Slovak Agricultural Research Centre, Research Institute for Animal Production Slovak University of Agriculture, Faculty of Agrobiology and Food Resources NutriVet Ltd.

# 13<sup>th</sup> INTERNATIONAL CONFERENCE

# FORAGE CONSERVATION



3<sup>rd</sup> - 5<sup>th</sup> September 2008 NITRA - SLOVAK REPUBLIC

Slovak Agricultural Research Centre, Research Institute for Animal Production, Nitra, SK Faculty of Agrobiology and Food Resources, Slovak University of Agriculture Nitra, SK NutriVet Ltd., Pohorelice, CZ

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Proceedings

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Research Institute for Animal Production Nitra

3<sup>rd</sup> - 5<sup>th</sup> September 2008

# 13th International Conference **FORAGE CONSERVATION** Research Institute for Animal Production in Nitra 3<sup>rd</sup> - 5<sup>th</sup> September 2008

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OPTIMÁLNA FERMENTÁCIA SILÁŽE - VYŠŠIA KONCENTRÁCIA ENERGIE - LEPŠIA STABILITA

PROFITUJTEZKVALITYAPRODUKČNEJÚČINNOSTI VAŠICHSILÁŽÍVYROBENÝCHSOŠPIČKOVÝMI **MOVERENÝMIPRÍPRAVKAMIBONSILAGEODFIRMY** 



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# PREFACE

Development of production efficient agriculture, which will provide purposeful and effective management of land resources in the country and availability of raw materials of good quality for processing industry, is of top priority in EU countries. The present agriculture in developed countries has not only production functions but also increasingly important position in elimination of negative agro-anthropogenic influences, preservation of biodiversity and creation of cultural landscape. Demands of the society as a whole on creation of food raw materials of good quality with simultaneous maintenance of ecological principles of utilization of renewable resources can be realized only by using scientific and research knowledge. It is obvious that effective transfer of this knowledge from research laboratories into agricultural practice will be limiting factor in further development of agro-food sector.

From the viewpoint of present needs in users and consumer spheres are most of the agricultural production capacities aimed at production of food raw materials of good quality. This production is influenced by basic inorganic and organic inputs into processes in primary production. With regard to losses that arise in the process of transformation making more effective the change of nutrients in feeds to high quality raw materials of animal origin has more and more important position. Important factors in decrease of losses in matter and nutrients during the production of feeds are the new methods of conservation. Within them it will be inevitable to develop new means to achieve marked decrease in losses of matter and nutrients in conserved feeds. By means of new ensilaging additives, microbiological flora, anti-mycotic preparations and new methods it will be possible to achieve minimum differences between native and conserved feeds.

Within this context is the 13<sup>th</sup> international conference on feed conservation a continuation of the tradition of previous meetings of experts from institutions, universities and users aimed at exchange of new knowledge in the sphere of technological viewpoints of forage crops production, optimum structure and choice of forage crops for particular localities and production objectives, technical and agrotechnical problems of growing, treatment, harvesting and conservation, microbiological control of fermentation process as well as improvement of nutritive value in feeds and techniques of feeding.

Participation of outstanding native and foreign specialists is a guarantee that the conference will contribute not only to development of new knowledge but it will contribute also to more effective transfer of scientific and research information into the user's sphere of service and primary production.

Nitra, 5<sup>th</sup> August 2008

doc. RNDr. Ján Rafay, CSc. Managing Director of the Research Institute for Animal Production in Nitra

# PLENARY PAPERS

## FORAGE PRODUCTION AND UTILIZATION IN AN ERA OF CLIMATE CHANGE

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#### **INTRODUCTION**

Climate change is a topic that is high on the international political agenda and it has entered the public consciousness as a subject of global environmental concern. Uncertainties about the extent and seriousness of the effects of future climate change and its implications for agriculture, especially when set against a background of growth in world population and the need to reduce dependence on fossil fuels, has led to food security becoming a new priority for policy makers (Defra, 2008; FAO, 2008a).

Projected population and socio-economic growth is predicted to double the current food demand by 2050 (FAO, 2008b). World prices of grain and other livestock feedstuffs have risen appreciably in recent years, propelled by rising demand from developing countries and exacerbated by production deficits linked to drought and to the switch to biofuel crops in exporting countries. In the context of European agriculture the economic value of forage crops for ruminants is likely to increase in line with agricultural commodities, especially if more grain and other alternative feeds are required for direct human consumption or as feed for pigs and poultry, rather than for cattle. Thus, the possible effects of climate change on forage production in Europe in the 21st century need to be considered in the context of 'an era of climate change'. This is likely to be an era characterized not just by climate change itself, but by changes in the global demand for food and non-food crops, with potentially major disruption to agriculture in some of the world's most populous regions and agriculturally marginal areas. Agriculture will need to embrace new technologies and adapt to policies and practices aimed at raising production and mitigating the causes and impacts of climate change, and that incorporate measures to maintain the sustainability of water, soil and energy resources, while at the same time delivering on increasing standards for environmental protection and food quality. In this paper we review the evidence on the causes of climate change, its possible impacts on forage production, quality and utilization in Europe, the opportunities for changes in integrated management to both adapt to the effects of climate change and to reduce the emissions of 'greenhouse gases' associated with forage and ruminant production. Finally, we consider the impact of indirect effects of associated measures, such as global markets and land requirements for non-forage crops.

## Recent and future climate change and the role of greenhouse gases

Climate change is not a new phenomenon. Europe has experienced many periods of alternate warming and cooling during the Quaternary era, including several episodes during recent centuries. North Africa and the Levant were agriculturally productive areas in the Roman period, and an early Medieval warm epoch which was followed by the Little Ice Age occurred as recently as the sixteenth century (see review by 't Mannetje (2007)). Evidence that warming of the earth's atmosphere is now taking place seems unequivocal, although the causes remain uncertain. The Third and Fourth Assessment Reports of the Intergovernmental Panel on Climate Change (IPCC, 2001a,b; 2007) conclude that most of the increase in global average temperature since the mid-twentieth century are 'very likely' (i.e. >90% probability) due to increased concentrations of greenhouse gases (GHGs) - principally carbon dioxide, methane and nitrous oxide – anthropogenic emissions of which have increased greatly since the nineteenth century. These reports detail the extent of recent global change. Key indicators of change (increased mean temperatures, changes in patterns of precipitation, increased cloud cover, more frequent floods and other extreme events) are also widely accepted to be due mainly to the radiative forcing effects of increased concentrations of atmospheric GHGs, exacerbated by land-use changes. Carbon dioxide (CO<sub>2</sub>) is the principal GHG by virtue of its relatively high atmospheric concentration (at the present time 379 ppmv, having increased by over 30% since ca. 1750). Methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O) have also increased by similar proportions over the same period. Although they are less abundant in the atmosphere at 1774 ppbv and 319 ppbv, their global warming potential is respectively 23 and 296 times greater than  $CO_2$  over a 100-year timescale, and a high proportion of their emissions are derived from agriculture. Improving agricultural management to reduce emissions of these gases is therefore part of the overall package of measures to limit the extent of future climate change.

It is estimated that during the past century there has been a 0.74°C increase in global average temperature. The effect in recent years has been most pronounced with 11 of 12 years during the period 1995-2006 ranking among the top 12 warmest years since instrumental records began in 1850. A series of model-based projections for the future has been developed, based on different socio-economic emissions scenarios (IPCC, 2001a,b). For all scenarios, over the next two decades a temperature increase of 0.2°C per decade is projected, with an associated increase in the frequency of warm spells, heat waves and heavy rainfall events considered very likely (90% probability), and an increased incidence of droughts and cyclones considered likely (60% probability). Taking the example of the B1 scenario (relatively low emissions and characterized by rapid economic growth with resource-efficient technologies and a large service economy but population increasing to 9 billion by 2050) a 21st century increase in temperature of 1.8 °C is estimated, leading to a sea-level rise of 18-38 cm. However, higher emissions-scenarios lead to projections of far greater increases in global temperatures and associated climate impacts. The term 'dangerous climate change' has been adopted to

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describe possible outcomes in which ecosystems and food productions systems cannot adapt and sustainable economic development is threatened (discussed by Scheider and Lane, 2005).

### Direct impacts and adaptations of future climate change for forage production

First, let us remind ourselves of some fundamental biology: the main limiting factors of soil and climate that affect forage production and quality. Most temperate crops, including forages, require an adequate soil moisture (either from rainfall or irrigation, the supply and retention of which depends on soil texture), solar radiation and daylength, daytime temperatures in the range 6-20°C during the growing period, an adequate supply of nitrogen and other essential soil nutrients, and atmospheric CO<sub>2</sub> as the carbon source for photosynthesis. Low values or deficits in any of these will invariably limit photosynthesis and growth. At present the number of grass-growing days per year can vary from <200 to >300 over relatively short distances depending on oceanicity/ continentality and on soil moisture deficits linked to soil texture and rainfall distribution (Lazenby, 1981; Peeters and Kopec, 1996). Excesses of temperature (heat waves) and precipitation (heavy rain storms) can cause physical crop damage and prevent utilization. Future changes in temperatures, rainfall and CO<sub>2</sub> concentration and their effects need to be considered in this context.

### Effects of elevated temperature and CO<sub>2</sub>

If temperatures are sub-optimal while soil moisture and all other crop requirements are non-limiting, then temperature is the limiting factor and increasing temperature will increase the rate of plant growth. However, if all other crop requirements are optimal then atmospheric CO<sub>2</sub> may be the limiting factor. Several reviews have concluded that elevated CO<sub>2</sub> concentrations stimulate photosynthesis, leading to increased plant productivity and modifying water and nutrient cycles (Kimball *et al.*, 2002; Nowak *et al.*, 2004; Soussana and Lüscher, 2008). Experiments under optimal growing conditions have shown that doubling CO<sub>2</sub> concentration can lead to a 0.30-0.50 increase in leaf photosynthesis in C<sub>3</sub> plants, and an increase of 0.10-0.25 in C<sub>4</sub> plants, with above-ground canopy increases in excess of 0.17 occurring in grassland ecosystems (research reviewed by Soussana and Lüscher, 2008). Plant functional groups also differ in their response to enhanced CO<sub>2</sub> and both legumes and non-legume forbs are reported to be more responsive grasses (Lüscher *et al.*, 2005). Thus, climate- changed-induced effects of higher temperatures and enhanced CO<sub>2</sub> in situations where water is not the limiting resource, as commonly occurs in northern and north-west Europe, could be generally positive for forage production. Increased concentrations of CO<sub>2</sub> may also modify the plant species balance of grasslands and grassland responses to the effects of changes in temperature and in water availability.

#### Effects of changes in precipitation and extreme weather events

For most parts of Europe changes in mean annual precipitation are expected to be small (Table 1) and under most emissions scenarios these are within the range of natural variability. However, shifts towards a higher proportion of the annual rainfall in winter, and less in summer, have the potential to increase the frequency of years with summer drought stress, leading to reduced security of crop yields on non-irrigated land. This is exacerbated by higher temperatures that lead to increased evapotranspiration. More important for strategic planning, both at the farm scale and in a regional context, is the increased frequency of extreme events: rainfall occurring in storms showers of greater intensity resulting in crop damage, problems with cultivation or harvesting and rapid run-off on slopes (soil erosion and flooding), and increased leaching of nutrients, especially N. Increased frequency of exceptional prolonged hot, dry spells also pose risks for feed budgeting where the normal management plan is to conserve surplus summer forage to meet livestock requirements during the winter housed period within a one-year cycle.

#### Net climate change effects for European forage production

Table 1 summarizes the key features of 21st century climate change for Europe and highlights some of the geographical variation in anticipated effects. The values for increased temperatures vary considerably according to the emissions scenarios; best estimates are that by 2100 global temperatures will increase by between 1.4 and 4.0°C (based on low and high emissions of GHGs, respectively) and that CO<sub>2</sub> concentrations could reach 500-900 ppmv IPCC (2001a; 2007). Estimates of sea-level rise due to thermal expansion of sea water and from melting of Polar ice range from 18-38 cm (under low GHG emissions) and 26-59 cm (under high emissions). The implications of sea level rise are greatest for areas currently close to sea level (particularly the valuable grassland areas of Netherlands and parts of eastern England) and land bordering estuaries in northern Europe). In general terms, agricultural vulnerability in Europe is likely to be less than in many other drought-prone or flood prone areas of the world. However, impacts are potentially most serious in Mediterranean and southern Europe due to summer heat and drought, and also at the highest latitudes and elevations, where natural ecosystems such as wetlands, tundra and periglacial vegetation are threatened, and changes in snowfall affect summer water availability (Schroter et al., 2005). Climate-change-induced effects such as coastal and river flooding, droughts, and threats to some vulnerable habitats are likely to be felt locally. However, the effects of higher temperatures and enhanced CO<sub>2</sub> in situations where water is not the limiting resource will generally be beneficial and the limits of productive agriculture will be extended northwards. In contrast, in parts of southern Europe, water shortages are likely to lead to increased risks of harvest failures and possible further abandonment of agriculture.

# Table 1. Some Key Features of Climate Scenarios for Europe

# Temperature

- Annual temperatures increase by 0.1-0.4°C per decade. Effect greatest over southern Europe (Spain, Italy, Greece) and northeast Europe (Finland, western Russia) and least along the Atlantic coastline.
- Rapid winter warming (0.15–0.6°C per decade) of continental interior of eastern Europe. In summer, southern Europe warming 0.2-0.6°C per decade and northern Europe warming 0.08-0.3°C per decade.
- Less frequent cold winters (i.e. 1-in-10-year events during 1961–1990) and disappearing almost entirely by the 2080s. Hot summers become much more frequent. Under the 2080s scenario, nearly every summer is hotter than the 1-in-10 hot summer as defined under the present climate.
- All model simulations show warming in the future across the whole of Europe and in all seasons.
- Alternative scenario, at present considered low-risk (but with a high potential impact) of regional cooling due to shutdown of Atlantic thermohaline.

# Precipitation

- Increased annual precipitation in northern Europe (+1-2% per decade), small annual decreases across southern Europe (maximum -1% per decade), and small or ambiguous changes in central Europe.
- Marked contrast between winter and summer patterns of precipitation change. Most of Europe gets wetter in the winter season (+1-4% per decade), except in Turkey and Balkan region. Pronounced summer north-south gradient: northern Europe increases by 2% per decade, and southern Europe decreases (up to -5% per decade).
- Only for the A2-high GHG emissions scenario are there substantial areas (Fennoscandia and north-west Europe) where precipitation changes by the 2020s are larger than might occur within natural climate variability. Even for this scenario with rapid global warming, not all regions in Europe have well-defined precipitation signals from GHG-induced climate change by the 2080s.

# Weather Extremes

• It is considered likely that frequencies and intensities of summer heat waves will increase throughout Europe; that intense precipitation events will increase in frequency, especially in winter, and that summer drought risk will increase in central and southern Europe; and possible that gale frequencies will increase.

### Sea Level

- Although global-mean sea level may rise by up to 70 cm during 21st century, due to thermal expansion and glacial melt, regional effects in Europe will vary because of post-glacial tectonic movements.
- Potential interactions of sea-level rise with increased storms and tidal surges.

Sources: Kundzewicz and Parry (2001); Hulme et al. (2002); Wood et al. (2005); IPCC (2007)

# **Regional impacts and potential adaptations**

### Northern, north-western and central lowland Europe

The effects of higher temperatures and enhanced  $CO_2$  in situations where water is not the limiting resource could be generally positive for grassland and most forage crops. A UK study involving sward growth experiments under elevated  $CO_2$  and its interaction with temperature, linked to regional and livestock enterprise scenarios applied to the LEGSIL grassland model, has revealed the likelihood of several impacts (Topp and Doyle, 1996; Hopkins *et al.*, 2003; Harmens *et al.*, 2004; Hopkins, 2004; Scholefield *et al.*, 2005). These showed:

- Increased herbage growth potential: compared with ambient CO<sub>2</sub> and temperature, dry matter (DM) yield from permanent grassland swards cut frequently was enhanced in the short-term by elevated temperature, elevated CO<sub>2</sub> and elevated CO<sub>2</sub> + temperature by 0.30, 0.46 and 0.56, respectively.
- There are changes in herbage quality with higher content of water-soluble carbohydrate and lower N content (crude protein) at a given yield.
- The overall effects of future climate change will lead to a proportionately greater production increase from legume forages, particularly red clover and lucerne, than grasses. However, there could be reduced opportunities for utilization (harvesting machinery or grazing) during periods of high rainfall, especially on poorly drained soils.
- An increased incidence of summer drought would, at its most serious, off-set the advantages that may arise.
- Nutrient leaching may be increased due to increased winter rainfall, but with some potential for improved N use by the growing sward and the animal.
- Farm-scale adaptive responses could include greater reliance on conserved food for housed livestock, increased use of maize, greater use of forage legumes in place of N-fertilized grass, increased need for manure storage and improved applications, increased demand for irrigation (although with greater pressure on water resources this option may be unavailable in many areas). Feed budgeting for dry seasons could require alternative forage

species / mixtures adapted to drought (*Cichorium intybus, Medicago sativa*, and grasses such as *Dactylis glomerata* and *Festuca arundinacea* and managed accordingly, or for introducing traits into existing widely used grasses and legumes).

Adaptive responses have further implications for N<sub>2</sub>O and CH<sub>4</sub> emissions (discussed in section on mitigation responses).

Effects of seasonal changes in precipitation will vary according to soil type; on sandy soils where temporary grassland is commonly sown the seasonality of production could be greater with a mid-season feed deficit. Conserved forage could be split between a high quality spring cut and a poorer quality late-summer cut, with some potential management issues with machinery and soil compaction (Søegaard et al., 2007). An increase in the use of forage legumes in response to warmer conditions that enable earlier growth and rhizobial-N fixation would also reduce the reliance on mineral N fertilizers. The high protein values of legumes can also further reduce dependence on non-forage proteins. These advantages apply under grazing as well as crops intended for cutting and conservation. There is already considerable scope, based on multi-site experiments (Doyle and Topp, 2001; Halling et al., 2001) and on economic modelling (Topp and Doyle, 2001) for profitably increasing the role of forage legumes for silage conservation in this region of Europe. There is also potential for increasing the area used for maize (Zea mays) silage where improved growing conditions allow this, as shown by outcomes from several modelling studies; e.g. Holden and Brereton concluded that in Ireland, where maize has until recently been a marginal crop, under future climate change scenarios it could become a major crop. In recent years there has also been an expansion in the use of whole-crop silages in northern and north-west Europe, based on wheat or combinations of grain crops and peas (Pisum sativa). These have the potential for early season growth in response to warmer winter and spring temperatures, and some flexibility at the time of harvest, which fits well within the potential for extended growing season as presented by climate change.

Irrigation to overcome short-term soil moisture deficits may continue as a management option in areas where water supplies are available. However, it seems likely that water security will become an increasing problem in an era of climate change, and the environmental implications and well as the economics of its use to support forage are likely to prevent its widespread use.

On permanent grasslands shifts in botanical composition are likely, especially in response to summer droughts. Deep-rooting forb species such as *Rumex obtusifolius* can respond vigorously to drought situations, developing woody stems. If not controlled, their contribution in a second or third silage cut in a dry season can be very high, leading to very poor overall feed quality and physical damage to the clamp seal or the film of wrapped bales.

Extensive areas of peaty soils and other wet grasslands remain in areas of central and eastern Europe, often with very high botanical diversity and nature conservation importance (Zimkova *et al.*, 2007). Overall, this region holds great potential for increased agricultural productivity. However, cultivation and drainage, or drying out of the peat under warmer and drier weather conditions, could lead to their loss as a fodder resource, or greatly reduced forage yield potential. Of greater significance in the overall context of climate change could be the loss of the carbon sink that these areas represent. Their future management that balances their ecosystem functions with agricultural use is therefore an important consideration.

#### The Mediterranean zone

In many areas of the Mediterranean region there is already a problem in supplying forage during the summer dry season. This is likely to become greater with hotter, drier and more variable conditions ('t Mannetje, 2007). Water limitations will prevent potential benefits of CO<sub>2</sub> enhancement from being realized. Irrigation offers a practical measure for greatly increasing the production of forage legumes (especially lucerne, Medicago sativa) and grasses, but under future scenarios with higher temperatures and more frequent droughts, combined with other demands on water supplies, this cannot be relied upon as a sustainable option. The use of annual legumes to supply feed in the winter growing season, with increased areas allocated for conserved forage where the terrain allows, can help meet the summer feed gap. A number of alternative legume forage species have been used in other areas of the world (e.g. Australia) that have a Mediterranean climate (see review of Sulas (2005)). An integrated land management approach will be needed to maintain agriculture in the Mediterranean zone. This will need to incorporate soil and water protection, management to reduce the risk of wildfires in shrub and browse communities, to ensure high quality silage is available to support utilization of low quality forage in dry periods. New forage resources are required that are adapted to higher temperatures and increased  $CO_2$ . In addition to new legumes a shift to communities with more  $C_4$  grass species is a likely successional outcome in semi-natural Mediterranean grasslands but their feeding value is lower than C<sub>3</sub> species ('t Mannetje, 2007). There is a need to develop strategies for incorporation of  $C_4$  grasses, ideally with improved germplasm, into ruminant production systems.

#### Alpine and sub-alpine grasslands

The Alps and other montane areas of Europe face particular problems in maintaining sustainable mountain farming systems, with large areas facing abandonment for economic reasons. Even in an era of global food shortage the higher cost of production in the mountains means that products have to compete on quality and distinctiveness linked to the feed source, rather than on price. Warmer conditions are already showing changes in botanical composition, and reduced permafrost at high elevations carries increased risks of natural hazards. A number of studies have shown that alpine grasslands experience greatly reduced biomass yield in response to summer drought conditions (as occurred in

2003) and in experiments with rain-out shelters that simulate the precipitation-reduction element of future climate change (Gilgen and Buchmann, 2008). Reductions in biomass on the scale reported by Gilgen and Buchmann (2008) (from 1800 kg to 550 kg/ha) would have serious consequences for the economic sustainability of high alpine grasslands. On the other hand, the wetter grassland soil conditions that are more common in the pre-Alps are less susceptible to drought. Adapting alpine agriculture needs to incorporate a range of objectives, and a climate-change adapted agriculture in the Alpine areas needs to be based on meeting a range of ecosystem services and public goods (Nösberger and Biala, 2006).

### Management to mitigate Greenhouse Gas emissions

Grassland-based agricultural systems contribute to the biosphere-atmosphere exchange of radiative-forcing gases, with fluxes intimately linked to management practices (Soussana *et al.*, 2004). The FAO report *Livestock's Long Shadow* (FAO, 2006) concluded that the world's livestock industry contributed greatly to GHG emissions, through forest clearance (in Latin America) and emissions of  $CH_4$  and  $N_2O$ , but that there are large opportunities for adapting management to mitigate these emissions. Governments, advisers and farmers are likely to come under increasing pressure to reduce the environmental impact of ruminant production, and changes in forage-based ruminant agriculture are likely to be required to mitigate  $CH_4$ ,  $N_2O$  and  $CO_2$  emissions.

#### Carbon dioxide

Reduced direct emissions can be achieved through reduced or more efficient use of mechanical operations, and indirect  $CO_2$  emissions can be cut by reducing the inputs of artificial fertilizers. On the other hand, the growing of crops and pasture, the maintenance of other farmland vegetation (hedges, trees, scrub etc.) and the accumulation of carbon as organic matter in soils can all contribute to the temporary removal, and in some cases to the long-term sequestration, of  $CO_2$  from the atmosphere (Freibauer *et al.*, 2004; Smith, 2004). Measures to reduce net- $CO_2$  emissions include improving efficiency of animal manures and crop residues, reducing soil disturbance, maximizing the C returns in manure, use of deeper rooting species, application of sewage sludge or compost to land, irrigation, extensification, and improved management to reduce wind and water erosion. Permanent pasture and non-till systems are favoured over annual cultivations. High organic-matter soils (peats) are a particularly important carbon sink and their management to avoid C loss will be essential. The increase in soil C content after a shift from arable to grassland is partly explained by a greater supply of C to the soil under grass, mainly from the roots, but also from the shoot litter (Soussana *et al.*, 2004). Whereas this rate of increase of soil C after conversion to grassland is slow, the rate of C disappearance from soil after returning grassland to arable is rapid. A grassland sward aged more than 20 years may no longer act as a C sink (Frank, 2002). Introducing short duration leys tends to have an intermediate C sequestering potential between crops and permanent grasslands (Soussana *et al.*, 2004).

#### Methane

Enteric fermentation is the main agricultural source of methane in Europe, with emissions from livestock manures accounting for most of the rest. Methane is produced as a by-product of digestion of structural carbohydrates, due to the action of rumen microbes (bacteria, fungi and protozoa). During this digestion, mono-saccharides are fermented to  $H_2$ , CO<sub>2</sub> and volatile fatty acids (VFAs). As part of this stage of ruminant digestion some of the microbes (methanogens) produce CH<sub>4</sub>. Several studies have formulated abatement strategies to mitigate CH<sub>4</sub> emissions. Mitigations aimed at enteric fermentation may be addressed at three different levels (Jarvis 2001): dietary changes, direct rumen manipulation, and systematic changes. The dietary changes involve measures which enhance the efficiency of feed energy use, and this is one area which has potential implications for forage use in the future (Cardenas et al., 2007). Even assuming a constant percentage of methane loss, this strategy will decrease methane loss per unit of product and probably decrease CH<sub>4</sub> emissions in the long term (Johnson and Johnson, 1995). The most natural way to depress CH<sub>4</sub> production would be to manipulate the diet to give high rates of fermentation and/or passage through the rumen, affecting rumen VFAs. These changes in VFA proportions have been associated with a decrease in the fibre content of the diet (by including maize silage). Ingestion of organic acids (aspartate, malate and fumarate) and yeast culture have been associated with reduced emissions in total CH<sub>4</sub> per cow and also with beneficial increases in animal product (Hopkins and Del Prado, 2007). The use of some plant extracts (i.e. tannins, saponins) has also been associated with CH<sub>4</sub> reduction (Sliwinski et al., 2002; Hess et al., 2003; Carulla et al., 2005; Hu et al., 2005; Puchala et al., 2005). There may be considerable potential for tanniniferous legumes such as *Lotus* species, but further research is needed on their effectiveness.

There are some drawbacks to using dietary supplements. The organic acids are not yet commonly used, and they may also trigger pH problems in the rumen. Plant extracts may also have anti-nutritional effects and even be toxic (Teferedegne, 2000). For instance, in a study by Hess (2005), extracted tannins had a positive effect on feed rates and hence a possible reduction of  $CH_4$  per kg product, whereas the use of shrub legumes rich in tannins resulted in decreased feed rates. Yeast culture, on the other hand, although variable, may be promising as a successful mitigation option as it is already in common use.

Direct rumen manipulation may offer an alternative to dietary change; for instance, defaunation of protozoa may decrease the number of methanogenic bacteria as an important proportion of rumen methanogenic bacteria are parasitic to protozoa (Takahashi, 2005). However, there are many drawbacks including risks of metabolic disorders. The ingestion of ionophores acts as propionate enhancers and hence increases the ratio of propionate: acetate. Their use is

very limited as they are antibiotics. Some changes in the dietary fat contents of the ration have been described to reduce  $CH_4$  emissions from ruminants (Johnson *et al.*, 2002; Giger-Reverdin *et al.*, 2003) as some fats alter the ruminal microbial ecosystem and, in particular, the competition for metabolic  $H_2$  between the  $CH_4$  and propionate production pathways (Czerkawski, 1972). Clearly, many research challenges exist before these approaches can be implemented.

Systematic changes may involve identifying animal breeds which result in a reduction of  $CH_4$  output per animal, though so far no clear evidence has been found (Münger and Kreuser, 2005). Increasing productivity per head (i.e. milk yield per cow), or increasing the number of lactations for which the average cow remains economically productive, would decrease  $CH_4$  production per unit of milk, and within the framework of production targets would decrease total  $CH_4$  emissions. Although more intensive forms of animal production tend to decrease total  $CH_4$  output, they might not be compatible with other policy targets.

Mitigations aimed at manure management include opportunities to decrease total  $CH_4$  outputs from farming systems are limited to either increasing the  $O_2$  supply to restrict methanogenesis, minimizing the release of  $CH_4$  to the environment (e.g. covered lagoons) or using anaerobic digesters to produce more  $CH_4$  in a controlled environment and hence use this  $CH_4$  as a source of energy. This last technique could represent a sustainable option, and if the issues of high capital cost can be overcome this may become an important feature of future forage-based systems compatible with low  $CH_4$  emissions.

#### Nitrous oxide

Nitrous oxide is formed in the soil through nitrification and denitrification and is controlled by a number of soil factors, including moisture content (del Prado et al., 2006c), temperature (Hatch et al., 2005), fertilizer additions (del Prado et al., 2006c), pH (Merino et al., 2000), organic matter content (Smith et al., 1997; Chadwick et al., 1998), nitrate and ammonium (Tiedje, 1988; Granli and Bockman, 1994).

Nitrate and ammonium in the soil are subject to several process dynamics (Hopkins and del Prado, 2007). In general,  $N_2O$  emissions can be reduced by implementing practices aimed at enhancing the ability of the crop to compete with processes that lead to the escape of N from the soil-plant system (Freney, 1997). For instance, there are several methods for increasing the efficiency of the crop to remove mineral N from the soil. These include improving fertilizer efficiency (Brown *et al.*, 2005), optimizing methods and timing of applications (Dosch and Gutser, 1996), using ammonium-based fertilizers rather than nitrate-based ones (Dobbie and Smith, 2003) and employing nitrification chemical inhibitors (Dittert *et al.*, 2001; Merino *et al.*, 2002; Macadam *et al.*, 2003).

Increasing the soil aeration may significantly reduce  $N_2O$  emissions. Improving drainage would be particularly beneficial on grazed grassland (Monteny *et al.*, 2006). Hence, avoiding compaction by traffic, tillage (Pinto *et al.*, 2004) and grazing livestock may help to reduce  $N_2O$  emissions. Housing system and management will also influence  $N_2O$  emissions, e.g. straw-based manures result in greater  $N_2O$  emissions than slurry-based ones (Groenestein and Van Faassen, 1996). Minimizing the grazing period is likely to reduce  $N_2O$  emissions as long as the slurry produced during the housing period is uniformly spread. Livestock diets also affect the  $N_2O$  emissions from slurry subsequently applied to land (Cardenas *et al.*, 2007).

#### The need for integration

There is need to develop useful tools in order to explore agricultural systems in a holistic way. The previous section has considered that although forage-based agriculture contributes to the total of GHG emissions there are many practical and potential solutions that can help reduce these impacts. Agriculture is also subject to other pressures, including environmental (biodiversity, eutrophication, erosion, and acidification), socio-economic and sustainability issues. Identification of 'win-win' strategies requires development of appropriate modelling systems together with the acquisition of field and farm data (Scholefield *et al.*, 2005).

Modelling studies have been developed to (i) assess the effects of different dietary strategies on the sustainability of a grassland system (del Prado *et al.*, 2006a), (ii) evaluate the economic cost for implementing mitigation strategies for GHGs (Jarvis, 2001), (iii) evaluate the impact of NO<sub>3</sub> leaching abatement measures on N<sub>2</sub>O, NH<sub>3</sub> and CH<sub>4</sub> emissions (Brink *et al.*, 2005; del Prado *et al.* 2005), (iv) assess successful mitigation strategies for GHGs (Schils *et al.* 2005), and (v) evaluate not only environment (N<sub>2</sub>O, CH<sub>4</sub>, NH<sub>3</sub>, NO<sub>3</sub><sup>-</sup> and P leaching) and economics, but other attributes which define the sustainability of a farm.

For instance, using the SIMS<sub>DAIRY</sub> model (del Prado *et al.*, 2006b) we compared environmental losses, milk yield and milk properties in two typical dairy farms in the UK (2 LU ha<sup>-1</sup>, 30.5 ha grazed grass, 16.5 ha cut grass and 3 ha maize) which differ only in terms of their past grassland management. Whereas the baseline farm had a history of longterm grassland with old swards (>11 years), the second farm had a history of short-term grass leys with new swards (2-3 years). As indicated in the previous sections, the baseline farm's past history would offer more opportunities for C sequestration than would the history of the second farm. However, if we compare the predicted environmental losses of the SIMS<sub>DAIRY</sub> model, we find that NO<sub>3</sub><sup>-</sup> leaching (24%) and N<sub>2</sub>O (6%) per L of milk were significantly lower from the second farm than from the baseline farm. Milk yield and milk properties (butterfat and protein) were also slightly improved, which may have a modest impact on the economy of the dairy farm.

However, greenhouse gas and other emissions account for only a part of the whole challenge facing grassland and forage-based systems as we move into an era of climate change. Policy makers are increasingly becoming concerned with energy security, food security and management of water resources. Climate change effects that occur outside of Europe have implications for European food policy, especially if world commodity prices continue to increase.

### CONCLUSIONS

There is strong evidence that the climate is warming and that this is associated with increased atmospheric concentrations of carbon dioxide, methane and nitrous oxide. Uncertainties remain about the effects and timescales of climate change, and its regional variations. The effects may result in increased forage yields, particularly of legume crops, mainly through warmer temperatures and higher carbon dioxide concentrations. However, these gains could be offset by more frequent droughts and other extreme weather events affecting both production and utilization. Low-lying coastal areas are at risk of inundation from rising sea levels and could be lost permanently. Reductions in forage yield are likely to be greatest in semi-arid areas especially in the Mediterranean region, and on some Alpine sites, and integrated management policies are needed to ensure a future for sustainable agriculture in these areas. At the farm scale, the adoption of locally appropriate forage resources, including  $C_4$  species, will be essential. Legumes are likely to have a greatly increased role and their use will offset the needs for other protein feeds and mineral N fertilizers. Irrigation opportunities are likely to be limited. Management should ensure seasonal feed budgets through adequate reserves of standing crops or conserved forage, particularly of high quality silage, with a planned carry-over of feed between seasons to allow for unexpected feed deficits. Well-made wrapped baled silage offers an ideal solution to this requirement.

Grasslands, particularly permanent grasslands, represent an important sink for the sequestration of carbon dioxide. This could be lost if there are pressures to increase the area sown with cereals, maize or other arable crops. There is, however, likely to be pressure for measures to reduce the emissions of methane and nitrous oxide from ruminant production. Knowledge on this topic is increasing but many research challenges remain and measures to reduce greenhouse gas emissions need to be considered as part of an integrated approach to environmental and agricultural objectives. Manipulation of livestock diets in this context may restrict future forage production options. At national and regional scales, policy makers need to ensure that decisions on land and environmental management, and on future food security, are based on evidence-based research and expert advice in order to ensure a sustainable balance of forages, arable crops and biofuel crops, consistent with broader economic and environmental objectives. Research funders also need to ensure that climate change implications are factored into future projects.

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## CONTROL OF THE FERMENTATION PROCESS AT THE CONSERVATION OF FEEDS

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#### INTRODUCTION

All actions to control the fermentation process during the storage period have to be taken in advance, before filling the silo. All actions except one, the creation of anaerobic conditions through packing and sealing the silo. That is, of course, when we deal with desired fermentations processes. There is also a definite way to control the fermentation process by suppressing it totally: with further drying or by the addition of chemicals. These measures can halt the deterioration process in a feed that is not completely dry. This paper, however, deals with feed conservation based on the spontaneous fermentation process and how it can be controlled.

We have several aims with our wish to control the fermentation process. One is to minimize the energy losses during the storage period. By controlling the chemical pathways taking place when carbohydrates are transformed to fatty acids under anaerobic conditions, losses could be manipulated. Another aim is to influence the degradation of proteins and other nitrogenous compounds in a way so they are favourable in relation to the nutrient needs of the animals at feeding. Yet another aim during the fermentation process is to avoid the development of microbes that could be detrimental to the animal or to products such as milk or meat.

In the present paper we will discuss some of the well-known factors that influence the fermentation process. We will do it from the practical viewpoint and illustrate it with research results from our part of the world, northern Europe, where we mainly ensile perennial leys of grass and clover.

#### INFLUENCING THE FERMENTATION PROCESS IN PRACTICE

Of course the crop is of basic interest the type of fermentation process that can be expected. The substrate content and buffering capacity of the crop influence the basic conditions for ensiling together with the physical structure of the material. However, the choice of crop is often based on other motives than its ensilability and our mission is to preserve the crop as well as possible. The aim is therefore to try to control the fermentation in the best possible way for the given crop.

#### Dry matter

The most dominating, and therefore most important factor in controlling the fermentation in a given crop is the dry matter (DM) content of the crop. It is also a factor that is possible to affect. Increased DM content involves an increased substrate concentration in crops low in sugars such as clover and lucerne facilitating the ensiling process. Increased DM content also favours the growth of lactic acid bacteria (LAB) and, in particular, restricts the development of *Clostridia tyrobutyricum*. The earlier works by Silliker et al. (1980) stating that *Cl. tyrobutyricum* is inhibited at water activity below 0.94 and Hengeveld (1983) showing that DM contents above 40-45 % inhibits clostridia growth in silage are well confirmed by recent experiments by Kaiser et al (2005) and Pauly et al (2008), both clearly showing that a high DM content inhibits the development of *clostridia*.

When discussing pre-wilting systems one often come into the debate whether it is better to pre-wilt in windrows or pre-wilt with the crop wide-spread. By wide-spreading the desired DM content is reached faster but the system implies a greater hazard involving soil contamination of the crop at tedding and by driving over the harvested crop with the tractor. The current recommendation to farmers is to leave the crop in windrows after the mower-conditioner and let it wilt to the desired DM content without moving it until collecting it with a chopper, loader wagon or baler. Recent research indicates, however, that even when manure is spread on the ley in the spring, the use of wide-spreading technique does not contribute to the contamination of the silage (Nysand et. al., 2008). Own experiments also clearly demonstrate the advantages with the wide-spreading technique. A typical development of the DM content in the crop is illustrated in Figures 1 and 2.

The consequence of wilting in windrows without spreading and tedding is that the bottom layer of grass will keep its original high DM content for a long time. Protected from sun radiation and close to the soil the environment stimulates the growth of detrimental microbes. Elevated counts of *enterobacteria* were found in the bottom layer of the swaths already after 24 h (Spörndly, unpublished data). The wide-spreading technique either leads to a harvest at higher DM or to a shorter wilting time. In the example, illustrated in Figure 1, the harvest of the wide-spread and swathed crop was made simultaneously, and a better silage quality was obtained in the wide-spread treatment as can be seen in Table 1.





**Figure 2.** DM content in the upper layer (Top) and the under layer (Bottom) in wide-spread and the swathed crop during the wilting process.



	Wide-spread		Swathed		
	Mean	SD	Mean	SD	Sign. $(P <)$
DM content, g/kg	416	24.2	285	17.2	0.001
pH	5.2	0.09	4.8	0.13	0.001
NH <sub>3</sub> -N (g/kg N)	65.1	11.38	109.6	14.87	0.001
WSC, g/kg DM	86.0	11.92	43.2	15.33	0.001
Lactic acid, g/kg DM	15.4	4.69	39.6	10.74	0.001
Acetic acid, g/kg DM	3.6	0.90	8.8	2.89	0.001
Butyric acid, g/kg DM	0.4	0.08	7.3	4.35	0.001
Ethanol, g/kg DM	12.2	3.35	14.7	2.54	0.001
2.3-butanediol, g/kg DM	3.1	0.56	13.9	3.21	0.001
Density, kg DM/m <sup>3</sup>	181	12.8	137	9.7	
DM loss, % of initial DM	1.9	0.61	4.5	12.8	

 Table 1. Silage quality, density and DM losses in round bales produced from a grass crop wilted for 24 hours either wide-spread or in swaths (Spörndly et al., 2008).

#### **Mechanical treatment**

Pauly et al. (1999) showed how increasing mechanical treatment enhanced the homogeneity in the silage mass, increased the lactic acid production and decreased the pH thus steering the fermentation process in the right direction, probably due to a more efficient release of the cell contents. Several studies with a more intensive maceration at mowing have been described (Koegel et al., 1992) but the tested systems, with the mat technique as en extreme (Shinners et al, 1988), have not come into practical use in silage making in northern Europe.

Another form of harder mechanical treatment, the bagged silage technique, has increased in popularity during the last years. With this method, the forage is commonly harvested with a precision chopper and then further mechanically treated by the packing rotor during filling of the bag. Sundberg and Pauly (2005) has performed a number of fermentation studies where the grass has been ensiled in experimental silos before and after it has passed the filling rotor. They have shown that the drop in pH was faster and reached a lower level in the crop that had passed through the filling rotor confirming that mechanical treatment has a beneficial effect on the fermentation process.

A question that is often raised by farmers is how the fermentation is affected by a loader wagon versus a chopper. During the last years several studies have been performed comparing loader wagons (rotor cutters) with precision choppers. Lingvall & Knicky (2008) made a comparison of a Pöttinger loader wagon with a rotor cutter and a Taarup precision-chop wagon. The forage was ensiled in two tower silos. The particle sizes, the silo densities achieved and the fermentation results are illustrated in Table 2. It is evident that no differences between the two mechanical treatments could be found regarding fermentation properties.

Harvest year:	20	04	2005		
Wagon:	Pöttinger	Taarup	Pöttinger	Taarup	
Nominal length, mm	34	25	34	25	
Average length, mm	58	25	56	35	
Highest 25%	99	38	57	39	
Lowest 25%	34	16	20	16	
DM, %	24	23	40	40	
pH	4.0	4.0	4.4	4.4	
Butyric acid, g/kg DM	<0.7	<0.7	< 0.2	< 0.2	
NH <sub>3</sub> -N, g/kg total N	83	78	48	45	
Density, kg DM/m <sup>3</sup>	167	157	275	273	

 Table 2.
 Particle size, density and fermentation result when the same crop was harvested by a Pöttinger rotor cutter wagon or a Taarup precision chopping wagon. From Lingvall & Knicky 2008.

#### **Using Additives**

Many farmers opinion is that the easiest way to control the fermentation is to apply an additive. There are uncountable numbers of inoculants and chemical additives on the market in Europe and the arguments from the industry to use additives are sometimes overwhelming. But additives are costly and in some European areas they are not used very much and in certain areas the use of additives has actually decreased (Mayne and O'Kiely 2005). In Sweden, farmers pay great interest in additives, probably driven by the increasing demands of high milk quality from the dairy industry. The Swedish farmers get price deduction of the milk payment when passing 150 000 somatic cells per ml and when clostridia spores are found in the milk.

Several comprehensive reviews are available, e.g. Kung et al. (2003) and McDonald et al. (1991), covering silage additives. Silage additives with the aim to affect the fermentation process are often classified into categories of their origin and how they act: *Inoculants, Chemicals* and *Nutrients*. From the user's point of view it might however be easier to make the choice by looking at the problem that has to be solved. Does he have problems with *clostridia* spores in the

silage, does he have problems with the aerobic stability getting spoiled silage at unloading the silo, does his silage have too high pH or does he have problems with mould on the silage and is afraid of mycotoxins? Another effect of additives is that they decrease fermentation losses, which is an important advantage for the farmer but often difficult to measure.

#### Additives against clostridial growth

*Clostridia* can multiply in grass silages up to a DM content of 30–40% depending on how evenly the moisture is distributed in the forage (Jonsson et al., 1990). We think that clostridial growth often starts in wet or untreated pockets within the forage. Strong acids such as formic or lactic acid could be very effective in suppressing clostridial growth. The main drawback is that they have to be very evenly distributed within the crop to be effective. The common application technique that sprays the additive on top of the swath just when the crop is fed into the pick-up unit is not able to mix the additive evenly enough into the forage (Sundberg & Pauly, 2000). The distribution in a precision-chop harvester is much better and the treatment effect is usually improved. Excellent, spore-free forage in 90% of the silage is not sufficient if the remaining 10% allow *clostridia* to proliferate. If *clostridia* would multiply to about 10<sup>6</sup> spores / g fresh matter (FM) in only 1% of the silage, then the dairy farmer would have to face a spore problem.

Among chemical additives the ones containing nitrate or nitrite are known to have an inhibiting effect on clostridial growth (nitrate is degraded to nitrite by various common bacteria). Even forage crops contain nitrate particularly after application of N fertilizer. The advantage of nitrite is that it is degraded into different nitrous gases (mainly NO and  $NO_2$ ) that have a strong anti-clostridial activity (Spoelstra, 1985). Gases spread well in silage even if the additive is not evenly applied (e.g. in big bales). That explains the relative stable effect of nitrite-containing additives in big bale silage.

An application of homofermentative lactic acid bacteria (LAB) can inhibit clostridia if growth conditions for LAB are favourable (nutrients available, oxygen-free environment) and numbers of competitive microorganisms (e.g. clostridia, enterobacteria) are low (Honig, 1990). From experience we can estimate the content of fermentable carbohydrates by assessing plant species, weather conditions during the last days and targeted DM content, but a picture of the composition of epiphytic microorganisms on the crop would be difficult to get at the day of harvest. This makes it difficult to estimate the effect of a LAB application. However, if the crop is not contaminated with slurry, manure or soil, if wilting conditions are favourable (less than 2 days, no rain) and if heating during silo filling is avoided, a LAB application usually produces a high fermentation quality.

#### Additives designed for wet silages

The intensity of fermentation is high in wet silages and changes in microbial populations happen fast due to a large variety of competing microorganisms with high growth rates. Strong organic acids such as formic acid have the advantage to slow down the fermentation process, inhibit the bacterial degradation of amino acids and peptides and inhibit plant respiration or heating during silo filling (McDonald et al., 1991). If thoroughly distributed in the forage (e.g. in precision-chop harvester) the effect against clostridial spores is good. This is the reason why formic acid has been extensively used in the Scandinavian countries in the past. Today wet silages are made only when weather conditions do not allow a proper wilting phase. It makes it possible to harvest the ley at its ideal stage of maturity even when weather conditions are unfavourable. Waiting for better weather implies lower energy content and lower digestibility, particularly with grass crops.

Effluent losses can be controlled either by a short wilting period (preferably wide-spread) to reach a DM content above approx. 25-30% (Bastiman & Altman, 1985) or by adding large amounts of rolled grain (approx. 100-160 kg/ton FM) that absorbs the excess moisture (Spörndly, 1986).

#### Additives designed for dry silages that prolong aerobic stability

Particularly drier silages are prone to aerobic instability and often tend to heat during unloading from the silo. These problems can be reduced or eliminated entirely by proper compiling, sealing and covering of the silo. Many farmers rely, however, on additives that inhibit fungal growth (moulds and yeasts) and in that way prolong the aerobic stability of their silages.

Formic acid-based additives are sometimes supplemented with propionic acid to add anti-fungal activity and widen the recommended DM range. Compared to benzoic and sorbic acid, much larger amounts of propionic acid are needed to give comparable anti-fungal effects (Woolford et al., 2001).

Most chemical additives or supplements that aim to improve the aerobic stability, are based on Na-benzoate or Ksorbate, two additives that are found in many berries and that have been used a long time in the food industry for their anti-fungal activity. The main anti-fungal effect of these compounds is related to the amount of undissociated acid, which in turn is pH-dependant (Baird-Parker, 1980). The lack of water in dry silages reduces the formation of fermentation acids leading to high pH values. When silage pH rises to 4.5 (Na-benzoate) or 5.0 (K-sorbate) only about 1/3 of the acid is in the active, un-dissociated form. Large amounts of sorbate or benzoate are therefore needed to maintain the antifungal activity if pH rises above these limits. That makes the additive expensive and economically less competitive. The addition of homofermentative, osmotolerant LAB (tolerate dry conditions) might help to acidify the silage and thus increase the efficiency of the benzoate and sorbate. However, osmotolerant LAB differ in their degree of osmotolerance and in their competitiveness against the epiphytic flora in the crop. Not all LAB that claim to be osmotolerant are able to do a good job in dry silages. Homofermentative LAB alone often make excellent silages but the lack of aerobic stability is often becoming a problem during unloading of the silo (Muck & Kung, 1997).

A group of heterofermentative LAB, mainly *L. buchneri* strains, have been shown to improve the aerobic stability of silages (Oude Elferink et al., 1999). To develop its full potential, it is important to store these silages for at least 2 months so that the antifungal compounds (mainly acetic acid) have time to build up. The limitation of this type of additive lies in: i) uncertainty of the inoculant's osmotolerance (efficient up to which DM content?) and ii) its limited effect if larger numbers of competing microorganisms are present in the crop.

#### Additives to decrease losses

An anoxic environment and a rapid acidification of the forage lead to low losses of DM or energy in silages. DM or weight losses are positively correlated to the moisture content of silages or to the intensity of fermentation. Losses are therefore much lower in dry than wet silages (Savoie & Jofriet, 2003).

The application of a strong organic acid (e.g. formic acid) gives an instant effect and inhibits plant respiration (spares fermentable carbohydrates, impedes heating) and inhibits the degradation of amino acids and peptides. The epiphytic LAB usually recover from the 'acid shock' after a few days and grow then with very little competition from other microbes.

If homofermentative LAB are applied, the acidifying effect is slower than in acid-treated silages because a few days are needed to build up the lactic acid concentration. But the acidification is usually considerably faster and losses lower than in untreated silages (Muck & Kung, 1997).

It is often stated as a disadvantage that inoculants based on heterofermentative LAB (such as *L. buchneri*) produce larger DM losses than homofermentative inoculants. These increased losses derive mainly from the formation of  $CO_2$  when lactic acid is transformed into acetic acid (Driehuis et al., 2001). However, the  $CO_2$  formed contains very little energy and will therefore not cause any measurable energy losses. If, however,  $H_2$  is produced (as during clostridial fermentation), energy losses are substantial because  $H_2$  contains a lot of energy. In well-fermented silages energy losses are much lower than DM losses. Even if energy losses would be a far more relevant unit to use, they are laborious and difficult to determine with precision. Data on energy losses are therefore hard to find.

#### Contamination

Spore forming *Clostridium* bacteria are one of the most common microorganisms in wet silages that compromise forage quality and eventually contaminate dairy products. The bacteria are common in soil and it is inevitable to contaminate the crop. Avoid spreading of manure in the growing crop is, however, one action that can be taken to decrease the problems (Rammer et al., 1994). In Sweden and elsewhere researchers have devoted a lot of efforts to the understanding of clostridial growth. One statement we believed had been clearly established was that high DM contents of the crop stop the development of clostridia. In spite of massive information to farmers the problem of clostridia spores in milk coming to the dairies did not decrease. In all ensiling experiments at our department during the last decade, e.g. testing additives, wilting methods and air leakage, we have used a special strain of Clostridium tyrobutyricum as challenge organism to contaminate the crop in order to be sure to stress the fermentation. The strain was isolated by the Swedish Dairy Association from blowing cheese in the late 1980's. In this way all knowledge about how to control clostridia was based mainly on one strain. In order to check the sensitivity of the used strain, 24 strains of Cl. tyrobutyricum, Cl. butyricum, Cl. bifermentans, Cl. sporogenes and Cl. sticklandi in 10 different cocktails were used as challenge flora testing the growth in grass silage from two harvests at two DM levels, 29 and 39 % DM. All strains were selected from dairy products, from butyric acid fermented silage or from dairy cow faeces and were provided by the Swedish Dairy Association. The strains developed differently, some were restricted by the high DM level, others not. Some strains developed relatively high numbers of spores but no butyric acid. The challenge strain used originally was one of those with the highest activity both in forming spores and producing butyric acid and will continue to be used as a suitable challenge organism for experiments focusing on the inhibition of clostridial activity in silages (Pauly et al., 2008)

#### Temperature

The effect of pre-ensiling temperature on the fermentation process is somewhat forgotten. In the 1960's Dutch, British and Japanese authors (Wieringa, 1961; McDonald et al., 1966 and Takahashi, 1969) reported that clostridial fermentation was favoured on behalf of lactic fermentation when the silage was made at 40°C compared to 20°C. In practice most crops keep the ambient temperature after mowing or slightly lower due to evaporating water as long as it stays on the field wide-spread or in thin windrows. After being chopped or cut by a loader wagon the temperature immediately starts to rise since the plant respiration continues and the enzymatic activity is high. The time until the crop heats to about 40°C in a heap waiting to be moved into the silo can be estimated to between 2 and 4 hours for untreated green crops. The heating process is faster in wilted crops because they contain less water to heat. In practice, this can be a very short time when technical problems or brake-downs occur. Particularly when ensiling in tower silos the ensiling system is sensitive to disturbances.

To avoid the crop getting heated in case of delayed filling of the silo, chemical additives based on acids like formic acid and/or propionic acid work as preventers as they inhibit plant respiration. Other additives such as inoculants, nutrients or non-acid chemicals do not have this effect.

The importance of temperature on the fermentation process is an area where we want to increase our research efforts. Not only about high temperatures, also the effect of ensiling at low and variable temperature is of interest for

many North European countries. In areas where the maize cultivation has its northern limit, the harvest often takes place at a time of the year when frosts can occur. This means that the maize is ensiled under conditions where the ambient temperature is around 0°C.

## Seal integrity

The silo has to be airtight of course. Therefore the seal integrity is of outmost importance. In tower silos the plastic seal is a minor part of the surface, in bunker silos it is of great importance and in round bales the stretch film makes the whole silo. The established recommendation for round bale ensiling in Scandinavia is to wrap the bales with six layers of stretch film of 0.025 mm thickness. But even with 6 layers of film, the round bale is a delicate piece that has to be handled with great care. Many bales have to be discarded after bad fermentation due to a leaking seal. Measurement of bale seal integrity can be made by measuring the time from an applied under-pressure of -200 Pa (vacuum) to -150 Pa. The under-pressure is produced with a hand pump, which is placed above a special one-way valve. Before that the valve is pushed through the film and sealed tightly against the film as described by Jonsson (2001). The results of an experiment investigating the effect of time of lifting and handling bales after wrapping on seal integrity are illustrated in Figure 3. The experimental design derives from a popular hypothesis that handling the bales should be made immediately after wrapping or several days later. Handling the bales later during the day of wrapping or the coming day should be avoided because the lamination between the film layers might be disturbed. The results indicate that this is not the case, telling that the seal integrity is not impaired by any of the tested handling times compared to bales not handled at all. The equipment used for handling was a Trima Quadrogrip (Trima, 2008)





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#### PRECAUTIONS TO AVOID MYCOTOXINS IN SILAGES AND OTHER FORAGE

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#### ABSTRACT

Our own investigations into hay and silage show that mycotoxins to avoid the assessment of microbiological pollution are a requirement. In a good professional practice, the feed spoil would be avoided. The sampling requires a great deal of understanding once the costs and secondly a result, which allows to control the process chain to prevent mycotoxins and especially feed to avoid losses. Microbiological testing is an important element. Both orientation value to assess soundness for commercial feed as well as to forage in their own operation. The data base is of the VDLUFA for commercial feed. The assessment prepared for this framework is now in the forage (hay, straw, grass silage, corn silage) and wet feedstuffs applied. First preliminary results led to provisional orientation values, to prevent mycotoxins, if not prevented, then reduce. It is verydifficult to estimate to the animal health, only in the presence of pathogenic germs. For the farmer is usually sufficient to reduce the losses during storage of animal feed to contribute to be avoided the formation of mycotoxins.

#### **INTRODUCTION**

Total bacteria, fungi and yeasts show the incipient spoilage of feed. These nutrients are lost and both the concentration of nutrients as well as the feed intake decreases. Each feed ration design therefore needs a feed hygienic proper feed. This means the best feed conservation and storage, for the period from the storage until feeding avoids the mould. It covers the objective of the farmer as little as possible losses during storage acceptable with the necessary conservation and storage conditions, which prevent mould growth. To the farmer advisory assistance have been in recent years a number of microbiological investigations into hay [11] and silage [10]. Several key assessments led to different results for the practice [13, 17, 8]. It was first shown that mycotoxins of moulds in hay and silage formed and can be [1, 5, 6, 15, 16, 18]. The risk of deterioration of animal health is linked to the presence of mycotoxins in feed is high, but the evidence and the production of a link almost impossible. Either the animal is ill or the associated feed no longer exists, or vice versa. For the farmer is therefore the application of good practices for safe conservation and storage easier. An additional assistance is a microbial testing of feed containing one for the farmer understandable assessment. To achieve this goal is first data collected and evaluated and if necessary with a targeted sampling of the framework for an assessment scheme will be developed. A contribution will make the following compilation.

#### **MATERIAL AND METHODS**

Hay samples were first in cooperation with three offices with the proviso that, depending District 5 samples very well, 10 samples of medium and 5 samples moulded be considered. The sample was compared with a questionnaire. It was loose hay approximately 1 kg per sample in a paper bag and transported to Grub, here with the sample cutter cutted. The first two-thirds were rejected and used to cleaning up the cutter from the last third was the test for microbial investigation considered. This study was done in the laboratory with the usual plate count technique and to differentiate species. Were bales sampled, these were cut up or wrapped and the sample drawn proportionately. From the 2002 harvest was so Haylage sampled.

The microbiology for silage was at TUM Munich [5], which were associated fermentation parameters in the laboratory in Grub. The silos have been divided into two office districts under the service business largely randomly sampled. It was the first sample from the silo bleed considered as unencumbered views of farmers. The silage was taken to a laboratory silo 1 l pressed and hermetically sealed. After that a mould nest was pressed in a silo and also sealed. There were 2 each laboratory silos with N means for normal and 2 each laboratory silos with S moulded definitions. The interim storage place in the refrigerator. One laboratory silo N and S are following the sample collected and sent to the microbiology and / or the provision of fermentation parameter.

#### **RESULTS AND DISCUSSION**

#### Hay:

Evaluation of microbiological investigations was in accordance with requirements of the sampling. The samples with visible mould, so the moulded samples were on mean at  $2.5 \times 10^5$  cfu<sup>-1</sup> g and would be taking into account the key of the Animal Health Service (TGD) classified as spoiled. When the samples with the middle and very good quality would be assessed the mean values of the mould levels already in the quality must be reduced. According to current knowledge, the key is not fully applicable to hay. Table 1 is the frequency of the families of the fungi identified. They

are often simplified rules and only the family of fungi named. Only the Aspergilla show a significant decrease towards better quality.

From VDLUFA, a comprehensive assessment key for cereals and compound feeds and is soon to be proposed in the animal nutrition provisions. It is also here that the division into product as well as show wasted mould within the same family and hence an indication of the family no clear statement.

Genera of Mould	Quality good	Quality middle	Quality bad
Eurotiales	90	100	100
Mucorales	70	85	90
Aspergilla	40	20	80
Penicillin	0	5	0
Scopulariopsis	0	10	45
Walemia	50	75	65
Dematiaceae	40	45	0
Cladosporien	30	5	20
Fusarien	20	3	0
n	10	40	9

 Table 1. Occurrence of mould in hay according to the class quality (frequency %)

An assessment of samples in regard to animal health in a dairy farm is not possible and should also be avoided because, besides the operations division in simplified product specific mould and deteriorate mould, the toxin production even within the kind of mould of stem to stem varies. Furthermore, there is no correlation between the degree of moulding the produced toxins, both individually and to several occur. On the other hand, already the fungal spore's allergies in humans and animals. As a first step towards microbial quality assessment of hay were provided the samples for the amount of mould and germ figures ranked the 5 best and the five worst as the basis for this assessment. The average grades were classified in between.

Bale	Total germs	Total germs	Mould	Mould	Yeast	Yeast
No	Out	In	Out	In	Out	In
1	$4.0 \ge 10^6$	8.8 x 10 <sup>6</sup>	< 10 <sup>3</sup>	< 10 <sup>3</sup>	<nwg< td=""><td><nwg< td=""></nwg<></td></nwg<>	<nwg< td=""></nwg<>
2	1.6 x 10 <sup>5</sup>	$2.4 \times 10^3$	< 10 <sup>3</sup>	< 10 <sup>3</sup>	<nwg< td=""><td><nwg< td=""></nwg<></td></nwg<>	<nwg< td=""></nwg<>
3	6.4 x 10 <sup>4</sup>	$2.0 \times 10^4$	< 10 <sup>3</sup>	< 10 <sup>3</sup>	<nwg< td=""><td><nwg< td=""></nwg<></td></nwg<>	<nwg< td=""></nwg<>
4	2.8 x 10 <sup>6</sup>	2.4 x 10 <sup>5</sup>	< 10 <sup>3</sup>	< 10 <sup>3</sup>	<nwg< td=""><td><nwg< td=""></nwg<></td></nwg<>	<nwg< td=""></nwg<>
5	7.7 x 10 <sup>5</sup>	1.4 x 10 <sup>4</sup>	5.6 x 10 <sup>3</sup> n. d.	< 10 <sup>3</sup>	<nwg< td=""><td><nwg< td=""></nwg<></td></nwg<>	<nwg< td=""></nwg<>
6	1.1 x 10 <sup>5</sup>	1.7 x 10 <sup>5</sup>	$5.4 \ge 10^3$ A. fumigatus	< 10 <sup>3</sup>	6.1 x 10 <sup>5</sup>	$2.4 \times 10^3$
7	3.8 x 10 <sup>5</sup>	1.1 x 10 <sup>5</sup>	< 10 <sup>3</sup>	< 10 <sup>3</sup>	<nwg< td=""><td><nwg< td=""></nwg<></td></nwg<>	<nwg< td=""></nwg<>

**Table 2.** Microbiological contamination of Haylage [14]

Another example is the 2002 harvest. Haylage bale been studied (Table 2). Of seven bales the slide was removed and the bales cut up.

The front page was designated as the outside and a disc in the middle of the rectangular bales from the edges and thus free to cutting. As mentioned above, samples were processed and then frozen to the investigation. The results show a low to no exposure to mould. If fungi have been confirmed, then there was a film as injury and even bales from these higher levels yeast with a clear influence from outside the core. This also shows the total germs, it was therefore in the sampling of bales to be taken into account.

# **Figure 2.** Frequency of samples with different mould content in grass and corn silage with (S) and without (N) visible mould [15].



#### Silage:

What to do if mould in feed noticed? With what risks can be expected and how harmless can be disposed of? This question was the basis of the sample drawing on the silo. It was obvious (meaning Testing) good silage first sampled and then the corresponding mould nest. To the timing differences to leveling the air samples in the laboratory silos. The results showed that the most frequently occurring mould *Penicillium roqueforti* is in corn silage and *Monascus ruber* and *Aspergillus fumigatus* was in Grassilage [12].

Similar occurrences are also used by other authors called [2]. Health risk assessments to be only on the presence of mycotoxins. This was a set of methods to the detection of those mycotoxins in silage allow (5, 6, 7, 16, 18]. Also *Monascus ruber* toxins these methods have been developed [15]. The detection of mycotoxins alone is not sufficient guidance for direct threat to animal health. Here are feeding trials necessary that have not yet identified. There may be but preventive measures to avoid the mould goals.

Part of the samples of [15] in the laboratory to Grub fermentation parameter investigated and, as in Figure 1 now evaluated again to grass and maize silage, without (N) and with (S) visible mould. This shows that just for the hint moulded feed discarded for the visible mould good selection options. But a statement on the still usable silage burdened with high uncertainty.

The work group "feed Microbiology of the Division of the feed VDLUFA was an orientation value scheme developed, which is also for cereals, coarse and liquid feed application. These values should guide suggestions as to the microbiological quality of a feed ordered [20].

From the presence of fungi of the genera *Aspergillus*, *Penicillium*, *Scopulariopsis*, *Wallemia*, among other things, and the *Mucorales* and yeasts can be references to deficiencies in the storage derived [13]. To describe the quality of, 4 levels of quality (QS I to IV). They result inevitably from the respective determined number of bacteria levels.

 Table 3.
 Quality assessment of feed to germ count stages (GCS)

GCS I - for all groups of bacteria	QS I	Normal
GCS II - at least one microbial group	QS II	Slightly or moderately reduced
GCS III - at least one microbial group	QS III	Reduced or significantly reduced
GCS IV - at least one microbial group	QS IV	Spoiled

An excerpt from the evaluation scheme is shown in Table 3. The unit is in bacteria and fungi always in "colonies forming units (cfu<sup>-1</sup>.g).

Field flora (black fungi, Fusarium and others)					
Corn	$\leq$ 40,	000			
Wheat, Rye	$\leq$ 50,	000			
Barley	$\leq 60,$	000			
Oat	$\leq$ 70,	000			
Secondary flora for	or maize, wheat, rye, barley, o	pats			
Penicilli, Aspergilli a. o.	Mucor	Yeasts			
≤ 30,000	$\leq$ 2,000	≤ 50,000			

Table 4. Orientation values (OV) for microbial count (only mould in cfu<sup>-1</sup> g) for QS I in grain

The differentiation of the germ count stages number of bacteria levels based on the classification of identified germ count (Table 5). Always the highest determined GCS is crucial for classification of a certain quality, regardless of whether the GCS only for one or more groups of bacteria has been identified. The final quality assessment is always taking into account both the existing fungi as well as the bacterial flora.

Table 5. Classification of identified germ count for individual groups of germ count stages

Germ count of one group exceeds the orientation value	Germ count stages	Evaluation of the germ count
- Not	GCS I	Normal
- Up to 5 times	GCS II	Increase slightly or increased
- Up to 10 times	GCS III	Increased significantly
- More than 10 times	GCS IV	Excessive or highly exaggerated

Mykotoxin producer are among the product-typical fungi, for example, the *Fusarium* as indicated in the spoilindicating fungi to find. To estimate the reduction of forage suitability of toxic metabolic products may therefore mycotoxin analyses is necessary. The results of germ counting and germ differentiation can be used to decide whether and how it should be mycotoxins, can make a difference.

#### Assessment of the Mycological flora of green feed, silage, hay and straw

The epiphytic (naturally guarantees) microflora on forage crops in general varies from 0.1 to 100 million  $cfu^{-1}$  g for bacteria, the black fungi 10,000 to 1 million  $cfu^{-1}$  g. The presence of even higher numbers of bacteria is not excluded. When yeast is orders of magnitude from 10,000 to 500,000  $cfu^{-1}$  g. The epiphytic stocking lactic acid bacteria, depending on the weather and the harvest time in grass about 50,000  $cfu^{-1}$  g in corn and about 200,000  $cfu^{-1}$  g. For the *Fusarium* fungi are of particular importance because it is not only in the cereals and straw but also on grass mycotoxins can form. Also for hay and straw were meanwhile orientation values with quality levels developed from Table 6 below, but is still regarded as provisional handle. From the orientation values indicator germ (Table 6) for each germ groups, the number of bacteria levels (Table 5) and the resulting quality levels (Table 3). Therefore a germ or a germ group is not on the quality of the entire feed to close.

#### Example

In a hay sample be 200,000  $\text{cfu}^{-1}$  g sample spoil indicating mould (such as *Penicillium verrucosum*). 100,000  $\text{cfu}^{-1}$  g than normal (= OV). The OV closed at twice exceeded. The result leads to the GCS II, as the highest result is this QS II also moderately reduced.

GC	Germ Group	Typical germ in this group (Toxin producer)	Corn silage	Grass- silage	Нау	Straw
1	Product- typical bacteria	Yellow germ Enterobacteria a.a Bazillus,	5 x 10 <sup>5</sup>	2 x 10 <sup>5</sup>	30 x 10 <sup>6</sup>	100 x 10 <sup>6</sup>
2	Spoil-indicating bacteria	Mikrococcus, Staphylococcus	3 x 10 <sup>5</sup>	3 x 10 <sup>5</sup>	2 x 10 <sup>6</sup>	2 x 10 <sup>6</sup>
3	Streptomycetes	- Black mould,	3 x 10 <sup>4</sup>	3 x 10 <sup>4</sup>	1,5 x 10 <sup>5</sup>	1,5 x 10 <sup>5</sup>
4	Product typical Mould and black fungi	Acremonium, Verticillium, Fusarium Aspergillus	5 x10 <sup>3</sup>	1 x10 <sup>4</sup>	2 x 10 <sup>5</sup>	2 x 10 <sup>5</sup>
5	Spoil indicating Mould and black fungi	Penicillium, Scopulariopsis, Wallemia	1 x 10 <sup>4</sup>	1 x 10 <sup>4</sup>	1 x 10 <sup>5</sup>	1 x 10 <sup>5</sup>
6	Mucorales	-	3 x 10 <sup>3</sup>	5 x 10 <sup>3</sup>	5 x 10 <sup>3</sup>	5 x 10 <sup>3</sup>
7	Spoil indicating Yeasts	-	1 x 10 <sup>6</sup>	5 x 10 <sup>5</sup>	1,5 x 10 <sup>5</sup>	4 x 10 <sup>5</sup>

Table 6. Indicator germ and provisional orientation value (POV) forage [13 changed].

#### CONCLUSION

The application of value orientation scheme [20] for quality assessment and to distinguish the microbiological status of compound feed and grain (commercial feed) to asses soundness or the deterioration of feed on the forage as well as on wet or liquid feed, is possible in principle. The application requires for each group of this feed its own orientation values (OV). This guidance values in order to better the quality, the sooner the samples used collective, which means the samples for which feed group is representative. For silage and liquid feed are still OV to develop and for hay and straw, they are still provisional.

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# CHANGE OF FEED VALUE DURING THE PROCESS OF ENSILAGE, WITH MAIN FOCUS OF FIBER, PROTEIN QUALITY AND CAROTIN

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# **INTRODUCTION**

During the ensilage process we usually focus on the change of carbohydrate fraction and energy (e.g. water soluble carbohydrates  $\rightarrow$  acetic acid, lactic acid, butyric acid). On the opposite, the change of crude protein quality is often neglected. However during the process of ensilage some substantial rebuilding and degradation processes for the crude protein happen which is important for the feed value too. During the ensilage the microorganism initiates an extensive degradation of the green plant's true protein. This process is called proteolysis. Little is known about the possibility to influence the extension of proteolysis throughout the ensilage process (intensity and quality). The reduction of true protein, with negative consequences for the rumen undegraded protein (RUP or UDP) and the utilizable crude protein (uCP), significantly impacts on the feed value of silage.

Licitra et al. (1996) divide the crude protein into five different fractions (A, B1, B2, B3, C). Fraction A is the NPN, B1 is the buffer soluble crude protein (fast degradation), B2 is the buffer insoluble crude protein (variable degradation), B3 is the protein insoluble in neutral detergent (slowly degradation) and C is the protein insoluble in acid detergent (no degradation). Shannak et al. (2000) and Kirchhof et al. (2006) found some strong correlation between in situ UDP content for different feed stuffs and the crude protein fractions. The UDP content of different feedstuffs can be calculated for three rumen solid outflow rates  $(2\% h^{-1}, 5\% h^{-1}, 8\% h^{-1})$  with the help of different formulas.

Several studies investigated the changes of the crude protein fractions during the harvest and ensilage process (Richardt and Steinhöfel, 2000, Gruber et al., 2004, Kofahl et al., 2007). Richardt and Steinhöfel (2000) found an increase of fraction A (from 276 g/kg CP to 631g/kg CP) through the ensilage of clover-grass (see Figure 1). The true protein (Fraction B1, B2, B3, C) decreases in the same quantity. The main decrease can be seen with fraction B2 and B3.



Figure 1. Change in crude protein fractions through ensilage of clover-grass (Richardt and Steinhöfel, 2000)

# Crude protein fractions and success of conservation

The first study focused on the correlation between crude protein quality and success of conservation. The samples of grass silage have been analyzed in the forage lab of the Milk Recording Association of Saxony between 2001 and 2005. The crude protein fractions have been analyzed according to Licitra et al. (1996), the other nutrients according to the VDLUFA Methodology, Vol. III. The success of conservation was evaluated according to the DLG score (Weissbach and Honig, 1991), the UDP according to Kirchhof et al. (2006). The grass silages were divided into two groups depending on the DLG score (see Tab. 1).

Parameter		Success of conservation (	Р	
		1 and 2	4 and 5	
		n = 246	n = 33	
А	% CP	52.4 (11)	47.3 (12)	p < 0.01
С	% CP	6.7 (2.8)	9.4 (3.3)	p < 0.0001
UDP5	% CP	25.3 (10.1)	35.5 (13.0)	p < 0.0001
NH <sub>3</sub> -N	% total N	6.8 (2.1)	16 (8.1)	p<0.0001
piCP	% CP	23.6 (6.3)	30.8 (9.6)	p<0.001

Table 1. Success of conservation and crude protein quality (Richardt and Steinhöfel, 2007)

UDP5 calculated according to Kirchhof et al. (2006) for a rumen solid outflow rate of 5%/h

We found some significant differences for the crude protein fractions A and C, for the rumen undegraded dietary protein (UDP), for the pepsin-insoluble crude protein (piCP) and for the ammonia depending on the DLG score. Silages with a score of 4 or 5 (bad and very bad) have a reduced fraction A (-5.1%), a higher fraction C (+2.7%) and a higher UDP (+10.2%). Richardt et al. (2002) found some strong correlation between UDP content for grass silage and the pepsin-insoluble crude protein (r= 0.69). The reason for this may be due to the higher process temperature of bad fermented silages resulting in a higher content of heat damaged proteins. The higher values of pepsin insoluble protein and the fraction C clearly point to heat damages. A low content of fraction A and a high content of UDP alone are not sufficient indicators for a good fermentation and a high protein quality.

#### Influence of additives in grass silage

In the second study we examined the influence of additives (lactic acid bacteria) on the proteolysis. We tried to find out whether it is possible to reduce the proteolysis during the ensilage resulting in a higher UDP content. Table 2 and 3 show the results of 25 ensilaging trials.

 Table 2. Influence of inoculation with lactic acid bacteria to energy and nutrients in grass silage (Richardt and Steinhöfel, 2007)

Parameter		Control	Inoculated	Р
		n = 25	n = 25	
Crude ash	g/kg DM	99	98	n.s.
Crude protein	g/kg DM	166	165	n.s.
Crude fibre	g/kg DM	270	268	n.s.
ADF	g/kg DM	283	284	n.s.
Sugar	g/kg DM	21	38	n.s.
NEL	MJ/kg DM	5,9	5,9	n.s.

Energy according to (GfE, 1998), n.s. = not significant

 Table 3. Influence of inoculation with lactic acid bacteria to UDP and some crude protein fractions in grass silage (Richardt and Steinhöfel, 2007)

Parameter		Control	Inoculated	Р
		n = 25	n = 25	
Fraction A	% CP	52 (19.0)	50 (18.3)	p<0.05
True protein	% CP	48 (19.0)	50 (18.3)	p<0.05
Fraction C	% CP	4.3 (1.9)	4.7 (1.9)	n.s.
piCP	% CP	19.1 (3.8)	19.5 (4.7)	n.s.
UDP5	% CP	18.6 (7.7)	20.6 (8.0)	p<0.05

UDP5 calculated according to Kirchhof et al. (2006) for a rumen solid outflow rate of 5%/h

There were no significant differences between the two groups for energy and nutrient content (see Table 2). However, there were some significant differences with respect to fraction A, true protein and UDP (see Table 3). The group with inoculation shows a slightly lower degree of proteolysis (true protein 50% vs. 48%) and a small increase of UDP (20.6 vs. 18.6). With respect to the high preservation success of all samples, these results must be considered remarkable.

#### Influence of wilting period

With the third study we focused on the influence of the wilting period on the proteolysis. Table 4 shows the connection between the length of the wilting period and the crude protein fractions.

 Table 4. Influence of the wilting period on the UDP and some crude protein fractions in grass silage (Richardt and Steinhöfel, 2007)

Wilting period	DM	СР	piCP	ADF	А	С	UDP5
D	g/kg	g/kg DM	g/kg DM	g/kg DM	% CP	% CP	% CP
0	149	170	17	244	25	5	29
0.5	177	170	20	242	21	7	35
1.5	268	170	18	241	21	7	34
3	401	163	18	247	31	6	29
5 (1 d rain fall)	287	188	19	259	34	6	28

UDP5 calculated according to Kirchhof et al. (2006) for a rumen solid outflow rate of 5%/h

The longer the wilting period lasts the more the fraction A increases which points to a more extensive proteolysis. On the opposite fraction C shows no influence of the wilting period. The differences in fraction A and ADF result in a remarkable change of the UDP content.

#### Influence of inoculation of lactic acid bacteria in alfalfa

It has been shown that the duration of the wilting process, the quality of the conservation process and the inoculation have a significant influence on the quantity of the proteolysis and on the content of the UDP. However, it remains unclear at what time of the fermentation those differences appear.

Therefore, with a fourth study we investigated the influence of the wilting period, the inoculation and the fermentation time on the crude protein fraction in alfalfa  $(2^{nd} \text{ cut})$ .

There are two variants: the first without inoculation (control) and the second with inoculation (Lactobacillus plantarum DSM 3676, Lactobacillus plantarum DSM 3677, propionic acid bacterium sp. DSM 9576, Propionic acid bacterium sp. DSM 9577, with 400,000 cfu/g forage). We measured the different variables at the time of harvest (Fresh), after 12 hours of the wilting process and after 3 days of the ensiling process. Table 5 and 6 display the average values of three samples of each variable.

Table 5.	Influence of wilting and	l inoculation on nutrients.	, alfalfa, 2 <sup>nd</sup> cut	n = 3	(Richardt and Steinhöfe	l, 2007)
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Parameter		Fresh	Wilting 12 h	Ensilage 3 d	
				Control	Inoculated
Dry matter	g/kg	322 <sup>a</sup> (26.4)	534 <sup>b</sup> (13.6)	511 <sup>b</sup> (6.8)	547 <sup>b</sup> (17.0)
ADF	g/kg DM	$276^{a}(8.1)$	316 <sup>b</sup> (22.3)	298 <sup>ab</sup> (16.3)	284 <sup>ab</sup> (23.3)
Crude protein	g/kg DM	206 (7.5)	206 (13.9)	191 (11.2)	205 (17.6)

a, b = different letters point to significant differences (p < 0.05)

The dry matter of fresh alfalfa was very high (322 g/kg DM) and even significantly increases throughout the wilting process (534 g/kg DM). The ADF content also significantly rises during the wilting process (316 g vs. 276 g). In contrary there were no significant differences to be found between the ADF content of fresh alfalfa and the alfalfa silage (298 g and 284 g vs. 276 g).

The changes in crude protein fractions are remarkable (see Table 6). Fraction A significantly increases both during the process from fresh alfalfa to wilted alfalfa (36% vs. 43%) and during the process from wilted alfalfa to ensilaged alfalfa (43% to 52%). However, the latter holds true only for the control group. With the inoculated group fraction A only slightly and insignificantly increases. This clearly shows that the proteolysis already starts during the wilting period and during the early state of the ensilage process.

The fresh alfalfa had a normal content of fraction A (36%) which is in line with other studies (Richardt and Steinhöfel, 2000, Kofahl et al., 2007). The content of 52% for fraction A in the control group after 3 days of ensilage is a typical value for grass and legume silage (see Figure 1 and Table 1 and 3). With the inoculate variant fraction A only slightly increases to 47% and shows no significant difference to the wilting material (43%). We assume that the inoculation with lactic acid bacterial reduces the quantity of proteolysis in the early stage of the ensilage process. The lower content of fraction A with the inoculate variant results in a higher amount of fraction B2 and B3. There were no differences to be found in fraction C between fresh and ensilage and between the control group and the inoculate group.

Parameter		Fresh	Wilting 12 h	Ensilage 3 d	
				Control	Inoculated
Fraction A	% CP	$36^{a}(0.6)$	$43^{b}(3.2)$	$52^{c}(1.6)$	$47^{bc}(3.1)$
Fraction B1	% CP	$5^{a}(1.3)$	$4^{ab}(2.1)$	$2^{b}(0.2)$	$2^{b}(0.3)$
Fraction B2	% CP	$44^{a}(1.3)$	$41^{ac}$ (3.8)	$31^{b}(0.3)$	$33^{c}(0.7)$
Fraction B3	% CP	$9^{a}(0.7)$	7 <sup>b</sup> (1.0)	$8^{ab}(1.2)$	$11^{ab}(2.2)$
Fraction C	% CP	$6^{ab}(0.7)$	$5^{a}(0.4)$	$6^{b}(0.2)$	$6^{b}(0.2)$
UDP5	% CP	25 (1.9)	22 (2.7)	22 (1.2)	23 (1.0)

Table 6.	Influence of wilting and inoculation of crude protein fraction and UDP, alfalfa, 2 <sup>nd</sup> cut, n =	= 3
	(Richardt and Steinhöfel, 2007)	

a, b = different letter means significant differences (p < 0.05)

UDP5 calculated according to Kirchhof et al. (2006) for a rumen solid outflow rate of 5%/h

#### CONCLUSIONS

We could show that the inoculation with lactic acid bacteria reduces the proteolysis results in a lower content of fraction A and in a higher content of UDP (see Table 3 and 6). The proteolysis starts with the time of harvest. So, the wilting period and the early stage of fermentation must be considered most important in this context (see Table 4 and 6). It seems that the inoculation reduces the proteolysis during the early stage of ensilage, but further investigations are necessary in order to improve our knowledge about this process. A low content of fraction A and a high content of UDP alone are not sufficient indicators for a good fermentation and a high protein quality. Silages with a DLG score of 4 or 5 show a high content of UDP because of a high content of heat damaged protein (see Table 1). Consequently, we need two kinds of parameter: one for proteolysis (fraction A and UDP) and one for the quality of the ensilage process (piCP, fraction C and NH3-N).

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Príjem sušiny, Úžitkovosť, Fermentácia siláže, Výživná hodnota, Chutnosť, Ziskovosť, Stráviteľnosť, Produkcia mlieka, Uchovávanie dusíkatých látok

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# PRODUCTION OF FORAGE CROPS AND CLIMATIC CHANGES

#### **RESISTANCE AND RESILIENCE OF PERMANENT GRASSLAND INFLUENCED BY FERTILISATION**

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#### ABSTRACT

An experiment was established on permanent grassland (Festuco-Cynosuretum association) site in a mountainous region of Slovakia (the Low Tatras mountain range). This type of permanent grassland was influenced by four mineral fertilisation levels. Drought is a severe natural disturbance with negative effects on dry matter production. Based on dry mater production, resistance and resilience were calculated. The resistance of permanent grassland to perturbation and the resilience, as the speed of recovery, are two important components of stability. The research results suggested that the increasing intensity of fertiliser application stabilised dry mater production that is the grassland ecosystem was more resistant and more resilient.

#### INTRODUCTION

One of the ecological tenets justifying conservation of biodiversity is that diversity begets stability. The preservation of biodiversity is essential for the maintenance of stable productivity in ecosystems (Tilman and Downing, 1994) and biodiversity reduces variability in ecosystem productivity through compensatory effects; that is, a species increases in its abundance in response to the reduction of another in a fluctuating environment. Undoubtedly, ecosystem stability depends not only on community composition but also on disturbance, nutrient supply and climatic condition (Bai *et al.*, 2004). Tilman and Downing (1994) used the change in biomass following a severe drought to measure the stability of prairie grasslands and found that biomass on more species-rich plots was both more resistant and more resilient than biomass on species-poor ones. Bai *et al.* (2004) found that stabilising effects of the compensatory interactions between species or plant functional groups involved were also corroborated by the relatively high drought resistance and resilience of the steppe communities. These authors reported the resistance and resilience (based on dry matter production) in grassland as a primary vegetation cover, both in prairie and steppe communities, but only the ecological approach to the problem was presented in their works. Under temperate conditions of Slovakia however, grassland is a secondary vegetation cover, fertilised and used as a source of nutrition for livestock. The paper presents resistance and resilience of grassland ecosystem as calculated in species-rich sward influenced by fertiliser application.

#### MATERIALS AND METHODS

Forest is a primary vegetation cover in mountain regions of Slovakia (the Low Tatras mountain range). Since the  $17^{\text{th}}$  century, there has been human settlement in the investigated area (the village of Liptovská Teplička) resulting in deforestation and creating arable land and pastures. The research trial was established at the research site (altitude 960 m) where the arable land had naturally returned to grassland and created the plant community of *Festuco-Cynosuretum* association. In 1992, the permanent grassland comprised 32 vascular plant species and four nutrition levels at the following fertiliser rates were applied: Treatment 1:  $N_0 P_0 K_{0}$ ; Treatment 2:  $N_0 P_{30} K_{60}$ ; Treatment 3:  $N_{90} P_{30} K_{60}$  and Treatment 4:  $N_{180} P_{30} K_{60}$ . Total above-ground dry matter (DM) production was determined at three cuts over the growing season in four randomised replicates. On the basis of DM production data recorded during 10 years (1992 - 2001), the resistance (resistance of a grassland ecosystem to perturbation) and resilience (as the speed of recovery) were calculated as follows:

Resistance =  $(B_{drought} - B_{pre-drought})/B_{pre-drought}$ Resilience =  $B_{post-drought}/B_{pre-drought}$ 

Where: drought is natural perturbation; B  $_{drought}$  is DM production in the year with the drought occurred, B  $_{pre-drought}$  is DM production in the year before the drought event, and B  $_{post-drought}$  is DM production in the year after the drought event. The resistance values closer to zero imply greater drought resistance and the resilience values nearer to 1.00 entail faster negotiation with some disturbance.

#### **RESULTS AND DISCUSSION**

In the initial research years of 1992 - 1994, the rainfall was below and the temperature was above the long-term average, respectively, but the data on DM production of 1991 are not available (Table 1). That is why the research data on the resistance and resilience are given only for the years 1998 and 2000. The development of botanical composition (species numbers) is not presented here but can be found in Britanak *et al.* (2007). The fertiliser application induced both the parameters investigated. The PK and also the N<sub>180</sub> PK fertiliser treatments were not influenced by the drought. In 1998, the grassland resistance to drought was as follows: PK fertilisers > N<sub>180</sub> > N<sub>90</sub> + PK > zero-fertilised control. Treatments 1 and 2 had a positive valence (Table 2). The resilience had a modified order: PK fertilisers > N<sub>90</sub>PK >

 $N_{180}$ PK > zero-fertilised control. Only one value higher than 1.00 was recorded at PK treatment (1.16), see Table 2. The values were lower than 1.00 at the other treatments (from 0.82 to 0.87).

More intensive effects of the drought were found in the year 2000. The highest resistance of sward (- 0.15) to this natural disturbance was recorded at the treatment with the highest N rate applied. The resistance dropped at the treatment with the lowest N rate (- 0.39) followed by the control (- 0.53) and the treatment with only the PK fertiliser application (- 0.68). The identical sequence was found for the values of resilience, too. North America's natural vegetation resistance value was about - 0.35 for experimental species-rich ( $\geq$  11 species) grassland plots (Tilman and Downing, 1994). Bai *et al.* (2004) reported the resilience level of - 0.27 for steppe grassland dominated by *Leymus chinensis* (a perennial rhizomatous grass) and - 0.22 for that with dominance of *Stipa grandis* (a perennial caespitose grass).

On average, the stability of dry matter production was rising with increasing fertiliser rates. The resistance values, on average, increased with higher fertiliser rates (r = 0.6465; P = 0.0231). The resilience - another character of grassland ecosystem - acted similarly (r = 0.6225; P = 0.0362). One more evidence for this argument was the lower coefficient of variation at the dry matter production. The parameter was increasing with decreased fertiliser rates (r = -0.63). However, this interpretation must be manipulated carefully because the experiment has lasted only for a short time. After an initial period of  $\approx$  40-year adjustment of the application of fertilisers, the botanical composition of the plots reached equilibrium (Silvertown *et al.*, 1994). Only a ten-year experiment is presented here.

 Table 1. Sum of rainfall (mm) and mean daily temperature (° C) throughout the growing season and DM production (t/ ha) at permanent grassland influenced by fertiliser application

Years	R	t	Treatments			
			1	2	3	4
1992	317.6	13.15	2.85	3.75	4.73	6.35
1993	349.5	12.54	1.91	3.75	4.93	6.04
1994	337.5	13.41	2.30	4.61	6.44	7.91
1995	477.0	12.43	2.98	4.43	6.91	8.32
1996	592.4	12.01	1.67	2.63	5.26	7.24
1997	383.6	11.94	2.30	3.59	6.23	7.53
1998	353.6	13.93	1.77	3.83	5.57	7.83
1999	425.8	13.52	1.89	4.16	5.44	6.31
2000	400.3	13.30	0.89	1.35	3.31	5.34
2001	607.5	12.88	1.26	1.68	3.79	4.80
Long-term ave	rage 392.0	12.20				

**Table 2.** Resistance (R 1) and resilience (R 2) of grassland to natural disturbance and coefficient of variance (CV) at dry matter production influenced by fertiliser application (1992 - 2001)

Treatments	1998		2000		Mean		CV	
	R 1	R 2	R 1	R 2	R 1	R 2	%	
1	- 0.23	0.82	- 0.53	0.67	- 0.38	0.74	32.8	
2	0.07	1.16	- 0.68	0.40	- 0.30	0.78	33.2	
3	- 0.11	0.87	- 0.39	0.70	- 0.25	0.78	21.5	
4	0.04	0.84	- 0.15	0.76	- 0.06	0.80	17.14	

# CONCLUSIONS

Increasing intensity of fertiliser application could stabilise grassland dry matter production over a period of drought (resistance). However, grassland needs a longer period of time for reaching such level of dry matter yields as before this natural disturbance occurred.

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# THE BOTANICAL CHANGES IN PERMANENT GRASSLAND AFTER RESTORATION OF NITROGEN FERTILIZING

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# **INTRODUCTION**

Nutrition and fertilizing as intensification factor sharply interfere into floristic composition of ground cover. Thereby directly or indirectly influencing the production process. Holúbek et al. (1997) introducing that diversity of species, at the natural grass biome, may be substantial: in semi-natural semiarid flora we can find about 50 species of vascular plants. in sub-humid it can be over 200 species. Nösberger and Kessler (1997) assigning importance to high level of biodiversity from position of its stability role and ecosystem resistance against disturbance. Different species responding differently on the combination of disturbs and from this point of view result also stability of non-maximal production depending from biodiversity.

By Krajčovič et al. (1985) with increasing level of fertilizing is the floristic diversity express by the ratio in the count of species and decreasing two-times up to four-times. Similarly Jančovič (1999) observing, that floristic composition and its changes are affected in the first range by the fertilizing and by the intensity of exploitation. He also emphasizes big impact of reference point of grassland.

The aim of this study was to investigate impact of grassland fertilizing and utilization on the botanical changes after restoration of suspension nitrogen fertilizing.

#### **MATERIALS AND METHODS**

Experimental investigations were carried out in a long-term grassland trial. There was studied in the years 1986–1993 the effects of different fertilization intensity. In 1994–1996 no fertilization took place and the vegetation was cut once a year with the aim of maximum above-ground phytomass production (Rychnovská *et al.*, 1987). In the period 1997–2002 the same fertilization regimes were performed as in the years 1986–1993.

The site is situated at the altitude of 640 m a. s. l. in the locality of Chvojnica in the mountain of The Strážov Hills (48°53'N. 18°33'E). It belongs climatically to a mildly temperate region. Region mildly dry with prevailing cold winter. According to long-year measurements the average year temperature is 7.5 °C. within growing season 11.1 °C. Long-term average of the whole year sum of precipitation represents 848 mm and that of growing season 431 mm. Soil-forming substrate is formed by crystalline rocks with predominance of granite and crystalline slates on which the brown acidic sandy–loam soil (Cambisol) has developed.

The trial was originally established by a block method in four replications with the area of an experimental plot 10  $m^2$  and protective 0.5 m wide belt. Nutrient application and dosing are stated in Table 1.

Treatment	Year									
	1997	1998	1999	2000	2001	2002				
1	_	_	_	_	-	-				
2	РК	РК	РК	РК	РК	РК				
3	PK + 60 N									
4	PK + 120 N									
5	PK + 240 N									

**Table 1.** Treatments and nitrogen rates /kgN.ha<sup>-1</sup>/

Grasslands were exploited by three cuts:

 $1^{st}$  cut – at the beginning of dominant grass earning

 $2^{nd}$  cut – for to 5–6 weeks after the 1st cut

 $3^{rd}$  cut – 8 weeks after the  $2^{nd}$  cut

Dominance of the botanical groups and individual species were determined in both managements by Regal (1956).

# **RESULTS AND DISCUSSION**

The original grasslands represent *Lolio-Cynosuretum* R. Tx. 1937 association. From the viewpoint of botanical species occurrence grass species (73%) prevailed in the grassland; leguminous plants formed a 2% portion and other meadow herbs a 25% portion. there were no blank places. There were recorded dynamic changes in floristic composition of vegetation in period before our investigation. The cover of floristic units in trial period is presented in Tables 2 and 3. More intimately results are mentioned at the work of Vozár (2003).

There was already in first year (1997) strong reaction of grassland on applied nutrients. By graduated nitrogen fertilization intensity the portion of botanical grass species was increased in comparison with control variant. Most in

ground cover with 240 kgN.ha<sup>-1</sup> of fertilizer.

On treatments with PK-nutrition there were increasing the degree of legumes. Nitrogen fertilizing decreased portion of legumes to minimum (from hint to 0.75% on variant 5). In the next time there were continuing dynamic changes in the grassland canopy. On the treatments with N-fertilizing was increasing the attendance of grass species to 89.5% in  $3^{rd}$  cut in the 1999. Leguminous species were increasing their proportion mainly on variant number 2 to maximum 49% in  $2^{nd}$  cut in the 1999.

Species, which was determined physiognomy of ground cover. With bigger canopy was on survey location *Festuca rubra* L. On variants fertilized with nitrogen was *Festuca rubra* L. unequivocally dominantly, where its cover was increasing with rising dose of N to 78.25% (treatment 10. 1999). Dominant position was decreasing just in treatments 2 fertilizing with PK. where the dominant placement had gradually becoming *Trifolium repens* L. Similarly also on treatment 1, where was increasing the part *Nardus stricta* L. and *Sieglingia decumbens* (L.) BERNH. What is indicating about oligotrophic character of location. Regal and Sindelářová (1970) described *Sieglingia decumbens* (L.) BERNH. Like a very submissive competitive capability species, which can exert only in an extreme locations, where rising higher grassy species. What is confirming our results.

Floristic groups	Cut		Treatment				
Plotistic groups	Cut	1	2	3	4	5	
	1	46.00	51.00	59.25	70.50	75.50	
Grasses	2	48.00	55.00	69.00	78.00	80.25	
	3	46.00	55.50	68.00	72.00	81.25	
Leguminous	1	2.00	4.50	2.00	1.25	0.50	
	2	3.75	10.75	3.25	0.50	0.75	
	3	2.75	13.50	7.00	1.25	+	
	1	26.00	18.50	16.25	25.75	16.50	
Other herbs	2	22.25	19.75	19.25	21.00	19.00	
	3	28.75	22.00	17.00	25.75	18.75	
	1	26.00	26.00	22.50	2.50	7.50	
Blank places	Cut         1         2 $1$ 46.00         51.0 $2$ 48.00         55.0 $3$ 46.00         55.5 $1$ 2.00         4.50           eguminous $2$ 3.75         10.7 $3$ 2.75         13.5 $1$ 26.00         18.5 $2$ 22.25         19.7 $3$ 28.75         22.0 $1$ 26.00         26.0 $2$ 25.50         14.5 $3$ 22.50         9.00	14.50	8.50	0.50	-		
	3	22.50	9.00	7.00	1.00	-	

 Table 2.
 Dominance of floristic groups in the year 1997 (%)

+ - Rarely

**Table 3.** Dominance of floristic groups in the year 2002 (%)

Eloristic groups	Cut			Treatment		
Floristic groups	Cut	1	2	3	4	5
	1	37.50	37.00	65.50	60.50	80.00
Grasses	2	46.00	51.00	36.50	58.00	60.00
	3	51.50	39.50	46.50	65.00	88.00
	1	2.00	15.00	1.00	+	+
Leguminous	2	9.00	19.50	3.50	1.50	+
	3	3.00	28.00	16.50	30	+
	1	32.50	19.00	26.00	32.50	16.50
Grasses Leguminous Other herbs Blank places	2	27.50	19.00	22.50	28.00	20.00
	3	37.50	32.00	35.50	30.00	11.00
	1	28.00	29.00	7.50	7.00	3.50
Blank places	2	18.00	10.00	37.50	12.50	20.00
	3	8.00	0.50	1.50	2.00	1.00

+ - Rarely

# CONCLUSION

Experimental investigations were made on a long-term grassland trial in the area of Strážov Hills (Middle Slovakia; height above see level 640 m; association *Lolio-Cynosuretum* Tx. 1937; experiment time 1997-2002).

With rising intensity N fertilization the floristic group of grass species increased. The spread of this species started on the detriment of legumes and other herbs.

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The list of references can be requested from the first author.

#### ACKNOWLEDGEMENTS

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# DRY MATTER DEGRADATION OF FRESH GRASS *IN SITU* DEPENDING ON VEGETATIVE STAGE AND GROWTH NUMBER

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#### **INTRODUCTION**

During vegetation radical morphological and chemical changes in fresh grass occur. Therefore, vegetative stage is the crucial influencing factor on the feeding value of green fodder. It determines the proportion and composition of the cell wall components and the nature of the cell ingredients. The stem proportion increases at the expense of the leaf fraction accompanied by a considerable decrease in digestibility due to continuous formation of cell wall constituents and their lignification (Minson, 1990; Van Soest, 1994; Südekum et al., 1995; Gruber et al., 1997). Consequently, the feeding value and energy content decline and feed intake is reduced.

In a large research project the influence of the vegetative stage of fresh grass on digestibility, feed intake and milk production was investigated during three complete growing seasons. In this paper the dry matter degradation in the rumen – determined with the nylon bag-technique *in situ* – is presented.

# MATERIALS AND METHODS

#### Experimental design

A meadow with a homogeneous botanical composition was divided into three sections to examine the vegetative process in the three growths. The time table for harvesting is shown in Table 1. To account for climatic changes the duration of the study was set for three years (2000, 2001, 2002). Every growth was harvested over a period of 7 weeks using the fresh grass of different maturities for the feeding trials.

<b>Table 1</b> . Experimental design of the harvest dates
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<b>Feeding trial 1<sup>st</sup> growth</b> 1 <sup>st</sup> cut: 4 <sup>th</sup> week of May 1 <sup>st</sup> cut: 4 <sup>th</sup> week of May	
8 8	
Feeding trial $2^{nd}$ growth $2^{nd}$ cut: $4^{th}$ week of July	
Feeding trial 3 <sup>rd</sup> growth	
May June	
2     3     4     1     2     3     4       July     August	
1 2 3 4 1 2 3 A. September Oc	xt.
	2

#### *In situ*-investigations

The *in situ*-investigations were based on the specifications of Orskov et al. (1980), Michalet-Doreau et al. (1987), Madsen and Hvelplund (1994), Huntington and Givens (1995) and NRC (2001). For incubations four runnially fistulated steers were used. The diet consisted of 75 % forage ( $\frac{1}{3}$  hay,  $\frac{1}{3}$  grass silage,  $\frac{1}{3}$  corn silage) and 25 % concentrates and was offered four times per day at energy maintenance level. The incubation times were 0, 3, 6, 10, 14, 24, 42, 65, 92 and 120 h (Mertens, 1993). Data were analysed according to the model of Orskov and McDonald (1979): deg = a + b × (1 - exp(-c × (t - lag))) for t > lag

Effective degradabilities (ED2, ED5, ED8) for rumen outflow rates of 0.02, 0.05 and 0.08 (per h) were calculated according to the specifications of McDonald (1981) modified by Südekum (2005):  $ED = a + [(b \times c) / (k + c)] \times exp(-k \times lag)$ 

#### **RESULTS AND DISCUSSION**

The results of the *in-situ*-investigations are shown in Table 2 and Figure 1. As expected the vegetative stage influenced the degradability of DM of the fresh grass significantly. However, growth number also had an impact on degradability. There were significant interactions between growth number and vegetative stage regarding many degradation parameters according to Orskov & McDonald (1979) i.e. influence of vegetative stage was different in the three growths.

When accounting for all vegetative stages, potential degradability (a + b) was higher for the 1<sup>st</sup> growth than for growths 2 and 3 (79.2, 76.7 and 76.7 %, respectively). Whereas e.g. potential degradability of vegetative stages 7 differed marginally (71.4, 70.9 und 71.4 %), degradability of vegetative stage 1 from the 1<sup>st</sup> growth was higher than of 2<sup>nd</sup> and 3<sup>rd</sup> growth (85.7, 80.2 und 75.8 %). I.e. the higher nutritive value of fresh grass from the 1<sup>st</sup> growth, as stated in this study, is strongly influenced by the values of early vegetative stages. Consequently in terms of feed value it is particularly worthwhile to harvest the first growth early. However, research at our institute has shown that this comes at the expense of DM yield (Gruber et al., 2000; Gruber et al., 2006). On average of all growths the potential degradability decreased from 80.6 to 71.2 % during vegetation.

On average of all vegetative stages the proportion of fraction a (rapidly and completely soluble) slightly declines

with growth number (22.0, 19.3 and 18.4 %) while there are marginal differences between proportions of the insoluble, potentially degradable fraction b (57.1, 57.4 and 58.3 %). As in potential degradability there are interactions regarding parameters a and b. During vegetation the decrease is higher for the 1<sup>st</sup> growth than in the 2<sup>nd</sup> and 3<sup>rd</sup>. On average of all growths fraction a decreased from 20.4 to 18.1 % during the 7 weeks of vegetation, fraction b from 60.2 to 53.1 %. Influence of vegetative stage was most obvious in degradation rate c. It decreased from 6.7 % per hour at vegetative stage 1 to 4.6 % at vegetative stage 7. In the 1<sup>st</sup> and 2<sup>nd</sup> growth this decline was comparatively linear while in 3<sup>rd</sup> growth this was the case only at the beginning and the degradation rate subsequently remained almost 4.5 %.





The effective degradability ED is a function of parameters a, b and c and accounts for passage rate of feed in the rumen, i.e. being lower than the potential degradability. E.g. ED5 (medium outflow rate) decreased from 54.2 to 42.5 % during vegetation, this decline being more pronounced in the  $2^{nd}$  and  $3^{rd}$  growth. On average of all vegetative stages ED5 for the three growths was 51.3, 48.0 and 44.9 %, respectively.

	a	b	c	lag	a + b	ED2	ED5	ED8
1 <sup>st</sup> growth								
Week 1	22,3	63,4	0,075	0,00	85,7	72,4	60,4	53,0
Week 2	25,4	59,2	0,057	0,00	84,5	69,1	56,8	49,9
Week 3	21,5	59,2	0,060	0,00	80,7	65,8	53,7	46,8
Week 4	22,0	57,5	0,051	0,00	79,5	63,4	51,1	44,5
Week 5	19,5	58,8	0,043	0,43	78,3	59,4	46,2	39,5
Week 6	22,3	51,6	0,042	0,00	74,0	57,3	45,9	40,1
Week 7	21,2	50,2	0,044	0,00	71,4	55,7	44,7	39,0
2 <sup>nd</sup> growth	1				<u>.</u>			
Week 1	20,6	59,6	0,065	0,00	80,2	66,3	54,4	47,4
Week 2	21,0	58,7	0,066	0,00	79,7	66,0	54,3	47,5
Week 3	20,2	58,0	0,053	0,00	78,2	62,3	50,0	43,3
Week 4	19,6	58,5	0,048	0,21	78,0	60,6	47,8	41,1
Week 5	19,1	58,2	0,051	0,33	77,4	60,7	48,1	41,3
Week 6	17,7	54,5	0,038	0,23	72,2	53,3	41,0	34,9
Week 7	16,5	54,4	0,045	1,39	70,9	53,2	40,6	34,0
3 <sup>rd</sup> growth	l							
Week 1	18,2	57,7	0,060	1,29	75,8	60,4	47,8	40,6
Week 2	19,1	60,0	0,048	1,46	79,0	60,1	46,3	39,0
Week 3	18,5	59,7	0,042	1,93	78,1	57,3	43,1	36,0
Week 4	19,4	59,8	0,045	1,05	79,1	59,8	46,2	39,1
Week 5	18,1	58,5	0,046	1,76	76,6	57,4	43,7	36,6
Week 6	18,7	57,8	0,045	0,75	76,5	58,3	45,2	38,5
Week 7	16,7	54,7	0,050	1,36	71,4	54,8	42,3	35,7

Table 2. Results of the in-situ-investigations (parameters of the degradation kinetics according to Orskov, 1979)

#### CONCLUSIONS

Based on the results of the study it can be concluded that during vegetation especially for the 1<sup>st</sup> growth there is a strong decrease in degradability of DM and consequently a significant interaction between growth number and vegetative stage exists. Degradability of the later growths is lower.

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The list of references can be requested from the first author.

# CHANGES IN FORAGE QUALITY BY ANIMAL FERTILIZERS' APPLICATIONS AND BY DIFFERENT **GRASSLAND MANAGEMENT**

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#### ABSTRACT

A long-term small plot trial the locality Rapotin. It consists of treatments with application of three doses of different fertilizers (no fertilization and cattle fertilizers) and with three levels of intensity of cutting (2, 3 and 4 cuts per year). Three levels of fertilization correspond with stocking rate of 0.9, 1.4, and 2 LU.ha<sup>-1</sup> (LU=livestock unit). The results obtained during two years of monitoring are reported in this paper. The chemical composition of the forage was evaluated on the basis of Czech state standard and there was also estimated the organic matter digestibility (OMD) in vitro. As for the fodder quality, it was found that the concentration of crude protein  $(93 - 170 \text{ g.kg}^{-1} \text{ DM})$  and the organic matter digestibility (63-72 %) were higher in treatments with higher intensity of cutting. We also found a significant decrease of concentration of crude fibre (297-224 g.kg<sup>-1</sup> DM). There was also monitored the concentration of crude protein.

#### **INTRODUCTION**

In the Czech Republic an expansion of the permanent grassland areas is expected. In this situation it is necessary to identify the most suitable methods of grassland management for the future. Permanent grassland management for cattle breeding is the most rational attractive. It is important to provide the necessary quality of the feedstuffs to satisfy the animals' requirements. A major part of the Czech farms that are engaged both in crop and animal production utilises mineral fertilizers for this purpose. However, grassland enables to utilise effectively also natural fertilizers (manure, dung-water, slurry etc.). Natural fertilizers are important base for rational agriculture.

#### **MATERIALS AND METHODS**

A long-term small plot trial was established in 2004 on permanent grassland sites in the locality Rapotín. The trial is situated in 390-402 m above see level. Average values for temperature during the vegetation period (March-October) were 11.1 °C in 2005 and 12.2 °C in 2006 and the rainfall during the vegetation was 399.0 mm in 2005 and 460.2 mm in 2006. The grassland vegetation on the experimental stands was classified as Arrhenatherion. The dominant species in the permanent sward at the beginning of the trial were Poa pratensis, Dactylis glomerata, Lolium perenne, Elytrigia repens, Taraxacum sect. Ruderalia and Trifolium repens.

Experimental treatments simulating cattle grazing were as follows:

N0-2 – no fertilization, 2 cuts per year

N0-3 – no fertilization, 3 cuts per year

N0-4 – no fertilization, 4 cuts per year

Man 0.9-2 – cow manure + dung water with the load of 0.9 LU.ha<sup>-1</sup> (2 cuts per year and 54 kg.N.ha<sup>-1</sup>)

Man 1.4-3 – cow manure + dung water with the load of 1.4 LU.ha<sup>-1</sup> (3 cuts per year and 84 kg.N.ha<sup>-1</sup>)

Man  $2.0-4 - \text{cow manure} + \text{dung water with the load of } 2.0 \text{ LU.ha}^{-1}$  (4 cuts per year and 120 kg.N.ha<sup>-1</sup>)

Slu 0.9-2 – slurry with the load of  $0.9 \text{ LU.ha}^{-1}$  (2 cuts per year and 54 kg.N.ha<sup>-1</sup>) Slu 1.4-3 – slurry with the load of  $1.4 \text{ LU.ha}^{-1}$  (3 cuts per year and 84 kg.N.ha<sup>-1</sup>) Slu 2.0-4 – slurry with the load of  $2.0 \text{ LU.ha}^{-1}$  (4 cuts per year and 120 kg.N.ha<sup>-1</sup>)

The cow manure is dosed in the autumn, dung water after the first cut; half of slurry is aplied in the spring and second half after the first cut. The samples from these plots 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. The in vitro organic matter digestibility (OMD) was determined by the Tilley and Terry method (1963) modified according Resch (1991). The ME (metabolisable energy), NEL (net energy of lactation), NEV (net energy of fattening), PDIE (ingested digestive protein allowed by energy), PDIN (ingested digestive protein allowed by nitrogen) was predicted by means of the regression equations (Pozdíšek et al., 2001) and by means of the equations that mentions Petrikovič et al. (2000). The evaluation of the nutritive value in system NE, PDI is officially used in the Czech Republic and Slovakia. This system corresponds with the system INRA (Jarrige et al., 1989). The results were statistically evaluated by analysis of variance (ANOVA).

### **RESULTS AND DISCUSSION**

The effect of different number of cuts on the evaluated parameters was statistically significant (Table 1). Tendency to decrease was found only in soluble nitrogen fraction (FNtca), particularly for variant N0-2 and N0-4. The results concerning contents of nutrients correspond with the results published previously (Pozdíšek et al., 2006 and other authors).

For the optimal cattle nutrition it is suitable to use permanent grassland with PDIN/PDIE ratio that is approximately 1. Treatments N0-4, Man 0.9-2, Man 1.4-3, Slu 0.9-2, and Slu 0.9-3 meet this requirement (see Fig. 1). Analogous to Pozdíšek *et al.* (2006) we can consider as suitable the treatments utilised with three cuts per year and fertilised with medium dose of fertilisers.

	ENItoo	CP	CF	OMD	ME	NEL	PDIN	PDIE
AVG	rinica [0/]	[g.kg <sup>-1</sup>	[g.kg <sup>-1</sup>	[g.kg <sup>-1</sup>	[MJ.kg <sup>-1</sup>	[MJ.kg <sup>-1</sup>	[g.kg <sup>-1</sup>	[g.kg <sup>-1</sup>
	[70]	DM]	DM]	DM]	DM]	DM]	DM]	DM]
N0-2	16.8	93.3	296.7	632	8.65	5.03	58.5	73.4
N0-3	21.5	116.7	263.8	680	9.25	5.44	73.2	80.5
N0-4	23.1	144.3	236.0	717	9.74	5.79	90.1	85.9
Man 0.9-2	21.1	125.1	266.5	634	8.63	4.98	77.9	80.6
Man 1.4-3	21.7	133.8	246.6	674	9.20	5.38	83.7	84.1
Man 2.0-4	22.7	169.0	223.8	682	9.21	5.38	104.7	88.1
Slu 0.9-2	22.4	128.3	267.7	642	8.77	5.08	80.0	81.7
Slu 1.4-3	22.1	138.0	263.7	653	8.93	5.19	85.9	83.3
Slu 2.0-4	22.5	169.9	232.1	677	9.12	5.33	105.2	87.5
ANOVA F - ratio								
Cutting	1.91	71.89++	29.49++	$29.05^{++}$	15.08++	$14.92^{++}$	72.95++	45.96++
Fertilization	1.72	14.16++	4.06+	2.53	1.95	2.53	14.13++	9.22++
Year	0.01	0.11	0.44	0.01	0.44	0.45	0.15	0.03

Table 1. Chemical composition, digestibility and content of nutrients (mean of samples from 2005 and 2006)

 $^{+}P < 0.05 ^{++}P < 0.01$ 

FNtca - nitrogen fraction soluble in trichloroacetic acid 10% w/v in water, OMD - organic matter digestibile



# CONCLUSIONS

It is possible to influence significantly the amount and the quality of the fodder by means of the grassland management, i.e. via number of cuts and fertilization. Our findings presented in this paper are important for the nutrition of cattle and for efficient grassland management. The other enlargement of the knowledge in this issue is still necessary.

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#### CULTURE, HARVEST AND ENSILING OF SOME GRAIN LEGUMES

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#### **ABSTRACT**

Culturing grain legumes is interesting to obtain a more sustainable agriculture, with positive effects on energy balance and benefits of crop rotation (less soil borne pathogens, a better soil structure, ...). Also the dependence on import of soybeans decreases. In the period 2004-2007, field trials with lupins and intercrops such as barley-pea and wheat-faba beans were conducted. Different aspects of culture (control of weed and plant pathogens) and ensiling of moist grain were optimized. The course of the silage process and the quality parameters were monitored.

#### **INTRODUCTION**

Europe has an enormous shortage of vegetable protein sources since the general ban of feeding animal meal in the EU. As consequence, the European countries must import 78 % of their protein needs for food as well as for feed processing (A.P.P.O., 2005). Legumes are the most interesting source of vegetable proteins. They produce substantial yields without any requirement for nitrogen fertiliser (Fan et al., 2002; Honeycutt, 1998). Intercropping of cereals with legumes is an alternative for monocropping and has a number of advantages, for example, lower inputs, lower costs of production and better silage quality than monocrop systems (Carruthers et al., 2000). Harvesting legumes as dry grain, can be a problem under north western European climate conditions. Ensiling moist grain can be a solution to obtain a culture with less risks.

#### MATERIALS AND METHODS

During the years 2004, 2006 and 2007, several field trials with grain legumes and mixed cultures were conducted at the Experimental Farm of the University College Ghent, Merelbeke, Belgium on a sandy loam soil. All trials were set up as a randomised block design with 3 replicates except for the lupin trials (4 replicates). The trials were harvested with a Wintersteiger field trial tresher. The yields were determined and a sample was taken to determine the dry matter (DM) content and the chemical composition at ensiling. If it was a mixed culture, the sample was separated into the different components. The components were dried and the composition was expressed on a DM basis.

The harvested grain was crimped and homogenised well. A 15 kg sample was treated or with a control solution or with a silage additive. All solutions were vaporised as a fine mist over the crimped grain (100 ml solution/10 kg grain). Two additives were used: Pioneer 1188 (Pioneer Hi-Bred, Johnston): *Lactobacillus plantarum* 2.4 x  $10^{10}$  CFU/g+ *Enterococcus faecium* 0.6 x  $10^{10}$  CFU/g (applied dose:  $3.3 \times 10^{-3}$  g/kg FW) and Crimpstore (Spider Solutions Bvba, Waarschoot): 50 % formic acid + 50 % propionic acid (applied dose:  $6 \, l/1000$  kg FW).

The treated and crimped grain was ensiled in microsilos with a content of 2.75 l and equipped with an air seal. Four silos per treatment and per culture were preserved. The silos were opened after 10 weeks and two samples were taken. One to determine the chemical composition and one for the determination of the volatile fatty acids with the method of Lepper (1938). The silage quality was determined with the combined scale as described by Vanbelle (1981). The scale is a combination of the Flieg score (Flieg, 1938) and the protein conservation (Lepper, 1938).

#### **RESULTS AND DISCUSSION**

**Yields and cropcomposition.** The results of the yields are shown in Table 1. Intercropping of grain legumes with cereals do have significantly higher (P < 0.001) yields. During the growing season 2004, the yield of the pea/wheat intercrop was significantly higher (2920 kg DM/ha) than the yield of the monoculture of pea (2175 kg/ha). In 2006 the same tendency was visible for the culture of faba bean and the mixed culture of faba bean and summer wheat. The faba bean production was 2355 kg DM/ha, which was significantly lower than the yield of the intercrop with wheat (2748 kg DM/ha). During the growing season 2007, the mean yields of *Lupinus albus* (1770  $\pm$  910 kg DM/ha) and those of *L. angustifolius* (1827  $\pm$  477) were comparable.

**Fermentation characteristics.** In table 2 the silage characteristics are shown. All silages have a good quality, no butyric acid was detected. The crude protein (CP) content of *Lupinus albus* (374g/kgDM) and of *Lupinus angustifolius* (351g/kgDM) were higher than those of faba beans (285g/kg DM), peas (186g/kgDM) and pea/barley (156g/kgDM).

The low NH<sub>3</sub> content in all silages indicates that the protein conservation was very good.

The CP content of *L. angustifolius* silages was comparable with the results described by Fraser et al. (2005) and was much higher than the protein content of whole crop silage of *L. angustifolius*. The CP content of the lupin silages was comparable with the CP content of the dry seeds (Sujak et al., 2006). Adding an additive did not have a significant influence on the pH in the case of the pea/barley silages, but did significantly decrease the pH (p < 0.05) in the case of faba bean silages. Also the NH<sub>3</sub> content was significantly lower if an additive was applied in pea/barley and faba bean silages. The influence of Crimpstore on the silage quality of *Lupinus* spp. was minimal.

# Table 1. Yield and dry matter content

Year	Variety	Species	Yield (kg DM/ha)	DM (g/kg)
2004	Harnas/Flandria	pea/barley	2920	769
	Harnas	pea	2175	833
			** *	
2006	Diana/Tybalt	faba bean/wheat	2748	676
	Diana	faba bean	2355	670
			***	
2007				
	(a)	Lupinus angustifolius	$1827\pm477$	$763\pm130$
	(a)	Lupinus albus	$1770 \pm 910$	$700 \pm 40$

(a) means for different varieties

\*\*\* p < 0.001

#### Table 2. Fermentation characteristics

Year	Variety	Species	silage additive	DM (gkg FM)	рН	NH3 (g/kg)	CP (g/kg DM)	Acetic acid (g/kg FM)	Butiric acid (g/kg FM)	Lactic acid (g/kg FM)
2004	Hamas/Flandria	pea/barley	-	807	5.18	0.010	157	0.080	ND	0.292
	Hamas/Flandria	pea/barley	1188	807	5.30	0.003	156	0.043	ND	0.345
	Hamas	pea	-	825	5.42	0.001	186	0.015	ND	0.460
				*	NS	***	NS	NS		NS
2006	Diana/Tybalt	faba bean/wheat	-	643	4.30	0.007	270	0.050	ND	0.128
	Diana	faba bean	-	643	4.20	0.013	285	0.058	ND	0.268
	Diana	faba bean	Crimpstore	646	4.08	0.005	273	0.038	ND	0.213
				NS	**	**	**	NS		*
2007										
	(a)	Lupinus angustifolius	-	$695 \pm 84$	$4.95\pm0.60$	$0.010\pm0.01$	$350\pm30$	$0.132 \pm 0.023$	ND	$0.554 \pm 0.612$
	(a)	Lupinus angustifolius	Crimpstore	$703 \pm 79$	$4.79 \pm 0.81$	$0.006 \pm 0.005$	$346 \pm 32$	$0.157 \pm 113$	ND	$0.569 \pm 0.231$
	(a)	Lupinus albus	-	$660 \pm 7$	$4.95\pm0.32$	$0.019 \!\pm\! 0.013$	$374\pm42$	$0.148 \pm 0.089$	ND	$0.501\pm0.003$
	(a)	Lupinus albus	Crimpstore	$670 \pm 15$	$4.99\pm0.23$	$0.014 \pm 0.011$	$368\pm28$	$0.12\pm0.057$	ND	$0.402 \pm 0.028$

(a) means for different varieties

\*\* p < 0.05 \*\*\* p < 0.001

#### CONCLUSIONS

Harvesting moist grain, crimping and ensiling it, can be a good alternative for the dry grain harvest in years when climate during harvest period is instable. Adding an additive can, in some cases have a positive influence on the silage quality.

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# ECONOMIC ANALYSIS OF PROTEIN SILAGES PRODUCTION

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### ABSTRACT

We have cooperated with the agricultural enterprise "SEMAT s. r. o." in Trnava in order to obtain the production the production of protein fodder for beef cattle and carrying capacity of game surroundings. The experiments were carried out on the degraded backland of the maize field. The pea was used as the cover crop for the lucerne. The cultivation technology of low inputs was used to obtain new knowledge about production and nutrition parameters of the lucerne - pea silage with the positive economic indicator. The direct costs for 1 ha were not higher than 17.800, - Sk (592  $\epsilon$ ) and the direct costs for 1 ton of silage were 556, - Sk (18.45  $\epsilon$ ). Therefore, this production of fodder is economically effective for livestock and fodder can be used as food for game as well.

Forage crop	1st cut	2nd cut	3rd cut	Total
Pisum sativum + Medicago sativa in sowing year	310-320	70-80	40-50	420-450
<i>Medicago sativa</i> in 1st yield year	160-180	90-120	60-80	310-380
<i>Medicago sativa</i> in 2nd yield year	160-180	90-120	60-80	310-380
Average	210.00-226.67	83.33-106.67	53.33-70.00	346.67

 Table 2.
 Yields of whole-crop pea and lucerne in t.ha<sup>-1</sup> (3 years average)

Table 3.	The quality of whole-crop pea and lucerne silage
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Parameter	Original matter	Dry matter
Dry matter %	35.06	100
Crude protein %	5.93	16.91
RDP %	4.88	13.91
Fat %	1.31	3.75
Crude fibre %	8.02	22.87
Structural fibre %	5.52	15.74
Volatile fibre %	3.40	9.69
Ash %	3.56	10.15
Nitrogen-free extract %	16.24	46.31
ME for cattle MJ/kg	3.34	9.52
NEL (550 kg - 15 l/-4% fat) MJ/kg	1.96	5.60
NEV (350 kg - 0,9 kg/animal/day) MJ/kg	1.90	5.43
Q (ME/BE) %	52.22	-
PDIN g/kg	35.20	100.41
PDIE g/kg	28.03	79.96

			Costs per 1	ha in Sk /€/		
Work operation	1 <sup>st</sup> utili	ty year	2 <sup>nd</sup> utility vear	3 <sup>rd</sup> utility vear		
work operation	Pea + Lucerne 1 <sup>st</sup> cut	Lucerne 2 <sup>nd</sup> & 3 <sup>rd</sup> cuts	Lucerne sum of cuts	Lucerne sum of cuts	Total	Average
Stubble breaking by CASSE	6′ /22	70 .24/	-	-	670 /22.24/	223 /7.40/
Deep ploughing by CASSE	22 /73	.00 .02/	-	-	2200 /73.02/	733 /24.33/
Dragging by Z 160	18 /5.	80 97/	-	-	180 /5.97/	60 /1.99/
Cultivating by Z 160	20 /6.	00 64/	-	-	200 /6.64/	66 /2.19/
Fertilizer (NPK15-15-15) 200 kg.ha <sup>-1</sup>	20 /66	00 .39/	-	-	2000 /66.39/	666 /22.16/
Fertilizer application 200 kg.ha <sup>-1</sup>	12 /3.	20 98/	-	-	120 /3.98/	40 /1.32/
Seeds 250 kg.ha <sup>-1</sup> pea + 15 kg.ha <sup>-1</sup> alfalfa	36 /120	025 0.32/	-	-	3625 /120.32/	1208 /40.09/
Sowing by Z 160	37/12	75 .45/	-	-	375 /12.45/	125 /4.14/
Rolling by Z 80-120	1: /4.	50 98/	-	-	150 /4.98/	50 /1.65/
Protection against <i>Aphis</i> by TALSTAR 10 EC (0.1 %)	90 /30	908 /30.14/		-	908 /30.14/	-
Cutting by Petinger Classe	540 /19.58/	1180 /39.17/	1770 /58.75/	1770 /58.75/	5260 /174.60/	1753 /58.18
Raking by Z 180-120	120 /3.98/	240 /7.97	360 /11.94/	360 /11.94/	1080 /35.84/	360 /11.94/
Harvest by Class Jaguar	400 /13.28/	800 /26.56/	1200 /39.83/	1200 /39.83/	3600 /11949/	1200 /39.83/
KemiSile application TM (4 l.t <sup>-1</sup> )	4992 /165.70/	-	-	-	-	-
Silage transport	850 /28.21/	1700 /50.43/	2550 /84.64/	2550 /84.64/	7650 /253.93/	2550 /84.64/
Silage compaction	470 /15.60/	940 /31.20/	1410 /46.80/	1410 /46.80/	4230 /140.40/	1410 /46.80/
Sum of direct costs	17800 /592.48/	4860 /161.32/	7290 /241.98/	7290 /241.98/	37240 /1236.14/	12413 /412.03/
Production overhead	1762 /58.48/	-	1762 /58.48/	1762 /58.48/	5286 /175.45/	1762 /58.48/
Operating overhead	1852 /61.47/	-	1852 /61.47/	1852 /61.47/	5556 /184.42/	1852 /61.47/
Sum of own expense	21414 /712.43/	-	10904 /361.94/	10904 /361.94/	43.222 /1434.70/	14407 /478.22/
Own expense per 1 ton silage	669.18 /22.4/	-	316.05 /10.49/	316.05 /10.49/	1301.28 /43.194/	433.76 /14.39/
Silage yields (t.ha <sup>-1</sup> by 35 % DM yield)	32.00	11.00	34.50	34.50	112.00	37.33

# Table 1. Costs of forage crops growing and protein silages production

### ECONOMIC USE OF GRASS ELYMUS ELONGATUS

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#### **INTRODUCTION**

*Elymus elongates var. ponticus* coming from the Mediterranean region with its dry summers, this is a cool-season grass that grows mainly from autumn round to the spring and then can become dormant in the summer. It has been reported to be promising even in the arid zone of South Australia where rainfall is 12,5-20cm annually. The plant prefers light, medium and heavy soils. According to the phytomass files, annual productivity from 2 to 15t/ha. Phytomass could be converted to alcohol or methane (Duke 1983).

### MATERIALS AND METHODS

Experiment was conducted from April to September 2007. Materials used were *Elymus elongatus var. ponticus* perennial grass species. Agro technical part of experiment was conducted in Botanical Garden of PBAI in Bydgoszcz. Plants were collected every seven-day at following phases of development:

- Vegetative phase (VS) at 18-67 day of vegetation (days starting from 1st April),
- Beginning of heading -74-116 day of vegetation,
- Beginning of flowering -123 day of vegetation,
- Full seed maturity -186 day of vegetation.

For each development phases, green forage was collected in 4 replicates from area of  $1m^2$ . Forage was cut by hand collector ca. 3 cm above ground. Analytical part of experiment was conducted in Department of Animal Nutrition and Feed Management Economy, Faculty of Animal Breeding and Biology of University of Technology and Agriculture in Bydgoszcz. After drying the main components was determined (dry matter - DM, crude ash – CA, crude protein –CP, Crude fat – CFa, crude fiber – CF) according to standard procedures [AOAC 1990]. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to Goering and van Soest [1979]. Water-soluble carbohydrates (WSC) were determined according to Polish Standard [1998]. Buffer capacity of forage (BC) and forage fermentation coefficient was determined according to Weissbach [1992] and Weissbach [1998].

#### **RESULTS AND DISCUSSION**

Dry matter in grass in vegative phase was noted on average level 20.64 % (from 18.66 5 to 23.59 %). Dominant component of dry matter was crude fibre. Amount of this component was on average 31,85 % (from 25.10 % to over 38 %). Crude protein contents was 19.24 % of dry matter (from 27.24 % to 11.83 %) (Table 1).

In beginning of heading phase was noted increase of dry matter content in grass to 33,80% (from 26.69 % to 41.00%). *Elymus elongatus var. ponticus* species in beginning of heating was subject to strong lignifications process. Crude fibre contents in dry matter were in average 43 % (from 38.60% to 46.39%). Average contents of crude protein in dry matter (table 1) were reduced to the level 7.72 % (from 6.53% to 10.80%). In next phase of vegetation increase of dry matter and higher contents of fibre deposition was stated in plants. Crude protein contents were lower. Plants in the beginning of flowering (table 1) contained about 39.78% dry matter (from 38.76% to 40.57%). The fibre contents was 47.60% of dry matter (from 47.38% to 48.66%). The level of protein was lower and amount 7.07% of dry matter (from 6.35% to 8.31%). Grass in full seed maturity phase was characterized by average contents of dry master on the level 49.50% (from 46.01% to 50.92%). Contents of protein were 5.16% of dry matter (from 4.40% to 5.69%). Slightly lower contents of crude fibre were noted in this phase. Number of this component in dry matter was 40.71% (from 38.28% to 43.64%). Forage fermentation coefficient in vegetative phase (table 1) was low and amounted to 25.85 (from 20.74 to 33.50). In next phases of *Elymus elongatus var. ponticus* species development contents of this coefficient (table) subject to increase what guarantee correct process and direction of fermentation in ensilage grass [Weissbach 1998]. Fermentation coefficient under 35% allows classifying this green forage as a plant easy to ensiling [Weissbach 1998]. However the high contents of crude fibre limit use of this species as a fodder for breeding animals.

Composition of regrowth was similar to the composition of plant in main crop. High contents of crude fibre did not allow using it as potential fodder source (table 1). High potential of plants yield, ovet 12 t dry matter from 1 ha, in full seed maturity phase was similar to value presented by Duke [1983].

#### CONCLUSIONS

1. Value of forage fermentation coefficient *Elymus elongatus var. ponticus* from heading phase allows classifying green forage as easy ensiling plant.

- 2. High contents of structural hydrocarbons in *Elymus elongatus var. ponticus* grass limit its use as a fodder.
- 3. High contents of structural hydrocarbons show the potential use *Elymus elongatus var. ponticus* species as power plants.

Spaceification	DM					Con	tent in c	dry mat	ter (g/k	g)				EC
specemeation	DIVI	CA	CP	CFa	CF	NDF	ADF	HEM	ADL	NFE	WSC	BC	WSC/BC	гC
				Vege	tative s	tage (18	8-67 day	s of ve	getatio	n)				
N=32														
х	206.4	75.2	192.4	33.3	318.5	564.3	334.1	230.2	30.7	380.6	57.9	89.2	0.65	25.84
±	21.5	15.7	52.6	6.9	51.7	73.7	51.3	27.3	6.9	30.3	12.5	13.2	0.17	3.10
Min	177.2	50.4	114.6	21.2	243.5	465.3	270.1	176.5	22.7	312.6	38.6	68.6	0.38	20.74
Max	252.1	105.6	284.5	45.2	384.8	735.7	451.6	284.1	46.5	448.4	88.3	120.0	1.29	33.50
					Begi	inning o	of headi	ng (74-	116 da	ys o f v	egetatic	m)		
N=28														
х	338.0	47.7	77.2	21.0	436.7	724.5	440.9	283.8	56,9	417.4	73.1	54.9	1.36	44.65
±	50.3	7.2	15.7	3.1	24.5	29.4	23.8	21.4	7.9	12.5	15.7	6.3	0.37	7.47
Min	276.7	36.0	56.9	13.9	381.3	657.7	391.1	246.8	43.2	388.2	44.9	41.8	0.87	35.17
max	425.8	61.8	110.3	26.4	467.4	783.6	477.2	333.3	75.1	434.6	105.9	65.9	2.14	57.39
					Begint	ning of	floweri	ng (123	day of	vegeta	tion)			
N=4														
х	397.8	42.2	70.7	16.7	476.0	721.2	455.4	268.9	63.4	394.4	93.7	52.5	1.78	54.02
±	6.5	2.4	7.4	1.0	6.6	25.4	25.2	15.6	2.9	39.6	16.3	2.0	0.29	2.56
Min	387.6	39.3	63.5	15.2	473.8	693.0	437.3	255.7	61.2	473.8	70.4	50.3	1.69	53.48
Max	405.7	49.6	83.1	18.0	486.6	781.9	491.1	290.8	68.5	486.6	116.5	55.3	2.18	58.01
					Full	seed m	aturity	(186 da	y of ve	getatio	n)			
N=4														
х	495.0	39.4	51.6	17.4	407.1	723.8	460.8	262.9	78.7	484.5	94.0	37.9	2.48	69.34
±	20.2	6.3	5.0	1.3	19.0	24.0	19.5	12.7	2.8	20.7	14.6	0.9	0.39	3.28
Min	460.1	32.0	44.0	16.3	382.8	703.5	439.2	244.6	75.4	459.1	72.5	36.9	1.91	66.20
max	509.2	47.3	56.9	19.7	436.4	764.5	492.2	272.3	81.2	509.1	103.9	39.0	2.62	73.35

**Table 1.** Chemical composition of *Elymus elongatus var. ponticus*

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### EFFECTS OF HEAVY METALS ON THE YIELD AND APPLICATION OF OILSEED RAPE SEEDS FOR ANIMAL FEEDING

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#### **INTRODUCTION**

Heavy metal contamination in soil is one of the world's major environmental problems, posing significant risks to human health as well as to ecosystems. Recently, phytoremediation-using plants to remove metal pollutants from contaminated soil, has been developed as a new method for the remediation of contaminated land. This environmentally friendly, cost-effective and plant-based technology is expected to have significant economic, aesthetic and technical advantages over traditional engineering techniques (Salt et al., 1998; Garbisu and Alkorta, 2001). A number of highbiomass plants have been used in phytoremediation studies. The ideal plant species to remediation a heavy metal contaminated soil would be a high biomass crop that can both tolerate and accumulate the contaminants of interest (Cunningham and Ow, 1996). Among them the Brassicaceae family, to which most metal hyper accumulator species belong, represents a potential and promising solution to the problem (Marchiol et al., 2004; Rossi et al., 2004).

The experiments were carried out to assess the influence of heavy metals on the yield and potential application of oilseed rapeseeds for animal feeding.

#### **MATERIALS AND METHODS**

A pot experiment was settled in 2006 to investigate effects of zinc, copper, lead and cadmium on the growth of spring oilseed rape. The following doses of heavy metals were used: zinc - 300 and 600 mg/kg soil (Zn-I; Zn-II), copper - 80 and 160 mg/kg soil (Cu-I; Cu-II), lead - 400 and 1600 mg/kg soil (Pb-I; Pb-II), and cadmium - 2 and 6 mg/kg soil (Cd-I; Cd-II). The soil not contaminated with the heavy metals under study was the control. The total content of the metals in the control samples was: Zn ( $39.8 - 51.8 \text{ mg} \text{kg}^{-1}$ ), Cu ( $6.8 - 7.7 \text{ mg} \text{kg}^{-1}$ ), Pb ( $10.8 - 13.0 \text{ mg} \text{kg}^{-1}$ ), and Cd < 0.2mg.kg<sup>-1</sup>, typical for unpolluted soils.

The collected seeds were analyzed for protein (Kjeldahl) and fat content (according to PN - EN ISO 659) and the percentage of some fatty acids (palmitic, stearic, oleic, linolic, linoleic, with the use of gas chromatography) in the sum of extracted oil. The seeds were dried at 105°C to a constant weight and than mineralized in the HNO<sub>3</sub> and  $H_2O_2$ mixture. The total content of heavy metals under study was assayed by emission spectrometry with plasmic excitation.

#### **RESULTS AND DISCUSSION**

The studies dealing with the use of oilseed rape in phytoremediation have not evaluated possibilities for the influence of the intake heavy metals on the impairment of fatty acids synthesis and potential qualitative and quantitative changes in the amount of the oil extracted from the seeds. Similarly, there is only a few reports on the openings for the use of post-extraction meal. Therefore, the idea of studying these problems arose. The results demonstrated that Brassica napus accumulated zinc, copper, lead and cadmium in the seeds. The content of heavy metals in seeds was observed: zinc - from 80.12 to 101.31 mg/kg, copper - from 2.06 to 3.51 mg/kg, lead - from 3.018 to 3.85 mg/kg, and cadmium - from 0.00 to 0.81 mg/kg in the dry matter (Tables 1-4). The seeds collected from the control pots contained zinc (22.83 mg/kg dw) and copper (1.37 mg/kg dw), but they did not show any traces of lead and cadmium.

Accumulation of heavy metals in the seeds over the phase of their maturation was observed by Rossi et al. (2004), who used zinc and copper in the doses 300 and 600 mg/kg soil and found that the content of the elements under study significantly increased in the maturing seeds of oilseed rape. This relationship was confirmed in our study, but the content of zinc assayed in seeds was higher in our study (Tables 1 and 2). However, the concentrations of lead and cadmium in the seeds (Tables 3-4) allowed considering the use of the post-extraction meal for feeding animals (Sapek 1993).

As compared with the control, no significant effects of the metal doses used in the experiment on the percentage content of protein were noted. However, a non-significant increase of protein concentration was shown in the seeds collected from plants grown in pots with copper applied in the doses 80 and 160 mg·kg<sup>-1</sup> soil (by 24.12 % and 23.97 %, respectively) (Table 2), and a considerable increase in the case of cadmium used in the doses 2 and 6 mg·kg<sup>-1</sup> soil (22.90 % and 23.30 %, respectively) (Table 4).

Combination	Seed yield	Zn content	Protein	Fat content	Fatty acids content [%]				
	per pot [g]	[mg/kg d.w]	content [%]	[%]	Palmitic	Stearic	Oleic	Linolic	Linoleic
Control	5.19	22.83	23.47	43.05	4.52	2.25	70.47	16.22	5.67
Zn-I	5.13	80.12	23.50	43.67	4.55	2.15	69.67	15.95	5.57
Zn-II	5.33	101.31	23.82	43.12	4.40	2.27	71.67	15.45	5.32
$LSD_{n=0.05}$	n.s.	8.78	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

**Table 1.** Concentrations of zinc in seeds, seed yield and qualitative fodder parameters of spring oilseed rape seeds.

Combination	Seed yield	Cu content	Protein	Fat content	Fatty acids content [%]					
	per pot [g]	[mg/kg d.w]	content	[%]	PALMITIC	Stearic	Oleic	Linolic	Linoleic	
			[%]							
Control	5.19	1.37	23.47	43.05	4.52	2.25	70.47	16.22	5.67	
Cu-I	5.09	2.06	25.12	42.17	4.45	2.40	70.62	15.97	5.62	
Cu-II	5.40	3.51	23.97	43.07	4.37	2.42	70.57	15.97	5.70	
LSD <sub>p=0,05</sub>	n.s.	0.41	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	

Table 2. Concentrations of copper in seeds, seed yield and qualitative fodder parameters of spring oilseed rape seeds.

Table 3. Concentrations of lead in seeds, seed yield and qualitative fodder parameters of spring oilseed rape seeds.

Combination	Seed yield	Pb content	Protein	Fat content	Fatty acids content [%]				
	per pot [g]	[mg/kg d.w]	content	[%]	PALMITIC	Stearic	Oleic	Linolic	Linoleic
			[%]						
Control	5.19	0.00	23.47	43.05	4.52	2.25	70.47	16.22	5.67
Pb-I	5.11	3.02	23.55	43.37	4.75	2.27	70.65	15.87	5.62
Pb-II	4.53	3.85	23.50	44.37	4.40	2.30	70.60	15.85	5.92
LSD <sub>p=0,05</sub>	n.s.	2.35	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

**Table 4.** Concentrations of cadmium in seeds, seed yield and qualitative fodder parameters of spring oilseed rape seeds.

Combination	Seed yield	Cd content	Protein	Fat content	Fatty acids content [%]				
	per pot [g]	[mg/kg d.w]	content	[%]	PALMITIC	Stearic	Oleic	Linolic	Linoleic
			[%]						
Control	5.19	0.00	23.47	43.05	4.52	2.25	70.47	16.22	5.67
Cd-I	5.12	0.00	22.90	43.80	4.45	2.15	70.97	15.92	5.70
Cd-II	4.48	0.81	23.30	43.22	4.47	2.37	70.97	15.85	5.52
LSD <sub>p=0,05</sub>	n.s.	0.15	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

n.s. - not significant

Similarly, no significant effects of the doses of heavy metals used in the investigations on the percentage concentration of fat were recorded (Tables 1-4). However, a significant influence of the increase of the cadmium dose from 2 to 6 mg·kg<sup>-1</sup> soil on the content of stearic acid in the oil extracted from the rapeseeds. After the use of 6 mg cadmium·kg<sup>-1</sup> soil it increased by 0.22 % (Table 4). As for the other doses of the heavy metals studied no effects were observed on the concentrations of fatty acids in the oil extracted from the rapeseeds

# CONCLUSIONS

Application of heavy metals under study in specific doses did not affect significantly the parameters of oilseed rapeseed yield collected from the pots.

The seeds accumulated small amounts of heavy metals; therefore the post-extraction meal can be used for feeding animals.

The doses of heavy metals did not influence parameters of oilseed rape seed, such as percent of protein and fat as well as fatty acids in the oil.

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# EFFECT OF OVERFLOWING AND DRY TO BOTANICAL COMPOSITION OF PERENNIAL SWARD IN DRY POLDER BEŠA

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#### **INTRODUCTION**

Dry polder Beša was based for retention of flood water from area of The East Slovak Lowland (ESL). Non-regularly ecological stability of polder Beša is disturbed by its artificial overflowing in time of special flood situations. Artificial overflowing of area significantly effect on botanical composition of sward in followed years, abandonment signs and followed processes of secondary succession. Similarly processes exist also in other localities of Slovak Republic (Novák, 2006; Vorobel', 2006; Hanzes et al., 2007).

#### MATERIALS AND METHODS

Dry polder Beša is situated to south-eastern part of the East Slovak Lowland near village Beša. Polder Beša has area 1 568 ha and capacity 53 million  $m^3$  and is the greatest polder in middle Europe. Subject of research was perennial swards, which have area 638.41 ha (81.38 % from agriculture land). Total area of perennial swards was divided into four parts – northern, central, southern and eastern and each part was individually valued. At perennial swards (PS) abandonment signs were valued by advancement index as follows: 0 - starting dilapidation; 1 - advanced dilapidation; 2 - marked dilapidation based on overgrowing with self-seeded forest and shrubs. Botanical composition of perennial swards was made by method of reduce projective dominance (Braun-Blanquet, 1964). On sites with advanced dilapidation was not determined.

#### **RESULTS AND DISCUSSION**

On 31<sup>st</sup> March 2006 polder Beša was saturated and it had very important effect to its research. Polder 11 million m<sup>3</sup> of water was loaded and it was 20 % from its retention capacity. Greater parts of polder are overflowed till in time of evaporating of water. Several parts of polder are overflowed during whole year and that in year 2006 ground survey was not realized. In year 2007 by ground survey botanical composition of sward and abandonment signs by index of advancement were made. Advancement of sites was visible by overgrown of forest and shrubs. Valuation of sward using in concrete year was made on September 2007. Results are shown in Table 1.

Da	romatar		Part of	polder	
1 a	Tameter	northern	central	southern	eastern
Area of perennia	l swards [ha]	165.42	124.92	218.69	129.38
Altitudes [m]		98.19 - 101.59	97.93 - 98.30	97.43 - 100.00	98.13 - 101.09
Signs of abandor	nment [ha]	0	0	71.9	73.61
Advancement	0 [ha]	0	0	0.98	17.70
index	1 [ha]	0	0	70.92	55.91
	2 [ha]	0	0	0	0
Botanically evalu	uation of sites [ha]	165.42	124.92	146.79	55.77
Botanical	grasses	70.5	52.7	76.8	63.8
composition	legumes	4.2	5.0	2.5	0.7
[%]	other herbs	24.4	38.2	15.3	27.3
	bare ground	0.9	4.1	5.4	8.2
Utilized areas [ha	a]	165.42	108.72	146.79	38.71
No-utilized areas	s [ha]	0	16.20	71.90	90.67

**Table 1.** Valuation of swards in polder Beša by parts in 2007 year

In northern part of polder perennial grassland present 165.42 ha. At using of swards by cutting processes of negative succession was not determined. Hay was taken from sites. In swards grasses were in majority and were on 75 % of area after cuttings. Grasses in sward from 95 till 99 % had *Alopecurus pratensis* L. From other grasses was identified *Poa compressa* L. (only 1 - 3 % presence). From legumes only *Vicia cracca* L. was presented. From other herbs very importantly were presented as follows: *Galium boreale* L., *Ranunculus repens* L., *Carex ssp., Taraxacum officinale* Weber in Wiggers and *Cirsium arvense* L.Scop. In central part of polder from total area 124.92 ha for cutting 108.72 ha were used. Only on acreage 16.2 ha was not made no pratotechnics arrangements. Botanical composition of sward was less favourable than in northern part of polder. Presence of grasses was 52.7 % and other herbs had high content – 38.2 % and it was enough high. At grasses dominant components was *Alopecurus pratensis* L. (70 – 80 %), further *Poa compressa* L. (10 – 15 %) and grasses from family *Juncaceae*. From *Fabaceae* in this part of polder were as follows:

*Vicia cracca* L. (75 - 90 %), *Trifolium pratense* L., *Trifolium repens* L. The other herbs, as follows *Galium boreale* L., *Tithymalus lucidus* Waldst. et Kit., *Leucanthemum vulgare* lamk., *Aristolochia clematitis* L., *Lychnis flos - cuculi* L., *Carex ssp.*, *Pulicaria vulgaris* Gaertn., had significant representation. Abandonment signs were marked in southern part of polder, where from total area 218.69 ha on 71.9 ha and process of succession was shown and from it on 70.92 ha advanced stage of dilapidation was determined. On used sites, in comparison with other part of polder, the highest presence of grasses (76.8 %) was determined. But low presence of legumes (2.5 %, only *Vicia cracca* L.) and no presence of clover crops were determined. Grass *Alopecurus pratensis* L. was dominanted (75 – 90 %) in this part of polder. From other grasses were presented only *Poa compressa* L. and grasses from family *Juncaceae*. In northern part of polder, so as in central part, the same species of herbs were dominated.

The greatest abandonment signs were ascertained in eastern part of polder. From total area 129.38 ha of perennial swards no-favourable tendencies of abandonment and follow succession of wood species on acreage 73.61 ha (56.7 % from area) were determined. In higher stage of succession is visible on 55.91 ha. Botanical evaluation was made on 55.77 ha. In typical float grass sward minimum presence of *Fabaceae* (only *Vicia cracca* L.) was determined. From grasses *Alopecurus pratensis* L. (90 – 98 %) and less *Juncus ssp.* were presented.

At evaluation of botanical composition of sward favourable share of grasses of sward was determined. Very low presence of legumes (optimal level 20 - 25 %) was ascertained (in average 3.1 %). On the other hand Novák (2006) for Západné Karpaty locality determined presence of legumes on level 12.55 %. Similarly Hanzes et al. (2006) in first year of observation noted in cadastre Liptovská Teplička village presence of 5 % legumes in sward. In polder Beša after three years of cut using of swards share of legumes increased to 13 %. Low legumes share in polder Beša had connected with overflowing of this area in year 2006.

### CONCLUSION

In year 2006 research of dry polder Beša was influenced by critical situation on river of the East Slovak Lowland and followed saturating polder with water. On account of stationary water almost during vegetation season ground survey was not possible. It was determined, that perennial swards are created by foxtail stands from association *Alopecuretum pratensis*. Ground survey connected also in year 2007. Abandonment signs of sites in southern part of polder on area 71.9 ha and in eastern part on area 73.61 ha were ascertained. For other areas of perennial swards botanical composition of stands was determined. Favourable presence of grasses components in northern, southern and eastern parts of polder (63.8 - 76.8 %) was ascertained. Low presence of legumes (*Fabaceae*) was noted. *Vicia cracca* L. created almost 100 % and it was occurred on 0.7 - 5.0 % of acreage.

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# INTERACTION BETWEEN STAND STRUCTURE AND FORAGE QUALITY OF ALFALFA IN THE FIRST CUT

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#### ABSTRACT

The aim of this study was to quantify the effect of stand structure on quality traits in the first cut of alfalfa. There were used six stand structure parameters as explanatory variables for six qualitative traits in CANOCO software. It was confirmed stand height as the most important stand structure parameters connected with all evaluated qualitative traits whilst count and weight of stems was connected only with crude fiber, crude protein and fat content. It is possible to conclude that stand density also played a role in qualitative traits formation but this influence was lower in comparison with stand height. All stand structure parameters explained about 20 % of variability of forage quality.

# INTRODUCTION

Morphological characteristics of lucerne are significantly correlated with yield and quality of dry matter (Katić *et al.*, 2003). In practice, a single plant of lucerne has no interest, because the exploitation concerns not a plant but the stand. Indeed, an optimal structure of stand is necessary but not sufficient to secure a high forage quality (Rotili *et al.*, 1999). The significant effect of stand structure on fiber content in alfalfa forage was confirmed by Hakl *et al.* (2006) who described the stand height as the most important stand structure factor connected with fiber content. According to Šantrůček (1989), also stand density could influence the fiber content but this effect was not confirmed within close and dense stand (Hakl *et al.*, 2006). In their experiment, the count of plants per  $m^2$  and total stems count per  $m^{-2}$ , ranged approximately from 200 to 400 and from 750 to 1600, respectively. In this condition, these variables and count of stems per plant did not affect the quality of lucerne forage. Generally, the effect of stand height and density on forage quality of alfalfa was described but this effect was not still more quantified. The aim of this study was to quantify the effect of stand structure on quality parameters in the first cut by means of multivariate statistical method.

#### MATERIALS AND METHODS

In 2006, a field experiment with 15 alfalfa germplasms was established at the research station of the Czech University of Life Sciences in central Bohemia (405 m above sea level, 50°04' N, 14°10' E). The long-term annual temperature of the experimental site is 7.9 °C and precipitation 526 mm.

The following 15 germplasm were used: Czech varieties Pálava, Morava, Vlasta, Magda, Jarka, Zuzana, Jitka, Niva, Oslava, Kamila, French variety Europe, and Czech candivars ŽE XLII, ŽE XLVII, ŽE XLVII, ŽE XLVIII. In 2007, the sampling was realized in the first cut in the late bud stage. The samples were taken in square 200 x 200 mm with three replicates by each lucerne germplasm. The stems counts, maximal stems length (MSL), average stem weight (ASW), and alfalfa dry matter yield (DMY) was assessed for each sample. Lastly, the crude protein (CP), crude fiber content (CF), organic matter digestibility (OMD), netto energy of lactation (NEL), nitrogen free extract (NFE), and fat content were determined. The variable abbreviations, their descriptions and obtained ranges in the first cut are summarized in table 1. The influence of alfalfa germplasm was statistically evaluated by analysis of variance (ANOVA). This statistical analysis was performed using Statistica 6.1 (StatSoft 2003). For evaluation of stand structure effect on forage quality, the data analyses were performed by redundancy analysis (RDA) in Canoco 4.5 software. The RDA is a linear ordination method based on PCA. An RDA ordination diagram can display scores for explanatory quantitative variables and explained variables (represented by arrows). The identical orientations of arrows mean positive correlation, an opposite a negative correlation (Lepš and Šmilauer, 2003).

Table 1.Descriptions and ranges of variable evaluated as stand structure or quality parameters in alfalfa stand in<br/>the first cut (n = 45, SD = standard deviation).

Variable	Description	Mean	SD	Min	Max
S over 20	Count of stems longer than 20 cm (pcs.m <sup>-2</sup> )	770	200	300	1300
S to 20	Count of stems to 20 cm ( $pcs.m^{-2}$ )	93	55	0	93
TSC	Total stems count (pcs.m <sup>-2</sup> )	863	199	425	1350
ASW	Average stem weight (g. pcs <sup>-1</sup> )	1.5	0.6	0.5	3.2
MSL	Maximal stem length (cm)	78	8	61	94
DMY	Dry matter yield $(g.m^{-2})$	1232	445	468	2438
CP	Crude protein (g.kg <sup>-1</sup> )	174	12	153	210
CF	Crude fiber $(g.kg^{-1})$	286	20	243	329
OMD	Organic matter digestibility (%)	65	2	61	70
NEL	Netto energie of lactation (MJ.kg <sup>-1</sup> )	5.1	0.2	4.7	5.7
NFE	Nitrogen free extract (g.kg <sup>-1</sup> )	405	18	340	456
Fat	Fat $(g.kg^{-1})$	23	2	90	119

#### **RESULTS AND DISCUSSION**

Based on ANOVA results, we did not record any significant differences among germplasms in all evaluated quality traits therefore this factor was not included in following analyses.

From all explanatory stand structure parameters were selected those with best predictive values. The relationships among selected stand structure parameters and forage quality are shown in Fig 1. All ordination axes explained 19 % of measured parameters variability (P=0.0200). The first canonical axe shows the positive correlation between MSL and CF content and negative correlation with remainder of qualitative parameters. This first axe explained 13.7 % of measured parameters variability (P=0.0360) so the MSL value could be recognize as the most important factor connected with forage quality. The second canonical axe explained over 3 % of variability and displayed positive correlation among count of stems over 20 cm, CF and fat. These parameters were negatively related to ASW, count of stems to 20 cm and CP. It seems that count and weight of stems influenced mainly CP, CF and fat content whilst MSL was connected with all evaluated qualitative traits. In contrast to experiment in dense stand (Hakl *et al.*, 2006), it is possible to conclude that density influenced forage quality when ranged from 425 to 1350 stems per m<sup>2</sup>. It is in accordance with Šantrůček (1989) that stand density could influence the fiber content but this influence was lower in comparison with stand height.

Figure 1. An RDA ordination diagram (for description of variables see table 1.)



# CONCLUSION

With limited data from one year, this study confirmed stand height to be the most important stand structure parameter connected to forage quality. Stand density played also a role in formation of qualitative parameters but this influence is lower in comparison with stand height. It seems that count and weight of stems influenced mainly CP, CF and fat content whilst MSL was connected with all evaluated qualitative traits. All stand structure parameters explained about 20 % of variability of forage quality. The experiment is continuing so results can not be definitive.

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# FEED VALUES OF GREEN PEA PLANTS DURING VARIOUS STAGES OF GROWTH

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# INTRODUCTION

The use of feed from the whole pea plant as roughage for ruminants is not very broad. Under certain conditions, like official support for areas with legumes or in connection with use of a following plant grown after harvesting the pea plants, this can be an interesting alternative. To get more knowledge on yield and feeding value of pea plants, there has been made a sampling of the pea plants in the different stages of growing and digestibility trials with pea plants.

# MATERIALS AND METHODS

The peas has been sown in the 2002 season on a field of 1.72 ha in southern Bavaria (sowing date 18<sup>th</sup> of March, 220 kg seed/ha). Starting on 21<sup>st</sup> May up to 16<sup>th</sup> July every week there has been taken samples of the green plants from an area of 4 or 2 square meters. From these samples the crude nutrients has been analysed and with the weigh and dry matter content of the green material the yield of dry matter could be calculated.

To find out the digestibility of the crude nutrients, digestibility trials has been done. Green material of pea plants taken at 3<sup>rd</sup> June (and frozen) and two bales of ensiled pea plants silage harvested on 3<sup>rd</sup> and 19<sup>th</sup> July were proofed with 4 or 5 male sheep. These results of digestibility were combined with data of pea feedstuffs in the tables of feed values (DLG-Futterwerttabellen für Wiederkäuer, 1997), to fit regression functions for the digestibility of crude protein on the content of crude protein in organic matter and digestibility of crude fat, crude fibre and N-free extract (NfE) on the content of crude fibre in organic matter. These regression functions were applied to the samples of the growing trial, to estimate the digestibility of crude nutrients and calculate the energy content and energy yield of the pea plants.

#### **RESULTS AND DISCUSSION**

The contents of crude nutrients of the pea plants in the weeks with sampling and the dates is shown in the following table (in g/kg DM).

Week	Date	Crude ash	Crude protein	Crude fat	Crude fibre	N-free extract
1	21 <sup>st</sup> May	126	277	18	210	369
2	28 <sup>th</sup> May	107	276	44	221	352
3	3 <sup>rd</sup> June	94	226	39	244	397
4	11 <sup>th</sup> June	86	224	25	284	378
5	18 <sup>th</sup> June	87	179	18	328	388
6	25 <sup>th</sup> June	88	182	19	305	406
7	1 <sup>st</sup> July	74	163	11	319	433
8	9 <sup>th</sup> July	67	165	12	303	453
9	16 <sup>th</sup> July	68	145	17	323	448

With the weigh of the green material of the sampling area and there dry matter content the yield per ha has been calculated. In the following table is shown the dry mater, the yield as fresh mater (FM) and dry mater (DM) in dt/ha. The energy as MJ ME/kg DM and MJ NEL/kg DM and the yield of energy as GJ ME/ha also is reported.

Week	DM	Yield	Yield	ME MJ	NEL MJ	Yield
	g/kg	dt FM/ha	dt DM/ha	/kg DM	/kg DM	ME GJ/ha
1	109	145	15.8	10.75	6.52	17.0
2	92	188	17.3	11.06	6.67	19.1
3	119	275	32.7	10.65	6.38	34.9
4	119	425	50.6	10.12	6.00	51.2
5	131	430	56.3	9.11	5.30	51.3
6	129	350	45.2	9.38	5.50	42.4
7	210	338	71.0	9.17	5.35	65.1
8	258	338	87.7	9.47	5.55	83.1
9	332	271	89.9	9.11	5.30	81.9

The measured feed values has changed during growing in an usual way for such kind of feed. After week 4 the crude fibre was on a high level and the content of ME was relatively low. Because the yield of DM increased, the yield of ME increased too.

The peas not used for the feeding trials were harvested as corn on  $30^{\text{th}}$  July. The sampling at this time gave a yield of 46.5 dt DM corn/ha and 42.9 dt DM straw/ha.

To describe the development of parameters with smoothed lines during the time of growing, functions of linearquadratic regressions on week were fitted. The number of week, written in the first column in the tables above were used as independent (X-) variable. The resulting equations are listed here:

Crude ash (g/kg DM)	$= 132.5 - 12.47 * \text{week} + 0.589 * \text{week}^{2}$
Crude protein (g/kg I	$DM) = 315.7 - 31.25 * week + 1.408 * week^{*2}$
Crude fat (g/kg DM)	$= 33.4 - 1, 23 * \text{week} + 0.147 * \text{week}^{*2}$
Crude fibre (g/kg DN	1) = 160.1 - 41.44 * week + 2.698 * week**2
NfE (g/kg DM)	= 358.3 + 3.50 * week + 0.848 * week**2
Dry matter (g/kg)	= 140.6 - 31.4 * week + 5.780 * week * 2
Yield DM(dt/ha)	= 5.80 + 8,44 * week + 0.121 * week*2
ME (MJ/kg DM)	$= 11.67 - 0.548 * \text{week} + 0.0298 * \text{week}^{*2}$
Yield ME (GJ/ha)	$= 8.95 + 7.65 * \text{week} + 0.074 * \text{week}^{*2}$

The feedstuffs proofed in digestibility trials had the crude nutrients (g/kg DM) and gave the digestibility coefficients (DC) in % with there s.d. as shown in the following table.

Material		OM	Crude Protein	Crude fat	Crude fibre	N-free extract
Green, 3 <sup>rd</sup> June	Crude nutrients	913	225	30	244	414
	DC	77.5	81.4	64.7	63.7	84.3
	s.d.	2.5	1.6	2.8	5.0	2.3
Silage, 3 <sup>rd</sup> July	Crude nutrients	925	172	24	291	438
	DC	70.9	73.3	72.2	55.0	80.4
	s.d.	1.4	2.5	2.5	2.6	0.4
Silage, 19 <sup>th</sup> July	Crude nutrients	936	147	1.4	338	437
	DC	66.1	64.6	60.7	55.4	75.1
	s.d.	0.8	2.1	1.5	0.8	0.8

The measured DC are well in agreement with the data listed in tables of feed values (DLG-Futterwerttabellen für Wiederkäuer, 1997) for pea plants with corresponding crude nutrients.

To estimate the DC of the crude nutrients, these data combined with data of pea feedstuffs from the tables of feed values (DLG-Futterwerttabellen für Wiederkäuer, 1997), gave the following regression functions for DC based on crude nutrients (g/kg DM):

DC Crude protein (%) = 25.2 + 240.8 \* crude protein / OM

DC Crude fat (%) = 77.92 - 73.5 \* crude fibre / OM

DC Crude fibre (%) = 65.3 - 17 \* crude fibre / OM

DC N-free extract (%) = 110.4 - 119,2 \* crude fibre / OM

The resulting DC of the crude nutrients are used in the formula for calculation of ME:

ME (MJ) = Crude protein \* DC (Crude protein) / 100 \* 0.0147 + Crude fat \* DC (Crude fat) / 100 \* 0.0312

+ Crude fibre \* DC (Crude fibre) / 100 \* 0.0136 + NfE \* DC (NfE) / 100 \* 0.0147 + Crude protein \* 0.00234

#### CONCLUSIONS

The measured data of feeding values of pea plants give remarkable lower values after middle of June, when the plants where on end of flowering. After this time, the yield of DM increased more, but the concentration of energy was relatively low, so such feed is not appropriate for high yielding animals (cows, bulls).

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#### **SUMMARY**

With the data of samples of pea plants the development of feeding values during growing is described. A procedure is given to calculate the content of energy (ME) in pea plants, based on estimating the digestibility coefficients depending on the content of crude nutrients.
## INFLUENCE OF WEED INFESTATION TO THE CONTENT OF SELECTED ELEMENTS IN SILAGE MAIZE BIOMASS

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#### ABSTRACT

Experiment with silage maize was set on experimental area of CULS Prague (sugar-beet growing region, altitude: 286 m) in 2007. Maize stand with weed infestation to the stage of 5<sup>th</sup>, 7<sup>th</sup>, and 9<sup>th</sup> leaf, stand without weed treatment, and stand with post-emergent herbicide treatment were evaluated. Mechanical removal of weeds was realized in treatments 1 - 3. Contents of selected elements (N, P, K, Ca, and Mg) were analyzed in maize biomass sampled in period of silage maturity. Based on results of chemical analysis there was found statistically significant decrease ( $\alpha = 0.05$ ) of N content caused by weed infestation in maize stand. Content of this element in maize was determined in interval 9.98 – 12.61 g/kg depending on period of infestation. Plants from herbicide treatment contained 11.82 g of N/kg. Based on statistical evaluation, weed infestation does not influence the content of P, K, Ca, and Mg in maize plants. Statistically significant difference among tested treatments in total uptake of N, P, K, and Ca by maize was proved.

#### **INTRODUCTION**

Monitoring of duration of weed and crop competition is frequently used for study of influence of weed infestation on yield and other quantitative and qualitative parameters of a crop. The competition between plants is for essential resources especially for water, light, space and nutrients, which are in interaction (Rajcan and Swanton, 2001, Lehoczky, 2002). One of monitoring methods is study of the effect of weed infestation duration for defined periods and after each of the phases, the crop is then kept weed free for the rest of the growing season. Many articles described this period in wide interval 2 - 8 weeks after emergence of maize plant. The data for each study are accurate but because environment and other impacts play such an important role, the data cannot be applied across different geographic regions (Zimdahl, 2004). Our aim was study the influence of weed infestation to the nutrient content and nutrient uptake of the silage maize at weed infestation of stand to the stage of 5<sup>th</sup>, 7<sup>th</sup>, and 9<sup>th</sup> leaf of maize and to the end of vegetation period in conditions of sugar-beet growing region in Czech Republic.

#### MATERIALS AND METHODS

Field plot experiments with silage maize were based on 25 April 2007 on area of CULS Prague. Experimental plot is located in sugar-beet growing region in altitude 286 m and according to agrometeorological characteristics belongs to temperate warm and predominantly dry climatic region. Duration of vegetation period is 172 days. Average year temperature is 7.9 °C (14.0 °C per vegetation period) and long-term year sum of precipitation is 526 mm (364 mm per vegetation period). Hybrid Kuxxar (FAO 300, Stay green, Sc) was tested in this experiment. Seeding rate was 85 000 plants per ha with 0.7 m distance between rows. Fertilization with 120 kg of N/ha (ammonium sulphate), 45 kg of P/ha (triple superphosphate), and 120 kg of K/ha (potassium salt) was used. Experiment was designed by method of random blocks. Plot area was 20 m<sup>2</sup>. We evaluated five treatments in three replications: maize stand with weed infestation to the stage of 5<sup>th</sup>, 7<sup>th</sup>, and 9<sup>th</sup> leaf, stand without weed treatment, and stand with post-emergent herbicide treatment (Titus 60 g/ha + Lontrel 0.4 l/ha + Starane 250 EC 1.5 l/ha – 1 June 2007). Mechanical removal of weeds was realized in treatments 1 - 3 (5 June 2007, 19 June 2007, and 29 June 2007, respectively). Maize plants were sampled from two central rows of each plot in 1.5 m of length in the silage maturity period. Dry matter of maize was determined by drying to constant weight at 80 °C. Based on results of maize yield and analysis of contents (g/kg) of selected elements (N, P, K, Ca, and Mg), nutrients uptake (kg/ha) by maize were calculated. Analysis of variance in Statistica 8.0 software ( $\alpha = 0.05$ ; Tukey HSD) was used for statistical evaluation of the data.

#### **RESULTS AND DISCUSSION**

The highest yield of dry matter of maize (Table 1) was found in treatment 1 with weed infestation to the stage of 5<sup>th</sup> leaf of maize (17.71 t/ha) and treatment 5 with herbicide application (16.73 t/ha). Yields of these treatments statistically significant differed ( $\alpha = 0.05$ ) from treatment 4 (12.17 t/ha) without weed removal. Yields of maize in treatments with weed infestation to the stage of 7<sup>th</sup> and 9<sup>th</sup> leaf were 14.81 t/ha and 14.52 t/ha, respectively. Our results correspond for example with conclusions of Martinková and Honěk (2001). Content of dry matter in maize plants in harvest period was not significantly affected by treatment (Table 1). Data of weed spectrum and production of weed biomass were published by Fuksa and Hakl (2007). Figure 1 shows results of chemical analysis of maize biomass sampled in harvest. Statistical analysis did not proved influence of weed infestation to content of P, K, Ca, and Mg in maize biomass. Similarly, Andreasen *et al.* (2006) did not found an influence of weed infestation to changes in P content in spring barley. Significant effect was found in evaluation of N content in our experiment. Content of this element ranged 9.98 – 12.61 g/kg in maize plants depend on period of weed infestation. Plants from treatment with herbicide application contained 11.82 g N/kg. Statistically significant influence of weed infestation to N content in maize plants was described by Promsakha Na Sakonnakhon *et al.* (2006). Table 1 shows total uptake of nutrients by maize stand. Statistically significant differences among treatments were found for all tested elements except Mg.

		, ,		1	1	U	5		
Treatment	GM	DM	DMC			Nutri	ent uptake (k	(g/ha	
	(t/l	ha)	(g/kg)		Ν	Р	K	Ca	Mg
1	50.63 <sup>a</sup>	17.71 <sup>a</sup>	350.0 <sup>a</sup>		223.40 <sup>a</sup>	33.65 <sup>a</sup>	164.50 <sup>a</sup>	47.24 <sup>a</sup>	20.68 <sup>a</sup>
2	41.95 <sup>ab</sup>	14.81 <sup>ab</sup>	354.2 <sup>a</sup>	1	67.86 <sup>abc</sup>	29.58 <sup>ab</sup>	136.28 <sup>ab</sup>	41.28 <sup>ab</sup>	17.30 <sup>a</sup>
3	41.32 <sup>ab</sup>	14.52 <sup>ab</sup>	351.3 <sup>a</sup>	1	153.31 <sup>bc</sup>	$27.07^{ab}$	134.29 <sup>ab</sup>	43.17 <sup>ab</sup>	17.03 <sup>a</sup>
4	35.62 <sup>b</sup>	12.17 <sup>b</sup>	341.6 <sup>a</sup>		122.16 <sup>c</sup>	21.55 <sup>b</sup>	110.78 <sup>b</sup>	29.11 <sup>b</sup>	13.38 <sup>a</sup>
5	49.56 <sup>a</sup>	16.73 <sup>a</sup>	337.0 <sup>a</sup>	1	197.67 <sup>ab</sup>	29.59 <sup>ab</sup>	139.36 <sup>ab</sup>	$45.40^{a}$	20.19 <sup>a</sup>
p-value	0.0046	0.0099	0.7606		0.0028	0.0102	0.0149	0.0285	0.0724

Table 1.Yield of maize in green (GM) and dry matter (DM) (t/ha), dry matter content (DMC) (g/kg), and uptake<br/>of N, P, K, Ca, and Mg (kg/ha) by maize plants in period of silage maturity

Figure 1. Content of N, P, K, Ca, and Mg	g (g/kg) in maize biomass i	in period of silage maturity	(p-value = 0.0124)
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#### **CONCLUSION**

Different duration of weed infestation during the vegetation did not influenced contents of P, K, Ca, and Mg in biomass of maize. On the other side, difference in N content was found. Treatment without weed removal showed about 16 % decrease, treatment with weed infestation to the stage of 9<sup>th</sup> leaf about 11 % decrease and to the 7<sup>th</sup> leaf about 4 % decrease in comparison to control treatment (herbicide application). Total uptakes of N by maize stand were lowered about 38 %, 22 %, and 15 %, respectively. Treatment with weed infestation to the stage of 5<sup>th</sup> leaf of maize demonstrated the highest content of N as well as uptake of N, but differences were not statistically significant in comparison with control treatment. Uptakes of P, K, and Ca were significantly lower in plants without treatment. Published one-year results are specific for defined experimental area in Czech Republic. Our experiments continue so results can not be definitive.

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## CELL WALL COMPOSITION OF RED CLOVER (*TRIFOLIUM PRATENSE* L.) STEMS AND LEAVES DIFFERING IN MATURITY

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#### ABSTRACT

Content of CP and cell wall constituents in leaves, stems and whole plant of red clover (Trifolium pratense L.) with advancing maturity was investigated. Sampling was done in 7 day intervals in second cut. As the stems mature their nutritive value declined. The nutritive value of total herbage also declined. Changes in NDF, ADF, CP and lignin contents were rapid in stems than in leaves. Two methods – ADL and KL for determining lignin concentration were compared. Each of these methods gave different lignin values.

#### INTRODUCTION

In Serbia, red clover (*Trifolium pratense* L.) is one of the most important leguminous plant grown for forage production. Compared with grasses, red clover is usually higher in concentrations of pectin, lignin, N and some minerals. Plant cell wall is the major source of dietary fiber for animals. Fiber, measured as neutral detergent fiber (NDF), usually accounts for 30-80% of the organic matter in forage crops. The remaining organic matter, cell solubles, is almost completely digestible. The nutritional availability of fiber to livestock, however, varies greatly, depending on its composition and structure (Buxton and Redfearn, 1997). Lignin has been identified as primarly responsible for limiting digestibility of fiber, but fiber utilization is also limited by physical constraints at the cellular organization level.

#### MATERIALS AND METHODS

Three stages of growth of red clover (*Trifolium pratense* L.) cv Viola were examined after second cutting. The first stage was cut on June 17<sup>th</sup>, at full boot stage, another one on June 24<sup>th</sup> (around 40 % flowering), and a third one in full flowering on July 1<sup>st</sup>. Dry matter was determined by drying out samples at 65°C and grinding and sieving them to 1 mm particle size.

Crude proteins were computed indirectly from the amount of total nitrogen, measured by the Kjeldahl method modified by Bremner (1983). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined as the insoluble part of a neutral detergent solution and acid detergent solution. The amount of hemicellulose in samples was determined as a difference between the amounts of NDF and ADF and lignin content in ADF was determined as lignin insoluble in 72 % H<sub>2</sub>SO<sub>4</sub>, applying the method of Van So*est and Robertson*, (1980). Klason lignin was determined according to the method of Theander and Westerlund (1986). Data were processed by the analysis of variance in a randomized block design. The significance of differences between arithmethic means was tested by LSD test.

#### **RESULTS AND DISCUSSION**

General observation that a decrease in contents of crude protein coincides with plant aging was confirmed in this study (Table 1). As plant growth advances, there is a greater accumulation of cellulose and lignin in the stem, while the content of crude proteins decreases. Content of crude protein in leaves and whole plants was the highest (332.2 g.kg<sup>-1</sup> DM and 266.7 g.kg<sup>-1</sup> DM, respectively) at the first stage of development and the lowest (272.8 g.kg<sup>-1</sup> DM and 181.5 g.kg<sup>-1</sup> DM, respectively) at the third stage of development. The rate of decline in crude protein content with advancing maturity was faster in stems than in leaves.

The results of this study show that contents of cell walls increase differently in plant organs of this legume species as plants age (Table 1). The highest content of NDF was recorded in stems in the third stage of development (625.7 g.kg<sup>-1</sup>DM), while NDF in leaf was lower (from 293.9 g.kg<sup>-1</sup> DM to 343 g.kg<sup>-1</sup> DM) from first to third development stage. The stem fraction of red clover generally had much larger proportions of NDF, ADF and lignin than did the leaf fractions of red clover. Fiber profiles of whole plant tissue reflected the relative proportions of each tissue type and were consistent with other findings for red clover. The highest increase of NDF content was recorded in stems (100.4 g.kg<sup>-1</sup> DM, after the second development stage) and after the first development stage in leaves (28 g.kg<sup>-1</sup> DM). Content of cell walls in leaves increased with plant age only up to 10%, so that declining quality of fibre feeds is closely related with a decreasing proportion of leaves in total plant biomass and reduced quality of stems. Higher fiber concentrations in stems occur in part because stems contain more structural and conducting tissues than leaves, whereas a larger portion of leaves is occupied by thin-walled mesophyll cells. During growth and development, the content of hemicelulose was found to increase in the plant organs examined.

Overall, as red clover matured, lignin increased in both investigated anatomical fractions, with differences between stages of growth (Table 1). The two methods of determining lignin content resulted in different amounts of residue, with KL values consistently higher than ADL values for all forage samples. Both methods reflected maturity trends, although the magnitude of the change was different. Klason lignin concentrations ranged from 74.8 g.kg<sup>-1</sup> DM to 89.5

g.kg<sup>-1</sup> DM and from 82.1 g.kg<sup>-1</sup> DM to 107.7 g.kg<sup>-1</sup> DM, in leaves and stems respectively. The KL residue concentrations were on the order of 2 to 3.5 times greater than the ADL residues. It is generally considered that the major limitation of the KL method for forage samples is the inclusion of protein in the insoluble residue, resulting in artificially high lignin values (Fukushima and Dehority, 2000).

			Stage of growth		LS	SD
	Plant parts	Ι	II	III	0.05	0.01
	Leaf	332.2	319.8	272.8	1.72	2.85
Crude	Stem	174.9	139.8	126.4	1.87	3.10
Protein	Whole plant	266.7	224.0	181.5	1.84	3.05
	Leaf	293.9	321.9	343.0	7.14	11.84
NDF	Stem	475.6	525.3	625.7	50.36	83.51
	Whole plant	369.8	430.0	527.9	32.99	54.71
	Leaf	168.3	178.1	191.1	7.19	11.92
ADF	Stem	342.2	399.1	422.1	1.43	2.38
	Whole plant	240.9	295.7	341.3	4.16	6.89
	Leaf	125.8	143.7	151.9	11.51	19.08
Hemicellulose	Stem	133.4	126.2	203.6	50.61	83.92
	Whole plant	128.9	134.4	186.6	34.71	57.76
	Leaf	27.3	38.6	43.7	6.97	11.56
ADL	Stem	45.2	59.0	71.1	7.09	11.77
	Whole plant	34.8	IIIII $0.05$ $0.$ $319.8$ $272.8$ $1.72$ $2.$ $139.8$ $126.4$ $1.87$ $3.$ $224.0$ $181.5$ $1.84$ $3.$ $321.9$ $343.0$ $7.14$ $11$ $525.3$ $625.7$ $50.36$ $83$ $430.0$ $527.9$ $32.99$ $54$ $178.1$ $191.1$ $7.19$ $11$ $399.1$ $422.1$ $1.43$ $2.$ $295.7$ $341.3$ $4.16$ $6.$ $143.7$ $151.9$ $11.51$ $19$ $126.2$ $203.6$ $50.61$ $83$ $134.4$ $186.6$ $34.71$ $57$ $38.6$ $43.7$ $6.97$ $11$ $49.5$ $61.7$ $5.85$ $9.$ $74.9$ $89.5$ $7.22$ $11$ $97.1$ $107.7$ $6.87$ $11$ $86.7$ $106.1$ $2.05$ $3$	9.71		
	Leaf	74.8	74.9	89.5	7.22	11.98
KL	Stem	82.1	97.1	107.7	6.87	11.39
	Whole plant	77.9	86.7	106.1	2.05	3.41

**Table 1.** Cell wall constituents in leaf, stem and the whole plant of red clover  $cv - Viola (g.kg^{-1} DM)$ 

NDF – neutral detergent fiber, ADF – acid detergent fiber, ADL – acid detergent lignin, KL – klason lignin First store of growth – sorth bud Second store of growth – 40% (lowering Third store of growth – full bloc

First stage of growth - early bud, Second stage of growth - 40% flowering, Third stage of growth - full bloom

#### CONCLUSION

General observation that a decrease in contents of crude protein coincides with plant aging was confirmed in this study. As plant growth advances, there is a greater accumulation of cellulose and lignin in the stem. The highest content of NDF and ADF was recorded in stems in the third stage of plant development. The highest increase of NDF and ADF was in stem, after the first stage of development and after the second stage of development, respectively. Various methods of determining lignin content resulted in different amounts of residue. Lignin values determined from sulfuric acid are usually lower than the values determined by Klason lignin.

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## THE INFLUENCE OF WEATHER CONDITIONS OF POLAND ON THE YIELDING OF SPRING BARLEY CULTIVATED WITH UNDERSOWN CROPS

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#### ABSTRACT

This work presents researches from years 2004-2006, the aim of the present research was to compare the yielding of spring barley cultivated in pure sowing and with undersown crops in changeable weather conditions. In the experiment the following combinations of undersown crops were taken into account: control object (pure sowing of spring barley), white clover, white clover +Italian ryegrass, black medic, black medic +Italian ryegrass, Italian ryegrass. The harvest of spring barley was made in the stage of full maturity of grain. During the harvest, on every field the grain field was determined, and in collected samples the content of true protein was also determined. Weather conditions significantly modified the grain yield and efficiency of true protein from spring barley grain. Undersown crops, especially papilionaceous crops with Italian ryegrass caused important increase of grain yield and efficiency of true protein from spring barley grain.

#### **INTRODUCTION**

The development of level-headed agriculture inclines to fill the crop rotation with interharvests, which enable to utilize the natural factors stipulating the yielding of crop plants (Lesak and Sverakova 1999, Reynolds et al. 1994, Zając and Witkowicz 1999). The most valuable group of intercrops are undersown crops, which are recommended to sow into spring barley. In agricultural literature we exactly know the interaction of agrotechnical factors on spring barley yielding cultivated in pure sowing, whereas there is a few experimental datas defining the interaction of undersown crops on protective plant yielding - spring barley. So there is the requirement of conducting that type of researches. The aim of this researches was comparing the yielding of spring barley cultivated in pure sowing and with undersown crops in weather conditions of Poland.

#### MATERIALS AND METHODS

The field experiments were carried out at the Experimental Station in Zawady on a very good cereal complex soil. The experiment was a three-replicate split blocks design. In the experiment the following combinations of undersown crops were taken into account: control object (pure sowing of spring barley), white clover 18 kg/ha, white clover +Italian ryegrass 9+15 kg/ha, black medic 20 kg/ha, black medic +Italian ryegrass 10+15 kg/ha, Italian ryegrass 30 kg/ha. Spring barley was sown in amount of 120 kg/ha in the first decade of April. Undersown crops were sown in a day of sowing of protective plant. The harvest of spring barley was made in the stage of full maturity of grain. During the harvest, on every field the grain yield was determined, and in collected samples the content of true protein was also determined. Received results of researches were drawn up statistically.

#### **RESULTS AND DISCUSSION**

Statistic analysis indicates the important influence of conditions of vegetation period, undersown crops and their cooperation on grain yield of spring barley and the efficiency of true protein from the grain of spring barley (Table 1 and 2).

Undersown crop	2004	2005	2006	Means
Control object	5.21	4.75	3.57	4.51
White clover	5.84	5.21	4.03	5.03
White clover + Italian ryegrass	6.07	5.52	4.26	5.28
Black medic	5.79	5.12	3.98	4.96
Black medik + Italian ryegrass	5.92	5.41	4.21	5.18
Italian ryegrass	5.28	4.80	3.63	4.57
Means	5.69	5.14	3.95	-
LSD <sub>0.05</sub>				
Years				0.27
Undersown crop				0.14
Interaction				0.33

Table 1. The grain yield of spring barley, in t/ha

The highest yield of seeds of spring barley and the highest efficiency of true protein was noticed in 2004. The

deficiency of falls in 2005 has caused important decrease of grain yield and efficiency of true protein from spring barley grain. However, significantly the lowest grain yield and efficiency of true protein from spring barley grain were noticed in dry and warm year 2006. Mazurek (1999) also showed that changeable weather conditions in particular years caused considerable disparities in cereals yielding. In carried experiences, similarly to researches of Ceglarek (1982) the positive influence of undersown crops on protective plant yielding was noticed, even in dry years. The highest yield grain and the highest efficiency of true protein was achieved from spring barley cultivated with undersown crops, especially with mixtures of papilionaceous crops with Italian ryegrass. Also Zając and Witkowicz (1999) made a note of favourable influence of undersown crops on protective plant yielding. In carried out experiment, similarly to Pisulewska and Zając (1997) and Zając and Witkowicz (1999) papilionaceous crops caused important increase of true protein concentration in grain of protective plant. In personal researches higher efficiency of true protein from the point of view of greater yield was achieved from spring barley cultivated with papilionaceous crops and Italian ryegrass than with papilionaceous plants. An interaction was found and it indicated that the highest yield of seeds with the highest efficiency of true protein was achieved from spring barley cultivated with papilionaceous crops with Italian ryegrass in 2004, and the lowest yield of seeds with the highest efficiency of true protein was achieved from spring barley cultivated with papilionaceous crops with Italian ryegrass in 2006.

Undersown crop	2004	2005	2006	Means
Control object	579	497	418	498
White clover	684	596	524	601
White clover + Italian ryegrass	766	649	580	665
Black medic	672	572	511	585
Black medik + Italian ryegrass	750	634	569	651
Italian ryegrass	583	508	426	506
Means	672	576	505	-
LSD <sub>0.05</sub>				
Years				21
Undersown crop				27
Interaction				36

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I able 2.	The efficiency	v of true protein	of spring	barley grain.	in kg/na
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#### CONCLUSIONS

It should be claimed that weather conditions significantly modified the grain yield and efficiency of true protein from spring barley grain. Undersown crops, especially papilionaceous crops with Italian ryegrass caused important increase of grain yield and efficiency of true protein from spring barley grain.

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## THE YIELDING OF FIELD PEA AND SPRING TRITICALE MIXTURE IN CLIMATIC CONDITIONS OF POLAND

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#### ABSTRACT

This work presents researches from years 2000-2002, the aim of the present research was to compare the yielding of mixtures of field pea and spring triticale depending on participation of components in mixture in weather conditions of Poland. In the experiment the following combinations with field pea were taken into account: (100, 80, 60, 40, 20, 0%) and with spring triticale (0, 20, 40, 60, 80, 100%) in the mixture. The collection of mixtures cultivated on seeds was taken in the stage of full maturity of grain of spring triticale. During the harvest, on every field grain field was determined, and in collected samples the content of true protein was also determined. The conditions of vegetation period significantly modified the yield of seeds and efficiency of true protein from the seeds of field pea and spring triticale mixtures. The biggest yield of seeds was obtained from mixtures of field pea and spring triticale of components content 60 + 40% and the highest efficiency of true protein was obtained from field pea seeds cultivated in pure sowing.

#### INTRODUCTION

The legume-cereal mixtures are the source of wholesome feedstuff, which farmers can produce in their own farms. In comparison with pure sowing, in that conditions, legumes grow up faster, ripen evenly, give yield faithfully, are resistant on lodging and weed infestation and reached feedstuff includes richer array of feed components and it is better qualitatively (Ceglarek et al. 2002, Rudnicki i Wenda-Piesik 2002). Extremely valuable component of those mixtures is field pea (Kotecki 1990). Putting the spring triticale into the cultivation, cereal with relatively large seed production and high fodder value, it makes the requirement of its estimate as the component of mixtures (Rudnicki i Wenda-Piesik 2002). It shows that there is the requirement of conducting the researches which aim is to compare the yielding of mixtures of field pea with spring triticale depending on participation of components in mixture in weather conditions of Poland.

#### MATERIALS AND METHODS

The field experiments were carried out in 2000-2002 at the Experimental Station in Zawady on a very good cereal complex soil. The experiment was a three-replicate split blocks design. In the experiment the following combinations with field pea were taken into account: (100,80, 60, 40, 20, 0%) and with spring triticale (0, 20, 40, 60, 80, 100%) in the mixture. The participation of plant was normalized relatively to the number of sowed seeds in pure sowing, it means 100 seeds on  $1m^2$  of field pea and 400 grains of spring triticale. The collection of mixtures cultivated on seeds was taken in the stage of full maturity of grain of spring triticale. During the harvest, on every field the grain yield was determined, and in collected samples the content of true protein was also determined. Received results of researches were drawn up statistically.

#### **RESULTS AND DISCUSSION**

The grain yield of field pea and spring triticale mixtures was significantly modified by the conditions of vegetation period, the participation of components in the mixture and their cooperation (Table 1). Year 2002 was the most favourable to cultivate mixtures, and in this year the grain yield was the highest. Significantly lower grain yield was noticed in 2001 about 5.7%, and the lowest in 2000. Plants cultivated in mixed sowings have bigger immunity on unfavourable course of weather than in pure sowing (Ceglarek et al. 2002, Kotecki 1990, Rudnicki 1999). An interaction was found and it indicated that the highest yield of seeds was achieved from the mixture of field pea with spring triticale of components content 20 + 80%, and also from spring triticale cultivated in pure sowing in 2000.

Statistic analysis indicates that important influence of conditions of vegetation period, the participation of components in the mixture and their cooperation on true protein efficiency from seeds of field pea mixtures with spring triticale (Table 2). The highest efficiency of true protein was determined from mixtures cultivated in 2002, relatively smaller in 2001, and the smallest in dry and warm year 2000. it should be explain by the fact that in years in which the highest yield of seeds was obtained also the highest efficiency of true protein was noticed. The participation of components in the mixture also significantly diversified the efficiency of true protein. The highest efficiency of true protein was obtained from field pea seeds cultivated in pure sowing. In discussed experiment, just as in researches of other authors (Ceglarek et al. 2002, Green et al. 1985, Kotecki 1990) the legume-cereal mixtures provided more true protein from seeds than spring cereals.

Interaction

Participatio	n of components, %	2000	2001	2002	Means	
Pea	Spring triticale					
100	0	3.52	4.37	4.68	4.19	
80	20	3.65	4.50	4.82	4.32	
60	40	3.87	4.65	4.97	4.50	
40	60	3.56	4.48	4.73	4.26	
20	80	3.31	4.27	4.52	4.03	
0	100	3.36	4.30	4.48	4.05	
Means		3.55	4.43	4.70	-	
LSD <sub>0.05</sub>		•				
Years	0.21					
Participation of	of components in the mi	xture			0.16	
Interaction	Interaction					

Table 1.	The grain	yield o	of field	pea and	spring	triticale	mixtures,	t/ha
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Table 2.	The efficiency of true protein	from field pea and spring tritica	ale mixture, kg/ha
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Participation o	f components, %	2000	2001	2002	Means
Pea	spring triticale				
100	0	816	1046	1099	987
80	20	769	983	1032	928
60	40	737	893	966	865
40	60	602	791	821	738
20	80	492	661	692	615
0	100	436	577	594	536
М	eans	642	825	867	-
LSD <sub>0.05</sub>					
Years	27				
Participation of c	42				
Interaction					48

#### **CONCLUSIONS**

The conditions of vegetation period significantly modified the yield of seeds and efficiency of true protein from the seeds of field pea and spring triticale mixtures. The biggest yield of seeds was obtained from mixtures of field pea and spring triticale of components content 60 + 40%, and the highest efficiency of true protein was obtained from field pea seeds cultivated in pure sowing. The mixtures of field pea with spring triticale provided more true protein from seeds than spring triticale cultivated in pure sowing.

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#### YIELD AND CHEMICAL COMPOSITIONS OF FABA BEAN-SPRING WHEAT MIXTURES

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#### ABSTRACT

The objective of the research (2004-2006) was to determine an impact of faba bean and spring wheat shares (respectively 0, 25, 50, 75, 100% and 100, 75, 50, 25, 0%) in a mixture on the grain yield and chemical composition of the mixtures. The highest grain yield was produced by the faba bean-spring wheat mixture whose components share was 25 + 75%, and by spring wheat cultivated in pure stand. The highest crude protein yield was obtained from cultivation of faba bean in pure stand and a faba bean-spring wheat mixture characterized by the components share of 75 + 25%. Increasing the faba bean share in a mixture with spring wheat was followed by an increased crude protein, crude fibre, and crude ash contents, and by reduced crude fat and nitrogen-free extracts contents in grain.

#### INTRODUCTION

Leguminous-cereal mixtures may be cultivated for grain, used as a material to produce concentrated mixtures, for green forage fed directly to animals, or for dried fodder or silage (Ostrowski and Daczewska, 1993; Borowiecki and Księżak, 2000). They are a source of feed characterized by a beneficial chemical composition, as far as the protein:energy ratio is concerned (Brundage and Klebesadel, 1970; Ostrowski and Daczewska, 1993). Moreover, they produce a high level of reliable yields (Borowiecki and Księżak, 2000). Species and cultivar selection, in addition to determination of the proportions of mixture components, greatly influences both yields and nutritive value of the mixtures (Ostrowski and Daczewska, 1993; Szczygielski, 1993; Siuta et al., 1998; Borowiecki and Księżak, 2000; Kotwica and Rudnicki, 2004; Ceglarek et al. 2006).

The aim of this researches was defining the yielding and the content of chemical compounds in faba bean-spring wheat mixtures, which were cultivated on grains in dependence of composition's presence in mixtures.

#### **MATERIALS AND METHODS**

A field experiment was carried out in the years 2004-2006 at the Experimental Farm in Zawady. It was situated on a grey-brown podsoilc soil formed from strong loamy sand, characterized by neutral pH and medium available phosphorus, potassium and magnesium contents. The soil belongs to the rye very good complex, quality class IVa. A one-factor experiment was designed as a randomized block assignment in three replications. The area of one plot for harvest was 20 m<sup>2</sup>. Different shares of faba bean (Vicia faba L.) (0, 25, 50, 75, 100%) and of spring wheat (Triticum aestivum L.) (100, 75, 50, 25, 0%) in a mixture constituted the experimental factor. The species share in the mixture was established in relation to the number of grains sown in pure stand that is 70 grains of faba bean and 400 grains of spring wheat per 1 m<sup>2</sup>.

Phosphorus and potassium fertilizers were applied in the autumn in the amounts adjusted to soil fertility. In the spring a nitrogen fertilizer was applied on all the plots prior to planting at the rate of 30-kg N/ha. Additional rates of 50 and 30 kg N/ha were applied at spring wheat shooting on the plots where, respectively, spring wheat only and a mixture of faba bean and spring wheat in the amounts of 25 + 75% were sown. Faba bean cv. Titus, spring wheat cv. Pasteur and mixtures of the above crops were planted in the first decade of April. Oats was the previous crop for both plants. Crops, cultivated to obtain grains, were harvested together at the full maturity stage.

The research included determination of weight of seed (grain) in the yields of the species a mixture was made up of, dry matter by the by the oven-drying gravimetric method, crude protein by the Kjeldahl method, crude fat using a Soxhlet apparatus, crude fibre by the Henneberg-Stohmann method, and crude ash by burning plant material at the temperature of 600°C in an electric oven. A percentage share of faba bean seeds in the mixture seed yield, crude protein yield and the content of nitrogen-free extracts (NEF) were calculated. The experimental data were subjected to statistical analysis. Significance of differences was checked by Tukey test at the significance level of  $\alpha$ =0.05.

#### **RESULTS AND DISCUSSION**

Grain and crude protein yields of faba bean-spring wheat mixtures as well as the content of chemical compounds in the mixture grains were significantly influenced by the components share in the mixture (Table 1).

An effect of the components share on grain yields of mixtures of leguminous plants and spring cereals has been confirmed by many authors (Szczygielski, 1993; Siuta et al., 1998; Noworolnik, 2000; Ceglarek et al., 2006). The grain yield of the faba bean-spring wheat mixture whose components share was 25 + 75% did not differ significantly from the yield of spring wheat cultivated in pure stand. However, it was significantly higher than the grain yield of the mixtures whose components shares were 50 + 50% and 75 + 25%, and faba bean grown in pure stand. Also studies by Siuta et. al. (1998) indicated that grain yield of leguminous-cereal mixtures was higher than the grain yield of leguminous plants, but lower or similar to the cereal grain yield. Under the conditions of the experiment discussed increasing the faba bean share in the mixture with spring wheat was followed by a significant drop in the mixture grain yield. Similar inferences were made by Szczygielski (1993). The faba bean share in the grain yield harvested of the mixtures with spring wheat (25 + 75, 50 + 50, 75 + 25%) was respectively 21.6; 45.2 and 71.9 %.

The significantly lowest crude protein yield was produced by spring wheat cultivated in pure stand whereas the

significantly highest yield was harvested when faba bean was grown in pure stand or a faba bean-spring wheat mixture was cultivated and its components share was 75 + 25%. The crude protein yield of faba bean-spring wheat mixtures was on average by 106 to 234 kg/ha higher than the crude protein yield of spring wheat cultivated in pure stand. Similarly, in the opinion of Kotwica and Rudnicki (2004) as well as Ceglarek et al. (2006) cultivation of leguminous-cereal mixtures produces more crude protein compared with spring cereals. In the present study the crude protein yield of faba bean-spring wheat mixture. Similar relationships have been reported in the works by Szczygielskiego (1993), Noworolnika (2000) and Ceglarka et al. (2006).

Components share in a		Seed yield (t/ha)		Crude	The content of chemical compounds in the seeds					
miz	xture (%)	50	protein (g/kg D)			DM)	) M			
faba	Spring wheat	faba	Spring	Mixture	yield	Crude	Crude	Crude	Crude	Nitrogen-
bean	Spring wheat	bean	wheat		(kg/ha)	protein	fat	fibre	ash	free extracts
0	100	_	3.21	3.21	435	138.2	20.6	24.4	20.1	796.7
25	75	0.70	2.54	3.24	541	170.1	19.8	35.9	24.0	750.2
50	50	1.38	1.67	3.05	620	206.6	18.6	48.6	28.4	697.8
75	25	1.97	0.77	2.74	669	247.8	16.6	63.0	32.6	640.0
100	0	2.45	_	2.45	709	293.1	14.7	80.1	37.6	574.5
LSD <sub>0.05</sub>				0,16	42	14.1	1.0	4.3	1.9	47.4

Table 1. Yields and chemical composition of faba bean-spring grain mixtures (means form the years 2004-2006)

In the experiment discussed, grains of faba bean-spring wheat mixtures (25 + 75, 50 + 50, 75 + 25%) contained more crude protein (from 31.9 to 109.6 g/kg DM), crude fibre (from 11.5 to 38.6 g/kg DM) and crude ash (from 3.9 to 12.5 g/kg DM) than the grain of spring wheat cultivated in pure stand. Simultaneously, they contained less crude fat (from 0.8 to 4.0 g/kg DM) and nitrogen-free extracts (from 46.5 to 156.7 g/kg DM). Increasing the faba bean share in the mixture was followed by an increase in crude protein, fibre, and ash contents, and reduction in crude fat and nitrogen-free extracts contents. An effect of components share in the mixture on grain chemical composition of leguminous-cereal mixtures was also found by Szczygielski (1993) and Ceglarek et. al. (2006). Changes in grain chemical composition of the mixtures result from the share of mixture components in the grain yield harvested as well as species variability (Brundage and Klebesadel, 1970; Szczygielski 1993).

#### CONCLUSION

The components share in the mixture significantly influenced grain yield, crude protein yield and chemical compound content in the grain of faba-bean-spring wheat mixtures. The highest grain yield was obtained from cultivation of a faba bean mixture whose components share was 25 + 75% as well as cultivation of spring wheat in pure stand. Faba bean-spring wheat mixtures (25 + 75, 50 + 50, 75 + 25%) grown for grain produced more crude protein than spring wheat cultivated in pure stand. Increasing the faba bean share in the mixture with spring wheat was followed by increased crude protein, crude fibre and crude ash contents. However, it reduced crude fat and nitrogen-freed extracts contents in grains of the mixtures.

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## BOTANICAL COMPOSITION AND MIXTURE COMPONENTS QUALITY FOR GAME IN LOWLAND REGIONS

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#### ABSTRACT

In experimental years 2002 - 2006 (location Nitra) the development of botanical composition and some parameters of quality in two multi-species mixtures for game (M1 – Avena sativa, Fagopyrum esculentum, Secale sylvestre, Trifolium pratense, Medicago sativa, Dactylis glomerata, Festulolium seeded on 17 April 2002; M2 – Fagopyrum esculentum, Brassica napus conv. napus, Sinapis alba, Secale sylvestre, Trifolium pratense, Medicago sativa, Dactylis glomerata, Festulolium seeded on 20 July 2002) were observed. Annual forage crops provided the grazing and shelter for game during the year of sowing. From the second year vegetations stands were developed, as legumes-grass mixtures with marked dominancy of Medicago sativa. In this paper contents of nitrogenous substances, fibre and mineral elements in individual components of mixtures were evaluated.

#### INTRODUCTION

Deficiency of natural food resources forces game to attend field and forest cultures in which it causes substantial damages. It is possible to be prevented by establishment of plots with forage mixtures with suitable composition (Libosvár, 1996; Hell, Slamečka and Gašparík, 2000; Gregorová and Ďurková, 2001; Moser, 1997 and others). The plots fulfil many ecological functions (Fritz, 1979). The aim of this paper is evaluation of two perennial multi-species mixtures for game according to botanical composition, production and some parameters of quality.

#### MATERIAL AND METHODS

The field experiments were realized in location Nitra in 2002 - 2006 (south-west part of Slovakia, 160 m a. s. l., annual rainfall 561 mm; during the growing season 327 mm; average annual temperature 9,7 °C; during the growing season 16,5 °C). The soil was clay-loam fluvisoil with fluctuating level of underground water. There were seeded two perennial mixtures in experiment: mixture M1 – seeded on 17 April 2002 (*Avena sativa* 15 kg/ha + *Fagopyrum esculentum* 6 kg/ha + *Secale sylvestre* 7 kg/ha + *Trifolium pratense* 4n 10 kg/ha + *Medicago sativa* 3 kg/ha + *Dactylis glomerata* 2 kg/ha + *Festulolium "Perun"* 2 kg/ha) and mixture M2 – seeded on 20 July 2002 (*Fagopyrum esculentum* 5,75 kg/ha + *Brassica napus conv. napus* 5 kg/ha + *Sinapis alba* 0,75 kg/ha + *Secale sylvestre* 6 kg/ha + *Trifolium pratense* 4n 9 kg/ha + *Medicago sativa* 1,5 kg/ha + *Dactylis glomerata* 2 kg/ha + *Festulolium "Perun"* 2 kg/ha). Plot size: 9 m<sup>2</sup>; replications: 4; row spacing: 125 mm (M1), 150 mm (M2). Mixtures were neither fertilized nor irrigated. They were mowed twice (M1), respectively once (M2) in year of sowing; in further years they were mowed four times per year – May, June, August and October.

#### **RESULTS AND DISCUSSION**

Annual forage crops provided grazing and shelter for game during the year of sowing (tab. 1 and 2). Legumes dominated in mixture M1 already in the second half of growing season and in mixture M2 they dominated in the second growing year. First *Trifolium pratense* predominated over *Medicago sativa* in mixtures. From summer of the second year *Medicago sativa* predominated over *Trifolium pratense*. *Secale sylvestre* had relatively high portion on yield in the first mowing in 2003 (35 %) and in 2004 (11 %) only in mixture M2; it was presented there only sporadically in mixture M1. Portion of weeds on yield was high in both mixture M1 and mixture M2 in year of sowing (34 % and 29 %, respectively). Portion of weeds markedly decreased by mowing and they were almost fully eliminated from plots from half of year 2004. Grasses were spread only gradually, with maximum in 2005; more in mixture M2. Drought-tolerant *Dactylis glomerata* predominated in grass part of mixture.

Dry matter yields in mean of years 2002 – 2004 11.16 t/ha (M1) and 9.36 t/ha (M2) were achieved without fertilization (tab. 3). Avena sativa and Fagopyrum esculentum provided high shelter in spring mixture (M1) from June in the year of sowing; Sinapis alba and Fagopyrum esculentum in summer mixture (M2) from August; Medicago sativa and Brassica napus conv. napus from September. Grasses with predominance of Dactylis glomerata provided high shelter from the second year of growing, especially in the first mowing and Festulolium "Perun" in the second mowing, too.

*Brassica napus conv. napus* and legumes had high content of crude protein and low content of fibre (tab. 4). The highest Ca content was in *Sinapis alba*, then in *Brassica napus conv. napus*, then in *Medicago sativa* and finally in *Trifolium pratense* with values more than 10 mg/g of dry matter. Monocotyledonous plants had low content of Ca and Mg equally. *Fagopyrum esculentum* had the highest content of Mg. Phosphorus content varied from 2.38 to 4.09 mg/g of dry matter. Weeds and *Brassica napus conv. napus* cumulated most of potassium according to expectation. Sodium content did not reach 1 mg/g of dry matter. Only *Avena sativa* had several times higher content of sodium.

#### CONCLUSION

Knowledge of mixture components quality and botanical changes during growing enables to manage the establishment and using of mixtures so that they were at disposal in every time of vegetational period forage crops with

different nutritious value. It brings possibility to reach the permanent offer of suitable pasture and possibility of shelter for game. It is possible to be reached by regular renovation of plots part, combination of grazing, mulching and mowing with preservation of fodder surplus for winter period.

Forage crops	Su.(1)	A.(2)	Sp.(3)	Α.	Sp.	Α.	Sp.	A.	Sp.
	2002	2002	2003	2003	2004	2004	2005	2005	2006
Avena sativa	33	-	-	-	-	-	-	-	-
Fagopyrum esculentum	28	-	-	-	-	-	-	-	-
Secale sylvestre	+	+	-	-	-	-	-	-	-
Trifolium pratense	2	50	58	7	1	+	+	-	-
Medicago sativa	+	35	23	91	70	96	81	73	76
Grasses	3	4	8	+	16	4	15	27	21
Weeds	34	12	11	2	13	+	4	+	3

Table 1. Portion of components on yields in % (M1)

1)summer; 2)autumn; 3)spring

Table 2. Portion of components on yield in % (M2)

Forage crops	Su.(1)	Sp.(2)	A.(3)	Sp.	А.	Sp.	А.	Sp.
	2002	2003	2003	2004	2004	2005	2005	2006
Fagopyrum esculentum	32	-	-	-	-	-	-	-
Sinapis alba	20	-	-	-	-	-	-	-
Brassica napus conv. napus	5	-	-	-	-	-	-	-
Secale sylvestre	+	35	+	11	-	+	-	-
Trifolium pratense	6	35	18	6	6	+	-	-
Medicago sativa	6	11	72	60	88	68	50	56
Grasses	2	13	7	9	6	32	50	44
Weeds	29	6	3	14	+	+	+	-

1)-3) as in table 1

#### **Table 3**. Yield of the mixtures (t/ha)

Mixtures	2002		2003		2004		Average	
	Green	Dry	Green	Dry	Green	Dry	Green	Dry
	matter	matter						
M1	45,31	8,33	49,58	12,24	60,05	12,92	51,65	11,16
M2	16,5	4,13	39,15	10,20	58,97	13,74	38,21	9.36

Table 4. Quality of mixtures components (average of mixtures and years) mg/g of dry matter.

Forage crops	Crude	Crude	Ca	Mg	Р	K	Na
	protein	fibre					
Fagopyrum esculentum	145,46	229,66	9,54	5,08	3,53	22,66	0,50
Secale sylvestre	138,70	298,30	5,10	2,36	3,19	20,94	0,52
Avena sativa	130,00	299,50	6,77	1,93	4,00	21,62	1,95
Brassica napus conv. napus	232,05	158,50	14,85	4,73	4,09	33,73	0,94
Sinapis alba	170,00	291,50	15,66	3,46	3.26	21,89	0,74
Trifolium pratense	203,75	195,50	12,52	4,04	3,13	25,23	0,26
Medicago sativa	223,94	211,50	14,61	3,64	3,38	21,24	0,61
Grasses	120,76	283,00	4,58	2,67	2,38	18,84	0,62
Weeds	154,10	249,80	13,41	6,44	3,13	32,48	0,22

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## THE INFLUENCE OF NITROGEN FERTILIZATION ON YIELD AND FODDER VALUE OF SPRING WHEAT STRAW

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#### **INTRODUCTION**

In spite of low feed value, straw is quite generally applied in animals feeding. To feeding purposes straw of spring cereals is the most often used, which is softer, richer in feed components, it contains less fibre and it characterizes with higher digestibility of feed components in comparison to the straw of winter cereals. Applied in ruminants feeding (cattle, sheep) and horses has relatively low feed value, which first of all depends on a sort of plant. Feed value of straw fluctuates from 0.17 to 0.52 oat units, and the content of true protein from 4 to 45 g in 1 kg (Gradziuk, 1995). The content of basic components in straw depends also on applicable agrotechnology. The aim of researches was to estimate the influence of different doses of nitrogen fertilization on straw yield, the yield of true protein and the content of true protein in straw of several varieties of spring wheat.

#### MATERIALS AND METHODS

The field experiments were carried out in 2002-2004 at the Experimental Station in Zawady belonging to the University of Podlasie in Poland. This experiment was established on a very good cereal complex soil, belonging to the quality class IV a. The soil pH was neutral. The experiment was a three-replicate split blocks design. Two factors were taken into consideration:

- I factor fertilization rate of nitrogen: 0 control object, 40 N kg/ha, 80 N kg/ha, 120 N kg/ha, 160 N kg/ha
- II factor spring wheat cultivars: bread type-Eta, Henika, Banti, quality type-Jasna.

Every dose was divided into two -50 % before sowing, 50 % in the phase of shooting. Spring wheat was cultivated on the position after potatoes. The time of sowing was first decade of April and the amount of planting was 250 kg ha<sup>-1</sup>. During the vegetation of wheat the mechanical and chemical nurturing was applied. The harvest of spring wheat was made in the stage of full maturity of grain. During the harvest the straw yield was determined. In a laboratory the content of true nitrogen was determined by using the rate equal 5.7. The yield of true protein was determined. Received results of researches were drawn up statistically by using the Tucker's test by the level of essentiality  $\alpha = 0.05$  (Trętowski and Wójcik, 1988).

The years of conducting researches characterize with considerable diversity of weather conditions (table 1). Year 2002 was humid and warm. Intensive falls made difficult to harvest the seeds with proper humidity. In 2003 there were considerable deficiency of falls, which has caused that the weather conditions were the least favourable for cultivation of spring wheat. In contrast with 2002 and 2003, vegetation period of 2004 characterized with favourable conditions for growth and development of spring wheat plants and in the effect it favoured its yielding.

Specification	Veare		Months								
specification	1 cais	III	IV	V	VI	VII	VIII	III-VIII			
	2002	3.9	9.0	17.0	17.2	21.0	20.2	14.7			
Temperature (°C)	2003	1.3	7.0	15.5	18.3	20.0	18.4	13.4			
	2004	2.0	8.0	11.7	15.4	17.5	18.9	12.3			
1987-2000		1.4	7.8	12.5	17.2	19.2	18.5	12.9			
	2002	15.9	12.9	51.3	61.1	99.6	66.5	307.2			
Rainfall (mm)	2003	7.0	13.6	37.2	26.6	26.1	4.7	115.2			
	2004	10.5	35.9	97.0	52.8	49.0	66.7	321.9			
1987-2000		27.8	38.6	44.1	52.4	49.8	43.0	320.5			

 Table 1.
 Weather conditions in the period of spring wheat vegetation at the Experimental Station in Zawady in years 2002-2004

#### **RESULTS AND DISCUSSION**

About the yield of straw importantly decided the doses of nitrogen fertilization and researched varieties (table 2). Important differences in straw yield were showed, which was collected from control object – without nitrogen fertilization, and between objects fertilized with the following doses: 40, 80, 120 and 160 and in the yield collected from the object fertilized with the dose of 40 kg N ha<sup>-1</sup> and fertilized with the dose of 160 kg N ha<sup>-1</sup> From the researched varieties the highest yield of straw was obtained from the Banti variety – 6.76 t ha<sup>-1</sup>, the lowest straw yield had Henika – 5.45 t ha<sup>-1</sup>. Received results are in accordance with Makarewicz (2004), Mazurek and Kuś (1991), Mazurek et al. (1992) and Mazurek and Sułek (1996) results. The yield of true protein collected with straw depended significantly on the level of nitrogen fertilization, and significant differences in yield were showed between control object and objects fertilized with growing dosese of

nitrogen (table 2). Mazurek i Kuś (1991) noted the highest increase of true protein yield in spring wheat grain fertilized with the dose of 80 kg N ha<sup>-1</sup> but Fatyga et al. (1994) after using the dose of 120 kg N ha<sup>-1</sup> In personal researches also noted the increase of true protein yield with the increase of nitrogen doses. Just as in case of true protein yield, also the content of protein in spring wheat straw depended on nitrogen fertilization doses (table 2). The highest content of true protein was noted in fertilization with the highest dose 160 kg N ha<sup>-1</sup> Similar results noted Makarewicz (2004), Mazurek and Kuś (1991) and Liszewski (1997).

**Table 2.**Straw yield and true protein yield and the content of true protein in grain depending on nitrogen<br/>fertilization doses and varietes in t ha<sup>-1</sup>, mean from years 2002-2004

Specification	Yield of straw	Yield of total protein	Content of total protein in
Specification	t/ha	t/ha	straw %
0	4.22	0.13	3.07
40 N kg/ha	5.11	0.17	3.27
80 N kg/ha	6.13	0.21	3.36
120 N kg/ha	6.91	0.24	3.49
160 N kg/ha	7.28	0.26	3.60
mean	5.93	0.20	3.36
LSDp=0.05 for dose if nitro	gen 1.82	0.06	0.27
Banti	6.76	0.22	3.26
Henika	5.45	0.18	3.35
Jasna	5.59	0.19	3.41
Eta	5.90	0.20	3.42
mean	5.93	0.20	3.36
LSDp=0.05 for cultivation	0.66	n.i.	n.i.

#### CONCLUSIONS

About the straw and true protein yield and the content of true protein in straw of spring wheat decided mainly weather conditions in years of researched experiments and the nitrogen fertilization. Increasing doses of nitrogen fertilization caused systematic increase of analyzed yields and the content of true protein in spring wheat straw. Among researched varieties the highest yield had Banti, and the most true protein was observed in straw of variety Eta.

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## ENSILAGE CAPACITY AND QUALITY OF SILAGE FROM A RANGE OF DROUGHT-RESISTANT GRASS AND LEGUME CULTIVARS

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#### ABSTRACT

Over 2005 - 2007, monocultures of drought-resistant grass and legum varieties were sampled in the research trial plots with the aim to determine their ensilage capacity at three cuts. A low monosaccharide and high water-soluble carbohydrate contents were found in herbage at all the years and cuts. The ensilage capacity was higher at the  $1^{st}$  cut than at the other cuts during the trial years. Only the silage from two treatments at the  $1^{st}$  cut was classified as the Quality Class 2, the silage ranked to the Quality Class 1 at all the other treatments and cuts.

#### INTRODUCTION

Considering the changes in climate as recorded during recent years, the composition and growing of forage crops are requiring adjustment, because farms need to produce a sufficient amount of feeds even if the climatic conditions are not favourable. At ensiling, there are many factors having effects on the final quality of preserved forage that must be taken into account, such as the forage crop species and composition, the phenological stage at harvesting, weather conditions, agronomic practices, harvesting techniques, conservation procedures and storage. The feeding value of silage, the character and scope of losses are consequently linked to fermentation and other processes in preserved forage (Čunderlíková and Polák, 2003). Successful fermentation depends on a sufficient content of carbohydrates - mainly glucose, fructose, fructosans, saccharose and maltose - and has a positive impact on palatability of ensiled forage (Loučka and Pozdíšek, 1998).

#### MATERIALS AND METHODS

Drought-resistant grass and legume varieties were sampled in the research trial plots at three cuts over 2005 - 2007. The objective was to determine the ensilage capacity of herbage. The research treatments were as follows: A - *Festuca rubra* L. cv. Levočská; B - *Phleum pratense* L. cv. Lema; C - *Bromus inermis* Leyss. cv. Tabron; D - *Agrostis gigantea* ROTH. cv. Levočský; E - *Onobrychis viciifolia* SCOP. cv. Višňovský; F - *Trifolium pratense* L. cv. Astur; G - *Medicago sativa* L. cv. Alpha and H - *Trifolium repens* L. cv Tasman. In the fresh herbage samples, the content of water-soluble carbohydrates (WSC) - by the Luf-Shoorl method - and buffering capacity (BC) were determined. Consequently, the ensilage capacity of herbage was determined as the ratio of water-soluble carbohydrates to crude protein (CP) as well as the WSC to BC ratio (coefficients of ensilage capacity). Prior to ensiling, fresh herbage was analysed in the laboratory to determine the dry matter (DM) and organic nutrients in compliance with the "Decree of the Ministry of Agriculture of the Slovak Republic. No. 1491/1/1997-100 on feeding materials used to manufacture feeding mixtures and fodder". Herbage of the cultivars from the treatments was chopped and wilted to an optimum DM content and filled into plastic containers of 1000 ml volume (n = 3). The full and pressed containers were hermetically sealed and stored in a room with constant temperature. After the fermentation process had finished (42 days), samples were taken from the preserved herbage and silage quality was determined as the Quality Class according to the modified Flieg-Zimmer scale.

#### **RESULTS AND DISCUSSION**

From the viewpoint of suitability of grasses and legumes for preservation as silage, the WSC content is one of the limiting factors. The research data and analyses showed reduced monosaccharide content and increased WSC content at all the treatments and cuts (Table 1). The carbohydrates in plants can be defined as the structural (pectins, hemicelluloses and celluloses) or the non-structural (monosaccharides, fructosans and starches) ones. From these, only the free monosaccharides (glucose and fructose) and fructosans can be hydrolysed by the lactic acid bacteria during ensiling (Merry and McAllan, 1989). The carbohydrate content and composition markedly participate in the course and the quality of fermentation process. Their amount can be increased by breaking down into simpler sugars through hydrolysis and enzymes (Gallo and Petrikovič, 2002). The content of WSC, comprising the total monosaccharides and soluble sugars in herbage, was significantly influenced by the trial years, the order of the cut and the species at the treatments. Moreover, the WSC content in forage crops is fluctuating during the day (maximum in the afternoon and minimum in the morning), because it is influenced by the intensity of sunshine as well as by the soil moisture content (Loučka and Pozdíšek, 1998). The significantly higher WSC content ( $r = 0.31^{++}$ ) was recorded at the 1<sup>st</sup> cut by comparison with the other cuts. The content of WSC was significantly higher in the grass treatments than in the legume ones. The highest WSC content was found with Phleum pratense at the 1st cut. Over all the three research years, the highest BC (2.68 - 5.22) was recorded at the legume monocultures. Considering the fact that WSC content in the species fluctuates and CP content is more stabile, there are differences in the coefficient of ensilage capacity between the species. The WSC/CP coefficient was higher at the 1st cut than at the other cuts. The significantly lowest WSC/CP was recorded at the legume monoculture treatments during all the research years. The highest WSC/CP ratios were found in *Phleum pratense* at the 1<sup>st</sup> and 2<sup>nd</sup> cuts, and in *Bromus inermis* at the 3<sup>rd</sup> cut. The lowest ensilage capacity (0.32 - 0.39) was recorded in Medicago sativa at all the cuts. The ratio of WSC to BC (WSC/BC) was another of the

coefficients assessed. The highest WSC/BC was recorded at the 1<sup>st</sup> cut, but the differences between all the cuts were not significant. At all the cuts, the significantly highest WSC/BC was recorded at the grass monoculture treatments (3.08 - 5.25). There were significant differences in lactic acid content between the cuts and the species. A positive correlation was found between the content of WSC and lactic acid ( $r = 0.21^{++}$ ). An increased content of acetic acid was recorded in the legume monocultures at all the cuts. The content of butyric acid was higher at the 1<sup>st</sup> cut and it was decreasing in the silages with the order of the cuts. The silage quality was classified in compliance with the Flieg-Zimmer modified scale of Quality Classes, in accordance with the points allotted by the content of lactic, acetic and butyric acids as per cent, respectively. At the first cut, increased proportion of butyric acid was found at the *Agrostis gigantea* and *Trifolium pratense* treatments, so these silages ranked to the Quality Class 2. All the other treatments ranked to the Quality Class 1 at all the cuts.

		DM in	Carbohy	Irates			Coeff	Coeff	Aci	d content	(%)	Quality
Cuts	Treatm	fresh	Monosac	WSC	CP	BC	WSC/BC	WSC/CP	1101		(,,,)	Class
		herbage	wionosae.	wbc			indende	wbe/er	Lactic	Acetic	Butyric	Cluss
1 <sup>st</sup>	Α	225.85	56.22	69.23	126.76	2.53	5.25	1.01	21.31	6.24	0.30	1
	В	213.66	59.10	88.88	130.31	4.18	3.65	1.21	21.14	6.52	0.37	1
	С	225.82	53.74	75.66	129.23	3.77	3.56	1.04	17.55	6.58	0.21	1
	D	210.38	46.45	69.44	138.64	2.60	4.49	0.85	27.66	7.17	0.44	2
	E	211.60	30.95	58.08	169.67	2.68	3.31	0.55	14.95	5.81	0.07	1
	F	194.93	36.03	45.34	182.50	3.71	2.30	0.46	14.34	6.12	0.48	2
	G	218.76	31.45	43.75	199.11	4.75	2.03	0.38	14.92	6.68	0.24	1
	Н	187.23	40.70	50.55	219.77	3.63	2.51	0.42	18.54	7.52	0.10	1
2 <sup>nd</sup>	Α	302.72	32.62	65.05	119.88	2.40	4.22	0.82	24.12	5.97	0.05	1
	В	289.26	50.30	74.29	124.29	3.36	3.83	1.01	24.32	7.63	0.04	1
	С	269.66	53.55	71.90	125.56	3.18	4.09	1.00	36.92	9.65	0.08	1
	D	318.39	42.05	55.37	119.40	2.47	3.98	0.82	25.84	7.98	0.09	1
	Е	264.76	32.59	48.05	149.88	2.73	2.97	0.54	23.97	8.35	0.36	1
	F	240.70	38.48	43.38	155.76	4.03	2.04	0.53	26.02	8.39	0.21	1
	G	251.81	25.20	42.01	174.07	4.57	1.74	0.39	31.78	11.01	0.03	1
	Н	261.18	35.24	47.65	191.41	3.40	2.52	0.44	38.06	12.50	0.39	1
3 <sup>rd</sup>	А	271.90	33.73	68.37	109.60	2.87	3.89	0.95	25.36	3.92	0.00	1
	В	271.16	36.43	63.52	115.33	3.25	3.08	0.87	23.19	4.60	0.04	1
	С	252.23	40.29	69.30	112.48	2.84	4.26	0.97	26.28	3.75	0.44	1
	D	270.39	31.18	64.59	109.35	2.94	3.30	0.88	29.86	4.23	0.22	1
	Е	216.29	24.90	53.47	151.97	3.11	2.68	0.52	19.52	4.67	0.09	1
	F	209.80	35.55	48.92	172.05	3.44	2.46	0.49	26.50	8.85	0.25	1
	G	235.81	18.70	38.06	179.82	5.22	1.10	0.32	18.29	6.48	0.18	1
	Н	208.03	35.15	47.08	185.74	2.90	2.89	0.45	17.74	5.40	0.03	1
Tukey (P	$P < 0.05)^+;$	Tukey (P	$< 0.01)^{++}$									
Years		_	++	++	+	_	_	++	++	++	++	-
Cuts		++	++	++	++	_	_	+	++	++	+	++
Species		++	++	++	++	+	++	++	++	+	-	+
Treatmen	nts	-	+	-	-	-	-	-	-	-	-	-

Table 1.	Ensilage	capacity ar	nd silage	quality	(g kg <sup>-1</sup>	DM)
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#### CONCLUSIONS AND RECOMMENDATIONS

The research results confirmed the variability of WSC and CP content, i. e. the main parameters of ensilage capacity. High ensilage capacity was found at the 1<sup>st</sup> cut. The highest ensilage capacity was recorded with *Phleum pratense* at the 1<sup>st</sup> and 2<sup>nd</sup> cuts, and with *Bromus inermis* at the 3<sup>rd</sup> cut. The lowest ensilage capacity was found in *Medicago sativa* at all the cuts. In compliance with the modified Flieg-Zimmer scale, only the silage from *Agrostis gigantea* and *Trifolium pratense* treatments ranked to the Quality Class 2 at the 1<sup>st</sup> cut, all the other treatments ranked to the Quality Class 1 at all the cuts. Considering the observed climate change effects, the use of drought-resistant grass and legume varieties or their mixtures enables better sward development and increased forage production, thus improving the stability of forage production for animal husbandry.

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## PRODUCTION AND QUALITY OF *FESTULOLIUM* AND *ARRHENATHERUM ELATIUS* FORAGES IN THE CONTEXT OF CLIMATE CHANGES

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#### **INTRODUCTION**

Grasslands can be used by applying the method of rotation. Crop from the first cut is harvested for conservation and the sward can be subsequently used until the end of the growing season for grazing. Winter feed ration is based on the conserved fodder from the first cut and serves also as additional feed in the autumn. The requirement of conserved feeds in extending the grazing season is difficult to estimate because it depends on the general character of weather (Achilles et al., 2002). The above mentioned method of grassland use is particularly suitable for hybrids of *Festuca arundinacea x Lolium multiflorum* (Opitz von Boberfeld, 2005, Opitz von Boberfeld and Banzhaf, 2006).

Suitable for conservation is the first cut of permanent grasslands (Opitz von Boberfeld, 1994) that features the highest content of sugars. As compared with haymaking, ensiling represents a much lower weather risk, which reflects favourably in working costs and low conservation losses (Achilles et al., 2002). Prerequisite for high-quality silage are not only bacteria of lactic fermentation but also a clean and healthy phytomass. Important for a sound course of fermentation is also the ratio of carbohydrates and N-substances. Water-soluble carbohydrates affect the stability of ensilage. Individual graminaceous species show great differences in their ensiling capacity (Holúbek et al., 2007).

The paper objective is to assess yields and quality of *Festulolium (Festuca arundinacea* x *Lolium multiflorum)* and *Arrhenatherum elatius* forages in the summer period and possibilities of conserving first-cut forage. Further to the intensity of use in summer, forage quality is evaluated at the end of the growing season together with a possible use of the two species for extension of the grazing period.

#### MATERIALS AND METHODS

The small-scale experiment was established in 2004 in the Bohemian-Moravian Upland at an altitude of 560 m a.s.l. In 1970-2000, mean annual precipitation was 617 mm and mean annual temperature amounted to 6.9 °C. The experimental plots were fertilized each year with nitrogen (50 kg N/ha), phosphorus (30 kg P/ha) and potassium (60 kg K/kg). The grassland was used in summer either as a one-cut sward (June) or as a two-cut sward (June and the end of July), and subsequently harvested at the end of the growing season (October-December).

The first experimental factor was the grass species (S). The monitored species were *Festulolium* (FS) cv. Felina, *Dactylis glomerata* (DGS) cv. Vega, and *Arrhenatherum elatius* (AES) cv. Median. The second experimental factor in the summer period was the cut order (C). Monitoring was made of forage production and quality in the first cut (1C) and in the second cut (2C). Another experimental factor at the end of the growing season was the term of preparatory cut (P). Monitored parameters were production and quality of swards with one preparatory cut in June (1P) and with two preparatory cuts in June and at the end of July (2P). The experiment was repeated in three subsequent years (Y) 2005 (1Y), 2006 (2Y) and 2007 (3Y).

Monitored quantitative characteristics were dry matter production in summer and at the end of the growing season. Characteristics monitored in summer were contents of crude proteins (CP), crude fibre (CF), water-soluble carbohydrates (WSC), net energy of lactation (NEL) and the WSC/CP ratio. The contents of nutrients were established by using the NIRS method. Characteristics monitored at the end of the growing season were the CP content established by the method according to Kjeldahl, CF and ADF contents established by ANKOM Fiber analyzer, and the NEL content calculated from the ADF content (NEL =  $9.23 - 0.105 \times ADF$ ).

The obtained results were analyzed using ANOVA and by subsequent verification based on the Tukey test.

#### **RESULTS AND DISCUSSION**

The first-cut yields of AES were higher than in FS. The second-cut yields of the two species were comparable. The species (S) as well as the cut order (C) significantly (P<0.05) affected dry matter yields. In the second cut, AES had a significantly higher (P<0.05) CP content than in the first cut. Moreover, the CP content in the first cut was in FS significantly higher (P<0.05) than in AES. The WSC content was not significantly affected by the cut (C). The WSC content in FS was significantly higher (P<0.05) than in AES. The WSC content was not significantly affected by the cut (C). The WSC content in FS was significantly higher (P<0.05) than in AES. For successful ensiling, the content of water-soluble carbohydrates should be higher than 20 g/kg (Holúbek et al., 2007). Haigh (1995) mentions even a WSC content of 37 g/kg. Both values were exceeded by the two studied species in the first cut as well as in the second cut. As claimed by Holúbek et al., 2007, the WSC/NL coefficient should be higher than 0.7. Its value in our experiments was over 0.6 in FS while a maximum value in AES was only 0.48. The coefficient could be increased in the first cut by earlier harvest of stands. An essential influence on ensiling capacity has the vegetation stage of plants at harvest. The ensiling capacity decreases with the ageing of the grass stand (Holúbek et al., 2007). Effect of year on the surveyed characteristics is obvious in most qualitative characteristics. Higher sward quality was recorded especially in the second year (2Y) with the even precipitations in spring. In contrast, years 1Y and 3Y exhibited irregular precipitations and water deficit for the growth in the spring months.

Yields towards the end of growing season are affected by the number of preparatory cuts (P). Stands 1P showed significantly (P<0.05) higher yields than Stands 2P. The term of preparatory cut (P) affected the forage quality, too.

Significantly higher (P < 0.05) CP and NEL contents were observed in Stands 2P than in Stands 1P. On the other hand, the CF and ADF contents were higher in Stands 1P than in Stands 2P.

Table 1.	Dry matter yields (t/ha), contents of CP, CF, WSC (g/kg), NEL (MJ/kg) and WSC/CP ratio in Festulolium
	(FS) and Arrhenatherum elatius (AES) in the first (1C) cut and in the second (2C) cut in 2005-2007.

Factor		DM yields	СР	CF	WSC	NEL	WSC/CP
Species (S)	and Cut (C)						
FS	1C	6.48 <sup>a</sup>	105.4 <sup>a</sup>	288.3 <sup>a</sup>	64.0 <sup>a</sup>	5.04 <sup>ab</sup>	0,61
	2C	2.39 <sup>b</sup>	106.3 <sup>ab</sup>	275.5 <sup>b</sup>	67.4 <sup>a</sup>	5.07 <sup>b</sup>	0,63
AES	1C	7.18 <sup>c</sup>	79.5°	331.0 <sup>c</sup>	38.2 <sup>b</sup>	4.90 <sup>a</sup>	0,48
	2C	2.30 <sup>b</sup>	117.7 <sup>b</sup>	260.2 <sup>d</sup>	42.0 <sup>b</sup>	5.68 <sup>c</sup>	0,36
Year (Y)			•				
1Y		4,74 <sup>a</sup>	104.3 <sup>a</sup>	293.7 <sup>a</sup>	51.3 <sup>a</sup>	5.08 <sup>a</sup>	0.49
2Y		4,93 <sup>a</sup>	104.4 <sup>a</sup>	279.9 <sup>b</sup>	60.6 <sup>b</sup>	5.31 <sup>b</sup>	0.58
3Y		4,09 <sup>b</sup>	98.0 <sup>a</sup>	292.6 <sup>a</sup>	46.7 <sup>c</sup>	5.12 <sup>a</sup>	0.48

**Table 2.** Dry matter yields (t/ha), contents of CP, CF, ADF (g/kg), NEL (MJ/kg) in *Festulolium* (FS) andArrhenatherum elatius (AES) in dependence on preparatory cut (P) at the end of the growing season in 2005–2007.

Factor		DM yields	СР	CF	ADF	NELADF
Species (S)	and Preparato	ory cut (P)				
FS	1P	2.09 <sup>a</sup>	62.6 <sup>a</sup>	296.9 <sup>b</sup>	349.2 <sup>a</sup>	5,56 <sup>a</sup>
	2P	$0.70^{b}$	85.0 <sup>b</sup>	259.5 <sup>a</sup>	300.2 <sup>b</sup>	6,08 <sup>b</sup>
AES	1P	2.72 <sup>c</sup>	69.8 <sup>a</sup>	331.2 <sup>c</sup>	385.1 <sup>c</sup>	5,19 <sup>c</sup>
	2P	0.93 <sup>b</sup>	106.1 <sup>c</sup>	266.9 <sup>a</sup>	309.3 <sup>b</sup>	5,98 <sup>b</sup>
Year (Y)						
1Y		2.04 <sup>a</sup>	77.3 <sup>a</sup>	277.5 <sup>a</sup>	337.9 <sup>ab</sup>	5.68 <sup>ab</sup>
2Y		2.05 <sup>a</sup>	85.0 <sup>b</sup>	292.8 <sup>b</sup>	344.7 <sup>a</sup>	5.61 <sup>a</sup>
3Y		0.74 <sup>b</sup>	80.4 <sup>ab</sup>	295.6 <sup>b</sup>	325.2 <sup>b</sup>	5.81 <sup>b</sup>

#### CONCLUSION

Forage yields and quality were in the studied species affected by the vegetative stage of plants. Namely in the first cut, the *Arrhenatherum elatius* (AES) exhibited yields higher than the *Festulolium* (FS). Thanks to the higher WSC content and the higher WSC/CP ratio, the FS appeared to be a better species for ensiling. Yields and quality of the forage at the end of the growing season were affected by the intensity of grassland use in summer. The effect of year was not negligible, too. Although the more intensively used grasslands exhibited lower yields, the quality of forage was higher. The CP content was at the end of the growing season higher in AES. However, based on the CF and ADF contents, the species more suited for extended grazing at the end of the growing season appeared to be FS. Thanks to its drought-resistance in summer and resistance to low temperatures in autumn and winter, the FS may find many useful applications in changing climate conditions.

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#### **SUMMARY**

The paper presents assessment of the yield and quality of Festulolium (FS) and Arrhenatherum elatius forages (AES) in the summer period and possibilities of conserving forage from the first cut. Further to the intensity of use in summer, forage quality is evaluated at the end of the growing season together with a possible use of the two species for extension of the grazing period. The grassland was used in summer either as a one-cut sward or a two-cut sward. Parameters monitored in summer were dry matter yields and contents of CP, CF, WSC and NEL. The WSC content at ear stage was 64 g/kg in FS and 38.2 g/kg in AES. The WSC/CP ratio (0.61), too, points to FS as a species more suitable for ensiling. Towards the end of the growing season, quality was affected namely by the intensity of use in summer with AES showing higher yields and a higher CP content, but with FS exhibiting lower CF and ADF contents.

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## NUTRITIONAL VALUE AND DEGRADABILITY OF NUTRIENTS IN SELECTED MORPHOLOGICAL PARTS OF FLINT X DENT MAIZE

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#### **INTRODUCTION**

According to the present valid system of feeds quality evaluation (Sommer et al., 1994) is effective nutrients degradability an important indicator, which characterizes quality of food for ruminants. Quality of maize plant can be influenced by the purpose of growing – hybrids suitable for ensiling or for grain (Verbič et al., 1995). It is necessary to know the quality of composition and nutritive value of the growing assortment of maize hybrids.

The aim of our work was to assess the content of selected nutrients and degradability of dry matter and organic matter in the whole plant, in leaves and stalks with flower head of selected maize hybrids of flint x dent type.

#### MATERIALS AND METHODS

In our experiment we used whole plants, leaves and stalks of four maize hybrids of flint x dent type – Ecrin, Diplomat, Bellevue, Gavott and Gavott fertilized with Zn additive, with different FAO (210/210, 220/220, 250/250, 270/260). Sampling was performed at the stage of milk-wax ripeness. Plants were separated to stalks with flower head without leaves and leaves after the sampling; we assessed content of dry matter, and green matter was lyophilized. In sacco experiments were performed as described by Harazim et al. (1999). Content of nutrients (tab. 1) was assessed in whole plants, stalks and leaves and content of dry matter (DM) and ash in residues after incubation was determined according to the Decree of MA SK 2145/ 2004-100. Effective degradability and parameters were calculated as described by Ørskov and McDonald (1979) at the outflow rate  $k=0.06.h^{-1}$ ). Content of nutrients was statistically processed by one way variance analysis and significance of differences was tested by Tukey test.

#### **RESULTS AND DISCUSSION**

Differences among hybrids in content of nutrients in whole plants beginning with dry matter is not caused only by current differences among morphological parts but also by the proportion of individual morphological parts and their ripeness at time of harvest (Verbič et al., 1995) as it was in our experiment (tab. 1).

Hybrid		Ecrin <sup>1</sup>	Diplomat <sup>2</sup>	Bellevue <sup>3</sup>	Gavott <sup>4</sup>	$Gavott + Zn^5$	Significant differences
Dry mottor	WP	341.5	366.2	344.5	355.4	372.9	
Dry matter	S	188.7	165.2	149.5	191.2	181.8	
	L	267.8	192.4	211.3	271.0	280.0	
Crudo protoin	WP	93.9	86.7	87.8	71.0	93.8	4:(1,2,3,5)**, 1:(2,5)*
Crude protein	S	41.7	44.5	38.3	27.8	51.7	4: (1,2,3,5)**, 5: (1,2,3)**, 2:3*
	L	139.8	145.3	129.0	128.1	145.8	
Starch	WP	264.5	304.2	277.4	339.7	356.7	4,5:(1,2,3)***, 2:(1,3)**
	WP	212.0	211.1	184.6	180.3	175.0	5:(1,2,3,4)**, 1:4*
ADF	S	376.0	391.7	395.5	399.0	343.2	3:5**, 1: (3,4)*, 4:5*
	L	267.5	268.3	316.3	271.3	266.5	3: (1,2,4)**, 3:5*
	WP	477.0	414.7	403.1	360.2	339.0	1:(2,3,4,5)**,2:(3,5)**, 3:(4,5)**, 4:5*
NDF	S	613.1	612.5	637.4	614.9	577.3	3: (1,2,4,5)**, 4:(1,5)**, 2:4*
	L	524.2	535.0	555.6	522.3	489.4	3:(4,5)**, 3:1*, 2:5*
	WP	11.9	3.0	5.6	10.9	10.9	1: (2,3)**, 2: (3,4,5)**
Lignin	S	31.4	26.0	31.5	38.0	54.0	5: (1,2,3,4)**
	L	20.4	23.5	16.1	26.4	28.9	5:3*
	WP	6.81	7.02	7.03	7.06	7.05	1:(2,3,4,5)**, 5:(1,2,3,)**
NEL (MJ.kg <sup>-1</sup> )	S	5.27	5.07	4.78	5.13	5.04	
	L	5.76	5.71	6.35	5.87	5.43	5: (1,2,3,4)**, 3:(1,2,4)**, 4:1*
NEV (MJ.kg <sup>-1</sup> )	WP	6.94	7.19	7.20	7.24	7.23	1:(2,3,4,5)**, 5: (1)**
	S	5.08	4.80	4.49	4.88	4.77	
	L	5.73	5.66	6.35	5.88	5.35	3: (1,2,4,5)**, 5: (1,2,4)**, 1:4*

Table 1. Content of nutrients in morphological parts of selected maize hybrids (g.kg<sup>-1</sup>)

WP - whole plants, S - stalks, L - leaves

We noticed only small differences among hybrids in DM of whole plants, with the exception of Gavott hybrid + Zn. This hybrid had higher content of crude protein in stalks (P<0.01) and leaves, and lower content of NDF and ADF in leaves, stalks and the whole plant (P<0.01) compared with other hybrids. The crude protein content in leaves of maize is approximately three-fold in comparison with stalks (tab. 1). Out of the compared hybrids flint x dent and their leaves and stalks was the highest content of crude fibre, ADF, NDF and lignin in stalks as detected Tolera and Sudstøl (1999), followed by leaves and then whole plants of maize (tab. 1). In hybrids tested by Verbič et al. (1995) was at lower DM of plants totally higher content of NDF, ADF and lignin in stalks and leaves than in ours. We found the highest starch

content in the hybrid Gavott (P<0.001), mainly in the hybrid Gavott+Zn (356.7 g.kg<sup>-1</sup> DM).) The nutritive value of maize plant is negatively affected mainly by ADF concentration Gross and Pesche (1980; it increses with ripening of plant. The level of NEL should be in whole maize plant in the period of milk-wax ripeness 6.30 MJ.kg<sup>-1</sup> DM (Biro 2001), and the level of NEV shall represent 6.2 MJ.kg<sup>-1</sup> DM (Štráfelda, 1999). We found higher energy concentration with the highest NEL value in the hybrid Gavott (P<0.01) and the lowest one in the hybrid Ecrin (P<0.01). Similar tendency was also with the NEV concentration (tab. 1). Effective degradability of organic matter (OM) and DM and parameters of their degradability are in table 2. Phipps and Weller (1979) explain higher soluble fraction "a" in stalks at the stage of milk-wax ripeness by higher concentration of soluble saccharides in stalks. We can do it also. Degradation rate "c" of the non-soluble fraction "b" was higher in leaves and the highest in the whole plant, with the exception of the hybrid Ecrin. We found the lowest degradation rate of DM and OM in stalks for the hybrid Gavott + Zn; they had also the highest concentration of ADF and especially of lignin. It became evident also in the lowest effective degradability of DM and OM of stalks. Verbič et al. (1995) reach the similar results. The obtained results are significant from practical point of view because according to effective degradability of dry matter and organic matter it is possible to predict their digestibility in maize silage and to choose suitable hybrid for silage production.

	Hyb	rid	Ecrin	Diplomat	Bellevue	Gavott	Gavott + Zn
	WP	OM	34.5	38.0	39.7	41.2	42.2
		DM	36.7	39.4	40.9	41.5	42.9
a (%)	S	OM	38.3	33.5	31.4	35.8	40.5
		DM	39.7	35.1	35.7	36.7	41.7
	L	OM	19.7	26.8	31.6	23.5	24.9
		DM	22.6	29.0	27.2	25.1	28.1
	W/D	OM	55.2	49.2	48.7	45.2	50.0
	VV F	DM	53.3	47.7	47.2	44.3	49.0
h (%)	ç	OM	48.6	33.6	47.1	40.6	39.3
0 (70)	2	DM	46.1	32.4	43.5	40.0	37.8
	L	OM	61.2	62.6	60.0	63.8	63.7
		DM	61.2	59.5	61.7	60.3	59.6
	WP	OM	0.037	0.033	0.033	0.032	0.024
		DM	0.037	0.033	0.033	0.032	0.024
$c (\frac{0}{h^{-1}})$	S	OM	0.011	0.037	0.021	0.019	0.016
C (70.11)	3	DM	0.012	0.037	0.022	0.019	0.017
	т	OM	0.040	0.031	0.029	0.034	0.032
	L	DM	0.041	0.029	0.029	0.032	0.031
	WD	OM	55.5	54.8	56.8	57.0	55.8
	VV F	DM	56.7	56.1	57.0	57.3	56.1
EDg (%)	S	OM	46.1	46.4	43.1	45.6	48.9
EDg (70)	3	DM	47.4	47.4	46.6	46.3	50.1
	т	OM	46.3	47.9	51.2	47.0	47.1
	L	DM	47.2	48.0	46.8	46.8	47.8

Table 2. Parameters of degradation and effective degradability of organic matter and dry matter

a – soluble and degradable fraction, b – non-soluble and degradable fraction, c – speed of degradation of fraction b EDg – effective degradability, OM – organic matter, DM – dry matter

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## QUALITY PARAMETERS IN A RANGE OF GRASSLAND TYPES WITHIN THE NÍZKE TATRY NATIONAL PARK

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#### **INTRODUCTION**

Grassland management is an activity having a positive impact, and the produced above-ground biomass finds its most efficient utilisation as forage for livestock (KAŠPAROVÁ and ŠRÁMEK, 2005). The quality of grassland is influenced not only by the botanical composition, but also by the phenophase of individual species, the water and nutrition regimes, sward exploitation, herbage preservation and storage (VESELÁ and MRKVIČKA, 2002). The management of grassland in the protected areas puts the accent on maintaining the species diversity and on the natural landscape value what presumes limitations in the agricultural production. The objective of this research was to make a comparison between the quality parameters of two grassland types located in the protected area of national park.

#### MATERIALS AND METHODS

The parameters of quality were investigated in two types of grassland in the *Nízke Tatry* National Park during two years. The first research site (*Zálom*) was 1052 m above sea level with the sward type of alliance *Polygono - Trisetion* Br.-BL. et R. Tx. ex Marshall 1947. The second research site (*Panská hoľa*) was at 1350 m altitude and the sward type was determined as the alliance *Nardo - Agrostion tenuis* Sillinger 1933. The sites were utilised by two cuts per year. The quality of dry matter (DM) was determined at each of the cuts. The following parameters were analysed in the laboratory: DM (gravimetry), crude protein (Kjeldal x 6.25), fibre (Hanneberg-Stolmann), P, K, Na, Ca, Mg (STN 46 7093, i. e. the Slovak Technical Standard). The nutritive and energy values - PDI (protein digested in the intestine), NEL (net energy for lactation) and NEV (net energy for fattening) - were calculated using the recorded laboratory data and the appropriate equations. The parameters were submitted to the analysis of variance and to the non-parametric Kruskal - Wallis test.

#### **RESULTS AND DISCUSSION**

#### Zálom site

Over the research years, the fibre content (Table 1) ranged from 164.40 g kg<sup>-1</sup> (1<sup>st</sup> cut in 2004) to 256.58 g kg<sup>-1</sup> (1<sup>st</sup> cut in 2002). Except for the year 2003, increased crude protein (CP) content was recorded at the 2<sup>nd</sup> cuts, respectively, but only once the CP content rose over the level of 160 g kg<sup>-1</sup> (2<sup>nd</sup> cut in 2004).

A close observation of mineral elements content is important both from the viewpoint of ecosystem (mineral cycling) but also from that of herbage quality (KRAJČOVIČ, 2002). There was the nutrient P deficiency. The content of K markedly fluctuated over the years. In 2002, however, the K content was more than the optimum value of 20 g kg<sup>-1</sup>. The content of Na ranged between 0.16 and 0.45 g kg<sup>-1</sup>, so consequently there was Na deficiency. According to the animal requirements, all the recorded Na content values were above the bottom level of the optimum interval defined as  $2 - 2.5 \text{ g kg}^{-1}$  by HOLÚBEK (1986).

The lowest PDI content (63.47 g kg<sup>-1</sup>) was found at the  $2^{nd}$  cut in 2003 and the highest PDI (83.51 g kg<sup>-1</sup>) was recorded at the  $2^{nd}$  cut in 2004. There were minimum differences in NEL and NEV and the values were in agreement with the sward condition and the utilisation intensity.

#### Panská hoľa site

The content of fibre was decreasing over the years (Table 2). The highest fibre content (264.58 g kg<sup>-1</sup>) was recorded at the 1<sup>st</sup> cut in 2002 and the lowest one (156.94 g kg<sup>-1</sup>) at the 2<sup>nd</sup> cut in 2004. The crude protein content above the level of 100 g kg<sup>-1</sup> was found only once (2<sup>nd</sup> cut in 2003). The content of P was low throughout the research period. The content of K reached nearly an optimum level only at the 1<sup>st</sup> cut in 2002. The contents of Ca and Mg were appropriate, however, they were decreasing over the years - a situation similar to that found with K content. The lowest Na content was found in herbage DM in 2003, presumably due to this more dry year showing effects on the content of Na in herbage (MÍKA, 1980). Despite the differences between the years, there was the Na deficiency.

In 2003, the lowest PDI content (55.66 g kg<sup>-1</sup>) was found at the 1<sup>st</sup> cut as well as the highest PDI (64.16 g kg<sup>-1</sup>) was recorded at the 2<sup>nd</sup> cut. These data were less than a bottom level of the optimum range 67 - 76.4 g kg<sup>-1</sup> defined by HRABĚ *et al.* (2005). Any notable differences between NEL and NEV were not found.

The statistical comparison between the two research sites showed that all the parameters studied were higher at the *Zálom* site - except for Mg and NEV (without the significant differences). The highest differences were found at crude protein (P = 0.00649) and also at PDI (P = 0.00640). The differences on the alpha level of significance ( $\alpha = 0.01$ ) were recorded at phosphorus (P = 0.01041). Any statistical differences were not found at the other parameters, namely fibre (P = 0.52184), K (P = 0.14954), Na (P = 0.17349, Ca (P = 0.52184) and NEL (P = 0.81018).

Years	Cuts	Fibre	СР	Р	Κ	Na	Са	Mg	PDI	NEL	NEV
2002	1 <sup>st</sup>	256.58	115.98	2.35	22.33	0.29	8.01	2.80	72.30	5.51	5.27
2002	$2^{nd}$	199.20	126.58	2.43	20.99	0.45	9.93	4.02	76.44	5.44	5.20
2002	1 <sup>st</sup>	214.37	140.43	2.02	19.93	0.16	5.67	2.57	80.15	5.50	5.25
2003	$2^{nd}$	232.62	101.82	1.77	14.37	0.18	7.40	2.97	63.47	5.48	5.25
2004	1 <sup>st</sup>	164.40	136.96	2.22	14.39	0.34	4.97	2.34	80.59	5.55	5.30
2004	$2^{nd}$	175.08	160.83	2.76	16.57	0.45	6.15	2.75	83.51	5.47	5.21

Table 1. The quality and energy parameters of herbage dry matter - Zálom site

Table 2. The quality and energy parameters of herbage dry matter - Panská hoľa site

Years	Cuts	Fibre	NL	Р	Κ	Na	Ca	Mg	PDI	NEL	NEV
2002	1 <sup>st</sup>	264.58	98.05	1.86	19.22	0.20	7.12	2.84	61.12	5.53	5.30
	2 <sup>nd</sup>	214.12	91.41	1.61	15.27	0.23	5.58	3.00	56.98	5.40	5.18
2002	1 <sup>st</sup>	187.88	89.29	1.41	14.81	0.17	9.28	3.31	55.66	5.45	5.23
2003	2 <sup>nd</sup>	188.03	102.93	1.61	12.73	0.16	7.08	3.00	64.16	5.55	5.32
2004	1 <sup>st</sup>	174.92	100.00	1.68	14.67	0.22	5.28	2.23	62.33	5.54	5.31
	$2^{nd}$	156.94	98.71	1.83	13.18	0.21	5.16	2.49	61.53	5.40	5.18

#### CONCLUSIONS

The comparison between the quality parameters of herbage DM showed that 8 of 10 parameters were better at the  $Z\dot{a}lom$  site. The individual parameters were relatively appropriate and well-balanced, and consequently, this site is predetermined for the production functions. Only two of the parameters (Mg and NEV) were better at the other research site (*Panská hoľa*), but the differences between them were not significant, albeit the quality of DM was markedly poorer at this site. The grassland located within the National Park territory is limited by its defined conservation status, but in addition to a range of non-production functions, it can fulfil also the production functions. Besides the other factors, in this case the differences in the quality parameters were related also to the site altitudes.

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#### **SUMMARY**

The parameters of quality were investigated in two types of grassland in the Nízke Tatry National Park during two years. The swards were utilised by two cuts per year - the 1<sup>st</sup> cut at the ear emergence of dominant grass species. At the Zálom site. The parameters were relatively appropriate and well-balanced, and consequently, this site could be utilised for the production functions. At the Panská hoľa research site, only two of the parameters (Mg and NEV) were better, but the DM quality was notably lower here. The grassland located within the National Park territory is limited by its defined conservation status, but besides the non-production functions, it can fulfil also the production functions.

#### SOWN GRASSLAND GROWING AT MOUNTAIN REGIONS IN SLOVAKIA

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#### **INTRODUCTION**

Sown grassland represents an important part in forage production. These established swards allow to select the species and varieties and therefore their productivity is better and also the forage quality is more efficient than that provided by seminatural grassland. The sown grassland can be grown under a range of systems, such as annual forage crops, perennial forage crops, temporary grassland. More offen now, the system of perennial forage crops is using not only a monoculture of red clover (*Trifolium pratense* L.), but also simple (binary) grass/legume mixtures with intergeneric grass hybrids (x *Festulolium* ASCHERS *et* GRAEBN.). Many authors had reported that growing the grass/legume mixtures is more efficient from the production point of view (Klimeš *et al.*, 2001; Ilavská, 2002 b; Ilavská and Vorobeľ, 2006; Ilavská *et al.*, 2007; Vorobeľ *and* Ilavská, 2007), and also from the viewpoint of forage quality, easier drying and ensiling (Gejguš and Kováč, 2000; Čunderlíková and Polák, 2003). The paper presents the implementation of red clover and its simple mixtures under the conditions of northern Slovakia.

#### MATERIALS AND METHODS

Research trials were established at Liptovská Teplička site and the following legume varieties were investigated: red Research trials were established at Liptovska 1 epitcka site and the following legume varieties were investigated: red clover (*Trifolium pratense* L.) - cv. Beskyd (Treatment1), cv. Amos (Treatment 2) and cv. Dolina (Treatment 3); lucerne (*Medicago sativa* L.) cv. Syntéza (Treatment4) as well as four simple (binary) grass/legume mixtures (Treatment5 to 8) composed of the clover and lucerne cultivars with the intergeneric grass hybrid (IGH) (x *Festulolium* ASCHERS *et* GRAEBN), newly-bred HZ 13 DK. The site characteristics had been specified earlier by Ilavská *et al.* (2007). The seed rates were 20 kg ha<sup>-1</sup> at clover and 18 kg ha<sup>-1</sup> at lucerne monocultures. The seed rates of the grass/legume mixtures were 26 kg ha<sup>-1</sup> with 40 % legume and 60 % grass proportions (at 100 % seed utility value). The swards were utilised by 3-cut system (the 1<sup>st</sup> cut of legume monocultures at the onset of flower heads and the 1<sup>st</sup> cut of grass/legume mixtures according to the dominant plant group; the 2<sup>nd</sup> cut followed 4 - 5 weeks after the 1<sup>st</sup> one; the 3<sup>rd</sup> cut was carried out after 7 - 8 weeks).

**RESULTS AND DISCUSSION** In the 1<sup>st</sup> harvest year, a higher proportion of herbs and non-sown grass species was found in the legume In the 1<sup>st</sup> harvest year, a higher proportion of herbs and non-sown grass species was found in the legume monocultures, mainly rough meadow-grass (*Poa trivialis* L.) and shepherd's purse (*Capsella bursa-pastoris* (L.) MED.). The proportion of sown species at the treatments and cuts ranged between 56 % (1<sup>st</sup> cut - *M. sativa*) and 96 % (3<sup>rd</sup> cut - *T. pratense* cv. Dolina). Decreased proportion of legumes was observed also at the grass/legume mixtures and position of *M. sativa* in sward was obviously weaker than that of T. *pratense*. The following two cuts were characterised by increased proportion of the sown components and marked reduction of herbs in sward. Bare spots were nearly none or only 1 % of ground cover. In the 2<sup>nd</sup> harvest year, the legume proportion in the monocultures notably decreased. According to the species and cultivars, the ground cover ranged from 21 % (*M. sativa*, 1<sup>st</sup> cut) to 85 % (*T. pratense* cv. Dolina, 2<sup>nd</sup> cut). Their position was occupied by *P. trivialis*, native white clover (*Trifolium repens* L.) and some other herb species. There was an interesting increase in the proportion of *T. pratense* and especially that of *M. sativa* in the grass/legume mixtures. Thus, reports of some other authors were confirmed that legumes find a better application in their simple mixtures with grasses than as monocultures (Lesák and Svěráková, 1992; Gejguš and Kováč, 2000; Ilavská and Ratai, 2002 a; b; Ilavská, 2003). In the legume monocultures, the lowest dry matter production (DM) 2000; Ilavská and Rataj, 2002 a; b; Ilavská, 2003). In the legume monocultures, the lowest dry matter production (DM) was recorded with *M. sativa* (Tab.1).

was recorded with *M. sativa* (1ab.1). There were significant differences between *M. sativa* and the other legume species ( $LSD_{0.05} = 1.125$ ). The low DM production of the *M. sativa* in monoculture was also found in its mixture with the intergeneric grass hybrid HŽ 13 DK, but the significant difference was found only in relation to the mixture of HŽ 13 DK + *T. pratense* cv. Dolina ( $LSD_{0.05} = 1.603$ ). In the 2<sup>nd</sup> harvest year, the sward proportion of *T. pratense* decreased what resulted in reduced DM yield by 1.66 - 2.15 t ha<sup>-1</sup>. However, an increase in DM by 0.67 t ha<sup>-1</sup> was found at *M. sativa*. The decrease in DM yield was not so dramatic at the grass/legume mixtures in the 2<sup>nd</sup> harvest year ( the decrease ranged from 0.3 to 1.29 t DM ha<sup>-1</sup> at three of the treatments). Under the mountain conditions, the tetraploid varieties of *T. pratense* showed better performance, their DM production was higher both in their monocultures and in their simple mixtures with IGH than the DM production of *M. sativa* swards. Similar conclusions had been reported at an earlier research (Ilayská and Rata) 2002 a: 2002 b). As to M. sativa swards. Similar conclusions had been reported at an earlier research (Ilavská and Rataj, 2002 a; 2002 b). As to a suitability of species and mixtures for preservation as silage, the content of water-soluble carbohydrates (WSC) is one of the most decisive factors. Analyses showed low content of monosaccharides and high content of WSC in all the legume monocultures and their mixtures at all the cuts (Table 2)

The WSC content was influenced mainly by the order of the cuts and then by the species in the treatments. The highest WSC (and also crude protein) content was recorded with the legume monocultures at the 2<sup>nd</sup> cut. There was more variability in the content of WSC and more stability in the content of crude protein (CP), consequently, there were significant differences in the coefficient of ensiling capacity between the cuts. The significantly highest coefficients of ensiling capacity were found at the 1<sup>st</sup> cut and the lowest ones at the 3<sup>rd</sup> cut. The differences between the cuts and the species were very significant for all the parameters of ensiling capacity. At all the cuts, the lowest coefficients were recorded with the legume monocultures what is related - in addition to other factors - with high CP content, as reported also earlier (Cunderliková and Polák, 2002; 2003). Losses in DM and CP were found in the ensiled herbage during the also earlier (Cunderliková and Polák, 2002; 2003). Losses in DM and CP were found in the ensiled herbage during the fermentation process. At all the silages, there was increased content of fat, ash and fibre related to the fermentation, i. e. with the utilisation of nutrients and the production of substances by the existing microflora. Increasing the content of DM by wilting before ensiling showed positive effects on the final quality rank of silage, because good conditions for fermentation were provided. There were very significant differences in the lactic acid content between the cuts and species, while differences between the treatments were not significant. The highest lactic acid content was recorded in legumes at the 2<sup>nd</sup> cut and in the mixtures at the 3<sup>rd</sup> cut. The silage quality was classified in compliance with the scale of points allotted for the content and percentage proportion of the lactic, acetic and butyric acids. High content of butyric acid was recorded at the treatments with cv. Beskyd and cv. Amos at the 1<sup>st</sup> cut, therefore the silage quality from these ranked to the Quality Class 2. The same was true of the treatment with cv. Beskyd at the 2<sup>nd</sup> cut as well as for the ranked to the Quality Class 2. The same was true of the treatment with cv. Beskyd at the  $2^{nd}$  cut as well as for the mixtures of cv. Dolina + HZ 13 DK and cv. Syntéza + HZ 13 DK at the  $3^{rd}$  cut. The sensory parameters of colour, smell, dust and consistency did not show significant differences between the species and treatments. All the silages were characterised by brownish colour, aromatic or slightly acidic smell and firm texture apparent, in agreement with the Quality Classes 1 and 2. The nutritive value of silage (Table 3) was in correspondence with its quality and the

content of nutrients. The highest PDIN (protein digested in the small intestine when nitrogen is limiting) was recorded in the legume monocultures at all the cuts. The highest NEL (net energy for lactation), NEV (net energy for fattening) and metabolisable energy (ME) were found in the legume/clover mixtures at all the cuts.

Table 1. Dry matter production (t ha	$\iota^{-1}$
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Year	-	Tre	eatments		Year	Treatments				
	Legumes	Σ	Mixtures	Σ		Legumes	Σ	Mixtures	Σ	
	1	8.482	5	9.220		1	6.385	5	7.925	
2004	2	8.476	6	9.034	2005	2	6.324	6	9.335	
	3	8.197	7	9.372		3	6.537	7	8.160	
	4	4.844	8	7.736		4	5.514	8	6.568	

#### **Table 2.** Mean ensilage capacity $(g kg^{-1} DM)$

	Treat	Fresh	Carbohy	drate content			WSC / CP
Cuts	ments	herbage DM	Mono-	WSC	Σ	СР	coefficient
	1	121.20	71.66	00.20	161.06	150.02	1.02
	1	131.30	/1.00	90.20	101.00	159.02	1.02
	$\frac{2}{2}$	137.02	08.82	80.49	155.51	155.89	1.00
	3	134.04	67.96	88.50	150.52	140.81	1.07
1 <sup>st</sup>	4	1/4.08	54.89	95.88	150.76	134.08	1.12
-	5	150.05	57.15	106.57	163.71	96.37	1.70
	6	159.10	53.40	85.77	139.16	93.76	1.48
	7	156.87	54.20	78.94	133.13	92.32	1.44
	8	177.98	40.88	74.64	115.52	80.80	1.43
	1	99.51	69.34	93.65	162.99	213.12	0.76
	2	102.45	75.65	96.05	171.70	193.83	0.89
	3	102.41	70.79	92.67	163.46	196.05	0.83
$2^{nd}$	4	152.24	50.05	79.28	129.34	181.26	0.71
2	5	112.10	68.73	84.52	153.25	168.34	0.91
	6	112.22	67.01	86.70	153.71	163.30	0.94
	7	115.34	61.08	75.99	137.07	175.03	0.78
	8	168.09	41.26	51.25	92.59	109.30	0.85
	1	238.24	51.76	79.91	131.67	170.64	0.77
	2	231.34	38.90	56.10	94.90	192.53	0.49
	3	232.29	44.04	62.83	106.87	174.88	0.61
2rd	4	233.33	40.37	73.14	113.51	209.97	0.54
5	5	232.54	38.70	54.06	92.76	181.96	0.51
	6	232.96	45.95	62.65	108.60	176.80	0.61
	7	231.41	48.31	74.78	123.09	177.32	0.69
	8	232.34	41.13	58.49	99.62	156.55	0.64

**Table 3**. Mean nutritive value of preserved legumes and grass/legume mixtures

Cute	Treatments	PDIN <sup>(1)</sup>	$PDIE^{(2)}$	NEL <sup>(3)</sup>	NEV <sup>(4)</sup>	ME <sup>(5)</sup>
Cuts	Treatments	g k	.g		MJ kg <sup>-1</sup>	
	1	94.94	70.00	5.22	4.97	8.96
	2	90.33	69.02	5.25	5.01	9.00
	3	80.27	66.07	5.20	4.96	8.92
1 <sup>st</sup>	4	83.50	62.17	4.86	4.51	8.40
1	5	65.19	69.07	5.73	5.65	9.65
	6	58.47	65.22	5.72	5.61	9.64
	7	61.76	67.60	5.72	5.60	9.66
	8	58.95	67.38	5.73	5.61	9.67
	1	122.06	67.58	5.03	4.75	8.67
	2	119.91	68.31	5.07	4.79	8.45
	3	115.63	66.17	5.09	4.81	8.98
and	4	108.73	68.69	4.77	4.42	8.32
2	5	96.58	75.09	5.53	5.37	9.39
	6	92.33	71.43	5.44	5.26	9.26
	7	103.48	75.60	5.53	5.36	9.40
	8	70.69	64.23	4.92	4.62	8.50
	1	106.68	67.13	5.21	4.94	8.97
	2	115.56	68.19	5.21	4.94	8.98
	3	101.43	67.53	5.19	4.92	8.94
2rd	4	125.93	70.73	4.87	4.50	8.50
3	5	115.12	69.00	4.83	4.47	8.42
	6	106.30	66.47	4.84	4.49	8.44
	7	95.83	62.33	4.83	4.49	8.41
	8	97.37	73.28	5.41	5.21	9.23

#### CONCLUSIONS

**CONCLUSIONS** Under the mountain conditions, low yield of *M. sativa* indicated a more successful use of *T. pratense* that achieved higher and more evenly distributed DM yields. By comparison with these, there was better application of grass/legume mixtures because their yields ranged between 7.737 t DM ha<sup>-1</sup> (the mixture with *M. sativa*) and 9.372 t DM ha<sup>-1</sup> (the mixture with *T. pratense*). The research results confirmed the variability in the content of WSC and CP as the main parameters of ensiling capacity. The lowest coefficients of ensilage capacity were found with the legume monocultures at all the cuts. The increased DM content achieved by wilting the herbage prior to ensiling showed positive effects on the fermentation process at all the cuts and treatments. A greater part of the silages was classified as Quality Class 1 according the modified Flieg-Zimmer scale. The highest PDIN content was found at the legume monocultures, the highest NEL, NEV and ME were recorded with the grass/legume mixtures.

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The list of references is available at the authors

## HERBAGE CHEMICAL COMPOSITION AFTER THE CESSATION OF FERTILIZER APPLICATION MOWN GRASSLAND

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#### INTRODUCTION

Fertilization of permanent and temporary grassland change the chemical composition of grass matter and other properties of grassland site. Dry matter nutrient concentration always reflects the changes in botanical composition and the actual effect of applied fertilizers (Holúbek, 1991; Jančovič, 1996).

However, at present the problems of structural changes in permanent grassland chemical composition after the complete cessation of mineral fertilization seem to be topical. Botanical diversity of these grasslands has been changing over the years, with dicotyledonous plants tending to acquire a dominant position (Lichner, 1973), which can adversely affect the quality of above - ground biomass and its convenience for conservation (Nőssberger and Kessler, 1997).

The goal of this contribution is to changes in chemical composition of permanent grassland grass matter after the cessation of mineral fertilization.

#### MATERIAL AND METHODS

The changes in chemical composition of grass matter were observed on permanent grassland (association *Lolio-Cynosuretum cristati*) on a site in the Strážov Hills (central part of Slovak Republic). A field trials were established with four replicates (area of the harvest plot was 10 m<sup>2</sup>). In the years 1986-1989, grassland was harvested at the grazing stage four times a year, in the years 1990-1993 at the hay-making stage (2-3 cuts). During the years 1994-2006 fertilization was omittet and grassland was non-utilized. Before each cut, botanical analysis was assessed on particular treatments by the method of reduce projective dominance (Regal, 1956) for purpose of determining changes in botanical composition of grassland. Fertilizer treatments in years 1986-1993 were as follows: 1. K – non-fertilized control, 2. PK - constant rate of P and K (35 kg/ha P and 70 kg/ha K), 3. N<sub>60</sub> – 60 kg/ha N was applied in early spring, 40 kg/ha N after the 1st cut (+PK), 5. N<sub>240</sub> – 100 kg/ha N was applied in early spring, 80 kg/ha N after the 1st cut and 60 kg/ha N after the 2nd cut (+PK).

It was found that in the twelfth year of ceasing fertilization (2006) and in the last year (1993) of eight-year periodical fertilization the average values of mineral nutrition concentrations (N, P, K, Ca, Mg, Na) corresponded with the values specified by the Slovak technical standard (STN 467007).

#### **RESULTS AND DISCUSSION**

In 1993 year (the last year of mineral fertilization) higher concentrations of nitrogen in the grassland dry matter (DM) were found in the treatment with N-fertilization (22.03–26.89 g/kg) than in the unfertilized control (15.12 g/kg) and treatment with PK-fertilization (17.24 g/kg). In contrast, phosphorus concentration decreased with increasing nitrogen doses. However, the lowest phosphorus concentration was revealed in the unfertilized control treatment (1.31 P g/kg DM). It is known from a number of works that as a consequence of increasing nitrogen dose long-term fertilization, the potassium concentration in dry matter decreases (Holúbek, 1991; Holúbek et al., 1990; Velich, 1986). This was also confirmed by our experiment. After the application of 240 kg/ha N, the potasium dry matter concentration (14.06 g/kg) decreased below the level of the unfertilized control treatment (16.19 g/kg). A similar tendency was also found in the calcium concentrations having an enormously low value in the treatment fertilized with 240 kg/ha N (2.63 Ca g/kg DM). In reason for this was the entire reduction of legumes and the subsequent propagation of dicotyledonous herb and grass species generally characterized by a low calcium concentration was nearly the same in all treatments, only in treatment PK and N<sub>60</sub> decreased below 3 Mg g/kg DM. Interestingly, in all treatments natrium concentration was kept at the same level, 0.31 Na g/kg DM on average (Tab. 1).

Twelve years after the cessation of fertilization (2006), the dry matter nitrogen concentration in experimental treatments PK,  $N_{120}$  and  $N_{240}$  decreased, while increasing in the unfertilized control treatment (15.41 g/kg) and  $N_{60}$  treatment (23.11 g/kg). A rapid reduction in nitrogen concentration from 1.87 to 4.83 N g/kg DM found mainly in treatment with application of nitrogen  $N_{120}$ ,  $N_{240}$  and PK-fertilizing. However, phosphorus concentration in all treatments increased in addition to treatment with PK application. PK concentration decreased below the level of the treatments with nitrogen application ( $N_{60}$  and  $N_{240}$ ). Twelve years after the cessation of PK-fertilization, the effect of applying phosphorus in the form of mineral fertilizers on its concentration in meadow plants became weaker, which is also related to the changes in botanical composition of grasssland. Potassium concentration rapid increased in all treatments after this period what is the consequence of dicotyledonous plants extension (*Taraxacum officinale, Alchemilla xanthochlora, Pimpinella saxifraga*) and other plants with the greater potassium – fixing capacity. Calcium DM levels increased in all treatments and especially high increase of calcium content was found in the treatment with 240 kg/ha N (increasing 8.87 g/kg/ha), whereas magnesium concentration in dry matter decreased in all treatments (Tab. 1). Markedly decreased sodium concentration in dry matter (by 0.26 g/kg on the average). The relative concentration reduction was the most pronounced with sodium (36%), but also with nitrogen and magnesium (11%); the increase in potassium concentration (61%), but also calcium (22%) and phosphorus (14%).

The changes in chemical composition of grass matter, taking place in period without mineral fertilizing, reflect especially the structural and functional changes in grass phytocenosis. The twelve years period following the cessation of mineral fertilization significantly influenced the botanical composition of grassland (Fig. 1). At the same time it should be emphasized that befor this cessation the grassland were utilized by 2-3 cuts (the first cut represented 46–70% of total yield-average from eight years), but a long period without utilization when the species resistant to low trophic levels could propagate than influencing the concentration of mineral elements in the dry matter of grasslands. Similar results in nutrient concentration changes documents Gáborčík et al. (1997), but his results, though are two years only, indicates rapid changes already in short-term period after cessation of fertilization. This author further claim that another factor which can influence the mineral element concentration i grass biomass is the gradual accumulation of root matter, particularly in the second year after ceasing mineral fertilization. Supposedly, the more intensive

accumulation of root matter also causes the retention of some nutrients (K, Na) in the roots, which may be adversely reflected in their concentration in the above - ground biomasss. In the case of nitrogen this was unambiguously caused by its exclusion from fertilization (Gáborčík et al., 1997).

#### CONCLUSIONS

From the results obtained with cessation of mineral fertilization can be drawn the following conclusions and recommendations:

- The most decrease was detected in sodium (by 36%), slight decrease in nitrogen (by 11%) and magnesium concentration (by 11%).
- A expressive increase was found i potassium (by 61%), calcium (by 22%) and phosphorus concentration (by 14%).
- Changes in chemical composition seem to be a consequences of the cessation of mineral fertilizing, the botanical changes in grassland composition, non-utilized grassland and the resulting more intensive accumulation of nutriens in root system of meadow and pasture plants.

 Table 1.
 Nitrogen and mineral nutrient concentrations in grass dry matter in treatments with (1993) and without mineral fertilization (2006)

Traatmont	Fertilization	Year	Concentration of nutrients (g/kg)								
Treatment	rentinzation		Ν	Р	K	Ca	Mg	Na			
N <sub>0</sub>			15.12	1.31	16.19	14.26	3.05	0.31			
PK	According to		17.24	1.93	18.88	13.68	2.61	0.31			
N <sub>60</sub>	treatments of	1993	22.03	1.84	19.26	12.65	2.83	0.33			
N <sub>120</sub>	fertilization		26.89	1.65	16.09	9.50	3.27	0.30			
N <sub>240</sub>			26.25	1.62	14.06	2.63	3.09	0.31			
	Average		21.50	1.67	16.89	10.54	2.97	0.31			
N <sub>0</sub>			15.41	1.75	22.50	15.29	2.60	0.06			
PK	Connetion of		15.37	1.81	25.00	15.09	2.53	0.04			
N <sub>60</sub>	fortilization	2006	23.11	1.88	28.75	12.15	2.60	0.06			
N <sub>120</sub>	Tertilization		22.06	1.81	31.25	10.57	2.69	0.05			
N <sub>240</sub>			22.06	2.31	28.75	11.50	2.94	0.06			
	Average		19.20	1.91	27.25	12.92	2.67	0.05			
% (1993 = 100)	% (1993 = 100 %)		89	114	161	122	89	164			

Figure 1. Dominance and botanical composition of grassland after long-term fertilization



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#### PRODUCTION AND QUALITY PARAMETERS OF GRASSLAND INFLUENCED BY SELECTED STRIP-OVERSOWN SPECIES

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#### ABSTRACT

In 2001, this trial was established in a mountain region of Slovakia (the Low Tatras mountain range, Liptovská Teplička site). Production of grassland and its quality can be improved by different ways. It involves improved management, fertiliser application, over-sowing with legumes and grasses. In a field trial, permanent grassland was oversown with grass and clover cultivars. The effects were studied of oversown cultivars, mineral fertiliser application and increased cutting frequency on dry matter (DM) production and some of the quality parameters. As drought during a part of or throughout the growing season can negatively influence DM production, our aim was to stabilise the production of DM by the management interventions. The results suggested that the DM production was stabilised by introducing strip-oversown Trifolium pratense as well as grass cultivars with nitrogen fertiliser applied to permanent grassland.

#### **INTRODUCTION**

Grassland improvement in an agricultural context entails the use of management to increase primary and secondary production. Common improvement strategies involve the introduction or encouragement of plant genotypes of high nutritional quality, high yield, and long stand life and the use of animal genotypes with efficient feed conversion. The goals of improvement depend upon the status of ecosystem. For productive grasslands, improvement goals are likely aimed to maintain economic or environmental viability (KEPHART *et al.*, 1995). The aim of agronomists in mountain areas is to achieve long-term high forage production (HOLÉCY and BARTKO, 1991). This is important also from the viewpoint of expected global climatic change.

#### MATERIALS AND METHODS

Two assortments of main grassland components were tested at *Liptovská Teplička* site (a mountain village). Cultivars of legumes (Group 1 - Treatments 1 and 2: *Trifolium pratense*, cvs. Nodula and Rezista, respectively; Treatment 3: *Medicago sativa* cv. Zuzana; Treatment 4: *Lotus corniculatus* cv. Polom) and grasses (Group 2 - Treatment 5: *Festuca arundinacea* cv. Kora; Treatment 6: *Dactylis glomerata* cv. Niva; Treatment 7: *Festuca rubra* cv. Tagera and Treatment 8: *Arrhenatherum elatius* cv. Median) were strip-oversown into permanent grassland. The main site characteristics were as follows: average annual rainfall 900 mm (500 mm during the growing season) and average annual temperature 4 °C (9.5 °C during the growing season). Over the growing seasons of 2002-2005, the total rainfall was 462.6, 322.0, 510.9 and 459.2 mm, respectively, and the average temperature was 13.56, 13.82, 12.39 and 13.12°C, respectively. On  $31^{st}$  July 2001, the grass and legume cultivars were strip-oversown using the sowing machine SPP 6 (made in the Czech Republic). The sowing machine made two strips per treatment (dimensions of a plot: 10 m x 1.5 m; four randomised replicates) and cultivated a strip of soil 0.10 m deep and 0.15 m wide, 0.5 m apart.

For all the treatments, P and K fertilisers were applied at the rates of 30 kg P ha<sup>-1</sup> and 50 kg K ha<sup>-1</sup>. For Group 2, i.e. over-sown grasses, nitrogen was applied at the rate of 120 kg N ha<sup>-1</sup>, divided into two dressings. Total dry matter yield was determined in three cuts over the growing season (the cutting dates: early June, early July and late September). In the pre-experiment period, this grassland was utilised by a one-cut system with the aftermath rarely grazed.

#### **RESULTS AND DISCUSSION**

The above-ground DM production, fibre (F) and crude protein (CP) content at the treatments are given in Table 1. Between 2002 and 2005, dry matter production was decreasing with the rising mean temperature in the growing season (r = -0.56; P < 0.001). On the contrary, DM production was enhanced by expanded amounts of rainfall in the growing period (r = 0.77; P < 0.001). Above-ground DM production at the treatments over-sown with grasses was higher than at the ones with legumes ( $\chi^2 = 5.64$ ; Df = 1; P = 0.018; Table 2). Statistically significant differences between the subsequent years were observed ( $\chi^2 = 17.91$ ; Df = 3; P < 0.001; Table 2). In 2003, production of DM was reduced by 32 % in comparison with 2002, due to the drought in the summer, as reported by CIAIS *et al.* (2005). If the drought had not occurred throughout the growing period (e.g. sufficient spring rainfall to the first cut), different results could have been recorded. As shown in Table 1, the application of nitrogen fertiliser stabilised DM production of the over-sown grasses. Sufficient spring rainfall gave an advantage of DM over-yielding to *Trifolium pratense, Medicago sativa* and *Lotus corniculatus* and grasses. Kruskal-Wallis non-parametric test (i.e. rank-sum) has provided statistically significant results in differences between treatments ( $\chi^2 = 21.79$ ; Df = 7; P < 0.003; n = 4). However, statistically significant differences between the tested cultivars were not found ( $\chi^2 = 0.07$ ; Df = 1; P = 0.792; n = 16). The significant differences between the treatments were not found, neither one-way ANOVA (Model F<sub>7, 24</sub> = 1.305), nor Kruskal-Wallis test of ANOVA ( $\chi^2 = 8.47$ ; Df = 7; P = 0.293; n = 4).

The fibre content in herbage was positively correlated with increased temperature (r = 0.53; P < 0.01) and a negative interaction was observed with increased amount of rainfall (r = -0.23; P = 0.20), in the growing season. The fibre content was significantly higher in the over-sown grasses than in the legumes ( $\chi^2 = 5.11$ ; Df =1; P = 0.023; Table 2). Improved grassland management resulted in a gradual and statistically significant decrease in mean fibre content (Table 2). One-way ANOVA did not show any differences among treatments. The crude protein content in herbage was influenced negatively by increasing temperatures (r = -0.47; P < 0.01), but positive effects of increasing rainfall were found (r = 0.55; P < 0.01). In contrast to fibre content in DM, the content of P was significantly higher in the over-sown legumes than in the grasses ( $\chi^2 = 8.42$ ; Df = 1; P < 0.004; Table 2). It was despite the application of mineral N fertiliser to the over-sown grasses. Because of higher-than-average temperature and lower-than-average rainfall in 2003, the lowest content of CP in DM was detected. Subsequently in 2004, the meteorological conditions were inverse to those of 2003 and the highest CP content in DM was recorded. As to fibre and crude protein content, any statistically significant differences between the research treatments were not found.

**Table 1.** Dry matter production (t ha<sup>-1</sup>) and the content of fibre and crude protein in DM (g kg<sup>-1</sup>) for the factor treatment (averaged over four years) and rank-sum Kruskal -Wallis test ANOVA and ANOVA test DM production in 2003.

Treatments	Dry matter $\pm$ SE	Fibre ± SE	Crude protein $\pm$ SE	Rank-sum DM '03	DM '03
1	$4.96 \pm 0.36a$	$199.06 \pm 8.73a$	$136.41 \pm 6.08a$	109	4.28a
2	$5.09 \pm 0.29a$	$186.60 \pm 5.00a$	$136.86 \pm 5.25a$	106	4.25a
3	$4.04 \pm 0.45a$	$204.91 \pm 9.34a$	$129.38 \pm 9.83a$	17	2.70c
4	$4.07 \pm 0.42a$	$196.83 \pm 7.10a$	$134.42 \pm 5.41a$	25	2.83c
5	$5.38 \pm 0.58a$	$211.50 \pm 11.43a$	$123.98 \pm 2.18a$	67	3.66b
6	$5.46 \pm 0.60a$	$215.10 \pm 11.05a$	$120.71 \pm 2.53a$	74	3.74ab
7	$5.27 \pm 0.53a$	$208.65 \pm 7.90a$	$125.27 \pm 2.57a$	71	3.71ab
8	$5.20 \pm 0.62a$	$211.66 \pm 11.72a$	$121.57 \pm 1.93a$	59	3.46b
Р	0.290	0.421	0.167	0.0028	< 0.001

 $\pm$  SE – standard error of mean; the same letter designates statistically not significant differences between two treatments at level *P* < 0.05 (Tukey *t* - test)

**Table 2**. Dry matter production (t ha<sup>-1</sup>) and the content of fibre and crude protein in DM (g kg<sup>-1</sup>) for the factors years and type of grassland

Year	$DM \pm SE$	$F \pm SE$	$CP \pm SE$	Туре	$DM \pm SE$	$F \pm SE$	$CP \pm SE$
2002	$5.27 \pm 0.21a$	225.02±6.15a	131.62±4.02ab	1	4.45±0.21b	196.85±3.9b	134.23±3.2a
2003	$3.58 \pm 0.21b$	208.95±4.97ab	119.27±2.42b	2	5.33±0.26a	211.73±4.8a	122.88±1.1b
2004	$5.51 \pm 0.27a$	192.10±3.09bc	136.48±4.26a				
2005	$5.38 \pm 0.25a$	191.08±3.33c	126.93±2.05ab				
Р	< 0.001	< 0.001	0.008		0.027	0.022	0.002

 $\pm$  SE – standard error of mean; the same letter designates statistically not significant differences between two treatments at level P < 0.05 (Tukey t - test); DM – dry-matter; F – fibre; CP – crude protein; Type – 1 grassland over-sown with legumes, 2 – grassland over-sown with grasses

#### CONCLUSIONS

The research results indicated that DM was stabilised after strip-oversowing to permanent grassland, either by introducing varieties of *Trifolium pratense* or by nitrogen fertiliser applied with the tested grasses. In the qualitative characteristics, the excessive amount of rainfall was favourable for crude protein concentration and *vice versa*. Improved sward management by increased cutting frequency has successfully reduced the content of fibre in DM.

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## PRODUCTION POTENTIAL OF RESTORED MOUNTAIN PASTURE IN RELATION TO DIVERSITY INDEX OF GROWTH

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#### ABSTRACT

Production potential of restored pasture in relation to diversity index was observed under effect of all year round using by beef cattle. The experiment was situated on a site at Diel in the district of Poltár at an altitude of 920 m a.s.l. in Slovakia. The highest yields ( $\emptyset$  5.53 t.ha<sup>-1</sup>) and the lowest diversity index (0.85 and 0.80) were found out on a plot, which had burned before started experiment. However plot improved by reseeding had high yields ( $\emptyset$  5.48 t.ha<sup>-1</sup>), but also this plot had higher diversity index (1.81 and 2.52). We found out low dependence production potential to diversity index on the pasture (r = -0.2). The effect of diversity index influence to yields is discussed. The hay production can form principally species, which can produce a lot of phytomass which does not depend to increase diversity index on the pasture.

#### **INTRODUCTION**

Begon *et al.* (1997) introduce that as for increase productivity of environment lead to bigger extent accessible of source so this lead probably to bigger species richness too. However, more productive environment can contain more quantity source or input source but its can not affect source of diversity. As a consequence of this in the environment can occur more individuals of a single species but not more a number of species. The authors observe that many experimental works show that diversity does not increase with productivity definitely and as well that species richness is the biggest at middle level of productivity. Increased production with higher diversity can be explained by the chance through the "sampling effect", whereby a greater number of species is more likely to include individual species that are highly productive, such as legumes (Thompson *et al.*, 2005). Dulinger (2003) state that grazing has strong impact to plants species composition often and this has positive effect to species richness.

#### **MATERIALS AND METHODS**

The experimental area used had not been grazed  $50^{\text{th}}$  years, and had experienced a period of nationalization with encroachment of trees and shrubs forming area of woodland. The experiments were established on a restored mountain pasture in 2005. The cattle are retained on the experimental area all year round, with no period housing, and with supplementation feed provided during the winter. The stocking rate was  $0.30 - 0.60 \text{ LU.ha}^{-1}$  per annum. The experiment was situated on a site at Diel in the district of Poltár (48° 25' N, 19°34' E) at an altitude of 920 m a.s.l. in Slovakia. Average yearly temperature was 5.1 °C and during the growth period averaged 10.51 °C. Average precipitation was 926.72 mm and the average for the growth period was 629.81 mm. Soil profile was clay-sandy throughout the whole of the soil depth. The experimental variants (Tab. 1) were organized into randomized incomplete blocks with three

Tab	le 1	Experimental	variants
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Variants	Charakteristics of variants
BG	burned places (autumn 2005) and grazing plot
UM	un-management plot
G	only grazing plot (control)
CG	cutting in Jun and after grazing plot
RG	reseeded (5 <sup>th</sup> May 2006) and grazing plot

repetitions after the experimental area was cleared of encroaching woodland. Above ground phytomass was taken twice a year (Jun, September) and analyses of floristic composition were done by method reduce projectively dominance (D %) according to Klapp (1965). On a base of botanical composition (D %) was calculated Shannon diversity index by the formula  $H = -\sum_{i=1}^{s} p_i \ln p_i$  (Begon *et al.* 1997). Results were

determined by statistical analysis using Pearson correlations analyze and ANNOVA by software Statistica 7 (2002).

#### **RESULTS AND DISCUSSION**

Plant diversity is associated with production potential of growth very often. Bullock *et al.* (2007) introduce that experimental work over the last decade has conclusively demonstrated positive effects of greater plant species richness on some ecosystem functions, especially through increasing biomass production (Hooper *et al.*, 2005 In Bullock *et al.* 2007). Yields and diversity index of experimental variants are documented in table 2.

A plant community had the highest hay production affected reseeding of valuable plant species for fattening ( $\emptyset$  5.48 t.ha<sup>-1</sup>) and plant community affected burned of growth together with rest of encroachment wood ( $\emptyset$  5.53 t.ha<sup>-1</sup>), as its shown no significant effect variants on the yields. However, growth had the lowest diversity index on the variant BG (0.85 and 0.80) whereas diversity index was increased on the variant RG to 2.52 in year 2007. Hay production was increased as well as value of the diversity index on the variant CG influenced cutting and grazing in the year 2007. However, yields were not increase but the diversity index was increased on the variant G influence only grazing by beef cattle during couple of years.

The correlation relation between hay production and its diversity were explored with Pearson analyze (Figure 1). This show low dependence increase of yields to diversity (r = -0.2). We put suggestion that diversity of experimental plots have not affected hay production after restoration of pasture in the first two years. This is not to say that only

diversity can be designed to create of high hay production.

		Variants									
Years	Sampling	BG		UM		G		CG		RG	
		$(t.ha^{-1})$	Н								
	1	0.82	0.68	2.40	2.56	2.15	1.78	0.71	2.03	2.01	1.75
2006	2	4.11	0.84	0.40	2.41	1.84	1.96	0.98	1.99	3.62	1.86
	$\sum$ yield / ø H	4.93	0.85	2.80	2.49	3.99	1.87	1.69	2.01	5.63	1.81
	1	5.33	0.76	2.00	2.02	2.73	2.17	2.53	2.46	4.20	2.58
2007	2	0.80	0.83	1.73	1,76	1.00	2.16	1.60	2.40	1.13	2.46
	$\sum$ yield / ø H	6.13	0.80	3.73	1.89	3.73	2.17	4.13	2.43	5.33	2.52
	ø yield	5.53	-	3.27	-	3.86	-	2.91	-	5.48	-

 Table 2.
 Yields and diversity index (H) of grassland of experimental variants

Figure 1. Correlation analyze of yield to diversity index of grassland



Hay production can be creating above all species which produce lot of phytomass independently for plant diversity. This is noticeable on the variant BG with predominance Trifolium repens. Savadogo et al. (2007) investigated species richness and phytomass production on the burned and no burned pastures. They found out inverse relation between diversity and phytomass production. And the quantity of plant litter on the ground, in other words the old plant litter from previous years as well as fresh plant litter from the year under investigation, varied depending on the intensity of grazing and fire treatment. Bullock et al. (2007) shown that restoration of species-rich communities of conservation value can increase agriculturally relevant hay production and this effect is maintained over at several years. On the other hand Thompson et al. (2005) suggest that over time the species richness of community will by determined by the soil fertility: so productivity, determined by the soil fertility, will affect diversity rather than vice verse.

#### CONCLUSIONS

From the results gained from this experiment, looking at the yield and diversity index on the pasture at an altitude of 920 m a. s. l.; we can make the following observations:

- 1. Plant diversity affected production potential minimally on the pasture all year round using by beef cattle after restoration pasture in the first two years,
- 2. Reseeding of valuable species for fattening increased production potential of pasture as well as plant diversity on the pasture.

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#### ASSESMENT OF WILD GENETIC RESOURCES OF MEDICAGO SPECIES

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#### INTRODUCTION

In consequence of climatic changes the need for growing crops and varieties with higher tolerance to biotic and abiotic factors raises. This fact imposes superior demands on breeding programs and investigation of germplasm with specific traits and properties. In this context wild relatives of cultivated plants are important sources of genes with resistance to pests, pathogens and abiotic stresses. Wild germplasm grow in different environments with contrasted edaphic and climatic constraints; consequently, they are likely to display a broad range of tolerance to abiotic factors. These have direct applications in the extension of crop cultivation to new and eventually less favourable environments (Prosperi et al., 2006). The various cultivars of grasses and forages are indigenous to primary wild forms, e.g. Czech variety of crown vetch Eroza. Several studies indicated that wild genetic resources of *Medicago sativa*, *Trifolium pratense* and others forages are an immense reservoir of variability for breeding of this species in the future (Crochemore et al., 1998, Marshall et al., 2003).

The objectives of the study described here were to evaluate agronomic and some forage quality traits of wild *Medicago* species, to compare these wild populations to cultivated alfalfa varieties, and to increase the interest of wild forms in research and breeding of alfalfa.

#### MATERIAL AND METHODS

The field experiment was established in 2006 at the experimental station of SARC - Research Institute of Plant Production. Natural populations of *Medicago* species (*M. sativa, M. varia, M. romanica, M. falcata*), collected in various regions of Slovakia, Czech Republic, Poland, Kazakhstan and Ukraine were studied (Tab. 1). These germplasm were evaluated with control varieties Palava and Vanda under completely random block design, replicated four times. The plots were cut two times in the first and four times in the second years. At each harvest plant height, plant weight, stem number and regrowth intensity were observed. The leaf/ stem ratio, crude protein and mineral concentration were determined in the first production year by the second cut. For experiment analysis of variance was performed to determine variations and differences between genotypes.

#### **RESULTS AND DISCUSSION**

Differences between natural populations and cultivated controls were significant for all agronomic traits (P < 0.05). The mean values of evaluated variables for natural populations and cultivated controls are given in Table 1. Natural populations of *M. varia, M. falcata* and *M. romanica* showed poor agronomic performance in comparison with controls, mainly for plant height or plant weight. Nevertheless, a remarkable variability occurs within *Medicago sativa* populations and the best ones may challenge the controls. A variance analysis revealed significant differences between control varieties and populations SVNPIR01-217 and SVNPIR01-227 for plant weight stem number and regrowth intensity. The most of natural populations were characterised by higher number of stems per plant (from 22.77 to 32.37) in comparison to control varieties (Palava 23.48, Vanda 22.39).

The differences between populations and varieties were significant (P < 0.05) for leaf/ stem ratio, content of crude protein, phosphorus, potassium, calcium and magnesium (Tab. 2). Leaf/stem ratio ranged from 43.29 to 76.96 %, with 46.52, resp. 48.77 % for control varieties. The highest leaf/stem ratio showed populations of *M. romanica* UKRKRY98-288 and *M. falcata* SVKPOV96-40, SVKSIT971-10, what confirmed results from previous studies that genotypes with low forage yield are outstanding in the leaf/stem ratio (Drobná, 2006). Crude protein contents of natural populations were higher as compared to the control cultivars, the highest contents were found by populations with the highest leaf/ stem ratio. Regarding mineral concentration, the highest variability was found in content of potassium. The control varieties were bellow-averaged in concentration of all minerals. Assessment according to particular species showed, that populations and varieties of *Medicago sativa* had the lowest mineral contents.

#### CONCLUSIONS

Evaluation of agronomic and some forage quality traits of wild *Medicago* species showed good agronomic performance of *M. sativa* SVNPIR01-217 and SVNPIR01-227 and high crude protein and mineral contents of *M. romanica* UKRKRY98-288 and *M. falcata* SVKPOV96-40, SVKSIT971-10 populations. The results indicate that there is a potential for utilization of wild *Medicago* species populations in future research and breeding improvement.

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Variety/ population	Species	Origin	Plant height	Plant weight	Number of	Regrowth
			(mm)	$(g.plant^{-1})$	stems	intensity (mm)
Palava	M. sativa	CZE	766.55	214.63	23.48	413.75
Vanda	M. sativa	SVK	763.03	192.15	22.39	403.25
CZEPOD00-18	M. sativa	CZE	714.95	183.37	26.68	377.45
SVNPIR01-256	M. sativa	SVN	685.63	187.44	27.03	364.93
SVNPIR01-198	M. sativa	SVN	628.55	176.17	27.05	322.45
SVNPIR01-217	M. sativa	SVN	747.25	253.87	32.37	427.33
SVNPIR01-227	M. sativa	SVN	759.45	221.33	27.68	433.90
POLKIE99-8	M. varia	POL	647.5	193.85	30.82	336.40
SVKNTAT01-320	M. varia	SVK	500.05	131.53	29.97	270.83
SVKNTAT01-497	M. varia	SVK	529.93	114.74	26.50	205.45
UKRKRY98-288	M. romanica	UKR	243.53	25.22	10.68	87.55
SVNPIR01-83	M. falcata	SVN	472.95	77.89	22.77	215.50
KAZACH90-0124H	M. falcata	KAZ	632.03	121.37	21.19	302.78
KAZACH90-0140H	M. falcata	KAZ	583.80	158.79	28.12	293.28
SVKPOV96-40	M. falcata	SVK	318.25	47.22	19.73	102.45
SVKSIT971-10	M. falcata	SVK	310.00	50.54	20.04	97.78
Average			581.46	146.88	24.78	290.94
HSD (0,05)			42.55	35.93	4.69	27.39

Table 1. Agronomic traits of evaluated varieties and wild Medicago species

Table 2.	Leaf/stem ratio,	crude protein	n and mineral	contents of	evaluated	genotypes
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Variaty/ population	Leaf/stem	СР	Р	K	Ca	Mg
variety/population	ratio (%)	$(g.kg^{-1})$	$(g.kg^{-1})$	$(g.kg^{-1})$	$(g.kg^{-1})$	$(g.kg^{-1})$
Palava	46.52	214.07	2.87	11.69	16.79	3.29
Vanda	48.77	200.01	3.00	12.00	16.14	3.11
CZEPOD00-18	46.65	197.19	2.73	9.87	14.81	3.00
SVNPIR01-256	49.97	211.88	2.99	9.26	17.32	3.12
SVNPIR01-198	43.29	218.76	3.13	12.60	17.93	3.53
SVNPIR01-217	46.49	223.75	2.87	9.87	16.55	3.06
SVNPIR01-227	47.45	202.19	2.82	11.28	17.67	3.20
POLKIE99-8	51.52	212.82	3.06	11.51	16.88	3.06
SVKNTAT01-320	54.32	230.63	3.39	15.10	16.85	3.52
SVKNTAT01-497	59.41	239.07	3.31	12.47	16.05	3.09
UKRKRY98-288	76.96	241.57	3.31	11.91	22.74	4.14
SVNPIR01-83	58.03	213.75	3.32	12.98	15.32	3.69
KAZACH90-0124 H	50.44	215.63	3.23	11.22	15.70	3.17
KAZACH90-0140 H	52.19	225.94	3.24	10.95	15.95	3.37
SVKPOV96-40	72.80	238.13	3.51	14.56	18.68	3.94
SVKSIT97-110	75.02	251.25	3.44	13.90	18.29	3.83
Priemer	54.99	221.04	3.14	11.95	17.10	3.38
HSD (0,05)	15.56	47.63	0.63	3.35	4.21	0.87

#### **SUMMARY**

In a field experiment established in 2006 at the experimental station of SARC - Research Institute of Plant Production the differences in agronomic performance, leaf/ stem ratio, crude protein and mineral concentration between wild populations of Medicago species (M. sativa, M. varia, M. romanica, M. falcata) and alfalfa varieties (M. sativa) were investigated. The differences between natural populations and cultivated varieties were significant for all variables. Natural populations of M. varia, M. falcata and M. romanica showed poor agronomic performance in comparison with controls, but they were characterised by the highest leaf/stem ratio, crude protein and mineral contents. Remarkable variability in agronomic traits occurs within Medicago sativa populations; the most of them exceeded control varieties, mainly genotypes SVNPIR01-217 and SVNPIR01-227. The populations and varieties of M. sativa had lower leaf/stem ratio, crude protein and mineral contents as compared to other Medicago species.

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# CONTROL OF FERMENTATION PROCESS, MICROBIOLOGY AND HYGIENIC QUALITY OF CONSERVED FEEDS
# DESCRIPTION OF SIGNIFICANT INFLUENCING FACTORS ON BUTYRIC ACID CONTENT OF GRASS SILAGE BY MEANS OF A MULTI-FACTORIAL LINEAR MODEL

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#### INTRODUCTION

The content of butyric acid is an important indicator for the quality of fermentation of grass silages, therefore detailed knowledge of how various influencing factors (dry matter (DM), crude fibre (XF), crude protein (XP), crude ash (XA), type of forage, growth, silage system, particle length, silage additives etc.) have an impact on the butyric acid content is of great importance in consulting and agricultural practice.

# MATERIAL AND METHODS

Within the framework of farm mentoring, a nationwide silage project (Resch, 2008) with consistent and comprehensive samplings, chemical analyses (examination using standardized methods in the Laboratory Rosenau of the agricultural chamber of Lower Austria) and interviews concerning silage management on dairy farms, was conducted in the years 2003, 2005 and 2007 in order to build up a database, both up-to-date and statistically evaluable, for mentoring. By means of a General Linear Model (GLM) categorical and quantitative effects of 1,429 Austrian grass silages were statistically analysed concerning the parameter butyric acid at the LFZ Raumberg-Gumpenstein. Analysis by means of GLM modelling enables the coordination and lock-out of fix effects and regression variables, for this reason this method is well suited for the evaluation of multiple influences on a dependent variable. All calculations were carried out with the software package Statgrafics, Version 5.1.

### **RESULTS AND DISCUSSION**

The GLM data-examination of 1,429 grass silages of Austrian farms showed an explanation of variance ( $r^2$ ) of 40.4 % and a standard error of the model for butyric acid in the amount of +/- 7.1 g/kg DM for the dependent variable butyric acid content [g/kg DM].

As can bee seen in table 1, DM content [g/kg FM] exerts a highly significant and also the strongest influence (P-Value 0.000, F-Ratio 427.8) on the content of butyric acid. At a constant content of XP (148.1 g/kg DM), XF (265.5 g/kg DM) and XA (103.4 g/kg DM) as well as the lock-out of the factors type of forage, growth, silage system, particle length and silage additives, content of butyric acid decreases by about 0.06 g/kg DM, when dry matter increases by 1 g/kg FM. There is also highly significant influence of the regression variables XF and XA on the content of butyric acid and a positive correlation with the content of butyric acid. The proportion of butyric acid content increases by 0.06 g/kg DM (XF-effect) and by 0.04 g/kg DM (XA-effect), when the content of XF resp. XA are increased by 1 g/kg DM. Using coordination and lock-out of the mentioned factors, also the XP content exerts highly significant influence on the content of butyric acid. An increase of the content of XP by 1 g/kg DM causes a decrease in the butyric acid content by 0.04 g/kg DM.

Source	F-ratio	P-value*	r-squared (r <sup>2</sup> )	Res. standard error
<b>Categorical factors</b>			40.4	7.1
Type of forage	5.5	0.0042		
Growth	11.4	0.0000		
Silage system	11.7	0.0000		
Particle length	18.6	0.0000		
Silage additives	21.4	0.0000		
Quantitative factors			Average of reg. variable	Regression coefficient
Dry matter	427.8	0.0000	382.4	-0.0566
Crude protein	14.2	0.0002	148.1	-0.0429
Crude fibre	55.2	0.0000	265.5	0.0638
Crude ash	20.7	0.0000	103.4	0.0400

 Table 1. Description of significant influencing factors on butyric acid content of grass silage by means of a multi-factorial linear model (data source: Austrian silage project 2003/05/07)

Apart from the clear influences of the above mentioned quantitative factors, highly significant influences by fix effects were verified by means of the GLM model. For instance, content of butyric acid can be decreased to a level of 6.3 g/kg DM using lactic acid bacteria and coordination of DM, XP, XF and XA, whereas untreated silage shows a butyric acid content of 9,5 g/kg DM (see Table 2).

Particle length of the harvested forage also has a highly significant influence on the butyric acid content. Grass with a length not exceeding 3 cm contained at constancy of quantitative factors less than 4 g butyric acid/kg DM while long

grass that was not cut or chaffed contained 11.6 g butyric acid/kg DM. Comparison of various silage systems shows a highly significant influence on the content of butyric acid. Big bales had the lowest contents of butyric acid (6.5 g/kg DM), bunker silos showed average contents of 9.9 g butyric acid/kg and silage heaps reached top contents of 12.2 g butyric acid/kg DM.

Analyses proved a highly significant influence of the factor growth regarding the level of butyric acid content. The first growth contained at constancy of quantitative variables explicitly higher values of butyric acid (10.9 g/kg DM) than the following cuts (8.1 to 8.4 g/kg DM). In the GLM model, type of forage was also tested regarding its effect on the content of butyric acid. Forage of permanent meadows shows significantly higher contents of butyric acid at a level of 9.9 g/kg DM than forage of intensive ley farming areas (red clover, red clover - grass, lucerne, lucerne-grass, etc.) at a level of 8.4 g butyric acid/kg DM.

Categories	Count	Mean	Stnd. error	Confidence interval 95 %	
				Lower limit	Upper limit
Grand mean	1429	8.9	0.65	7.7	10.2
Forage					
Permanent grassland (pg)	982	9.9	0.66	8.6	11.2
Ley faming (lf)	310	8.4	0.73	7.0	9.8
Mixture of pg/lf	137	8.6	0.85	6.9	10.2
Growth					
First	1041	10.9	0.60	9.8	12.1
Second	190	8.3	0.77	6.8	9.9
Third	44	8.1	1.22	5.7	10.5
Combination of two or	154	8.4	0.82	5.8	10.0
more	134	0.4	0.82	5.8	10.0
Silage system					
bunter silo	1004	9.9	0.58	8.7	11.0
Silage heap	28	12.2	1.45	9.3	15.0
Monolith silo	65	7.2	1.01	5.2	9.2
Big bales	332	6.5	0.71	5.1	7.9
Particle length					
Less 3 cm	114	3.8	0.94	1.9	5.6
3,1 to 6 cm	626	9.2	0.69	7.8	10.5
6,1 to 10 cm	385	10.2	0.71	8.8	11.6
10,1 to 20 cm	222	10.0	0.81	8.4	11.6
Long grass	82	11.6	1.04	9.5	13.6
Silage additives					
Without additives	1135	9.5	0.56	8.4	10.6
Acid or salts	44	11.0	1.19	8.7	13.3
Microbiogical inoculants	250	6.3	0.71	4.9	7.7

 Table 2.
 Statistical values of categorical factors influencing the content of butyric acid of grass silage (data source: Austrian silage project 2003/05/07)

# CONCLUSIONS

Statistical data analysis of 1,429 Austrian grass silages using the GLM model shows that 5 categorical (silage additives, particle length, silage system, growth, type of forage) and 4 quantitative variables (dry matter, crude fibre, crude ash, crude protein) exert a highly significant influence on the content of butyric acid [g/kg DM]. The multiple model explains 40.4 % (r<sup>2</sup>) of the variance of the butyric acid content.

In Austria, tolerance limit for butyric acid in grass silage was set at a level of < 3 g/kg DM. The current situation of silage quality in Austria is in need of improvement as there are too many grass silages showing butyric acid contents above the tolerance limit. The illustrated GLM model facilitates a multiple validation of reliable recommendations in order to reduce the content of butyric acid of grass silages in agricultural practice and is therefore of great importance for official consulting.

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# ENSILING OF INTERCROPS WITH LEGUMES

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# ABSTRACT

A more sustainable agriculture can be achieved by incorporating legumes in the crop rotation. Harvesting and ensiling of these crops, however, requires special attention. In the period 2003-2007, several trials were conducted at the experimental farm of the University College Ghent (Belgium) with different legumes: intercrop barley-pea, intercrop grass-white clover, intercrop grass-clover-pea, intercrop alfalfa-pea, intercrop grass-alfalfa and whole crop of lupins. Characteristics of intercrop yield under Flemish conditions and the possibilities of ensiling with or without inoculants were examined. Intercrops with legumes harvested as whole crop gave promising results, however feeding value is sometimes too low to be interesting for the practice. It is advisable to use an inoculant in order to secure a good silage quality.

# **INTRODUCTION**

Intercropping systems have been largely researched and they do have a lot of potential. Intercropping grain legumes with cereals produces maximal biomass with an optimal use of light, water and nutrients (Hauggaard–Nielsen, 2006). Intercropping systems do also have a better weed control with reduced use of herbicides (Hauggaard-Nielsen, 2006) and a higher Land Equivalent Ratio (LER) (Anil et al., 1998). The LER is defined as: "the relative surface that monocrops need in order to produce the yield of intercrops; a value higher than 1 indicates a more productive system (Willey, 1979). According to Willey (1979), the wheat–faba bean LER was 1.4, and the maize-soybeans LER 1.18. Nitrogen fixation from the air by the legume component makes nitrogen fertilizer redundant (Jensen, 1996). Other positive aspects are: a faster soil covering and a better rooting of the plants are an advantage in case of erosion problems (Anil et al., 1998; Hauggaard-Nielsen, 2006). These interesting benefits, local interest of farmers and encouragement of the Flemish community (with support of the European community) lead to a lot of research on legumes in the period 2003-2007. Since three years, an additional research program on *Lupinus spp*. is going on. Characteristics of yield, nutritional aspects and ensiling properties were examined. During this research the use of silage additives (inoculants) was also studied, as the benefits on silage quality are well known (Driehuis et al., 2001).

### MATERIALS AND METHODS

Several intercrops with several varieties were studied: barley-pea, pea-English ryegrass-clover, faba bean-summer wheat and pea-alfalfa. Field trials were conducted on a sandy loam soil in Bottelare (6), a sandy soil in Bocholt (2) and a clay soil in Koksijde (2), in a randomised block design with 3 replicates. The whole crop harvest was done with a Haldrup plot harvester. The yield was determined and subsamples were taken. The subsamples were separated into the morphological components. The components were dried and the plant composition was determined on a dry matter (DM) basis. Determination of the cellulose digestibility (De Boever, 1999) and Weende analysis were carried out on the different pure components and re-calculated on the proportions in the mixture. In 2005, several varieties of lupins (Lupinus luteus, L. angustifolius and L. albus) were studied in a field trial in Bottelare and harvested as a whole crop. The chopped forage was randomly separated into 15 kg lots and treated with the appropriate solutions (control solution - additive solution). All solutions were vaporised as a fine mist over the crops in an amount of 100 ml per 10 kg of fresh material, with thorough mixing in-between. PVC microsilos (content: 2.75 l), all equipped with an airlock, were used. An ensiling period of at least two months was taken into account. Four inoculants were used : (1) Pioneer 1188 (Pioneer Hi-Bred, Johnston): Lactobacillus plantarum 2.4 x  $10^{10}$  CFU/g + Enterococcus faecium 0.6 x  $10^{10}$  CFU/g; dosage 3.3 x 10<sup>-3</sup> g/kg FW, (2) Bonsilage Plus (Schaumann, Pinneberg): Lactobacillus plantarum 3.5 x 10<sup>10</sup> CFU/g + Pediococcus pentosaceus 0.5 x 10<sup>10</sup>/g + Lactobacillus buchneri 6.0 x 10<sup>10</sup>/g; dosage 1x10<sup>-3</sup> g/kg FW, (3) Ecosyl (Ecosyl, Yorkshire): Lactobacillus plantarum 8.4 x 10<sup>10</sup>/g, dosage 3 x 10<sup>-3</sup>g/kg FW and (4) a pure strain of Lactobacillus plantarum, dosage  $3 \times 10^{5}$ /g FW. The silage additives were prepared by adding plain water.

# **RESULTS AND DISCUSSION**

**Characteristics of intercrop yield**. The results of the intercrop barley-pea<sup>1</sup> varied over the years 2003-2007 and soil types. In Bottelare, yield varied from 6.8 to 12.6 ton DM/ha, with a dry matter content also varying from 267 to 445 g/kg (the latter depending on variety combination and harvesting date). In a clay soil, yields varied from 16.6-18.2 ton DM/ha in 2005 to 9.3-11.4 ton DM/ha in 2006. Mean CP (120 g/kg DM) varied from 93 g/kg to 142 g/kg of DM, depending on pea content. The energy value was estimated about 750-850 VEM<sup>2</sup>. The intercrop of pea-English ryegrass-clover<sup>3</sup> gave promising results in 2006 with yields varying from 8.2 to 10.9 ton DM (mean DM: 304 g/kg) in Bottelare, 7.8-9.7 ton DM/ha (mean DM: 350 g/kg) in Koksijde, to 6.7-7.2 ton DM/ha (mean DM: 450 g/kg) in Bocholt.

<sup>&</sup>lt;sup>1</sup> Example of seed mixture: 160kg/ha of pea+40kg/ha of barley

<sup>&</sup>lt;sup>2</sup> VEM : net energy for lactation, comparison: it takes 442VEM for 1 kg of standardized milk

<sup>&</sup>lt;sup>3</sup> Example of seed mixture: 160kg/ha of pea + 35kg/ha English ryegrass+clover

Mean CP content varied from 135 g/kg - 164 g/kg and the energy value varied between 850-950 VEM. The advantage of this intercrop is a good development of the clover grass turf and the possibility to harvest up to 4.5 ton DM/ha during late summer and autumn in 2006. The intercrop of faba beans-summerwheat<sup>4</sup> gave also interesting results. Yield (whole crop) varied from 3.4 ton/ha (Bocholt, sandy soil, 2005) over 9 ton DM/ha (Bottelare, 2006) to 15.6-18.6 ton DM/ha (Koksijde, 2005 and 2006). CP content varied from 120 g/kg to 154 g/kg on DM basis. The energy value however was low and varied between 785-845 VEM. Finally the intercrop pea-alfalfa was examined in 2007 and gave a DM yield of 7.6 ton DM/ha (DM: 229 g/kg, harvested too early!), with a CP content of 169 g/kg and an energy value of 844 VEM. The aftergrass gave an additional yield of 2.3 ton DM/ha.

*Lupinus luteus* had a yield of 10.7 ton DM/ha (DM: 207 g/kg, CP: 15.7% of DM), *L. angustifolius* 10.8-11.3 ton DM/ha (DM: 360 g/kg, mean CP: 173 g/kg of DM) and *L. albus* 6.9-8.5 ton DM/ha (DM: 230 g/kg; mean CP: 159 g/kg of DM). The digestibility (*in vitro* by cellulase) was deceiving because of high crude fibre contents (up to 285 g/kg). **Ensiling results.** The results are displayed in Table 1. Without a silage additive, legume silages often showed moderate results, illustrated by a high ammonia fraction and the presence of butyric acid. Addition of inoculants of LAB resulted mostly in a significantly lower pH, ammonia fraction and butyric acid content.

year	intercrop/crop	silage additive	DM (g/kg DM)	Hq	NH3 fraction (% of N)	butyric acid (% FW)
2003	pea-barley	-	387 a <sup>(1)</sup>	5.4a	11.9a	1.4a
		Pioneer 1188	411b	4.1b	6.6b	06
		Ecosyl	413b	4.1b	6.2b	0.1b
	pea-grass	-	363	5,3	9,8	1,6
2005	Lupinus luteus	-	264	4.1 a	7.8a	(2)
	Lupinus luteus	Bonsilage Plus	286	3.7ъ	6.3a	(2)
	Lupinus angustifolius	-	309	4,5	8,9	0,25
	Lupinus albus	-	282	4,7	12,2	0,9
	pea-barley	-	343a	4.68a	7.5a	0.6a
		Pioneer 1188	390ъ	4.17b	65	0.1b
	pea-grass	-	550a	4.7a	3.6a	0.4a
		Pioneer 1188	545b	4.0ъ	2.5b	0.016
2006	pea-grass-clover variety	-	292a	5.3a	12.8a	1.3a
	pea-grass-clover variety	Pioneer 1188	302a	4.6b	7.1b	0.7a
	faba bean-summerwheat	-	462a	4.5a	5.3a	0.2a
	faba bean-summerwheat	Pioneer 1188	483b	4.1b	3.3ъ	0.2a
2007	pea-alfalfa	-	286a	4.7a	10.3a	3.3a
	pea-alfalfa	L. plantarum	303a	4.5b	8.6b	2.0a
<sup>(1)</sup> values foll	owed by different letters are signific	antly different (P<0.05)				
(2) concentrat	ion too low to take into account					

Table 1. Fe	rmentation c	haracteristics	of who	le crop	silages
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### CONCLUSION

Intercrops with legumes harvested as a whole crop gave promising results under Flemish conditions, however the feeding value is sometimes too low to be interesting for the practice. It is advisable to use an inoculant in order to secure a good silage quality. Ensiling of *Lupinus* spp. as a whole crop gave less promising results.

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 $<sup>^{4}</sup>$  Example of seed mixture: 87kg/ha of summer wheat + 104kg of faba beans (both 50% of the normal seed density as single crop

# CONTROL OF FERMENTATION PROCESS BY CHEMICAL ADDITIVES AT ENSILAGING OF LUCERNE WITH LOW CONTENT OF DRY MATTER

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Forage crops of proteinic character are deficient in easily fermentable saccharides that are necessary for intensive production of lactic acid in the process of ensilaging (Biro et al., 2008). High buffer capacity is another factor, which makes ensilaging of lucerne more difficult. If is such feed ensilaged at low content of dry matter, there are created conditions for onset and quick development of undesirable microflora. The consequence of it is often undesirable course of fermentation process, which is attended with high production of volatile fatty acids, high losses of dry matter and increased proteolysis.

The objective of this work was to detect the effect of application of chemical ensilaging additives on quality of fermentation process in lucerne at low level of dry matter.

# MATERIAL AND METHODS

Lucerne stand was cut at the stage of budding and wilted subsequently. Lucerne with content 23 and 27 % dry matter was chopped and ensilaged in laboratory conditions. We created three variants of silages on both levels of dry matter in feed. The first variant was the control silage, which was not treated with ensilaging preparation. The other two variants were treated with chemical additives:

- >  $T_1$  contained 24.4 % natrium nitride, and 16.3 % hexamethylentetramine, applied amount was 3.5 litres per tonne of ensilaged lucerne.
- T<sub>2</sub> contained 42.5 % formic acid, 30.3 % ammonium formate, and 10.0 % propionic acid, applied amount was 4 litres per tonne of ensilaged matter.

Each treatment consisted of six replicates. The laboratory silos (1.7 l) were placed in a dark room at  $21 \pm 1$  <sup>0</sup>C. Silage losses of dry matter were determined regularly at 21-days intervals. After 105 days of silage fermentation was the experiment finished. All nutrition and fermentation parameters were determined according to actual norm (Výnos MP SR, 2004) in samples of lucerne original matter, and silages. Content of WSC was determined by the Luff-Schoorl titration method, ph electrometrically. Lactic acid, acetic acid, propionic acid, butyric acid, valeric acid and capronic acid levels were determined by gas chromatography, alcohol and NH<sub>3</sub> by the micro-diffusion method according to Conway. Total volatile fatty acids, total acids were calculated out of the determined concentrations. Energy concentrations in the silages were calculated as mentioned by Sommer et al. (1994). The results were statistically evaluated by one-factorial analysis of variance, and compared by Student-t test.

### **RESULTS AND DISCUSSION**

The climatic conditions often do not enable to provide sufficient wilting and therefore it is necessary to ensilage the feed at low level of dry matter. Lucerne in our experiment contained 20 % dry matter at harvest. Because of cold and cloudy weather was the course of wilting very slow. After 24 hrs of wilting was content of dry matter 23 % and after 48 hrs of wilting 27 %. Table 1 contains parameters of nutritive value in feed after cutting and before ensilaging. We observed decrease in content of crude protein, total and reducing sugars and fat in lucerne matter owing to wilting. Marked decrease occurred in nitrogen-free extract after 48 hrs of wilting. On the contrary, we found increase in concentrations in crude fibre, ADF, NDF, hemicelluloses and ash compared to fresh matter.

Wilting	Dry matter	Organic matter	Crude protein	Crude fibre	ADF	NDF	Nitrogen-free extract	Sugar total	Sugar reduced	Fat	Ash
	(g)		(g.kg <sup>-1</sup> dry matter)								
0 hour	202.5	899.7	223.1	229.8	265.0	333.7	412.6	74.0	49.6	34.2	100.3
24 hrs	233.1	894.3	216.1	239.3	622.2	392.3	411.2	61.1	45.0	27.7	105.7
48 hrs	270.1	893.8	212.4	289.6	342.5	414.0	364.5	51.2	36.9	27.3	106.1

Table 1. Chemical composition of original lucerne matter before and after wilting

Results of fermentation process in lucerne silage at both levels of dry matter content are in table 2. Silages produced without additives showed very high losses in dry matter. Content of dry matter in control variants of silages decreased to 174 and 217 g compared to the ensilaged matter. High moisture supported the activity of undesirable microorganisms during fermentation. Intensive butyric fermentation took place in control silages instead of lactic fermentation. The result of ensilaging was feed, which was not suitable for feeding the animals.

The fermentation process stabilized by applying the chemical preparation on the basis of salts of acids (T1) and on the basis of organic acids (T2), and the expected lactic fermentation took place in silages. The result was silage of very good quality on both levels of dry matter content in lucerne.

Application of chemical additives increased statistically highly significantly the content of lactic acid and decreased the content of acetic, propionic and butyric acids in silages. It reflected positively on pH level, which varied from 4.17 to 4.44 in treated silages. Highly significant was the decrease in content of alcohol and ammonium nitrogen expressed in % out of total nitrogen. In silages produced from lucerne with 23 % content of dry matter was proteolysis almost

three times higher in the control silage than in the treated silages, and in silage from lucerne with 27 % content of dry matter were these differences greater more than two-fold.

High proteolysis in untreated silages corresponds with a number of works. According to Owens et al (2002) pH over 5 is optimum for the course of proteolysis in lucerne silages.

The overall extent of the proteolysis is pH dependent because pH influences the activity and stability of red clover and lucerne proteases. Quick reduction of pH during ensiling is advantageous to reduce proteolysis of the ensiled legumes (Finley et al., 1980; McKersie, 1985; Jones et al., 1995).

Differences in fermentation process of silages treated with preparation T1 and T2 were not statistically significant. Fermentation process was better in treated silages, which were made from lucerne containing 27 % dry matter compared to silages produced of lucerne with 23 % dry matter. They contained higher concentrations of lactic acid, lower concentrations of butyric acid, and the level of proteolysis was slightly lower also.

ad				Losses				Ac	ids			Alcohol	NH <sub>3</sub> -N
tin			DM	DM	nH	Lactic	Acetic	Propion.	Butyric	Valeric	Capron.	Alcohol	of total
Wil			(g)	(%)	pii			(g.k	g <sup>-1</sup> dry matte	r)			N (%)
	П	$\overline{x}$	174.70**	26.80**	6.12**	12.33**	19.68	11.89 <sup>a**</sup>	85.70**	3.83**	0.06	8.54**	27.03**
0	U	S	5.09	2.17	0.51	3.90	3.33	7.43	12.33	1.08	0.01	0.84	1.42
unou	T <sub>1</sub>	$\overline{x}$	203.94	12.73	4.44	68.78	18.57	$0.92^{b^{**}}$	1.42	0.41	0.05	2.24	9.93
24 h	• 1	S	0.93	0.41	0.09	5.69	0.62	0.40	0.98	0.25	0.01	0.15	0.51
	T <sub>2</sub>	$\overline{x}$	201.95	13.50	4.17	70.19	11.70**	2.49	2.27	0.58	0.05	1.45	9.95
	12	S	3.31	1.37	0.05	2.38	2.14	0.22	1.22	0.13	0.01	0.13	1.05
	U	$\overline{x}$	217.00**	21.24**	5.34**	32.01**	34.13 <sup>a**</sup>	$2.04^{a^{**}}$	28.53**	1.00	0.29	8.87**	15.73**
s	Ũ	s	4.42	1.61	0.22	13.79	3.19	0.94	4.29	0.52	0.27	0.66	1.21
nour	T <sub>1</sub>	$\overline{x}$	241.62	10.75	4.40	78.17	19.40 <sup>b**</sup>	$0.42^{b^{**}}$	0.24	0.40	0.64	2.07	7.06
48 b	- 1	S	1.25	0.45	0.05	4.50	0.61	0.11	0.15	0.33	0.41	0.14	1.00
	T <sub>2</sub>	$\overline{x}$	240.00	11.36	4.25	81.25	12.49	1.13	0.07	0.45	0.32	1.68	6.95
	- 2	S	2.43	0.90	0.01	5.58	1.00	0.10	0.04	0.18	0.22	0.33	0.30

**Table 2.** Fermentation parameters in lucerne silage

n = 6, U – untreated, T1 - chemical additive, T2 - chemical additive,

Statistical significance of difference - \* P < 0.05 \*\* P < 0.01 the statistics is performed for the individual trials only.

#### CONCLUSIONS

Ensilaging of lucerne with low content of dry matter without silage additives caused high losses of dry matter, which occurred during the fermentation process; namely 26.8 and 21.2 %. Butyric fermentation took place instead of lactic fermentation and it caused higher pH 6.12 and 5.34, high concentration of butyric acid (85.7 and 28.5 g.kg<sup>-1</sup> DM) and volatile fatty acids (120.4 and 66.0 g.kg<sup>-1</sup> DM). Undesirable course of fermentation reflected also at the level of proteolysis, which represented 27.0 and 15.7 % NH<sub>3</sub> -N of total nitrogen. Silages made from lucerne with 23 and 27 % dry matter content without the use of ensilaging additives were not suitable for feeding the animals. The worse parameters were in silage that was produced of lucerne with 23 % dry matter content.

Application of chemical additives on the basis of organic acids as well as on the basis of salts of acids improved and stabilized the course of fermentation process and the quality of produced silages. Chemical ensilaging additives statistically highly significantly decreased pH, increased content of lactic acid, and decreased content of acetic, propionic and butyric acids. Course of fermentation influenced positively also the level of proteolysis.

On the basis of obtained results we consider the application of chemical additives in ensilaging of lucerne with low content of dry matter for an inevitable part of technological process at ensilaging.

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# APPLICATION EFFECT OF VARIOUS CHEMICAL ADDITIVES ON FERMENTATION QUALITY OF RED CLOVER SILAGE

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#### INTRODUCTION

Red clover is a frequent crop in the north of Slovakia. In spite of good production results, the conservation of this crop is rather difficult since it is negatively influenced by frequent rains occurring during the period of the first and the last harvest. The aim of this investigation was to state possibilities of using acid- and salt-based chemical additives in the conservation of red clover with low amount of dry matter in ensilaged mass.

# MATERIALS AND METHODS

The experiments were run with red clover from the third harvest (tab. 1) with high amount of crude protein, low amount of dry matter, roughage and sugar. Cut forage was preserved 24 hours after harvesting. Fermentation was observed in untreated control (K) and in experimental variants treated with acid- and salt-based chemical additives.

We used liquid preparative ( $P_1$ ) based on formic acid and propionic acid at a volume of 5 litres per 1 tonne of ensilaged matter, and pulverized preparative ( $P_2$ ) based on hexamethylentetraamine, sodium nitrite and calcium formiate at a volume of 3 kg per 1 tonne of ensilaged matter. The experiment took place in laboratorial silos with a volume of 1.7 litres. Filled silos were placed in a dark room at 25<sup>o</sup> C. The level of nutrients and basic indices of the fermentation process were determined after 151 days.

#### **RESULTS AND DISCUSSION**

The fermentation process of untreated red clover was bad. Forage had high pH and NH<sub>3</sub>-N on total N. The amount and ratio of acids was unsuitable. The application of acid- and salt-based chemical additives showed very positive effects on the fermentation process. Differences were significant and highly significant (tab. 2). Both silages showed good pH and NH<sub>3</sub>-N on the total N, as well as amount of acids. More positive amount of acids as well as their ratio was stated in silages treated with preparative P<sub>2</sub>. The amount of butyric acid in P<sub>2</sub> silages was significantly lower than in P<sub>1</sub> silages. For this reason it can be stated that fermentation in P<sub>2</sub> silages is better. The positive effect of silage additives' application showed in reduction of DM losses in silage and its nutrition structure.

Also in this case we realised high significant differences between untreated and treated silages. The differences between treated silages were unsignificant.

Based on these realised results it can be stated that addition of the chemical additives showed very positive upon the fermentation process of made silages. Improved course of a fermentation process in treated silages showed positive effects on the reduction of mass losses of DM and their nutrition structure.

Hetta (1999) and Fichan (2002) obtained similar results, as we did, during the conservation of clover and grass crops. They mention at monitoring of the influence of DM and lactic acid bacteria on the fermentation of red clover also similar results; however the mass they conserved contained higher sugar level than in our observation. In spite of that there are higher levels of lactic acid and also NH<sub>3</sub>-N on total N realised in our experiment. This author also mentions in treated red crop silage decrease in pH, acetic acid, and NH<sub>3</sub>-N on total N. On the other hand Winters et al. (2002) observed the influence of biological preparative on the fermentation of red clover with decreased sugar levels; in comparison with our values mention worse results of the fermentation process in red clover silage. In agreement with our previous report (Gallo et al. 2001) we found better results of the red clover conservation with chemical additives than in untreated silages.

Pessi and Nousiainen (1999) found by the grass silage production with low volume of DM and by using chemical additive lower pH and better fermentation process as in untreated control. In similar experiment O'Kelly (1999) mentions that application of biological additive showed improvement in the fermentation process parameters of made silage in comparison with untreated control, but chemically treated silages showed not only improvement in fermentation process but also in digestibility of organic mass and higher volume of proteins, which was also proved in our observation.

As in our previous reports (Gallo et al. 2001, and Gallo et al. 2001) also in this one was confirmed the positive effect of using ensilaging preparatives in the conservation of red clover with decreased DM content.

# CONCLUSION

The treatment of red clover by chemical preparatives showed in decreased loss of DM and improvement of fermentation process in silages. Because of lower butyric acid level showed the silages treated by preparatives based on hexamethylentertaamine and sodium nitrite better fermentation than silages treated by acid-based preparatives.

Wilting	Dry matter	OM	Crude protein	Crude fibre	Nitrogen- free extract	Sugar total	Fat	Ash	ME	NEL
	(g)				$(g.kg^{-1}DM)$				(MJ.kg	<sup>1</sup> DM)
24 hours	180.7	887.2	238.4	209.4	409.3	36.0	30.1	112.8	9,9	5,8

 Table 1.
 Red clover - fresh matter

Table 2.	Nutrient com	position and	parameters	of the	fermentation	in red	clover silage
		p 0 0					

n=	= 6	DM	Losses	СР	CF	Ash	NEL	nН	Acid lactic	Acid acetic	Acid butyric	NH3-N	
11 -	-0	(g)	%	(	g.kg <sup>-1</sup> DI	(N	MJ.kg <sup>-1</sup> DM	pm	(g.kg <sup>-1</sup> DM)		M)	N in %	
II	$\frac{1}{x}$	156.7	15.1	261.0	181.0	117.2	5.82	5.2	22.1	36.0	11.4	13.8	
0	s	4.9	2.6	5.2	6.0	4.7	0.03	0.2	7.3	2.5	3.4	3.7	
D	$\frac{1}{x}$	178.6	2.9	270.8	152.7	105.4	5.88	4.2	40.5	22.9	7.6	7.6	
11	s	3.9	1.2	8.3	6.3	2.1	0.02	0.1	10.5	1.7	5.4	0.7	
D	$\frac{1}{x}$	185.1	1.5	268.0	154.1	116.4	5.87	4.2	59.2	19.5	0.8	8,0	
12	s	1.5	0.6	5.2	6.7	5.8	0.04	0.04	13.5	1.5	0.1	0.6	
*											P <sub>1</sub> :P <sub>2</sub>		
**		U:P <sub>1</sub> ,P 2	U:P <sub>1</sub> ,P 2		U:P <sub>1</sub> ,P 2	P <sub>1</sub> :U,P 2	$U:P_{1}P_{2}$	U:P <sub>1</sub> ,P 2	U:P <sub>1</sub> ,P 2	U:P <sub>1</sub> ,P 2	U:P <sub>2</sub>	U:P <sub>1</sub> ,P <sub>2</sub>	

U – untreated, DM – Dry matter, CP – crude protein, CF – crude fibre, NEL – Netto energy of lactation Statistical significance of differences \* P<0.05, \*\* P<0.01

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### **SUMMARY**

The application effect of chemical preparatives on fermentation process and nutrition structure of clover silages was observed in this experiment with red clover. The application of silage additives reduced fermentation losses, improved indicators of made silage fermentation process and their nutrition structure. Treated silages in comparison with untreated silages showed lower pH, higher volume of lactic acid, lower volume of acetic and butyric acid and  $NH_3$ - N on total N. The silage treated by salt-based additive had lower volume of butyric acid than silage treated by acid-based additive.

# FERMENTATION CHARACTERISTICS AND AEROBIC STABILITY OF WHOLE-PLANT MAIZE SILAGE WHEN ADDING AN INOCULANT

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#### ABSTRACT

Whole-plant maize was harvested at the waxy stage from three different fields and conserved in laboratory silos. The whole-crop maize was ensiled without an additive (control) or treated with a bacterial mixture of Pediococcus acidilactici, Lactobacillus plantarum Milab 393, Lactococcus lactis SR 3.54 and Enterococcus faecium M74® applied at 5.5x105 CFU/g of ensiled maize. Three replications were made for each treatment from each field and the silos were opened after 2 days and after 49 days of ensilage. According to DLG procedures for approval in category 2, silage was aerated on days 28 and 42. Treated silage had a better fermentation compared with untreated silage. Treated silage had lower ammonia-N and consequently lower proteolysis, less butyric acid, lower DM losses and slightly better aerobic stability. Whole-crop maize silage treated with the additive had a higher (P<0.05) lactic acid concentration and a lower (P<0.05) ethanol concentration compared with control silage. The inoculation increased (P<0.05) the number of LAB and reduced (P<0.05) enterobacteriaceae count and numerically decreased yeast count relative to the control treatment. Mould count was unaffected by treatment. Control silages deteriorated at the fastest rate. At 18-36 h of exposure to air untreated silage had temperature significantly higher compared with inoculated silage.

# INTRODUCTION

Whole-plant maize is a popular source of silage for ruminant animals in Europe because of its high digestibility and energy content and high yields at a single harvest. Classical microbial inoculants containing homolactic lactic acid bacteria (e.g., *Lactobacillus plantarum*) are often added to silage because they produce large quantities of lactic acid very rapidly, which lowers the pH of silage (Muck and Kung, 1997). ). However, most homolactic lactis acid bacteria have little or no effect on aerobic stability as lactic acid alone is not particularly anti-fungal. While heterofermentative lactis acid bacteria do not have as good pH-reducing effects and result in higher DM losses compared with homofermentative lactis acid bacteria, they have a relatively good effect against fungal growth. More recently, newly identified homofermentative lactis acid bacteria with anti-fungal properties have been identified. The combination of efficient fermentation and antifungal properties is consequently superior to the effect of the heterofermentative lactic acid bacteria. The objective of the present study was to examine the possibility to improve fermentation and aerobic stability during maize (*Zea mays*) silage preparation with *Lactobacillus plantarum* Milab 393, which has proven antifungal properties in combination with *Pediococcus acidilactici, Lactococcus lactis* SR 3.54 and *Enterococcus faecium* M74® compared with untreated silage in laboratory experiments.

#### MATERIALS AND METHODS

Whole crop maize (*Zea mays* - c.v. AUXXEL FAO 190; BAXXAO FAO 180; COXXIMO FAO 220) was harvested at the waxy stage of maturity from three different fields. Whole-plant maize was chopped by a forage harvester "Massey Ferguson 5130" under farm conditions to pieces of 1 - 2 cm, and subsequently transported in a polyethylene bag to the laboratory. In the experiment, 3-liter silos were used. The silage additive (*Pediococcus acidilactici, Lactobacillus plantarum* Milab 393, *Lactococcus lactis* SR 3.54 and *Enterococcus faecium* M74<sup>®</sup>) was applied at 5.5x105 CFU/g fresh crop. Gases were allowed to escape from the laboratory silos during the initial 5 to 7 days of extensive fermentation and later, during silage storage, they were hermetically sealed. According to DLG approval in category 2, the silages were aerated on day 28 and 42. In addition, to determine the reduction in pH-value 2 days after ensiling, chopped whole-crop maize untreated and inoculated were ensiled in 0.7-litre laboratory silos (glass jars).

The SAS statistical package was used to analyse the data. Separation of untreated and treated means has either been done for each field (per field) or in a collected analysis in which fields was used as one factor (over fields). Three replications (glass jars) were used per additive treatment. Silos were analysed as a randomised complete block.

#### **RESULTS AND DISCUSSION**

The ensilability of the whole-plant maize was good because WSC/BC (water soluble carbohydrates to buffering capacity) ratio was 7.5 and herbage had a low concentration of nitrate. Differences in a range of fermentation parameters were found between the treatments. The inoculant-treated whole-crop maize silage had lower (P<0.05) crude fibre concentration than untreated silage. The NFE concentration was higher in treated (P<0.05) than untreated silage. Starch, residual WSC and crude protein concentrations were unaffected by the inoculant (Table 1). Treated whole-crop maize silage had a higher (P<0.05) lactic acid concentration and a lower (P<0.05) ethanol concentration compared with control silage. The inoculation resulted in lower (P<0.01) pH-value after 2 days and numerically lower pH-value after 49 days of ensilage. Therefore, it may be concluded that inoculation results in a more rapid and extensive acidification. Differences were found among treatments for the concentrations of total fermentation acids, butyric acid, ammonia-N and DM losses. However, the differences were not significant. Concentrations of the butyric acid and

ammonia-N and DM losses in inoculated silages were numerically lower.

 Table 1. Mean chemical composition, fermentation parameters and microbial counts in untreated control and additive-treated maize silage from three fields

Measured parameters	Control	Inoculant	Average	LSD0.05	EMS1	Sign.
Dry matter (DM), g/kg	298	302	300.2	11.745	116.733	NS
Crude protein, g/kg DM	84	87	84.4	6.569	36.516	NS
Crude fibre, g/kg DM	217	194	205.6	20.855	368.064	*
NFE, g/kg DM	637	660	648.4	21.358	386.036	*
WSC, g/kg DM	6	5	5.8	4.058	13.934	NS
Starch, g/kg DM	32	32	31.7	3.223	8.789	NS
Total fermentation acids, g/kg DM	119	138	128.9	29.135	718.317	NS
Lactic acid, g/kg DM	51	71	60.8	17.043	245.80	*
Acetic acid, g/kg DM	66	66	66.4	18.463	288.455	NS
Butyric acid, g/kg DM	2.3	0.9	1.6	2.124	3.817	NS
Propionic acid, g/kg DM	0.06	0.03	0.04	0.1	0.008	NS
Ethanol, g/kg DM	7	6	6.4	1.165	1.149	*
Ammonia N, g/kg total N	47	41	44.1	7.085	42.475	NS
pH after 49 days	3.96	3.80	3.88	0.204	0.035	NS
pH after 2 days	4.37	4.14	4.25	0.169	0.024	*
DM losses, g/kg DM	82	74	77.6	17.041	245.742	NS
Yeast, cfu g	6.4 x 106	2.5 x 105	4.5 x 106	6.75E6	3.638E1	NS
Yeast, log cfu g	4.97	4.81	4.89	1.319	1.473	NS
Mould, log cfu g	1.48	1.63	1.55	0.356	0.107	NS
LAB, log cfu g	4.96	6.01	5.49	0.737	0.460	*
Enterobacteriaceae log	1.77	1.0	1.38	0.737	0.459	*
DON mg/kg	0.059889	0.056433	0.058	0.007	0.000048	NS
Afl B 1 mg/kg	0.00134444	0.00126667	0.0013	0.0001	1.347E-8	NS
Sum aflatoc. B1, B2, G1, G2 mg/kg	0.0024189	0.0022533	0.002336	0.006	2.75E-7	NS

t0.05=2.306; Error df=8 1EMS=Error mean square; \* denotes significant at level 0.05.

The inoculant increased (P<0.05) the number of LAB and reduced (P<0.05) enterobacteriaceae count and numerically decreased yeast count relative to the control treatment. Mould count was unaffected by treatment.

Control silages deteriorated at the fastest rate. At 18 to 36 h of exposure to air untreated silage had temperatures significantly higher compared to inoculated silage. Therefore, it may be concluded that bacterial mixture results in more aerobically stable silage (fig. 1).



# Figure 1. Aerobic stability of inoculated (I) treated and untreated (C) whole crop maize silages. \* denotes significant at level 0.05.

#### CONCLUSIONS

Inoculation resulted in a better fermentation compared with untreated silage. Treated silage also tended to have lower proteolysis, butyric acid and DM losses and improved aerobic stability. Herewith, maize silage treated with a bacterial inoculant had a better chemical composition.

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# FERMENTATION AND AEROBIC STABILITY OF WET CRIMPED CORN ENSILAGED WITH ORGANIC ACIDS CONTAINING PRESERVATIVE

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# **INTRODUCTION**

Crimping was developed in Finland in the end of 1960s. In 1918 British researchers had established that grain attains its peak nutritional value when the moisture content of the grain is between 35% and 45%. Traditionally, grain is not harvested until it is dry enough to be ground by a hammer mill. In crimping, the grain will break and flatten Crimped grain is stored in storage silos or plastic bags as silage. Crimping technologies of grains getting more popular, worldwide just in a past decade (Gálik 2008, Wagner et al. 2006) The reason should be: rises of energy costs of drying and the traditional feed preparation, the earlier harvesting, and over the reduce in-field losses, the less weather depended. Practical experiments by farming and livestock research institutions in Finland, Sweden, UK and Hungary and elsewhere have confirmed, that crimped feed has higher nutritional values, it increases the animals' production, improves the animals' health, and in addition, helps cut costs. For the good fermentation, in order to ensure the protection of nutrients and for the aerobic stability of the feed it is advantageous to use organic acid additives, as preservatives The objective: to study the effect of organic acid containing preservative on the fermentation of 2 varieties of corn and aerobic stability of wet crimped corn silages.

#### **MATERIALS AND METHODS**

The experiment was carried out by the University of Szeged Faculty of Agriculture Hódmezővásárhely pilot farm and laboratory and the Gorzsai Mezőgazdasági ZRt cattle farm Two varieties of corn DEKALB and PIONEER were ensilaged in itself (control) and with organic acids containing preservative in their biological ripening stage of maturity with 30-33% moisture content in 200 litre of capacity model scale silos. Two variety of corn together with preservative was ensilaged in large farm scale clamp silo (3000 ton) at that time (the varieties were located in different layers separately in the silo according to the rank of harvesting from different plots

The acid composition of preservative: formic acid 62.1 %, propionic acid 20 %, sorbic acid: 2,5 %

Dosage of applied organic acid: 4.5-litre/ ton row material

Chronological order of experiment:

0<sup>th</sup> day. Filling of the silos, and laboratory analyses of row materials

56<sup>th</sup> day of storage: farm scale wet corn silage sampling for laboratory analyses.

70<sup>th</sup> and 315<sup>th</sup> day of storage: model scale silages sampling for laboratory analyses and start of aerobic stability study *Analyses day 0 on fresh corn, as ensiled:* 

DM, pH, water-soluble carbohydrates (WSC), starch, crude protein, crude fibre, crude ash, crude fat, NDF *Analyses day 56, 70 and 315* 

DM, pH, water-soluble carbohydrates (WSC), starch, crude protein, (crude fibre, crude ash, crude fat, NDF) lactic, acetic, propionic and butyric acids, ammonia-N, ethanol,

*Procedure of aerobic stability study* based on the Völkenrode System (Honig 1990) The temperature of samples was measured automatically by hourly basis. The time a silage is supposed to be stable is given till the registration unit shows a temperature rise of 3 °C above ambient temperature We applied two different temperature and monitoring time: Short time (70 days) storaged samples were monitored on  $20\pm2$  °C ambient room temperature and the samples were exposed to air for 7 days. Long time (315 days) storaged samples were monitored on  $30\pm2$  °C room ambient temperature and the samples were exposed to air for 9 days. We wanted to simulate the Summer time feeding conditions in Hungary this way.

# RESULTS

#### Row material

The chemical composition of ensilaged corn was parallel with biological ripening DEKALB and PIONEER varieties. The DM content was 67% of both varieties.

It can be stated, that the crude protein content of DEKALB variety is higher with 13 % than the PIONEER one, while the crude fat content of PIONEER is higher. This is why there is no relevant difference in netto energy contents between the two varieties. There was no significant difference between the chemical compositions of two varieties.

The pH shows the success of -treatment with preservative: (4,2-4,6 pH)

Wet corn silages in model silo 70 storage days DEKALB variety:

There is no reasonable, significant difference between untreated control and treated silages, regarding the nutrient content and nutritive value. The control wet corn went through lactic acid fermentation and originated higher amount of ethanol, while the treated corn had no lactic acid and acetic acid content. It seemed to be blocked the fermentation by added organic acids until that time

The only fermentation product is ethanol, but there is considerabely and significantly less amount of it than in control one (P=1%).

The NH<sub>3</sub> content of untreated samples is essentially higher than that of treated silages. The difference is significant *PIONEER variety:* 

The tendencies are similar with DEKALB, but the difference in  $NH_3$  contents is significantly less (P=5%) between untreated and treated silages.

# 315 storage days

### DEKALB variety

There is no reasonable difference between untreated control and treated silages regarding nutrient content and nutritive value.

The control samples contain significantly more lactic acid (P=1%) while the treated silages have considerabely more acetic acid content.

The untreated wet corn has higher ethanol and much more NH<sub>3</sub> content than the treated one.

# PIONEER variety

There is no difference between untreated control and treated silages regarding nutrient content and nutritive value. But the lactic acid - acetic acid - ethanol - and  $NH_3$  contents of control are considerabely higher than in treated ones. Remark: The acetic acid content of treated silages was higher than the control in the samples of 70<sup>th</sup> days storage.

# Wet crimped corn silage in farm scale silo

# 56 days of storage

The quality of wet corn silage was uniform good.. The chemical composition and nutritive value was similar with model scale samples.

The only difference was the P=0,1% significantly higher ethanol content of silage from the upper layer compare to the feed storaged in the medium layer. It should be the reason of stronger expose to air because of weaker compactness on the upper side.

# Aerobic stability of wet corn silages

### 70 storage days

The untreated control silages were essentially less stable than the treated silages. There were differences between the stability of DEKALB and PIONEER varieties. The PIONEER lasted their stability only for less than 3 days (~60 hours). When temperature of the forage rises more than 3 °C compared to ambient temperature, it signals the start of deterioration. The peak temperature was registered in the  $87^{\text{th}}$  and  $93^{\text{rd}}$  hours. The new wave of spoilage started from 160-163 hours on expose to air. The DEKALB control samples started to deteriorate on the  $3^{\text{rd}}$  day of exposure to air, from the  $68^{\text{th}}-72^{\text{nd}}$  hours. The 1<sup>st</sup> peak temperature was detected in the  $95^{\text{th}} - 97^{\text{th}}$  hours, and after declining a newer deterioration started from the  $160^{\text{th}}-163^{\text{rd}}$  hour of exposure to air.

All treated wet corn samples (both varieties) protected their stability for 7 days.

#### 315 storage days

The aerobic stability of wet corn silages on high temperature) was the following.

The untreated samples have spoiled, while the treated samples remained healthy, avoided deterioration during the 9 days of experiment on the contrary of high  $(30\pm2 \ ^{\circ}C)$  room ambient temperature.

PIONEER control samples started spoilage in the 91<sup>st</sup> - 92<sup>nd</sup> hour while the DEKALB later from the 202<sup>nd</sup> hour

### CONCLUSION

There was no significant differences between the chemical composition and nutritive value of DEKALB and PIONEER corn varieties neither their row materials nor their silages The organic acid containing preservative is able to drive the fermentation of wet crimped corn ( $\sim$ 70% DM) and help to achieve a good quality of silage. It reduces the proteolysis and ethanol production significantly. Its effect on aerobic stability of wet corn silages is excellent both in normal (20 °C) and high (30 °C) temperature within aerobic conditions. It was proven that the silo could be open, and the treated crimped wet corn silage is ready for feeding after 8 weeks of fermentation.

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# INFLUENCE OF ELEVATED TEMPERATURE AND OXYGEN SUPPLY ON THE SILAGE FERMENTATION CHARACTERISTICS AND AEROBIC STABILITY OF MAIZE, ARTIFICIALLY INFECTED WITH *PENICILLIUM ROQUEFORTI* AND *P. PANEUM* SPORE SOLUTION

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#### ABSTRACT

The influence of elevated temperature in combination with oxygen supply (directly after ensiling or after stabilization of the silage) on maize silage was studied using micro-silos with a content of 2.75 liter. Maize (DM: 365 g/kg) was artificially infected with spores of P. roqueforti and P. paneum ( $1.10^6$  spores/g), and two silage additives were applied: Propiosil (propionic acid) and Sil-All Fireguard (homofermentative LAB, enzymes, heat delayers). The micro-silos were desiled after 15, 40 and 76 days. The aerobic stability of the desiled material was determined by the Honig protocol. Microbial and chemical analysis were performed according to the standard methods. Results show that elevated temperature in the presence of oxygen after silage stabilization promotes mould development, and dramatically decreases the aerobic stability of the desiled material. Also a rise in pH could be observed, noting that the silage additive Propiosil had a direct pH-lowering effect. Presence of oxygen has a clear influence on the fermentation fatty acid pattern: it decreases the lactic acid content, and increases the amount of acetic and butyric acid.

#### **INTRODUCTION**

In practice, completely airtight sealing of silos is very hard to accomplish. Since moulds require a minor amount of oxygen, this can possibly lead to mould development inside the silo (Woolford, 1990). Other factors (temperature, acidity, ...) play also a role in mould development. During summer, higher temperatures, often in combination with reduced feed-out rate, could promote mould growth (Bonner and Fergus, 1960). To investigate the effect of oxygen and elevated temperature on maize silage quality and aerobic stability, a microsilo experiment was performed. Prior to ensiling, the maize was treated with either *P. roqueforti / P. paneum* spore solution (1.10<sup>6</sup> spores/g maize) or an equal amount of control solution (sterile distilled water). The effect of two heat-delaying silage additives was also assessed (van Vuuren et al., 1989; Weinberg and Muck, 1996): Propiosil (Comptoir de Gives - Rotterdam; 99% propionic acid) was applied at 4,5 liter/ton and Sil-All Fireguard (Alltech – Lexington; *Lactobacillus plantarum, Enterococcus faecium* and *Pediococcus acidilactici* complemented with enzymes (cellulose, hemicellulase and pentosanase), potassium sorbate and sodium benzoate) at a rate of 150 g/ton. Three different temperature regimes were studied: one part of the microsilos was heated up to 32°C during 4 days immediately after ensiling; a second part of the microsilos was kept at ambient temperature during the whole experiment; a third part of the microsilos was given aerobic stress 57 days after ensiling, followed by 4 days at 32°C. The first and second part were desiled after 15, 40 and 76 days (9 microsilos ensiled per treatment). The third part (4 microsilos per treatment) was desiled after 76 days.

#### **MATERIALS AND METHODS**

**Ensiling**. Suspensions of *Penicillium* spores were prepared by flooding 10 days old cultures on PDA petri dishes with sterile peptone water and adjusting the concentration after counting with a Thoma cell. The silage additive suspensions were prepared by adding sterile distilled water. The chopped forage was randomly separated into 20 kg lots and treated sequentially with the appropriate solutions (control solution – spore solution – additive solution). All solutions were vaporised as a fine mist over the maize in an amount of 100 ml per 10 kg of maize, with thorough mixing in-between.

PVC microsilos (content: 2.75 l), all equipped with a CO<sub>2</sub>-slot, were weighed before and after ensiling with maize (DM: 365 g/kg). Treatments were: 1) no infection – no additive, 2) no infection – Propiosil, 3) no infection – Sil-All Fireguard, 4) *P. paneum* – no additive, 5) *P. paneum* – Propiosil, 6) *P. paneum* – Sil-All Fireguard, 7) *P. roqueforti* – no additive, 8) *P. roqueforti* – Propiosil, 9) *P. roqueforti* – Sil-All Fireguard. For each of these treatments, 9 microsilos were placed at 32°C immediately after ensiling (part I). Nine microsilos for the treatments 1, 4 and 7 were kept at ambient temperature (part II). Part III consisted of 4 microsilos per treatment. All microsilos were ensiled at a density of 160 kg DM/m<sup>3</sup>.

**Aerobic stability**. Aerobic stability was defined as the number of hours before the temperature of the desiled material rises 3°C above ambient temperature (20°C). During 7 days after desiling, the desiled material was subjected to the Honig protocol (Honig, 1985): an equivalent of 100 g DM was put loosely in a recipient (content 1 l) and placed in an insulating box allowing gas exchange. In the centre of the recipient, the temperature was registered every 2 hours.

**Microbial analysis**. Besides visual observation of mould growth, mould counts were performed. An amount of 10 g of silage was diluted in 90 ml of sterile peptone water and homogenized for 2 minutes at 230 rpm in a stomacher. Aliquots of 0.1 ml from 5 serial dilutions were surface spread on MEA supplemented with 200 ppm chloramphenicol. Petri dishes were incubated at ambient temperature for enumeration of total moulds.

**Chemical analysis**. Following analyses were performed: 1) dry matter content by drying 600 g fresh material at 60 °C to constant weight, 2) pH from an extract based on demineralised water, 3) lactic acid (LA), butyric acid (BA) and acetic acid (AA) content using HPLC.

**Statistical analysis.** Statistical analysis was performed using the SAS 4.1 software package. The normally distributed variables were subjected to one-way ANOVA with Tukey as *post hoc* test. The variables which had no normal distribution were statistically analysed by non-parametric one-way ANOVA with Wilcoxon as *post hoc* test.

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# **RESULTS AND DISCUSSION**

Aerobic stability. The aerobic stability of part I and part II was not affected by the duration of the ensiling period. Over the three desiling moments, part I showed a significantly lower aerobic stability  $(77.07\pm38.55 \text{ h}; \#: 81)$  than part II  $(108.94\pm39.77 \text{ h}; \#: 27)$ . In part III, which was heated and received oxidative stress after silage stabilization, a dramatic reduction of the aerobic stability was observed  $(22.60\pm11.76 \text{ h}; \#: 36)$ . The artificially infected microsilos  $(18.38\pm7.80 \text{ h}; \#: 24)$  were significantly less stable than the uninfected microsilos  $(31.04\pm14.02 \text{ h}; \#: 12)$ , as could be expected. The use of silage additives didn't significantly improve the aerobic stability.

#### Microbial analysis.

*Visual observations*. Visual mould growth was only observed at desiling after 76 days. One microsilo which was heated directly after ensiling, infected with *P. paneum* and treated with Propiosil showed visual mould development. From the 36 microsilos heated and subjected to oxygen after stabilization of the silage (part III), 16 microsilos were visually mouldy (mostly belonging to treatments T5, T7 and T8).

*Mould counts*. The mould counts on the desiled material of part I and part II were not influenced by the duration of the ensiling period, nor by the temperature regime. Evidently, artificial infection with fungal spores lead to significantly higher amounts of moulds  $(3.94\pm1.11 \log_{10} \text{ CFU/g maize}; \#: 72)$  compared to the uninfected microsilos  $(2.53\pm0.67 \log_{10} \text{ CFU/g maize}; \#: 36)$ . The use of silage additives resulted in lower mould counts, but the effect was not significant.

The mould counts performed on part III were significantly higher:  $5.22\pm1.75 \log_{10} \text{CFU/g}$  maize (#: 36), confirming that the presence of oxygen combined with elevated temperature promotes mould development. Again, the mould counts were significantly higher for the artificially infected microsilos ( $5.75\pm1.75 \log_{10} \text{CFU/g}$  maize; #: 24) than for the uninfected microsilos ( $4.16\pm1.24 \log_{10} \text{CFU/g}$  maize; #: 12). The silage additives didn't have a significant effect on the artificially infected microsilos treated with Propiosil ( $3.48\pm0.05 \log_{10} \text{CFU/g}$  maize; #: 4) showed lower mould counts compared to the untreated microsilos ( $5.24\pm1.67 \log_{10} \text{CFU/g}$  maize; #: 4), due to the inhibitory effect op propionic acid on mould growth (Glancey, 1998). Use of Sil-All Fireguard lead to lower mould counts ( $3.76\pm0.66 \log_{10} \text{CFU/g}$  maize; #: 4), but the difference was not significant.

#### Chemical analysis.

*Dry matter content*. For part I and part II, the DM content at desiling after 76 days (346.41±5.78 g/kg; #: 36) was lower than after 15 (351,56±10.36 g/kg; #: 36) and 40 days (350.92±3.56 g/kg; #: 36). For the microsilos desiled after 76 days, there are significant differences in DM content between part I and part II compared to part III (341.85±10.24 g/kg; #: 36). Presence of oxygen seems to cause secondary fermentation (Woolford, 1990), leading to DM losses. Artificial inoculation didn't influence the DM content, nor did the silage additives.

*pH*. For part I and part II, the pH was not influenced by the duration of the ensiling period, temperature regime and artificial infection. Use of the silage additive Propiosil, which has a directly pH-lowering effect, resulted in a significantly lower pH ( $3.76\pm0.07$  g/kg; #: 27) compared to the untreated microsilos ( $3.83\pm0.05$  g/kg; #: 54) and those treated with Sil-All Fireguard ( $3.80\pm0.06$  g/kg; #: 27). Oxidative stress combined with heating after silage stabilization resulted in a significantly higher pH ( $3.99\pm0.22$  g/kg; #: 36), not influenced by artificial infection or additive use.

Fermentation fatty acids. The fermentation fatty acid pattern was significantly influenced by oxidative stress in

		Part I & II	#	Part III	#
	LA	16.71±1.43	36	13.05±2.74	36
	Untreated	5.08±0.60 a	18	6.02±1.38 a	12
AA	Propiosil	3.65±0.30 b	9	4.28±0.38 b	12
	Sil-All Fireguard	5.14±0.66 a	9	6.21±2.07 a	12
	BA	$0.004 \pm 0.022$	36	0.097±0.160	36

**Table 1.** Fermentation fatty acids (g/kg fresh material)

combination with heating after silage stabilization, resulting in a higher BA and AA content, and a lower LA level. Heating directly after ensiling had no significant effect. Table 1 shows the LA, AA and BA content 76 days after ensiling for part I and II compared to part III. Artificial infection had no significant effect on the fatty acid pattern. The LA and BA content were not influenced by the use of а silage additive. but Propiosil significantly reduced the AA level.

#### CONCLUSION

The results of the microsilo experiment confirm that the presence of oxygen plays a crucial role in microbial and chemical silage quality, even after silage stabilization. Use of silage additives cannot prevent the damage due to oxygen.

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# THE OCCURRENCE OF MYCOTOXINS IN HIGH MOISTURE CRIMPED CORN

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#### INTRODUCTION

Mycotoxins are secondary metabolites of fungal pathogens with different levels of toxicity. 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 (Nedělník and Moravcová, 2006). Feed can contain broad spectrum of mycotoxins which deteriorate hygienic quality of feed (Bíro et al. 2006) and cause physiological and metabolic diseases of animals (Kačániová et al., 2005). The most important mycotoxins, based on worldwide occurrence, are aflatoxins, deoxynivalenol, ochratoxins, fumonisins and zearalenone. Moulds normally associated with these mycotoxins belong to the genera *Fusarium*, *Aspergillus* and *Penicillium*. *Aspergillus* and *Penicillium* are associated with storage while members of *Fusarium* are usually associated with crops while still under cultivation in the field. It is estimated by the Food and Agricultural Organization that 25 % of the world's crops are contaminated with mycotoxins (Naicker et al., 2007). An experiment was carried out to know the extent of mycotoxins contamination of fresh and conserved high moisture corn.

#### **MATERIALS AND METHODS**

We conserved in experimental conditions high moisture corn (*Zea mays L.*) variety Pardi. Maize grain was gathered when content of dry matter was 700 g and consequently mechanically processed by crusher. We took from the fresh maize grain average samples. Mechanically processed high moisture grain without additives we ensiled into silos with volume 15 dm<sup>3</sup> and hermetic sealed. During the fermentation process were silos stored by temperature of 18-20 °C. After 3 months of storing we took average samples for analysis the content of mycotoxins. Samples of fresh and conserved high moisture corn were analyzed for mycotoxins by the Laboratory of feed conservation using enzyme-linked immunosorbent assays (Elisa Reader, Noack SR) methodology for the analysis of total aflatoxins (AFL), total fumonisins (FUM), total ochratoxins (OT), zearalenone (ZON), deoxynivalenol (DON) and T-2 toxin (T-2). The principle of testing is in identifying of toxin concentration on spectrophotometer by 650 nm wave-length. Before determination of concentration were samples extracted in distilled water (DON) and in methanol (70% concentration for FUM, ZON and AFL, 50% concentration for OT and T-2). We followed the methods of the machine provider by determination of mycotoxins. That were quantitative tests.

# **RESULTS AND DISCUSSION**

In fresh high moisture corn we detected occurrence almost of all mycotoxins, in exception of AFL, with the highest concentration of FUM (464.5  $\mu$ g.kg<sup>-1</sup>). FUM reduce the milk production in dairy cattle as well as feed consumption (Diaz et al., 2000). Concentration of DON was from 381.4 to 445.4  $\mu$ g.kg<sup>-1</sup>. Results of Charmley et al. (1993) showed that cows consuming DON contaminated diets tended to produce less milk. DON has been associated with reduced flow of utilizable protein to the duodenum (Danicke et al., 2005). Amount of ZON in fresh corn did not exceed the limited maximum amount (Regulation of government SR 438/2006). The same results observed Golian et al. (2006) by testing the concentration of ZON in 36 samples of cereals grown in SR.

Indicators of	f	DON	ZON	T-2	FUM	AFL	OT			
contaminati	on			μg.kg <sup>-1</sup>						
	Average	412.2	102.8	56.7	464.5	0.0	0.43			
Fresh corn	Min.	381.4	88.6	43.9	399.2	0.0	0.2			
	Max.	445.4	119.1	65.2	401.5	0.0	0.7			
Conconved	Average	510.1	269.0	272.8	163.9	0.1	1.4			
com	Min.	489.2	167.4	243.4	121.2	0.0	1.1			
COILI	Max.	540.9	325.3	294.2	208.9	0.3	1.6			

Table 1. Concentrations of tested mycotoxins in fresh and conserved high moisture crimped corn

DON: deoxynivalenol, ZON: zearalenone, T-2: T-2 toxin, FUM: fumonisins, AFL: aflatoxins, OT: ochratoxins

The content of dry matter in conserved grain was 690.5 g, it was higher as stated Doležal and Zeman (2005). Content of mycotoxins was higher in ensiled grain like in grain before conservation, excepting FUM (Table 1). AFL and OT in conditions of Slovakia are produce by species of storage fungi. We did not detect the occurrence of AFL in fresh grain, but in conserved grain was the concentration 0.1 µg.kg<sup>-1</sup>. Cows consuming diets containing 30 µg of

aflatoxins can produce milk containing aflatoxins residues above the level of 0.5  $\mu$ g. Aflatoxins appears in the milk rapidly and clears within three to four days (Diaz et al., 2004). The average concentration of OT was 0.43  $\mu$ g.kg<sup>-1</sup> in fresh grain and 1.4  $\mu$ g.kg<sup>-1</sup> in conserved with interval from 1.1 to 1.6  $\mu$ g.kg<sup>-1</sup>. In conserved high moisture grain without additives we detect the highest content of DON (510.1  $\mu$ g.kg<sup>-1</sup>) and the second highest content of T-2 toxin (272.8  $\mu$ g.kg<sup>-1</sup>). T-2 toxin is a very potent *Fusarium* produced mycotoxin (Russell et al., 1991). In conserved grain was concentration of T-2 toxin almost 5-multiple higher like in fresh grain. Content of ZON fluctuated in interval from 167.4 to 325.3  $\mu$ g.kg<sup>-1</sup> in conserved grain. Concentrations of mycotoxins in fresh and in conserved high moisture corn did not exceed the limited values (Regulation of government SR 438/2006).

# CONCLUSIONS

In fresh high moisture corn with content of dry matter 700 g we detect occurrence of all mycotoxins, excepting aflatoxins. The highest concentration we determined in content of fumonisins and deoxynivalenol. In total occurrence of *Fusarium* toxins was substantial. In conserved high moisture corn in comparison with fresh grain we detect higher content of all mycotoxins, excepting fumonisins, with the highest concentration of deoxynivalenol. Concentration of T-2 toxin was almost 5multiple higher like in grain before conservation. Content of mycotoxins produced by species of storage fungi (aflatoxins and ochratoxins) was in conserved corn the lowest.

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# EFFECT OF MICROBIAL INOCULANT ON THE QUALITY OF BREWER'S GRAIN ENSILAGE

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# **INTRODUCTION**

At a dry matter content of 200-220 g/kg, brewer's grains can be effectively used for direct feeding to cattle, or alternatively stabilized for subsequent feeding after ensiling (Küntzel, 1991; 1992; Lohnert et al., 1996; Nishino et al., 2003; Doležal et al., 2003 and others). Buchgraber and Resch (1997) suggest that in order to prevent the discharge of silage effluents, the fresh brewer's grains can be pressed to a higher dry matter content of 350-400 g/kg, or ensiled in combination with various sorbent materials (Pereira et al., 1998; Tanaka et al., 2001). Carbohydrates predominant in the grains are namely maltose, raffinose (Nishino et al., 2003) and glucose. Küntzel (1991, 1992) informs that low (negligible) production of lactic acid in the silage of brewer's grains occurred (in anaerobic conditions) at higher temperatures (above 45-50 °C), and a small increase in the lactic acid production was observed only at temperatures below 45 °C. However, at lower temperatures, an increased production of acetic acid and ethanol occurred. During the storage of brewer's grains ensilage, the content of lactic acid decreases and the contents of acetic acid, ethanol and pH value increase. Stability of brewer's grains silages can be achieved at a pH value below 4.5 and by using silage additives. Küntzel (1992) described beneficial influence of sodium benzoate, potassium sorbate and/or sodium propionate on the aerobic stability of silages. Nishino et al. (2003) state that the production of lactic acid in model silages rapidly increased immediately after ensiling and that a maximum amount (50 g/kg DM) was measured after 20 days. Similarly, Buchgraber and Resch (1997) claimed that lactic acid bacteria + sugar and Maize Kofasil -when used as additives in pressed brewer's grains- resulted in a better quality of fermentation, not significantly as compared with the control, though.

Brewer's grains are a feed that readily deteriorates, particularly in summer months. Küntzel (1992) informs that all groups of microorganisms were represented in grains. Gruber et al. (1997), Doležal et al. (2006) observed that fresh and unconserved grains could keep in feedable condition usually for max. 48 hours. Longer storage results in sensory, nutritive and namely microbial changes.

The objective of the model experiment was to study the effect of microbial inoculant on the quality of fermentation process in brewer's grains.

# MATERIALS AND METHODS

In the model experiment fresh brewer's grains were used with an average DM content of 216.73 g/kg; moisture sorbents were malt sprouts and wheat bran. The percentage ratio of grains and sorbents was 89:7.33:6.7. Average (n = 6) resulting dry weight of ensiled stock was 283.02 g/kg (ranging from 280.58 – 287.51 g/kg). Established were four model experimental variants with two different storage times (56 and 112 days). The subject of this paper is to assess and compare the effect of inoculant with the untreated control variant. Variant  $A_c$  – control silage without silage additive; Variant  $A_{BF}$  – treated with inoculant (BF) containing as active substance bacteria of lactic fermentation (*Lactobacillus paracasei* (DSM 16245), *Lactobacillus lactis* (NCIMM 30160) and *Pediococcus acididlactici* (DSM 16243) in a total concentration of CFU 1×10<sup>11</sup>/g applied at a dose of 2 g/ton. The model silages were stored in the laboratory at an average laboratory temperature of 26-28 °C and sampled on Day 56 and Day 112 after ensiling. Parameters measured in assessing the fermentation process quality on the mentioned dates were: silage DM content, pH, titrating acidity (TA), amounts of lactic acid, acetic acid, propionic acid, butyric acid, contents of alcohol and ammonia. DM content was established by desiccation at 103±2 °C to constant weight. Analytical procedures were described in our earlier work (Doležal, 2002). Results were statistically processed by using the method of variance analysis and differences between the individual experimental groups were analyzed by F-test.

# **RESULTS AND DISCUSSION**

Dry matter content in silages made of brewer's grains ranged after 56 days of storage from  $293 - 297.67\pm0.52$  g/kg while all silages stored 112 days showed a decreased DM content. During the storage of up to 56 days, the supplementation of moisture sorbent – malt sprouts and wheat bran in Experiment resulted in a discharge of effluents converted per 1 ton at an amount of 17.5 resp. 24.2 l/t of silage with the inoculated silage showing higher losses. The effluents were released also after 56 days of storage, which is illustrated by total amount of effluents in the last sampling (32.5 litres in the control silage and 39.2 litres in the inoculated silage). The assessment of the effect of silage additive on the fermentation process quality demonstrated the relation to the pH value of silage. Due to higher DM content, a significantly decreased pH value was observed in the experimental silages in the second sampling. The results corroborate findings of other authors (Küntzel, 1992 and others). Such a pH value was apparently a sufficient guarantee for the inhibition of butyric fermentation. This was corroborated by the analysis, which did not reveal any content of butyric and propionic acids. Titrating acidity (TA) of respective variants treated with the silage additive correlated with the sum of fermentation acids. In inoculated silages exhibited the value of titrating acidity higher in all cases.

Demonstrated was a trend of higher TA in the later sampling term, which relates to partly reduced (slowed down) fermentation process due to higher DM content. Differences in TA values between the control and inoculated silages were significant.

Our experiments corroborated findings published by Küntzel (1992) concerning the increasing production of acetic acid and ethanol with the longer storage of silages. On the other hand, the addition of inoculant reduced the generation of ethanol during extended storage and stabilized the amount of acetic acid in the silage. Production of lactic acid has a significant influence on the fermentation quality. Our findings indicate that great differences existed between the control and experimental silages in the production of lactic acid. As compared with the experimental results published by Nishino et al. (2003), who state a rapidly increasing production of lactic acid in model silages immediately after ensiling and with a maximum value of 50 g/kg DM after 20 days, our results showed that the production of lactic acid was not at all so rapid as described by this author. Its production in the control silage of was 56.2 g/kg DM on Day 56 of storage and up to 69.6 g/kg DM on Day 112 of storage. In the inoculated silages, its production was positively stimulated by the inoculant, and on Day 56 and Day 112, its concentrations were  $93.71 \pm 3.24$  g/kg DM and  $95.29 \pm 6.56$  g/kg DM, respectively. Nishino et al. (2003) report that with the extending storage time the lactic acids is metabolized into the acetic and propionic acids, which was not unambiguously demonstrated in our experiment as no propionic or butyric acids were detected.

Variant	A <sub>c1</sub>		A <sub>BF1</sub>		A <sub>c2</sub>		A <sub>BF2</sub>	
Parameter	Average $\pm se$	VC (%)	Average $\pm se$	VC (%)	Average $\pm se$	VC (%)	Average $\pm se$	VC (%)
DM [g/kg]	$293.00\pm3.90$	1,33	$297.67\pm0.52$	0.17	$289.33\pm2.73$	0.94	$291.33\pm2.88$	0.98
pН	$3.52 \pm 0.02$	0.42	$3.70\pm0.02$	0.58	$3.81 \pm 0.03$	0.90	$3.52 \pm 0.01$	0.30
Titrating acidity [mg KOH/100g]	$1280.83 \pm 104.41$	8.15	$1402.17 \pm 102.87$	7.34	$1584.50 \pm 34.52$	2.20	1794.33 ± 12.44	0.69
Lactic acid (g/kg)	$56.20 \pm 11.25$	20.03	$69.60 \pm 4.12$	5.92	$93.71 \pm 3.24$	3.46	$95.29\pm6.56$	6.89
Acetic acid (g/kg)	$11.67 \pm 0.50$	4.28	$13.27 \pm 0.54$	4.08	$17.92 \pm 1.70$	9.47	$17.68 \pm 1.71$	9.65
Propionic acid (g/kg)	$0\pm 0$		$0 \pm 0$		$0\pm 0$		0	
Butyric acid (g/kg)	$0\pm 0$		$0\pm 0$		$0\pm 0$		$0\pm 0$	
Sum of acids	$67.87 \pm 11.68$	17.21	$82.87 \pm 4.45$	5.48	111. 63 ± 4.34	3.89	$112.97 \pm 8.16$	7.22
LA : AA	$4.80 \pm 0.78$	16.26	$5.24 \pm 0.21$	3.99	$5.27 \pm 0.51$	9.63	$5.40 \pm 0.22$	4.07
Ethanol (g/kg)	$1.42 \pm 0.25$	17.80	2.41 ±0.25	10.56	$2.48 \pm 0.25$	9.95	$2.00 \pm 0.26$	13.18
Ammonia (g/kg)	$1.54 \pm 0.19$	12.24	$1.85 \pm 0.18$	9.96	$2.71 \pm 0.25$	9.14	$2.92 \pm 0.19$	6.52

Table 1. Fermentation characteristics of BG silages after 56 and 112 days of storage (in DM)

AK1-Control silage in Group A after 56 days of storage; ABF1-Inoculated silage in Group A after 56 days of storage; AK2-Control silage (in Group A) after 112 days of storage; ABF2-Inoculated silage in Group A after 112 days of storage

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# **SUMMARY**

Experimental results show that the addition of microbial inoculant led to the increased LA production, to the extended LA/AA ratio and to the reduced ethanol production. The pH value of inoculated silages sampled in the later term was lower than in untreated silages. The addition of moisture sorbent to brewer's grains resulted in the trend of slower fermentation, which showed in a higher content of lactic acid in silages sampled in the later term (Day 112). The contents of alcohol and ammonia exhibited an increasing trend with the increasing storage time, too, with higher values found in the control silage variants. None of experimental silages showed production of butyric and propionic acids.

# INFLUENCE OF EFFLUENT AND STORAGE TIME OF SILAGE QUALITY OF WET BREWER'S GRAINS **ENSILED IN PLASTIC SILAGE BAGS**

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### **INTRODUCTION**

Since 2004 a new ensiling technology for wet brewer's grains was developed – the truck bagging technology – where the wet brewer's grains are tipped out directly from a truck into a silage bag. This new storage method spread rapidly since its introduction. 2007 more than 200.000 t of wet brewer's grains were filled into bags in Germany by truck baggers.

Up to now there was a common recommendation for wet brewer's ensiling to ensure the unhindered flowing of effluent from piles or bunker silos. Different authors reported on increasing silage losses and bad silage quality caused by effluent accumulation (SCHOCH 1957; WYSS 1997 and 2002). By using the truck bagging technology the wet brewer's grains are filled into the clean bag without touching the ground. The bags are air and liquid tight and the effluent could be hold within the bag. Therefore different praxis trials were done to analyse the influence of effluent on the quality of wet brewer's grains silage in bags. Besides also the influence of storage time was investigated. There were several reports in literature on worsen silage quality of wet brewer's grains with increasing storage time (BECKHOFF 1985; SCHNEIDER et al. 1995). Parallel to the praxis trials wet brewer's grains were also filled into minibags to investigate the dynamic of effluent flowing and to determine the factors affecting the effluent amounts more detailed.

#### **MATERIALS AND METHODS**

In October 2006 two bags were filled each with 25 t wet brewer's grains from the same brewery by the truck bagging technology on same day. One bag (bag 1) was tipped at ground level, the second (bag 2) on ground with slight gradient. Bag 1 was closed immediately and the effluent was held into the bag during the whole storage time of six month. The effluent from bag 2 could flow permanently through two tubes into collecting containers. This whole system was kept airtight. Both bags were opened after 178 silage days and samples were taken in three layers ("top" = 20 cm below the bag surface, "below" = 20 cm above the bag ground, "ground" = layer on bag ground 3 cm thick) with three repeats.

Parallel to the praxis trial the same material was filled into minibags (one layer bags with 20 cm diameter) with three filling heights (50 cm, 70 cm and 90 cm). Besides three more DM-steps were produced from the delivered wet brewer's grains by manual pressing and also filled into minibags with three filling heights. All minibags were placed erected in a special device. The effluent of each minibag was collected in separate measuring cups during the storage time of 84 days.

Pos.	effluent	DM	pН	NH3-N	Lactic acid	Acetic acid	Propionic acid	Butyric acid	Ethanol	Propanol
		g/kg					g/kg DM			
	With	248	4,0	1,0	19,3	3,4	0,0	0,0	0,0	0,4
	(bag 1)	15	0,1	0,2	4,5	2,3	0,0	0,0	0,0	0,7
top	Without	254	4,1	0,8	28,4	4,1	0,0	0,0	0,0	1,3
	(bag 2)	7	0,1	0,2	9,6	2,4	0,0	0,0	0,0	0,3
	sign. <sup>1)</sup>	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	With	198	4,4	1,3	3,8	13,8	0,0	0,0	0,7	6,6
≥	(bag 1)	12	0,0	0,2	0,9	7,4	0,0	0,0	1,1	2,7
elo	Without	201	4,4	1,2	6,6	12,8	0,0	0,0	1,4	6,0
þ	(bag 2)	3	0,1	0,2	3,3	7,2	0,0	0,0	1,3	4,0
	sign. <sup>1)</sup>	n.s.	n.s. <sup>2)</sup>	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	With	204	4,2	1,4	17,8	13,6	3,8	0,0	0,4	4,9
рг	(bag 1)	9	0,1	0,2	26,5	2,5	1,5	0,0	0,7	1,6
ino	Without	201	4,3	1,3	5,5	14,4	1,2	0,0	0,8	6,8
ад	(bag 2)	17	0,1	0,1	0,5	6,2	1,1	0,0	1,0	2,7
	sign. <sup>1)</sup>	n.s.	n.s.	n.s.	n.s. <sup>2)</sup>	n.s.	n.s.	n.s.	n.s.	n.s.

Table 1. Comparison of effluent influence of fermentation quality of wet brewer's grains silage after 178 ensiling days in different positions (n=3, t-test in case of variance homogeneity)

<sup>1)</sup> sign. = significant differences of one factor (n.s. = non significant: p>0.05; \* = significant:  $p\leq0.05$ )

<sup>2)</sup> approximative t-test in case of variance inhomogeneity

#### **RESULTS AND DISCUSSION**

Table 1 is showing that the silages of both bags (bag 1 = with effluent and bag 2 = without effluent) didn't contain

any butyric acid after 178 ensiling days. Comparable contents of fermentation acids and alcohol were found as well in the upper as in the lower positions in bag 1 and bag 2. Mainly lactic acid was produced in the upper layer and a little more acetic acid and traces of alcohols in the lower positions. The low NH<sub>3</sub>-content indicates that the protein degradation was low during the six month storage time even though the wet brewer's grains are rich in protein. Yeasts and moulds were determined only at one position with two repeats. No moulds were found in both bags and only one sample from bag 1 with yeasts with 10<sup>4</sup> CFU /kg FM. The quality of the wet brewer's grains silages after 178 ensiling days were consequently good. No significant differences were found between the variants with and without effluent.

Table 2 is showing that the amount of effluent flow is mainly influenced by dry matter content. Already a DM increase of 10 g/kg caused significant lower effluent amounts. An increasing filling height also raised the effluent flow significantly from DM-content of 222 g/kg if the filling heights differed about 40 cm (compare table 2, filling height 50 and 90 cm)

The amounts of effluent for not pressed wet brewer's grains (DM = 207 g/kg) measured in the minibags correspond to the amounts found in the literature for effluent flow in laboratory experiments and in practise (SCHOCH 1957; VYSKOCIL et al. 2006). Figure 1 compared the effluent flow from the minibags with the real effluent amounts developed from silage bags. For the comparison minibags with the same material as in the silage bag (not pressed brewer's grains, DM = 207 g/kg and a filling height of 70 cm was selected. The height of the silage bag was also 70 cm. Figure 1 shows that less effluent flowed out of the silage bag than from the minibags. However the dynamics of effluent flow were comparable. During first days after ensiling the effluent flow was highest. But it never stopped until end of trial.

				Effluent flow in l/t FM										
			Fil	ling heig	ght	Fil	lling heig	ght	Filling height			Filling height		
			50 cm	70 cm	90 cm	50 cm	50 cm   70 cm   90 cm   5		50 cm	70 cm	90 cm	50 cm	70 cm	90 cm
	n		DM-ste	ep 0 (20'	7 g/kg)	DM-st	DM-step 1 (222 g/kg)		DM-step 2 (234 g/kg)		DM-step 3 (244 g/kg)			
$\mathbf{M}\mathbf{V}^{(1)}$	2	X	177 <sup>a</sup>	158 <sup>a</sup>	161 <sup>a</sup>	118 <sup>a</sup>	121 <sup>ab</sup>	133 <sup>b</sup>	68 <sup>a</sup>	70 <sup>a</sup>	100 <sup>b</sup>	27 <sup>a</sup>	31 <sup>ab</sup>	42 <sup>b</sup>
101 0	5	S	17	24	4	7	3	5	10	4	10	3	2	7
$MV^{2)}$	٥	X		165 <sup>D</sup>			124 <sup>C</sup>			79 <sup>в</sup>			33 <sup>A</sup>	
IVI V	-	S		17			8			17			8	

 Table 2.
 Effluent flow after 84 ensiling days

1)

mean value of each filling height and DM-step, <sup>2)</sup> mean value of each DM-step marks significant differences; p $\leq$ 0,05; Tuckey-HSD , <sup>ABC</sup> marks significant differences; p $\leq$ 0,05; Dunett T3

Figure 1. Comparison of effluent amounts collected by ensiling of wet brewers grains in silage bags and minibags with same material (DM = 207 g/kg TM) and same filling height (70 cm)



#### CONCLUSIONS

The effluent didn't influence the silage quality of wet brewer's grains by using the truck bagging technology. Effluent could be hold inside the bag and must be not wasted until opening the silo. The amount of effluent in silage bags is plainly lower compared to collected effluent from minibags. Minibag trial shows that the DM content has the main influence of effluent flow.

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# EVALUATION OF QUALITY AND AEROBIC STABILITY OF GRASS SILAGE TREATED WITH BACTERIAL INOCULANTS CONTAINING LACTOBACILLUS BUCHNERI

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### INTRODUCTION

Obtaining a good fermentation quality and high digestibility of nutritive components in grass silage ensures the stimulation of the ensilage process by adding biological preparations. One of the consequences of increasing the proportion of lactic acid is the negative impact on aerobic stability. It is accepted that well preserved, high quality silages, inoculated only with homofermentative lactic acid bacteria (LAB) are more prone to aerobic spoilage than untreated (Weinberg *et al.*, 1993). Aerobic deterioration of silage can affect both the efficiency of nutrient utilisation and its hygienic quality. The recent developments have focused on the use of heterofermentative LAB, particularly *Lactobacillus buchneri* (Driehuis *et al.*, 2001), which produces acetic acid inhibiting the yeast development, that may improve silage stability after bales are opened (Danner *et al.*, 2003). The aim of this study was to investigate the influence of two bacterial additives containing the homo- and heterofermentative LAB on the fermentation quality, aerobic stability and nutritive value of grass silage in big bales.

#### MATERIALS AND METHODS

During the years 2005 – 2006 the study on the influence of two bacterial silage additives applied under farm-like conditions on the fermentation quality, aerobic stability, nutritive value of grass silage and animal performance was conducted. Silages were made from a meadow sward composed of 80 % grasses (Poa pratensis, Alopecurus pratensis, Dactylis glomerata, Arrhenaterum etatius, Lolium perenne) and 20 % weeds and herbs. The meadow was cut three times a year: first at full heading of Dactylis glomerata and then at nine weeks intervals. The herbage was mown with a rotary mower -conditioner and pre-wilted to a dry matter (DM) concentration of approximately 400-450 g kg<sup>-1</sup>. The meadow herbage was ensiled either without treatment (control silage), with the addition of commercial inoculate containing: Enterococcus faecium M74, L. casei, L. plantarum, Pediococcus spp. L. buchneri (treatment K1) or with another commercial inoculate containing: L. plantarum K, L. plantarum C, L. brevis, L. buchneri (treatment K2). Both additives were applied at the rate of  $2 \text{ l t}^{-1}$  fresh herbage by spraving during bale rolling in a variable bale chamber baler. The big bales (about 400 kg/bale) were wrapped in four layers of stretch film after transport to their place of storage. In January every next year the bales were opened for feeding and sampling. Two feeding trials, one in 2005 and one in 2006 were performed. Twenty seven heifers (mean initial live weight of 215±22 kg in 2005 and 198±35 kg in 2006) were blocked into three groups of nine according to live weight. The heifers (black-white breed) were offered silage, either with additives (treatment K1 or treatment K2) or without the additives ad libitum and supplemented with a controlled amount of concentrate for a period of 91 (2005) and 92 (2006) days. The silages from all 3 cuts were fed successively. During the feeding experiment silage samples were taken for determination of: DM (oven method), pH, ammonia-N concentration (distillation method), organic acids (enzymatic method), nutritive components (NIRS technique), moulds and yeast counts. Aerobic stability was analysed by monitoring the temperature changes in silage samples stored in aerobic conditions (ambient temperature about 21 °C) for 12 days. Stability was measured as the time necessary to increase silage temperature by 1 °C over air ambient temperature. Data concerning the chemical composition of silage were analysed using analysis of variance in ANOVA 3. Silage intakes and animal performance were compared using ANOVA 2. Differences between treatments were tested using the Student's t-test.

# **RESULTS AND DISCUSSION**

The addition of both bacterial inoculants improved fermentation quality. Inoculate treated silages displayed higher (P<0.05) lactic acid and lower butyric acid concentration than the untreated silage. The increase of acetic acid content was significant (P<0.05) only in case of K1 treatment. The ammonia-N concentration was lower (P<0.05) in K1 silage than in control silage. The silages prepared with inoculants obtained more (P<0.05) Flieg-Zimmer scores than control silage (Table 1). The addition of additives increased (P<0.05) aerobic stability of silage from 6.5 days (control) to 9.4 days (K1 treatment). The aerobic spoilage of silage depended also on the time of harvest (cut) and the year. The inoculate-treated silages had lower (P<0.05) yeast and mould counts than the untreated silage. The reason of this effect is an anaerobic degradation of lactic acid to acetic acid by *Lactobacillus buchneri* (Oude Elferink *et al.* 2001) that inhibits yeast and mould development (Danner *et al.*, 2003). Inoculate treatment had an evident impact on the nutritive value of the feeds. The total protein, WSC and NEL content was higher (P<0.05) and crude fibre lower (P<0.05) in inoculated silages than in control silage. The differences (P<0.05) were found in nutritive components concentration between silages produced from swards harvested at different. In spite of high concentration of acetic acid in inoculate

treated silages, even in the case of K1 silage, a decline of silage intake was not observed. However, no differences between live weights among animals were found. Despite lower initial body weight, heifers offered the tested silage in 2006 showed higher (P < 0.05) daily live weight gain than those offered the silage in 2005.

Examined parameters	Trea	atment (	T)		Cut (C)		SEM	Year	r (Y)	SEM	Significance <sup>1)</sup>			
Examined parameters	Control	K1	K2	Ι	II	III	SEIVI	2005	2006	SEIVI	Т	С	Y	TxC
DM (g kg <sup>-1</sup> )	472	422	477	463 <sup>a</sup>	513 <sup>a</sup>	395 <sup>b</sup>	5.094	467	447	4.159	NS	**	NS	NS
pН	5.01 <sup>a</sup>	4.49 <sup>b</sup>	4.74 <sup>ab</sup>	4.56 <sup>b</sup>	4.86 <sup>a</sup>	4.83 <sup>ab</sup>	0.082	4.91 <sup>a</sup>	4.59 <sup>b</sup>	0.067	**	*	**	NS
NH <sub>3</sub> -N (g kg <sup>-1</sup> total N)	48.6 <sup>a</sup>	31.8 <sup>b</sup>	44.7 <sup>ab</sup>	41.9 <sup>a</sup>	27.9 <sup>b</sup>	55.3 <sup>a</sup>	4.722	40.5	42.9	3.856	*	**	NS	NS
Lactic acid (g kg <sup>-1</sup> DM)	23.0 <sup>b</sup>	39.0 <sup>a</sup>	37.1 <sup>a</sup>	36.4 <sup>a</sup>	25.1 <sup>b</sup>	37.7 <sup>a</sup>	2.088	34.7	31.4	1.705	**	**	NS	NS
Acetic acid (g kg <sup>-1</sup> DM)	10.2 <sup>b</sup>	14.5 <sup>a</sup>	10.1 <sup>b</sup>	12.8	10.9	10.9	0.872	7.4 <sup>b</sup>	15.6 <sup>a</sup>	0.712	**	NS	**	*
Butyric acid (g kg <sup>-1</sup> DM)	1.2 <sup>a</sup>	$0.4^{b}$	0.3 <sup>b</sup>	0.4	0.8	0.7	0.115	0.5 <sup>b</sup>	0.8 <sup>a</sup>	0.094	**	NS	*	NS
Flieg-Zimmer score	55.8 <sup>b</sup>	86.3 <sup>a</sup>	89.9 <sup>a</sup>	83.4 <sup>a</sup>	67.0 <sup>b</sup>	81.6 <sup>a</sup>	3.211	86.7 <sup>a</sup>	68.0 <sup>b</sup>	2.622	**	**	**	NS
Yeast $(\log_{10} \text{cfu g}^{-1} \text{ FM})$	2.53 <sup>a</sup>	1.54 <sup>b</sup>	1.59 <sup>b</sup>	1.79 <sup>b</sup>	1.93 <sup>ab</sup>	1.94 <sup>a</sup>	0.041	1.81 <sup>b</sup>	1.97 <sup>a</sup>	0.033	**	*	**	NS
Moulds $(\log_{10} \text{cfu g}^{-1} \text{ FM})$	2.99 <sup>a</sup>	1.81 <sup>b</sup>	1.89 <sup>b</sup>	1.96 <sup>b</sup>	2.34 <sup>a</sup>	2.40 <sup>a</sup>	0.053	1.94 <sup>b</sup>	2.52 <sup>a</sup>	0.043	**	**	**	**
Stability (days)	6.50 <sup>c</sup>	9.39 <sup>a</sup>	7.61 <sup>b</sup>	8.44 <sup>a</sup>	8.17 <sup>a</sup>	6.89 <sup>b</sup>	0.350	8.63 <sup>a</sup>	7.04 <sup>b</sup>	0.286	**	**	**	*
Total protein (g kg <sup>-1</sup> DM)	127 <sup>b</sup>	143 <sup>a</sup>	137 <sup>a</sup>	126 <sup>b</sup>	129 <sup>b</sup>	152 <sup>a</sup>	2.618	133	138	2.138	**	**	NS	**
Crude fibre (g kg <sup>-1</sup> DM)	264 <sup>a</sup>	257 <sup>ab</sup>	255 <sup>b</sup>	256 <sup>b</sup>	278 <sup>a</sup>	242 <sup>c</sup>	2.323	254 <sup>b</sup>	263 <sup>a</sup>	1.897	*	**	**	NS
WSC (g kg <sup>-1</sup> DM)	118 <sup>b</sup>	125 <sup>a</sup>	125 <sup>ab</sup>	133 <sup>a</sup>	112 <sup>c</sup>	123 <sup>b</sup>	2.314	132 <sup>a</sup>	113 <sup>b</sup>	1.889	*	**	**	NS
NEL (MJ/kg DM)	5.16 <sup>b</sup>	5.19 <sup>ab</sup>	5.25 <sup>a</sup>	5.26 <sup>a</sup>	5.10 <sup>b</sup>	5.24 <sup>a</sup>	0.025	5.19	5.21	0.020	*	**	NS	NS
DM Intake (kg/d)	6.27	5.88	6.32	-	-	-	0.216	5.94	6.37	0.176	NS	-	NS	-
Daily gains (kg)	0.80	0.87	0.82	-	-	-	0.033	0.76 <sup>b</sup>	0.90 <sup>a</sup>	0.027	NS	-	**	-

Table 1. Chemical composition, aerobic stability and nutritive value of silages

<sup>1)</sup> There was a TxY and TxCxY interacion (P<0.05) only for yeast count and stability; SEM-standard error of means; Values in rows with different superscripts differ significantly (P<0.05); NS – not significant; \*,\*\* - significance of differences and interactions at P<0.05 and P<0.01 respectively

# CONCLUSIONS

The addition of bacterial inoculants containing homo- and heterofermentative LAB improved the fermentation quality and aerobic stability of grass silage made in big bales. The additive treatments did not increase silage intake and had no effect on daily live weight gain.

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# APPLICATION OF NATURAL ANTIBACTERIALS IN PRESSED PULP SILAGE PRODUCTION

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#### ABSTRACT

During the application of natural antibacterials – products based on hop components, tree resins and fatty acids – in the extraction area of sugar factories, it was observed that these substances are effective against clostridia in very low concentrations. These findings led to considerations to apply natural antibacterials for preventing silage spoilage caused by clostridia. In systematic laboratory test series with pressed pulp and grass, the plant material was contaminated with clostridia by soil addition. The outcome was that additions of natural antibacterials to contaminated laboratory silages result in drastically lowered butyric acid contents compared to untreated silages. On the other hand, the activity of lactic acid bacteria was not restricted. Based on these results it can be concluded that natural antibacterials support a rapid drop in pH for silage conservation parallel to a selective growth-inhibition of clostridia.

# INTRODUCTION

During the application of natural antibacterials – products based on hop components, tree resins and fatty acids – used in the extraction area of sugar factories, it was observed that these substances are effective against clostridia in very low concentrations. With suitable dosage applications it was possible to suppress a clostridia infection without negatively affecting the favoured flora of lactic acid bacteria (Pollach et al, 2002, Hein et al, 2006). These results led to first considerations to apply natural antibacterials for preventing silage spoilage, especially caused by clostridia (DLG, 2006).

The aim of the study was to develop a laboratory model to determine the effectiveness of natural antibacterials against clostridia in pressed beet pulp silage in order to evaluate the general potential of the substances in the field of silage production.

#### MATERIALS AND METHODS

For the laboratory trials pressed pulp was collected during the 2006 campaign in Tulln and stored in a deep freezer. The application of soil, natural antibacterials and commercial silage additives (by means of a sprayer) and, for some variants, carbonate, was done in a concrete mixer. After careful mixing the treated material was transferred into 1 L glass jars and incubated at 25 °C. Variants were prepared in duplets for every sampling date. Samples were taken after 0, 2, 7, 14, 30, 60 and 90 days and analysed for the parameters dry matter, pH and organic acids by means of HPLC. Sampling was performed in duplets from every glass jar for a specific incubation time. Mean values in the figures are based on 4 single-values of two glass jars of each variant at a specific incubation time and are calculated on dry matter. Standard error for pH-determinations is  $\pm 1$  %, for HPLC-determinations  $\pm 5$  %.

#### **Investigated substances**

50:50 mixture of hop beta acids & rosin acids prepared of the following products:

- Hop beta acids: BetaStab 10A (BetaTec Hopfenprodukte GmbH, D Nuremberg)
- Rosin acids: PileStab 20A (Zuckerforschung Tulln GmbH, A Tulln)
- Silage additives [applied according to the recommendations of the producer]
  - LagroSil pH Granulat: mixture of lactic acid bacteria (Garant Tierernährung GesmbH, A Pöchlarn)
  - Bonsilage Forte Flüssig: mixture of lactic acid bacteria (H. Wilhelm Schaumann GmbH & Co KG, A Brunn am Gebirge)

# **RESULTS AND DISCUSSION**

Fig. 1 shows lactic acid, acetic acid and butyric acid contents in soil contaminated pressed pulp silages to which 50:50 mixtures of hop beta acids and rosin acids (ppm on fresh matter) were applied. Despite rather high butyric acid contents in the control, treated variants are nearly free of butyric acid. Fig. 2 depicts the content of organic acids in soil contaminated pressed pulp silages to which natural antibacterials and commercial silage additives (one of them with known effectiveness against clostridia) were applied. The silages were additionally treated with carbonate (10 g/kg dry matter) for pH buffering. The control contains large amounts of butyric acid – also variants treated with silage starter cultures show rather high butyric acid contents. Natural antibacterial treated variants limited butyric acid formation and obviously clostridia growth.

#### CONCLUSION

Additions of natural antibacterials to soil contaminated laboratory silages result in drastically lowered butyric acid contents compared to untreated silages. On the other hand, the fermentation activity of lactic acid bacteria was not

20000

10000

90

restricted. Based on these results so far, it can be concluded that natural antibacterials support a rapid drop in pH for silage conservation parallel to a selective growth-inhibition of clostridia. Further test series also indicate equal effectiveness to commercial silage starter products. Due to the fact that soil contamination is of special importance in the field of grass silage, and particularly in the field of organic farming, a strong potential is seen in this field.











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# TALL OAT GRASS (*ARRHENETHERUM ELATIUS*) SILAGE QUALITY DEPENDENCE ON STAGE OF GROWTH AND WILTING

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#### **INTRODUCTION**

Tall oat grass is plant species that is more raised in Serbia, because of satisfying nutrition value and high yield (Vučković, 2004). Most common way of its usage is in form of hay. However, weather conditions in May, (when first cut of perennial legumes and grasses is ready in Serbia), are often very unsuitable for natural drying. Therefore silage technology is very significant, because herbal material can be conserved with high contents of humidity. However abundant rainfall in this period disables field works and functioning of mechanisation, so optimal deadline for harvest cannot be fulfilled, and also, suitable aerating cannot be achieved. For that reasons, influence of different stages of cutting and different level of dry matter on chemical composition and silage quality of tall oat grass was studied in this experiment.

### MATERIALS AND METHODS

In the experiment tall oat grass from first cut was silaged. This research was set as two factorial experiment, by scheme 2x2, in three repetitions, examined factors were: A= growth stage ( $a_1$  =stage of earring and  $a_2$  = stage of blooming; in interval of 7 days) and B= level of dry matter ( $b_1$ = mass with natural content of humidity and  $b_2$  = wilted mass). From silage sample were researched chemical composition and quality after ending of fermentation; statistical calculations were made using software Statistics 6 (2003)

# **RESULTS AND DISCUSSION**

Sugar quantity and buffer capacity are crucial for persistence of silaging and silage quality. Tall oat grass biomass derived from this experiment have had more sugar and more suitable buffer capacity in relation to optimal values for perennial grasses that are pointed to by Beyer at al. (1982), ant which are 115 gkg<sup>-1</sup> DM for sugar, and 47 meqv LA 100g<sup>-1</sup> DM for buffer capacity. According to that, with respect to all of other major factors, forming of quality silages can be expected.

Parameters	$A_1$	$A_2$	$B_1$	$B_2$	LSD 0.05	LSD 0.01
Total sugar (S), gkg <sup>-1</sup> DM	187,5a	159,3b	182,3a	164,5b	13.90	21.50
Buffer capacity (BC),	32.82a	29.83b	34.18a	28.47b	2.36	3.57
meqv LA100g <sup>-1</sup> DM						
S/BC	5.79a	5.35b	5.79a	5.36a	0.73	1.10
DM, gkg <sup>-1</sup>	308.3b	322.5a	233.8b	392.0a	4.37	6.63
Crude proteins, gkg <sup>-1</sup> DM	156.0a	138.2b	144.2b	150.0a	2.91	4.40
Crude fat, gkg <sup>-1</sup> DM	50.05a	48.2a	49.3a	48.9 a	9.2	13.9
NDF, gkg <sup>-1</sup> DM	690.1a	704.4a	711.7a	691.0a	19.51	29.51
ADF, gkg <sup>-1</sup> DM	338.7a	340.3a	328.8a	335.2a	13.43	20.30
Hemicelluloses, gkg <sup>-1</sup> DM	351.3a	364.1a	382.8a	355.8a	13.30	20.20
Ashes, gkg <sup>-1</sup> DM	104.4a	97.6b	102.8a	99.2a	4.69	7.10
Ca, gkg <sup>-1</sup> DM	5.4a	4.9a	4.7b	5.6a	0.78	1.18
P, gkg <sup>-1</sup> DM	3.53a	3.05b	3.33a	3.25a	0.27	0.40

Table 1. Suitability for biomass silaging and chemical composition of silages

<sup>a,b</sup> Values in the same row for the same factor marked by different letter are statistically different (P<0.05)

Later cutting and earring have brought to significant increase in silages (table 1.). Tall oat grass silage that was cut during blooming stage, have had significantly less crude proteins, ashes and phosphorus, but this factor have not had influence on quantities of fat, NDF and ADF fractions of fibbers, hemicelluloses and calcium (table 1.). Quantity of proteins, calcium and phosphorus was higher than average values for this species that are presented by Swedrzynsky and Kozlowski (1998), and significantly higher values for proteins (166-180gkg<sup>-1</sup>DM) and lower values for NDF (523-530gkg<sup>-1</sup>SM) and ADF (254-265 gkg<sup>-1</sup>SM) were assessed by Aufrére et. al. (2008). Silage made from wilted material have had significantly higher content of proteins , which can be explained by lower quantity of ammonium nitrogen and by lower losts of evaporable nitrogen matter during drying of samples for chemical analyses.

Fermentation in silages made from wilted material was of smaller scale, but with higher ratio of lactic acid in total acid matters and with total absence of butyric acid. In the same treatments, significantly higher pH value caused by

reduction of fermentation was determined. In opposite to that, significantly lower quantity of ammonium nitrogen as an index of final degradation of proteins was determined in the silages made from wilted material (table 2.). Same silages have had significantly lower quantities of soluble nitrogen, which is result of adequate conditions for silaging, mostly lower humidity, which is significant for activity of microorganism enzymes (McDonalds at al. 1991.).

For grading silage quality Flieg's, DLG and Zelther's methods were used. According to Flieg (based on acids ratio), later cutting and earring have brought to increase of quality for one class. Same tendencies were determined by using Zelter's method (which considers quantity of ammonium nitrogen, acetic and butyric acid). When DLG method was used (content of ammonium nitrogen and relative ratio of acids), no differences in silage quality were determined, and all of them ere graded as I class.

Later cutting of tall oat grass, which can be consequence of unfavourable weather conditions, have negative influence on protein quantity, while it has not any significant parameter changes, which define digestibility of silage. Aerating reduces fermentation, but has beneficial influence on relative production of lactic acid and reduces proteolysis. Usage in later stages and aerating by Flieg and Zelter brings to increase of silage quality for one class. Regardless on indicated variations, all silages had been useful.

Parameters	$A_1$	A <sub>2</sub>	$B_1$	$B_2$	LSD 0.05	LSD 0.01
pH	4.38a	4.13b	4.00b	4.50a	0.14	0.22
Lactic acid, gkg <sup>-1</sup> DM	52.78a	56.58a	68.30a	41.07b	7.06	10.70
Acetic acid, gkg <sup>-1</sup> DM	18.88b	21.45a	24.50a	15.83b	1.79	2.72
Butyric acid, gkg <sup>-1</sup> DM	7.55a	0.0b	7.55a	0.0b	0.68	1.03
Lactic/total	0.67	0.73	0.68	0.72	-	-
NH <sub>3</sub> -N, gkg <sup>-1</sup> N	92.6a	93.6a	113.6a	72.6b	1.98	3.00
Soluble N, gkg <sup>-1</sup> N	685.2a	632.8b	725.4a	592.6b	3.84	5.82
Quality class by Flieg	II	Ι	II	Ι	-	-
Quality class by DLG	Ι	Ι	Ι	Ι	-	-
Quality class by Zelter	II	Ι	II	Ι	-	-

Table 2. Biochemical changes in the silages

<sup>a,b</sup> Values in the same row for the same factor marked by different letter are statistically different (P<0.05)

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# EFFECT OF ADDITIVE SUBSTANCES IN THE GRASS SILAGES

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# INTRODUCTION

Silage additives have an ability of improve the fermentative process in the present of nutrient losses minimalization during conservation. Current aspects of grass silage quality assessment and use of silage additives described Richardt 2007. Essentials and principles of silage treatment described Pahlow, 2007. Addition of lactic fermentation bacteria caused on file of observed silages the increase in lactic acid and pH reduction (Hejduk a Doležal 2004). Biological additives caused faster pH decrease compared with a control silage without additives, especially in inoculates with LAB or with an addition of saccharolytic enzymes (Woolford 2004). It conduce to the rapid decline of coliform bacteria, higher pH decrease intensity and to the proteolysis finish. In the silages preserved by means of the biological additives, there is the fermentative process faster finished and it is possible to feeding them earlier than the silages without preserving agents and chemical additives.

# MATERIALS AND METHODS

In the experimental observation there was 45 samples of grass silages from the working conditions in a piedmont area. Samples were divided into three groups. There was 15 silage samples from the first cutting on every group. In the first group there were silages without preserving agents, the second group presented silages with bacterial preparation and in the third group there was used bacterial - enzymatic preparation. Nutrient analyse, acids content in silage, pH, degree of proteolysis was determined by ÚKZUZ methods. Acids in silage were determined by means of isotachoforetical analyzer. Composition of used preservatives is noted in the table No. 1 and 2.

Species (strains) of microbes and	their minimum	Enzymes and their minimum act	ivity in	Other
quantity in additive (CFU/g)		additive (nkat/g, nkat/ml)	components	
L. plantarum (CCM 3769), L. casei (CCM 3775), E. faecium (CCM 6226), P. pentosaceus (CCM 3770)	1,5 x 10 <sup>10</sup>	cellulase and hemicellulase glukosaoxidase	31 000 4 800	dry whey, saccharose, lactose
L. plantarum (CCM 3769), L. casei (CCM 3775), E. faecium (CCM 6226), P. pentosaceus (CCM 3770)	1,5 x 10 <sup>10</sup>	cellulase and hemicellulase glukosaoxidase	28 000	dry whey, saccharose

 Table 1. Composition of bacterial-enzymatic additives for preservation of roughages (grass)

 Table 2.
 Composition of bacterial additives for preservation of roughages (grass)

Species (strains) of microbes and their minimum quantity in add	litive (CFU/g)	Other components
L. rhamnosus (NCIMB 30121), E. faecium (NCIMB 30122)	R: 1 x 10 <sup>11</sup>	R: dry whey
	G: $2 \times 10^8$	G: calcium carbonate
L. plantarum (CCM 3769), L. casei (CCM 3775), E. faecium	$1 \ge 10^{10}$	dry whey,
(CCM 6226), P. pentosaceus (CCM 3770)		sacharose, lactose
L. plantarum (DSM 4784, DSM 4785, DSM 4786, DSM	R: 1,35 x 10 <sup>11</sup>	R: maltodextrin, sodium
4787), E. faecium (DSM 4788, DSM 4789)	G: 2 x 10 <sup>8</sup>	aluminium silicate, sodium
		thiosulfate, color E133
		G: calcium carbonate

L = Lactobacillus, E = Enterococcus, P = Pediococcus, CFU = colony forming unit, R = soluble, G = granulated

### **RESULTS AND DISCUSSION**

Table No. 3 shows the results of statistical examination of fermentative characteristics, lactic acid, acetic acid, pH values, degree of proteolysis and nutritive value characteristics NDF, NEL. Statistically evidential differences in lactic acid content between the control group and the group with bacterial additive (P<0.05) and bacterial - enzymatic additive (P<0.05) was found. In pH values and degree of proteolysis, evidential differences between the control group and the group with bacterial additive (P<0.05) was found. In pH values and degree of proteolysis, evidential differences between the control group and the group with bacterial additive (P<0.05) was found. Other fermentative and nutritive value characteristics (NDF and NEL) in silages, where the bacterial and bacterial - enzymatic additive was used are statistically nonsignificant. Woolford (2004) described, that the biological additives caused faster pH decline compared with the control silage without additives especially in

inoculants with LAB alone or with saccharolytical enzymes addition. It conduce to the fast coliform bacteria decline, higher intensity of pH decline and to the proteolysis stopping.

	Control group without additive	Bacterial additive	Bacterial fermentative additive
Dry matter in g.kg <sup>-1</sup>	395	398	396
Lactis acid in g.kg <sup>-1</sup> DM	35.53 a	58.57 b	74.07 c
Acetic acid ing.kg <sup>-1</sup> DM	13.44 a	16.88 a	17.91 a
pH	4.74 a	4.25 b	4.29 b
Degree of proteolysis %	10.23 a	6.8 b	7.3 b
NDF in g.kg <sup>-1</sup> DM	504.3	496.1	489.8
NEL in MJ.kg <sup>-1</sup> DM	5.12	5.26	5.32

Table 3.	Mean values of fermentation	characteristics,	NDF, NEL (	(n = 15)
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a,b, means with the others indexes, significant difference in rows (P< 0.05)

### CONCLUSION

Fermentation characteristics, NDF and NEL values in the grass silages ranked into three groups were evaluated. The first group was without preserving agents. The grass silage in the second group was treated with bacterial additive, the third group was treated with bacterial - enzymatic additive. Statistically evidential differences in lactic acid content between the control group and the group with bacterial additive (P<0.05) and bacterial - enzymatic additive (P<0.05) and bacterial - enzymatic additive (P<0.05) was found. In pH values and degree of proteolysis, evidential differences between the control group and the group with bacterial additive (P<0.05) and also between the control group and the group with bacterial - enzymatic additive (P<0.05) was found. Nonsignificant reduction of NDF values associated with conservation of energy was detected.

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# THE INFLUENCE OF CHEMICAL AND BACTERIAL ADDITIVES ON QUALITY AND AEROBIC STABILITY OF ALFALFA SILAGE

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#### INTRODUCTION

Alfalfa (*Medicago sativa*) is more and more popular herbage for silage production in Poland. Alfalfa silage forms a substantial part of diets for farm animals, particularly on farms with shortage of feeds from grasslands. Alfalfa silage has high nutritive value (Pyś, 1997). It is rich in crude protein and exogenous amino acids and has profitable mineral nutrients composition (Gaweł, Brzóska, 2001). But alfalfa herbage is generally considered difficult to ensilage because of low concentration of water soluble carbohydrates (WSC) and high buffering capacity (McDonald et al. 1991). This combination of factors requires that the crop should be wilted to a dry matter (DM) content of a least 300 g/kg before harvesting in order to concentrate the available WSC for successful fermentation. The previous results have shown that fermentation quality of legumes can easily be improved by using additives containing bacterial inoculants or chemical additives (Jatkauskas et all., 2008; Tyrolova & Vyborna, 2006) or when legume-grass mixtures are ensiled (Lättemäe & Tamm, 2005). The objective was to study the effect of bacterial-enzymatic inoculate in comparison with chemical additive on quality and aerobic stability of alfalfa silage.

#### MATERIAL AND METHODS

The trial was conducted under farm conditions at the Experimental Station at Falenty near Warsaw in Poland in 2005. The alfalfa (Medicago sativa) field was cut at the initial flowering stage three times: 20.06 (2<sup>nd</sup> cut), 20.07 (3<sup>rd</sup> cut) and 25.08 2005 (4<sup>th</sup> cut). The first cut and the fifth cut were not studied. The herbage was mown with a rotary mower -conditioner and before harvest was pre-wilted to a dry matter (DM) concentration of approximately 350 g/kg. During each harvest alfalfa silage was produced in big cylindrical bales (about 400 kg). Herbage before ensiling was treated with two commercial silage additives. In the first group of silage the chemical additive containing: propionic acid, formic acid and ammonium was used. The bacterial inoculant containing homo- and heterofermentative lactic acid bacteria (two strains of L. plantarum, L. brevis, L. buchnerii) with enzymes (endo1,4-beta-glukanaza, endo-ksylanase, glukoamylase) was used in the second group of silage. The additives were put into the herbage during bale rolling in a variable bale chamber baler in amounts recommended by manufactures. The big bales were wrapped in four layers of stretch film after transport to their place of storage. Obtained silage were analysed for: DM (oven method), pH, lactic acid, butyric acid, acetic acid (enzymatic tests), and nutritive components content by Near Infra Red Spectroscopy (NIRS) technique. Aerobic stability was evaluated through changes in temperature in silage samples (about 10 kg) stored in aerobic conditions at ambient temperature (about 20°C) for 12 days. Stability was measured as the time necessary to increase silage temperature by 1 °C over air ambient temperature. Data concerning the chemical composition of silage were analysed using analysis of variance in ANOVA 2.

# RESULTS

The quality of herbage used for silage production was good. Because the weather conditions during wilting period and harvest were very good the dry matter level in alfalfa herbage at ensilage was over 350 g/kg and was the highest in the 4<sup>th</sup> cut (>480 g/kg). The crude protein content in herbage was over 200 g/kg DM and crude fibre content - usually below 300 g/kg DM (Table 1).

Harvest	$2^{nd}$ cut		3 <sup>rd</sup>	cut	$4^{th}$ cut		
Additive	Chemikal	Bacterial-	Chemikal	Bacterial-	Chamikal	Bacterial-	
Additive	Chemikai	enzymatic	Chemikai	enzymatic	Chemikai	enzymatic	
Dry matter [g/kg]	408.1	430.0	308.0	342.2	484.3	489.3	
Total protein [g/kg DM]	222.2	225.1	238.3	224.5	215.3	211.1	
Crude fiber [g/kg DM]	291.1	305.6	294.0	310.8	292.1	292.9	
Crude ash [g/kg DM]	108.0	102.3	85.1	145.8	106.4	103.4	
Crude fat [g/kg DM]	26.4	25.1	25.5	27.0	26.9	25.8	

 Table 1.
 Alfalfa herbage composition at ensilage

The quality of all silages was very good. The additive treatment had the influence only on lactic acid concentration in DM of silage. Silages treated with inoculate had higher lactic acid concentration (P>0.01) than the silage with chemical additive. The acetic acid and butyric acid of silage with biological additive was lower and obtained higher Flieg-Zimmer scores than in second treatment but the differences were not significant (Table 2). The time of harvest (cut) influenced the DM content in silage, lactic acid concentration and crude fat content in DM. The silages from the  $3^{rd}$  harvest contained less DM and more lactic acid than silages from the remaining cuts. The aerobic stability of tested silages was similar for both treatments (> 7 days), but a bit better for silages from the  $2^{nd}$  harvest (8 days). The similar results in aerobic stability can be explained by acetic acid concentration that did not differ between treatments (Table 2). Applied additives and time of harvest had no influence on nutritive value of silage except the crude fat content, that was lower in alfalfa silage prepared with bacterial inoculant and made of herbage coming from second cut.

	Additi	ve (A)		Harvest (B)		Significance		
Examined parameters	chemical	bacterial- enzymatic	2 <sup>nd</sup> cut	3 <sup>rd</sup> cut	4 <sup>th</sup> cut	А	В	AxB
Dry matter [g/kg]	438.1	426.8	458.7	339.7	498.8	NS	**	*
pH	4.86	4.87	4.68	4.99	4.94	NS	NS	NS
Lactic acid [g/kg DM]	37.74	49.49	39.17	54.87	36.82	**	**	NS
Acetic acid [g/kg DM]	10.29	8.47	8.03	10.95	9.15	NS	NS	NS
Butyric acid [g/kg DM]	0.43	0.27	0.40	0.52	0.13	NS	NS	NS
Scores in Flieg-Zimmer scale <sup>1)</sup>	90.7	95.8	89.5	94.2	96.0	NS	NS	NS
Evaluation	very good	very good	very good	very good	very good	-	-	-
Stability	7.33	7.89	8.0	7.17	7.67	NS	NS	**
Organic matter [g/kg DM]	886.1	873.4	892.9	868.8	877.5	NS	NS	NS
Total protein [g/kg DM]	214.7	225.2	215.2	222.2	222.4	NS	NS	NS
Crude fiber [g/kg DM]	300.0	302.3	311.5	295.5	296.4	NS	NS	NS
Crude ash [g/kg DM]	113.9	126.6	107.1	131.2	122.4	NS	NS	NS
Crude fat [g/kg DM]	22.2	19.8	22.6	17.4	23.1	*	**	*
JPM	0.80	0.79	0.80	0.79	0.80	NS	NS	NS
JPZ	0.71	0.70	0.71	0.70	0.71	NS	NS	NS
BTJE	80.86	81.71	81.38	81.17	81.31	NS	NS	NS
BTJN	122.08	128.07	122.39	126.39	126.46	NS	NS	NS

Table 2. Fermentation characteristic and nutritive value of alfalfa silage

<sup>1)</sup>100-score scale where 81-100 scores – very good, 61-80- good, 41-60 – satisfactory, 20-40 – poor, 0-20 – bad quality. NS – not significant; \*,\*\* - significance of differences and interactions at P<0.05 and P<0.01 respectively

Figure 1. Aerobic stability of silage with inoculate and chemical additives.



# CONCLUSIONS

Both additives positively influenced the fermentation of alfalfa silage and can be used for improvement of fermentation process in alfalfa silage. In the result all evaluated silages had very good quality. Silage prepared with biological additive contained significantly more lactic acid and crude fat than silage prepared with chemical additive. The differences in aerobic stability between treatments and harvests were rather small. The content of nutritive components in obtained silages was similar to the content in ensilaged herbage. Biological additive occurred to be as effective as chemical additive but is more environmental friendly and can be used in organic system of agriculture production.

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# THE EFFECTIVENESS OF DIFFERENT ADDITIVES ON ENSILING OF BREWERY'S GRAIN

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# INTRODUCTION

Brewery's grain is valuable by-product of brewery industry. With regard for high content of protein with high biological value (Hernandez et al., 1999) and relatively low price, this feed is often use in cattle nutrition (Murdock et al., 1981; West et al, 1994). Unfortunately, in fresh brewery's grain very easy come to process of spoiling (Wyss, 1997). According to FAO recommendations, the maximal storage period for this feed is from one month in winter to only five days in summer. The conservation by ensiling is good way to solution of this problem, but brewery's grain is not ideal material for fermentation process – the water-soluble carbohydrates level is low and moisture is high (Murdock et al., 1981). The very important factor for production good quality brewery's grain silages is rate of pH value decrease (Vyskocil i wsp., 2006). Some additives can accelerate acidification process. The aim of presented study was researched the effectiveness of different additives on ensiling of brewery's grain.

# MATERIALS AND METHODS

The brewery's grain was ensiled without any additive (negative control group), with formic acid and molasses (positive control group), with two pure inoculants (inoculant A: *Enterococcus faecium, Lactobacillus casei, Lactobacillus plantarum, Pediococcus ssp., Lactobacillus buchneri*; inoculant B: *Lactobacillus casei, Lactobacillus plantarum, Lactobacillus lactis, Enterococcus faecium, Pediococcus acidilactici, Lactobacillus casei OSMO 254*) and with one inoculant combined with chemical additive (*Enterococcus faecium*, two strains *Lactobacillus plantarum*, two strains *Pediococcus acidilactici*, potassium sorbinate, organic acids), as well as with two chemical preservatives (preservative A: ammonium formate, formic acid, bezoic acid, propionic acid; presevative B: potassium sorbinate, sodium benzoate). There was experiment on laboratory scale – 3,5 litre microsilos (glass jars), three repetitions for treatment. The chemical composition of silages, quality, as well as aerobic stability (temperature test) were determined. Additionally, the organic matter digestibility coefficients according to Kesting (*in vitro*) and dry matter and protein losses were calculated. Obtained results were statistically analyzed by one-way analysis of variance and multiple-range Duncan's test using the software StatSoft (2001).

# **RESULTS AND DISCUSSION**

Applied additives affected some chemical components of silages (tab 1). Generally, all additives (without one – inoculant + chemical additive) decreased the level of fibre fractions and increased N-free extract. However, the concentration of protein and fat, as well as OM digestibility were less differential.

	Dry matter	Crude	Crude	Crude fibre	NDF	ADF	Crude	N-free	OM
Additive	g/kg	ash	protein				fat	extract	digestibility
				g/]	kg DM				%
Control – no	218,2ABab	37 94 Ba	284.64Ba	182 84 Bab	766 0Aab	272 7h	47.9	446 8ab	74
additive		57,7ADa	204,0ADa	102,0ADa0	700,0740	272,70	ч7,7	++0,0a0	/4
Formic acid + molasses	211,7BCbc	45,1Cc	265,5Ab	164,9Cc	689,1Bc	246,7ab	60,1	464,4b	77
Inoculant A	223,9Aa	37,2Aa	283,6ABa	165,2Cc	755,8ABab	220,4a	42,0	472,0b	76
Inoculant B	218,8ABa	37,3Aa	283,9ABa	166,7BCc	754,0ABab	238,2ab	47,4	464,7b	77
Inoculant +	221 / A Ba	41 7BCh	280 0Ba	103 5 4 2	786 2 1 2	255 0ab	16.5	120 12	76
chemical add.	221,4ADa	41,7DC0	289,0Da	195,5Aa	780,2Ad	235,040	40,5	429,4a	70
Chemical	206.800	36.61.2	283 QABa	160 0BCc	712 6 A Bbc	221.22	54.4	156 Jah	76
preservative A	200,800	30,0Aa	205,9ADa	109,0000	/12,0AD00	221,2a	54,4	430,240	70
Chemical	216 9ABab	37 0 A a	284 4 A Ba	175 5BCbc	748 2 A Bab	240 1ah	49.5	453 5ab	76
preservative B	210,7ADa0	57,0Ma	204,4ADa	175,5000	740,2ADa0	2-10,140	ч,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	ч <i>ээ</i> , <i>э</i> ао	70

Table 1. Chemical composition and organic matter digestibility of brewery's grain silages

A,B values in the rows with different letters differ significantly ( $P \le 0.01$ )

a,b values in the rows with different letters differ significantly  $(P \leq 0,05)$ 

Interesting results in the case of dry matter and protein losses were stated (tab. 2). After use the chemical additives, the losses were clearly lower, what could be explained by suppression of fermentation intensity and reduction of effluent release. On the contrary, the significantly differences for acid concentrations were not observed, however, silages with formic acid + molasses and with preservative A were characterized by lowest pH values (tab.2). Moreover, silage with chemical additive A contained very high level of ammonia nitrogen (tab. 2), what probably was connected

with restriction of effluent losses, which is rich in soluble ammonia nitrogen. This thesis corroborate lowest concentration of dry matter, observed for this silage (207 g/kg) - tab. 1. Another reason could be a fact, that preservative A contained ammonium formate.

All additives improved aerobic stability (fig. 1). The ranking of time of stability (number of hours, after which temperature increase two degrees over temperature of environment) was following: control -8 hr, silage with formic acid + molasses -30 hr, silage with chemical additive B -48 hr, another silages were stable to the end of experiment, i.e. during 120 hours.

Additive	Dry matter losses	Protein losses	Acetic acid	Butyric acid	Lactic acid	pН	N-NH <sub>3</sub> % N total
	%	%		g/kg DM			
Control Control – no additive	15,69Aa	13,69Aa	29,4	0,29	25,4	4,88Aa	4,3A
Formic acid + molasses	3,74DEe	3,16Ccd	50,7	0,00	44,4	4,43Cc	5,6A
Inoculant A	14,52ABa	9,43ABab	24,0	0,00	38,0	4,67ABb	4,6A
Inoculant B	9,66BCbc	7,08BCbc	31,7	0,00	33,0	4,73ABab	6,7A
Inoculant + chemical add.	12,12ABCab	10,24ABab	32,6	0,00	11,6	4,65Bb	7,5A
Chemical preservative A	1,19Ee	0,95Cd	51,5	0,00	55,5	4,36Cc	31,3B
Chemical preservative B	7,78CDc	6,27BCbc	36,0	0,00	46,0	4,87Aa	6,1A

Table 2.	Dry matter and	protein losses di	ring production	of brewery's	s grain silages	, as well as quali	ty of these silages
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A,B values in the rows with different letters differ significantly ( $P \le 0.01$ ) a,b values in the rows with different letters differ significantly ( $P \le 0.05$ )

24 23 ပ 22 21 20 0 20 40 60 80 100 120 hours - control chemical additive II chemical additive I formic acid+molasses inoculant+chemical additive × inoculant I - inoculant II

# Figure 1. Temperature changes of brewer's grain silages' samples during aeration experiment (environment's temp.=21°C)

# CONCLUSIONS

The use of chemical additives on ensiling brewery's grain suppressed fermentation intensity and could reduced effluent losses. The additives: both inoculants, inoculant + chemical additive and preservative A very effectively protected brewery's grain silages against aerobic spoilage.

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# THE CHEMICAL COMPOSITION, QUALITY AND AEROBIC STABILITY OF SILAGE MADE FROM FIELD BEAN-MAIZE MIXTURES

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# INTRODUCTION

The studies concerning evaluation of silages made from whole crop maize and legumes more and more frequently appear on literature (Andrighetto et al., 1992; Baskay and Vetesi, 1995; Hart et al., 2003; Szyszkowska et al., 2007). Two-components silages from these plants are characterized by better energy : protein ratio and mineral composition. The aim of study was evaluation the effect of different, multi-components microbial additives on chemical composition, quality and aerobic stability of maize silages, as well as filed bean-maize silages.

# MATERIALS AND METHODS

The harvest of whole crop field bean was carried out after twelve weeks of vegetation. At this moment maize crop was in wax stage of maturity. Six variants of silages was prepared: 1. maize without additives, 2. maize with inoculant A (*Enterococcus faecium, Lactobacillus casei, Lactobacillus plantarum, Pediococcus ssp., Lactobacillus buchneri*) 3. Maize with inoculant B (*Lactobacillus casei, Lactobacillus plantarum, Lactobacillus lactis, Enterococcus faecium, Pediococcus acidilactici, Lactobacillus casei OSMO 254*) 4. Maize + Field bean (50:50 % on the basis of fresh matter) without additives, 5. Maize + Field bean with inoculant A, 6. Maize + Field bean with inoculant B. There was experiment on laboratory scale – 3,5 litre microsilos, three repetitions for treatment. The chemical composition of silages, quality, as well as aerobic stability (temperature test) was determined. Obtained results were statistically analyzed by one-way analysis of variance and multiple-range Duncan's test using the software StatSoft (2001).

### **RESULTS AND DISCUSSION**

Higher dry matter concentration was observed in two-components silages as compared with maize silages (tab.1). It was caused by higher content of dry matter in field bean green crop at the harvest time. Maize-field bean silages without additives and with additive A were significantly higher ( $P \le 0.05$ ) crude protein level as compared with maize silage without additives. Although the trend to decrease of crude fibre and increase crude fat in silages with microbial additives was observed, significantly differences were not stated. Similar results were obtained in another study (Filya et al., 2006), in which the effect of biological additive on protein and fibre concentration in maize silages was not confirmed.

	Dry matter	g/kg DM						
Silage	o/kg	Crude	Crude fibre	Crude fat	N-free	Crude ash		
	5/16	protein	Crude nore	Crude lat	extract	Cruce asi		
Maize without additives	319,2	62,0a	286,7	17,0	576,0	58,3		
Maize with additive A	305,1	63,5ab	264,0	22,9	595,8	53,9		
Maize with additive B	320,5	64,8ab	273,9	20,2	583,2	57,9		
Maize + Field bean without	240.2	78.2h	271.1	12.4	506.0	67.2		
additives	540,5	78,50	271,1	13,4	390,9	07,5		
Maize + Field bean with	364.6	70.0b	249.6	20.4	501 /	50.7		
additive A	504,0	79,00	249,0	20,4	391,4	39,1		
Maize + Field bean with	366.8	73 8ab	270.0	22.0	572 7	60.7		
additive B	500,8	75,840	270,0	22,9	572,7	00,7		

Table 1.    Chemical of	composition	of silage
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a,b values in the columns with different letters differ significantly ( $P \le 0.05$ )

The use of additive B to maize silage had an influence on decrease  $NH_3 - N$  level in comparison to both silages without additives. Maize-field bean silage was characterized by lowest lactic acid concentration (P $\leq$  0,01). The pH value was lowest in maize silage without additives (P $\leq$  0,01).

Both silages without additives were unstable in air condition: maize silage after 41 hours had lost the stability (number of hours, after which temperature increase two degrees over temperature of environment), maize-field bean silage – after 50 hr. Both microbial additives improved aerobic stability (fig. 1) - silages with these preparations were stable to the end of experiment, i.e. during 120 hours.

# CONCLUSIONS

Production of two-components silages from maize and field bean whole crop (in share 50:50%) is good method to increase concentration of protein in feed. Quality of these silages is not worse in comparison with maize silages.

Used biological additives very significantly affected increase of lactic acid level in two-components silages made from maize and field bean whole crop. Applied biological additives effectively increased aerobic stability of maize silage, as well as maize-field bean silage.

Silage	NH <sub>3-</sub> N % N total	Acetic acid g/kg DM	Lactic acid g/kg DM	Butyric acid g/kg DM	pН
Maize without additives	6,4a	38,4 ab	34,88 A	0	3,4A
Maize with additive A	5,1ab	42,8 a	38,2 A	0	4,4B
Maize with additive B	4,6b	26,6 b	36,08 A	0	4,3B
Maize + Field bean without additives	6,6a	48,05 a	22,0 B	0	4,5B
Maize + Field bean with additive A	5,1ab	19,34 b	34,37 A	0	4,4B
Maize + Field bean with additive B	5,3ab	39,8 a	37,49 A	0	4,4B

A,B values in the columns with different letters differ significantly ( $P \le 0.01$ ) a,b values in the columns with different letters differ significantly ( $P \le 0.05$ )



Figure 1. Temperature changes of silages' samples during aeration experiment (environmet's temp. = 21°C)

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# QUALITY AND CHEMICAL COMPOSITION OF TWO-COMPONENTS SILAGES FROM WHOLE PLANT OF SOYABEAN AND MAIZE

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# INTRODUCTION

The increase of farming animals number involve more effective exploitation of farmland. The cultivation two plant species on the same area at the same time (for example as a bicropping) is the possibility for intensification of feed production (Dinic et al., 1999). From these reasons the studies concerning evaluation of two-components silages made from whole crop maize and legumes more and more frequently appear on literature (Andrighetto et al., 1992; Baskay and Vetesi, 1995; Hart et al., 2003; Szyszkowska et al., 2007). The aim of study was evaluation the effect of different whole plant maize ration to whole plant soybean on chemical composition and quality of two-components silages.

# MATERIALS AND METHODS

Five variants of silages was prepared – on the basis of fresh matter, the percentage proportions of weight maize to soyabean were: 100:0, 75:25, 50:50, 25:75, 0:100. There was experiment on laboratory scale – 3,5 litre microsilos, three repetitions for treatment. The chemical composition of silages and quality were determined. Obtained results were statistically analyzed by one-way analysis of variance and multiple-range Duncan's test using the software StatSoft (2001).

# **RESULTS AND DISCUSSION**

All silages were characterized by similar concentration of dry matter, crude fibre and crude ash(tab. 1). Significantly differences (P $\leq$ 0,01) were observed in protein level for maize silage and other silages. The lowest concentration of crude fat was noted in soyabean silage (14,5 g/kg DM), the highest – in maize-soyabean silage (75:25). Moreover, significantly difference (P $\leq$ 0,05) between monospecies silages (maize vs. soyabean) for N-free extract levels was stated.

Compound	Percentage ratio of maize to soyabean in silages							
Compound	100:0	75 : 25	50:50	25:75	0:100			
Dry matter g/kg	268,0	257,7	236,0	263,5	244,9			
Crude protein g/kg DM	52,0 B	77,2 A	82,4 A	85,7 A	85,5 A			
Crude fat g/kg DM	19,4 ab	21,3 a	15,9 ab	16,1 ab	14,5 b			
Crude fibre g/kg DM	252,1	270,4	242,0	252,4	280,5			
N-free extract g/kg DM	624,7 b	566,9 ab	595,4 ab	577,1 ab	541,4 b			
Crude ash g/kg DM	51,9	64,3	64,3	68,7	78,1			

**Table 1.** Chemical composition of silages made from maize-soyabean mixtures

A,B values in the rows with different letters differ significantly ( $P \le 0,01$ ) a,b values in the rows with different letters differ significantly ( $P \le 0,05$ )

The quality parameters (VFA, N-NH<sub>3</sub>, pH) were very differential (tab. 2). The best acid profile and quality was observed in silage made from mixture 25% maize - 75% soyabean. However, the worst quality parameters were noted in soyabean silage, which was characterized by high level of butyric acid and its quality was bad (only 10 points in Flieg-Zimmer scale). In this silage dissolution of protein to ammonia was the highest (almost 14%) and pH value exceeded 5,3, also. Unexpected, high concentration of acetic acid was observed in pure maize silage.

# CONCLUSIONS

Two-components silages made from whole plant maize and soyabean contain 20-30 g/kgDM more protein as compared with pure maize silage. It is possibility to improve quality of soyabean silage through combined ensiling with maize. The best option for two-components silages made from whole plant maize and soyabean is mixture in percentage weight proportion 25 to 75.

Table 2. Quality of silages made from maize-soyabes	an mixtures
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Compound	Percentage ratio of maize to soyabean in silages							
Compound	100:0	75 : 25	50 : 50	25 : 75	0:100			
Lactic acid g/kg DM	4,7 Aa	11,0 ABa	23,3 ABa	61,3 Bb	28,4 ABab			
Acetic acid g/kg DM	117,4 a	96,3 ab	63,7 b	28,1 b	28,9 b			
Butyric acid g/kg DM	0,0 A	0,0 A	0,0 A	0,2 A	44,1 B			
Score acc. to Flieg-Zimmmer scale	50 Ab	54 Aab	55 Aab	64 Aa	10 Bc			
N-NH <sub>3</sub> %N total	7,56	5,58	8,29	7,56	13,9			
Ph	4,36 Aa	4,38 Aab	4,37 Aab	4,56 Ab	5,34 Bc			

A,B values in the rows with different letters differ significantly (P  $\leq$  0,01)

a,b values in the rows with different letters differ significantly ( $P \le 0.05$ )

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StatSoft . Statistica - Data Analysis Software System. Version 6. (2001): Available at: www.Statsoft.com.
# ENSILAGE OF WILTED LUCERNE TREATED WITH DIFFERENT TYPES OF BIOLOGICAL PRESERVATIVES

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# INTRODUCTION

Lucerne is one of the most valuable protein-feedstuff of Hungary. 60-65% of the lucerne is consumed by farm animals as fresh forage or hay, while the remaining 35-40% is used for production of haylage or silage. The dry matter and fermentable carbohydrate content of lucerne is relatively low at harvesting. Besides, its buffer capacity is high because of the high protein content and cation concentration. Due to these facts lucerne belongs to the food that is not easily fermentable. Because of these disadvantages for silaging of lucerne biological additives are frequently used. Recently biological additives containing lactic acid bacteria have been used. Due to this the fermentation processes become advantageous: the amount of lactic acid increases, while the acetic acid content, pH and NH<sub>3</sub>-N decreases in silage. Larger part of biological preservatives contains not only one but more genus and strains of lactic acid bacteria. These microbes complement each other's activities during the fermentation processes. *Enterobacter* sp. ferment sugars to acetic acid and initiate the fermentation but are not tolerant for acids at all. The *Streptococcus* sp. grows fast but are relatively weak acid producers. The *Lactobacillus* and *Pediococcus* grow more slowly but are able to produce copious quantities of acid (Woolford, 1984).

# MATERIALS AND METHODS

The ensilage-experiment was carried out by the Animal Nutrition Science Department of University of Szeged, College of Agriculture Hódmezővásárhely, in Hungary in the year 2006. The basic row material originated from 2<sup>nd</sup> cut lucerne. The lucerne was cut with rotation scythe in the mid of blooming/early flowering maturity. The chopping was carried out with Jaguar-chopper. The chop length was 2-4 cm. The temperature for wilting on the swath was fit for the season (there was no rain during the harvesting).

#### Treatments:

- **T0** Untreated control
- T1 Lactobacillus plantarum + Pediococcus pentosaceus  $(9,1 \times 10^{10} \text{ CFU/g inoculant})$  1 g/ 1 tonne wilted lucerne
- **T2** Lactobacillus pentosus DSM 14025 (1 x 10<sup>11</sup> CFU/g inoculant) + Pediococcus pentosaceus DSM 14021 (2,5 x 10<sup>10</sup> CFU/g inoculant) 1 g/ 1 tonne wilted lucerne
- **T3** Lactobacillus pentosus DSM 14025 (5 x  $10^{10}$  CFU/g inoculant) 2 g/ 1 tonne wilted lucerne

On micro-size ensiling evenly spread chopped and wilted lucerne on plastic foil (100 kg / treatment) was used, then vaporized with the silage additives and finally mixed them. For each treatment 24 pcs small sized containers of 4.2 l cubic capacity was filled and closed with screwed hat. The average weight of filled containers was 3,6-3,7 kg. The filled micro containers were stored for 100 days. Storage took place at 23-25C° interior temperatures, in dark room. The containers were open on the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 6<sup>th</sup>, 14<sup>th</sup> and 100<sup>th</sup> days following the day of ensilage and laboratory analysis were performed. Samples taken from the starting material of ensiling was considered as 0 day samples and examined in laboratory immediately: dry matter, buffer capacity, carotene, and pH. After further preparations the following analysis were performed: Weende analyses (crude protein, crude fat, crude fibre, NDF, crude ash) and water-soluble carbohydrate (WSC).

The examination on 1<sup>st</sup> -14<sup>th</sup> days was focused primarily on the products and the dynamics of fermentation. The following factors were measured: dry matter, organic acids (lactic acid and volatile fatty acids), pH, NH<sub>3</sub> –N, water soluble carbohydrates (WSC) and ethanol (only on 14<sup>th</sup> day).

The chemical analyses of silages on 100<sup>th</sup> day after ensilage were as follows: dry matter, pH, Weende analyses (crude protein, crude fat, crude ash, crude fibre) to give ME, NE (m), NE (l), NE (g), NDF buffering capacity, water-soluble carbohydrates (WSC), carotene, lactic, acetic, propionic and butyric acids, ammonia-N, ethanol, dry matter losses, begin aerobic stability study by HONIG method (1986).

# **RESULTS AND DISCUSSION**

The dry matter content of starter row material was 45.1 %. Its buffer capacity was 55.1 g/kgDM. This figure is significantly lower than the buffer capacity of green lucerne, according to the literature in general (75 g/DM). It means that wilting improved the fermentability of the row material considerably. The 62 g/DM WSC content refers to a medium amount of respiratory loss during wilting. The chemical composition (crude protein, metabolisable protein (MPE, MPN), crude fat, crude fibre, NDF, crude ash) and the calculated netto energy contents (NE<sub>m</sub>, NE<sub>g</sub>, NE<sub>l</sub>) were

similar to the early flowering maturity standard of lucerne. Carotene content was reasonable, 108 mg/kg DM.

<u>**T0** (Control) silage</u>: The starter 6,0 pH decreased slowly but gradually until 4.8 pH to the  $14^{th}$  day of fermentation, and settled down on 4.7 pH at the end. This level was detected on the  $100^{th}$  day of storage. The lactic acid production increased gradually until the  $3^{rd}$  day of fermentation. During the following period of the fermentation the lactic acid production fluctuated, and 2.9 %/DM was detected on the  $100^{th}$  day of fermentation.

Volatile fatty acid production started powerfully. The growing of acetic acid production continued on the first phase of fermentation, 4.9 DM % was detected on  $14^{th}$  day. There was no increase in volatile acid production during the following period, and the propionic acid dissolved presumably. A very small amount of butyric acid was shown on the  $14^{th}$  day only (0.1 DM%) and that amount disappeared at the end. The NH<sub>3</sub> content increased gradually during the fermentation and storage. The ethanol content of silage was small both on the  $14^{th}$  and 100th day.

<u>**T1 treated silage:**</u> The pH decreased gradually from 6 to 4.64 pH for  $14^{th}$  days of fermentation and remained almost the same during 100 days of storage. The lactic acid production increased slowly during 3 days of fermentation but increased powerfully by the 6<sup>th</sup> day (more than double amount of the 3<sup>rd</sup> day's production could be detected, which was 2.8 %). It means that the most powerful activity of lactic acid bacteria was between the 3rd-6th days of fermentation. After 6 days the lactic acid production hardly increased and kept on 3.2 DM % until the end. The acetic acid production was vigorous from the beginning to the end, and it was similar to the control samples. There was no butyric and propionic acid production. Ethanol content of the silage on the  $14^{th}$  and  $100^{th}$  days of storage was similarly low like in the control. The NH<sub>3</sub> content increased during the storage, but it was significantly lower than in the control samples.

<u>**T2 treated silage:**</u> The pH of T2 treated lucerne decreased until the  $6^{th}$  day of fermentation and remained on the same level during further fermentation and storage. The fermentation activity of microbes was strong from the beginning to the end. The production of lactic acid continuously increased to the  $6^{th}$  days at stayed in the same level until the 100<sup>th</sup> days. The acetic acid production was intensive from the beginning and increased to some degree up to 3.9 DM %. There was neither propionic acid nor butyric acid production during the 100 days of the experiment. The ethanol production was higher than in the control on the  $14^{th}$  day of fermentation, but the NH<sub>3</sub> production was considerably less during the 100 days of storage.

<u>T3 treated silage</u>: The silages achieved the standard final pH 4.7 on the 6<sup>th</sup> day of fermentation. On the first day the pH reduction was originated from the acetic acid production, while the lactic acid was produced from the second day. The highest level of lactic acid production was detected on the 6<sup>th</sup> day of fermentation (3.7 DM %) which decreased to 2.7 DM % at the end of 100 days storage. There was no butyric or propionic acid production at all. The ethanol production was similar to the control silages, but the NH<sub>3</sub> content was significantly less (P=0.1 %) in the treated silages than in control. pH of the T3 treated silage was significantly lower compared to the control.

The statistical evaluation of data compared to control proved quicker pH reduction (P=5%, 0.1%) and less NH<sub>3</sub> production (P=0.1%) during fermentation. Both the control and all treated silages remained stable during 7 days according to the result of the aerobic stability experiments.

# CONCLUSION

The characteristics of ensilaged row material were similar to the early flowering maturity medium wilted (45 % DM) lucerne. The initial fermentation was the strongest by the T2 treatment (*Lactobacillus pentosus* + *Pediococcus pentosaceus*). The T3 treatment (*Lactobacillus pentosus*) increased the lactic acid production effectively from  $2^{nd}$  day to 6 day. The lactic acid production of T1 (*Lactobacillus plantarum* + *Pediococcus pentosaceus*) treated lucerne was slow on the first 3 days of fermentation, but it was higher than the untreated control on the  $6^{th}$  day. The pH of T2 and T3 treatment than that of the control. The carotene loss was less in treated silages and considerably less with *T3* treatment. This difference between the control and T3 treatment was significant. There were some differences in the other fermentation products and in the nutritive value of silages, but these were not significant. All silages remained stable on the 7 days aerobic conditions. It can be concluded that the inoculants applied were able to help the domination of Lactobacillus bacteria during the first phase of fermentation and resulted healthy silages with good quality.

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# THE EFFECT OF SILAGE ADDITIVE ON THE QUALITY OF BREWER'S GRAINS ENSILED WITH THE SUPPLEMENTATION OF MOISTURE SORBENT

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#### INTRODUCTION

Brewer's grains as a remainder after leaching of crushed malt in beer brewing represent an important protein feed the annual production of which amounts to about 380 thousand tons in the Czech Republic. Dried brewer's grains are valuable raw material in the production of fodder mixtures and fresh grains with DM content of 200-220 g/kg are used either for the direct feeding of cattle and pigs, or for ensiling (Lohnert et al., 1996; Nishino et al., 2003; Doležal et al., 2005 and others). Chemical composition and digestibility of brewer's grains were studied by many authors (Amari and Purnomoadi, 1996; Lohnert et al., 1996; Daccord et al., 1997 a.o.). Brewer's grains feature high nutritive value and different ruminal degradability of proteins (Costa et al., 1995; Costa et al., 1994). Biological value of proteins depends on the content of aminoacids in malting barley and is further enriched by the activity of yeasts.

Prevailing carbohydrates are maltose, mellitriose (Nishino et al., 2003) and glucose, too. Net energy content ranges from 6.1 – 6.7 MJ NEL/kg DM (Lohnert et al., 1996; Spann, 1993). Costa et al. (1994) claim that 1 kg of brewer's grains DM contains 161.9 g/kg fibre, 386.3 g/kg BNLV, 486.0 g/kg NDF and 188.3 g/kg ADF. Brewer's grains have excellent dietary characteristics relating namely to the higher content of group B vitamins (Spann, 1993).

A specific property of high-quality brewer's grains is their beneficial influence on the rumen environment in dairy cows, namely on microbial activity in the rumen and on the production of microbial protein. Daccord et al. (1997) state that average ruminal degradation of proteins from the grains is 65%.

Brewer's grains are fodder that readily deteriorates, especially in summer months. Gruber et al. (1997) and Doležal et al. (2006) report that fresh, non-conserved grains keep in feedable condition as a rule 48 hours at the longest. During storage, serious sensory, nutritional and particularly microbial changes occur in the grains. The low content of dry matter in the fresh grains causes extensive release and discharge of effluents. Wyss (1997) claims that in the first week after ensilaging, an amount of up to 160 litres is released from each ton of conserved grains. Similar results are reported by Vyskočil et al. (2006). In order to prevent the discharge of silage effluents, Buchgraber and Resch (1997) recommend that fresh brewer's grains be pressed to a higher DM content of 350-400 g/kg or ensilaged in combination with the addition of various absorbents (Pereira et al., 1998; Tanaka et al., 2001).

The objective of this model experiment was to establish the effect of the supplementation of various silage additives onto the quality of the fermentation process in brewer's grains with the addition of moisture sorbent.

# MATERIAL AND METHODS

Material used in the model experiment was fresh brewer's grains at a DM content of 222 g/kg. Malt sprouts were used as moisture sorbent. The grains: sorbent ratio was 4.68: 1. The final dry matter content of ensiled material ranged from 320-330 g/kg. Established were four experimental variants in three repetitions: Variant A – control silage without the supplementation of silage additive, Variant B – treated with the ensilage additive based on organic acids (formic acid at 435 g/kg, propionic acid at 100 g/kg, ammonium formate 309, benzoic acid at 22 g/kg and water) at a dose of 3.5 litre/ton, Variant C – treated with a preparation based on NaNO<sub>2</sub>, E250 and hexamethylentetramine E 239 and water (ratio 244 + 163 + 593 g/kg) at a dose of 2 litres/ton. Active substance in Variant D was bacteria of lactic fermentation (*Lactobacillus paracasei* (DSM 16245), *Lactobacillus lactis* (NCIMM 30160) and *Pediococcus acididlactici* (DSM 16243) at a total concentration of CFU 1×10<sup>11</sup>/g.

Model silages were stored in the laboratory at average laboratory temperature of 26-28 °C for 56 days. Parameters assessed to establish the quality of the fermentation process after the 56 days were as follows: DM content of silage, pH, water extract acidity (KVV), amounts of lactic acid, acetic acid, propionic acid, butyric acid, contents of alcohol and ammonia. The content of dry matter was established by desiccation at a temperature of  $103 \pm 2$  °C to constant weight. Analytical procedures were described in our earlier work (Doležal, 2002). Results were statistically processed by using the analysis of variance and differences between individual groups were analyzed by F-test according to Snedecor and Cochran (1967). Data in the text are presented as average  $\pm$  standard deviation.

## **RESULTS AND DISCUSSION**

Dry matter of silages made of brewer's grains sampled after 56 days of storage ranged from  $326.85 \pm 0.54$  g/kg in Variant D to  $326.85 \pm 4.04$  g/kg in Variant B. Thanks to the use of moisture sorbent – malt sprouts, none of the model silages showed discharge of effluents.

The assessment of fermentation process quality corroborated the efficacy of silage additive on the pH value of silages. The lowest pH value  $(3.91 \pm 0.07 \text{ pH})$  was found in the control silage and the highest pH value  $(4.23 \pm 0.03 \text{ pH})$ 

was detected in Variant D. Statistically significant differences between the respective variants are presented in Table 1. The KVV value in variants treated with the silage additive correlated with the pH value. In spite of the high pH value, Variant D exhibited the highest KVV (1866.33  $\pm$  22.33 mg KOH/100 g). A statistically highly significant difference (P<0.01) was found between Variant B with the lowest KVV (1574.33  $\pm$  30.31 mg KOH/100 g) and all the other variants. A statistically highly significant difference (P<0.01) was found between Variant difference (P<0.01) was found between Variant difference (P<0.01) was found between Variant B and all the other variants also in the amount of lactic acid, which was established in Variant B at merely 54.40 g/kg of dry matter, and in the total amount of fermentation acids, which amounted to 69.48  $\pm$  2.78 g/kg of dry matter. Total amounts of fermentation acids in Variant D were 99.56  $\pm$  7.41 g/kg DM, 103.02  $\pm$  2.49 g/kg DM, and 106.20  $\pm$  3.13 g/kg DM, respectively. The difference between Variant A and Variant D was statistically significant (P<0.05).

The amount of acetic acid was lowest in Variant B, too  $(15.45 \pm 0.42 \text{ g/kg DM})$  and highest in Variant D  $(28.55 \pm 1.26 \text{ g/kg DM})$ . Statistically highly significant differences (P<0.01) were found between all studied variants.

In Variant D, the propionic acid was detected at an amount of  $1.79 \pm 0.18$  g/kg DM. Nishino et al. (2003) observed that metabolization of lactic acid into acetic acid and propionic acid occurs with the storage time. In the assessment of the fermentation process quality with respect to the ratio of the amount of lactic acid to volatile fatty acids, statistically highly significant differences (P<0.01) were found between all studied variants. The lowest and the highest ratio was found in Variant D ( $2.50 \pm 0.08$ ) and in Variant A ( $3.77 \pm 0.16$ ), resp.

Highly significant differences (P<0.01) between all studied variants were found also in the amount of ethanol, which ranged from  $7.45 \pm 0.24$  g/kg of dry matter in Variant D to  $15.75 \pm 0.84$  g/kg of dry matter in Variant B.

Although the amount of ammonia in the respective silages was relatively equable (see Table 1), the amount established in Variant D was highly significantly (P<0.01) higher than in Variant A or Variant B. The difference between Variant D and Variant C was statistically significant (P<0.05).

Variant	Α		В		С		D	
Parameter	$Av. \pm stand.dev.$	Note						
Dry matter [g/kg]	$321.69 \pm 3.88$	B,c,D	$326.85 \pm 4.04$	A,D	$325.19\pm0.7$	a,D	$306.5\pm0.54$	A,B,C
pH	$3.91\pm0.07$	B,C,D	$3.99\pm0.05$	A,D	$4.04\pm0.02$	A,D	$4.23\pm0.03$	A,B,C
KVV [mg KOH/100g]	1847.33 ± 175.75	В	$1574.67 \pm 30.31$	A,C,D	$1764.17 \pm 68.8$	В	$1866.33 \pm 22.33$	В
Lactic acid	$78.69 \pm 6.37$	В	$54.03 \pm 2.46$	A,C,D	$78.78 \pm 1.71$	В	$75.86 \pm 2.1$	В
Acetic acid	$20.87 \pm 1.2$	B,C,D	$15.45\pm0.42$	A,C,D	$24.24 \pm 1.05$	A,B,D	$28.55 \pm 1.26$	A,B,C
Propionic acid	$0\pm 0$	D	$0\pm 0$	D	$0\pm 0$	D	$1.79 \pm 0.18$	A,B,C
Butyric acid	$0\pm 0$		$0\pm 0$		$0\pm 0$		$0\pm 0$	
Sum of acids	$99.56 \pm 7.41$	B,d	$69.48 \pm 2.78$	A,C,D	$103.02\pm2.49$	В	$106.2 \pm 3.13$	a,B
KM: KTM	$3.77 \pm 0.16$	B,C,D	$3.5 \pm 0.11$	A,C,D	$3.25 \pm 0.11$	A,B,D	$2.5 \pm 0.08$	A,B,C
Ethanol	$10.82 \pm 0.5$	B,C,D	$15.75\pm0.84$	A,C,D	$8.77 \pm 1.02$	A,B,D	$7.45 \pm 0.24$	A,B,C
Ammonia	$2.64 \pm 0.3$	D	$2.5 \pm 0.25$	D	$2.72\pm0.24$	d	$3.1 \pm 0.18$	A,B,c

Table 1. Quality of the fermentation process in brewer's grains silages (g/kg DM)

KVV... water extrakt acidity, KM... lactic acid, TKM ... volatile fatty acids, Variants in capitals differ (P < 0.01); variants in lower case differ (P < 0.05).

# CONCLUSION

The objective of the model experiment was to establish the effect of the supplementation of various silage additives onto fermentation process quality in brewer's grains with the addition of malt sprouts as moisture sorbent. The results indicate that the dose of silage additive in Variant B was high because the fermentation process was suppressed, which is further corresponded to –in spite of low pH- by a demonstrably (P<0.01) low content of fermentation acids. Variant D showed a demonstrably higher (P<0.01) pH value at which higher amounts of acetic acid, propionic acid and ammonia were generated. Variant C had a demonstrably higher pH and at the same time, a higher content of acetic acid than the control silage. The silage exhibited a reduced production of ethanol, too.

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The list of references can be requested from the first author.

# ACKNOWLEDGEMENT

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# RUMEN DEGRADATION OF ORGANIC MATTER AND PROTEIN OF SILAGES MADE FROM RED CLOVER AND ALFALFA ENSILED WITH DIFFERENT ADDITIVES

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### INTRODUCTION

In nutrition of diary caws basic components of ration, apart silages from maize are silages from legumes, grasses and their mixtures. Protein from ensiled legumes is unfortunately quickly degraded in the rumen, and because of that some of them are lost. It is possible that some additives can decrease susceptibility to degradation of this feeds, but results of researches on this fields are equivocal. Some researches results indicate that formic acid decreased degradation of nitrogenous compound in rumen (Brzóska et al., 1999], but others not confirm that (Gąsior and Brzóska, 1999). This researches was done to determine the rumen degradation of alfalfa and red clover silages made with chemical and biological additives.

# MATERIALS AND METHODS

Alfalfa and red clovers green forages was cut in stage of budding, wilted till 30% DM content and ensiled in micro silo at capacities 3 dm<sup>3</sup> without any supplements (KP) as well as with three different additives: formic acid and molasses mixtures (KMM) (applied 0,3% and 2% respectively), microbiological (MI) and microbiological-enzymatic additives (ME) (applied in accordance with recommendation of manufacturer to rich the level of bacteria in ensiled crop 100 000 cfu/g and enzymatic activity 0,65 HEC/g. Microbiological preparation contained culture of lactic acid bacterium: *Enterococus faecium, Lactobacillus casei, Lactobacillus plantarum, Pediococcus spp.* and *Lactobacillus buchneri*, whereas microbiological-enzymatic additive contained bacteria's strain: *Pediococcus acidilactici* and *Lactobacillus plantarum* and cellulase and xylanase enzymes too.

Research was conducted by *in sacco* method on four ruminaly fistulated oxen (~500kg BW), kept on a constant diet of meadow hay to concentrate (70:30) as per INRA (Jarrige, 1993), to meet their maintenance requirement. After a preliminary feeding period (21 days), 2 g of the previously dried and ground samples were weighed into nylon bags (50x 90mm size with pore size  $50\pm10$  microns) and incubated into rumen for 2, 4, 8, 16, 24 and 48 hours. After incubation bags was washed in washing machines, dried and weighted. Bag residues were analyzed for dry matter and protein (Kiejdahl method) content (AOAC, 1990). The ruminal effective degradability (ERD) of CP and DM was calculated for a speed of rumen outflow of  $0,06h^{-1}$ , according to the Ørskov and McDonald equation (1979).

# **RESULTS AND DISCUSSION**

Used additives did not influence onto quality of silages, qualities all of them was very good.

#### Silages organic matter in sacco degradation

Rate of organic matter and protein degradation are an important factor in feeding value of fodder estimation. The result for organic matter can be seen on figure 1 and 2.



Figure 1. In sacco organic matters degradation in alfalfa silages

Figure 2. In sacco organic matters degradation in red

For alfalfa silages slowest degradation rate was observed in silages prepared with molasses and formic acid mixtures (KMM). After 16<sup>th</sup> hour incubation lower than 50% of this silages was degraded. Silages prepared with biological additives (MI & ME) was degraded in almost same rate. In contrast to alfalfa silages applied additives increase a little

degradation of red clover silages. In incubation process all additives affect on degradation process in the same way but later (from 16<sup>th</sup> hour) silage with chemical preparation (KMM) was degraded slower than this with biological additives. In total red clover silages was degraded slower than alfalfa silages

# Ruminal degradation silages protein by in sacco method

Protein degradation in alfalfa silages was very dynamic (figure 3). The same like in others research (Brzóska i in, 2000). Highest degradation rate was observed in first two hour of incubation (65-72%). Almost for whole incubation time protein from alfalfa silages ensiled with formic acid and molasses was degraded in slowest rate. The same like for organic matter protein from alfalfa was degraded faster than this from red clover silages (figure 3 and 4). Effective degradability of red clover silages was on the level 60 -75%. The same ERD value was observed by Skiba at al (1996). Mixtures of molasses and formic acid had most profitable influence on degradation rate and effective degradability of protein in the rumen. However microbiological and microbiological additives had worse effect on this factors – protein of silages wit this preparations was degraded faster than this without any additives.







# CONCLUSION

Big relationship between organic matter and protein degradation in the rumen and kind of ensiled fodder as well as used additives was affirmed. Both organic matter and protein was degraded not only slower but in lower degree when silage was made from red clover than from alfalfa. From used additives mixtures of molasses and formic acid had most profitable influence on this factor.

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#### EFFECT OF INOCULANT ON FERMENTATION AND DIGESTIBILITY OF LUCERNE SILAGE

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## **INTRODUCTION**

Silage is the most variable and unstable feedstuff used in dairy cattle diets. It is difficult to ensile lucerne (*Medicago sativa*) due to its high buffering capacity and low content of water-soluble carbohydrates. In order to improve the nutritive value and hygienic quality of forage, several silage additives have been developed. A specific microbial additive in proper conditions improves ensiling of fresh material and increases the productivity of animals. Using of additives has decreased losses and improved the nutritive value of silage. Inoculants contain different lactic acid bacteria strains, which direct fermentation and preserve silage nutritive value. The study was aimed at finding out biological additives for the ensiling of lucerne, its effect on silage fermentation and digestibility in Estonian conditions.

# MATERIALS AND METHODS

The first cut of lucerne was wilted for 24h to a dry matter content of 309 g/kg. The material was chopped into 2 cm lengths, mixed and ensiled in 3-litre glass jars. In the trial silage was either untreated (control) or treated with four inoculants and chemical additive AIV Pro (Table 1). The additives were applied to fresh forage at the levels recommended by the manufacturers.

Number	Inoculant	Source		
1	L. plantarum, P. pentosaceus, L. rhamnosus, L. brevis, L. buchneri (Bonsilage)	Schaumann Agri Austria GmbH & Co KG		
2	L. plantarum (Biosil)	Dr. Pieper Ltd. Wuthenow, Germany		
3	L. plantarum, E. faecium, P. acidilactici, L. salivarius (Sil-All)	Alltech Biotechnoligy Centre, Co. Meath, Ireland		
4	L. plantarum MTD1 (Ecosyl)	Ecosyl, Yorkshire, UK		

Table 1. Inoculants used in the trials

After 90 days the jars were opened for analysis. The pH value was measured with a MP 120 Mettler Toledo pH meter, ammonia nitrogen was determined using an adjusted Kjeltec 2300 (FOSS) analyser. The ethanol, lactic acid and volatile fatty acids contents were determined chromatographically using a Perkin Elmer 900 gas-chromatograph with a column packed with 80/120 Carbopack B-DA/4 % carbowax 20 M.

Samples were dried for 20 hours at 60 °C, chopped into 1 mm-diameter-particles and analysed for the DM, crude protein, crude ash and crude fibre. For determining crude ash concentration, samples were reduced to ashes in a furnace at 550 °C for 6 hours. Crude protein was analysed by Kjeldahl method with Kjeltec 2300 analyser (FOSS Tecator Technology). The samples were analysed for *in vitro* dry matter digestibility (IVDMD) by the filter bag method, using a DAISY II incubator, fibre analysis equipment (ANKOM Technology, Fairport, NY USA). The NDF and ADF concentrations were determined with a fibre analyzer ANKOM 220. Water-soluble carbohydrates (WSC) were determined by the anthrone method and the buffering capacity (BC) by titration of lactic acid (LA) to pH 4.0.

Data were analysed by using GLM procedure of SAS. The effects of additives were tested by means of orthogonal contrasts.

# **RESULTS AND DISCUSSION**

Lucerne with the DM content of 309 g/kg was studied. The concentration of crude protein, NDF, ADF, N-free extractives and WSC was 182 g/kg, 410 g/kg, 306 g/kg, 409 g/kg, and 43 g/kg in DM, respectively, and the buffering capacity 93.3 LA g/kg DM. Fermentation coefficient of lucerne was 35 indicating that this material was difficult to ensile (Pahlow et al., 2002).

Inoculants as well as the chemical additive AIV Pro reduced lucerne silage dry matter losses by 10 to 42 g/kg compared with the control silage (Table 2). Difference between DM content of the experimental silages and that of the untreated control silage was higher when Bonsilage, Ecosyl or AIV treatments were used. Compared with fresh grass, in silages the content of crude protein- and fibre fractions was higher while that of N-free extractives lower. Nutrient concentrations in silage are altered by DM losses and mainly by the use of sugars by microorganisms during fermentation.

No significant differences were revealed between the DM digestibility of silages treated with inoculants and of that of the untreated silage. However, dry matter digestibility of the silage treated with AIV improved by 5.2 % units compared with that of the control silage (P<0.01). Inoculation had no effect on the digestibility of high quality silages (-.07) but it improved digestion of low quality forage by 1.57 units. Numerical effects of inoculation on digestibility were

smaller than the effects of formic acid for all types of comparisons shown by Harrison et al. (1994). Filya et al. (2007) have mentioned that only in 30% of trials the silage digestibility increased by using biological inoculants.

Treatment	Dry matter g/kg	Crude protein g/kg	NDF g/kg	ADF g/kg	N-free extractives g/kg	IVDMD %
Control	279	187	427	334	398	73.3
Bonsilage	293	179	424	335	395	74.0
Biosil	276	178	423	333	386	75.4
Sil-All	277	181	419	333	378	75.1
Ecosyl	298	188	413	328	377	74.9
AIV PRO	304	194	414	315	370	78.5
Significant difference, P						
C vs Bonsilage	0.006	0.050	0.144	0.311	0.493	0.280
C vs Biosil	0.084	0.023	0.053	0.211	0.151	0.027
C vs Sil-All	0.099	0.060	0.022	0.230	0.022	0.126
C vs Ecosyl	< 0.001	0.369	0.053	0.107	0.022	0.099
C vs AIV Pro	< 0.001	0.060	0.003	< 0.001	0.014	0.007

Table 2. Chemical composition (in DM) and in vitro digestibility (IVDMD) of Lucerne silages

 Table 3.
 Fermentation characteristics of lutserne silage (in DM)

Treatment	Dry matter losses, g/kg	рН	NH3-N of total N g/kg	Lactic acid g/kg	Acetic acid g/kg	Ethanol g/kg
Control	68	4.7	80	30.4	39.5	17.2
Bonsilage	35	4.6	76	29.8	35.8	13.6
Biosil	58	4.6	78	34.6	33.0	10.4
Sil-All	58	4.6	76	30.9	32.4	13.7
Ecosyl	42	4.6	77	39.3	28.7	11.1
AIV Pro	26	4.5	75	33.9	17.1	5.8
Significant difference, P						
C vs Bonsilage	0.011	0.008	0.199	0.347	0.009	0.002
C vs Biosil	0.044	0.008	0.433	0.177	0.008	< 0.001
C vs Sil-All	0.049	0.008	0.079	0.459	0.007	0.005
C vs Ecosyl	0.005	0.008	0.197	0.036	< 0.001	0.001
C vs AIV Pro	< 0.001	0.007	0.069	0.107	< 0.001	< 0.001

Fermentation characteristics, pH value,  $NH_3$ -N in total N, the content of lactic acid, acetic acid and ethanol in silage are given in Table 3. Butanediol and butyric acid were not found in any of the experimental silages. The pH value of all the additive-treated silages was lower than that of the untreated silage (P<0.01). The additives also decreased  $NH_3$ -N and ethanol content in silage.

# CONCLUSION

The use of inoculants for ensiling wilted lucerne improved fermentation and silage quality: pH, NH<sub>3</sub>-N and ethanol contents showed a decrease, and also reduced dry matter losses. All the commercial biological additives used - Bonsilage, Biosil, Sil-All, Biomax and Ecosyl - improved the fermentation of lucerne silage under stated conditions but did not increase silage organic matter digestibility. Digestibility of the chemically treated silage was improved by 5.2 %.

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# EVALUATION INFLUENCE OF PRESERVATIVE ON THE FERMENTATION PROCESS AND THE MICROORGANISMS GROWTH IN PRESERVED BREWERS GRAINS.

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### **INTRODUCTION**

Requirement to optimize the feeding rate of cattle faces breeders to always higher concentration of dosed nutriments and frequent inserting industrial by - products to the feeding rates (FR). Conventional by – product of brewing industry often inserted to the FR is brewer's grains (Daccord R, et al, 1997). Seasonal production and low immunity against negative incidence of the undesirable microflora are its main disadvantages. Submitted work lays behind aim evaluate the incidence of preservative agent even base on the sodium benzoate and sodium propionate on the process of fermentation of brewers' grains (BG) and growth of moulds and yeasts during it.

# MATERIALS AND METHODS

Two monitoring S1 and S2 were done. Fresh BG was treated by preservative – mixture of sodium benzoate (229 g/kg) and sodium propionate (83 g/kg). Both monitoring consists of three variants: P1 – BG was treated by dose 3 l of preservative on 1 t of mass, P2 – 6 l/t and K – BG was not treated. Treated or untreated BG was ensiled to the plastic tubes. Tubes were stored for a period of 119 (S1) and 89 (S2) days. There were monitored these indicators of fermentative process: pH scale values, aqueous leach acidity (ALA), concentrations of milk acid (MA), acetic acid (AA), propionate acid (PA) and ammonia (NH3) during the monitoring, further sum of volatile fat acids (SVFA), level of formalin titration (FT) and height of rate MA to SVFA were under consideration too. As well numbers of colony forming units (CFU) of moulds and yeasts were monitored too. Lababoratory analyses were realized in NutriVet s.r.o and SVÚ Jihlava. Indicators of fermentative process were given according to HARTMAN (1980), content of moulds and yeasts according to ANONYMOUS (2001). Results were evaluated with method of analyses of variances according to SNEDECOR and COCHRAN (1969).

# **RESULTS AND DISCUSSION**

Average values of fermentative process' indicators of treated and untreated BG from monitoring S1 are presented in table I. Referred – to data result that pH value of untreated BG silage (K) is statistically highly conclusive (P<0, 01) higher than that of silage P2. Alike pH of BG silage (P1) is statistically highly conclusive (P<0, 01) higher than that of P2. Introduced values of pH scale are not comfortable with values ascertained by MAJER (2006), which features pH at interval 4, 26 as far as 4, 35 at BG treated by mixture of organic acids before the fermentation. Likewise difference of ALA levels of groups K and P2 was statistically highly conclusive (P<0, 01). Statistically highly conclusive (P<0, 01) higher levels of FT values of group K (0,018±0,002 %) and group P1(0,018±0,003) against FT level of group P2 (0,012±0,002) show evidence of slower protein breakdown in BG silage P2. Average values of fermentative process' indicators of treated and untreated BG from monitoring S2 are presented in table II. Referred - to data result that average pH level of samples of groups K and P1 were statistically conclusive (P<0, 05) lower ten that of group P2. Introduced pH scale values of all three groups of BG after the process of fermentation (K= $5,620\pm0.052$ , P3=5,573±0,137, P6=6,400±0,047) are not comfortable with values ascertained by MAJER (2006). Further the statistically conclusive (P<0, 05) higher content of MA in BG silage P1 than that of K and P2 was ascertained and statistically highly conclusive (P<0, 01) lower content of AA than that of groups K and P2 was ascertained too during monitoring S2. Differences between formol titration levels of samples of all groups were not statistically conclusive (P<0, 05). Discrepancies between CFU numbers of yeasts and moulds in all of groups (K, P1, P2) in both monitoring (S1, S2), which are presented in tables III and IV, were not statistically conclusive (P<0, 05).

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Group	Group K		-	Р	1	P2		
Indicator	Units	Average	SX	Average	SX	Average	SX	
pН		5,788	0,052	5,733	0,137	5,480	0,047 ++	
ALA	g KOH	223,025	9,58	213,210	16,515	315,533	21,783 ++	
MA	%	0,228	0,079	0,223	0,106	0,388	0,136	
AA	%	0,445	0,083	0,470	0,064	0,438	0,043	
PA	%	0,168	0,078	0,160	0,141	0,108	0,038	
SVFA	%	0,613	0,159	0,630	0,196	0,545	0,068	
MA/SVFA		0,370	0,094	0,358	0,172	0,695	0,191 +	
NH3	%	0,011	0,002	0,025	0,01 +	0,017	0,004	
FT	%	0,018	0,002	0,018	0,003	0,012	0,002 ++	

Table 1. Summary of the fermentative process indicators S1

 Table 2.
 Summary of the fermentative process indicators S2

Group		K		P1		P2		
Indicator	Units	Average	SX	Average	SX	Average	SX	
pН		5,62	0,416	5,573	0,438	6,4	0,343 +	
ALA	g KOH	95,383	27,866	136,06	41,578	63,123	28,003 +	
MA	%	0,083	0,02	0,143	0,051 +	0,089	0,009 +	
AA	%	0,32	0,011	0,66	0,02 ++	0,22	0,001 ++	
PA	%	0,1	0,048	0,09	0,035	0,093	0,015	
SVFA	%	0,42	0,13	0,75	0,17 ++	0,313	0,031 ++	
MA/SVFA		0,205	0,029	0,195	0,075	0,285	0,037 +	
NH3	%	0,017	0,002	0,015	0,003	0,011	0,002 ++	
FT	%	0,005	0,002	0,008	0,003	0,004	0,003	

 Table 3.
 Summary of contents of yeasts and moulds S1

Group		K		Pl		P2	
Microorg.	Units	Average	SX	Average	SX	Average	SX
Yeasts	KTJ	12000000	3674688	13925000	2775337	12550000	1310216
Moulds	KTJ	50	17,321	63	26,3	60	29,439

 Table 4.
 Summary of contents of yeasts and moulds S2

Group		K		P1		P2	
Microorg	Units	Average	SX	Average	SX	Average	SX
Yeasts	KTJ	180	82,563	225	113,578	178,75	122,228
Moulds	KTJ	1625	1855,451	1360	722,403	2923	4526,827

# **SUMMARY**

Main aim of this paper was to evaluate incidence of preservative based on the sodium benzoate (229 g/kg) and sodium propionate (83 g/kg) on course of the fermentative process and growth moulds and yeasts after termination of the fermentation. Fresh brewer's grains (BG) were in double attempts treated by preservative solution graded tax 3 litres and 6 litres mixtures on ton BG - ensiled mass. Those were check by variant always be without treatment. There were monitored these indicators of fermentative process: pH scale values, aqueous leach acidity (ALA), concentrations of milk acid (MA), acetic acid (AA), propionate acid (PA) and ammonia (NH3) during the monitoring, further sum of volatile fat acids (SVFA), level of formalin titration (FT) and height of rate MA to SVFA were under consideration too. As well numbers of colony forming units (CFU) of moulds and yeasts were monitored in the BG masses. Discrepancy between numbers of FCU of moulds and yeasts in all attempts ware not statistically conclusive (P<0, 01). Monitoring of levels of fermentative process indicators has not unambiguous results. Ascertained levels of pH scale measured in groups of treated BG were higher than levels ascertained by MAJER (2006).

# NATURAL OCCURRENCE OF *FUSARIUM* MYCOTOXINS IN FORAGE OF CONVENTIONAL AND BT CORN IN TWO SUBSEQUENT YEARS

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# ABSTRACT

The objective of the study was to compare the nutritional value and natural occurrence of Fusarium mycotoxins between conventional corn hybrid (MONUMENTAL – C) and its near isogenic Bt hybrid (MON 810 – Bt) in two subsequent years. In the field experiments performed in the region of Ivanovice na Hané, CR, in 2005 and 2006, the corn crops of both hybrid were grown and harvested under identical conditions. Entire corn plants were harvested at the soft dough stage of maturity and ensilaged in microsilage tubes. Prior ensiling samples of fresh forage were analysed for content of nutrients and Fusarium mycotoxins. In 2005 content of DM, OM, CF and ADF determined in C was lower than that in Bt (P<0.05). Content of Fusarium mycotoxins (DON, FUM, AFL, ZON) did not differ significantly between hybrids (P>0.05). In 2006, content of mycotoxins and nutritional value of C and Bt corn did not differ significantly (P>0.05) except of CF content that was higher in Bt (P<0.05). The samples of C or Bt taken in 2005 had lower concentrations of DON, FUM, ZON and higher concentration of AFL than those taken in 2006 (P<0.05).

# **INTRODUCTION**

While most fungi only reduce the yield or nutritive value of the feed they infest, some fungi have the ability to produce toxic chemicals, mycotoxins. Mycotoxins are now more frequently being associated with crops like corn silage. Recently, mycotoxins in corn silage have been associated with dairy herd health problems during years. *Fusarium* species are common contaminants of corn (*Zea mays* L.) and are generally considered to be "field" fungi that are thought to proliferate before harvest (Christensen et al., 1977). Although ensiling may successfully eliminate fusaria from corn, many of their toxins, such as the trichothecenes and zearalenone, are stable in storage (Lepom et al. 1990a, b). Thus strategies to reduce *Fusarium* mycotoxins in silage should focus on the crop before ensiling.

The objective of the study was to compare the nutritional value and natural occurrence of *Fusarium* mycotoxins between conventional corn hybrid and its near isogenic Bt hybrid in two subsequent years.

#### **MATERIALS AND METHODS**

The field experiments were performed in 2005 and 2006 in the region of Ivanovice na Hané. The experimental field was divided into two areas of 10 m<sup>2</sup>. The control one (C) was sown with the conventional corn hybrid MONUMENTAL and the experimental one (Bt) with its near isogenic Bt-hybrid Monsanto MON 810, both grown, harvested and ensiled under identical conditions. Entire corn plants were harvested at the soft dough stage of maturity and ensilaged in microsilage tubes. Prior ensiling samples of fresh forage were analysed for content of nutrients and *Fusarium* mycotoxins. Obtained results were analysed using the Statgraphics 7.0 package.

#### Analytical procedures

Samples of corn forage were analyzed for DM, ash, CP, CF, ADF, NDF and fat. DM was determined by drying the samples at 55°C, followed by milling through a 1 mm screen and drying for another 4h at 105°C. Ash was determined by ashing the dried sample for 3 h at 550 °C. Crude fiber (CF) was analyzed according to Henneberg-Stohmann (Weende-method), neutral detergent fiber (NDF) and acid detergent fiber (ADF) was analyzed according to Van Soest et al. (1991) using the FIBERTEC system. Sulfite and heat-stable alpha-amylase were used for NDF analysis of all samples. Total N was analyzed using the Kjeldahl technique (KJELTEC 1030 AUTO analyzer). Concentration of CP was expressed as N x 6.25. Fat was determined by SOXTEC system by diethylether extraction.

Contents of *Fusarium* mycotoxins (DON, FUM, AFL, ZON) in fresh forage were analysed using imunoenzymatic method (ELISA, kit Veratox, natural toxins; Neogen Corp., Lansing. MI, USA) and expressed in units ppb (1 ppb = 1 g/kg).

#### **RESULTS AND DISCUSSION**

Table 1 compares the nutritional value of fresh corn forage of conventional corn hybrid (C) and its near isogenic Bt hybrid (Bt) in two subsequent years, 2005 and 2006. In 2005, content of DM, OM, CF and ADF determined in C was lower than that in Bt (P<0.05). In 2006, content of CF in Bt was higher (P<0.05) than in C, other determined parameters did not differ significantly between groups.

Table 2 compares natural occurrence of *Fusarium* mycotoxins in corn forage between C and Bt corn in 2005 and 2006. In the present study, DON and ZON were predominant mycotoxins in both of the forages in 2005 and 2006. These findings are in agreement with the e. g. Park et al. (1992). Within years, content of *Fusarium* mycotoxins (DON,

FUM, AFL, ZON) did not differ significantly between C and Bt corn (P>0.05). Between years, significantly higher concentration of DON, FUM and ZON and lower concentration of AFL was determined in 2006 in comparison to 2005 in both of the forages, either C or Bt (P>0.05). Nedělník et al. (2006) noted that almost all samples of silages analysed in their study were positive for mycotoxins, with highest levels of DON, ZON and T-2 toxin. As the majority of *Fusarium* mycotoxins in silage probably are produced in the field, strategies to reduce *Fusarium* mycotoxins in silage should focus on the crop before ensiling.

Parameter	unite	2005		20	06	SE	2005	2006
1 arameter	units	С	Bt	С	Bt	SE	C x Bt	C x Bt
Dry matter	g/kg	361.7	375.6	307.8	306.9	1.87	*	NS
Ash	g/kg	47.3	46.7	43.6	43.4	0.59	NS	NS
Organic matter	g/kg	314.4	328.9	264.2	263.5	1.90	*	NS
CF	g/kg	179.0	198.0	182.8	192.6	2.78	*	*
ADF	g/kg	216.7	229.1	210.3	213.1	2.78	*	NS
NDF	g/kg	419.6	432.1	414.5	416.5	7.00	NS	NS
СР	g/kg	65.9	61.7	75.4	72.7	1.39	NS	NS
Fat	g/kg	21.7	23.2	26.8	26.6	1.12	NS	NS

 Table 2.
 Natural occurrence of *Fusarium* mycotoxins in forage of conventional and Bt corn in years 2005 and 2006

		2005		20	2006		2005	2006	С	Bt
	units	C	<b>B</b> t	C	Dt.	SE	C x Bt	C x Bt	2005 x	2005 x
		C	Di	C	Di				2006	2006
DON	ppb	37.3	40.8	710.0	645.0	80.19	NS	NS	*	*
FUM	ppb	0.0	3.1	25.0	18.8	3.49	NS	NS	*	*
AFL	ppb	1.0	1.0	0.3	0.4	0.15	NS	NS	*	*
ZON	ppb	12.5	13.1	60.3	44.1	10.42	NS	NS	*	NS

DON - deoxynivalenol, FUM - fumonisins, AFL - aflatoxin, ZON - zearalenon

# CONCLUSION

In two subsequent years, 2005 and 2006, forage of control corn as well as Bt-corn was naturally contaminated with *Fusarium* mycotoxins, predominantly DON and ZON. As the majority of Fusarium mycotoxins in silage probably are produced in the field, strategies to reduce Fusarium mycotoxins in silage should focus on the reduction of mycotoxins contamination of crop before ensiling.

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# THE CONTENT OF YEASTS AND MOULDS IN SILAGE OF BREWER'S DRAFF WITH THE ADDITION OF VARIOUS SILAGE ADDITIVES

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#### INTRODUCTION

A brewer's draff is a residual part, remained after the leaching of extractive compounds of malt, rising up during the beer processing. For the optimal nutritive value, high digestibility of organic fraction and the economical availability, a brewer's draff became required secondary raw material utilized in the feed industry. The unadvantage is a short expiration date. It exists a possibility to enhance the expiration time in ensilaging with using of various additions, because of fact that brewer's draff is a poorly ensilage able material. Despite silaging additives usage, aerobic stability of brewer's draff remains an important problem. Besides bacteria also the yeasts (*Saccharomyces, Candida, Pichia, Hansenula*) can participate on decomposition processes. During catabolic processes in brewer's draff made silage, the activity of yeasts (*Saccharomyces, Candida, Pichia, Hansenula*) could be increased. According to Görner at Valík (2004) the yeast shows a tolerance to acidity as well as to the temperature. (The temperature tolerance is divided to interval of 0 - 45 °C, the optimal temperature is at about 30 °C). The counts of yeasts in draff should not exceed  $10^5/g$ , since otherwise they can be the cause of diarrhoeal disorders and disturbances of ruminal digestion. Moulds reproduce in silage more slowly than yeasts. The dangerousness of moulds rests in the first place on the possible formation of mycotoxines.

# MATERIALS AND METHODS

During the experimental period, four treatments of silages (originally 22 % of dry matter) with the addition of malt sprouts had been preservative by the specific additives made from brewer's draff, were examined. The ensilaging took 56 days (a final rate of the dry matter was 32 - 33 %). Four variants of fresh brewer's draff treatments with selected additives were studied: A (control) – no additives, B – preparation with formic acid, propionic acid, ammonium formate and benzoic acid, amount added 3 l/t, C - with addition of - E 250 (based on NaNO<sub>2</sub>) hexamethylenetetramine - E 239 and the water in concentration of 2 l/t,, D – (with the addition of malt sprouts longer time store) biochemical preparation containing lactic acid bacteria (*Lactobacillus plantarum, Pediococcus pentosaceus, Lactobacillus lactis, Enterococcus faecium*) in amount added of 2 g/t. Storage was taking place under aerobic conditions at 22 °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 °C.

# **RESULTS AND DISCUSSION**

From the results of microbial analysis of raw material (Table 1), consequenting a high numbers of the yeasts for brewer's draff and a short-term storaged malt sprouts. In result of low water activity, there were no yeast detected in malt sprouts at long term storaged. At the both cases, hight amounts of moulds have been observed. After 56 days, it was observed a presence of moulds and yeats. It is possible to bring out the fact, that during the 72 hours long exposition, showed the numbers yeast increasing and the numbers moulds decreasing tendency. The most evident was the increasing growth at C the alternate, the amount was  $4 \times 10^7$  CFU/g. However the pH level was 4.2 and a lower, the yeast and the spores of moulds had not been inhibited.

The yeasts are facultatively anaerobic so that thay can grow in anaerobical conditions. This can also clear up the appearance of ethyl alcohol in all of the varieties. Except of the present yeasts the heterofermentative lactic acid bacteria can participate on its creation. The moulds don't develop under anaerobic conditions, but its spores can stay alive and germinate during aerobical exposition. The usage of silage additive on the basis of organic acid (var. B) doesn't seem to be suitable. The formic acid (according to Jacobe et al., 1987) decreases pH and in non-dissociated form it has bactericidal effect, which can occur in the conditions of pH values 2.3 - 4.0. In lower doses it is ineffective, in higher doses it suppresses lactic fermentation. Benzoic acid is according to Jesenská (1987) effective against yeasts and moulds, but it is specified mainly for application on acidic matter. Drdák (1989) mentioned as extreme acidity for effective preservative usage the pH 4.5. In low doses it effectively inhibits aerobic bacteria, for suppression of yeasts and moulds higher doses should be used. Propionic acid is ineffective against yeasts according to Jesenská (1987) but it is effective against moulds. The least suitable seems to be the usage of nitrate and hexamethylenetetramine basis (var C). Sodium nitrate itself accordind to Jay et al., (2005) effectively supresses mainly *Clostridium*. According to Hrudková et al., (1989) it is ineffective against yeasts and moulds. The best results were achieved in the variety of D,

which showed the best microbiological stability. The total amount of yeasts and moulds was about  $10^3$  /g after 72 hours of cultivation. The low pH of created acids influences the development of undesirable microorganisms together with the competitive pressure and according to Tamine (2005) also some possible probiotic effects.

Sample	Hours	Sum	Yeasts	Мо	ulds
				Moulds	Geotrichum
А	0	$6.5 \times 10^3$	$5 \times 10^2$	$6 \ge 10^{3}$ *	$5.5 \times 10^3$
	24	$2.1 \times 10^4$	$4.5 \times 10^3$	1.6 x 10 <sup>4</sup> *	$1.5 \times 10^4$
	48	$9 \ge 10^3$	$4.4 \times 10^3$	$4.6 \ge 10^{3}$ *	$3.1 \ge 10^3$
	72	$9.9 \times 10^4$	$9.8 \ge 10^4$	6.7 x 10 <sup>2</sup> *	$3.3 \times 10^2$
В	0	$6.8 \times 10^4$	$5.9 \times 10^4$	$9 \ge 10^{3}$	0
	24	$7.5 \times 10^4$	$7.1 \times 10^4$	$4 \ge 10^{3}$ *	0
	48	$7.3 \times 10^4$	$6.9 \ge 10^4$	$4 \ge 10^{3}$ *	0
	72	$1.2 \times 10^5$	$1.2 \ge 10^5$	$3.3 \times 10^{2*}$	0
С	0	$1.2 \text{ x } 10^4$	$3.5 \times 10^3$	8.5 x 10 <sup>3</sup> **	$4 \ge 10^3$
	24	1.1 x 10 <sup>5</sup>	$1 \ge 10^5$	$4 \ge 10^{3}$	$2 \times 10^3$
	48	$2.3 \times 10^5$	$2.3 \times 10^5$	0	0
	72	4 x 10 <sup>7</sup>	4 x 10 <sup>7</sup>	0	0
D	0	$1.6 \times 10^3$	$3.7 \times 10^2$	$1.3 \ge 10^{3}$ *	$9.7 \times 10^2$
	24	$6 \ge 10^3$	$1.5 \ge 10^3$	$4.5 \ge 10^{3}$ *	$4 \ge 10^2$
	48	$9.1 \times 10^2$	$3.8 \ge 10^2$	5.3 x 10 <sup>2</sup> *	$3.7 \times 10^2$
	72	$1.7 \times 10^3$	$1.5 \ge 10^3$	2.7 x 10 <sup>2</sup> *	$1.6 \ge 10^2$
Brewer's draf	f	$4.7 \times 10^3$	$4.6 \times 10^3$	55	45
Malt sprouts – short term storage		6.7 x 10 <sup>6</sup>	6.6 x 10 <sup>6</sup>	9.5 x 10 <sup>4</sup>	$1.8 \ge 10^2$
Malt sprouts – long ter	m storage	$2.6 \times 10^3$	0	$2.6 \times 10^3$	0

Table 1. Content of yeasts and moulds in variants silage and in input raw material in CFU/g

\* Penicillium sp. \*\* Penicillium sp. and Aspergillus niger

# CONCLUSIONS

Fresh brewer's draff is practically sterile, but during transport and storage it can run through some secondary contamination. Low level of pH, contents of nutrients and high moisture makes it an ideal medium for microorganisms progression. One of the possibilities how to extend the time of durability is the ensilage but thanks its qualities it is hardly possible to use that technique. Also the malt sprouts used, as an absorbents during the ensilage is an important participant of moulds and in short-term storages also for yeasts. There was shown presence of yeasts and moulds after 56 days of storage in all of the varieties. Aerobic stability of silages of these types is quite low. The best results were shown in variety D, where there the stability was the highest in the light of microbiology. The usage of silage additives, in variety B, didn't seem to be very suitable, according to the analyse. Slight acidic conditions and low doses of used additive, high microbial contamination influenced negatively the preservative effect of organic acid, so it would be suitable to use higher concentration. The least suitable seems to be the usage of nitrate basis chemicals and chemicals on the basis of hexamethylenetetramine, variety C. High amount of microorganisms in this type of silage can bring some health risks. So it is not possible to recommend the usage of these chemicals.

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# WHOLE-PLANT PEA (PISUM SATIVUM) SILAGE WITH ADDITIVES

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## ABSTRACT

The cultivation of pea for feed includes a few advantages. These include reduced erosion, weed control, lower soil tillage and the possibility of a second harvest in the year. The cultivation of winter pea as a feed, result in a time offset and requires a maize cultivar with late seed tolerance. However it can also be used as whole plant pea silage. Given the appropriate availability of the seed, this time advantage by winter peas is further expanded. The target date is to harvest at the end of flowering until the kernels are dough maturing. The desired DM is 30 % with below 20 % being too wet and above 45 % being too dry. An undesirable DM leads to butyric acid containing silages and thus to badly fermented silages. In the extreme DM levels additives still show the effect, that chemical additives are safer. The optimal DM area can also draw on a use of additives be waived if double drum chopper and swath layer were used, with little soil and well-compacted, the whole crop pea would be fermented. If optimal DM is achieved in combination with little soil, good compaction and the use of a double drum chopper and swath layering, the use of additives can be avoided.

# **INTRODUCTION**

In Bavaria 14,000 ha are cultivated as kernel pea [2]. The cultivation of pea for feed includes a few advantages. The advantages are, reduced erosion, weed control, and the possibility a second harvest/year. Feed legumes were sown for cultivation in ecological farms [1]. The first harvest was successful with winter pea as whole-plant silage. With regards to the type of harvester, the two drum mowers jammed the least, out of several mowers tested. With regards to the windrowers the swath benefited with lower losses. The harvest with the round bale press is acceptable and the harvest losses could be reduced with a chopper. To investigate the fermentation of whole crop pea, two laboratory trials with two different harvest times were conducted.

## MATERIALS AND METHODS

Two harvests (2002, 2005) were used as well as two harvest times. As silos we used 1.5 litre capacity glasses. Different silo designs are used for day 3 pH, day 49 aerobic stability and day 90 fermentation studies, which run in parallel. To achieve anaerobically unstable untreated silage, 3 silos from each treatment will be air stressed by flushing the silages with air after 4 and 6 weeks of anaerobic storage for about 24 hours through 6mm holes in the bottom and lid of the silos. The silos were stored in a room adjusted to constant 25°C, in the dark. 3 replicates per treatment were opened after each of 3, 49 and 90 days storage. The trial was carried out in such a way that it conforms to DLG guidelines for silage trials and in line with the DLG silage additive approval scheme. The analysis was similar to the methods of VDLUFA. The fermentation parameters as pH and organic acids were analysed in the laboratory of the research centre.

# **RESULTS AND DISCUSSION**

Table 1 shows the level of crude nutrients of the two tests compared to results of other authors or standard table values. The harvest of 2002 was on 3rd July, in swath, prewilted to a dry matter content of 33.2 % and on 19th July to 56.3 % DM. The key is that the crude fibre content at this stage of maturity on 3rd July already stood at 29.1 % and in the 2nd harvest time had increase to 33.8 % DM; but the crude protein decreased from 17.2 % to 14.7 % based on DM.

Harvest time	DM %	CF	СР	CL	DOS	MJ
		% DM	% DM	% DM	%	NEL-1kg DM
Reference [1]	28.2	27.8	14.5	1.8	73.0	6.11
3rd July 2002	33.2	29.1	17.2	2.4	70.9	6.03
19th July 2002	56.3	33.8	14.7	1.4	66.1	5.48
13th July 2005	19.9	24.6	17.9	2.3	-	6.06
18th July 2005	27.8	28.4	17.7	3.0	-	6.00
Green peas *	15.0	25.0	20.0	3.0	-	6.08

Table 1. Harvest times and forage composition

(Table values)

This expresses itself clearly in the digestibility of the organic matter, and ultimately led to lower energy content.

This was, at the 1st harvest time, 6.03 MJ NEL per kg DM and decreased to 5.48 by the 2nd harvest time. This coincides with results from the literature and also agrees with the standard table values for green peas from Grub. The silage trial from the harvest of 2005 had similar harvest dates. Straight after harvesting it was transported to the institute for wilting. The DM levels were 19.9 and 27.8 %. As in the 2002 harvest it was expected that the fibre would increase and the protein would decrease with the second harvest. The digestibility was not determined but use of standard institute calculations estimated the energy content, as in the previous trial at 6.06 or 6.00 MJ NEL per kg DM respectively. Table 2 shows that a DM of more then 30 % gives a high fermentation coefficient as parameter for a good fermentation base. A DM that is too low also results in a low FC [4]. The primary question of the trial was whether this growth is suitable for silage. This ability is analytically about the fermentation coefficient (FC) expressed by the fermentable substances, such as water-soluble carbohydrates, and lactic acid production as affected by sugar. The FC depends on not only DM but also buffers against lactic acid, such as protein and soil (table 2). Comparing the crop years together, better FC is reflected in higher levels of DM. For the 2002 harvest the 2nd harvest date (56.3% DM) had a similar FC as the first harvest date, despite lower sugar content. The sugar content in the 2005 crop year was roughly the same between both the harvest dates, and the buffering capacity is also not far apart. The higher DM leads to a better FC, even when the fresh material in both cases is difficult to ferment.

Table 2.	Parameter	for	fermentation
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Harvest time	DM %	Sugar % DM	Buffer g LA/100 g DM	FC
3rd July 2002	33.2	12.6	5.3	61
19th July 2002	56.3	4.0	4.9	58
13th July 2005	19.9	7.8	6.2	30
18th July 2005	27.8	7.5	5.9	39

The results in table 3 show, that the fermentation quality is based on the DM. The aim is to have 30 % DM not < 20 % or > 45 % so as to avoid butyric acid fermentation, which is expected in the so-called area of wet or dry.

Harvest Time	pН	Acetic acid	Butyric acid	Lactic acid	Ethanol	NH3-N %	ASTA	GAS
	value	% DM	% DM	% DM	% DM	total N	day	points
Control03.07.02	4.3	3.2	0.0	10.4	0.3	5.7	> 9	90
Achem	4.3	3.4	0.0	9.4	0.5	8.5	> 9	90
Abiol	4.1	3.6	0.0	11.6	0.4	7.0	> 9	90
Control19.07.02	5.5	0.6	3.0	0.2	0.7	10.3	> 9	18
Abiol	5.1	1.4	0.5	1.1	1.3	4.2	> 9	77
Control13.07.05	5.2	3.2	4.5	1.2	0.6	17.2	2	0
Achem	4.6	5.4	0.0	4.7	0.6	13.8	14	60
Abiol	4.9	4.8	0.8	2.7	0.6	16.1	4	28
Control18.07.05	4.0	1.7	0.0	9.8	0.5	12.3	2	100
Achem	4.0	1.4	0.0	9.9	0.4	11.4	11	100
Abiol	4.3	2.9	0.0	6.6	0.4	13.7	10	95

**Table 3.** Fermentation Quality of whole crop peas (Achem = additive chemical; Abiol = additive biological (LAB)).

Table 3 shows the fermentation quality depending on harvest time. The better fermentation of the harvest 2002 on the 3rd of July with FC 61 leads to a butyric acid free silage, with high lactic acid and low protein degradation (NH3-N% Total N). Silage is assessed on a point system (100 being the best), using a new DLG-key [4], which results in the loss of 10 points for 3 to 3.5% acetic acid (AA) in DM. Samples without butyric acid, receive 90 points. At pH values - 4.5 in the DM-range 30 - 45%, +10 points total to 100 points. The fermentation quality is therefore a very good judge. When control was very good with no improvement additives be achieved. All silage of harvest 2002 was aerobically stable. At the 2nd harvest date with the very high DM content a high proportion of butyric acid bacterium with the quality label (1c, 2, 4b), it was a good quality to be raised. Will the 2nd trial from the 2005 crop year for comparison, is the big risk with this difficult fermentation classified as serious fresh forage. Even the use of a chemical additive leads to a Grade 3, improved. On the use of a biological additives should be waived, the DM-content of about 20 % is too low. Was prewilted, on the other hand, it is with the control already a very good silage produced, which also additives with no further improvement achieved.

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# EFFECT OF BONSILAGE MAIS ON THE FERMENTATION, AEROBIC STABILITY AND DIGESTIBILITY OF CORN SILAGE

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## **INTRODUCTION**

In silages, lack of oxygen and accumulation of lactic acid that results in a low pH, inhibits microbial metabolism and preserves nutrients. Exposure to air during feeding and storage can cause silages to spoil. In many studies lactate-assimilating yeast have been implicated as the primary cause of aerobic deterioration in silages. The objective of this study was to evaluate the effect of inoculating corn silage with Bonsilage Mais containing a mixture of homo- and hetero-fermentative lactic acid bacteria (LAB) on fermentation quality, aerobic stability and digestibility. The study has been carried out by the 'Landwirtschaftskammer Rheinland', Bonn, Germany.

# MATERIALS AND METHODS

#### **Plant material:**

Whole plant corn, cultivar Romario (KWS S240) harvested when 60% DM in grain corn was reached (approx. 36% DM in whole plant material). Prior ensiling the forage was homogenized in a fodder mixing trailer, one part was ensiled untreated (Control) another part was treated with Bonsilage Mais (Bonsilage M). The inoculum was diluted in water and applied at the rate of 1 g/t of forage with a sprayer.

# **Ensiling:**

18 samples of forage were ensiled in laboratory silos (1.5-L; 3x3 repeats per treatment) for 90 days; 12 samples were ensiled in 90-L macro silos (6 per treatment) for 125 days.

#### **Measurements:**

Forage material: Weende Analysis for crude nutrients, lactic-assimilating yeast (LAY), lactic acid bacteria (LAB), nitrate and volatile fatty acids (VFA) (VDLUFA Methods); Ensiled material: Crude nutrients, VFA, ammonia-N, pH-values 0, 3, 49, 90 and 125 days after ensiling, fermentation loss (FL, Weißbach Method) and aerobic stability (Honig Method).

# **Digestibility trial:**

10 "Schwarzkopf" breed wethers (5 per treatment) were used for the digestibility trial. Treatment period consisted of 14 days adjustment and 7 days collection. Wethers were fed 2.6 kg DM corn silage and 150 g of soybean meal per day. The procedure was adapted to GfE (1991) guidelines.

# **RESULTS AND DISCUSSION**

#### Fresh Forage:

The chemical and microbial composition of untreated (Control) and treated (Bonsilage M) corn forages were investigated. There were no differences detectable among treatments. Generally the LAY concentration of forage of both treatments was relatively low.

#### Silage composition and aerobic stability:

Silages fermented well and the pH3 and pH90 did not differ among the treatments. Both treatments showed relatively high concentrations of acetic acid. The Bonsilage M silage possessed markedly higher acetic acid contents with 5.2% to 4.5% in DM compared to Control silage. Furthermore the lactic acid concentration of Bonsilage M treated silage was approx. 50% higher in relation to Control silage with 1.8% to 1.2% in DM. Whilst the fermentation losses of ensiling process showed only slight differences between treatments (7.8% Bonsilage M vs. 7.6% in DM Control) there were distinct differences in aerobic stability (7 d Bonsilage M vs. 2.6 d Control) and aerobic fermentation losses (1.5% of DM Bonsilage M vs. 5.3% of DM Control) among treatments.

#### **Digestibility trial:**

The results of the digestibility trial are shown in table1. In the case of CF there were no differences in digestibility measureable whereas relative to CP and CL the Bonsilage M treated silage showed relatively better digestibilities. This resulted in a slight increase in total OM digestibility and a calculated higher energy content of Bonsilage M silage (7.44 Bonsilage M vs. 7.27 MJ NEL Control).

#### Table 1: Digestibility of crude nutrients in corn silage

		Control	Bonsilage M
dOM	% of OM	79.5 ± 1.6	80.4 ± 1.3
dCP	% of CP	61.0 ± 6.2	66.7 ± 4.2
dCL	% of CL	84.7 ± 2.3	88.4 ± 1.5
dCF	% of CF	72.7 ± 2.8	72.8 ± 2.7
NEL	MJ/kg DM	7.27 ± 0.18	7.44 ± 0.14

dOM, dCP, dCL, dCF - digestible OM, CP, CL, CF

# CONCLUSION

Although the lactate-assimilating yeast (LAY) in the forage material were already relatively low in number and in spite of equal fermentation losses of treated and untreated silages there were marked differences relative to total acid content and stability of silages under aerobic stress between Bonsilage Mais and Control silages. Therefore for silages treated with Bonsilage Mais the combination of a distinct enhancement in aerobic stability, lower fermentation losses when exposed to air and a tendency to a better energy supply has the potential to increase feed intake and therefore animal performance.

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# EFFECT OF HOMO- AND HETEROFERMENTATIVE SILAGE ADDITIVE ON THE METHAN YIELD OF MAIS SILAGE

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## INTRODUCTION

The maximisation of methane yields per agricultural crop land employed provides the basis for an economically successful operation of a biogas facility. This can be achieved by reducing avoidable losses.

The execution of the test considers unavoidable silage losses as well as avoidable ones. For this trial chaffed crop maize was ensiled with a homo/hetero fermentative silage agent as well as untreated crop as control under laboratory conditions (5 l bucket).

# MATERIALS AND METHODS

#### **Ensiling Material and Treatment**

For this trial chaffed crop maize was ensiled with a homo/hetero fermentative silage agent as well as untreated crop as control under laboratory conditions (5 l bucket). The determination of the aerobic stability after opening of the silage was determined according to Honig. Based on warming the net energy losses can be estimated by this procedure. The energy losses on the first cut of the silage stock and seal housing can be actually determined.

# **Potential of Biogas Production**

The biogas respectively methane yields can be determined in pursuing batch-tests according to VDI guideline 4630. The daily biogas yield is calculated on the sample of the gas produced less the zero sample of gas from inoculated mud. The design (contents of  $CH_4$ ,  $CO_2$ ,  $H_2S$ ) is based on infrared spectroscopy and electro-chemical methods. The volume of the biogas is converted to normal conditions afterwards.

# **RESULTS AND DISCUSSION**

The acetic acid content could be increased by 100 % compared to the untreated control silage by use of homo/hetero-fermentative silage additives. This leads to an improved aerobic stability and results in lower energy losses under ambient oxygen (Fig. 1).

Figure 1. Lactic acid and acetic acid concentration of the silage



The use of homo/hetero-fermentative silage additives increases the unavoidable silage losses, this effect is absorbed by an improved aerobic stability and thus more than compensated (Fig. 2).

# Figure 2. Silage losses and warming losses from untreated and treated silage



The positive influence of the acetic acid is clearly proved by the increased methane yields of the treated silage contrary to the control silage. Starting point for the increased methane production is the improved aerobic stability as well as the direct conversion of acetic acid into methane (Fig. 3).



Figure 3. Methane yield from untreated and treated silage

## CONCLUSION

The systematic regulation of the fermentative acid sample by silage starters leads to a depression of total avoidable losses. An improved methane yield per ton silage used could be proved in the lab test. Supporting tests on the examination of effects in practice have to be conducted.

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- poľnohospodárske a lesné traktory



 mobilné drviče vlhkého kukuričného zrna, technologické linky na výrobu kŕmnych zmesí



- konzervovanie krmovín do veľkoobjemových vakov



 kosačky, obracače a zhrňovače krmovín, obaľovačky, lisy, prepravníky balíkov, zberacie vozy



- valce na utláčanie siláže

# CONSERVED FEEDS IN ANIMAL NUTRITION AND NEW TECHNOLOGIES

# THE EFFECT OF INOCULANT APPLICATION ON LEGUME-GRASS SILAGE FERMENTATION, INTAKE, MILK QUALITY AND PERFORMANCE OF LACTATING DAIRY COWS

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# ABSTRACT

Low concentrate forage feeding systems for cattle are the most appropriate in Lithuania. Grass and legume silage is the basic feed in dairy cow rations. First cut red clover-grass mixture was ensiled in pits with inoculant (Lactobacillus rhamnosus +Propionibacterium freudenreichii ssp. shermanii) or without any additives. Addition of inoculant improved the fermentation profile of the silage by lowering pH (P<0.05), ammonia –N content (P<0.05) and butyric acid (P<0.01) content and increasing lactic acid concentration (P<0.01) compared with spontaneous fermented silage. The nutrient (DM) losses were lowered by 19.4% (P<0.01), and both organic matter digestibility and energy values were higher for the inoculated silage in comparison with the ordinary one. A feeding study was conducted using lactating dairy cows to compare red clover-grass mixture silage inoculated or not inoculated. The lactating dairy cows fed inoculated silage consumed on average 0.89 kg/DM more compared with feeding non-treated silage. The milk yield tended to increase when the inoculated silage was fed and, the yield of energy corrected milk (ECM) was higher by 2.1 kg<sup>-</sup>/day/cow. The output of milk fat and milk protein were higher respectively by 84.8 and by 58.6 g/day/cow for cows fed the inoculated silage compared with the untreated silage.

# INTRODUCTION

The advantages of legumes as one of the main nitrogen sources and valuable winter forage are still underused. In particular, legume-based systems are known to contribute to sustainable, environmentally-friendly and energy-efficient agriculture (Porqueddu *et al.*, 2003). Opportunities for promoting grassland utilisation are related to the positive health characteristics it gives to animal products. Obtaining good fermentation quality, digestibility of nutrients and high energy and protein value in silages, requires the regulation of the ensilage process, particularly for herbages with the higher values of buffering capacity. Studies (Playne *et al.*, 1966) have confirmed that clovers have approximately twice the buffering capacity of the ryegrass and this is clearly an important factor associated with the difficulties encountered in the ensilage of leguminous crop. The advantages of the use of biological inoculants, recently obtained bacterial additives, thanks to the suitable selection of lactic acid bacteria, have been stressed by many workers, and it is clear from the results that inoculants have a beneficial effect on the improvement of the fermentation quality of silages (Wrobel *et al.*, 2004). An experiment was conducted to compare the effects of ensiling red clover-grass mixture untreated and treated with biological additive on silage quality and to examine the nutritive value of the silages.

## MATERIALS AND METHODS

A legume-grass mixture was used in the experiment (64% to red clover (Trifolium pratense L.) cv. Arimaiciai, 12% - timothy (Phleum pretense L.) cv. Gintaras, 16% - meadow fescue (Festuca pratensis Huds.) cv. Kaita and 8% others) on second (2) year's use. The inoculant (based on two patented bacterial strains: Lactobacillus rhamnosus LC 705 (DSM 7061) and Propionibacterium freudenreichii ssp. Shermanii JS (DSM 7067), Finland) dosage 10<sup>6</sup> cfu g<sup>-1</sup> was applied using a commercial pump "HP-20" in the chopper. The herbages was ensiled in two ferro-concrete pits 100 t each (one - inoculant free, another - with inoculant). During the ensilage, samples of chopped grass were collected to determine its chemical composition. Five control bags of 1 kg weight each were put into each pit to determine dry matter (silage fermentation) losses. Silages were stored for a minimum of 92 days and from the pits were offered to animals. After withdrawal and weighing of the control bags, the chemical composition, fermentation quality and dry matter losses of silages were determined. Aerobic stability was measured using data loggers that recorded every four hours temperature readings from thermocouple wires placed in three replicate 200-g silage representative samples aerated in open plastic bags placed into open-top polystyrene boxes. Ten multiparous dairy milking cows divided in two groups were fed *ad libitum* one group ordinary made silage, another group – inoculated silage, during 120 days period, following a 16 days pre-period. Concentrates were offered at the some level to the both groups. The feed intake and milk yield were recorded for 2 days every 2 weeks. The results were analysed by one- way analysis of variance (ANOVA). The differences between treatment means were tested using the Fisher's Least Significant Difference (LSD) (Snedecor and Cochran, 1989). All differences quoted in the text are significant at the 0.05 levels unless stated otherwise.

# **RESULTS AND DISCUSSION**

The chemical compositions of the herbages and the silages are given in Table 1. The activity of the inoculant was evidenced in this experiment by higher water soluble carbohydrates (WSC) by 18.5g/kg (P<0.05), total acids by 9.81 g/kg (P<0.05) and lactic acid by 21.23 g/kg (P<0.01) and lower acetic acid by 10.41 g/kg (P<0.05), butyric acid by 1.03 g/kg (P<0.01) contents of the inoculated silage compared with the untreated silage. There were found (Driehuis et al., 2001) that silages inoculated with *L. buschneri* plus *Pediococcus pantosaceus* and *L. plantarum* had significantly higher concentrations of lactic acid and lower concentrations of acetic acid. As compared with the untreated silage, the inoculant reduced proteolysis. Lower protein breakdown occurred in the inoculated silage as indicated by the lower (P<0.05) ammonia –N content. Winters *et al.*, 2001 found that inoculation with *L. plantarum* improved silage quality and reduced the extent of protein breakdown during ensilage of red clover. Due to the higher fermentation quality the

nutrient (DM) losses were lower by 19.4% (P<0.01) in the inoculated silage compared with the untreated one. *In vitro* organic matter digestibility of inoculated silage was 760 g/kg DM and that of the untreated silage 748 g/kg DM. The inoculation had a positive effect on the nutritional value of silages, however, the digestible energy of the inoculated silage was higher by 0.67 MJ g/kg DM (P<0.01) compared with untreated.

Treatment	Herbage	Sila	ages	I SD. or	I SDaw	<b>S</b> -
Treatment	Therbage	С	Ι	LSD <sub>0.05</sub>	LSD <sub>0.01</sub>	S <sub>x</sub>
Dry matter, (DM) g/kg	327.8	318.60	331.5	27.377	39.835	2.582
In dry matter g/kg:						
Organic matter	938.5	933.80	937.5	5.303	7.716	0.174
Crude protein	124.4	125.80	130.1	14.872	21.640	3.564
Crude fibre	214.2	227.30	221.1	9.030	13.139	1.235
WSC	111.5	28.50	$47.0^{*}$	17.066	24.833	13.850
Total acids		67.68	77.49*	6.948	10.109	2.935
Lactic acid		40.01	61.24**	8.681	12.631	5.258
Acetic acid		26.25	$15.84^{*}$	9.103	13.245	13.260
Butyric acid		1.30	$0.27^{**}$	0.700	1.018	27.380
Ammonia N, g/kg total N		39.96	33.18*	4.810	6.999	4.033
pH		4.39	4.23*	0.133	0.193	0.946
ME, MJ/kg DM		8.21	8.88**	0.233	0.339	0.836
DM losses, g/kg DM		101.81	82.02**	8.302	12.080	2.770

Table 1.	Chemical com	position of h	erbage and	silages and	fermentation	quality of silages
		1	0	0		

\* and \*\* denotes significant at level 0.05 and 0.01 respectively.

The inoculant was not found to have a negative influence on air stability of the silage (Fig.1). Both inoculated and untreated samples increased in temperature by more than  $3^{0}$ C after 3 days from the start. The temperature of inoculated and untreated silages rose above the ambient temperature within 1 day, and the untreated silage had a temperature rise of more than  $2^{0}$ C within 1 day while the inoculated silage had a temperature rise of more than  $2^{0}$ C in more than 2 days. Other authors found that some inoculants can improve the aerobic stability in silages by inhibiting the growth of both yeast and moulds in silages (Driehuis *et al.*, 2001).





Inoculation gave the higher (by 0.89 kg/cow/day) dry matter intake than the untreated silage. Milk yield was affected due to the higher intake and the higher nutritive value of the inoculated silage. Average milk yield was higher by 13.2% for the inoculated silage diet compared with the untreated silage. Higher silage dry matter intake and better performance of animals were found by Winters et al., 2001. The results showed that milk fat and milk protein did not differ markedly between the treatments. Due to higher milk yield the output of milk fat and milk protein were higher respectively by 84.8 and by 58.6 g day<sup>-1</sup> cow<sup>-1</sup> for cows fed the inoculated silage compared with the untreated silage.

# CONCLUSIONS

Inoculation can improve forage quality of legume-grass mixtures and thus create financial economy at farm scale. The quality and nutritive value of the silage and obtained milk yield and milk composition of dairy cows demonstrate that ensilage of red clover-grass mixture with the bacterial additive was a better method of feed preservation than use of no additive when silage making.

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# **MECHANIZATION OF SILO COVERING**

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Up to now, the sealing of bunker silos is a lot of work. The purpose of sealing, namely the exclusion of air and water, is not always sufficiently obtained. A French dairy farmer, Gilbert Duhamel, has developed a mechanical sealing system, which reduces to a large extent the expenditure of work and allows – when properly applied – an airtight and watertight sealing.

# How the system works

The system works according to the following principles:

- First, to protect the sheet, a felt (synthetic textile) is used. This non-airtight felt is 0,5 cm thick, very resistent against mechanical damage, treated against ultra-violet rays and rather supple.
- Second, the felt is unrolled (to cover the silo) and rolled up (when silage is taken out). It is rolled up on a plastic tube. In reality, the tube consists of several 80 cm long plastic tubes connected by cardan joints. Normally, the sheet is unrolled and rolled up together with the felt. The tube is turned by a ratchet attached to the tube.
- Third, to ensure that the cover (felt and sheet) lies firmly on the silage stack and seals it permanently, special devices ("arms") are fixed at the top of the silo wall and with these arms the cover is pressed upon the stack. Silo bags are no more necessary, neither along the wall nor across the silo (the felt cover normally consists of several pieces glued together).

# Details of this arm are shown in the diagram.

Every 2,5 m, at the wall is fixed a rail-plate (10cm x 10 cm). In this rail-plate, a support (of T-form) is fixed. A tube slides in the horizontal part of this support. This tube ends in a guide in which another tube slides. This latter tube presses by means of an elastic rope a rail against the stack.

Air tightness is achieved in the following way. Before ensiling begins, a wall sheet is put over the wall. The upper part of this sheet is put on the stack. Then the sheet and the felt are unrolled on top of the stack. The main sheet and the wall sheet are overlapped. These two sheets are pressed against each other and upon the stack and this gives air tightness. The ratchet runs along the inner side of the wall, so that this covering system can be applied at every filling rate of the silo.

# The advantages of this system

It is labour saving and the work is much easier. The sheet is fully protected by the felt against mechanical damage (by birds, etc.). The felt has a life time between 10 and 15 years, and the sheet can be used several times (up to 5 times). Even the use of only a thin sheet (which is renewed every year) is possible, because it is fully protected by the felt.

# The system allows an airtight and watertight sealing.

With larger silos than 10 - 12 m wide, the system must be adapted to this condition. Instead of one piece of felt, several strips of felt are put on top of the stack alongside the silo. In this case, every strip of felt is unrolled and rolled up separately. The different strips are linked together (only for the time they lie on the stack) by special links.

In this case, the sheet cannot be rolled up as a whole. As a consequence, only a thin sheet is used which is renewed every year and not rolled up.

Because of the long lifetime of the felt and the repeated use of the sheet (or the use of a thin sheet only), the system is environmentally friendly, especially in connection with a better fermentation and lower losses due to the new system, which reduces the production of carbon dioxide.



# Holding device of Duhamel

Figure 1. The sheet and the felt (synthetic textile) are rolled up.



Figure 2. The sheet is protected by the felt. No tyres or bags are at the silo.



# TECHNOLOGICAL MEASURES FOR INCREASE OF QUALITY OF SILAGES AND GRASS HAY IN BIG ROUND BALES

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# **INTRODUCTION**

Big bale silage making technology has become popular among the farmers. However, the silage quality of big bale is often poor. (Lättemäe et al., 2003). For example O'Brien et al. (2005) found out at 35 farms of Central Ireland that at 17 farms, the dangerous *Schizophyllum commune* mould was detected (Killian et al., 2005); besides, 58 from 64 (i.e. full 90 %) of the tested bales were contaminated by mould colonies, mostly *Penicillium paneum*, *Geotrichum*, *Fusarium* a *Mucoraceous* species. O'Brien et al. (2008) obtained similar results in further years as well. Besides, silages stored in big round bale often show the big problem related to the presence of *listerias* and *clostridias* (Weissbach, 2005), mainly of those producing botulin of B type, which is particularly dangerous for horses. The significance of increased attention at production and storage of silages and hay in big round bales is emphasized also by the fact that such technology is often used in horse breeding. Horses are also particularly sensible to breathing difficulties caused by mould spores and dust and mould toxins bother them as well.

# MATERIALS AND METHODS

The goal of the work was to verify such technological measures in production of silages and hay in big round bales that would lead in line breeding of sheep and horses to improve their health, condition and performance; a secondary positive could be better use of fodders or reduction of the areas needed for cultivation of fodder plants.

The monitoring took place at the farm of Dibaq a.s. joint stock company from 2003 to 2007. The business farms in an LFA production area (submontane, less favourable, of Oa type), elevation of 410-470 m above see level, with average yearly temperature of 8°C and yearly precipitation of 730 mm. There are 154,85 ha of agricultural land available (from that amount, 111,38 ha arable land and 43,47 ha fodder plants). Besides, it has the possibility to gather fodder plants from local airport (18,4 ha) on the base of a contract. As airports have special regime – the height of vegetation must not exceed 30 cm, the fodder had to be gathered from that area earlier and more frequently than from other areas.

The year 2003 was check year. In the given period (2003-2007), the farm held 100 sheep of the Texel meat breed and 7 horses (English thoroughbreads, Czech warm-blooded horses, ponies) on average. In summer period, the animals were on pasture, in winter in stables.

All fodders intended for storage was stored into big round bales. Both the silages and the hay were packed into the stretch foil and stored in the farm rooms in the check year. There was little space, so the bales were stored even in places not suitable for such purposes, e.g. uneven, with weed vegetation, near to paths and fences where horses were grazing (they bent over the fence and bit holes in some bales; air penetrated into the bales through the holes, leading to degradation of the fodder stored inside). The bales were brought to the stable with a "bob-cut" with fork – the bale was placed on the straw with its flat side, the foil was removed from it and a metal feeding box was tilted over the bale.

The effect of the measures was ascertained on the base of the results of indicators of fertility of the mothers and further of the indicators of fattening ratio and slaughter quality of lambs (live weight gain in age of 100 days).

# **RESULTS AND DISCUSSION**

Measures in the first observed year:

- creation of space for storage of bales and levelling-out of surface (earthwork); so the bales could be stored on flat, dry surface free from sharp objects and vegetation
- use of microbial preservatives according to the condition of the gathered material
- all bales with their flat side to the side, i.e. with the round side down, not stacked
- the path sufficiently wide and near the fence, so that the horses cannot reach the bales over the fence lengthening of the stable roof so a space was created to store the hay under roof
- hay bales stored under roof without foil, stacked (full use of the space)
- signs of presence of moulds, fermentation, becoming stale, contamination and other degradation constitute clear motive to eliminate the fodder from the feeding dose.

Measures in the second observed year:

- all hay bales without foil, silage bales coated with six to eight layers of stretch foil (formerly with four)
- depositing of hay bales under roof with several-day delay (depending on weather) the hay can "breathe"
- at sorting, the bale with flat side to the side.
- Measures in the third observed year:
  - hay bales stored outside on a pallet, also hay bales under roof stored on a pallet, handling of bales on the pallet
  - hay bales in a row with their flat sides adjacent, in two rows with a gap between them

Measures in the fourth observed year:

• deratisation under bales (against rodents), desiccation of bale surroundings (against growth of weeds)

• hay bales stacked, but covered with canvas.

Completely in accordance with data stated in literature, e.g. Lättemäe et al. (2003), it was confirmed that insufficient care given to production and storage of silages and grass hay in big round bales lead to degradation of quality during storage and their subsequent feeding to sheep and horses can cause dietetic problems to the animals and degrade their performance.

The measures lead to improved condition of the animals; positive influence was observed also on the health condition of the animals (less visits of veterinarian, less death losses and forced slaughters). The indicators of fertility of mothers and indicators of fattening ratio of the lambs increased in inter annual comparison.

In general, the measures showed their effect in the differences between production and consumption of fodders and their write-offs because of bad quality.

Less fodder residues were removed to the dung (fodder write-offs dropped) because of mould contamination or other worsened quality (removal of surface layers of brown colour and slimy consistency).

Index	2003	2004	2005*	2006	2007
Big bales consumption of hay	371	266	252	238	183
Big bales consumption of silage	0	94	92	95	163
Nb. of DJ (500 kg live weight)	25.05	24.9	24.3	22.05	21.9
Consumption [kg DM/DJ/day]	10.2	9.4	9.1	9.5	9.2
Losses of DM [%] qualified estimation	18	12	11	8	7

Chart 1. Consumption of big bales of silage and grass hay

Index	2003	2004	2005*	2006	2007
Nb. of ewes in test	99	98	113	75	97
Fertility [%]	154.0	161.6	144.2	160.0	171.1
Breeding lambs [%]	97.6	145.4	119.3	133.7	155.1

Chart 3. Live weight gain of tested lambs in age of 100 days

Index	2003	2004	2005*	2006	2007
Nb. of lambs in test	105	131	128	85	139
Gain - Avg [g]	204.6 a	241.5 b	198.5 a	273.7 с	259.2 b,c
Gain – Sx	48.4	53.0	39.5	62.3	48.0

a, b, c: different lower case letters in a row show significant differences between variants (P<0,05)

\* In this year there were disease of lambs in the farm (Contagious ecthyma) - parapoxvirus (příměť pysková)

## CONCLUSIONS

During the five-year practice at the farm of horses and line-breed of sheep of Texel, distinctly meat-bringing breed, the technological measures in the sphere of obtaining and storage of silages and grass hays in big round bales (especially protecting from deterioration, storage in a pallet, and big bales of hay without foil) showed their positive effects in improved health condition, fitness and performance of the animals; the fodders were used better and saved – which could be seen in reduced write-off of the fodder and in use of the areas for other purposes than cultivation of fodder plants. The better general impression from the farm management is not insignificant either, because during the year it is visited by a lot of people and several students have their practical training there. The interannual increases of performance lambs (live weight gain in age of 100 days), especially among the first (control) year 2003 and the next years (2004, 2006, 2007), were statistically significant (P<0.05).

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# ALFALFA BALE-SILAGE: A NEW BALE-FORMING TECHNOLOGY

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## INTRODUCTION

The authors aimed to evaluate a new bale-forming system (bale parameters and fermentation characteristics) and dry matter limitation of the new baling technology. Authors investigated the effect of short (290 g/kg DM: 4 hours) and long term wilting (520 g/kg DM: 12 hours) on bale parameters and fermentation profile in the new-type alfalfa-bales.

# MATERIALS AND METHODS

Harvest was carried out in a second year alfalfa field with a self-propelled chopper harvester (Claas Jaguar 840). Alfalfa derived from the  $2^{nd}$  cut (June 2007) in bloomy phenophase (crude protein: 196 g/kg; NDF: 445 g/kg) and was mowed with theoretical chop length of 20-30 mm. Baling was carried out by a Göweil LT Master variable chamber baler-wrapper machine. Nominal size of bales was: 1.13 x 1.22 m. Pressurization: 150 bar. Film for wrapping was applied with thickness of 25  $\mu$ m in 7 layer carried out by 26 turn and 60% pre-stretch. Output and efficiency were the following: 18-20 bales / hour for alfalfa. The pH, lactic and volatile fatty acid composition were analysed on the 13<sup>th</sup>, 30<sup>th</sup> and 70<sup>th</sup> day of fermentation according to the Hungarian National Standards (Hungarian Feed Codex, 2004).

#### **RESULTS AND DISCUSSION**

Nutrient composition of un-wilted and wilted alfalfa, alfalfa haylage (520 g/kg DM) and alfalfa silage (290 g/kg DM) are shown in Table 1. There were not found significant difference in crude protein-, NDF-, ADF- and ADL-content of wilted alfalfa, alfalfa haylage and silage. It was found that relatively long term wilting (520 g/kg DM: 12 hours) reduced significantly the carotene content of wilted alfalfa and haylage compared to un-wilted and short term wilted alfalfa (un-wilted alfalfa:  $126.7\pm6.7$  g/kg DM, long term wilted alfalfa:  $24.3\pm2.0$  g/kg DM, short term wilted alfalfa:  $65.7\pm8.5$  g/kg DM).

It was found significant difference in wet weight (290 g/kg DM: 904 kg, 520 g/kg DM: 657 kg p $\leq$ 0.05, n=15) and weight based on dry matter content (290 g/kg DM: 262 kg DM; 520 g/kgDM: 342 kg DM, p $\leq$ 0.05, n=15) (Table 2). Better homogeneity of the new-type bales compared to the conventional bales, owing to chopped and mixed forage, was confirmed by the low variation in wet bale weight (coefficient of variation: 290 g/kg DM 1.4% and 520 g/kg DM 1.5%, respectively).

Extremely high density was carried out with the new technology: 213-278 DM kg/m<sup>3</sup> due to high pressurization (150 bar) and small particle size (20-30 mm) compared to conventional bales (90-200 DM kg/m<sup>3</sup>) in both treatment (Table 2). There was higher dry matter density in bales formed from alfalfa wilted longer time than in alfalfa bales with lower DM content (290 g/kg DM: 213 kg DM / m<sup>3</sup>; 520 g/kg DM: 278 kg DM / m<sup>3</sup>, p $\leq$ 0.05, respectively).

High density resulted extremely good anaerob circumstances for fermentation and organic acid composition was proven by fast pH-drop (pH on the 13th day: 4.87a vs 4.84a, respectively) and lactic acid dominated fermentation (lactic acid ratio on the 13th day: 73.7% vs 81.3%, respectively), in both treatment (Table 3).

It was found (Table 3) higher lactic acid concentration, significantly higher lactic acid: acetic acid ratio (LA:AA 13th day: 2.83a vs 4.46b; 30 day: 3.39c vs 4.17b; 70 day: 2.83a vs 4.46b, respectively) and lower acetic acid content during the whole fermentation period (on the 13<sup>th</sup>, 30<sup>th</sup> and 70<sup>th</sup> day of fermentation) in bales formed from alfalfa wilted longer time (520 g/kg DM).

# CONCLUSIONS

It was confirmed that the new baling system is able to form bales in a wide range of dry matter content (290-520 g/kg in alfalfa with theoretical chop size of 20-30 mm) with extremely high (213-278 DM kg/m<sup>3</sup>) density due to high pressurization (150 bar) and small particle size (20-30 mm) compared to conventional bales (90-200 DM kg/m<sup>3</sup>) in both treatment. Ideal wet weight range of alfalfa bales (1.1 x 1.2m size) is 750-800 kg (35-400 g/kg DM, 20-30 mm chop length, 40-45m g/kg DM NDF). Higher than 900 kg of bale weight (with nominal size of 1.1 x 1.2 m) due to low dry matter content (lower than 300 g/kg) can cause high challenge of effluent and bale deterioration. High density (effective and quick air exclusion) had beneficial effect on fermentation intensity and quality. It was proven by fast pH-drop (pH on the 13th day: 4.87a vs 4,84a, respectively) and early lactic acid dominated fermentation (lactic acid ratio on the 13th

day: 73.7% vs 81.3%, respectively), in both treatment. Long term wilting (520 g/kg DM: 12 hours) improved the lactic acid concentration ( $p \le 0.5$ ) and LA: AA ratio (lactic acid to acetic acid ratio: 13th day: 2.83a vs 4.46b; 30 day: 3.39c vs 4.17b; 70 day: 2.83a vs 4.46b, respectively), but significantly reduced the carotene content of wilted alfalfa compared to un-wilted and short term wilted alfalfa (un-wilted alfalfa: 126.7±6.7 g/kg DM, long term wilted alfalfa: 24.3±2.0 g/kg DM, short term wilted alfalfa: 65.7±8.5 g/kg DM).

Table 1.	Nutrient content of u	n-wilted alfalfa pla	nt, wilted alfalfa	, alfalfa haylage ar	nd silage (n=5).
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	C. protein	NDF	ADF	ADL	Carotene
Un-wilted alfalfa	196.0±10.4a	444.7±22.2a	354.8±31.1a	82.3±28.5a	126.7±6.7a
Wilted alfalfa 520 g/kg DM	197.6±5.9a	450.5±9.3a	347.4±5.6a	66.6±2.8b	24.3±2.0b
Alfalfa haylage 520 g/kg DM	199.8±4.2a	441.0±10.4a	353.1±11.2a	78.2±2.0a	10.9±0.7c
Wilted alfalfa 290 g/kg DM	192.7±7.4a	450.9±13.8a	342.8±18.5a	70.1±6.6a	65.7±8.5d
Alfalfa silage 290 g/kg DM	190.6±3.3a	428.1±10.0a	352.3±13.3a	73.5±8.6a	32.1±7.6b

Different letters show significant difference  $p \le 0.05$ 

 Table 2.
 Bale characteristics in alfalfa baled with the new baler system (n=15).

	290 g/kg DM	520 g/kg DM
Bale wet weight (kg)	904±25.1a	657±13.0b
Bale dry weight (kg DM)	262±7.3a	342±6.6b
Wet density $(kg/m^3)$	734±10.3a	534±7.9b
Dry density $(kg/m^3)$	213±3.0a	278±4.1b

Different letters show significant difference  $p{\leq}\,0.05$ 

Table 3. Fermentation characteristics in alfalfa baled with the new baler system (n=5).

Day of	Treatment	pН	Total acid	Lactic acid	Acetic acid	Butyric acid	LA:AA
lermentation		-	g/kg	g/kg	g/kg	g/kg	
13th day	290 g/kg DM	4.84a	28.9a	21.3a	7.6a	0.0a	2,83a
	520 g/kg DM	4.87a	30.0a	24.4a	5.5b	0.0a	4,46b
30th day	290 g/kg DM	4.60a	34.8a	26.9a	7.8a	0.0a	3,54c
	520 g/kg DM	4.79b	37.6b	30.8b	6.7a	0.0a	4,65b
70th day	290 g/kg DM	4.49a	32.9a	25.2a	7.6a	0.2b	3,39c
	520 g/kg DM	4.74b	35.1a	28.2a	6.8a	0.1a	4,17b

Different letters show significant difference  $p \le 0.05$ ; LA:AA = lactic acid to acetic acid ratio

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# **SUMMARY**

The authors aimed to evaluate a new bale-forming system (bale parameters and fermentation characteristics) and dry matter limitation of the new baling technology. Authors investigated the effect of short (290 g/kg DM: 4 hours) and long term wilting (520 g/kg DM: 12 hours) on bale parameters and fermentation profile in the new-type alfalfa-bales. Baling was carried out by a Göweil LT Master variable chamber baler-wrapper machine from chopped alfalfa (theoretical chop length: 20-30 mm, crude protein: 196 g/kg; NDF: 445 g/kg). Extremely high density was carried out with the new technology: 213-278 DM kg/m<sup>3</sup> due to high pressurization (150 bar) and small particle size (20-30 mm) compared to conventional bales (90-200 DM kg/m<sup>3</sup>) in both treatment. High density (effective and quick air exclusion) had beneficial effect on fermentation intensity and quality. It was confirmed that the new baling system is able to form bales in a wide range of dry matter content (290-520 g/kg in alfalfa with theoretical chop size of 20-30 mm). Long term wilting (520 g/kg DM content) had advantagous effect on organic acid composition (lactic acid concentration and lactic acid to acetic acid ratio: 13th day: 2.83a vs 4.46b; 30 day: 3.39c vs 4.17b; 70 day: 2.83a vs 4.46b, respectively), but significantly reduced the carotene content (un-wilted alfalfa: 126.7±6.7 g/kg DM, long term wilted alfalfa: 24.3±2.0 g/kg DM, short term wilted alfalfa: 65.7±8.5 g/kg DM).

# CONTROLLING OF SILAGE CROP COMPACTION FOR VALIDATION OF SILAGE QUALITY AND AEROBIC STABILITY IN CASE OF SILAGE BAGGING TECHNOLOGY

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# **INTRODUCTION**

The conservation of feed crops in silage bags has been developed as an alternative operating technique to clamp silos. Distinguishing is the flexible applicability without buildings and structures which reduces the fix costs significantly. In order to the development of the technology to higher throughput it can be employed in harvesting systems with high performance, which has already made the concept of silage bags relevant in the field of biogas crops conservation.

The crop compaction is one of the main factors with an important effect on silage conservation and the aerobic stability after silo opening. In order to Investigations of Honig (1987) the silage crop has to be compacted to a density reducing the gas exchange below 20 l/m<sup>3</sup> at the surface of the silo. According to this standard reference values for silage crop compaction were derived by Honig (1987) and Spiekers (1998). Measurements which were carried out at clamp silos by Spiekers (2004) and Thaysen (2006) came to the conclusion that only about 25 % of the analysed silages achieved a degree of density in the range of the reference values.

## **Objectives in project**

For analysing the silage crop compaction in case of bagging technology to compare it, the density in bags of different crops were measured. The questions were if there is a significant effect of the dry matter content on the compaction in silo bags and how different is the density across the transverse section of the bags.

The compaction of silage crops while bagging is controlled by brakes and tube extension. The controlling of the brake pressure to reach a maximal rate of compaction has to be done by the machine operator and depends a lot on his experience which makes intensive training necessary. One aim of the present project is to develop suitable control loops for optimised forage compression and for the reproducibility of the silage success by means of bagging machines.

# MATERIAL AND METHODS

For sampling of the cores to measure the density in the bags an electric driven drill with a diameter of 102 mm had been constructed. While drilling the trier into the silage crop the material inside the core bit runs into it. The forward movement is stopped at 50 cm depth of penetration. The results of the method was Validated by measuring the density of silo blocks taken with a block cutter. A very high correlation of the results could be found. Disadvantages of the big drill are the necessary driving power and the heaviness of the unit. The cores were taken at 10 positions as shown in figure 1 at the transverse Section of the tubes.

Figure 1. Filled drill (left) and positions of the cores at the transverse section of the tube (right)



The fresh mass density was collected by weighting the cores on place where as the dry matter density could be calculated after drying of the samples. To control the heating at the surface of the bags the temperature inside the holes was measured by using an electronic thermometer.

In order to compact the silage crop, it is pressed under constant mass flow into the tube using a rotor. During the process the tube should be seated on solid ground. While running more and more raw material is pressed into the bag. The pressure inside increases and according to this the force in driving direction pushes the machine forward. The compaction process is regulated by a brake system controlling the forward movement. In order to regulate the braking pressure the tube material's expansion has to be controlled at imprinted measuring strips on the tube film. The right braking pressure depends on feed crop, its DM-content and the ground the machine is used on. An additional braking effect is caused by the rolling drag of the bagger and the tractor which has to be pushed forward during the press process. The controlling of the brake pressure to reach a maximal rate of compaction has to be done by the machine operator and depends a lot on his experience which needs intensive training.

In the project sensors placed around the tube of an experimental bagger are used to control the tube extension while the pressing process.

The pressure of the hydraulic disk brake has to be adapted to the extension of the tube. It would be optimal to

control the brake in a way that the tube extension is always on a constant level.

**Figure 2.** Measuring of the tube extension by controlling the size of the bag



# RESULTS

In fig.2 the average density of sugar beet pulpe, maize and maize ear silage dependent on its dry matter content is shown. The storage density of maize in bags was in the arithmetic mean of the 10 samples at the same level like the density in clamp silos measured in former surveys. Related to fresh mass densities of 530 kg/m<sup>3</sup> to 680 kg/m<sup>3</sup> were achieved, which is equal to 180 kg/m<sup>3</sup> to 230 kg/m<sup>3</sup> at dry matter contends of 32 % to 38 %. In sugar beet pulpe of 22 % dry matter contends fresh mass densities about 800 kg/m<sup>3</sup> were found. The density in maize ear silages with dry matter contends from 52 % to 63 % was between 580 kg/m<sup>3</sup> to 800 kg/m<sup>3</sup>.

Figure 3. Density of dry matter and fresh matter depending on the dry matter content of the silage crop (arithmetic mean of 10 samples)







The maximal difference of density between centre, flanks and top of the bags at the transverse section is from 20 % to 30 %. In case of heavy and wet silage crops like sugar beet pulpe the pressure on the material in the centre while storage is higher than in silage of advanced dry matter contends which effects more compaction of the silage crop.

Reheating of the silage crop at the surface was nearly only to be found at bags where the tube film was perforated as result of picking birds. Because of this fact it is absolutely necessary to put bird nets on the top of the tube after bagging.

By using ultrasonic sensors the tube extension can be contactless detected online. Difficulties during the measurement can be caused by humpy contour of the tube. In order to use an electro hydraulic brake it is possible to adapt the pressure continuously. In further experiences the best way to control the brake pressure in order to achieve a constant tube extension has to be find out.

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#### **SUMMARY**

The compaction of feed crops in silage making has important effects on the feed quality at every kind of silage technology. In order to evaluate compaction in case of the bagging technology measurements of density in bags have been carried out at different crops. In another part of the project suitable control loops for the pressing process have to be developed. The results of the density measurements show that the crop compaction in bags is at similar level to clamp silos which were tested in former investigations. In order to control the pressing process trials with an experimental bagger equipped with sensors are in work. By using this technique the compaction should become more repeatable and the operators work less difficult.

# GALEGA FOR FODDER AND BIOGAS PRODUCTION

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# ABSTRACT

Galega (Galega orientalis Lam.) is fodder legume introduced in Latvia in last decades and have high productivity and capacity to fix atmospheric nitrogen in range 200 - 453 kg/ha. The 35 mixed (13 binary and 22 multi – species) swards were developed on Stagnic Luvisol and Gleyic Podzol soils during 20-years investigation period. Productivity of swards decreases by 35.2 %, if harvested four times compare to two times harvesting during the growing season. Pure galega swards provides 40 - 70 t/ha green biomass or 10.27 - 14.23 t/ha DM in a year, without application of pesticides and N-fertilizer. The average content of metabolizable energy was 10.9 or  $11.4 \pm 1.1$  MJ/kg<sub>DM</sub> for pure galega or mixed galega–grass swards respectively. Split fertilisation with commercial nitrogen in amount of 90 kg/ha decreases yield of pure galega swards by 1.0 t/ha.

Biogas yield from galega, and cow's manure as control was investigated in 4 laboratory scale digesters of 5 l volume. Methane yields obtained in digesters were 218.6  $l/kg_{VSd}$  from cow's manure and 384.2  $l/kg_{VSd}$ ; 309.2  $l/kg_{VSd}$ ; 244  $l/kg_{VSd}$  from different galega-water and cow's manure mixtures. Estimated volumes of biogas released in 2 digesters in anaerobic fermentation process of galega haylage-inoculum mixtures, having volatile solids 19.4 % or 11.1 %, were 116  $l/kg_{VS}$  or 244  $l/kg_{VS}$  after 64-days fermentation period respectively. Galega in pure stands or in mixtures with grasses is productive, persists for long period and is an acceptable plant for fodder or biogas production in climatic and soil conditions in Latvia.

# INTRODUCTION

Galega (*Galega orientalis* Lam.) is recently introduced in Latvia, due to its persistency and high yielding ability. Longlived legume survives in pure stands for 25 and more years and provides annual DM yields from 9.56 to 11.0 t/ha (Adamovich, 2006). Successful treatment of galega seeds with nodule bacteria results in fixation of atmospheric nitrogen from 200 to 453 kg/ha, thus can to decrease the need for commercial nitrogen fertilizers. The usage of the symbiotic potential of fodder galega, grown in mixtures with grass, contributes to the production of ecologically safe forage. Perennial herbaceous grasses and galega can be easily stored as haylage, thus providing its usage for fodder or for round year biogas production. Usage of perennial grasses, especially legumes, for biogas production can be important alternative for farmers, due to unstable animal breeding market in Latvia, where the number of cows decreased drastically during last decades. Aim of study was estimation of productivity of fodder galega-grass swards and investigation of biogas output from fodder galega and galega haylage in anaerobic treatment process.

# MATERIALS AND METHODS

Research on fodder galega was started at Latvia University of Agriculture in 1978. Field experiments were conducted during 20 years period (1986-2006), aiming to estimate continuous green forage production from fodder galega-grass swards in the stage of intensive growth. The 35 mixed (13 binary and 22 multi – species) swards were developed on stagnic – luvisol (pH<sub>KCl</sub> was 6.7, mobile P 52 and K 128 mg/kg of soil). Pure swards, binary- and multi-species seed mixtures were composed of fodder galega cv. 'Gale' and 13 grass species: *Alopecurus pratensis, Arrhenatherum elatius, Bromus inermis, Dactylis glomerata, Festuca pratensis, Festuca rubra, Festuca arundinacea, Phleum pratense, Lolium perenne, Phalaris arundinacea, Agrostis gigantea, Poa pratensis and Poa palustris. Stands were sown in early May in 1980, 1986, 1990 and 1997. Seeding rate was 1000 germinating seeds per 1 m<sup>2</sup>. The ratio of fodder galega:grass seeds mixtures was 50:50 in 13 binary mixtures (1986). In all experiment series (1990 and 1997) mixtures contained 40 % fodder galega and 60 % grass seeds: 7 binary mixtures 40:60, 14 three – component mixtures 40:30:30, 5 four – component mixture 40:20:20:20, 1 five – component mixtures 40:15:15:15:15 and 2 six - component mixtures 40:12:12:12:12:12:12. The botanical composition of the sward was determined at each cut for all treatments. The chemical composition of plants was determined only for the first cut by the following methods: dry matter (DM) – drying; crude protein (CP) – modified Kjeldahl; crude fibre (CF), neutral detergent fibre (NDF), acid detergent fibre (ADF) and nett energy of lactation (NEL) – Van Soest (1980); <i>in vitro* digestibility of the organic matter (IVOMD).

The biogas yield was investigated on original laboratory equipment B4 including 6 digesters in volume of 5 1 operated in batchwise mode and equipped with devices for regulation of temperature in digesters at  $37\pm1.0^{\circ}$ C or  $54\pm1.0^{\circ}$ C. Digesters were equipped with sensors for automated registering of pH and gas volume data in computer. The substrates used for anaerobic fermentation were galega mixtures with fresh cow manure at different proportions in 4 digesters and galega haylage mixtures with inoculum (fermented cow manure) in 2 digesters. Additional water was added in digester 6 to increase moisture, compare to digester 5 (Table). Substrates were analysed, using approved methods for organic matter, volatile solids and moisture content, before filling in and after extracting out of digesters. Accuracy of measurements were  $\pm 0.02$  for pH value,  $\pm 0.0025$  l for gas volume and  $\pm 0.1$  °C for temperature.

# **RESULTS AND DISCUSSION**

Average yields of dry matter DM and crude protein CP were 8.97 t/ha DM and 1.94 t/ha CP, obtained at early flowering on stagnic luvisol in a two-cutting management during 20 years of pure galega growing without reseeding. Pure galega swards provides 40 - 70 t/ha green biomass or 10.27 - 14.23 t/ha DM in a year, without application of pesticides and N-fertilizer. Fodder galega significantly surpasses other forage legumes in respect to productive longevity. Inclusion of a grass species in a mixture resulted in yield increase by 28-36 %. Split application of the 90 kg N fertiliser affected negatively the proportion of galega in a sward, resulting in the decrease of DM yields by 1.04 t/ha at two cutting management, compared to unfertilized plots. Average yields from pure galega stands were 9.50 t/ha DM or 6.16 t/ha DM at two-cutting or four-cutting management in 9 production years respectively. Average yields from three most successful galega-grass swards with no N fertilizer were 9.80 t/ha or 6.56 t/ha DM at two-cutting or four-cutting management of metabolizable energy was 10.9 or 11.4  $\pm$  1.1 MJ/kg<sub>DM</sub> for pure galega or mixed galega–grass swards respectively. Noncompetitive grasses in the mixtures, e.g. timothy, do not affect productive longevity of swards compared to pure galega yield.

Anaerobic fermentation process was weak in all 6 digesters working in batch mode during first 2-3 day period after the start, while hydrolysis and acidification processes were ongoing. Value pH decline and rise again in digester 1 and 4 after longer period, compare to digesters 2 and 3 due to too high organic load in these digesters. Results are shown in Table.

Parameter	Unit	Digester 1	Digester 2	Digester 3	Digester 4	Digester 5	Digester 6
Substrate composition	%	100 cm	25 cm	50 cm	75 cm	55 in	32 in
			75 g+w	50 g+w	25 g+w	45 gh	68 gh+w
Total substrate weight	kg	4.120	3.294	3.593	3.624	1.142	2.144
Total solids	%	14.7	5.83	9.41	12.6	20.9	11.9
Organic solids	%	12.8	3.4	7.2	10.5	19.4	11.1
Biogas yield	l/kg <sub>VSd</sub>	411	627.8	535	436		
Average methane content	%	53.2	61.2	57.8	56.1	40.1	49.0
Methane yield	l/kg <sub>VSd</sub>	218.6	384.2	309.2	244.2		
Conversion rate	%	62.5	68.3	64.6	63.8		

Table 1. Parameters of substrates and biogas obtained in anaerobic fermentation process.

Remarks: cm - cow manure, g+w - galega plus water, gh - galega haylage, in - inoculum (fermented cow manure), gh+w - galega haylage plus water, VSd - volatile solids degraded.

Methane yield in digester 6, having lower concentration of organic solids, is higher compare to that in digester 5. Average methane content in biogas was 41 % or 49 %, released from digester 5 or 6 respectively, during 64-day anaerobic fermentation period. Carbon dioxide concentration in biogas released from galega haylage changes from 45-55 % at the beginning of fermentation period to 23-25 % at the end of anaerobic treatment process. Anaerobic fermentation of galega haylage is ongoing more intensively in substrate having less total solids content. Estimated specific volume of biogas was 116  $l/kg_{VS}$  for digester 5 and 244  $l/kg_{VS}$  for digester 6, or by 52 % higher in digester 6 after 64-day fermentation period.

# CONCLUSIONS

- 1. Average pure galega yield was 8.97 t/ha DM or 1.94 t/ha CP on stagnic luvisol in a two-cutting management in 25 years.
- 2. Average metabolizable energy was 10.9 or  $11.4 \pm 1.1$  MJ/kg <sub>DM</sub> for pure galega or mixed galega–grass swards.
- 3. Split application of the 90 kg volume of biogas released after 64-day fermentation period from galega haylage, having volatile solids content in digesters 1N fertiliser, compare to unfertilized plots, affected negatively the proportion of pure galega in a sward, resulting in the decrease of DM yields by 1.04 t/ha at two cutting management.
- 4. Frequently cutting has a declining effect on the productivity of galega-grass mixtures, as the DM decreases by 35.2 % in average in all experimental plots at a four-cutting compare to two-cutting management.
- 5. Galega can to produce high (up to 384.2 l/kg<sub>VSd</sub>) biogas yield obtained despite to organic overloading in two digesters and working without mixing of substrate.
- 6. Estimated cumulative volume of biogas released in digester 5 or 6 was 116 l/kgvs or 244 l/kgvs respectively, after 64-day anaerobic fermentation period.

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#### QUALITATIVE AND CHEAP GRASS FEED

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#### INTRODUCTION

One of the main important tasks in animal feed production is increasing of protein income from one perennial grasses hectare.

According to statistics data consumption of plant and animal products increases. The prices of concentrated feed have a tendency to increase and it is reality in all world. Prime cost of animal food products increases in Latvia and producers profit decreases in such a way. Private farmers have possibility to solve this problem by producing as far as possible cheaper forage looking for new technologies and at maximum controlling production and feeding processes.

The more possibility to control different risk factors the higher possibility to get better result. During forage production process we can evaluate and control:

- What seed, where and how to sow,
- When and how to harvest crops,
- Choice of making way and technologies precise observing,
- Prepared forage corresponding storage,
- In sufficient amount and balanced feeding.

#### MATERIALS AND METHODS

Trial was carried out in Latvia middle area and following issues were investigated.

Three different botanic composition herbages (1 - grasses 100%; 2 - grasses 50% + papilionaceous 50%; 3 - corn 100%) were analyzed biochemically and microbiologically.

- Obtained forage (green mass, silage) analyzed biochemically and microbiologically.
- Harvesting time (shooting, blooming) and height (10 and 30 cm).

Economic effectiveness of one-hectare herbage was evaluated in two production systems (conventional and organic farming systems for herbage of  $2^{nd}$  botanic composition).

Data statistic processing was carried out (SPSS Programme). Obtained data were evaluated economically.

#### **RESULTS AND DISCUSSION**

During grasses harvesting for forage making it is very important to evaluate fibre, protein and carbohydrates outcome. Therefore plants growing phase and cutting height must be taken into account during cutting. Optimum cutting height for corn is approximately 30 cm, because plants lower part is poor with nutritive substances and not utilizable for animals.

By analyzing siloing mass ensiling ability we can choice appropriate making technology control process fulfilment and to anticipate a result. During conserving mass losses take place. Total mass of losses is in average 25 t on produced 1000 t of silage. If mass is right rammed then we can obtain approximately 800 kg/m<sup>3</sup> of silage for feeding. Mould and yeast that decompose nutritive substances and decrease silage quality (p<0,01) do not develop in right rammed mass. By evaluating forage production economic indices we compared 1 ha production express in conventional and organic farming systems.

Higher economic effectiveness indices we obtained in trial when qualitative grasses seed mixture was sawn in conventional farm, land was fertilized and grass cut correspondingly in definite vegetation period. Obtained forage was with higher yield in comparison with organic farming system fields. Forage production expenses did not changed essentially in  $1^{st}$  and  $2^{nd}$  variants but grass crop from conventional farming system fields was by 65 % higher.

Qualitative silage production (sufficient amount, tasty, full value and not containing mycotoxines) does not mean yet that farm will work with profit. Feed must be fed out in right way (silage >60% and concentrate feed <40%) ratio must be balanced. Fed out feed must promote the development of rumen microflora, that will improve taken in feed fibre decomposition and in such a way will increase it utilizability in organism and essentially (p<0,05) decrease obtained production prime cost.

#### CONCLUSIONS

To increase higher content of dry matter (DM) per ha, grasses need suitable fertilization. We must increase papilionaceous amount in grassland structure, grass must be harvest of plants with more suitable cutting height, growth stage demands of feed making technologies must be noticed and grassland area must be utilized at maximum completely.

	СР, %	UIP, %	NEL, %	ADF, %	NDF, %	Ca, %	P, %
Corn							
10cm	8,7	28	6,3	35,6	59,1	0,5	0,3
30cm	9,6	29	6,1	31,2	58,9	0,5	0,3
Grasses + papilionaceous							
10cm	13,0	20	5,8	37,5	57,4	1,0	0,3
15cm	14,9	21	5,9	35,9	50,4	1,0	0,3

#### Table 1. Cut height influence on grass mass feed value

Table 2.	Forage production	economic comparison	in conventional	l and organ	ic farming systems
	<b>U</b> 1	1		0	0,

	Expenses of	Expenses of	Expenses of	Yield
Farming system	Grass seed	fertilizers and its	Organic manure	in dry
Farming system	mixture	dispersion	and its dispersion	matter
	EUR per ha	EUR per ha	EUR per ha	t per ha
Conventional farming system	86.0	429.0	-	6,0
Organic farming system	86.0	-	500.0	3,9

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#### **SUMMARY**

Latvian farmers gradually turn on to the sustainable agricultural products production, where determining factors are agricultural lands maximum, but for environment friendly exploitation, balanced and even feeding and welfare of cows. It is interest of producers to increase income, by increasing amount of production and improving quality of milk and decreasing it expenses of production at the same time. Each forage production stage is significant from grasses growing till feeding. To make qualitative silage herbage must be thick, plain, and free of weeds, of multiform grasses, right fertilized. Grasses harvested in time are appetizing, have good digestibility and full value nutritive substances composition. Prepared forage must be in sufficient amount and with low expenses. The aim of work was analyzing economic and technological aspects of grass feed production to develop profitable milk branch.

## CLOSTRIDIA INHIBITION AND IMPROVEMENT OF FERMENTATION IN CHALLENGED GRASS SILAGE BY MEANS OF A BACTERIAL SILAGE ADDITIVE

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#### INTRODUCTION

Deterioration of fermentation quality in silage is caused mainly by spoilage bacteria like clostridia. In order to avoid anaerobic spoilage it is crucial to maintain silage conditions unfavourable for those organisms, the most important of which is an acidic environment. This can be achieved by either treating silage with chemical acidifiers, clostridia inhibitors or preparations of lactic acid bacteria (LAB), which pose a biological and cheaper alternative to chemicals which also lack their corrosive properties.

The aim of the following experiments was to challenge grass silage by means of buffering with calcium carbonate and/or elevated soil contamination and thereupon to test different treatments for their efficacy concerning clostridia inhibition and fermentation improvement. Treatments included Biomin<sup>®</sup>BioStabil Plus (a mixture of homo- and heterofermentative LAB), a preparation of hops products said to exhibit an anti-clostridial effect, or a combination of both. Products were compared to an untreated control.

#### MATERIALS AND METHODS

In two lab scale silage trials the following treatment groups were employed:

- 1. **BioStabil Plus:** Mixture of homo- and heterofermentative LAB at a dosage of  $1 \times 10^6$  cfu/g silage
- 2. **Product A:** preparation of anti-clostridial hops products
- 3. BioStabil Plus + Product A: Mixture of both products (100% dosage of each)
- 4. **Control**: untreated except for the respective challenge

Challenges were used in all trial groups including control and consisted of 2% soil in both trials. In one trial calcium carbonate was used in addition to the soil for elevation of buffering capacity and consequent hindrance of acidification. For each trial group 12 model silos (six with 0,5 kg, six with 2 kg) were prepared, of which three small ones were opened after 3 and 7 days, and three large ones after 35 and 90 days, respectively. The raw material used was meadow grass, first cut, wilted to DM-contents between 35 and 40%. Parameters analysed included pH, dry matter (DM) loss, organic acids (via HPLC) and determination of Clostridia spore count (employing the most-probable-number (MPN) method).

#### **RESULTS AND DISCUSSION**

**Figure 1.** DM loss after 90 day in buffered and unbuffered earth-contaminated grass silage (ECG) treated with LAB and/or hops products (n=3)



Figure 1 shows that even though DM-losses tended to be lower in buffered silage, losses were distinctively lower in the groups with BioStabil Plus treatment than in the control or combination groups. Next to proving that buffering with calcium carbonate was successful, figure 2 depicts very clearly the large improvement of acidification achieved by BioStabil Plus treatment in both trials. This effect was, however, impaired if the LAB were used in combination with the hops products. Figure 3 shows that BioStabil Plus-only treatment led to higher lactic acid contents than the other treatments and to the lowest ethanol and butyric acid contents. This indicates successful inhibition of both yeast and

clostridia growth. It is also shown that in these trials butyric acid was only found in buffered silages.

It can be deducted from figure 3 and table 1 that lower clostridia spore counts do not necessarily lead to detectable butyric acid levels. Furthermore it can be seen in all figures that combining BioStabil plus with Product A leads to a deterioration of the silage indicating that the product was not only inhibiting clostridia, but also lactic acid bacteria.





Figure 3. Lactic acid, ethanol and butyric acid contents in buffered or unbuffered ECG treated with LAB and/or hops products (n=3)



Table 1. Clostridia spore counts in both grass silage trials [cfu/g silage], (averages of triplicates)

	Control	BioStabil Plus + Product A	<b>BioStabil Plus</b>	Product A
buffered	1,10E+06	1,10E+06	1,10E+04	4,60E+05
unbuffered	2,40E+04	4,60E+04	2,40E+03	1,50E+04

It can be deducted from figure 3 and table 1 that lower clostridia spore counts do not necessarily lead to detectable butyric acid levels.

Furthermore it can be seen in all figures that combining BioStabil plus with Product A leads to a deterioration of the silage indicating that the product was not only inhibiting clostridia, but also lactic acid bacteria.

#### CONCLUSION

BioStabil Plus addition, in contrast to the hops preparation, led to more efficient acidification, lower DM-loss and more efficient inhibition of clostridia. Combining both additives impaired both LAB activity and led to the highest clostridia spore numbers. Therefore it is recommended that BioStabil Plus shall not be combined with other products for effective clostridia inhibition in this DM-range.

### THE INFLUENCE OF NUMBER OF FILM WRAP LAYERS APPLIED TO BALED GRASS ON SILAGE CONSERVATION, DRY MATTER LOSSES, QUALITY AND NUTRITIVE VALUE

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#### **INTRODUCTION**

The key factor in silage conservation is to ensure a good barrier to air and gas movements. This is particularly important in the case of big bales, as baled silage has six to eight times more surface area in contact with plastic film compared to clamp silage and about half of the ensiled volume is within 15 cm of the wrap film. The main factors influencing the fermentation process in bales are: the number of wrap layers applied (Forristal et all. 1999; Gudmundsson, 2001; Heikkila et all. 2002; Jacobsson et all. 2002; Nonaka et all. 1999; O'Kiely et all. 2000), film thickness (Forristal et all. 2002), and film colour (Gudmundsson 2001; Snell et all. 2003). In Poland, it is generally advised to apply 4 to 6 wrap layers when ensilaging grass with high dry matter (DM) content. However, in practice, farmers tend to use less than 4 layers of film wrap mainly due to cost considerations.

The aim of this study was to compare the impact of the number of wrap layers on fermentation process, dry matter losses, quality and nutritive value of grass silage specifically under Polish farm conditions.

#### MATERIALS AND METHODS

The study was conducted in 2007, under Polish farm conditions and climate. Silages were made from a first cut of permanent meadow sward mown on 22 May 2007. The herbage was wilted for 24 hours, raked and baled. Forty experimental big bales (about 400 kg/bale) were produced, in order to get ten bales allocated to each experimental treatment. The bales were wrapped after transport to their place of storage using 2, 4, 6 and 8 layers of 500 mm wide stretch film. After 3 weeks storage period each bale was tested to evaluate the effectiveness of film seal by creating a vacuum within bale and measuring the time taken for the pressure to drop. During November (190 days of ensilage) bales were opened. Immediately after unwrapping each bale was assessed for mould coverage. The representative sample of silage was taken from each bale for chemical analyses. Silages were examined for dry matter, pH, chemical composition and aerobic stability. The NEL concentration in silages was calculated. DM losses of silage were estimated by the differences between dry matter weight of ensilaged forage and dry matter weight of obtained silage.

#### **RESULTS AND DISCUSSION**

The quality of meadow sward used for silage production was good. The dry matter level in meadow sward at ensilage was 460.5 g/kg (SD 68.5), the crude protein content was 130 g/kg DM, crude fiber content - 269 g/kg DM and WSC content 161 g/kg DM.

The increased number of wrap layers improved film seal effectiveness. Significant differences were observed (P<0.01), between the number of wrap layers in the pressure to drop from 250 kPa to 150 kPa and from 350 kPa to 250 kPa. The most visible difference was between 2 and 4 layers treatment. The time taken for air to re-enter the 4 layered bales was 5-times longer than for the 2 layered bales (Table 1).

Increasing the number of wrap layers significantly reduced (P<0.01) the mould cover from 50% (2 wrap layers treatment) to 15.5% (4 wrap layers treatment) and 9.5% (6 layers treatment). In the case of bales with 8 layers of film, the mould cover was only 1.5%.

Increasing the number of wrap layers also significantly reduced (P<0.01) silage DM losses. Applying four layers of film resulted in 3-times lower DM losses then when wrapped with only two-layers, and 10-times lower DM losses when wrapped with six layers. Bales wrapped with eight layers, DM losses were very small and in many bales were nil.

Generally no significant differences in the silage chemical composition between the number of wrap layers were observed. Only the butyric acid concentration significantly increased (P<0.05) as the number of film layers went up from two to eight. Whilst no significant differences were observed between treatments in most remaining examined parameters, some trends were apparent. Silage DM, pH, butyric acid concentration and aerobic stability increased and ammonium, lactic acid and acetic acid concentration reduced in response to increase of layering. It can be explained by possibly more efficient and rapid process of lactic acid fermentation in silage with a higher number of wrap layers.

A significant impact (P < 0.05) of the number of layers was recorded on total protein and WSC concentration; a trend indicating an increase in NEL concentration in silage with increased number of wrap layers was also noticed (Table 1).

#### CONCLUSIONS

The study proves that wrapping silage bales in four to six layers of film provides a more robust oxygen barrier resulting in reduced loss of dry matter in silage from yeasts and moulds, as well as significant improvements in the quality and nutritive value and a more stable and consistent silage at feed out. The data confirms that applying two

layers of silage wrap film does not guarantee an effective film seal on bales, while 6 layers of film has been shown to give the greatest return. The number of wrap layers had no significant influence on silage chemical composition except butyric acid, total protein and WSC concentration. No significant differences were observed as to lactic acid fermentation levels.

	1	s	Significa		
Examined parameters	2	4	6	8	nce
FILM SEAL					
drop from 250 kPa to 150 kPa	21.2	104.2	148.0	207.1	**
drop from 350 kPa to 250 kPa	8.2	56.2	90.8	134.9	**
VISIBLE MOULDS COVERAGE AND DM LOSSES					
Mould cover (% of bale surface)	50.0	15.5	9.5	1.5	**
DM losses (%)	2.52	0.88	0.25	0.07	**
DM CONTENT AND CHEMICAL COMPOSITION					
Dry matter (g kg <sup>-1</sup> )	389	410	411	424	NS
pH	4.87	4.95	4.81	5.09	NS
$NH_3-N$ (g kg <sup>-1</sup> total N)	62.3	51.3	53.6	40.3	NS
Lactic acid (g kg <sup>-1</sup> DM)	41.7	38.1	42.3	29.9	NS
Acetic acid (g kg <sup>-1</sup> DM)	19.6	11.0	16.4	15.0	NS
Butyric acid (g kg <sup>-1</sup> DM)	0.11	0.23	0.22	0.86	*
Stability (days)	6.0	7.4	8.6	8.0	NS
NUTRITIVE VALUE					
Total protein (g kg <sup>-1</sup> DM)	123.8	130.7	130.6	118.0	*
WSC (g kg <sup>-1</sup> DM)	136.1	139.1	129.1	159.5	*
Ash $(g kg^{-1} DM)$	73.3	68.5	78.1	67.7	NS
NEL (MJ kg <sup>-1</sup> DM)	5.55	5.60	5.63	5.82	NS

NS - not significant; \*, \*\* - significance of differences and interactions at P<0.05 and P<0.01 respectively

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## PRESERVATION EFFICIENCY AND NUTRITIONAL QUALITY OF WHOLE-PLANT MAIZE SEALED IN LARGE PILE SILOS WITH AN OXYGEN BARRIER FILM (SILOSTOP) OR STANDARD POLYETHYLENE FILM

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#### **INTRODUCTION**

From 2001 to 2007 an average of 95.3 million metric tons of whole-plant maize was harvested annually for silage in the USA (United States Department of Agriculture, 2008) and about 83 percent of this silage was in walled bunker silos and unwalled piles. Bunker silos and piles are economically attractive for storing large amounts of ensiled maize, however they have large surface areas, which should be protected from air and water during the entire storage and feedout periods. Standard polyethylene, weighted with discarded full-casing tires or tire sidewalls, has been the most common method used to seal bunkers and piles, but OM losses in the original 0.75 m can exceed 300 g/kg (Berger and Bolsen, 2006).

The use of an oxygen barrier film (Silostop) (www.silostop.com) as an alternative to standard polyethylene for sealing bunker silos and piles was reported at the XII International Silage Conference in 1999 (Degano, 1999). It is well known that the use of different raw materials in the manufacture of plastic film provide a range of gas barrier properties, however until recently these characteristics have not been used in silage production. Degano (1999) stated that the permeability of Silostop film was 0.025 that of standard polyethylene film of the same thickness. Oxygen transmission rate (OTR) through standard polyethylene film using 100% oxygen is 1812 cm<sup>3</sup>/m<sup>2</sup>/24 h (American Society for Testing Materials, ASTM D3985), while OTR through Silostop film using 100% oxygen is 65.5 cm<sup>3</sup>/m<sup>2</sup>/24 h (ASTM D3985). Thus, the permeability of Silostop film was 0.036 that of the standard polyethylene film.

Wilkinson and Rimini (2002) reported virtually no visible surface mold or spoilage and lower percentage of inedible silage for Silostop film-sealed small-scale silos compared to single and double standard polyethylene film-sealed silos. The two trials presented here compared Silostop film to standard polyethylene film on large silage piles on commercial dairies.

#### MATERIALS AND METHODS

Silostop, 45 microns in thickness, was compared to standard polyethylene film, 125 micron in thickness, in two trials conducted with large unwalled silage piles on commercial dairies in California, USA. Both trials were with whole-plant maize silage, which was harvested at a DM content of 325 g kg<sup>-1</sup>, chopped to a theoretical particle size of 17 mm, and inoculated with a culture of lactic acid bacteria (www.lallemand.com) at the forage harvest. Single sheets of Silostop and standard black on white polyethylene films were applied side-by-side on 30 m wide x 12 m long areas of the silage pile side and top surfaces. The two types of film were weighted with discarded car tyre sidewalls. Because the Silostop film did not have protection from ultraviolet light, a sheet of standard polyethlene film was put over the Silostop film underneath the tyre sidewalls. At about 200 day post-filling, the sealing materials were removed and samples taken at 0 to 15, 15 to 30, and 30 to 45 cm from the surface at four locations across both the side and top of the piles. Representative samples were taken of the pre-ensiled forage going into the piles and also of the silage at about 2 m below the surfaces at the feedout face.

#### **RESULTS AND DISCUSSION**

Results of the trials are shown in Tables 1 and 2. There was very little visible discoloration or spoilage in the Silostop-sealed piles, however there was visible mold and extensive aerobic spoilage in the standard polyethylene-sealed piles, particularly between 0 cm and 30 cm from the surface. The lowest DM content, highest pH, and highest ash concentration was for maize silage on the side of the pile sealed with standard polyethylene in Trial 2, which suggests that large amounts of air entered the side of the silos during the 200 days of storage.

When averaged across sampling depth in the two trials (Table 1), silage on both the side and top of the piles under Silostop had better fermentation profiles (lower pH values and higher concentrations of lactic acid), higher nutritional quality (lower NDF and ADF concentrations and higher starch content), and numerically lower ash concentrations than silage under standard polyethylene. Estimated mean loss of OM between 0 and 45 cm from the surface were 318.2 and 241.8 g/kg under Silostop in Trials 1 and 2, respectively, vs. 401.2 and 378.2 g/kg under standard polyethylene in Trials

1 and 2, respectively.

#### CONCLUSIONS

Silostop film was more effective than standard polyethylene film in preventing the entry of oxygen into the ensiled material during the storage and feedout phases. This effect was observed both at the top locations (higher DM density) and the side locations (lower DM density).

Table 1.	Effect of standard polyethylene and Silostop on fermentation, nutritional quality, and estimated mean loss of
	OM at 0 to 45 cm from the surface at 200 days post-filling

	Trial 1		Tria	12
Item	Standard	Silostop	Standard	Silostop
DM, g/kg	297	312	252	315
pH	4.46	3.80	4.97	3.84
Estimated OM loss, g/kg <sup>1,2</sup>	401.2	318.2	378.2	241.8
		g/kg I	DM	
NDF	513.3	480.8	557.7	461.3
ADF	320.3	298.0	354.3	288.0
Starch	224	251	153	251
Ash	52.7	45.2	57.7	45.7
Lactic acid	21.0	34.2	13.2	38.7
Acetic acid	32.7	51.6	21.5	26.4

<sup>1</sup>Estimated loss of OM, calculated from ash content using the equations reported by Bolsen et al. (1993).

<sup>2</sup>Ash content of the pre-ensiled forage was 31.0 g/kg of DM in Trial 1; and 35.0 g/kg of DM in Trial 2.

**Table 2.** Effect of standard polyethylene and Silostop on fermentation, nutritional quality, and estimated mean loss of OM at 0 to 45 cm from the surface at 200 days post-filling

	Trial 1		Trial 2					
	Sic	le	To	ор	Side		Тор	
Item	Standard	Silostop	Standard	Silostop	Standard	Silostop	Standard	Silostop
DM, g/kg	292	314	301	309	192	318	312	311
pН	4.75	3.83	4.17	3.77	5.65	3.89	4.27	3.78
Est. OM loss, g/kg <sup>1,2</sup>	446.3	360.7	360.0	275.7	461.0	253.7	295.3	230.0
				g/kg	g DM			
NDF	531.3	489.3	495.3	472.3	635.7	457.0	480.0	465.7
ADF	331.0	300.7	309.7	295.3	407.7	284.7	301.0	291.3
Starch	205	250	243	252	71	262	236	240
Ash	57.3	48.0	48.0	42.3	66.3	47.3	49.0	45.0
Lactic acid	16.3	26.0	25.7	42.3	5.7	34.3	20.7	43.0
Acetic acid	40.3	72.4	25.0	30.7	20.4	28.1	22.6	24.4

<sup>1</sup>Estimated loss of OM, calculated from ash content using the equations reported by Bolsen et al. (1993).

<sup>2</sup>Ash content of the pre-ensiled forage was 31.0 g/kg of DM in Trial 1; and 35.0 g/kg of DM in Trial 2.

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