the preservation practice. The importace of D variant will increase in the ensilage of draff, which is the object of further studies. The development of moulds is distinctly slower in individual variants. The role of moulds is therefore of minor importace in the aerobic deterioration of draff. The amount of moulds exceeded in none of tested variants the limit of 10^5/g even after 72 hours of storage under aerobic conditions.

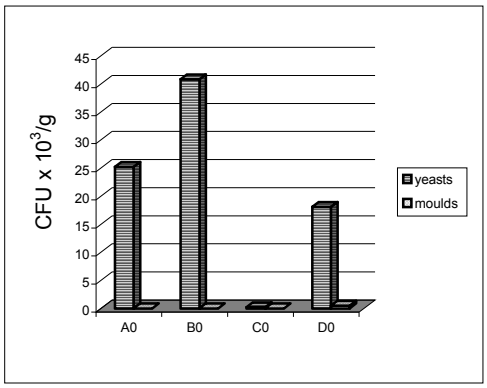

Fig. 1: Initial counts of yeasts and moulds.

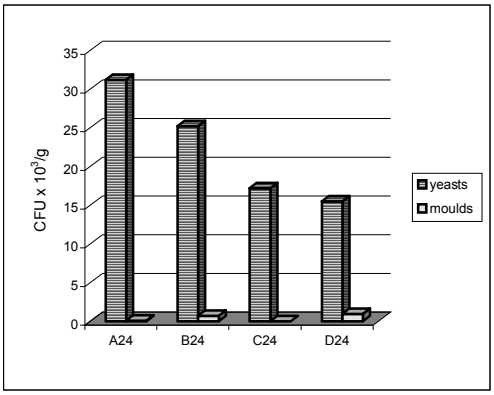

Fig. 2: Counts of yeasts and moulds after 24 hours.

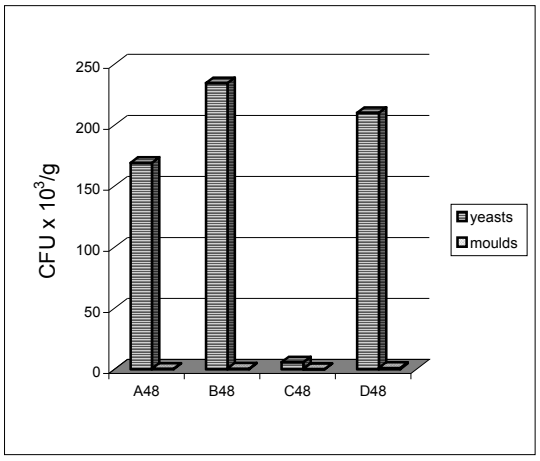

Fig. 3: Counts of yeasts and moulds after 48 hours.

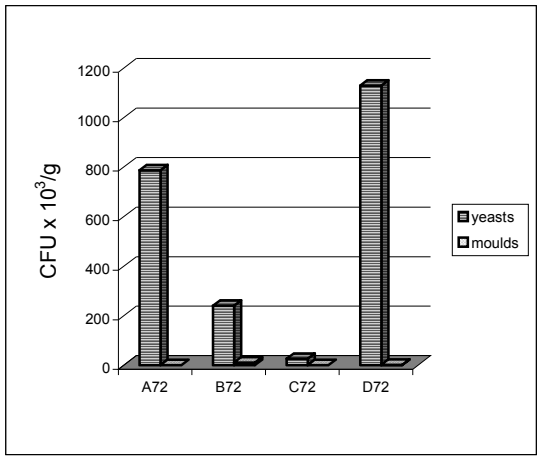

Fig. 4: Counts of yeasts and moulds after 72 hours.

Conclusion

Fresh brewer's draff is practically sterile. However, a secondary contamination takes place during the transportation and storage. The mildly acid pH, nutrient contents and a high humidity make it an ideal substrate for the development of microorganisms. The mildly acid pH and a low dosage of applied additive can negatively influence the preservative efficiency of organic acids. The use of preparations containing bacteria of lactic fermentation also appears as less favourable with respect to aerobic conditions in the course of storage and to competitive pressure of contaminant microflora. An efficient preservative effect for the whole duration of the experiment was demonstrated only in the C variant where the preparation containing formic acid, propionic acid and ammonium format was added in the amount corresponding to 6 l/t.

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The Effect Of Additives on Quality Of Silage Made Of Red Clover And Clover-Ryagrass Mixture

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Introduction

Red clover (*Trifolium pratense*) is one of the most popular legume in grassland cultivation. Red clover has a high nutritive value but on the other hand low ensiling properties. This is mainly due to high buffering capacity (BC) and low available sugar (WSC) concentration (McDonald, 1991). Furthermore, compared to other legumes, red clover has usually also lower dry matter (DM) concentration, and therefore resulting in high risk. This is characteristic in particular when tetraploid cultivars of red clover will be ensiled (Olt, 2003; Lättemäe et al., 2005). However, the additives may considerably reduce clostridial fermentation and DM losses. In order to improve fermentation properties of silage material it is also possible to use red clover-grass mixtures.

Materials and methods

The ensiling trial was carried out on 21th of Jyne in 2005. Silage material was first cut red clover cultivar ''Varte'' and red clover-hybrid ryegrass ''Molisto'' mixture. The chemical composition of red clover was as follows: dry matter concentration (DM) 120 g kg^{-1}, neutral detergent fibre (NDF) 443 g kg^{-1} DM, acid detergent fibre (ADF) 293 g kg^{-1} DM, water soluble carbohydrates (WSC) 53 g kg^{-1} DM and buffering capacity (BC) 107 g lactic acid kg^{-1} DM. The chemical composition of red clover-ryegrass mixture: DM 132 g kg^{-1}, NDF 470 g kg^{-1} DM, ADF 321 g kg^{-1} DM, WSC 72 g kg^{-1} DM and BC 97 g lactic acid kg^{-1} DM. By botanical composition the mixture consisted approximately of 80% of red clover and 20% of ryegrass. The silage was made of fresh cut crop. The crop was harvested by a mowing machine, chopped 4-8 chop-length and ensiled in 3 l glass jars. The following additive treatments were used in two replicates: untreated, Niben treated 5 l t^{-1} fresh matter (FM), Ammformprop treated 5 l t^{-1} FM, Kemisile treated 5 l t^{-1} FM and AIV-2000 treated 5 l t^{-1} FM. Niben is a chemical additive and based on sodium benzoate. In addition Niben consists also sodium nitrite. Other additives are chemicals and based on formic acid. Glass jars were sealed with 4 layers of plastic film (0,025 mm thickness) and these weights recorded. Thereafter, the jars were stored at room temperature18-25° C for 100 days, after which samples were taken for chemical analyses. In order to determine dry matter losses, the jar weights again were recorded prior opening the silo. The trial data were examined statistically by analysis of variance.

Results and discussion

The silage quality, fermentation results and dry matter losses are presented in table 1. The nutritive value of silage material was dependent on the mixture. Pure red clover resulted in higher crude protein concentration and nutritive value compared to mixture.

Table 1: The effect of using additive and red clover-grass mixture on silage fermentation and dry matter losses. The silage was made of fresh cut herbage.

Mixture	Dry matter (DM)	Crude protein	Crude fibre	pH	Ammonia	Butyric acid	DM losses
Additive	g kg^{-1}	-------g kg^{-1} DM--------			% of total N	g kg^{-1} DM	%
Red clover							
Untreated	117	189	282	5,9	36,5	53,8	14,4
Niben, 5l ton^{-1} FM	127	184	258	4,5	7,6	9,2	5,6
Ammformprop, 5l ton^{-1} FM	109	192	296	5,0	10,5	34,8	10,5
Kemisile,5l ton^{-1} FM	113	195	272	4,9	5,1	30,8	9,8
AIV-2000, 5l ton^{-1} FM	111	198	294	5,1	16,9	29,8	10,0
Red clover-ryegrass mixture							
Untreated	131	148	313	5,4	32,7	42,1	13,2
Niben, 5l ton^{-1} FM	142	151	296	4,4	6,7	3,9	3,6
Ammformprop, 5l ton^{-1} FM	129	173	303	5,3	15,0	43,1	14,2
Kemisile, 5l ton^{-1} FM	133	161	323	5,0	7,3	30,0	9,6
AIV-2000, 5l ton^{-1} FM	136	164	332	5,1	18,7	32,0	9,9
$LSD_{0.05}$		*24,7*	*36,4*	*0,4*	*11,6*	*18,4*	*3,6*

On the other hand mixture improved fermentation properties of silage material by increasing WSC concentration and decreasing BC. However, both direct cut red clover and red clover-ryegrass were very vet. The average DM concentration of red clover silage was 115 and mixture silage 134 g kg^{-1}. Such silage material favours the growth of clostridia and enterobacteria and results in high effluent losses.

The fermentation quality and dry matter losses were dependent on the use of additive and mixture. The silage, made of untreated red clover crop, had the lowest quality. It contained 53,8 g kg^{-1} DM butyric acid and 36,5% of ammonia nitrogen. Such silage is unsuitable for animal feeding. Dry matter losses were well correlated with butyric acid concentration in silage. Ryegrass improved fermentation properties of ensiling material, and therefore the silage quality was slightly better. However, the results varied and the silage quality was unsatisfactory in most treatments, except with Niben treated silage. It contained 3,9 g kg^{-1} DM and 6,7% of ammonia nitrogen. The DM losses were also the lowest of this treatment.

Conclusions

The nutritive value of silage was dependent on the mixture. Pure red clover ''Varte'' resulted in the higher crude protein concentration of silage. The silage fermentation quality and dry matter losses were dependent on the use of additive and herbage mixture. The silage, made of fresh red clover, had the lowest quality. Herbage

mixture slightly improved fermentation and silage quality. The silage additives improved fermentation whereas Niben was the most effective additive. However, the ensiling of tetraployed red clover is still problematical due to low dry matter concentration of this cultivar.

Acknowledgements

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Estimation of ensilability of alfalfa and whole crop triticale using an *in vitro* rapid test (Rostocker Fermentationstest)

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Introduction

For estimating the fermentability of different plant materials a simple and rapid *in vitro* method was developed by PIEPER *et al.* (1989) and ZIERENBERG (2000). As fermentation processes take place in the aqueous fraction of plants the principle of this method is the acidification of minced plant material in an aqueous mixture. The pH-value is measured at the beginning of the test and after a set time schedule during incubation. Fermentability can then be estimated considering the rapidity of acidification and the final pH-value. By using several treatments (sugar, lactic acid bacteria, enzymes or combinations) the necessity of an additive for ensilage can be evaluated. The aim of the study was to assess the fermentability of alfalfa and triticale, known to be difficult to ensile.

Materials and Methods

Alfalfa (*Medicago sativa*) at the end of flowering (18,9% dry matter) and whole crop triticale (*Triticosecale*) at the beginning of spike development (19,7% dry matter) were harvested and stored at -20°C. The frozen material was minced with a standard meat chopper and homogenized thoroughly. For preparing the aqueous mixture 50g of thawed plant material were mixed with 200ml of *aqua dest.*. Alfalfa was incubated either without additive (control) or treated with sugar (2% of fresh matter (FM)), lactic acid bacteria (LAB 1) ($3 \cdot 10^5$cfu/g FM, commercial product containing *Lactobacillus plantarum*) or a combination of sugar and LAB 1. Triticale was treated as follows: without additives (control), LAB 1 in combination with enzymes (β-glucanase, xylanase and glucoamylase, commercial product), LAB 2 ($2 \cdot 10^4$cfu/g FM, commercial product containing *Lactobacillus plantarum, Lactobacillus brevis* and *Lactobacillus buchneri*) in combination with enzymes or LAB 1 in combination with sugar (2% of FM). The mixtures were stirred well, covered with aluminium foil and incubated at 30°C. The pH-value was measured before incubation and after 14, 18, 22, 26, 38 and 46h, whereas only the pH-values at the beginning of the test, after 18h as a sign of rapidity of acidification and after 46h as a sign of fermentability were analysed in the following.

The data were interpreted by the analysis of variance and the significance of differences among means was tested with Duncan's multiple range test.

Results and Discussion

The pH-values of alfalfa and triticale during incubation with different treatments are shown in table 1 and 2.

Showing the same pH (5,85) at the beginning of the test, there is a rapid decrease after 18h within all treatments (table 1). Alfalfa being low in sugar content, the addition of fermentable carbohydrates leads to faster acidification than the variants without

sugar. The lowest pH-value is achieved by adding sugar in combination with LAB 1. At the end of the test (46h) pH increases again without additive and with LAB 1 treatment, which also indicates the lack of sugar in this forage crop. Adding LAB 1 in combination with sugar obtains a final pH-value of below 4,00 (3,57).

Comparing the pH-value in aqueous mixtures of triticale (table 2) after 18h with the initial pH only the variants with LAB 1 commit a distinct decline. After 46h there is no significant difference between control (3,57) and LAB 2 + enzyme (3,55). Furthermore no differences between LAB 1 in combination with sugar (3,43) or enzyme (3,40) can be stated.

Table 1: pH-values of aqueous mixtures of alfalfa whole crop during incubation (n=3)

	pH after incubation		
treatment	0h	18h	46h
control	5,85[a] *±0,01*	4,94[a] *±0,01*	5,32[a] *±0,08*
sugar	5,85[a] *±0,01*	4,35[b] *±0,03*	4,12[b] *±0,02*
LAB 1	5,85[a] *±0,01*	4,60[c] *±0,03*	5,13[c] *±0,11*
LAB 1 + sugar	5,85[a] *±0,01*	3,84[d] *±0,04*	3,57[d] *±0,01*

[a,b] different lower case letters within columns indicate significant differences between treatments (p<0,05)

Table 2: pH-values of aqueous mixtures of triticale during incubation (n=3)

	pH after incubation		
treatment	0h	18h	46h
control	6,10[a] *±0,02*	5,70[a] *±0,06*	3,57[a] *±0,02*
LAB 2 + enzyme	6,10[a] *±0,03*	5,79[a] *±0,05*	3,55[a] *±0,06*
LAB 1 + enzyme	6,09[a] *±0,05*	4,39[b] *±0,11*	3,43[b] *±0,01*
LAB 1 + sugar	6,14[a] *±0,02*	4,05[c] *±0,05*	3,40[b] *±0,00*

[a,b] different lower case letters within columns indicate significant differences between treatments (p<0,05)

Conclusion

The results of the *in vitro* rapid test show that the limiting factor for fermentation of alfalfa is the content of fermentable carbohydrates. The activity of the epiphytic microorganisms in the phyllosphere of alfalfa is sufficient to acidify the aqueous mixture supposed enough sugar is added.

In contrast the pH-values of untreated whole crop triticale indicate that fermentable carbohydrates do not limit acidification. The epiphytics are not able to decrease the pH rapidly enough regarding the pH after 18h, so that undesired fermentations might occur. Therefore the use of a suitable LAB-inoculant is advised. The need of adding an enzyme or another source of fermentable carbohydrates can not definitely be stated. Thus, further experiments have to be carried out.

The *in vitro* rapid test can be useful to estimate the need and choice of different additives, particularly LAB-inoculants, for sufficient acidification of green forages. Although based on the acidification in the aqueous fraction of plants the *in vitro* test is not an ensiling test, but it is simple to use and can give results in a relatively short period of time.

Summary

Estimating the fermentability of green forage prior to ensiling can help deciding which additives are to be used to achieve good quality silages. Therefore an *in vitro* rapid test was developed, based on measuring the pH-value after a certain time schedule as an indicator of the acidification in an aqueous mixture. The experiments were undertaken using alfalfa and whole crop triticale either without treatment or with addition of LAB, sugar, enzymes or combinations. The low sugar content is a known problem of alfalfa. Hence, the addition of fermentable carbohydrates to alfalfa leads to a rapid acidification and a low final pH-value which is only exceeded by the treatment

with sugar and LAB. Regarding the ensilability of whole crop triticale no additional sugar is needed, but for a rapid pH-decline the addition of LAB is recommended. Using the rapid test it could be deflected, that LAB 1 was more capable of acidifying triticale than
LAB 2. Thus, the *in vitro* method can also be used to test the efficiency of inoculants used for ensiling.

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Estimation of ensiling additives influence on fermentation processes in different silages of grasses

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In investigations we included grasses that grow in Latvia (cocksfoot –Dactylus glomerata, meadow fescue – Festuca pratensis, perennial ryegrass – Lolium perenne, timothy – Phleum pratense, meadow foxtail – Alopecurus pratense). Aim of investigations: to clarify the ensiling ability and fermentation of different grasses by using different for ensiling (biological inoculant SIL-All4x4 and chemical additive – AIV – 2Plus).

By analyses of grasses mass we cleared up that different grasses at schooting stage of maturity have different chamical content: sugar (101.7 – 164.3 g kg-1), crude protein (153.3 – 179.3), bufercapacity (270 – 590 mEq kg-1) and fermentation coefficient (28 – 44), which charactize green mass ensiling ability. That in it turn determines ensiling method and make possible to a quality of obtained silage. From ensimated grasses in experiment the most hard ensiling grasses were cocksfoot and meadow foxtail, but light ensiling were timothy, meadow fescue and perennial ryegrass. A grass silage feed value NEL and CP content are essentialy influenced ($p<0.01$) by grass species and ensiling way.

We can conclude, that

- for hard ensiling grasses the right fermentation processes beter provided biological inoculant SIL-All4x4 and chemical additive AIV-2Plus. They interrupted growth of undersirable microorganisms, improved fermentation (pH – 3.7 – 3,8 and 3.9 – 4.1, content of lactic acid from sum of acids 76 – 77 and 64 – 76 %, presence of butyric acid 0.22 – 0.27 and 0.33 – 0.49 %), in composition with control variant.
- The fermentation regulators SIL-All4x4 and AIV -2Plus additives ensure not only optimisation of fermentation, but essentially ($p<0.01$) improved preserving of energy and crude protein in grass silage too.

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Application Of Chemical Additives in Conservation Of Crimped Maize Corn With High Moisture

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Introduction

Maize grain is high-energetic and low-protein feed. High moisture corn was harvested between 25 and 35% moisture content and stored in a silo where it is preserved through fermentation. The optimum harvest moisture for good fermentation of high moisture corn is 28 to 32%. The aim of this technology is to get microscopicall ripeness of starch in cultivated corn not only for productional but also for nutrition reasons. Microscopically ripe corn is advantageous because of lower degradability in rumen of ruminants and greater passage into small intestine, where it is used more effectively (Čerešňáková et al., 2003).

Treatment of maize corn by chemical preparations on the basis of organic acids seems to be extraordinary effective (Bíro and Juráček, 2003). For the treatment of corn it is extremely efficient to use chemical additives based on organic acids (Volkov et al., 1999).

Aim of this work was to test the effect of two chemical preparations on the conservation of crimped maize corn with high moisture.

Material and Methods

The model experiment with crimped maize corn containing 648.46 g dry matter, 97.99 g crude protein, 652.13 g starch and 29.80 g sugar totally. The crimped maize corns were homogenized and filled into 1.7 l bottles. Three different groups were examined: a non-treated control and two experimental variants treated as follows:

- T_1 treated with a chemical additive consisting of 22.9 % natrium benzoate and 8.3 % natrium propionate, 4 l were applied per 1 t of feed
- T_2 treated with a chemical additive consisting of 42.5 % formic acid, 30.3 % formic ammonia and 10.0 % acid propionic. Again 6 l were applied per 1 t of feed.

The filled bottles were placed in a dark room at 20 – 25 ^{o}C. After 90 days of incubation the samples were examined for nutrient content, dry matter content losses in % of the original dry matter and electrometrically for silage extract pH. Lactic acid and volatile fatty acid levels were determined by gas chromatography, alcohol micro diffusion method. Dry matter was determined at 105 ^{O}C (drying till constant weight) according to the Slovak National Standard No. 2136/2004–100.

The results were statistically processed and compared by means of the one-factorial variance analysis using the Statgraphics 2.6 programme.

Results and Discussion

Application of chemical conservation preparations on the basis of organic acids and salts of organic acids manifested itself by decreasing the losses of dry matter compared with the untreated control (table 2.). The greatest losses were observed in non-treated control (0.75 %) and the lowest losses were in group T_2 (0.58 %). It became evident also in total content of dry matter that was higher in treated groups than in non-

treated control (646.92 and 646.97 versus 645.84 g.kg^{-1} dry matter). The lowest content of residual sugars was in un-treated maize corn and the highest one was in variant T_2, statistically highly significant differences being also between the treated groups T_1 and T_2. Application of chemical preparations manifested it self positively also in starch content in conserved maize corn. We observed the highest value in the group treated with preparation T_1 and the lowest value in the un-treated group (717.20 versus 689.85 g.kg^{-1} dry matter).

In group T_1 had the treated maize corn lower pH than the un-treated one (3.88 versus 3.81). The difference among groups was statistically highly significant. We noticed the statistically highly significantly highest pH in the treated group T_2. pH level corresponds with with the content of acids. Statistically highly significant content of lactic acid was in the corn conserved by preparation on the base of natrium benzoate and natrium propionic as well as in the un-treated control, and the lowest one was in the treated group T_2 (24.20 and 24.17 versus 1.50 g.kg^{-1} dry matter). Content of lactic acid in maize corn treated with the preparation on the base of formic acid and formic ammonia does not correspond with the results of Bíro and Juračka (2003), who applied mixture of two organic acids (propionic acid and formic acid) into moist maize corn and they found higher content of lactic acid in the treated group compared with the un-treated control.

Influence of application of chemical preparations manifested itself also in decrease of acetic acid content from 4.33 to 2.83 and 2.98 g.kg^{-1} dry matter ($P < 0.01$). Similar results detected also Filipovič and Ristič (2001), who applied propionic acid into moist maize corn. We found the highest content of alcohol in the un-treated control and in the first experimental group 1.44 and 1.07 g.kg^{-1} dry matter, respectively; the lowest one was in the second experimental group, namely 0.04 g,kg^{-1} dry matter ($P < 0.01$).

Table 1: Nutrient composition of maize corn with high moisture before conservation

Dry matter	OM	Crude protein	Crude fibre	Nitrogen-free extract	Starch	Sugar total	Fat	Ash	NEL	PDI
in g	in g.kg^{-1} dry matter								in MJ.kg^{-1} DM	in g.kg^{-1} DM
648.46	986.76	97.99	32.48	823.61	652.13	29.80	35.68	13.24	8.18	72.80

Table 2: Nutrient composition and parameters of the fermentation in maize corn with high moisture

Parameter n = 6	U		T_1		T_2		Significant difference	
	$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	$P < 0.05$	$P < 0.01$
DM in g	645.84	0.85	646.92	2.49	646.97	0.55		
Losses DM in %	0.75	0.13	0.60	0.29	0.58	0.09		
CP in g.kg^{-1} DM	96.96	0.93	96.15	1.12	99.82	1.28	1:2	K:2
CF in g.kg^{-1} DM	30.75	8.98	19.42	4.71	18.32	1.51		K:2
Starch in g.kg^{-1} DM	689.85	3.42	717.20	6.06	709.72	9.06	K:2	K:1
Sugar total in g.kg^{-1} DM	7.01	0.57	9.65	3.98	20.85	0.67		2:K,1
Fat in g.kg^{-1} DM	36.64	1.43	37.86	1.23	37.22	1.37		
Ash in g.kg^{-1} DM	13.55	0.08	13.76	0.27	13.33	0.48		
pH	3.88	0.02	3.81	0.01	4.05	0.05		K:1,2 1:2
pH on day 3 after fermentation	3.96	0.04	3.91	0.01	4.15	0.01		K:1,2 1:2
Lactic acid in g.kg^{-1} DM	24.17	0.33	24.20	0.66	1.50	0.12		2:K,1
Acetic acid in g.kg^{-1} DM	4.33	0.65	2.83	0.18	2.98	0.87	K:1	
Propionic acid in g.kg^{-1} DM	0.84	0.17	0.72	0.06	1.44	0.16		1:2
Butyric acid in g.kg^{-1} DM	0.32	0.06	0.10	0.05	0.69	0.22		1:K,2
Alcohol in g.kg^{-1} DM	1.44	0.07	1.07	0.16	0.04	0.01		2:K,1

Conclusions

Application of organic acids and salts of organic acids had positive effect upon the quality of the fermentation process and the nutrient composition in conservation of maize corn with high moisture. On the basis of results obtained at conservation of moist crushed maize corn we can state positive influence of the application of organic acids and salts on the conservation process that manifested itself in decreased losses of dry matter, decreased content of fibre, acetic acid and alcohol, and in increased content of starch.

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Effect of the stage of maturity on leaves percentage of alfalfa and the effect of additives on silage characteristics

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Keywords: alfalfa, leaves, silage, nutrients

Introduction

Alfalfa (*Medicago sativa*) can be utilized in the main for hay making and for silage production. Alfalfa, one of the major agricultural crops in the Czech Republic, is grown on 15.8% of the arable land. Alfalfa silage forms a substantial part of diets for farm animals. It is very important to choose a suitable term of harvesting for ensilage from the point of view of optimal nutrient content. The stage of maturity at cutting has large effects on each component, except crude protein (*Yu et al*., 2004). Herbage harvested at full bloom is expected to have a higher stem proportion than less mature herbage (*Fick and Holthausen*, 1975; *Kilcher and Heinrichs*, 1974).

Alfalfa can be difficult to ensile due to a high buffering capacity and a low WSC content. Addition of silage additive showed a positive effect on the fermentation process (*Gallo et al*., 2002).

Materials and methods

In the first trial we determined the effect of the harvest term on yield and quality of alfalfa herbage. The objective of this experiment was to examine changes in leaf and stem properties of alfalfa herbages. Alfalfa, cultivar Europe, was grown on the experimental field at the Research Institute of Animal Production in Prague (sugar beet growing region, 280 m above sea level). Alfalfa was planted at the seeding rate 18 kg/ha with wheat as a foregoing crop and legume-cereal mixture as a cover crop.The area 15x15 m was marked out on the 10 ha alfalfa field. The six samples (each 1x1 m) were cut in two stage of maturity (small buds and bloom). Collecting the samples at the bloom stage followed on the average twelve days after stage of small buds. The herbage mass from each sample was weighted. Finaly, each plant of alfalfa was fractionated into leaves and stems.

In the second trial, four different varieties of alfalfa silages were tested. The first silage group (C) was control (without additive). In the second group (I) bacterial inoculant (1 g/t) containing homo- and heterofermentative lactic acid bacteria (*L. rhamnosus, L. plantarum, L. brevis, L. buchneri, P. acidilactici* was used. The chemical additive (Ch) (containing formic and propionic acid) was used in the third group of silage in amount of 5 l/t. The bacterial inoculant (the same as for second group, 1 g/t) with the benzoic acid in the amount of 288 g/t was used in the fourth group (ICh). All additives were applied to chopped allfalfa forage at the time of ensiling.

Chopped forage (500 g) was packed into polyethylene bags. After sealing in vacuum, the bags were stored at the temperature 18-20 °C. After seven weeks, the bags were opened and analysed.

Table 1 Leaves percentage from whole herbage, whole herbage yield and leaves yield

	Unit	1st cut	SE	2nd cut	SE	3rd cut	SE
			% leaves from herbage				
Small buds	%	52.07 [a]	1.01	52.03 [a]	1.22	50.58 [a]	2.16
Bloom	%	46.62 [b]	1.95	44.70 [b]	1.10	46.26 [b]	1.04
			leaves yield				
Small buds	t/ha	1.86 [a]	0.23	1.23 [a]	0.25	1.03 [a]	0.13
Bloom	t/ha	2.30 [b]	0.32	1.55 [b]	0.11	1.60 [b]	0.19
			whole herbage yield				
Small buds	t/ha	3.57 [a]	1.43	2.37 [a]	2.48	2.03 [a]	0.65
Bloom	t/ha	4.93 [b]	2.51	3.47 [b]	1.52	3.43 [b]	0.93

[a,b] For the same observed characteristics, mean values in the same column with the different superscript are significantly different (P<0.05)

Table 2 Nutritive value and fermentation characteristics of alfalfa silane

	Unit	C	SE	I	SE	Ch	SE	ICh	SE
Dry matter	g/kg	317.7	7.50	313.7	5.04	313.8	4.51	310.7	4.76
Crude protein	g/kg	257.8	2.15	258.7	1.64	262.0	2.64	258.5	2.24
Crude fibre	g/kg	260.5	4.06	262.3	3.17	265.2	4.31	268.0	2.10
WSC	g/kg	3.39	0.46	4.97	0.56	4.11	0.64	3.37	0.17
Lactic acid	%	2.89	0.21	3.37	0.11	3.33	0.10	3.01	0.17
Acetic acid	%	1.14	0.12	1.22[b]	0.09	0.84[a]	0.03	1.43[b]	0.07
Propionic acid	%	0.29	0.09	0.10	0.02	0.19	0.01	0.16	0.01
Butyric acid	%	0.03	0.02	0.08	0.01	0.14	0.01	0.11	0.004
pH		4.45	0.04	4.47	0.06	4.38	0.03	4.47	0.03

[a,b] Mean values in the same line with the different superscripts are significantly different (P<0.05)
C = control, I = silage with bacterial inoculant, Ch = silage with chemical additive, ICh = silage with bacterial inoculant and benzoic acid

Results and discussion

The results of the first experiment are presented in the Table 1. The leaves percentage in the maturity stage of small buds was significantly (P<0.05) higher than in the stage of bloom. In the stage of small buds the leaves predominated above stems and the harvested mass was superior. In the time of bloom the protein content and WSC are reduced and crude fibre is increased (*Tyrolova and Vyborna, 2005*).

In the stage of bloom the yield of whole plants of alfalafa was significantly higher but this increase was totalled first of all by stems.

The results of chemical analyses carried out within of the second experiment are showed in the Table 2.

The pH of silage with the chemical additive was the lowest but the differences were not significant. The concentration of lactic acid was the lowest in the control silage. All additives positively influenced the concentration of lactic acid. Silage I contained the highest concentration of lactic acid.

Silages treated with inoculant which contained *L.buchner,* increased (P<0.05) the acetic acid concentration compared to the silage with the chemical additive. The observations similar to this study were reported by *Kung et al* (2003).

The pH of all silages declined below 4.55 and the low concentration of butyric acid and the high concentration of lactic acid suggested that all silages were of good quality.

Conclusions

All the additives improved the quality of alfalfa silage. All treated silages contained higher amounts of lactic acid. In the maturity stage of small buds the leaves percentage is higher than in the bloom stage. The harvested mass is of higher quality.

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Microbial Silage Additives: New Strategies For Development And Production

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Summary

The preservation of forage crops and grasses by ensiling can be supported by the addition of starter cultures consisting of one or more strains of lactic acid bacteria. Commercial preparations are often comparatively expensive and not always optimal. As a low-cost alternative, the authors report here a method for the production of high activity starter cultures "on the farm" requiring only simple technical efforts. Also different strategies for monitoring of the fermentation of starter cultures are presented with special respect to the application of molecular markers. In this context, PCR assays provide a versatile and valuable tool, which may lead to new and improved fermentation strategies. The economical aspects of these strategies are discussed.

Introduction

The preservation of herbage biomass with the aid of fermentative bacteria is a widely used method in production of animal forage. During this ensilage, the anaerobic microbial conversion of fermentable carbohydrates to organic acids lead to a reduction of the pH value and thus to a preservation effect. Ensiling of plant material usually starts even naturally through airtight storage and the proliferation of plant-associated lactic acid bacteria. However, in many cases this spontaneous process does not perform optimally. This may cause an insufficient acid production and thus this may lead to a decreased preservation and subsequent losses in forage quality. To prevent unwanted fermentation during ensiling, a commonly applied method is the addition of bacterial starter cultures to the harvested plant material (Seale 1996, Weinberg and Muck 1996).

Nevertheless, commercially available mixtures of starter cultures are comparatively expensive. A potential alternative is the production of starter cultures on-the-farm by the farmers themselves. To establish such strategies, not only a simple and inexpensive technical equipment will be required, but also a simple and easy-to-perform fermentation process will be needed.

Materials and Methods

Most experiments were conducted at the Leibniz-Institute for Agricultural Engineering Potsdam-Bornim, Germany, and, in part also at the Oak Park Research Centre of the Irish Agriculture and Food Development Authority (Teagasc), Ireland. A pilot plant for on-farm production of silage starter cultures was established at an agricultural cooperative at Niederschöna, Germany. Further detailed descriptions of material and methods are given by the cited references.

Results and Discussion

Strategies for an on-farm production of bacterial cultures

Based on laboratory scale experiments carried out at the ATB a method for the on-farm production of ensiling bacteria was developed including the fermentation of two lactic acid bacteria commonly used as silage starters, Lactobacillus plantarum and L. rhamnosus (Idler et al. 1998). Applying this concept, a pilot plant was successfully established (Idler et al. 2002). First results from the operation of this pilot plant were used to develop improved ensiling strategies.

The main parts of the production plant are a heatable double-skinned container for the sterilization of the nutrient medium, an intermittently driven mixer-fermenter and a control system for pH and temperature. To keep investment costs low, stainless steel dairy containers were modified in-house for use as fermenter and sterilization tank. The plant was constructed for an easy and safe operation. The production method includes the individual steps of fermentation preparation, fermentation and subsequent treatment of the product. The cultures can be stored without losses in activity for up to four weeks so that production can be timed for culture availability before the beginning of grass harvest. However, for the production of starter cultures single fermentation steps for each species included in the additive are still required.

Marker-assisted optimization of culture conditions

For the development of fermentation regimes for a co-cultivation of two or more species of lactic acid bacteria a reliable tool for monitoring of the participating species is needed. Conventional microbiological approaches are not sufficient to distinguish most lactic acid bacteria species because of the lack of polymorphic phenotypical characteristics. To overcome this limitations, we developed a 16S rDNA targeted multiplex PCR (polymerase chain reaction) assay for *L. plantarum* and *L. rhamnosus* (Klocke and Mundt 2004), which facilitates a species-specific and also semi-quantitative determination of these two species also in mixed cultures. Subsequently, the PCR assay was applied to establish a regime for a batch co-fermentation of both species (Klocke et al. 2005b). It was shown, that under distinct fermentation regimes the co-cultivation is possible and will result in nearly equal concentrations of both species.

In general, a third species can also be included in this PCR assay. As an example, we developed a PCR assay for a parallel detection of *L. plantarum*, *L. rhamnosus* and *L. buchneri* (Klocke et al. 2004). However, depending on the PCR process involved this approach is not even semi-quantitative. Nevertheless, it is a valuable tool for the determination of agar-plated colonies.

Recent developments of the monitoring of starter cultures in silages

In recent times, the limitation of PCR regarding their poor eligibility for quantification of initial DNA amounts were overcome by the development of the real-time PCR technique. The real-time PCR has proven its usefulness as a standard tool for detection and quantification of certain bacteria species in the analysis of both, microbial ecosystems and microbial contaminants in technical applications (e.g. water purification or food production).

The real-time PCR method was successfully applied to enable the detection of the most common lactic acid bacteria species *L. plantarum* (Klocke et al. 2005a, Klocke et al. 2006) but also for other bacteria associated with silages (Stevenson et al. in press). The developed assays have shown their ability for quantification of the regarded species in pure cultures (e.g. within a fermentation process) and furthermore for their

monitoring after application in the ensiling process (Klocke et al. 2005c, Klocke et al. 2006, Stevenson et al. in press). This assays enable a reliable determination of the concentration of one single bacteria species in the silage for the first time, which will allow the monitoring of the proliferation of starter cultures.

Economical aspects of an on-farm production of starter cultures

Based on the data obtained from the operation of the pilot-plant, the production of a two-component silage starter culture "on-the-farm" requires costs of approx. 0.50 €/ton silage (Idler et al. 2001). Within this total costs, variable costs for consumables (e.g. media compounds, salary, energy) has a considerably higher influence than the other cost factors, being approximately 55 % of the final costs. Thus, these cost factors hold a large potential for reduction.

Strategies comprising the co-fermentation of two or more fermentative bacteria species are suited to reduce the operation costs in principle (Idler and Klocke 2005). Depending on the number of species and the particular fermentation regimes a production of starter cultures seems to be feasible for costs of 0.35€/ton silage.

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The influence of LAB-enzyme inoculants on quality and nutritive value of big bale grass silage

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Introduction

The quality of silage depends on the herbage quality and moisture content at ensiling, type of fermentation and maintenance of anaerobic conditions. Obtaining good quality and high digestibility of nutritive components in grass silage requires the stimulation of the ensilage process. For many years chemical preservatives have been used for this purpose. Natural methods of ensilage need to be stimulated by adding biological preparations. Addition of bacterial inoculants with LAB improved the quality and aerobic stability of grass silage (Wróbel, Zastawny, 2004). The use of biological additives containing cell wall degrading enzymes may improve fermentation of silage as animal production (Żurek et all, 2004). The lack of aerobic stability of silage with usually applied biological additives is their main weakness. The improvement of air stability is important in case of ensilaging the long cut plant material with high DM content. Aerobic deterioration of silage at feed-out can be difficult to avoid, particularly when silage is used as a buffer feed during the summer. The aim of this study was to investigate the influence of bacterial additives containing lactic acid bacteria and celulolitic enzymes on the quality, air stability and nutritive value of meadow silage.

Materials and methods

This study on the influence of some bacterial-enzyme silage additives used in production conditions on the quality, aerobic stability and nutritive value of grass silage were conducted between 2003 and 2004. The experiment was carried out at the Experimental Station at Falenty near Warsaw in Poland on permanent meadow situated on mineral soil. Meadow sward composed of 80 % grasses (Poa pratensis, Alopecurus pratensis, Dactylis glomerata, Arrhenaterum etatius, Lolium perenne) and 20 % weeds and herbs was used for ensilage. The meadow herbage was ensilaged with the addition of two chosen commercial biological additives containing lactic acid bacteria and celulolitic enzymes (treatment K1 and K2) or without any additives (control silage). The meadow was cut three times a year. The first cut was made at full heading of Dactylis glomerata, the second and third cuts were taken at nine weeks intervals. Herbage was cut using a rotary mower that had a mounted conditioner. Before harvest the fresh grass was pre-wilted to a dry matter (DM) concentration of approximately 300-400 g kg-1. Bacterial-enzyme additives were put into the herbage by spraying during bale rolling in a variable bale chamber baler. The big bales (about 400 kg) were wrapped in four layers of stretch plastic film after transport to their place of storage.

During the 100 day feed experiment, silage was fed to three groups of 10 heifers (200 kg). The heifers were fed ad libitum. The daily feed intake and refusals were recorded. Live weights were determined at the beginning, in the middle and at the end of the study. During the feeding experiment silage samples were taken for chemical analyses.

The chemical composition and quality (according to the Flieg-Zimmer scale) of feed samples was determined. Silage was analysed for: DM, crude protein, crude fibre and crude fat concentration using the NIRS technique. Organic acids were determined with the enzymatic method. Stability was analysed by monitoring changes of temperature in silage samples placed in boxes in aerobic conditions (temperature about 21 °C) for 12 days. Changes of temperature were recorded twice a day in each group of silage. Stability of silage was measured as the time necessary to increase the temperature by l °C over air temperature.

Results

Mean DM content in examined silages hesitated from 367.8 g/kg (K1) to 415.5 g/kg (K2) and was the highest in case of silages harvested in the second cut. The mean pH values in silages were similar (about pH 4.5), but there was a tendency for higher pH values in the control silage. The concentration of organic acids in silage FM was different and depended on the treatment. Generally in all silage samples lactic acid dominated among other acids (12 3-16 4 g/kg FM). Its participation in the sum of all evaluated acids was over 80%. In case of K2 silage it was close 85%. The mean concentration of acetic acid in control silage was 2.4 g/kg FM (16% in sum of acids) and 3.0 g/kg FM in silage samples prepared with additives. The butyric acid content in relation to remaining acids was the highest in control silage (3.76%) and the lowest in K2 silage (0.15%). Addition of bacterial-enzyme inoculants containing improved silage quality. This was evident in the case of K1 and K2. The quality of those silages was very good while the quality of the control silage was good (Table l).

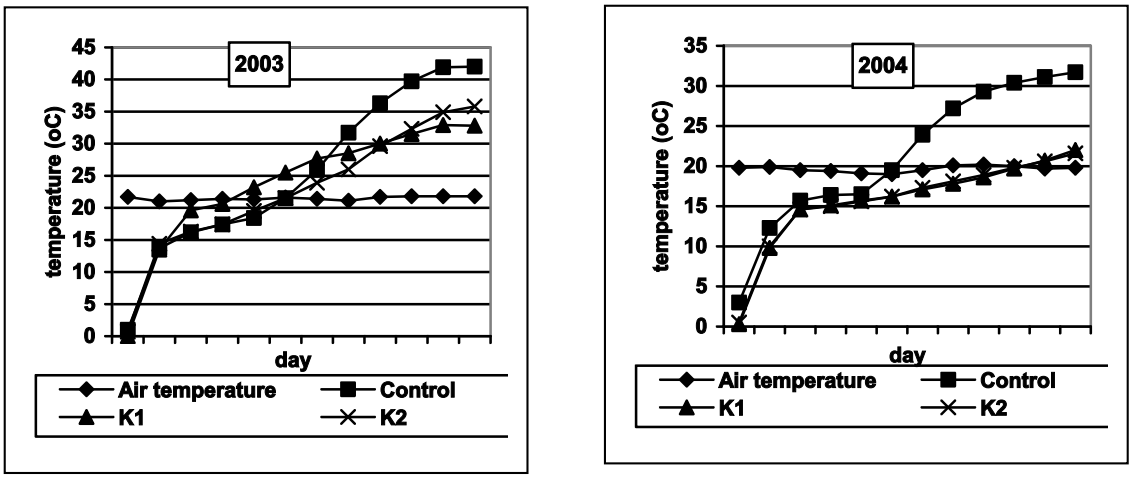
Table l: The quality and nutritive value of grass silage made with the addition of LAB-enzymes (2003-2004).

	Control		K1		K2	
	mean	SD	mean	SD	mean	SD
Dry matter (g/kg)	411.1	46.2	367.8	57.7	415.5	74.3
pH	4.86	0.27	4.38	0.21	4.50	0.34
Acids in FM of silage (g/kg)						
- lactic	12.3	2.9	15.0	3.1	16.4	2.7
- acetic	2.4	0.7	3.0	0.6	3.0	0.7
- butyric	0.6	0.5	0.2	0.1	0.0	0.0
Sum of acids	15.4	3.5	18.1	3.3	19.4	3.2
Acids in sum of acids (%)						
- lactic	80.34	2.96	82.28	3.81	84.37	2.16
- acetic	15.90	3.06	16.86	3.53	15.48	2.12
- butyric	3.76	2.75	0.87	0.39	0.15	0.08
Points in Flieg-Zimmer scale	76.00	14.52	98.33	1.51	99.00	1.10
Quality	good	-	very good	-	very good	-
Stability (in days)	6.33	2.66	7.33	3.39	7.17	2.64
Crude protein	141.50	8.40	151.10	2.40	139.40	8.50
Crude fibre	294.00	17.20	291.50	8.50	300.10	26.90
Crude ash	86.50	19.10	86.60	23.30	68.80	20.70
Crude fat	32.10	1.60	30.00	2.10	31.30	4.10
Intake of silage (kg d^{-1})	13.20	1.82	13.87	1.48	13.68	1.89
Intake of DM (kg d^{-1})	5.28	0.46	5.00	0.44	5.52	0.48
Daily gains (kg)	0.63	0.18	0.59	0.17	0.68	0.13

The addition of additives during ensilage improved aerobic stability of silage evaluated at a temperature of 21 °C. The mean air stability of the tested silages was 6.33 days (Control), 7.33 days with the addition of K1 and 7.17 days with K2 (Table 1). The changes of temperature in silage samples during aerobic stability test are shown on Figure 1.

Inoculant treatment had no evident influence on the nutritive value of the feeds. The concentration of crude protein ranged from 139.4 g kg-1 (K2) to 151.1 g kg-1 (K1). The mean concentration of crude fibre in all silage samples was about 290 – 3000 g kg-1. Heifers fed the tested silages consumed from 5.0 (K1) to 5.52 (K2) kg of dry matter daily (Table 1). Initial weight of the experimental heifers was over 170 kg. After the 100 day feeding period, the animals weighed on average about 240 kg. During the whole experimental cycle the highest average weight gains were obtained in 2004 with control silage (0.69) and K2 silage in both years (0.68 kg). The lowest gains (0.55 kg) were obtained in 2003 with heifers fed the control silage.

Figure 1. The aerobic stability of silage in 2003 and 2004



Conclusions

Addition of bacterial-enzyme inoculants improved the quality and aerobic stability of grass silage made in big bales. Among the inoculants compared the most effective was inoculate K2. The bacterial inoculants used had no influence on the content of crude components in the feeds.

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Effect Of Chemical Additives on The Quality Of Sugar Beet Pulp Silage

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Introduction

Pressed sugar beet pulp is a moist feed based on sugar beet residue direct from the sugar industry. It is a very palatable, economical energy source, which stimulates feed intake and can promote the intake of less palatable feeds. It can be used in complete diets or as a silage supplement when supplies are limited or quality is poor, and it makes an excellent buffer feed.

Wet beet pulp can be stored effectively in silage bags or in trench or bunker silos. Biological additives proved as not useful for beet pulp ensiling, is reported by Weber (2005). The positive effects on the quality of the fermentation process have only application of chemical additives.

In this study we investigated the quality of pressed sugar beet pulp silages treated by chemical additives in comparison to silage not treated.

Material and Methods

Wet beet pulp was ensilaged under laboratory conditions. Pressed sugar beet pulp contained 193.9 g/kg of dry matter (Table 1).

Table 1: Nutrient composition of sugar beet pulp before conservation

Dry matter in g/kg FM	Organic matter	Crude protein	Crude fibre	ADF	NDF	WSC total	Nitrogen free extract	Fat	Ash
	in g/kg DM								
193.9	921.6	202.29	257.19	239.9	419.4	49.2	595.3	5.3	78.4

Three different groups were examined, a non-treated control, and two experimental variants treated by chemical additives of company Addcon Agrar GmbH:

1. additive consisting of 22.9 % sodium benzoate, and 8.3 % sodium propionate. 4 l of solution was applied per 1 ton of feed.
2. additive consisting of 85 % propionic acid, and 15 % formic acid. 3 l of solution was applied per 1 ton of feed.

In each treatment we used 6 laboratory silos of 1.7 l cubic capacity. After 110 days of incubation the silages were evaluated.

Results and Discussion

The effects of chemical additive application on nutrient composition and fermentation parameters in sugar beet pulp silages can be seen in Table 2.

Quality of ensilaged feed in our experiment was according to reference of Petrikovič et al. (2000). Loučka et al. (1999) presented that content of dry matter for good fermentation of sugar beet pulp must be higher than 18 %.

During the fermentation process was undesirable butyric fermentation in non-treated forage. There was determined the degradation of feed consistency, and poor quality of silage, too. There was found low concentration of lactic acid (15.6 g/kg DM) but also high contents of butyric acid (30.0 g/kg DM), acetic acid (28.9 g/kg DM), and of alcohol (12.1 g/kg DM) in untreated silage. Dry matter losses were very high too (19.8 %).

The application of chemical additives in forage did not support improvement of lactic acid bacteria, and did not improve content of milk acid in silages. However these treatments prevent from ineligible butyric fermentation process. We determined that there were high significantly decreased contents of volatile fatty acids, alcohol and NH_3-N of total N in treated silages compared to non-treated silage. Fermentation losses markedly decreased, and there was sustained structure of forage.

Best differences were in content of fibre and its fractions from nutrient composition. The experimental silages had double content of hemicelluloses compared with the control silage. The high degradation of hemicelluloses effected high content of volatile fatty acids by butyric fermentation, reported Neuman et al. (1999).

The results obtained in our study coincide with those previously reported by Doležal (2002), Doležal (2003), and Jambor (2003).

Table 2: Nutrient composition and fermentation parameters in sugar beet pulp silane

Parameter		Control silage		Silages with additives				Statistical significance of differences	
				1		2			
		$\bar{x}$	s	$\bar{x}$	s	$\bar{x}$	s	P < 0,05	P < 0,01
Dry matter	in g.kg^{-1} FM	157.50	1.17	181.97	0.75	182.04	0.94		C : 1,2
Organic matter	in g.kg^{-1} DM	900.66	4.22	911.31	2.29	914.19	1.80		C : 1,2
Crude protein	in g.kg^{-1} DM	117.50	3.94	113.83	3.60	116.31	1.08		
Crude fibre	in g.kg^{-1} DM	228.66	6.43	204.45	4.06	201.71	5.86		C : 1,2
ADF	in g.kg^{-1} DM	267.71	1.89	241.98	6.42	238.84	10.28		C : 1,2
NDF	in g.kg^{-1} DM	344.88	12.00	410.40	16.46	414.00	17.73		C : 1,2
Hemicelluloses	in g.kg^{-1} DM	77.17	12.72	168.42	13.60	175.16	19.49		C : 1,2
Nitrogen free extract	g.kg^{-1} DM	528.65	9.05	587.96	6.31	590.85	5.61		C : 1,2
Sugar total	in g.kg^{-1} DM	4.73	1.36	1.36	0.45	1.01	0.02		C : 1,2
Fat	in g.kg^{-1} DM	5.29	1.35	5.07	0.74	5.31	0.83		
Ash	in g.kg^{-1} DM	99.34	4.22	88.69	2.29	85.81	1.80		C : 1,2
Losses dry matter	in %	19.81	0.61	6.39	0.41	6.34	0.47		
pH		3.73	0.07	4.05	0.08	3.70	0.05		1 : C,2
Acids	in g.kg^{-1} DM								
- lactic		15.57	1.98	14.39	3.22	15.96	3.24		
- acetic		28.88	2.47	7.03	0.30	5.47	0.61		C : 1,2 1 : 2
- propionic		1.85	0.23	1.82	0.16	2.45	0.52		
- butyric + isob.		30.02	3.66	1.81	0.19	1.43	0.32		C : 1,2
- valleric + isov.		0.27	0.06	0.09	0.03	0.24	0.23		C : 1
- capronic + isoc.		0.45	0.28	0.06	0.01	0.05	0.01	C : 1,2	
Total volatile fatty acids		61.46	3.01	10.81	1.16	9.64	1.42		C : 1,2
Total acids		77.04	3.52	25.20	3.43	25.60	3.19		C : 1,2
Alcohol	in g.kg^{-1} DM	12.07	1.34	9.05	0.51	7.16	0.72		C : 1,2 1 : 2
NH_3-N of total N	in %	9.44	0.94	7.46	0.51	7.76	0.87	C: 2	C: 1

Conclusion

The treatment of sugar beet pulp with chemical additives had a positive effect on the quality of the fermentation process. Improvement of fermentation parameters in treated silages is manifested in decrease of DM losses, butyric acid, acetic acid, alcohol and NH_3-N of total N content.

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Characteristics of ensiled brewers´ grains and the dynamics of effluent release

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Introduction

Brewers‘ grains are one of by-products of brewing industry. After the removal of wort it is that part of the mash that is a cheap and good-quality source of protein for farm animals. Fresh brewers' garins with the 20 % of DM contains approximately 5 % of crude protein, 4 % of crude fibre, 2 % of fat, 1 % of ash and 10 % nitrogešn-free extract. COSTA et al. (1994) **1 kg of** brewers' grains dry matter contains 16.19 % of crude protein, 38.63 % of nitrogen-free extract, 48.60 % NDF and 18.83 % ADF. Of saccharides, glucose and maltose are the most abundant sugars. The content of net energy ranges from 6.1 to 6.7 MJ NEL per kg DM (LOHNERT et al., 1996; SPANN, 1993). Brewers' grains can be fed either in the fresh conditon (within 48 hours) or ensiled. This feed can be also dried but this procedure is costly due to a high consumption of energy.

Silages made of brewers' grains can be given to dairy cows, bulls, pregnant sows and hogs. However, it is rather difficult to enile this feedstuff due to its low content of dry matter and water-soluble sugars and for that reason the problems associtated with its preservation are studied also in many EU countries.

A low content of DM in fresh brewers' grains is the main cause of a high formation of effluents. that there are only few data available about dynamics. runoff and composition of these effluents. The compostion of effluents is different from other ensiled materials and it must be said that they are also an environmental danger.

The aim of this model experiment was to describe dynamics of the runoff of effluents and to follow changes in some selected parameters of effluents originating from brewers' grains.

Material and Methods

In this model experiment fresh brewers' grains with 21.71 % DM was used. This material was transoported from the brewery Černá Hora to the laboratory of the Department of Animal Nutrition and Forage Production, Faculty of Agronomy (MUAF Brno) and treated with two preservatives. There were altogether five variants of this experiment: Variant A – untreated control; Variant B – treatment with the preservative Kemisile (a mixture of organic acids) in the dose of 3L/t; Variant C – treatment with Kemisile in the dose of 6 L/t; Variant D – treatment with the preservative Lactisil (a biochemical preparation with a microbial component + sodium benzoate) in the dose of 4 L/t; and Variant E – treatment with Lactisil in the dose of 6 L/t. All these variants were ensiled and stored in special hermetic containers enabling to measure the production of effluents. The number of containers was the same in all variants and the amount of ensiled material was also the same in all containers. Containers were stored in laboratoy at the temperature of 20 – 25 °C. Sampling and measuring of the amount of produced effluents were performed daily and the following parameters and values were recorded: DM, pH, acid water extract, , FT, ammonia, nitrogen and some

macroelements (Na, K, Ca, Mg and P). Measurements were performed on Day 1; 2; 4; and 8 after ensiling. DM content was estimated by drying to a constant weight at 103±2 °C do. Analytical methods used were peresented earlier (DOLEŽAL, 2002). Results were statistically analysed using the method of unifactorial analysis of variance (SNEDECOR and COCHRAN, 1969).

Results and Discussion

Results obtained in this study on dynamics of runoff of effluents formed in ensiled brewers' grains showed that the highest production of effluents occurred on Day 1 after ensiling in variants B and E. Thereafter the production of effluents gradually decreased. In variants C and D the maximum run off was recorded on Day . In variant D, the production of effluents thereafter gradually decreased till the end of the experiment while in varinat C the production of effluents increased from 60 ml (Day 5) to 112 ml (Days 6). As shown in Fig. 1, another slight increase in production of effluents occurred on Day 8. This figure also indicates that a slight increase in producton of effluents occurred on Day 2 (from 182 ml to 210 ml) in untreated control A; however, in variants C and D this increase was much more intensive, e.g. in variant D from 202 ml (Day 1) to 346 ml (Day 2). As compared wtih all other variants, the slowest production of effluents was observed in variant A.

When analysing the amounts of effluents produced on individual days after ensiling it is possible to conclude that 34 – 64 % of the total amount were produced within the first two days. As compared with control, a more intensive production of effluents was observed in all treated variants within the first few days of the experiment; however, on Day 8 altogether 81.55 % of all effluents were produced in untreated variant A while in variants B, C and D the corresponding abounts were 83.36 %; 82.06 % and 80.27 %, respectively. Till Day 15, the total production of effluents ranged from 92.05 to 98.62 %. The obtained results indicate that in preserved brewer's grains the highest production of effluents takes place immediately after ensiling and that this process is quicker than in the majority of other ensiled materials.

When evaluating the effect of additives on the volume of produced effluents it was found out that both chemicals increased their total volume. In variants B and C, which were treated with an additive containing organic acids, the total amounts of effluents produced per 1 ton of brewers' grains containing 21.71 % of DM were 136 and 137 litres, respectively. In variant E, which was treated with 6 L/t of a biochemical preparation, the total amount of effluents produced from one ton of ensiled material was only 127 litres and that was by 1 liter lower than in untreated control A. The lowest runoff of produced effluents was observed in variant D (123 litres).

Ass compared with control(1.54 ± 0.34 %), effluents produced in treated variants showed in average a higher content of DM: in variants B and C the average DM contents were 2.02 ± 0.05 % and C 2.41 ± 0.19 %, respectively. Higher pH values of effluents were recorded in variants treated with the biochemical preservative (pH valaues of variants D and E were 4.93 ± 0.5 and 4.84 ± 0.61, resp.). The lowest pH (4.22 ± 0.19) was observed in variant C; this corresponded with the additive dose. This variant, as well, showed also the highest acidity of water extract (5,015.0 ± 164.6 mg KOH/100 g).

The estimated contents of sodium, potassium, calcium, magnesium and phosphorus indicate that effluents produced in ensiled brewers¨grains contained 540.4 – 946.13 mg/kg of Ca; 464.2 – 711.27 mg/kg of Mg; 793.3–1,494.17 mg/kg of P; 25,5 – 116.67 mg/kg of Na and 69.37 – 109.14 mg/kg of K.

Conclusions

The rate of runoff of effluents produced in ensiled brewers' grains is high. In our experiment, up to 90 % of the total amount of effluents were produced within the first 15 days of the experiment. As far as the absolute numbers were concerned, the amount of effluents produced per 1 ton of brewers' grains ranged from 123 to 137 litres. The DM content of these effluents was always lower than 3 % and the titration acidity higher than 1,700 mg KOH/100 g. Contents of Ca, Mg and P ranged from 500 – 1500 mg/kg and were several times higher than those of Na and K (20 – 120 mg/kg).

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Determination of volatile components in silages using solid-phase microextraction (SPME)

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Summary

Volatiles from five samples of grass silages and eight samples of maize silages were analysed by gas chromatography-mass spectrometry (GC-MS) after static headspace solid-phase microextraction (SHS-SPME). Using a polyacrylate fibre, 23 volatile components were detected. Mainly phenolic and terpenic compounds were found in both maize and grass silages. These components were identified by Mass Spectral Library (Xcalibur NIST 98/EPA/NIH), only two of them were identified using standards: 4-ethylphenol and 2-methoxy-4-vinylphenol.

Introduction

Solid-phase microextraction is an extraction method involving adsorption of analytes on solid phase deposited on a silica fibre. The extraction of volatile components is achieved by immersing fibre into the liquid to be analysed (L-SPME), or by a simple contact with its headspace under the static conditions (SHS-SPME) (Lord and Pawliszyn, 1998; Cornu, 2001). Silages have been the most widely used bulk feed in developed countries. Volatile components of silages can affect palatability and voluntary intake of these important feeds by animals and moreover, some of them could be transferred to the milk (Toso et al., 2002; Stefanon and Procida, 2004).

Materials and methods

Maize and permanent-grass silages were prepared under laboratory conditions (Steidlová and Kalač; 2003, 2004). A silage sample of 2.5 g was weighed into a 7ml vial. The vial was sealed with a septum. Then, a SPME polyacrylate fibre (Supelco - Sigma Aldrich, CR) was exposed to the sample in the headspace of the vial for 20 min. Finally, the SPME fibre was introduced into hot inlet of the capillary gas chromatograph-mass spectrometer (Finnigan GCQ, Bellefonte, USA). The SPME fibre was held in the port for complete analysis. The components were identified by Mass Spectral Library (Xcalibur NIST 98/EPA/NIH), only two of them were identified using standards.

Results and discussion

Using the SHS-SPME method, 19 volatile components were detected (Tables 1 and 2). Mainly phenolic and terpenic compounds were found in both maize and grass silages. These components were identified by Mass Spectral Library (Xcalibur NIST98/EPA/NIH), only two of them were identified using standards: 4-ethylphenol and 2-methoxy-4-vinylphenol (Figures 1 and 2). Contents of these two components ranged between tens and hundreds mg/kg. Higher content of 4-ethylphenol was found in maize silages, while 2-methoxy-4-vinylphenol prevailed in grass silages. Both components are probably formed during fermentation process from forage constituents - genistein and/or relevant phenolic acids. 4-ethylphenol was identified in maize and grass silages also by Kami et al. (1987,1990) and Kibe and Kasuya (1979) using GC-MS. They isolated numerous volatiles from the silages.

The method proved to be a simple and fast procedure for identification of volatile components from silages.

Table 1 The identified components using SPME headspace method in maize silages

Retention time (min)	Components
3.65	benzaldehyde
4.30	phenylacetaldehyde
5.27	4-ethylphenol*
6.08	4-ethyl-2-methoxyphenol
7.70	azulene derivative

*identified using standard

Table 2 The identified components using SPME headspace method in grass silages

Retention time(min)	Components
2.77	butyric acid
2.85	*cis*-3-hexen-1-ol
3.02	isovaleric acid
3.20	valeric acid
3.65	benzaldehyde
3.93	caproic acid
4.32	methoxymethylbenzene
4.90	phenylethylalcohol
5.12	camphor
5.30	4-ethylphenol*
5.68	6-cyclocitral
6.12	2-methoxy-4-vinylphenol*
7.23	epi-bicyclosesquiphellandrene
7.38	cedrene
7.80	muurolene
8.02	cadina-3,9-diene
9.27	azulene derivative

*identified using standard

Figure 1 4-ethylphenol

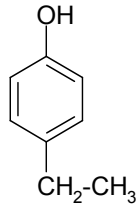
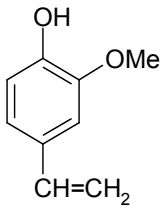


Figure 2 2-methoxy-4-vinylphenol



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