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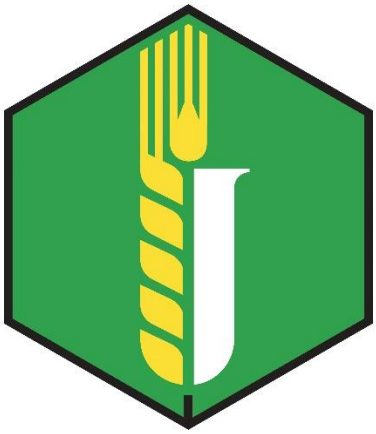

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CATTLE BREEDING IN THE AGE OF GENOTYPING

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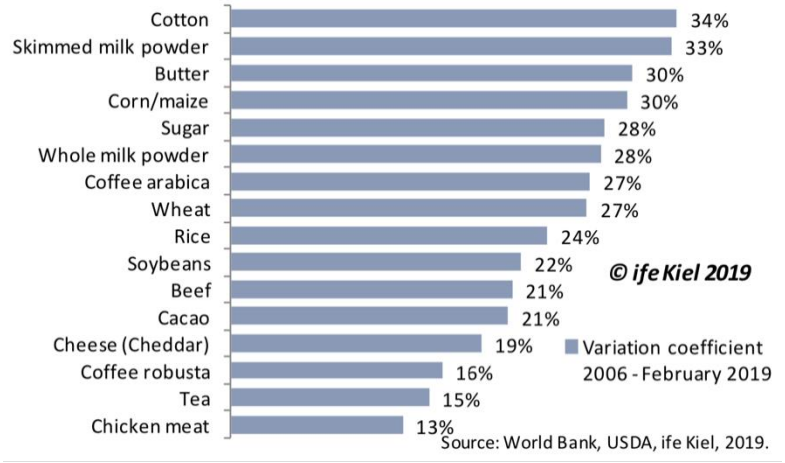
The main challenge of the current livestock systems is to produce high-quality food with a low ecological footprint and high standards of animal wellfare. It is very complex and difficult task, as the dairy products are even more volatile than other commodities. Thiele, Hilderbrand (2019) presented ten-years average of volatility of some main agricultural commodities. Dairy products belong to the most volatile one in the study: skin milk powder - 34%, butter – 30% , beef - 21 %, tee 15 %, chicken meat -13 %. Dairy price volatility become a "new normal" in Europe and will impact the dairy economics environment significantly in the future. In order to react on this circumstances, farmers need to invest more into new technologies and techniques, which are helping them to increase their competitiveness, resulting in high-value farms and an increasing necessity to guard, track and monitor all assets with the help of innovations.

Fig. 1: Volatility of some agricultural commodities



Those technologies need to seamlessly and proactively integrate to turn the industry into a smart and sustainable one. While the number of connected devices continues to rise simultaneously with the growing demands on the agri-food sector, particularly precision livestock farming requires reliable, affordable, low-power, wide-range network technologies and smart sensors. At the same time, involving multiple types of farms and connected supply chain stakeholders across different regions helps to further develop and deploy the latest innovations required to perform efficient and sustainable livestock farming and animal breeding.

Beside the implementation of the new technologies, the genetic improvement of the herds must be in the focus also, especially for a novel traits and/or traits with low heritability. In a progeny-testing program, the accuracy of selection depends largely on the number of offspring per sire and, hence, on the number of cows in progeny test herds that are available for mating to young, unproven bulls. With genomic selection, accuracy is primarily a function of the size of the reference population that is used to estimate SNP effects, which in turn are used to compute GEBV of selection candidates (Schefers, Weigel, 2012).

Genomic selection has changed the traditional breeding programs in cattle breeding and become to be a standard. Percentage of genomic bulls used in the breeding programs range between 30% (conservative approach) up to almost 100% is some breeding schemes/countries. In German Holstein population increased the percentage of genomic bulls from 27 % in 2011 up to 80 % in 2019.

Table 1: Number of genotyped animals (VanRaden, 2019)

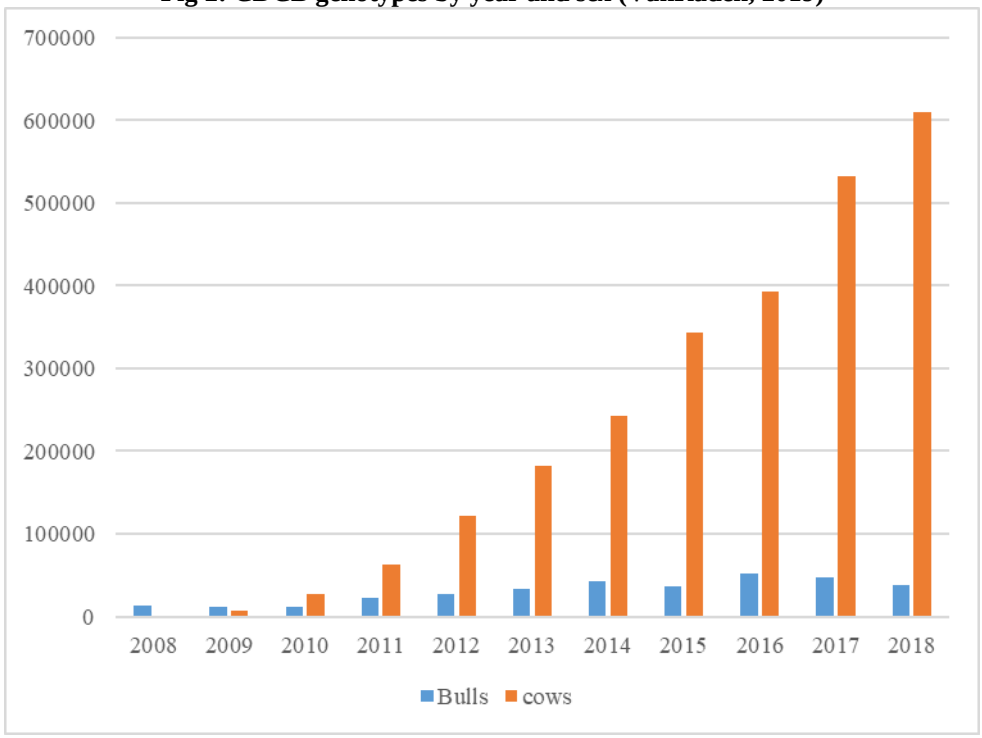
Species	Countries/Companies	Animals
Dairy	USA and Canada	3,020,000
	France	550,000
	Netherlands	465,000
	New Zealand	140,000
	Germany	785,000
Beef (Angus)	United States	550,000
Beef and dairy	Ireland	1,500,000
Poultry	Aviagen	1,000,000/year?

Farmers and breeders may choose bulls with higher reliability of the genetic predictions. Genomic evaluations were primarily implemented for traits with an established performance recording scheme providing phenotypes that were also used in conventional genetic evaluations ie production traits, fertility, longevity etc. The implementation of the genomic breeding values for this group of traits was relatively fast, as there are enough information available on the progeny of the progeny-tested bulls in the reference set. The above mentioned changing environment in the dairy (and beef) herds sector and progress in technology must react on new sources of data and new traits available for the evaluation. Mainly by a new traits only a limited number of sires will have daughters with phenotypic observations in the short to medium term even if a broad performance recording scheme will be established. In other cases, new and expensive traits will only be recorded on a sample of all cows of the breeding population, for example, only on cows that are milked in automatic milking systems or cows in specific herds.

Genotyping of the females is another way in which a larger reference population can be achieved. In August 2010, the only country including females in their reference population was the USA (Wiggans et al., 2011). However, genotyped females need to be incorporated cautiously, as there could be a risk that some of them are preferentially treated and therefore their phenotypes could be biased. Instead, directly targeting a group of randomly selected cows may be more beneficial. So adding genotyped females to the reference population could improve the reliability of breeding values. Nowadays many of the reference population in the main dairy breeds contain more female genotypes than males one, as shown on the example of US&Canadian population (Fig 2.)

The trend of female genotyping for farm & management purpose is supported by decreasing costs for genotyping. Farmers started to use the advantages of the female genotyping in very young age to precise the selection decision and therefore lower the rearing cost. As a "side-product" of the female genotyping we improve the quality of the parentage verification, which has positive impact on the overall reliability of the genetic evaluation and breeding value prediction and moreover help to better manage the problem of inbreeding.

Fig 2: CDCB genotypes by year and sex (VanRaden, 2019)



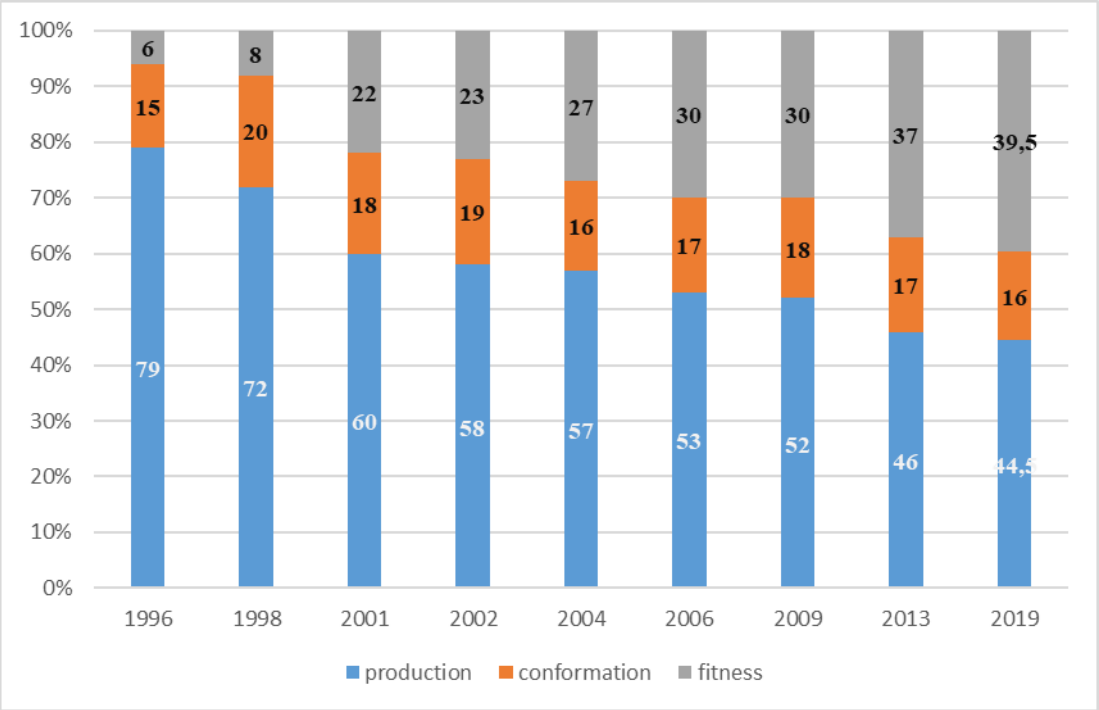
Genomic selection enriched for female genotypes is helping to exploit new phenotypes (new traits) observed usually on a small group of animal. These could be divided into few groups: scored by the breeder (milking temperament, farmer satisfaction/workability, calving difficult), scored by professionals (feet and leg ailments recorded by professional hoof trimmers, health treatment recorded by vets) or technological phenotypes (usually data which are automatically captured, like data from milking robots, weights, data from different sensors /rumination, movement etc/).

The increasing importance of fitness traits in the total merit indexes word wide is presented in the fig 3. A clear trend to decrease the share of the production traits within the TMI. The area of fitness, health, and management traits is the one where most scientific work is concentrate focused on few of trait groups which are gaining a lot of attention. Mastitis falls into this category. A breeding value for mastitis resistance has been available in regions like Scandinavia and the Netherlands for more than a decades, but in most leading Holstein countries the basic data has only reached a point recently that a direct breeding value for mastitis is able to

replace or work in tandem with the indicator trait of SCC for udder health. Another trait that has been gaining importance in recent years thanks to improved data is hoof health, which as a rule is calculated as an index of various hoof diseases. Like mastitis resistance, hoof health belongs in the classical area of health traits (Schneider, Hopman, 2019).

The most intensively researched at the moment is the theme of feed efficiency. This trait should expresses how efficiently feed is used for production and is calculated by dividing the production of a cow by dry matter intake. Current systems and knowledge do not allowe direct calculation for feed efficiency, therefore closely correlated traits are in the focus of the research. A good indicator is the trait "saved feed." This auxiliary trait gives how much less feed a cow needs during her lactation for maintenance. Also in the dual purpose the shift towards higher share of fitness traits is to be reported. Current TMI in the majority of Fleckvieh countries consist from 38-50 % of production, 15-25 % of beef traits and 30 – 50 % of fitness traits. In example within the join genetic evaluation Germany-Austria-Czech Republic the new TMI is based on 38% milk production, 18% beef traits and 46 % of fitness, whereas the most important traits within the fitness are fertility (14%), udder health (10%) and longevity (10%).

Fig 3: Construction of the "world wide" Holstein TMI (Schneider, Hopman, 2019)



Several statistics and forecast estimates that world's population will grow from the current 7.6 billion to 10.5 billion by the year 2067. This population growth will decrease the amount of land capable for crop production from 0.5 to an estimated 0.37 acres per person. Climate change will have a large impact on where dairy farms are located in the future and shift of the milk production industry is predicted for the main areas, where milk is produced nowadays. Increasing consumption of dairy products, especially in the transition economics, is a big challenge for dairy producers. Using the most recent biotechniques in combination with the modern technologies for the right management decision is the only possibility for the dairy operation hot to stay competitive for the future and reach all consumers and society needs and requirements in the future.

REFERENCES ARE AVAILABLE BY THE AUTHOR.

CORN HYBRID EVALUATION, NEW TECHNOLOGY AND NUTRITION VALUE QUALITY OF MAIZE SILAGES IN THE NORTHERN UNITED STATES

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The story for U.S. corn grain yield increases over time is well documented. Corn grain yields have been increasing at the rate of 126 kg ha^{-1} yr^{-1}. The trend for U.S. corn silage yields is not as well known. Since 1919, corn silage statistics have been collected for the northern tier of U.S. states and for states that have a large dairy or beef industry. For many years corn silage was made from fields that had other production stresses. Only recently has corn silage become more of a priority due to larger dairy/livestock operations. During 2014 to 2018, U.S. corn silage yield averaged 45 Mg ha^{-1} and varies significantly by county (Figure 1).

During the hybrid era (1930-1995) corn silage yields increased at the rate of 0.29 Mg ha^{-1} yr^{-1} (Figure 2). Many reasons are given for this dramatic increase including development of improved, adapted hybrids,

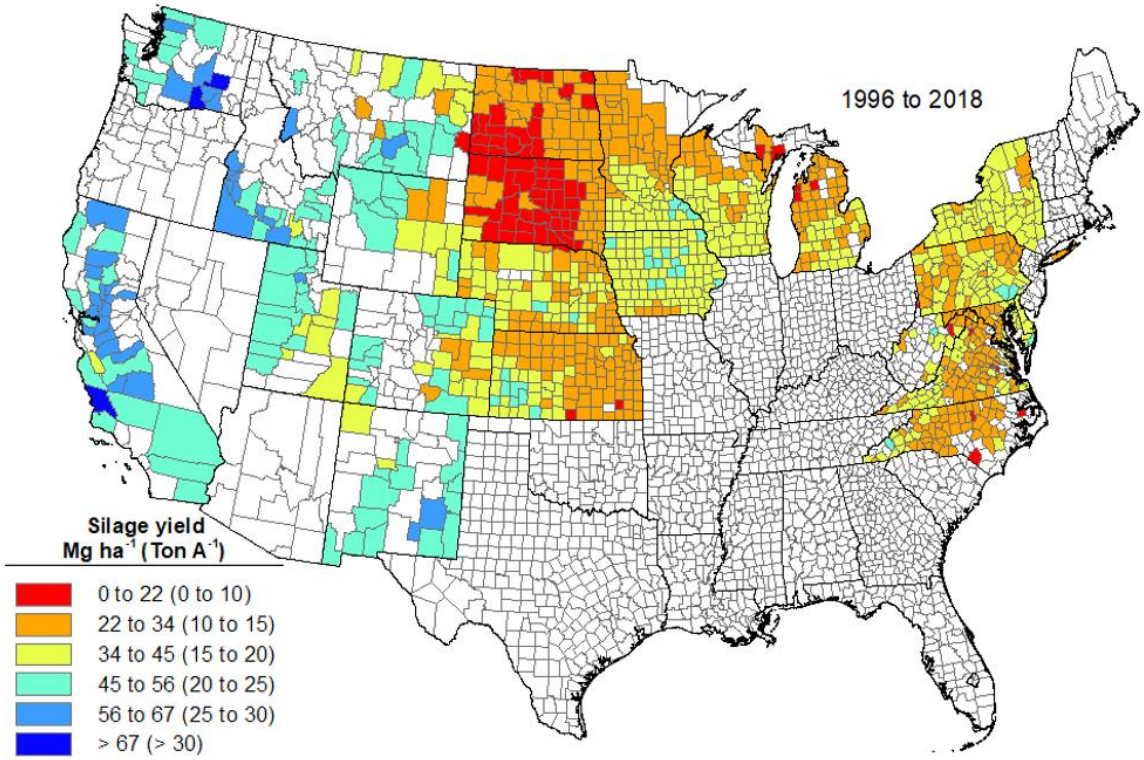


Figure 1. Corn silage yield for U.S. counties during the bio-engineered era. Data derived from USDA-NASS.

increased use of fertilizers and pesticides, machinery improvements for planting and harvesting, and improved management skills of corn silage growers.

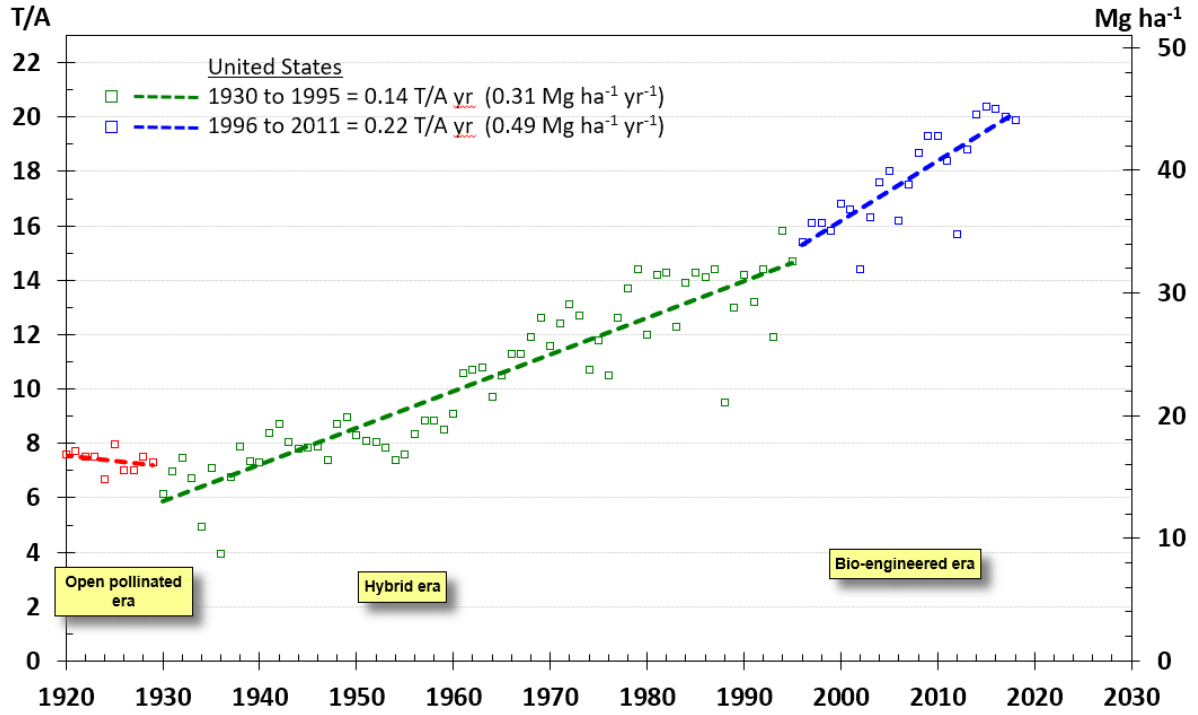


Figure 2. U.S. corn silage yield over time. Data derived from USDA-NASS.

Corn silage yield has continued to increase during the "Bio-engineered" era (1996 to present) at the rate of 0.52 Mg ha^{-1} yr^{-1}. Bio-engineered hybrids have insect and herbicide resistance traits that do well at "protecting" yield. In addition, end-users of corn silage have become much more diligent about management and silage yield and quality.

Not all U.S. counties that grow corn silage have an increasing yield trend (Figure 3). A total of 1001 of 3142 (32%) U.S. counties grow corn silage. Of the counties that grow corn silage 786 (79%) have a non-significant trend indicating that corn silage yield has not changed between 1996 and 2018. Of the 215 (21%) U.S. counties that have increased corn silage yield, 59 (6%) have increased corn silage yield by more than 897 kg ha^{-1} yr^{-1}, 129 (13%) have increased yield 448 to 897 kg ha^{-1} yr^{-1}, and 27 (2%) have increased yield 0 to 448 kg ha^{-1} yr^{-1}. Most yield increases have occurred in North Dakota, Minnesota, Pennsylvania and Wisconsin. Corn silage yields will likely continue to increase as new adapted hybrids are developed and as managers prioritize and acquire skills for producing high yielding quality corn silage.

Corn silage often makes up over 40% of the forage fed to dairy cows in the United States and is also an important feed in the beef finishing industry. Several characteristics of corn silage make it attractive to many livestock producers. It is a palatable forage with relatively consistent quality and higher yields and energy content than most other forages. Corn silage production requires significantly less labor and machinery time than other harvested forages because it requires only a single harvest activity. Hay and hay crop silage, on the other hand, often require multiple harvests. The cost per ton of dry matter also tends to be much lower for corn silage than for other harvested forage crops.

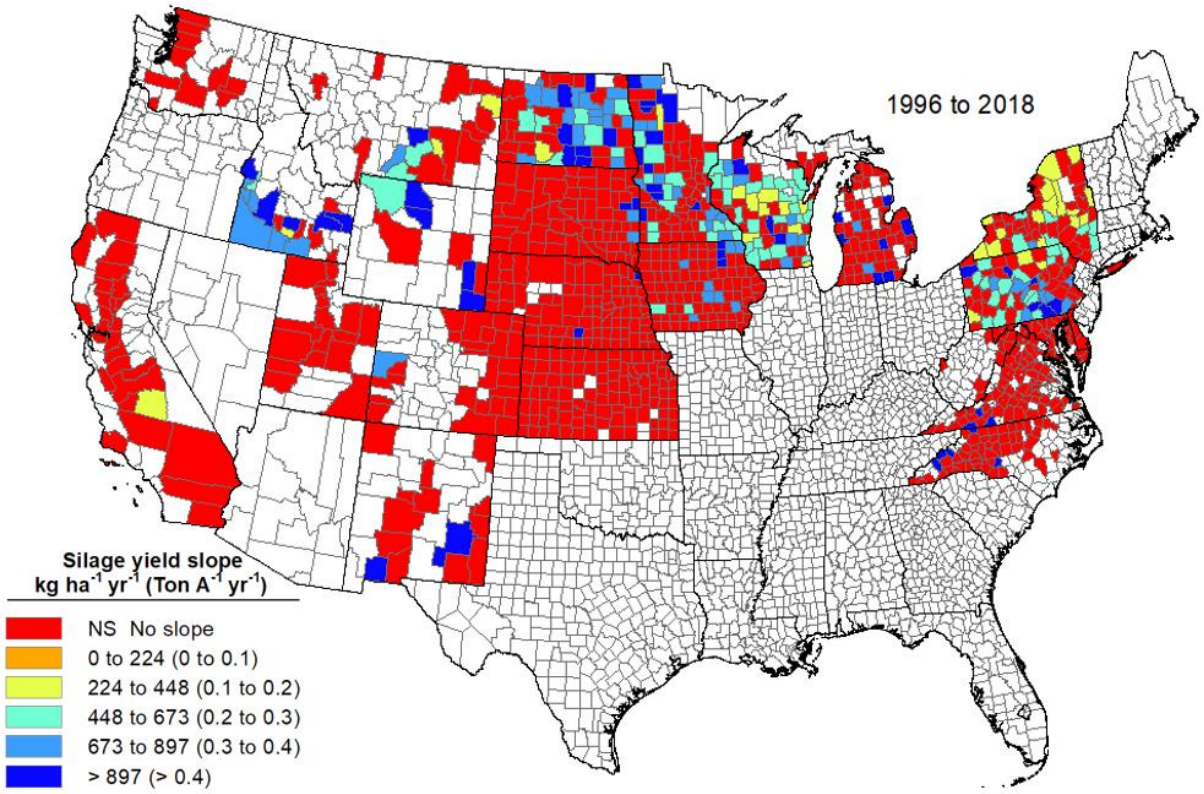


Figure 3. Corn silage rate of yield change over time for U.S. counties during the bio-engineered era. Data derived from USDA-NASS.

Offsetting these benefits of corn silage are some disadvantages relative to other forages. There are few established markets for silage sales in the Midwest and Northwest, and transportation costs are high so the crop must often be fed on or near the farm where it is produced. Storage systems for silage have reduced storage costs to reasonably competitive levels. In some situations, where corn is not well adapted, the cost of production may be too high to warrant corn silage production. Also, on erodible soils corn silage production may be limited because of soil conservation requirements.

Changes in crop management recommendations

The "double-peak" of corn silage

The 2019 crop production season has been challenging due to cool, wet conditions. Many farmers will be planting in July for "emergency" forages and cover crops. Corn is an excellent emergency forage and cover crop. Corn is deep-rooted and by the end of the end of the growing season can produce significant residue even when planted in July. Corn as an emergency forage is higher yielding than other emergency forages. Corn planted in July has produced yields up to 17 Mg dry matter ha^{-1}.

Like all forages, corn silage quality decreases after flowering (silking, R1). Unlike other forages, as grain development progresses, corn forage quality improves and achieves a second peak around 50% kernel milk (R5.5). The unique management decision involved with July planting dates is to plant a full-season or longer hybrid to hit the first NDFD quality peak around the R1 stage of development (Figure 4). Either let a frost kill the plant or cut with a haybine (higher ash likely) and let dry to the recommended moisture for the storage structure.

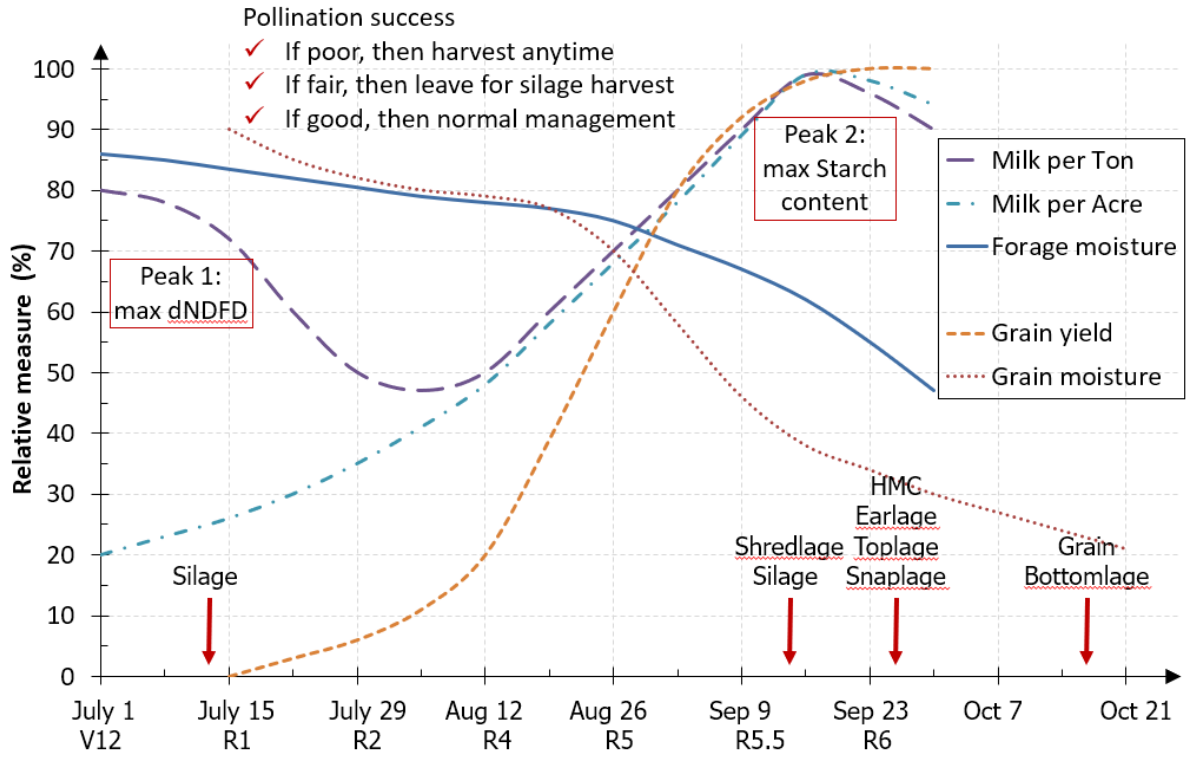


Figure 4. Normal pattern of corn forage and grain development

How thick should corn silage be planted?

Farmers are searching for ways to lower their corn production costs. Many management practices can be adjusted during "low-margin" years. However, farmers need to know the input level that provides maximum yield versus the input level required for the economic optimum. The input level for the economic optimum is lower than the level that provides maximum yield.

Corn seed costs have increased 5x over the last 20 years (USDA-ERS, 2017). Yet, farmers every year have been increasing harvested plant populations 756 plants ha^{-1} moving from 52,000 plants ha^{-1} in 1982 to over 74,000 plants ha^{-1} in 2018 (USDA-NASS, 2019). Several factors affect the plant population that produces maximum yield including hybrid, farm, field soil type and texture, environment, and management style. Factors that also affect the economic optimum include seed price and grain (or silage) price. A typical bag of corn seed costs $350 per 80,000 kernels or $4.38 per 1000 kernels.

From 2007 to 2016, staff at the University of Wisconsin have investigated corn yield and quality response to plant population. The plant population producing the maximum grain yield during this time was 96,000 plants ha^{-1} (Figure 5). The economic optimum plant population for grain yield was 84,000 plants ha^{-1}. The plant population producing maximum forage yield was 119,000 plants ha^{-1}, while maximum milk per ton was 18,000 plants/Acre, and maximum milk per acre was 111,000 plants ha^{-1}. All of these maximum yield and economic optimum plant populations are higher than the average current commercial plant populations of 74,000 plants ha^{-1}.

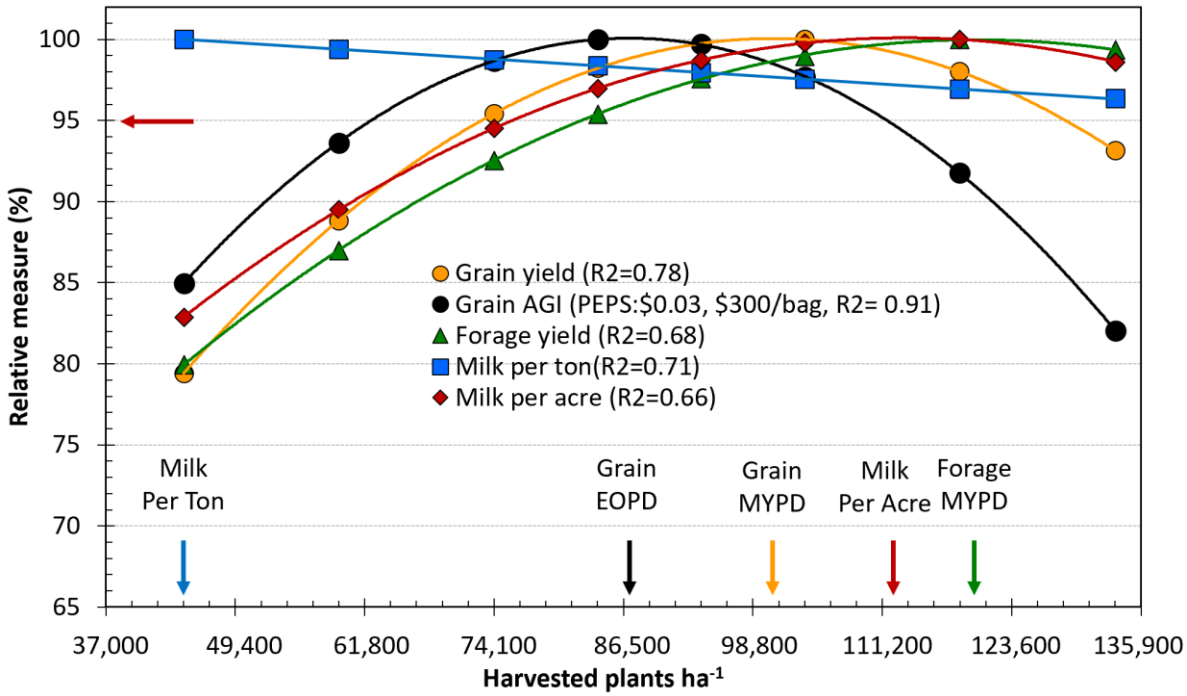


Figure 5. Relationship between corn plant density and grain yield, economic optimum (AGI), forage yield, Milk/Ton, and Milk/Acre. Data derived from Arlington (2008-2017).

The average plant density for maximum yield for one farm and one soil series is quite variable. For example, at Arlington on a Plano silt loam, the maximum yield plant density for grain varied from 72,000 plants ha^{-1} in 2011 to 106,000 plants ha^{-1} in 2013. For forage yield, the maximum yield plant density ranged from 86,000 to 124,000 plants ha^{-1}. Long-term data across 10 locations indicate that maximum yield plant density ranges from 74,000 to 99,000 plants ha^{-1}. Many researchers have documented hybrid X plant density interactions for maximum yield plant density (Assefa et al., 2016). Year, hybrid, and location play a significant role in determining how thick corn should be planted to maximize forage yield.

The way a farmer should approach the decision is to assume that maximum yield and economic optimum plant densities are increasing over time. For any field, choose what you think is the appropriate plant density for that field and plant most the field to that target density. For one round, increase plant density 10%. For example, if you feel that the economic optimum for a field is 74,000 plants/Acre, then for one round increase it to 82,000 plants/Acre. You should be able to pick up any plant density effects on a yield map during fall harvest.

Contracts for corn silage between dairy producers and grain growers

Understanding the relationship between corn grain and forage yield is important to dairy producers and grain farmers who often contract with each other for corn forage production. Arriving at a fair and equitable price for corn forage is difficult due to the number of factors involved that are dynamic and biologically variable.

The amount of corn grain in one Mg of forage (i.e. grain:forage ratio or grain equivalents) is often used in contracts. However, grain equivalents can be quite variable to the extent that one predetermined value should not be used in contracts between growers and dairyman. This variability is due to genetics, environment and management. For example, corn hybrids with the leafy and/or brown midrib trait have lower grain yield than bio-engineered hybrids. Some growing seasons can be too wet (or dry) and/or too cool (or warm) to maximize corn grain yield in a field even though stover yield is maximized. Management decisions like late planting date, plant population, and nitrogen rate can influence the amount of grain produced, while adjusting cutter bar height directly infleunces grain:forage ratios.

One of the earliest publications on grain equivalents in corn forage was written by Jorgensen and Crowley (1972). They found that the amount of grain in corn forage increased as grain yield of the field increased (Table 1). Corn open pollinated varieties and hybrids released to farmers through the 1900s had whole plant yield increases of 112 kg dry matter ha^{-1} yr^{-1}, while stover yield increased 224 kg dry matter ha^{-1} yr^{-1}. More

of the forage yield increase is due to an increasing grain yield. Starch content increased 0.15% yr^{-1} (Lauer et al., 2001).

Calculating value of modern corn forage fields

Some grain growers want to calculate the forage price based on corn grain yield (as the alternative harvestable crop) and some dairy producers want to calculate the price based on alternative forages (primarily alfalfa as the alternative forage source). In either case, the final price is affected by supply and demand of corn grain within a region.

Since 1997, the University of Wisconsin corn agronomy program has been using "paired" plots in experiments investigating the effect of management factors like hybrid, crop rotation, plant density, planting date, nitrogen rate, fungicide and row spacing on corn forage and subsequent grain performance in the same plot. In these studies, forage yield and quality were measured in four of eight rows in the plot. The four remaining rows were left for later grain yield and quality measurements.

Anything that affects grain or forage yield will affect grain equivalents. Depending upon grain yield level, grain equivalents of corn forage ranged from 259 to 437 kg grain at 15% moisture Mg^{-1} forage at 65% moisture (Table 1).

Table 1. Corn grain equivalents (at 15.5% moisture) per Mg of Forage (at 65% moisture). Data is derived from studies conducted by the University of Wisconsin Agronomy Program between 1997 and 2016.

Grain Yield	**Forage Yield**	**Starch content**	**Grain equivalents (1972)**	**Grain equivalents (Revised 2016)**	**Grain equivalents (Starch method)**	**difference**
Mg ha^{-1}	Mg DM ha^{-1}	%	kg Mg^{-1}	kg Mg^{-1}	kg Mg^{-1}	kg Mg^{-1}
< 5.7	8.5	20.9	140	143	123	20
5.7-6.9	12.1	27.3	154	185	162	22
6.9-8.2	13.5	29.0	168	199	171	28
8.2-9.4	15.0	30.4	182	210	179	31
9.4-10.7	16.4	31.4	196	218	185	34
10.7-11.9	17.7	32.2	196	227	190	36
11.9-13.2	19.3	32.6	196	232	193	39
13.2-14.4	20.9	32.6	196	238	193	45
14.4-15.7	22.2	32.4	196	241	190	50

To calculate the value of an acre of corn forage, the amount of grain equivalents is multiplied by the corn price and the forage yield. For example, if corn yielded 10.7 Mg ha^{-1} the grain equivalent is 218 kg Mg^{-1}. An average cornfield yield at 65% moisture is 56 Mg ha^{-1}. If corn is priced at \$138 Mg^{-1}, the value of the field is 218 kg Mg^{-1} x \$138 Mg^{-1} x 56 Mg ha^{-1} = \$1685 ha^{-1} or \$30 Mg^{-1} forage. Further negotiation would be needed over fertilizer removal and harvest, ensiling, and storage costs.

Factors that affect the grain equivalent calculation

Harvest timing can affect grain yield in the forage. Kernel milkline is a good indicator of development and remaining potential grain yield. For example, grain yield can still increase 5 to 12% when the kernel is at 50% kernel milk. No further yield increases occur after "black layer" formation at the kernel tip.

Moisture content in forage and grain has a major influence on this relationship and needs to be considered to accurately determine fair forage prices. For a field that has a grain yield of 9.5 Mg ha^{-1}, the grain equivalents range from 568 kg Mg^{-1} at 0% moisture to 179 kg Mg^{-1} at 70% moisture.

Environment can significantly affect the amount of grain in corn forage. Drought can reduce plant stature and affect pollination reducing both grain and forage yield. Sometimes early drought can reduce plant stature, but rains eventually occur relieving stress, and record grain yields are realized like the 2005 growing season. Following the 2005 growing season, farmers would sometimes talk about corn forage being 'hot' when fed to livestock, meaning that feed had too high grain equivalents in the forage. Depending upon year, grain equivalents have ranged from 179 to 263 kg Mg^{-1} at a 9.5 Mg ha^{-1} grain yield level.

Hybrid types evaluated have included bmr, leafy, bioengineered, and conventional hybrids. In a small study of six hybrids grain equivalents average 210 kg Mg^{-1} averaged across locations and years during the years tested. However, the range among hybrids for grain equivalents was 168 kg Mg^{-1} (min. hybrid= 126 kg Mg^{-1}, max. hybrid= 294 kg Mg^{-1}). Some locations produced consistently higher grain equivalents than others. Brown mid-rib hybrids had significantly lower grain equivalents than conventional or bioengineered hybrids.

A new way to calculate grain equivalents in corn forage

Ideally a field of corn intended for corn forage use is planned and managed to maximize grain equivalents. Often corn forage is harvested from the poorest fields. Fields that have been late-planted, weedy, affected by drought or flooding are the ones that end up being harvested for forage. We have measured wide differences among experimental treatments and the range is economically significant.

A better approach might be to pay for the grain yield produced and adjusted for fertilizer removal and soil erosion benefits. Corn forage is routinely tested for starch content as harvest and could be back calculated to determine grain equivalents on a field-by-field or load-by-load basis (Starch method in Table 1). This would allow for a much more accurate estimation of corn grain produced in a field regardless of circumstance and a fairer method for payment.

Clearly corn grain:forage ratios are changing over time. Forage moisture, hybrid and environment significantly affect grain equivalents and must be considered when negotiating a contract. Dairy producers and corn farmers need to understand the grain equivalent relationship when acres are contracted for forage production. This relationship is dynamic and, as we are learning, quite variable to the extent that one predetermined grain equivalent value should probably not be used in contracts.

Inter-seeding alfalfa in corn production systems for farms

Alfalfa and corn silage have complementary nutritional characteristics that benefit livestock when both are included in diets. Alfalfa has often been replaced in rotations by corn silage, in part because corn produces greater forage dry matter yield than alfalfa. First year yields of spring-seeded alfalfa are particularly low, often being one-half that of subsequent full production years.

One way to bypass the low yielding establishment year would be to inter-seed alfalfa into corn to enable full production of alfalfa the following year. Initial studies from 2008 to 2014 demonstrated that foliar applications of a growth retardant known as prohexadione on inter-seeded alfalfa in June increased seedling survival by 40 to 300% under high yielding corn grown at populations over 74,000 plants ha^{-1} (Osterholz et al., 2018). These studies also indicated shifting the seeding rate of alfalfa from 9 to 18 kg ha^{-1} increased alfalfa plant density by 32 to 50% following corn harvest. Other Wisconsin inter-seeding studies in 2015 and 2016 with 38 alfalfa varieties found substantial and consistent differences in plant survival and several conventional varieties had at least a 4-fold greater survival under corn than the poorest performing varieties.

When successfully established, first year dry matter yield of inter-seeded alfalfa was two-fold greater than conventionally spring-seeded alfalfa. In initial studies, alfalfa inter-seeding reduced silage corn yields by up to 15%, but shifting fertilizer nitrogen from the mid- to upper-end of recommended rates largely eliminated yield depression.

The alfalfa inter-seeding system also holds promise for protecting cropland and improving farm profitability. Rain simulator studies indicated alfalfa inter-seeding reduced runoff of both soil and nutrients by 40 to 80% during and after silage corn production compared to a conventional system where alfalfa was spring seeded after corn silage.

New technologies for corn silage production

Interpreting silage yield and quality data for hybrid evaluation

Developing better methods for interpreting silage yield and quality data are needed. A key concern is of Milk2006 is sensitivity to fiber digestibility and it may not fully account for improved intake and resulting milk production from some hybrids. Also, it is not possible to incorporate uNDF measurements in the prediction.

An alternative method for predicting milk response of corn hybrids would be to use a dynamic rumen model such as CNCPS 6.5.5 to estimate potential milk differences among hybrids. Cornell partners this approach estimated ME allowable milk yields for each hybrid using either a standard dry matter intake or a dry matter intake based on the uNDF240. They used a base ration with 12.7kg DM of corn silage and replaced each hybrid into the ration to calculate the individual values. When intakes were based on the uNDFD240, this resulted in potential milk differences among the hybrids up to nearly 9.1 kg day^{-1}.

We are all waiting on the new guidelines of the National Research Council. Hopefully, their guidance will be published soon and then Milk2006 and other milk estimation models will be updated.

Automated machine guidance and transport vehicle selection time-motion

Automated machine guidance has revolutionized production agriculture. For harvest machines, guidance is achieved either by following a row of the crop or by utilizing the Global Satellite Navigation System. Row based guidance consists of sensors placed on the header of the machine that determine the location of individual corn plants immediately prior to harvest. These sensors tell the steering wheels of the machine to move left or right based on the location of the crop row. These guidance systems work well for corn silage harvest but are not well suited for other crops that are planted on row spacing less than 76 cm or crops that are windrowed for harvest.

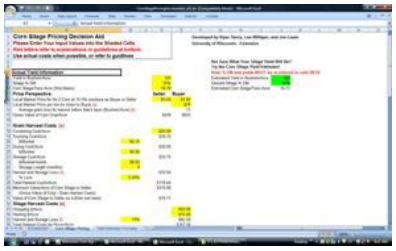
Transport vehicle and support machines play an important role in the corn silage harvesting process. Different transport vehicles have different characteristics, and these have an impact on the machines chosen to implement in an operation. Depending on how the forage harvester loads the transport vehicle will dictate the type of transport vehicles needed. Crop yield, forage harvester capacity, and distance to the storage site will also influence the type of transport vehicles and how many are required to keep harvested material away from the forage harvester. Transport vehicle capacity is generally the first thing considered when selecting transport vehicles. With higher capacity transport vehicles more of the field is harvested per transport vehicle load and unload cycle. Field and road speed of the transport vehicles must also be considered. Decreases in efficiency due to lower capacity transport vehicles can be minimized if the vehicles can travel to and from the storage site at higher speeds.

Kernel processing and theoretical length of cut

Monitoring particle size is very important in a high corn silage ration. In addition to looking at corn silage chop length (ideally about 19mm; longer if using a Shredlage™ processor); it is important to note the chop length and texture of the other forages in the TMR. Shredlage™ is harvested with a commercially-available self-propelled forage harvester fitted with aftermarket cross-grooved crop processing rolls. The self-propelled forage harvester is set for a longer theoretical length of cut than commonly used. The cross-grooved rolls used for processing cause greater damage to the coarse stover particles sufficient to allow for greater digestibility of the NDF and thus attenuate the negative effects of long forage particles on DMI.

Smartphone apps and computer spreadsheets

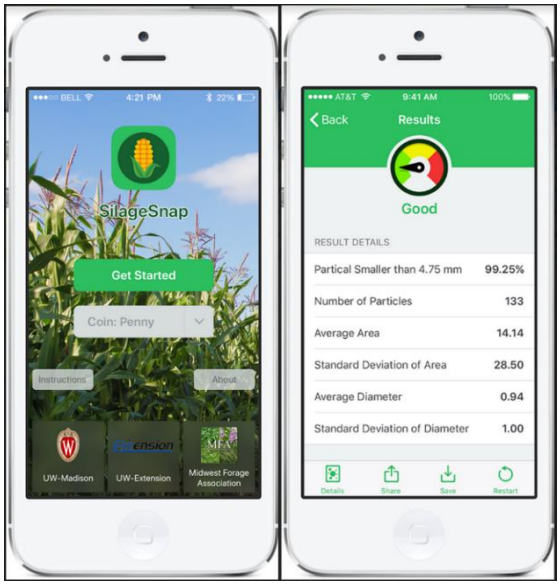
Negotiating the price of corn silage (spreadsheet)



A UWEX spreadsheet provides a framework for negotiating the price of corn silage. The spreadsheet develops a price from the seller's (minimum to accept) and buyer's (maximum to pay) perspectives. The seller will look at it from the standpoint of what is the value of the standing corn minus grain harvest costs. This would

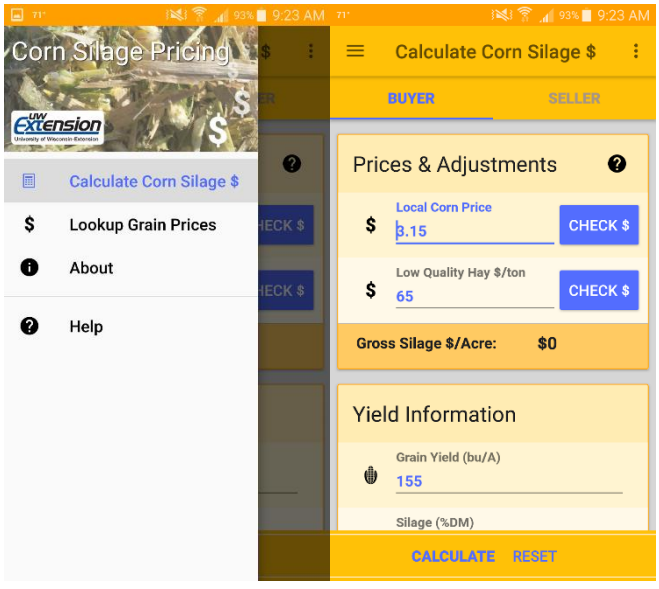
represent the same returns to the seller if the seller harvested the corn for grain. The price is adjusted for the value of the phosphorus and potassium harvested in the stover. The buyer will be looking at the price of standing corn in terms of quality and harvesting costs. This spreadsheet adjusts the value of corn silage for quality based on what it would cost to purchase corn and straw to replace the nutritional value of corn silage. This would represent the maximum price the buyer would be willing to pay. Because prices are likely to differ for buyer and seller, this spreadsheet is best suited to give a price range to start negotiations. Buyers and sellers need to consider local market conditions that would influence the final negotiated price. If the seller minimum is greater than the buyer maximum, it would be more economical to harvest the crop as grain versus silage. A Microsoft ™ Excel spreadsheet for calculating corn silage value is available at: http://corn.agronomy.wisc.edu/Season/DSS/UWEXCornSilagePricingDecisionAid_v2018Jun07.xls

Assessing kernel processor performance



Utilizing hydrodynamic separation, corn kernels can be isolated from stover material. An object of known size is included in an image that is analyzed by assessing samples at 1, 2, 3, and 4 mm processor gap settings. A smart phone application has been developed using this image processing method that dairymen, nutritionists, and machinery operators can use in the field to assess the performance of their kernel processors. The application accepts common United States coins or one Euro or one-half Euro coins. The application is called SilageSnap and is available on Android™ and iOS™ phone platforms.

Corn silage pricing



To help determine a fair price when buying or selling corn silage, UW-Extension developed an Android app that can quickly estimate the value of standing corn silage. The app includes links to current corn and hay market prices and then allows users to enter their own yield estimates and harvest costs, as well as the difference in the value of soil fertility removed from the field when harvested as whole plant silage versus corn for grain, to determine a standing value per acre.

Corn silage hybrids and management systems continue to evolve. As new technologies and knowledge become available, we continue to learn how to increase yield and better optimize this highly palatable, high quality forage crop for dairy/livestock systems.

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NUTRITIVE VALUE OF SILAGES (NIRS TECHNOLOGY) AND THEIR EFFECT ON HEALTH OF PRODUCTIVE ANIMALS

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INTRODUCTION

In the Central European countries, silages have become the predominant forages in dairy diets. In typical models of high yielding dairy cow feeding in Poland, the whole-plant corn silage provides 40-50% of DM and energy. On the other hand, wilted grass or legume silages, which in last decades have replaced the grass hay, provide not only 10-20% of DM and protein but also assure the proper physical structure of a diet. Since silages are so important part of the dairy ration, their nutritive value and fermentation quality affect significantly not only the productivity of cows, but also their health. For example, a low starch content of corn silage and/or its poor kernel processing may not only decrease the milk yield, but also create conditions for metabolic disorders, including ketosis. On the other hand, a high starch content of corn silage, and at the same time, a poor physical structure of a diet, predispose the cow to subacute rumen acidosis (SARA). Moreover, silage may contain several agents that are potentially hazardous to animal health. Since, the near infrared spectroscopy (NIRS) technology is not yet capable to determine them, they will not be discussed here. An excellent review on microbial hazards, plant toxins and chemical hazards as well as on foodborne pathogens have been published recently by Driehuis et al. (2018) and Queiroz et al. (2018), respectively.

Proper diet formulation, to obtain the most effective milk yield, but also to avoid health problems, needs the reliable methods of silage nutritive value and fermentation quality evaluation. In the study which we conducted in year 2015 (Sabatowicz et. al., 2019), on the representative sample of Polish dairy cow farmers (n=15 859; from farms involved in the milk recording system), 36.3% of them declared the regular chemical analyses of their feedstuffs, mostly silages. Taking into account that the mean herd size of these farms was only about 36 dairy cows, such a result we considered as unexpectedly good. A similar study conducted in year 2018 showed even higher percentage (41,5%; n=15 545; farm size 39 cows). There are several reasons for such an increasing tendency, but a much easier access to the laboratory nowadays than in the past seems to be the most import one.

In the past in Poland, the silages were analysed by a few regional labs, as well as by the labs of universities or research institutes, with only very few number of feed companies providing such a service. In these labs, the so called “wet” chemistry methods were used, making the analyses long lasting and expensive. In last decade, the access to the chemical analyses of silages has been increased dramatically due to the widespread introduction of the near infrared spectroscopy (NIRS) technology into practice.

NIRS FOR SILAGE ANALYSES – ADVANTAGES AND DISADVANTAGES

Since the early 1970s, especially since the early 1990s, the NIRS technology has been extensively applied for evaluating chemical composition, digestibility, rumen degradability and fermentation characteristics of forages, including silages. This technology has its advantages and disadvantages. The main advantage of this method over traditional “wet” chemistry methods is a fast generation of results (Park et al., 1998; Stuth et al., 2003). It is also much cheaper, does not destruct the sample and use of hazardous chemicals. The technology has been developed from lab advanced “machines” to portable devices which can now be used silo side, being an attractive tool for feeding advisors.

Typically, there are 4 parameters describing the precision of the NIRS calibration equations: R^2 (coefficient of determination), 1-VR (coefficient of determination of the cross-validation; 1-VR = 1 - (($SECV^2$) / (SD^2)), where SD is standard deviation of reference values), SECV (standard error of cross validation) and SEP (standard error of prediction). Since the NIRS calibrations base on the wet chemistry results (as the reference values), the error of estimation (SECV or SEP) by NIRS is a sum of errors of wet chemistry and NIRS itself. Thus, it is obvious that it has to be higher than the error of wet chemistry analyses, which is often between 1 and 5%. Lower precision of NIRS technology can be compensated by the multiplication (e.g. in time) of the analyses of the same silage.

The disadvantage of the NIRS technology is that not an "every important" silage parameter can be determined with reasonable precision, for example the mineral composition. The limitations come from the chemical nature of the indeterminable compound (-s), especially due to a lack of specific bonds, as well as due to its low, undetectable content (e.g., mycotoxins). Although the results obtained by Berardo et al. (2005) on mycotoxins in corn grain are promising, there have not been implemented in the NIRS calibrations for practical use in silage evaluation so far.

NIRS is successfully used for analysis of dry and ground feeds. However, the drying procedure of silage before NIRS analysis is time-consuming and could cause loss of volatile substances (McDonald et al., 1991). Moreover, for practical diet formulation for ruminants the analysis of fresh (wet) samples is much more desired. A fast determination of chemical composition of silages (or total mixed rations) allows for nearly immediate correction of feeding rations. Unfortunately, the NIRS analysis performed on fresh forage sample is less precise

as compared to dry and ground sample (Griggs et al., 1999). Firstly, a lack of homogeneity of fresh samples strongly contributes to the lower precision of analysis. A presence of particular parts of the plant in the sample, e.g. leaves, steams or kernels, affects final results of analysis. Secondly, water content in the sample interferes with most of spectra characteristics for particular nutrients (Coleman and Murray, 1993). An increase in precision of NIRS analysis conducted on fresh feed samples may be obtained by two ways, in most cases used simultaneously. Firstly, multiple scanning of the sample may be used with repacking of a scanning cup between measurements (Park et al., 2002). The mean of several replicates of such a performed analysis is calculated, being a final result of analysis. Secondly, mathematical pre-treatment of spectra may be used. A conversion of spectra to its derivatives is the most often used mathematical pre-treatment of spectra. As a result the signal quality is improved via separating superimposed spectra and elimination of intercept and slope from calibration equation (Heise and Winzen, 2006). In a case of whole crop maize silage the best accuracy of predicting of its chemical composition were obtained when conversion of spectra to second derivative was used (Butkute, 2005). Moreover, the methods which allow for removing of distortion of infra-red spectra due to its dispersion may effectively improve the precision of chemical composition analysis of feeds (Givens et al., 1992). Among them Standard Normal Variate (SNV), Standard Normal Variate + Detrend (SNVD) and Multiplicative Scatter Correction (MSC) have been the most often studied. It is commonly accepted that for purposes of chemical analysis of fresh feeds at least one of above mentioned methods of scatter correction should be used before calibration equations are calculated (Coleman and Murray, 1993). However, because the results of conversion of spectra depend on the type of analyzed feed (Butkute, 2005), it cannot be simply indicated which method to which extend improves an accuracy of estimation of chemical composition of whole crop maize silage, one of the most important forage for ruminants.

WHAT COULD BE DETERMINED IN SILAGES BY NIRS TECHNOLOGY

The parameters of chemical composition and silage fermentation of silages used in Poland are presented in Table 1 and 2, respectively. The example of the report for calibrations of corn silage is shown in Figure 1. Our calibrations for silages made of corn, grass, lucerne, corn grain (high moisture corn grain), beet pulp were developed for Foss NIRS devices, i.e. InfraXact, DA1650, DS2500. To obtain them we used the results of wet chemistry analyses of 100-800 representative samples which were collected from different regions of Poland, within 10 year time. The choice of parameters was related to the INRA (2007) system (e.g. crude fiber) which is used to diet formulation in Poland, to the needs of other systems alternatively used in Poland (DLG, NRC), and to the demands of feed advisors.

In Table 3 the nutritive value of silages and dOM (digestibility of organic matter) is presented. The reference data for these calibrations were obtained for each silage from the PrevAlim (3.22), a software used to calculate the INRA (2007) system parameters, such as energy units (UFL and UFV), PDI (PDIN and PDIE), and fill unit. PrevAlim calculates these values considering the chemical composition and table data, such as rumen protein degradability and intestinal digestibility of by-pass protein.

PARAMETERS OF SILAGE FERMENTATION AND NIRS TECHNOLOGY

According to Kung et al. (2018), data obtained from silage fermentation analyses (irrespective the method performed – added by Kowalski and Kanski) can be used to determine whether an excellent, average, or poor fermentation has occurred. Based on fermentation parameters, such as pH, ammonia, organic acids or alcohols, the management factors such as the speed of packing, pack density, type of additive used, chop length, covering management, and silo management during feed-out can be discussed. They may also explain poor nutritive value, low dry matter intake, and several health problems, like digestive or metabolic disturbances. For example, too acidic silages, with excessively high concentrations of lactic and/or acetic acids (two wet corn silages; <30% DM) may decrease the dry matter intake and in consequence induce metabolic problems, such as ketosis (Oetzel 2007). On the other hand, higher DM content increases pH, and the silage may spoil, especially when exposed to air. Less acids, especially of acetic increases the number of yeasts. It is still unclear whether the content of fermentation acids, especially acetic, determines the dry matter intake. In the meta-analysis study of Huhtanen et al. (2007), total acid and propionic acid concentration of silages were negatively correlated with dry matter intake in lactating cows. But in the opinions of authors, it was more the effect of poor silage fermentation, of which the above acids are the signs. Oliveira et al. (2017) summarised data from experiments in which silages were treated with homofermentative and facultative heterofermentative lactic acid bacterial inoculant. Inoculation increased dry matter intake and milk yield, but probably not by elevated lactate content, but by lower concnetration of butyric acid, ammonia, and biogenic amines.

Butyric acid is a product of fermentation of sugars, conversion of lactic acid to butyric acid performed by some clostridia (McDonald et al., 1991). In combination with ammonia, butyric acid may be a good indicator of the presence of amines and gamma-amino butyric acids. Butyric acid reduces dry matter intake. Since intake of high concentrations of butyric acid (more than 50–100 g/d) can induce ketosis in lactating cows (Oetzel, 2007), and since the presence of butyric acid testifies the actions of unfavorable microbes, including proteolytic activity, the determination of butyric acid would be desirable. Especially in very wet grass and/or legume silages.

Unfortunately, the precision of calibration equations to determine butyric acid was unacceptable poor. By the way, there is no need for such a calibration for corn silages, since its elevated content is less likely.

High concentrations of ethanol in silages (>3–4%) are often associated with high numbers of yeasts, and such silages usually spoil readily when exposed to air because some yeasts can assimilate lactic acid under these conditions (Kung et al., 2018). In this context, it is not the ethanol itself that is harmful to the cow, but the fact that the yeast caused the spoilage of the silage, which may reduce dry matter intake. The elevated ethanol level in silage is just an indicator of these changes. This is unlikely to occur in most commonly fed silages that the concentrations of ethanol is as high as to be toxic to the cow. According to Ethanol metabolizes into acetic acid in the rumen, in the intestines or in the liver (Weiss et al., 2003).

In some silages, an elevated concentration of 1,2-propanediol (propylene glycol) may be found, as a result of the action of some species of clostridia and yeasts (Sanchez et al., 1987). Also *Lactobacillus buchnerii*, which is naturally present in ensiling mass, converts lactic acid to 1,2-propanediol (Kung et al., 2018). It is thus obvious that *L. buchnerii* added in silage additive increases its content. Since propylene glycol is used in oral drenches as the glycogenic compound to increase the blood glucose content, one could assume that silages with elevated 1,2-propanediol content may be used in prevention of ketosis. Unfortunately, the amount consumed in the silage (if consumed....) is too low.

Determination of ammonia content, often as % of total nitrogen (protein), is common in NIRS calibrations, especially for grass or legume silages. High ammonia content shows the excessive proteolysis. Since fermentation processes increase soluble N, quite often up to 60% of total nitrogen, making it very degradable in the rumen, the determination of soluble N could also be of certain value to quantify the fermentation in the silo. In a study of Hermida et al. (2005) soluble N and NPN were well correlated with wet chemistry results.

As seen in Table 2, we developed the calibration for determination of acid detergent insoluble nitrogen (ADIN, % of total N), which is considered as a marker of protein heat damage caused by the Maillard (non-enzymatic browning) reaction (Goering et al., 1973). It is especially useful in the analyses of grass or lucerne silages with elevated DM content, in which production of heat (prolonged high temperatures above 45 to 50°C) changes the proteins structure, making them unavailable. In another study (Hermida et al., 2005) the prediction errors for ADIN in silages were unacceptable high. Heat damage of proteins needs to be considered in diet formulation to avoid protein and amino acid deficiencies.

Although the NIRS technology allows for the determination of main parameters used in evaluating of silage fermentation, the quantification of more sophisticated parameters related to silage fermentation is still practically impossible. Unfortunately, micotoxins, specific alcohols (methanol, propanol) and esters (e.g. ethyl lactate) and variety of nitrogenous compounds (such as amines, e.g., putrescine, cadaverine, tyramine, and histamine), so important for animal health, belong to these parameters. Some of them can adversely affect animal productivity and health. Unfortunately, NIRS technology does also not allow for the enumeration of microbes, including yeasts and molds, what may also be useful in evaluating of silage fermentation.

PARAMETERS OF SILAGE NUTRITIVE VALUE AND NIRS TECHNOLOGY

The contents of silage fiber, starch, and protein, their digestibility, as well as physical structure (particle size) affect dry matter intake, feeding behaviour, including sorting, and in consequence efficiency of milk production (Grant and Ferraretto, 2018). They have also a fundamental effect on the health of dairy cows. Unfortunately, the NIRS technology is not capable to determine the particle size distribution of silages (and diets). It is also not possible to determine the kernel processing quality.

On the other hand, there are examples of the NIRS calibrations for digestible NDF (NDFD) or NDF digestibility (dNDF). Oba and Allen (1999) in a literature survey revealed that for every one point increase in fiber digestibility there was a corresponding 0.37 lb increase in dry matter intake and 0.55 lb increase in milk production. Unfortunately, the quality of the NIRS calibrations used by the laboratories is still not good. Many calibrations are relatively new and may include relatively few samples. However, the most important limitation is still a poor standard of the reference method (wet chemistry).

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Table 1. Parameters of chemical composition of silages used in Poland, estimated by NIRS

Silages	Number of samples	Dry matter %	% as fed							pH
			Ash	Crude protein	Ether extract	Crude fiber	NDF	ADF	Starch	
Corn [1]	527	+	+	+	+	+	+	+	+	+
Corn grain [2]	101	+	+	+	+	+	+	+	+	+
Grass	802	+	+	+	+	+	+	+		+
Lucerne	122	+	+	+	+	+	+	+		+
Beet pulp	120	+	+	+	+	+	+	+		+
SECV [3]										
Corn		1,17	0,13	0,17	0,26	0,48	0,91	0,57	1,67	0,16
Corn grain		0,50	0,07	0,20	0,35	0,18	0,82	0,22	1,20	0,08
Grass		1,49	0,70	0,52	0,30	0,79	1,35	1,02	-	0,28
Lucerne		0,77	0,44	0,38	0,23	0,85	0,90	0,64	-	0,07
Beet pulp		0,29	0,10	0,06	0,05	0,13	0,42	0,17	-	0,08
1-VR [4]										
Corn		0,96	072	0,89	0,48	0,86	0,87	0,87	0,86	0,62
Corn grain		0,98	0,89	0,96	0,84	0,70	0,79	0,86	0,92	0,92
Grass		0,99	0,69	0,95	0,65	0,96	0,95	0,97	-	0,85
Lucerne		0,99	0,82	0,95	0,43	0,92	0,95	0,97	-	0,87
Beet pulp		0,99	0,85	0,96	0,34	0,93	0,94	0,93	-	0,91

[1] whole plant corn silage
[2] ensiled high moisture corn grain
[3] standard error of cross validation
[4] coefficient of determination of the cross-validation

Table 2. Estimation error (SECV) of fermentation parameters of silages used in Poland - NIRS technology

Silages	Number of samples	pH	Lactic acid (% of wet silage)	Acetic acid (% of wet silage)	Lactic acid (% of sum of acids)	Acetic acid (% of sum of acids)	Ethanol	N-NH3 (%N)	ADIN (%N)
Corn	134	+	+	+	+	+	+	+	+
Grass	384	+	+	+	+	+	+	+	+
Lucerne	147	+	+	+	+	+	-	+	+
SECV [1]									
Corn		0,16	0,33	0,52	6,2	6,7	0,10	1,0	1,7
Grass		0,28	0,64	0,34	15,0	15,0	0,03	2,2	1,1
Lucerne		0,07	0,63	0,35	8,0	8,8	-	1,7	1,5
1-VR [2]									
Corn		0,62	0,73	0,53	0,68	0,59	0,82	0,69	0,77
Grass		0,85	0,25	0,35	0,50	0,41	0,71	0,59	0,69
Lucerne		0,87	0,66	0,65	0,84	0,74	-	0,73	0,84

[1] standard error of cross validation
[2] coefficient of determination of the cross-validation

Table 3. Estimation error (SECV) of INRA system values in silages used in Poland – predicted directly by NIRS

Silages	Number of samples	UFL	UFV	PDIN (g)	PDIE (g)	Fill Unit (LFU)	dOM (%) [3]
Corn	155	0,02	+	+	+	+	+
Corn grain	147	0,01	+	+	+	-	+
Grass	359	0,02	+	+	+	+	+
Lucerne	198	0,02	+	+	+	+	+
SECV [1]							
Corn		0,02	0,02	1,3	1,3	0,01	1,04
Corn grain		0,01	0,01	1,3	0,49	-	0,38
Grass		0,02	0,02	3,9	1,9	0,02	1,20
Lucerne		0,02	0,02	9,8	2,2	0,02	1,07
1-VR [2]							
Corn		0,93	0,91	0,80	0,92	0,92	0,48
Corn grain		0,97	0,97	0,95	0,98	-	0,99
Grass		0,98	0,97	0,93	0,98	0,97	0,69
Lucerne		0,96	0,94	0,92	0,87	0,96	0,65

[1] standard error of cross validation
[2] coefficient of determination of the cross-validation
[3] digestibility of organic matter

Calibration set parameters

Constituent	n	min (%)	max (%)	average (%)	SEC	SECV	R^2
dry matter (DM)	361	19,45	57,18	35,37	1,26	1,37	0,96
Ash	342	0,14	2,84	1,48	0,18	0,19	0,47
crude protein	357	1,13	4,94	2,91	0,18	0,23	0,89
FAT	289	0,15	2,60	1,29	0,23	0,24	0,59
crude fiber	275	0,55	12,52	6,59	0,55	0,59	0,73
NDF	315	6,56	23,37	13,58	0,83	0,91	0,87
ADF	249	4,05	12,74	8,08	0,56	0,61	0,85
starch	274	4,49	24,65	12,66	1,79	1,88	0,73
pH	242	3,12	4,79	3,83	0,16	0,19	0,75

% in fresh feed

Validation (independent samples)

Constituent	n	SEP	SEP (C)	Bias	Bias limit	Slope
dry matter	23	1,243	1,261	-0,156	0,823	0,989
ash	23	0,206	0,206	0,042	0,114	0,724
crude protein	22	0,179	0,183	0,010	0,139	0,935
fat	23	0,174	0,178	0,011	0,143	1,031
crude fiber	24	0,539	0,541	0,103	0,356	0,992
NDF	23	0,746	0,762	0,045	0,544	0,947
ADF	23	0,523	0,533	0,043	0,368	0,967
starch	20	1,574	1,541	0,470	1,127	1,035
pH	23	0,109	0,112	0,001	0,115	0,796

n - samples used

Figure 1. Report for calibration for "wet" corn silage
for DA 1650™, Foss (University of Agriculture in Krakow)

Section 1: Production of forages – fertilization, quality and yield

sponzored by

IMPLEMENTATION OF PRECISION FARMING TECHNOLOGIES IN FODDER CROP MANAGEMENT

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INTRODUCTION

The Czech Republic has a specific land use defined by the highest average holding area in EU (over 130 ha per farm). The national statistical evaluation of agriculture sector (Ministry of Agriculture of the Czech Republic, 2015) shows that farm enterprises with acreage of managed land over 1000 ha cultivate 50.3 % of agricultural land in Czech Republic. Also, there is known large average size of fields - statistical evaluation of the size of land parcels shows that 60 % of arable land is located within the fields with the area over 20 hectares. Higher diversity of the relief and pedoclimatic conditions in combination with the size of land blocks occur in visible heterogeneity of land. This leads to an increased interest in the precision farming practices and technologies for site-specific crop management, where high quality of input geo-information about the land are required.

Precision farming or site specific crop management is internationally unified term for directions of land management using new technologies that began to be developed in the eighties and early nineties of the twentieth century. The aim of precision agriculture is an optimization of production inputs (fertilizers, pesticides, fuel, etc.) based on the local crop requirements and plants requirements. Crop management in this way can lead to the effective use of agrochemicals and avoid of environmental risks. Site specific management takes into consideration spatial variability within fields and optimizes the production inputs, thus fulfilling the objectives of sustainable agriculture (Corwin a Plant, 2005).

MAPPING OF SPATIAL VARIABILITY

The aspects of precision agriculture (PA) are described by Pierce and Nowak (1999). They defined precision agriculture as "the application of technologies and principles to manage spatial and temporal variability associated with all aspects of agricultural production for the purpose of improving crop performance and environmental quality". Gebbers and Adamchuk (2010) describe three goals of precision agriculture: 1. to optimize the use of available resources to increase the profitability and sustainability of agricultural operations, 2. to reduce negative environmental impact, 3. to improve the quality of the work environment and the social aspects of farming, ranching, and relevant professions.

Pierce and Nowak (1999) consider assessing variability as the critical first step because one cannot manage what one does not know. The factors and properties that regulate crop growth and yield vary in space and time. The higher is the spatial variability of a soil conditions (or crop properties), the higher is the potential for precision management and the greater its potential value. The degree of difficulty, however, increases with higher dynamics of temporal component.

The consequences of site variability are most reflected in the crop yield. The variability of yield represented by yield maps can serve as input information for decision about site specific management. If there is not known the cause of yield variability, the uniform crop management is suggested (Adamchuk et al., 2010, see Figure 1). Site specific management can be recommended if the spatial structure of yield differences are consistent over multiple years and correspond to some agronomically important phenomena (nutrient supply, topography, land use history, ...).

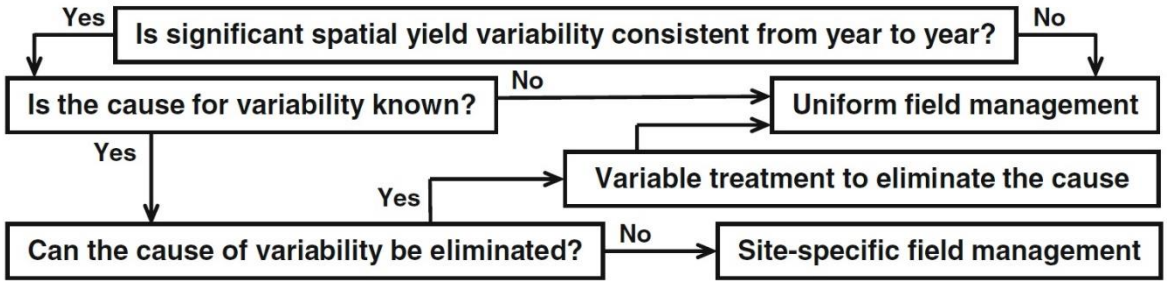

Figure 1 Yield-based decision making tree for application of site specific management (Adamchuk et al., 2010).

MODERN TECHNOLOGIES UTILIZED IN PRECISION AGRICULTURE

Basic principles of precision agriculture are not new, the spatial and temporal variability of soil and crop was recognized by farmers centuries ago. Smaller parcels with natural boundaries allow changing the agrotechnical treatments manually. With the merging of parcels and intensification of production and mechanization in the middle of the last century, it was no longer possible to take into account the spatial

variability. These technologies include global navigation satellite systems (GNSS), geographic information systems (GIS), information and communication technologies (ICT) and sensors.

Figure 2: Graphical list of modern technologies utilized by precision agriculture (Author: V. Lukas)

Global Navigation Satellite Systems (GNSS) provide autonomous geo-spatial positioning with global coverage. It allows to determine using GNSS receiver precise real-time localization on Earth surface. Enhanced position accuracy up to few centimetres is feasible with the use of Realtime kinematic (RTK) positioning for precise guidance of agricultural machinery on the field.

Geographical Information Systems (GIS) are widely used for processing and analysis of geospatial data and their representation in form of maps. In addition to spatial (graphic) representation of the object is important their description in the form of attribute table. Track logs of machinery, field boundaries, soil sampling data and yield maps – that are all spatial data, which are created and displayed using GIS. Besides commercial platforms also open-source software packages are available, such as QGIS, Grass, GDAL libraries, etc.

Sensor systems are an alternative to conventional (and expensive) techniques for mapping of soil and crop variability. Pierce and Nowak (1999) consider that sensors are critical to success in the development of a precision agricultural system for three important reasons: 1. Sensors have fixed costs, 2. sensors can sample at very small scales of space and time, and 3. sensors facilitate repeated measures. Disadvantage of sensor measurement is lower accuracy compared to laboratory procedures. However, it is compensated by more intense spatial coverage (Christy, 2008).

Proximal soil sensing

The conventional techniques of soil variability mapping are slowly replaced by indirect methods such as the on-the-go systems (see overview by Adamchuk et al., 2004) or remote sensing. These methods have more intense spatial coverage but are less accurate compared to laboratory procedures (Christy, 2008). Soil electrical conductivity (EC) has become one of the most frequently used measurements to characterize field variability for application to precision agriculture (Corwin a Lesch, 2003). The measurement of soil electrical conductivity is a cost-effective method complementing traditional soil survey, which provides rapid and non-invasive information on soil texture variability and available soil moisture (Godwin and Miller, 2003). According to the study of Corwin and Lesch (2005a), the most important factors influencing EC include the content of soluble salts in soil solution, relative moisture, soil water content and bulk density. The effect of these factors can be found in most of the studies cited here, but their significance varies with regard to specific site conditions. In agricultural areas where soil salinization is not a significant factor, EC measurements are the primary function of soil moisture and soil texture (Godwin a Miller, 2003). Finding the dominant soil characteristics on each plot is necessary for correct interpretation of EC maps (Brevik et al., 2006; Corwin a Lesch, 2003). In addition, the knowledge of the most important factors influencing the spatial variability of crop yield or production quality is required for utilization of EC in site specific crop management (Corwin a Lesch, 2005).

The advantage of the EC measurement is the vertical penetration of the electromagnetic or electric signal by the soil, and thus obtaining information of the soil profile. The result of the EC measurement is also not affected by the vegetation cover of the soil or crop residues (Brevik et al., 2003), which makes it possible to carried out measurement on bare soil or under vegetation cover.

Figure 3. *Measurement of soil EC by GF Instruments CMD devices at Mendel University in Brno*

Lukas et al. (2018) verified the use of on-the-go soil EC measurement by electromagnetic induction for mapping of within field spatial variability of selected physico-chemical properties of soil on 476 ha in ROSTĚNICE a.s. farm. Results from this mapping were compared with the soil sampling in non-regular sampling grid with the density one sample per 3 ha.

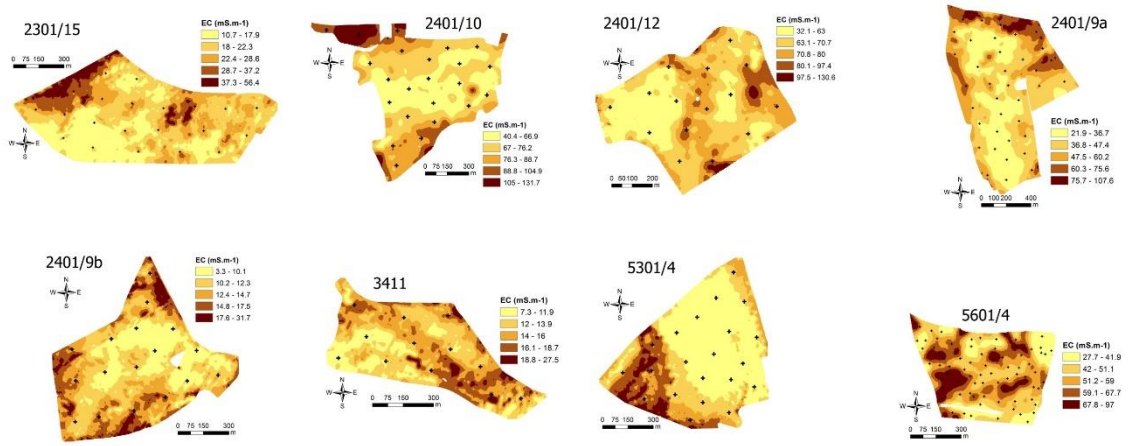

Figure 4. Maps of soil EC after spatial interpolation. Black crosses represent soil sampling points (Lukas et al., 2018)

The results of soil sampling showed different variability of the observed soil properties across the fields. The results of correlation analysis showed main sensitivity of EC measurement to the soil texture categories (clay, silt and sand) and content of soil organic matter. The correlation between EC and nutrients content in soil and pH value was almost not significant (except of K content). These results were obtained for individual fields, the aggregated evaluation showed lower relationships to EC. As the main advantageous of EC measurement is identification of main zones within the fields at high spatial level, which represents different soil properties. Recent studies showed that these zones can be used for directed soil sampling or to delineate the management zones for site specific crop management.

Remote sensing

For preparing of site-specific crop management treatments during crop vegetation, whole-area identification of plant status and capturing its spatial variability within the field is required. Diagnosis of plant status in the traditional way at the high spatial level is very time and cost labor. Therefore, remote sensing techniques are used to evaluate the spatial variability of crop parameters based on the spectral behavior of electromagnetic radiation, most often in the visible and near-infrared range. For the quantification of vegetation parameters, vegetation indices are often used, determined as the ratio of reflectance in defined bands of electromagnetic radiation. Recent studies provide a number of vegetation indices that were utilized for diagnosis of plant nutrition and stress (Fu et al., 2014; Li et al., 2014).

Remote sensing methods have been applied for two decades to assess crop stand conditions (Mulla, 2013). In addition to the already established aerial and satellite imaging, unmanned survey methods by remotely piloted aerial systems (RPAS) in precision agriculture are increasingly being enforced (Zhang a Kovacs, 2012). One reason could be significantly lower operational and investment costs (Pechanec et al., 2014) and low-altitude capability to capture data even when clouds are present above the scanned scene. On the contrary, the

disadvantage is the lower surface performance and limited load capacity, which determines the sensor equipment of the RPAS.

Figure 5. Photo of plant sampling within 50 x 50 cm square (left), DJI Matrice 600 in preflight preparation (middle) and detail on Micasense RedEdge-M camera (right, www.micasense.com)

Crop yield mapping

Differences of crop yield levels are often used as the indicator of field heterogeneity, if yield maps are available. Recent studies shown that there are many factors influencing the spatial variability of crop yields, such as evapotranspiration (Johnen et al., 2014), topographic attributes (Kumhálová a Moudrý, 2014) combined effects of soil fertility and weed control (Mallarino et al., 1999). Yield is the integrator of landscape and climatic variability and therefore provide useful information for identifying management zones (Kleinjan et al., 2007). Management zones represent in the context of precision agriculture areas possessing homogenous attributes in landscape and soil condition. These areas should lead to the same results in crop yield potential, input use efficiency and environmental impact (Schepers et al., 2004).

Delineation of management zones for site specific crop management, is usually based on yield maps over the past few years. Similar to the evaluation of yield variation from multiple yield data described by Blackmore et al. (2003), the aim is to identify high yielding (above the mean) and low yielding areas related as the percentage to the mean value of the field. Also the inter-year spatial variance of yield data is important for agronomists to distinguish between areas with stable or unstable yields. However, classification of management zones from time-series of yield maps appears limited because of the high frequency of erroneous data sets, systematic errors in the recorded data and their restricted yield predictive ability (Joernsgaard a Halmoe, 2003).

The study of Mezera et al. (2018) describe the processing of crop yields records from harvester. Aim of the study was to describe a methodology of yield data processing, spatial analysis and to indicate its interpretation for the decision support of agronomists. Yield data were recorded during harvest of 248 ha of winter wheat in 2016 at farm company SALIX MORAVA a.s. in Zdounky.

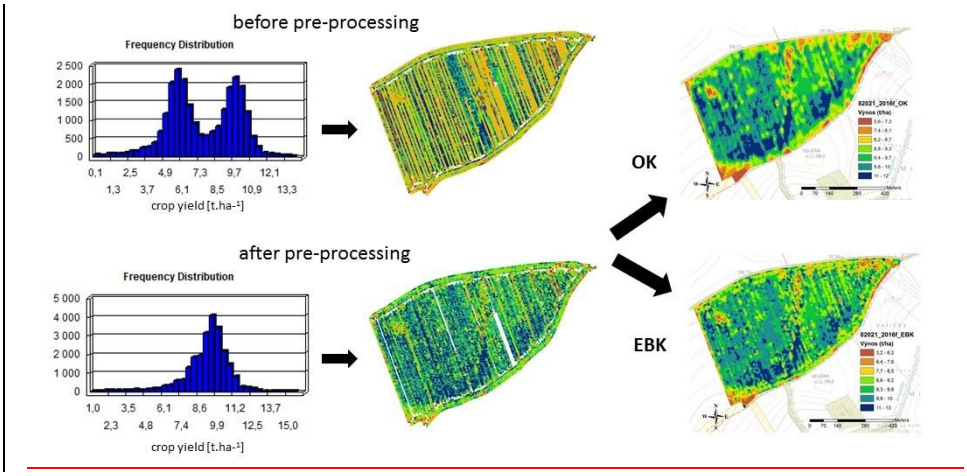

Figure 6. Crop yield map of 44-ha field expressed as the relative yield to the field average value (left) and area percentage of yield classes (right) (Mezera et al., 2018).

Yield mapping by grain flow sensors in harvesters provides crucial information for precision agriculture about the crop yield distribution within the fields. However, for reliable interpretation of the yield correct pre-processing of data must be carried out to correct a number of errors, such as outliers and spatial inconsistent values. As it was shown in the study, big challenge is the combination of datasets from two harvesters, especially in the case that both sensors were non-calibrated. Correction of these

effect enhance quality of final maps in the next step of data processing – spatial interpolation. In this study, geostatistical method Empirical Bayesian Kriging proved its ability for full automatization of yield maps creation in GIS environment.

Besides absolute values of crop yield also calculation of relative yield as the percentage to the field average value can be recommended. Identification of under- and over-average yield areas support the agronomist decisions on the crop treatment intensity in the form of variable rate application (fertilizers, crop protection, etc.). Recent studies show that aggregation of relative yield maps from multiple years is valuable information for further planning of site specific crop management.

IMPLEMENTATION OF SITE SPECIFIC CROP MANAGEMENT IN CZECH REPUBLIC

Precision agriculture is developed mainly in agriculturally advanced countries, but also can be seen worldwide trend of growing interest in this method of farming. In practice, the largest application is in the USA, which can be explained by specific agrarian structure (large area farms) and high level of utilization of technologies. Unlike Western Europe, the Czech agrarian structure suitable for the application of PA technologies –domination of large farms and fields, diversity of geological, pedological, hydrological and climatic conditions together with combination of topography). For all that the interest for PA technologies is increasing and also suppliers of agricultural machinery, fertilizers and pesticides take this system into account in near future. The focus is currently in providing the services for farmers.

Dissemination of site specific crop management is also supported by government by opening main agricultural geodata. There are available many sources of geodata in Czech Republic useful for farmers, agronomist or farm advisors, which are at the national level guaranteed by the state institutions. The most important information source is Land Parcel Identification System (LPIS) which contain the spatial registry of land parcel blocks. Database of land parcel boundaries and base set of land attributes is available for download also for non-registered users in form of shapefile, xlm or as web map services. Besides field boundaries, registered users (farmers) can download also polygons with restricted areas for agrochemicals application, in form of buffer zones near to water bodies for limited application of nitrate fertilizers or pesticides. Since 2017 soil unit maps in scale 1:5000 are available as open data in shapefile format with the basic information about soil properties of agricultural land for whole country. Also other geodata are available for processing or visualization in GIS such as various scales of topographic maps, cadastre maps and digital elevation models based on the aerial laser scanning.

Very important source of information for site specific crop management are Earth Observation data. Spatio-temporal analysis of vegetation indices images from satellites with combination of open vector data allows to carry out a large area survey with the identification of current crop status or field heterogeneity. An example of this approach is delineation of yield productivity zones within the fields based on the 8-year satellite imagery from Landsat (5, 8) and Sentinel-2. Main aim is to identify high yielding and low yielding areas related as the percentage to the mean value of the field, which are an input information for variable rate application. Final productivity map is provided with 5 m pixel size for each farm enterprise and has been used for variable application of nitrogen fertilizers for winter wheat and oil seed rape or for ISARIA sensor system as the yield potential map for map overlay mode.

ACKNOWLEDGEMENTS

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VARIABILITY OF NUTRITIVE VALUE OF FORAGE LEGUME LEAVES AS A PROTEIN SOURCE FOR ORGANIC PIG FARMS

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ABSTRACT

Present research in animal nutrition within the European region is looking for "home-made" protein sources to avoid high dependency on imported soybean protein. The main objective of this study was to investigate the variability of nutritive value of legume leaves growing under organic management. In 2017, forage was sampled in the common stands of a lucerne (*Medicago sativa* L.) – red clover (*Trifolium pratense* L.) mixture across four locations in the first cut. Number of stems, maximal stem length and leaf weight ratio on a DM basis was assessed for both species in the samples where total DM and leaf production were calculated. Results show high variability in productivity and stand structure of mixture stand among locations and species but low variability was observed for leaf qualitative traits. This research has indicated that a lucerne – red clover mixture can be an effective source of forage legume leaves where species and stand structure have higher impact on productivity than leaf nutritive value.

Keyword: forage, alfalfa, red clover, aminoacids

INTRODUCTION

The difference between high protein demands by the livestock sector and low protein production in the European Union (EU) has resulted in the fact that most of the protein for livestock production in the EU is imported in the form of the soybean meal (Van Krimpen *et al*., 2013). Apart from sensitivity of animal production to price volatility, most of the imported soya bean meal is genetically modified which can be a concern with regard to organic farming systems (Westhoek *et al*., 2011). For these reasons, present research in this scientific area is looking for "home-made" protein sources based on common field crops within the EU. Forage legumes play a key role in integrating livestock and crop production and are considered as a potential protein source for method of protein extraction (Solati *et al*., 2017) or leaf separation (Liebhardt *et al*. 2019). In line with these perspectives, the goal of the current project in the Rural Development Programme in the Czech Republic is the development of simple technology for legume leaf separation and their utilization as an organic protein source for pig nutrition. This paper summarises the initial part of this project and includes the first year results from a field experiment comparing the productivity of lucerne – red clover mixture across different fields and their leaf nutritive value.

MATERIALS AND METHOD

A field experiment was arranged in lucerne – red clover mixture growing under organic management in the first harvest. The organic farm is located at Sasov near Jihlava in the Czech Republic. In 2017, production of leaves per hectare was estimated through forage sampling in the common stands of a lucerne – red clover mixture (seeding ratio 50:50) at four locations differing in stand age (Table 1). Before first cut, samples were clipped using a hand scissors, to a height of 50 mm above the ground from an area of 12.5 x 50 cm in four replicates. The number of stems (stem density, SD m^2) and length of the longest stem (MSL, m) were determined for lucerne and red clover. Five of the longest stems of each legume species in the sample were selected and their leaves (blade, petiole, stipule) were separated by hand from the stems (stem, bud, flower). All samples were oven dried at 60°C to constant DM. The lucerne and red clover leaf weight ratio (LWR, g kg^{-1} DM), leaf dry matter yield and total dry matter yield (DMY, g m^{-2}) as well as percentage of weeds were calculated. Separated leaves were analysed for crude protein (CP, %) and crude fibre content (CF, %) in the laboratory of the Institute of Animal Science Prague. Field data were analysed by two-way analysis of variance (ANOVA) comparing differences in the contribution of lucerne and red clover in the mixture.

RESULTS AND DISCUSSION

The investigated localities varied significantly in their productivity (Table 1), where most productive were Vysílač and Zahrada. Observed variability corresponds with the lowest productivity in the oldest stands, together with increasing of weed proportion. Higher productivity of lucerne across fields correspond with expected higher lucerne persistence in comparison with red clover. It is in line with Marley *et al*. (2003), who published enhanced yield of this bi-crop with increasing seeded proportion of lucerne over three year period. It could be also associated with trend to lower seasonal precipitations in Central Europe. Hakl *et al*. (2018) reported variability of productive traits in this mixture in locality Vysílač over growing period 2017, where proportion of red clover declined over season. Summer and early autumn usually represents the drier period of the growing season for this area. According to Peterson *et al*. (1992), these two crops have complementary

production responses to climatic conditions, where lucerne is higher yielding in dry whilst red clover is higher yielding in wet conditions.

Contrast to variability in stand structure, leaf nutritive quality showed stable values across localities. There were significant differences between legume species where lucerne leaves were higher in CP and lower in CF. These inter-species differences correspond with Solati *et al*. (2018). Leaf DMY values ranged from 94 to 184 g m^{-2} and documents a high potential for leaf production, however only under optimal stand structure traits. Considering average yield from all cuts, it seems to could be a comparable to reported values for grain legume production under organic management. It is in accordance with Solati *et al*. (2018) who considered that red clover and lucerne are favourable species for protein production. However, for practical utilization of effective legume leaf production, adequate technology must be developed and tested in the field conditions (Liebhardt *et al*. 2019). The mixture with both species can give some advantages in this regard due to the higher productivity of lucerne and higher leaf proportion of red clover (Hakl *et al*. 2018).

Table 1: Effect of field and legume species in the mixture on the stand structure and leaf forage quality.

Localities	Stand age	Stem density [pcs/m^2]	Maximal stem length [cm]	Leaf weight ratio [%]	Forage DMY [g/m^2]	Leaf DMY [g/m^2]	Weed ratio [%]	Leaf CP [%]	Leaf CF [%]
Vysílač	2	752	64.5^{b}	43.6	404^{ab}	174	1.1^{a}	26.6	18.0
Střelnice	6	422	36.4^{a}	55.9	169^{a}	94	39.3^{b}	26.7	13.3
Jágrovo	3	634	41.5^{a}	49.9	232^{ab}	101	24.1^{ab}	25.6	15.4
Zahrada	3	750	53.6^{b}	44.0	398^{b}	168	23.1^{ab}	26.9	16.6
P		0.206	< 0.001	0.077	0.024	0.044	0.005	0.276	0.134
Red clover		284	42.8^{a}	49.5	238	108	-	23.7^{a}	17.8^{a}
Lucerne		356	55.3^{b}	47.2	363	160	-	27.7^{b}	13.9^{b}
P		0.252	< 0.001	0.532	0.069	0.067	-	< 0.008	< 0.026

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

CONCLUSION

It can be summarized that variability among fields was high in productive traits in relation to stand age. Leaf quality was stable across four locations. Contrast to red clover, lucerne provided leaves with higher CP and lower CF. Results supported idea that forage legume crops can be an effective field source of rich protein leaves under organic management where lucerne tends to higher productivity and nutritive value.

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THE INFLUENCE OF ORGANIC MANURES AND MINERAL FERTILIZERS ON BOTANICAL COMPOSITION, PRODUCTION AND FORAGE QUALITY UNDER FOUR-CUT REGIME USED IN PERMANENT GRASSLAND IN THE MALÁ HANÁ REGION

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INTRODUCTION

Permanent grasslands (PG) represent a significant production, stabilization (biodiversity, soil protection against erosion, etc.) and landscape forming element in the current farming systems in the Czech Republic. In the Czech Republic, the area of PG occupies approx. 1,011,000 ha, which is about 24% of the total agricultural land area / 4,215,000 ha / (CZSO 2018). PG's production significance is that it provides an important source of bulk glycid-protein feed for livestock, as well as a high-quality substrate for biogas plants (BPS) replacing corn seed (Nerušil et al. 2016). The most important factor affecting biomass production and at the same time changing species composition is PG fertilization (Hejcman et al. 2007). The botanical composition is of crucial importance to ensure the production and non-production functions of PG (Michaud et al. 2012), including the quality parameters of the forage produced / the forage quality is based on high digestibility, nutrient concentration and their mutual ratio (Gaujour et al. 2012). In the long term, forage quality, resp. the botanical composition can be influenced by the system of grassland use, ie the intensity and frequency of grazing, the alternation of mowing and grazing, or the number of mows during the year (Komárek et al. 2005, Hejcman et al. 2010; Nerušil et al. 2012). The aim of the study is to evaluate the effect of manure fertilization (manure, slurry) and mineral fertilizers (NPK) on yield characteristics, botanical composition (grasses, legumes, other herbs) and forage quality (CP, fiber, NEL, ME, OMD) in PG under 4-cut regime in the area of the Boskovice furrow (Malá Haná region).

MATERIAL AND METHODS

The research was conducted in a long-term experiment at PG (49.6282881N, 16.7317036E; CRI, GRS Jevíčko) founded in 2004 at 342 m above sea level, with an average annual temperature of 7.4 °C /vegetation period 13.4 °C / and average annual rainfall of 545 mm / growing season 347 mm / (Weather Station Jevíčko /1966–1995/ CHMI Ostrava). The soil type of the research areas is loamy haplic Luvisol (Němeček et al. 2011) and the geological basis of the territory is the rocks of the permocarbon of the Bohemian Massif. Botanical composition of the PG at the foundation of the experiment: grassland - oat type (*Arrhenatheretum*), dominant grass species - false oat-grass, cocksfoot, meadow foxtail, smooth meadow-grass, red fescue. Experimental variants: (1) control without fertilization /Control/; (2) mineral fertilization /NPK/; (3) manure /FYM/; (4) cattle slurry /CS/. Size of experimental plots of PG: 8 × 1.25 m = 10 m^2, 4 replications in each variant. Method of management - 4-cut regime (intensive), dates of mowing: 15 May; 30 Jun; 15 Aug; 30 Sept - 45 days increase. Dosages of mineral and livestock manure under the model load of PG by cattle at 2.0 CU ha^{-1} are reported by Komárek et al. (2005). Harvesting was carried out by a small-plot forage harvester MPZ-115. The representation of individual plant species, basic agrobotanical groups and the gaps of the studied PGs was performed by the method of reduced projective dominance in 2016 before the 1st cut (Horký et al. 2013). The dry matter production in the period 2011-2015 was determined on the basis of the green matter yield and the forage dry matter set by laboratory. Forage quality in the period 2011-2015 was determined using the NIR technique (FOSS NIRSystem 6500): crude protein (CP), fiber, net energy of lactation /NEL/, metabolizable energy /ME/, organic matter digestibility /OMD/. Statistical analyses including graphical outputs were performed in Statistica 13 (TIBCO Software Inc., Palo Alto, USA, 2018). Multidimensional exploratory analysis (EDA), ANOVA (HSD test), major component analysis (PCA) and factor analysis (FA) /Meloun et al. 2012/ were used for the evaluation. Statistical significance was tested at the significance level P = 0.05.

RESULTS AND DISCUSSION

In 4-cut utilization of PG (in the model load of 2 CU. ha^{-1}), grass species (> 70%) develop and dominate in the stand for all evaluated variants, including non-fertilized control at the expense of herbal and legume components. The most represented grass species in the control variant were smooth meadow-grass and false oat-grass (more than 80%). In the NPK variant there was a significant increase in the proportion of red fescue, meadow foxtail at the expense of smooth meadow grass and false oat-grass. The FYM variant revealed a higher proportion of tall fescue than NPK and CS variants. Fertilization of slurry (var. CS) contributed to a higher proportion of creeping grass compared to FYM and NPK. Dry matter production was statistically higher in fertilized variants (about 7 t ha^{-1}) compared to Control (4.5 t ha^{-1}). CP content is statistically higher in CS versus Control. NEL was found to be 5.8 to 5.9 without statistical significance, and similar results were found for the ME parameter. OMD is higher in FYM and CS versus NPK variant, see Tab. 1.

Only the first two axes (PC1xPC2), which together account for approximately 94% of the variability, are significant on the PC1, PC2 Component Scale Chart. A strong negative relationship between CP, Grass (r = -0.96–0.98) and NEL and Yield (r = -0.95–0.73) was found on the PC1 axis, followed by the positive relationship of Legumes (r = 0, 94). There is a very significant correlation with OMD on the PC2 axis (r = -0.93). There are

clearly divided variants along the PC1 axis in terms of the representation of Legumes, Yield, CP, NEL and the representation of Grasses. The PC2 axis clearly divides the OMD variants, see Figure 1.

The FA Factor 1 describes the yield properties, the Grasses and Legumes representation and the CP content, Factor 2 describes the property /character/ OMD.

Tab. 1: Basic characteristics: yield, forage quality in 2011-2015.

Variant	Yield DM (t ha^{-1})	CP (g kg^{-1})	Fiber (g kg^{-1})	NEL (MJ kg^{-1})	OMD (%)	ME (MJ kg^{-1})
Control	4.54^{a}	155.57^{a}	217.81^{a}	5.80	75.41	9.24
NPK	7.07^{b}	167.78	236.91^{b}	5.81	73.79	9.81
FYM	6.97^{b}	171.20	221.65	5.83	75.92	9.84
CS	7.06^{b}	182.20^{b}	224.70	5.91	75.50	9.96

Note.: CP - crude protein, NEL - netto energy of lactation, OMD - organic matter digestibility; ME - metabolizable energy, ^{a,b} statistically significant differences P = 0.05 (HSD test).

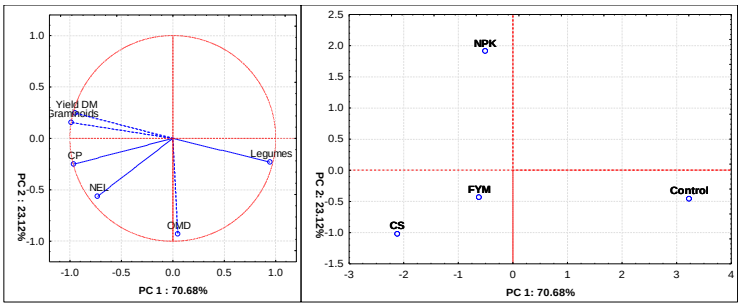

FIG. 1: Analysis of PCA parameters of yield characteristics, botanical composition and forage quality in 4-cut regime variants of PG management.

CONCLUSION

Results of multicriterial evaluation (multivariate analysis of PCA, FA) significantly differentiated between two categories in the evaluated parameters: (1) manure fertilization (FYM, CS): high dry matter production, high forage quality (CP, OMD); (2) mineral fertilization / NPK /: high dry matter production with lower forage quality.

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SILAGE MAIZE IN THE CZECH REPUBLIC

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The maize cultivation in the region of Czechia and Moravia was earliest mentioned in early nineteenth century while the former subtropical maize forms for long growing season were bred into earlier maize forms for colder regions including ours. Nevertheless, the maize breeding itself had not a big tradition in our region earlier. The Hungarian varieties of large-grain type and later early varieties from North America or Canada were used. As the hybridisation and using of inbred lines started here in mid-fifties of twentieth century the growing performance and yield started to increase.

Worthy to mention prof. Frimmel based in Mendelu in Lednice managed to breed the first hybrid here. Than maize breeding programs were running in breeding stations in Velké Pavlovice, Valtice, Čejč, Horní Moštěnice, Vyškov, Rašovice, Zlonice, Stupice a Lysá n. Labem. The first line hybrid „Lednicky rany" was approved in 1955. While specialization within breeding stations was requested the breeding station in Čejč began the only one focused on maize breeding in Czechia and Moravia in 1960.

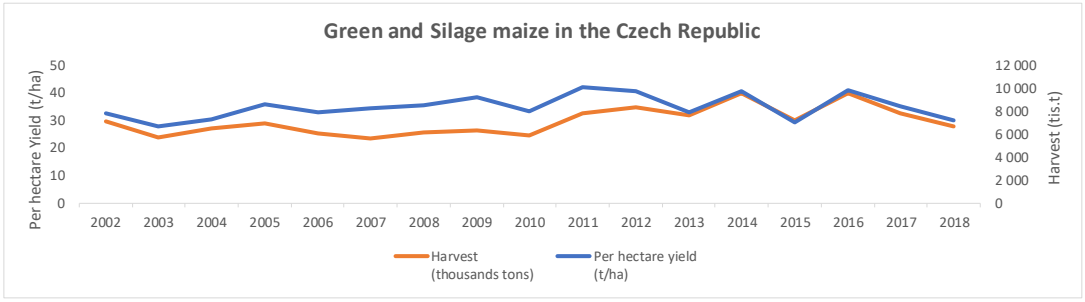
The successful breeding program, introduction of cytoplasmic pollen sterility with capability to restore fertility and also building of seed production facilities in Moravia led the former Czechoslovakia region was fully self-sufficing in maize seed production from mid-seventieth of last century.

Silage and grain maize hybrids also from abroad production started to be used widely by farmers after ninetieth of last century. Roughly average about 209.000 hectares were than used for silage maize sowing and production after 2000. The yield on dry matter is than changing in cycles based on yearly conditions and weather. Following table shows the history of cultivated area, harvested area, yield on dry matter per hectare and summary on yield dry matter.

Green and Silage maize in the Czech Republic

Year	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
Sowing area (thousands hectares)	219	215	216	211	191	180	180	180	182	198	215	219	236	231	242	225	224
Harvested area (thousands hectares)	n/a	n/a	n/a	n/a	n/a	n/a	n/a	166	179	186	205	234	237	245	234	223	224
Per hectare yield (t/ha)	32,39	27,55	30,26	35,69	32,66	34,41	35,33	38,15	33,04	41,79	40,6	32,66	40,37	29,1	40,7	34,8	29,84
Harvest (thousands tons)	7 083	5 707	6 462	6 870	6 066	5 570	6 144	6 333	5 902	7 782	8 328	7 635	9 578	7 134	9 545	7 777	6 687

Source: ČSÚ; MZe ČR



Company CEZEA – šlechtitelská stanice, a.s. (CEZEA – breeding station) based in Čejč, south Moravia, is the only one purely Czech company in Czech Republic whose main and only focus is a maize breeding and basic seed production of maize hybrids. Breeding a creation of new hybrids is based on traditional breeding methods. The breeding program includes process of making the initial genetic maize sources from who the new inbred lines are than bred. These new inbred lines where each inbred line population phenotype is stable, distinguishable and whose characteristics are fully observed, monitored and considered in next breeding phases are creating than the new base for new hybrid combinations. During the internal testing the new experimental maize hybrids are tested for grain yield as well as the selected ones also for silage usage where e.g. Dry Matter or Green (fresh) Matter yield, Dry Matter Ears, Plant size, etc. is fully considered.

Before selected and internally tested hybrid can be used widely it must go through registration process including two-years VCU (Value for Cultivation and Use) testing managed by national authority - Central Institute for Supervising and Testing in Agriculture (UKZUZ). These tests are necessary condition before the new hybrid is registered in National List of Varieties and National List & Plant Variety Rights Database and included also in list of registered hybrids within Europe Union. For farmers and other users of plant varieties, the National Listing is a guarantee of value for cultivation and use, the quality of the planting material, and also a guarantee that the variety is not dangerous to human health, animals, plants or the environment.

The other quality assurance testing of silage maize hybrids is managed by seed production company OSEVA a.s. based in Bzenec, south Moravia, which introduced a new brand for high-quality silage hybrids – Top Silage. The TOP silage brand is intended for the hybrids with the highest quality of silage matter that is known its high digestibility of fibre in the form of neutral detergent fibre (NDF). Cattle fed with maize silage with higher NDF digestibility will increase intake of dry matter, which leads to overall better cattle performance. These hybrids were monitored at minimum of three years for silage matter quality and only hybrids showing the best results of NDF digestibility and excellent yield and nutrition indicators were selected into brand. Currently there are ten selected silage maize hybrids in OSEVA a.s. portfolio with Top Silage brand, all of them bred in CEZEA breeding station in Čejč.

REFERENCE BY AUTHOR

CONTENT OF BIOGENIC AMINES AND COUNT OF MICROORGANISMS IN ALFALFA AND RED CLOVER SILAGES

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ABSTRACT

The aim was to compare the levels of biogenic amines in forage legumes, and also to compare the occurrence of microorganisms in silage and the influence of additives on the content of biogenic ammines in silage. The two varieties of *Medicago sativa* L. and one variety of *Trifolium pratense* L. were compared. Silage reaching up to 23 % of dry matter were evaluated and prepared using the bio-enzymatic additive and the chemical additive. The content of natural pollutants was mainly influenced by a mixture of organic acids. These silages had a tendency to reduce the risk of BA content.

Keywords: enterococci, tyramine, putrescine, cadaverine, spermine, biological additives, chemical additives

INTRODUCTION

The basis for biogenic amines (BA) formation is proteolysis, a naturally occurring process during ensilage comprising the enzymatic decarboxylation of amino acids by the action of plant proteases, peptidases and the enzymes of various Lac bacteria, clostridia and other genera (Scherer et al., 2015). Microorganisms of the genera *Clostridia*, *Bacillus*, *Klebsiella*, *Escherichia*, *Lactobacillus* or *Pediococcus* can also cause a deep decomposition of protein resulting in the formation of BA (Santos, 1996). The inoculation of *Lactobacillus casei* can lower the BA concentration, while the effects of *Lacobacillus buchneri* may vary considerably. The applied chemical additive reduced the level of amines in silages and *Enterobacteriaceae* counts (Selwet et al., 2013). The concentration of BA in silages mainly depends on the crop during harvest (Scherer et al., 2015). The risk of BA formation can be present when ensiling protein fodder, such as *Medicago sativa* L., especially when the appropriately dry matter cannot be provided, and the insufficiently wilted biomass is ensiled (Steidlova a Kalac, 2002). The aim of this work is to compare levels of BA in alfalfa and red clover fodder.

MATERIALS AND METHODS

Biomass was assessed from the first cut of alfalfa and red clover. The growths were harvested at the stage of deployment of flower buds. The crop was harvested in June. The species fodder was the first assessed factor with the levels, such as *Medicago sativa* L., with Holyna variety (1.1), *Medicago sativa* L., with Tereza variety (1.2) and *Trifolium pratense* L., with Amos variety (1.3). The second evaluated factor was the additive used in the production of silage (2) in the variants of untreated (2.1), treated with biological additives (2.2) and treated with a chemical additive (2.3). The biological silage additive contained bacteria of Lac fermentation, such as *Lactococcus lactis* (NCIMB 30117), *Lactobacillus plantarum* (DSM 16568), *Enterococcus faecium* (DSM 22502/NCIMB 11181) and enzyme xylanase EC 3.2.1.8. The composition of the chemical silage additive was in the ratio of 43 % of formic acid, 30 % of ammonium formate, 10 % of propionic acid and 2 % of benzoic acid. The part of biomass with dry matter content up to 23 % was preserved by the ensiling process. Representatives of fodder samples (6 kg) were filled into mini-silos using pneumatic press made of polyvinyl chloride and compacted with a pressure of 600 kg/m. At the end of the ensiling period (90 days), the silos were opened and samples were taken for the respective chemical analysis. Total number of microorganisms (TNM), Lac bacteria (lactic acid bacteria), bacteria of family *Enterobacteriaceae* and enterococci were evaluated. The results of analyses were expressed as colony forming units (CFU) per gram of silage. AAA 400 (Ingos, Prague, Czech Republic) IEC apparatus was used for the evaluation of BA content. The content of histamine (Him), putrescine (Put), cadaverine (Cad), spermidine (Spd), spermine (SPM) and the total content of BA were evaluated (Skladanka et al., 2017). The data were statistically processed using STATISTICA.CZ, version 10.0 (Czech Republic). Statistical significance was determined by examining the differences between groups using ANOVA and Scheffé's test.

RESULTS AN DISCUSSION

The use of mixtures of organic acids and their salts in the production of silage was reflected in a lower content of TNM, Lac bacteria, bacteria of the *Enterobacteriaceae* family (Table 1). Between the evaluated species, varieties respectively, no difference in the content of BA was detected (Table 2). However, in *Medicago sativa* L. especially in the Tereza variety there was evident a trend toward higher total content of BA, histamine, tyramine and cadaverine. Similarly, the effect of additives on BA content in produced silages was not possible to be confirmed but it was obvious that the use of mixtures of organic acids and their salts in the ensiling production aimed to reduce the total content of BA, tyramine, putrescine, cadaverine and spermidine, compared to control variant. The use of biological additive is reflected in an increase ($P < 0.05$) of Enterococcus counts, compared with the control variant and the variant treated with the mixtures of organic acids and their salts. This increase is not reflected in the content of BA. The use of biological additive practically did not affect the content of BA. Mainly the content of cadaverine in silages treated with chemical additive was decreased. Although, this reduction was not significant, detected tendency confirmed the fact that the chemical additives were more

suitable for preservation of biomass with low dry matter content rather than biological, with regard not only to the quality of the ensiling process but also the content of toxic products. The species was detected with lower counts of TNM, Lac bacteria, *Enterobacteriaceae* and especially enterococci. In the evaluated silages, tyramine, putrescine and spermine were mainly detected. Among the most harmful amines, histamine is included (Steidlova a Kalac, 2002). Our results indicated low histamine content in the evaluated silages. The highest content was found out in *Medicago sativa* L., especially in Tereza variety (16.5 mg/kg). Results showed the occurrence of histamine in alfalfa and red clover silage not only in untreated samples, but also in silages treated with chemical and biological additive. This fact could be given by a low content of dry matter of ensiling biomass.

Table 1. Total number of microorganisms (TNM), Lac bacteria, enterobacteria, enterococci in CFU/g in alfalfa and red clover silages

Factor	TNM	Lac bacteria	Enterobact.	Enterococcus
Species (S)				
Medicago sativa L. (Holyna)	$9{,}783 \times 10^{9}$	$6{,}027 \times 10^{8}$	$1{,}127 \times 10^{3}$	$1{,}714 \times 10^{4}$
Medicago sativa L. (Tereza)	$16{,}336 \times 10^{9}$	$11{,}804 \times 10^{8}$	$0{,}562 \times 10^{3}$	$2{,}152 \times 10^{4}$
Trifolium pratense L. (Amos)	$1{,}418 \times 10^{9}$	$0{,}237 \times 10^{8}$	$0{,}234 \times 10^{3}$	$1{,}725 \times 10^{4}$
p	0.5545	0.4495	0.4526	0.9386
Additives (P)				
Control	$22{,}096 \times 10^{9}$	$16{,}063 \times 10^{8}$	$0{,}422 \times 10^{3}$	$0{,}509 \times 10^{4\ a}$
Biological additive	$5{,}211 \times 10^{9}$	$1{,}829 \times 10^{8}$	$1{,}395 \times 10^{3}$	$5{,}015 \times 10^{4\ b}$
Organic acids	$0{,}231 \times 10^{9}$	$0{,}176 \times 10^{8}$	$0{,}106 \times 10^{3}$	$0{,}673 \times 10^{4\ a}$
p	0.2602	0.1722	0.1832	0.0023
SxP	0.5136	0.5935	0.9747	0.9915

Average values in the columns with superscripts (a,b) are significant at $P<0.05$.

Table 2. Content of histamine (Him), putrescine (Put), cadaverine (Cad), spermidine (Spd), spermine (Spm) and the total content of BA (mg/kg of dry matter) in alfalfa and red clover silages

Factor	Him	Put	Cad	Spd	Spm	BA
Species (S)						
Medicago sativa L. (Holyna)	4.5	241.0	21.7	2.5	219.5	722.7
Medicago sativa L. (Tereza)	16.5	211.0	71.5	1.8	204.8	965.8
Trifolium pratense L. (Amos)	9.0	356.8	50.8	3.0	225.5	872.2
p	0.2873	0.7087	0.3857	0.6857	0.8686	0.8045
Additives (P)						
Control	7.5	410.5	80.2	2.8	197.7	1174.8
Biological additive	12.5	282.7	63.7	3.0	225.8	1008.2
Organic acids	9.0	115.7	0.17	1.5	226.3	377.7
p	0.7882	0.3145	0.0992	0.4885	0.7200	0.1269
SxP	0.5617	0.4641	0.7238	0.4930	0.5826	0.5572

CONCLUSIONS

The Lac bacteria and enterococci were recorded in a higher numbers in produced silages. The content of natural pollutants was mainly influenced by a mixture of organic acids. Silages, treated with a chemical additive, a tendency to reduce the risk of BA content was obvious. Based on the results, it can be stated chemical additives (mixture of organic acids) are convenient to be used within ensiling process of the insufficiently wilted fodder of *Medicago sativa* L. and *Trifolium pretense* L.

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EFFECT OF MINIMIZED TILLING OF GRASSLAND ON MYCOTOXIN CONTAMINATION OF FRESH AND ENSILAGED FORAGES.

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INTRODUCTION

Increased milk production of dairy cows puts higher demands on the quality and biosafety of feeds. More attention has been paid to the optimal harvest time of forages and the fermentation quality of silages, but often mycotoxin-contaminated feeds might be a reason for the early culling of cows. Mycotoxins can be found in a wide variety of crops and the toxic effect of feed-associated mycotoxins in cattle also vary. Mycotoxins can reduce feed intakes and productivity, cause reproductive problems, immunosuppression and even death to the animals. In plant production, ploughing, minimum tillage or a no-till technique are used for soil cultivation, which may have effects on feed contamination with moulds and mycotoxins. Moulds can colonise forages and produce several mycotoxins in the field pre-harvest stage, during storage, or after ensiling during feed-out (Ogunade et al., 2018). Mould growth in suitable conditions is not necessary for mycotoxin formation. Moulds normally grow between 10 and 40 ºC, over a pH range of 4-8 and at a water activity level above 0.70, but toxin production usually occurs when moulds are stressed. Delayed harvesting, slow or delayed filling of the silo, insufficient compaction or sealing of the silo, air ingress due to damaged plastic cover and slow feed-out rates favour mould activity and mycotoxin production (Whitlow and Hagler, 2005). Soil cultivation techniques, physical damage to plants, rainfall and temperature extremes also influence mould growth and mycotoxin production. In northern countries, where there is a short vegetation period and overnight temperatures may fluctuate between warm days and cool nights, zearalenone (ZEA) and deoxynivalenol (DON) produced by *Fusarium* spp. are the most commonly encountered field-related mycotoxins. There are therefore several factors which can affect mould growth and mycotoxin production in forages. The objective of this study was to determine how mycotoxin contamination of forage is affected by minimized tilling of grassland and how it affects mycotoxin contents in silage.

MATERIALS AND METHODS

Permanent grasslands were planted under a conventional cropping system from one to four years before observation. A minimum tillage technique was used for soil cultivation. The same seed mixture (timothy, perennial ryegrass, meadow-grass and red clover) was sown on all of the investigated grasslands. Fresh grass samples were taken from first-, second-, third- and fourth year grasslands that had been cut, wilted, chopped and harvested by loader wagon, but before compaction in the silo. Samples were collected as one bulked sample from each 10 hectares per grassland. Silage was made in the bunker silo from the same fields as the collected grass forage samples. After 90 days of fermentation silage samples were collected using a 1.5m silage drill. Fresh grass and silage samples were frozen at -20 ºC and stored until analysed. All samples were analysed for the mycotoxins ZEA and DON by the ELISA (Enzyme-Linked Immunosorbent Assay) method (R-Biopharm AG test kits). Silage samples were analysed for chemical composition and pH. Ethanol and acids produced during fermentation were determined by gas chromatography.

RESULTS AND DISCUSSION

Considering that grass samples were taken from the silo after the pre-harvest stage, but before compaction, the mycotoxin contents were low in all grass samples (figure 1). On average, the ZEA concentrations were between 28-38 ppb and DON were 20-57 ppb per kg of fresh matter (FM). We did not find that minimized tillage had a negative effect on forage mycotoxin contamination. There was also no clear trend for mycotoxin content in the grasses when year of use of grassland increased. However, the concentration of mycotoxins in the silage increased considerably (table 1). ZEA and DON are produced by field fungi such as *Fusarium* spp (Driehuis, 2012), which do not grow at low pH nor in anaerobic conditions. However, all these silages had ideal fermentation parameters. The silage would not have such fermentation characteristics if there were air leakage into the silo. If the ZEA and DON are not storage-related mycotoxins, then one explanation is that they formed between filling, compaction and sealing of the silo.

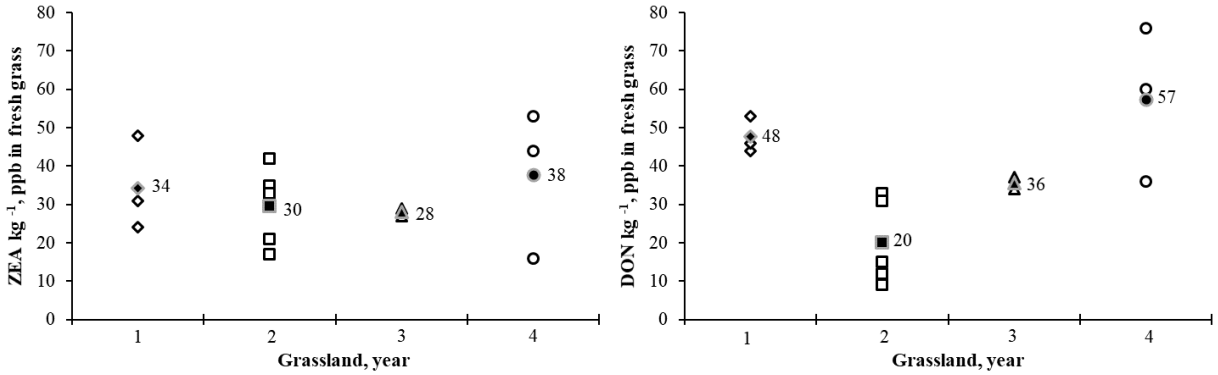

Figure 1. Mycotoxin concentration in fresh grass harvested from 1- 4-year grasslands. Empty symbols indicate the mycotoxin content of single grass samples per grassland, filled symbols with number are mean values.

Table 1. Quality parameters of silage samples**.**

Item	Silage			
	1	**2**	**3**	**4**
Dry matter, g/kg	318	318	255	268
Crude protein, g/kg in DM	135	143	140	136
Crude ash, g/kg/ in DM	72	73	80	67
Crude fibre, g/kg in DM	284	277	252	287
Ethanol, g/kg in DM	10	11	5	4
Acetic acid, g/kg in DM	24	19	21	20
Propionic acid, g/kg in DM	0	0	0	0
Butyric acid, g/kg in DM	0	0	0	0
Lactic acid, g/kg in DM	71	72	95	80
Total acids, g/kg in DM	95	91	116	100
pH	4.1	4.1	3.9	3.9
NH_3-N/total N, g/kg in FM	34	31	32	32
ZEA kg^{-1}, ppb in FM	343	370	412	466
DON kg^{-1}, ppb in FM	449	590	809	479

CONCLUSIONS

Mycotoxin concentrations were low in fresh forages harvested from minimized tilling grasslands and were unaffected by harvest year. However, during the storage stage, mycotoxin contents in silage increased, which suggests that the harvested forages were already contaminated with moulds. The rapid and efficient forage compaction and sealing of the silo accelerates fermentation and the pH drop may inhibit mould activity and reduce ZEA and DON production in the silo.

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ESTIMATION OF MYCOTOXIN CONTAMINATION OF HERBAGE IN MOUNTAIN LOCALITY EXPOSED TO RENEWED CATTLE GRAZING

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INTRODUCTION

Good health and production of farm animals depends on the feed quality. Green forage on pasture is exposed to many risk factors, which can have negative influences. One of the most important risks is the feed contamination by mycotoxins (Stryk and Křížová, 2013; Horký *et al*., 2014). Mycotoxins are the toxic secondary metabolites produced by organisms of the fungi kingdom, commonly known as moulds. Among these the moulds of the genus *Fusarium* are the most important under the central European agricultural conditions (Seeling and Dänicke, 2005). The most important *Fusarium* toxins from the point of view of animal health and productivity are deoxynivalenol (DON) and zearalenone (ZEA) (Fink-Gremmels, 2008). As Cheeke (1995) referred knowledge of various aspects of these toxins and toxicoses are necessary for optimal management and utilization of forage grasses. The aim of the study was to estimate the forage quality (including the content of *Fusarium* mycotoxins DON, T-2/HT-2 toxin and ZEA) in the locality of Švýcárna (1 304 m a.s.l., the Praděd National Natural Reserve), where the cattle grazing after the long-term management cessation was introduced in 2012.

MATERIAL AND METHODS

Our research was conducted in the mountain locality in surroundings of the Švýcárna lodge situated in the Hrubý Jeseník Mts. (1 304 m a.s.l.). In Praděd (1 491 m a.s.l., cca 2.5 km distant from the Švýcárna lodge) there is average annual temperature 0.9°C and annual precipitation 1 231 mm. From 2012, the rotational grazing system was conducted in the locality, which was divided by the road into two grazing plots differing in dominant grass species. The plot P1 (Nar) with dominance of *Nardus stricta* was situated above the lodge while the plot P2 (Des) with dominance of *Deschampsia cespitosa* was situated below the lodge. The whole plot area was 3.6 ha. The stocking rate was up to 1 livestock unit per ha and year. On average, there was found the extremely acid soil reaction (pH = 3.7) and simultaneously the low content of calcium (232.8 $mg.kg^{-1}$), phosphorus (10.0 $mg.kg^{-1}$) and magnesium (69.8 $mg.kg^{-1}$) in the soil. Five permanent plots (one plot area: 5 m x 5 m) were established in 2013 in different places of the locality (two in the sublocality P1 and three in the sublocality P2). From the plots there were conducted samplings of the forage in four dates (June, July, August, and September) during 2014. By the method of Weende analysis there was determined the content of crude protein (CP), ether extract (EE), crude fibre (CF) and ash (A) in $g.kg^{-1}$ DM (DM = dry matter). The content of nitrogen free extract (NFE) was calculated according to the equation: NFE = 1000 – (CP + EE + CF + A).

There was further estimated the content of macroelements (Ca, Mg, P, K) in $g.kg^{-1}$ DM and some microelements (Zn, Pb, Cd) in $mg.kg^{-1}$ DM. A competitive enzyme-linked immunosorbent assay (ELISA) was used to determine the presence of fusarium mycotoxins deoxynivalenol (DON), zearalenone (ZEA) and T-2/ HT-2 toxin in samples using the NEO 9303 Stat – Fax Reader 4700 apparatus and commercially available quantitative ELISA assay kits Veratox (both Neogen Corp., Lansing, MI, USA) according to the manufacturer's instructions. The two-factorial analysis of variance (with sublocality as factor A and date of harvest as factor B) and the Tukey test ($P < 0.05$) were used for statistical computations.

RESULTS AND DISCUSSION

Table I shows results of estimated qualitative parameters, including the statistics. The swards situated above the lodge (P1 Nar) differed significantly ($P < 0.05$) from the swards situated below the lodge (P2 Des) only in the content of ash, Ca and K. As for the content of crude fibre, it was on the level of 299.4 g/kg DM and 292.2 g/kg DM for the swards above (P1 Nar) and below (P2 Des) the lodge, respectively. The significant factor influencing the mean nutritive value of the swards in the locality was the date of harvest. In the course of the growing season the concentration of crude fibre significantly increased from 242.3 g/kg DM in June to 327.2 g/kg DM in September ($P < 0.05$). The concentration of crude protein simultaneously decreased from 172.3 g/kg DM in June to 98.4 g/kg in September ($P < 0.05$).

All samples analyzed in our study were positive for the estimated mycotoxines. Based on the month of sampling concentration of DON increased from 572.5 µg/kg DM in June to its maximum value of 792.5 µg/kg DM in July and then gradually decreased to 637.5 µg/kg DM in September. Similar trend in changes in DON concentrations in dependence on sampling terms was mentioned also by Skládanka *et al.* (2011). Mean concentration of T-2/HT-2 toxin was 44.88 and 55.04 µg/kg DM in P1 (Nar) and P2 (Des), respectively. Our values are close to lower values reported by Engels and Krämer (1996) who determined wide range of T-2 toxin concentrations (from 40 to 2 780 µg/kg) in several varieties of *Lolium* grasses. On the other hand, Ramirez *et al*. (2014) mentioned about 10 times higher concentration of T-2/HT-2 toxins in Argentinean pastures than those measured in our study. The effect of the term of sampling on T-2/HT-2 toxin content was not clear. In our study, the highest values of ZEA were found in July (97.82 µg/kg DM), and then from July till September, content of

ZEA gradually decreased in both groups. This is in agreement with Arslan and Essiz (2009) or Skládanka *et al*. (2011).

Tab. I Nutrient contents (in dry matter) in the forage sampled in surroundings of the Švýcárna lodge in 2014.

	Ash [g/kg]	CF [g/kg]	CP [g/kg]	EE [g/kg]	NFE [g/kg]	P [g/kg]	Mg [g/kg]	Ca [g/kg]	K [g/kg]	DON [µg/kg]	T-2/HT-2 toxin [µg/kg]	ZEA [µg/kg]
Sublocality (S)												
P1 (Nar)	43.26^{a}	299.4	116.4	38.83	502.0	1.588	0.949	1.594^{a}	9.70^{a}	715,0	44.88	54.65
P2 (Des)	68.23^{b}	292.2	121.5	39.62	478.5	1.506	1.171	3.716^{b}	14.03^{b}	620.6	55.04	78.72
Significance	0.004	0.451	0.344	0.936	0.055	0.597	0.090	0.005	<0.001	0.397	0.824	0.229
Harvest date (HD)												
June	60.12	242.3^{a}	172.3^{a}	86.68^{a}	438.6^{a}	2.049a	1.064	1.674	16.14^{a}	572.5^{a}	52.42	64.50^{a}
July	46.42	292.7^{b}	104.5^{b}	35.46^{b}	520.9^{b}	1.474^{b}	1.121	3.144	12.42^{b}	792.5^{b}	47.61	97.82^{b}
August	51.80	318.2^{bc}	102.5^{b}	17.85^{b}	509.6^{cb}	1.273^{b}	1.068	3.482	11.95^{b}	668.7^{c}	43.86	64.95^{a}
September	74.64	327.2^{c}	98.4^{b}	17.25^{b}	482.5^{c}	1.360^{b}	1.075	3.169	8.678^{c}	637.5^{c}	55.95	39.47^{c}
Significance	0.100	<0.001	<0.001	<0.001	<0.001	0.012	0.975	0.298	<0.001	0.025	0.865	<0.001

$^{a, b, c}$ Mean values in the same column with different superscripts differ significantly ($P < 0.05$).

CONCLUSION

Fusarium mycotoxins deoxynivalenol (DON), T-2/HT-2 toxin and zearalenone (ZEA) were present in all samples of pasture. While the effect of the term of sampling on T-2/HT-2 toxin content was not clear, concentration of ZEA in both groups increased from June to July, and then gradually decreased from July till September. In July, the highest mean concentrations of DON were noted, as well. Data published in this paper are important for the proper pasture management, for adequate cattle nutrition and for the following evaluation of changes in connection with the renewed cattle grazing. The issue of moulds and, thus, contamination with mycotoxins is very topical, particularly in connection with forages from grass stands used at the end of the growing season.

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CONTENT OF MYCOTOXINS IN GREEN MATTER AND SILAGE OF RED CLOVER AND ALFALFA

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ABSTRACT

The aim was to compare the mycotoxins in forage legumes, and also to compare the occurrence of mycotoxins in green fodder and subsequently produced silage and the influence of additives on the content of mycotoxins in silage. The two varieties of *Medicago sativa* L. and one variety of *Trifolium pratense* L. were compared. Green fodder and subsequently produced silage reaching up to 23 % of dry matter were evaluated and prepared using the bio-enzymatic additive and the chemical additive. Fungi were present in higher counts in anaerobic environment of green fodder and contaminating it with zearalenone and deoxynivalenol. However, lower counts of fungi were found out in silage, the zearalenone content was not changed. Lower content of deoxynivalenol was detected in silage, compared with green fodder.

Keywords: green matter, silage, enterococci, fungi, butyric acid, tyramine, putrescine, cadaverine, spermine, zearalenone, deoxynivalenol

INTRODUCTION

The production of fodder is also associated with the risk of mycotoxin occurrence that can also enter the food chain (Reverberi et al., 2010). Mycotoxins are dangerous metabolites that are often carcinogenic, and represent a serious threat to both animal and human health (Gravesen et al., 1994). Contamination of food and feed occurs in the field before harvest or during storage, despite the most strenuous efforts of prevention (Vasatkova et al., 2010). Deoxynivalenol (DON), zearalenone (ZEA) are ranked among the most frequently encountered mycotoxins (Dohnal et al., 2008). Trichothecenes, such as deoxinivalenol or nivalenol, are very large family of chemically related sesquiterpenic mycotoxins produced by various species of *Fusarium* (Driehuis et al., 2000). The aim of this work is to compare the occurrence of microorganisms and the levels of mycotoxins in green fodder and subsequently produced silages.

MATERIALS AND METHODS

The experimental plot was located in Troubsko and Vatín, the Czech Republic. Biomass was assessed from the first cut of alfalfa and red clover (*Medicago sativa* L., with Holyna variety, *Medicago sativa* L., with Tereza variety and *Trifolium pratense* L., with Amos variety). The growths were harvested at the stage of deployment of flower buds (butonization) in June. Immediately after harvest, the samples of green fodder were taken. The part of biomass with dry matter content up to 23 % was preserved by the ensiling process. Representatives of fodder samples (6 kg) were filled into mini-silos using pneumatic press made of polyvinyl chloride and compacted with a pressure of 600 kg/m. The filled silos (three repetitions per treatment) were sealed with a lid, stored in a room without direct light exposure at room temperature of 28 °C and stored for 90 days. At the end of the ensiling period (90 days), the silos were opened and samples were taken for analysis. Green fodder samples and silages were dried at 60 °C, grounded to a particle size of <1 mm, then analyzed for the content of the mycotoxins, such as deoxynivalenol (DON), zearalenone (ZEA) using the enzyme-linked immunosorbent assay (ELISA, Litolab, spol. s.r.o., (Czech Republic). For the determination of fungi, Chloramphenicol Glucose Agar (Biokar Diagnostics, France) was used and the incubation lasted 120 h at 25 °C. The data were statistically processed using STATISTICA.CZ, version 10.0 (Czech Republic). Box plots of multiple variables were used for graphical representation. Differences with $P < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Green matter was more contaminated by fungi then silage. Green fodder had already been contaminated by mycotoxins before ensilage (Figure 1). The occurrence of deoxynivalenol (DON) and zearalenone (ZEA) was confirmed. ZEA content ranged from 23.45 to 140.87 ppb in green fodder before silage. Similar ZEA content as in green fodder was also found out in silage, where reached values from 41.18 to 107.23 ppb. DON content ranged from 0.47 to 1.24 ppm in green fodder. In produced silages, the DON content decreased to 0.10 and then up to 0.35 ppb. The significant difference in the contents of DON and ZEA was not detected between the evaluated species. Fungi in alfalfa and red clover silage were detected in the order of magnitude 102 – 103 CFU/g. Scudamore and Livesey (1998) reported that the occurrence of fungi in higher counts than 104 CFU/g can induce animal health problems. Alonso et al. (2013) stated the occurrence of fungi is related to the lack of hygienic quality of silage leading to loss of nutrients and dry matter. Mycotoxins entered silage directly in the field. The difference in the content of mycotoxins between green fodder and silage was not observed. In the case of DON, a reduction of mycotoxin was apparent. Ensilaging can influence count of other microorganisms. The use of silage additives also has an effect (Skladanka et al., 2017).

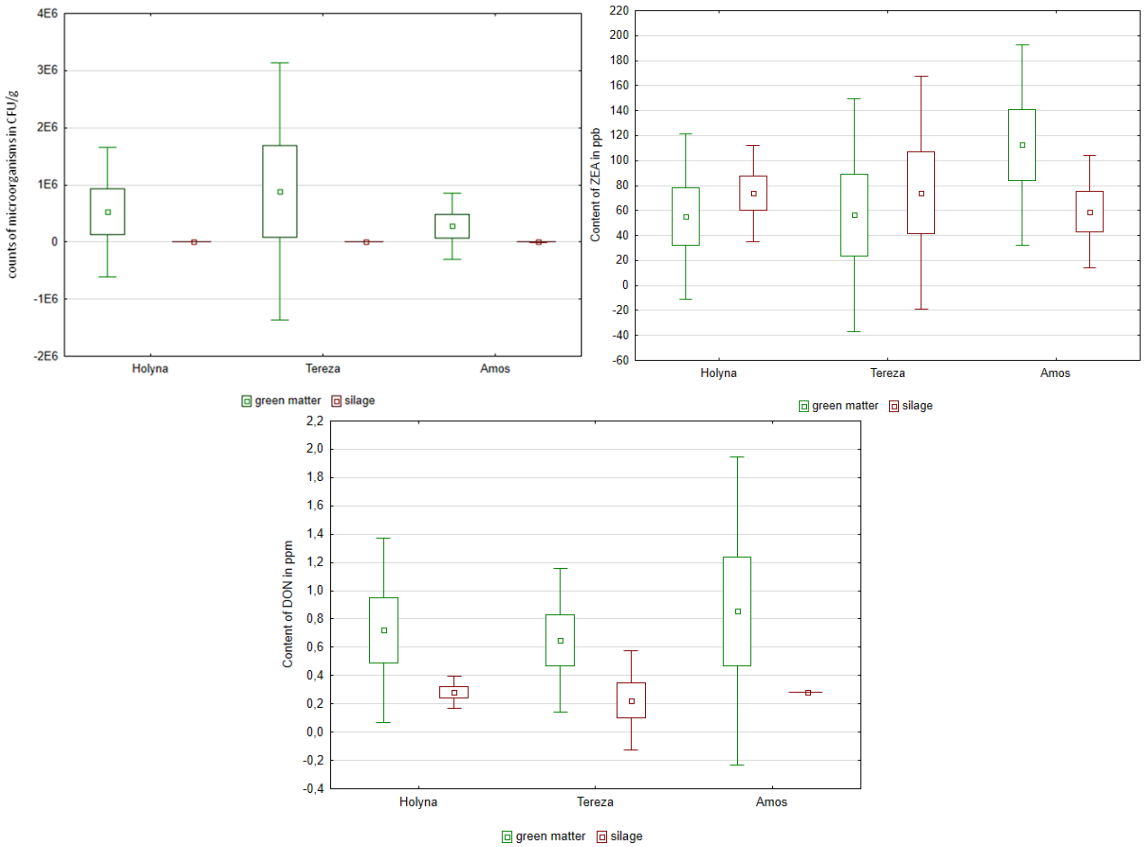

Figure 1. Comparison of fungi, content of zearalenone (ZEA) and deoxynivalenol (DON) in green mass and in silages of alfalfa and red clover.

CONCLUSIONS

Fungi were determined in higher quantities in an aerobic environment of green fodder. The counts of fungi were in silage detected lower, zearalenone content remained the same. In comparison with the green fodder, the content of deoxynivalenol decreased in silages. The contamination of mycotoxins was proven to originate from the field. The zearalenone content remained the same in the produced silage. On the other hand, the content of deoxynivalenol was reduced by the ensiling process.

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THE EFFECT OF HARVEST TIME AND WILTING ON QUALITY OF LUPINE SILAGES

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ABSTRACT

The aim of this study was to evaluate the effect of different harvest time and wilting on quality of lupine silages. Forage from *Lupinus albus* (cv. Amiga) was harvested in four different times. Forage was divided into two parts where one part was ensiled immediately and second part after wilting. Silages were stored for ten weeks and after opening analysed on chemical composition, quality of fermentation process, organic matter digestibility and dry matter losses. Younger forage had significantly lower dry matter content and fiber fractions in comparison with older ones. Digestibility of organic matter was affected by wilting. The highest effect of wilting was detected for dry matter losses, where differences among wilted and unwilted silages were since 4.5 to 23.5 %.

INTRODUCTION

Legume pulses such as field peas, faba beans and lupins are annual crops that are well suited for short crop rotations when harvested as ensiled forage (Borreani et al., 2007). Under organic conditions and on suitable soils, lupins offer an opportunity for the production of high protein forage, whilst at the same time providing some fertility in the form of nitrogen fixation (Hall et al., 2003). These short-term catch-crops harvested as silage produced a crude protein yield per hectare that should be considered rather high, because it represents around 0.35-0.40 of CP yield which is produced yearly by lucerne, stand in the same environment (Tabacco et al., 2002). The high moisture content at cutting makes these crops unsuitable for direct ensiling and they thus require a wilting period, in order to avoid poor fermentation and effluent production. In addition, wilting benefits the fermentation, as a higher DM affects the growth of desirable microorganisms and the rate of fermentation (McDonald et al., 1991).

The aim of this study was to evaluate the effect of different harvest time and wilting on quality of white lupine silages.

MATERIALS AND METHODS

White lupine (variety Amiga) was grown on field in Central Bohemia. Whole crop silages were made from forage harvested from three places of field. One part of forage was ensiled immediately after harvest and second part was wilted for 24 hours. Forage was two times mixed during wilting. Forage was cut for 2 cm before ensiling. Chopped forage was ensiled into plastic bag, air was sucked off and bags were hermetically closed. Silages were stored for 10 weeks. Then silages were weighted, dried and milled through 1 mm sieve. Samples were analysed for dry matter (DM), ash, ether extract (EE), crude protein (CP), crude fibre (CF) and neutral detergent fibre (NDF) (AOAC, 2005) content. Organic matter digestibility (OMD) was detected using *in vitro* pepsin cellulase solubility method with incubating of samples in Daisy Incubator (ANKOM Technology, Macedon, NY, USA). Results were evaluated using GLM procedure of SAS, where effects of harvest time, wilting and their interaction were used as fixed effect and repetition was random effect.

RESULTS AND DISCUSSION

Chemical composition, organic matter digestibility and parameters describing fermentation process of tested silages are presented in Table 1. As expected, silages made without wilting had lower dry matter content (112 to 251 g/kg) in comparison with wilted ones (240 to 356 g/kg). Borreani et al. (2009) didn´t find differences between wilted and unwilted lupine silages (110 vs 136 g/kg DM), but they had really short time of wilting (6 hours). Tested silages had the CP content within the range of 178 to 212 g/kg; the NDF content from 413 to 516 g/kg and OMD from 65 to 68 %. The wilting increased DM and OM contents and OMD. Differences among harvest times were detected at OM, EE, CF and NDF contents. The CP contents did not differ among harvests; however the highest value was observed in second harvest (203 g/kg). Increase in DM yield coupled with a limited decline of CP with maturity, make legumes more suitable for ensiling at advanced stages of maturity as reported by Cavallarin et al. (2006) for field pea. Mustafa et al. (2000) reported that whole pea silages, harvested at full pod stage, can successfully replace barley or alfalfa silage as a forage source for high yielding cows in early lactation. The CF and NDF contents increased during maturing of lupine. The organic matter digestibility did not significantly differ among harvests.

Some parameters of fermentative process were affected by wilting and also by maturing. All the silages showed a noticeable fermentation activity that was reflected in a high production of volatile fatty acids, as observed by Fraser et al. (2005) on white lupin silage. There were not any differences for pH and ammonia nitrogen values and acidity of water leach. Silages made without wilting had higher content of acids and mainly DM losses (21.96 vs 7.39 %). The wilting influenced only the amount of lactic acid in lupine silages in experiment of Borreani et al. (2009). The last harvest had the lowest acids content and DM losses. Butyric acid was not detected at any silage. High amount of this acid is normally associated with undesirable clostridial fermentations that usually occur when the DM content is lower than 300 g/kg (McDonald et al., 1991).

Table 1. The effect of harvest time and wilting on nutrition values and fermentation process of lupine

Harvest	**1**		**2**		**3**		**4**			**P**		
Wilting	**UW**	**WT**	**UW**	**WT**	**UW**	**WT**	**UW**	**WT**	**SEM**	**H**	**W**	**H*W**
Chemical composition												
Dry matter[1]	112^{e}	356^{a}	123^{de}	240^{c}	135^{d}	292^{b}	251^{c}	354^{a}	3.8	**	**	**
OM[2]	918^{d}	933^{ab}	920^{cd}	923^{cd}	920^{cd}	928^{bc}	937^{a}	935^{ab}	0.5	**	**	**
CP[2]	195	178	194	212	187	191	191	187	23.3	NS	NS	NS
EE[2]	13.4^{c}	11.6^{c}	14.5^{c}	16.7^{c}	16.9^{bc}	17.4^{bc}	31.8^{a}	28.0^{ab}	1.0	**	NS	*
CF[2]	315^{a}	287^{b}	366^{a}	353^{a}	379^{a}	357^{a}	354^{a}	360^{a}	32.8	**	NS	*
NDF[2]	424^{ab}	413^{b}	498^{ab}	469^{ab}	503^{ab}	490^{ab}	503^{ab}	516^{a}	66.3	**	NS	*
OMD[1]	66.1	68.1	65.9	67.3	65.0	66.3	66.3	65.8	8.3	NS	*	NS
Process of fermentation												
pH	4.87	4.98	4.82	4.86	4.94	5.07	4.86	4.79	0.01	NS	NS	NS
Lactic acid[2]	44.1^{a}	18.6^{c}	41.3^{ab}	23.0^{bc}	33.2^{abc}	17.6^{c}	17.1^{c}	21.8^{bc}	3.3	**	**	**
Acetic acid[2]	39.1^{a}	10.1^{d}	39.2^{a}	18.6^{bcd}	27.4^{b}	15.7^{cd}	24.3^{bc}	14.9^{d}	0.7	**	**	**
NH_3-N[3]	92.2	101.7	89.5	85.3	91.5	92.6	90.9	93.5	6.4	NS	NS	NS
KVV[4]	653	753	692	594	673	624	663	692	67.5	NS	NS	NS
DM losses[2]	26.3^{ab}	2.81^{de}	23.8^{b}	16.3^{c}	31.4^{a}	8.70^{d}	6.31^{de}	1.81^{e}	2.98	**	**	*

SEM – standard error of mean; ** $P < 0.01$; * $P < 0.05$; NS - nonsignificant - $P > 0.05$; SEM – standard error of mean; W – wilting; UW – unwilted; WT – wilted; H – harvest; Harvest 1 – pods and seeds light green, full seeds with green content; 2 – leaves begin get yellow, pods light green, full seeds with white husk; 3 – half of leaves yellow, full seeds with green husk; 4 – plants without leaves, half of pods get dry and seeds in wax ripeness. [1] g/kg; [2] g/kg dry matter; [3] g ammonia nitrogen per kg of total nitrogen; [4] Acidity of water leach - mg KOH/100g sil. [a, b, c, d] Means within rows with different superscript letters indicate a significant difference at $P < 0.05$ (Effect H*W).

CONCLUSIONS

Presented experiment proved that white lupine (variety Amiga) could be used for silage production with high amount of CP. Lupine forage had slow increase of CF and NDF contents without rapidly decrease of CP which provide more time for silage making. Results show also necessity to wilt whole crop chopped forage for decreasing of ensiling losses.

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THE POTENTIAL OF SORGHUM CULTIVAR "RUZROK" FOR PRODUCTION OF BIOMASS IN THE CZECH REPUBLIC

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ABSTRACT

"Ruzrok" is a multifunctional low-cost sorghum cultivar crop with a high potential for growing in relatively warmer and drier regions in Czechia (Czech Republic) due to its high tolerance to drought as a C4 crop. It can be grown in both conventional and ecological agricultural systems due to its minimum requirements on chemical protection.

Phytosanitary effects of sorghum - young sorghum plants synthesize cyanogenic glycoside durrhin which is released into the soil where it is decomposed to hydrogen cyanide. Hydrogen cyanide reveals fumigation effects on several plant pathogens such as fungal pathogens and nematodes. Sorghum cultivar "Ruzrok" thus can be utilized also as a phytosanitary intercrop in those crop rotations where crops such as sugar beet, potatoes and some vegetables (cabbage, garlic) susceptible to these pathogens are grown. Sorghum biomass has to be cut up, mulched and ploughed into the soil where hydrogen cyanide is released from cut up sorghum shoots (leaves).

Practical experience with sorghum cultivar "Ruzrok"

(a) SEED SERVICE is a company aimed at multiplication of seeds of forage and pulse crops, grasses, and other technical crops. The company also multiplicates the only Czech cultivar "Ruzrok". The advantages of cultivar "Ruzrok" with respect to growing conditions in Czechia (Czech Republic): (1) Fast growing rate and earliness in heading enable sowing of "Ruzrok" in later sowing terms to avoid spring frosts; (2) "Ruzrok" seems to be relatively more tolerant to low temperatures with respect to foreign sorghum cultivars; (3) Growth period of "Ruzrok" is relatively short thus unlike foreign sorghum cultivars, "Ruzrok" can provide stable grain yield in Czechia; (4)Thousand grain weight (TGW) of "Ruzrok" is relatively low (17 g) which is convenient for its utilization as a part of mixtures with other forage crops; (5) The plants of "Ruzrok" are relatively subtle and have relatively low number of tillers (1-3) which has a positive effect on good quality of foyer; (6) „Ruzrok" reveals a significant phytosanitary effect due to the presence of cyanogenic glycoside durrhin in young leaves and which is released from cut up plant biomass into soil.

b) AGROSPOL Knínice is the agricultural company focused on the crop and livestock production, operating in the area of Boskovice furrow (the Malá Haná region). In June 2019, an operating experiment with sorghum cultivar "RUZROK" was established. The experimental area covers approximately 20 ha and the preceding crop was Lucerne (Medicago sativa L.). The experimental site characteristics are: latitude 400 m a.s.l., the average annual temperature and precipitation is 8.6°C and 545 mm, respectively (Czech Hydrometeorological Institute, Jevíčko weather station). The soil type is modal Cambisols. To evaluate the yielding parameters, the forage quality (quality of grains), the root biomass production, and the basic soil physic-chemical properties, such as pH, soil organic matter content, nutrient content, and physical properties, we established 3 different cropping technologies, each technology covering 1 ha: (1) 1st cut – forage, 2nd cut – forage; (2) 1st cut – forage, 2nd cut – grain + straw; (3) no forage, only grain, and straw production.

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Section 2: Fermentation process of silages – harvesting, additives, conservation, stability and storage

MODULATION OF BACTERIAL COMMUNITY AND METABOLOME IN SILAGE BY INOCULATION HOMO- OR HETEROFERMENTERS

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INTRODUCTION

The global consumption of products derived from ruminants has been rising remarkably in the past two decades. Specialists predicted that about half of global ruminant meat and two thirds of global milk demand will come from developing countries by 2050, especially China and India (Rosegrant, 2009; Gerber, 2013). Silage is a kind of superior roughage to supply energy and indispensable fibre, which is storing green forages under anaerobic conditions with minimize loss of nutrients from harvest through storage (Mahanna and Chase, 2003; Grant and Ferraretto, 2018). Ensiled forage, especially corn silage, is an important composition of ruminant diet, particularly dairy cow worldwide (Ogunade et al., 2018). The dairy sector is growing fast. In 2016, the FAO predicted that world milk production is projected to increase by 177 million tonnes by 2025, at an average growth rate of 1.8% per annum in the next 10 years. There are about 133 million holdings keeping dairy cattle worldwide (Goals, 2013). That is to say, at least 665 million tonnes silage will be consumed per annum (except for silage consumptions of other ruminants). Corn silage is an important source of ensiled forage, it makes up over 40% of forage fed to dairy cows. Hence, high quality and safe silage is a crucial foundation and guarantee for developing ruminant husbandry to keep high yield and quality ruminant products.

The microbial population play various roles in ensiling (Pahlow, 1991), especially lactic acid bacteria (LAB). Advanced molecular biological techniques have been used to help understanding the complex process in structure of microbial community and succession (Parvin and Nishino, 2009; Pang et al., 2011) , which may reveal micro-ecology during ensiling. However, to the best of our knowledge, no study has been reported investigate the population dynamics of whole crop corn silage during ensiling at species level with PacBio single molecule, in conjunction with real-time sequencing technology (SMRT). The biochemistry of ensiling is complex because the interactions among plant enzymes and the activities of microbial species involved (Dwayne et al., 2003). Much of metabolites were produced during ensiling, and the metabolites also affect the growth and interaction of these microorganisms (Pahlow, 1991; Broberg et al., 2007). However, metabolites were conventionally investigated to assess the fermentation quality of ensiled forage, very few studies focused on animal health and welfare. To a great extent, the quality of silage depends on the microbial communities and their successions as well as the fermentative metabolites during ensiling. Therefore, improving understanding of silage fermentation from aspects of microbiome and metabolome will provide a scientific direction for making high-quality silages and making silages with components are good for ruminant health and welfare.

With additives, LAB strains are possible to modulate the structure of microbial community dynamics, end products during ensiling as well. Inoculants are divided into homofermentative and heterofermentatvie cultures based on the fermentation pattern. To our best knowledge, however, how the homofermentative or heterofermentative LAB affect the bacterial community and metabolites dynamics in whole crop corn silage is unclear. Therefore, homofermentative *Lactobacillus plantarum* and heterofermentative *Lactobacillus buchner* inoculants induce multimodal responses in microbiome and metabolome dynamics of the whole crop corn silage were profiled in the present study.

MATERIALS AND METHODS

Ensiling of corn samples. The whole crop corn (*Zea mays* L.) was manually harvested at the stage of half milk-line. *Lactobacillus plantarum* and *L. buchneri* were each added at 1×10^6 cfu/g FM. The application rate of each inoculant into the fresh forage was, and an equal volume of distilled water was sprayed onto the fresh corn for the control group. The effects of LAB inoculation and storage period were evaluated. The silos were then stored at ambient temperature (22-25°C) in dark conditions and sampled at 3, 7, 14, 45 and 90 days for fermentation. Silos were made in triplicate and stored at ambient temperature.

Microbial composition SMRT analysis. The DNA extraction of fresh and each treatment with a DNA isolation kit (Tiangen, DP302-02, Tiangen, China) according to the protocol instructions. The PCR amplification of the full-length 16S rRNA gene for SMRT sequencing was carried out with the forward primer 27F (5'-GAGAGTTTGATCCTGGCTCAG-3′) and reverse primer 1492R (5′-TACCTTGTTACGACTT-3′). The two primers contained a set of 16-nucleotide barcodes. The PCR program was as follows: 95°C for 3 min, 25 cycles of 98°C for 20 s, 57°C for 30 s and 72°C for 90 s, with a final extension of 72°C for 2 min.

Analysis of 16S rRNA amplicon sequencing data like build of 16S rRNA library, quality control for PCR amplifications, sequences pre-processing, species annotation and the alpha diversity were performed with the method of our previous study (Xu et al., 2019). After the comparison with the Silva (Release132 http://www.arb-silva.de) database (classified at a bootstrap threshold of 0.8) using the Mothur (https://mothur.org/wiki/Classify.seqs) software, the reads belong to unclassifiedd *Lactobacillus* using best BLAST hit method to gained species level information (Ovaskainen et al., 2010; Quast et al., 2013) (using

BLASR software). Samples ordination based on beta diversity was examined by means of principal coordinate analyses (PCoA) with phylogeny-based (UniFrac) unweighted and weighted distances (using QIIME). Linear discriminant analysis effect size (LEfSe) method was used to determine the genes most likely to explain differences between treatments by coupling standard tests for statistical significance with additional tests encoding biological consistency and effect relevance (Segata et al., 2011). Microbial networks were used to statistically identify keystone taxa and we recommend that the combined score of high mean degree and low betweenness centrality should be used as a threshold for defining keystone taxa in microbial communities (Berry and Widder, 2014). PICRUSt was used to predict the metagenome in terms of Kegg Orthology (KO) terms for each 16S rRNA sample (Langille et al., 2013). Microbiome functional shifts and phylotype-level contributions to functional shifts were obtained using the FishTaco framework (Manor and Borenstein, 2017).

metabolomics using GC-TOF-MS. The method of extraction of every sample was described in our previous study (Xu et al., 2019). The quality control (QC) samples consisted of partial extract (75 μl) from each sample. GC-TOF-MS analysis was performed using an Agilent 7890 gas chromatograph system coupled with a Pegasus HT time-of-flight mass spectrometer. The system used a DB-5MS capillary column coated with 5% diphenyl and cross-linked with 95% dimethylpolysiloxane (30 m × 250 μm inner diameter, 0.25 μm film thickness; J&W Scientific, Folsom, CA, USA). Samples (1 μl) were injected in split mode (split ratio 20:1), with helium used as the carrier gas at a flow rate of 1.0 ml min^{-1}. The oven temperature ramp was as follows: initial temperature was 50°C for 1 min, then raised to 310°C at a rate of 10°C min^{-1}, and finally kept at 310°C for 8 min. The injection, transfer line, and ion source temperatures were 280, 280, and 250°C, respectively. The energy was -70 eV in electron impact mode. The mass spectrometry data was acquired in full-scan mode with an m z-1 range of 50-500 at a rate of 12.5 spectra per second, after a solvent delay of 6.17 min.

Raw peak exaction, data baseline filtration and calibration of the baseline, as well as peak alignment, deconvolution analysis, peak identification and integration of the peak area were performed with Chroma TOF 4.3X software of LECO Corporation and the LECO-Fiehn Rtx5 database (Kind et al., 2009). Both the mass spectrum match and retention index match were considered in metabolites identification. Peaks with poor repeatability (< 50% of QC samples or RSD > 30%) in QC samples were removed (Dunn et al., 2011). The NIST (http://www.nist.gov/ index.html) and KEGG (http://www.genome.jp/kegg/) commercial databases were used to identify metabolites.

For statistical analysis, missing values are assumed to be below the level of detection. However, metabolites that were detected in all samples from one or more groups but not in samples from other groups were assumed to be near the lower limit of detection in the groups in which they were not detected (Theriot et al., 2014). We further normalized the entire set of 57 samples using SIMCA software (version 14, Umetrics AB, Umea, Sweden). Principal component analysis (PCA) models were tested for all samples. Welch's two-sample t-test was used to identify biochemicals that differed significantly between experimental groups. We defined significantly different compounds between treatment groups by the criteria of VIP > 1 (first principal-component of orthogonal projections to latent structure-discriminant analysis (OPLS-DA)) and *P*-value < 0.05.

Microbiome, metabolome and fermentation quality correlation analysis. We computed the Spearman's rank correlation coefficients for bacteria species and all identified metabolites with biofunctions (a total of 643 compounds). And we performed network construction in graph and annotated species by the phylum (*Proteobacteria* and *Firmicutes*) and well-predicted metabolites (similarity > 500) with biofumctions. To further characterize the effects of bacteria species and metabolites on fermentation quality, the Spearman's correlation analysis between bacteria species, fermentation quality and identified metabolites in different treatments have been computed and performed network plots by Cytoscape (v3.6.1). In order to show the plots clearly, we screened partial of data. Briefly, the well-predicted metabolites were selected with similarity > 500, and then the absolute value of correlation coefficients of bacterial species and fermentation quality is > 0.7; the absolute value of correlation coefficients of bacterial species and well-predicted metabolites is > 0.8; the absolute value of correlation coefficients of variables of fermentation quality and well-predicted metabolites is > 0.6.

RESULTS

The inoculants alter the structure of the corn silage microbiome. Our aim was to find out whether the composition of the silage microbiota was modulated by fermentation process and inoculants. Based on SMRT sequencing of the full-length 16S rRNA gene in silage bacteria, an average of 11948 Circular Consensus Sequencing (CCS) sequences were obtained from each sample. To identify factors that shape the differences between corn silage microbiomes (beta diversity), we applied the principal co-ordinates analysis (PCoA) based on unweighted UniFrac distances and weighted UniFrac distances. The results indicated a significant bacteria succession according to fermentation time regardless of treatments (Fig. 1a, 1b). The α-diversity analysis revealed decrease of the bacterial biodiversity from prolonged fermentation process in whole crop corn silage (Fig. 1c,). The L. buchneri increased the Shannon index at fermentation time 3, 7 and 30 days, compared with other treatments (*P* value < 0.013) and there was no difference at 90 days of fermentation (*P* value = 0.089). The *L. plantarum* decreased the Shannon index at fermentation time of 14 (*P* value = 0.023) and 45 (*P* value = 0.013) days compared with control group. The composition dynamics of microbiota was also altered by ensiling process

(Fig. 1d, 1e). At genus level (Fig. 1d), epiphytic microflora of fresh corn was mainly comprised by much of undesirable bacterial for ensiling like *Agrobacterium* (6.42%), *Microbacterium* (6.35%), *Sphingobacterium* (5.88%), *Chryseobacterium* (5.05%) and others (31%), these bacterial will be restrained promptly with start of fermentation. Most bacterial reads were derived from *Lactobacillus*, *Acinetobacter*, *Klebsiella*, *Methylobacterium* and *Citrobacter* after 3 days for fermentation in all treatments of silages. From day 7 to day 90 for fermentation, *Lactobacillus* mainly dominated the ensiling process. At species level (Fig. 1e), whole crop corn silages were dominated by *L. farciminis*, *L. parabrevis*, *L. brevis*, *L. parafarraginius*, *L. heilongjiangensis*, *L. acetotolerans* and *L. silagei*. In these dominated LAB species, *L. acetotolerans* and *L. silagei* dominated the process at 90 days of fermentation. Interestingly, the inoculants *L. plantarum* and *L. buchneri* did not dominate the whole fermentation process.

To explain the effect differentiating fermentation process of silages treated with or without inoculants by bacterial taxa, LDA Effect Size (LEfSe) analysis was conducted (Fig. 2a-2c). The bacteria dynamics contributed by different species with fermentation process in corn silages with or without inoculants. In control group, many of undesirable bacteria (even some pathogenic bacteria) were rich in early stage of fermentation (3 days), like *Proteobacteria*, *Enterobacteriales*, *Rhizobiaceae* and *Methylobacteriaceae*. Compared with control group, *L. plantarum*, *Clostridium beijerinckii* and *Agrobacterium* were significantly enriched in the silage inoculated with *L. plantarum* and *Bacteroidetes* and *Acinetobacter* were enriched in *L. buchneri* treated silage at 3 days of fermentation. When silages fermented for 7 days, *L. coryniformis* and *L. plantarum* were enriched in control group, *L. plantarum* and *L. xiangfangensis* were enriched in samples treated with *L. buchneri* and *L. plantarum*, respectively. At 14 days of fermentation, *L. farciminis*

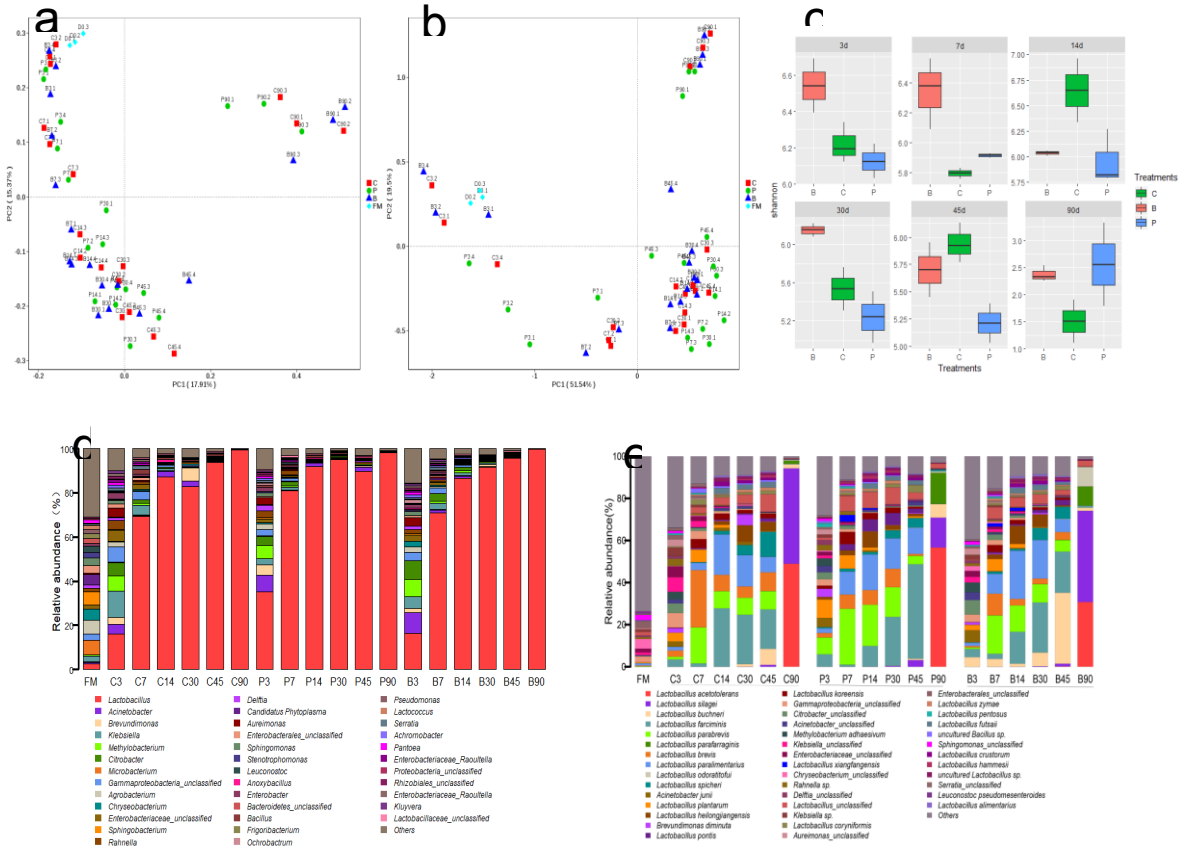

Fig. 1 Microbial community dissimilarities, and diversities of corn silage. (**a**) The community dissimilarities of the communities in different treatments and fermentation time, calculated by unweighted UniFrac distances, with coordinates calculated by principal co-ordinates analysis (PCoA). (**b**) The community dissimilarities of the communities in different treatments and fermentation time, calculated by weighted UniFrac distances, with coordinates calculated by principal co-ordinates analysis (PCoA). (**c**) The variations of community alpha-diversities (Shannon index). (**d**) Relative abundances of corn silage bacterial genus across different treatments and fermentation time. (**e**) Relative abundances of corn silage bacterial species across different treatments and fermentation time.

was enriched in silages without inoculants, *L. heilongjiangensis* was enriched in samples treated with inoculants and *L. pontis* also enriched in *L. plantarun* treated silage. At 30 days of fermentation, *L. heilongjiangensis* was enriched in control group, *L. farciminis* and *L. futsaii* were enriched in *L. buchneri* treated silage and *L. brevis* was enriched in *L. plantarum* treated silage. At 45 days of fermentation, *L. buchneri* and *L. pantheris* were enriched in control group, *L. buchneri* and *L. pontis* were enriched in *L. buchneri* inoculated silage and *L.*

farciminis and *L. coryniformis* were enriched in *L. plantarum* inoculated silage. At 90 days of fermentation, except for dominated species *L. acetotolerans* and *L. silagei*, *L. odoratitoful* and *L. parafarraginis* were enriched in *L. buchneri* inoculated silage and *L. parafarraginis* and *L. buchneri* were enriched in *L. plantarum* inoculated silage. The differences of bacteria species in different treatments at certain fermentation time indicated that inoculants modulated the bacteria dynamics with various species to dominate fermentation.

Microbial network was used to assess correlation between various species and which bacteria species was once statistically identified as keystone taxa to modulate in fermentation process. The results indicated that inoculants induced correlations between microbiota (Fig. 2d-2f). The putative drivers of keystone taxa in microbial communities of corn silages with different inoculants were defined with the combined score of high degree centrality and low betweenness centrality. The results showed that the *L. buchneri*, *L. parafarraginis*, *L. hammesii* and *Agrobacterium larrymoorei* can be considered as keystone taxa in silage without inoculants; *L. panis* and unclassified *Enterobacteriaceae* can be considered as keystone taxa in silage inoculated with *L. buchneri*; *L. crustorum* and *Agrobacterium larrymoorei* can be considered as keystone taxa in silage inoculated with *L. plantarum*.

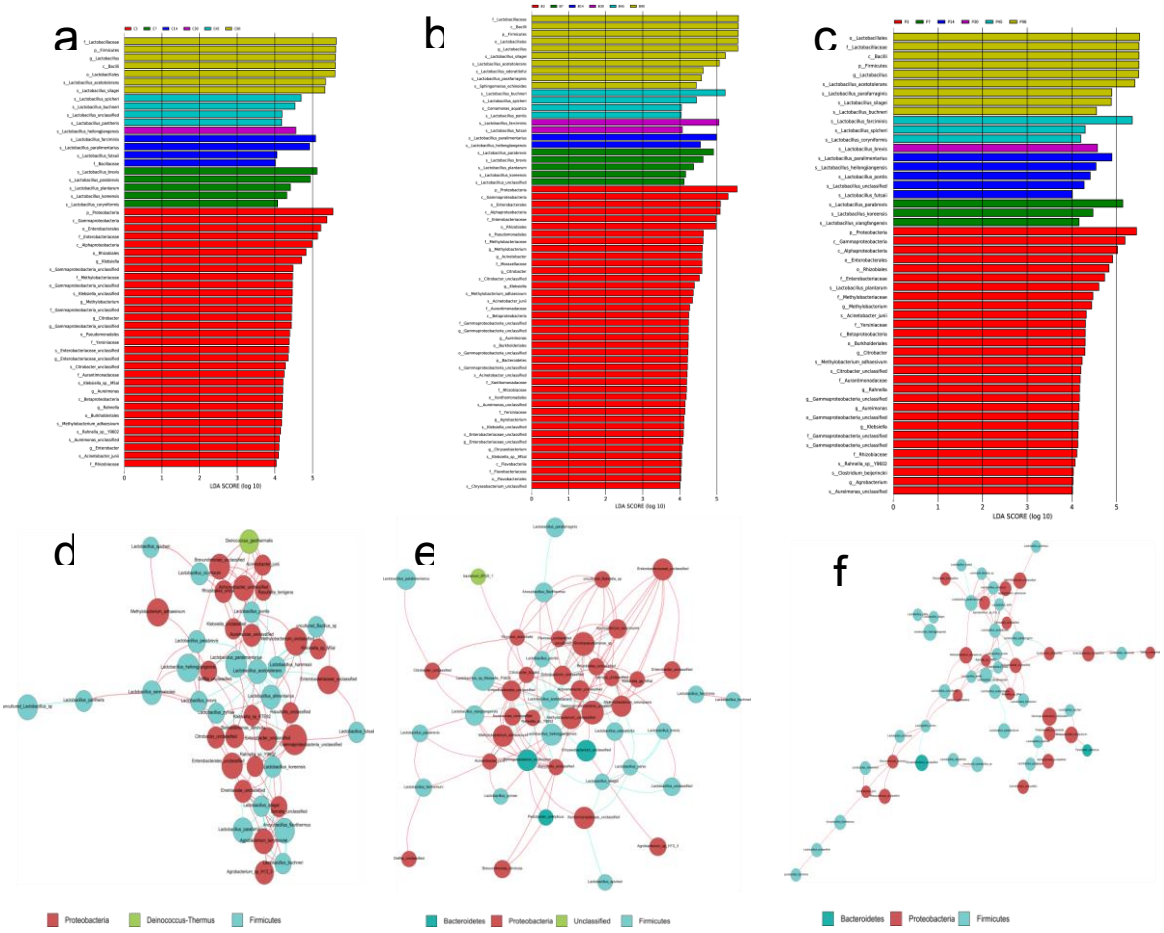

Fig. 2 Differences of bacteria taxa in corn silage with different treatments. LEfSe analysis of corn silage bacterial biomarkers associated with inoculants for different fermentation time (**a-c**). Histogram of the LDA scores computed for features differentially abundant among six fermentation time. LEfSe scores can be interpreted as the degree of consistent difference in relative abundance between features in the six fermentation time of analyzed microbial communities. The histogram thus identifies which bacteria taxa among all those detected as statistically and biologically differential explain the greatest differences between communities. (**a**) The corn silage without inoculants. (**b**) The corn silage inoculated with *L. buchneri*. (**c**) The corn silage inoculated with *L. plantarum*. Interaction networks of the whole crop corn silage microbiota (**d-f**). 16S rRNA gene-based correlation network of the whole crop corn silage microbiota, displaying statistically significant interactions with absolute value of correlation coefficients > 0.6. Node size is scaled based on the overall abundance of each taxa in the microbiota. Edge width is proportional to the strength of association between each metabolite-phylotype pair (as measured by the correlation), red edge indicted the positive correlation and green edge indicated the negatively correlation. (**d**) The corn silage without inoculants. (**e**) The corn silage inoculated with *L. buchneri*. (**f**) The corn silage inoculated with *L. plantarum*.

The inoculants alter the metabolome of the corn silage. To excavate the changes of the corn silage metabolome we used the untargeted metabolomic approach. In total, 643 metabolites were identified. The heatmap of sum of significantly different compounds in various treatments and various fermentation times is

shown in Fig. 3a. The plot showed many of metabolites produced after fermentation like amino acids, carbohydrate, organic acids, tocopherol, pantothenic acid and tyramine. Some metabolite disappeared after 45 days of fermentation in control group and the inoculants modulated the fermentation with these metabolites disappeared at 30 days of fermentation, like some amion acids, saccharic acid, polyhydric alcohols and other organics. The PCA specify in full of metabolome showed that the samples inoculated with *L. buchneri* were clearly separated by PC2, while, the differences with control were not significant (Fig 3b). The control group and samples inoculated *L. plantarum* were separated until 45 days of fermentation. The differences of fermentation process were contributed by PC1, which represented 61.7% in metabolites of ensiled forages were observed among samples with different fermentation times. The PC1 mainly contributed by amino acids like leucine, 4-aminobutyric acid, serine, alanine, phenylalanine, valine, isoleucine, proline L-allothreonine and glycine, and tyramine also contributed to the PC1. The PC2 mainly contributed by 4-aminobutyric acid, 5-aminovaleric acid lactam, methionine, stearic acid, trehalose, 2-hydroxypyridine and lysine.

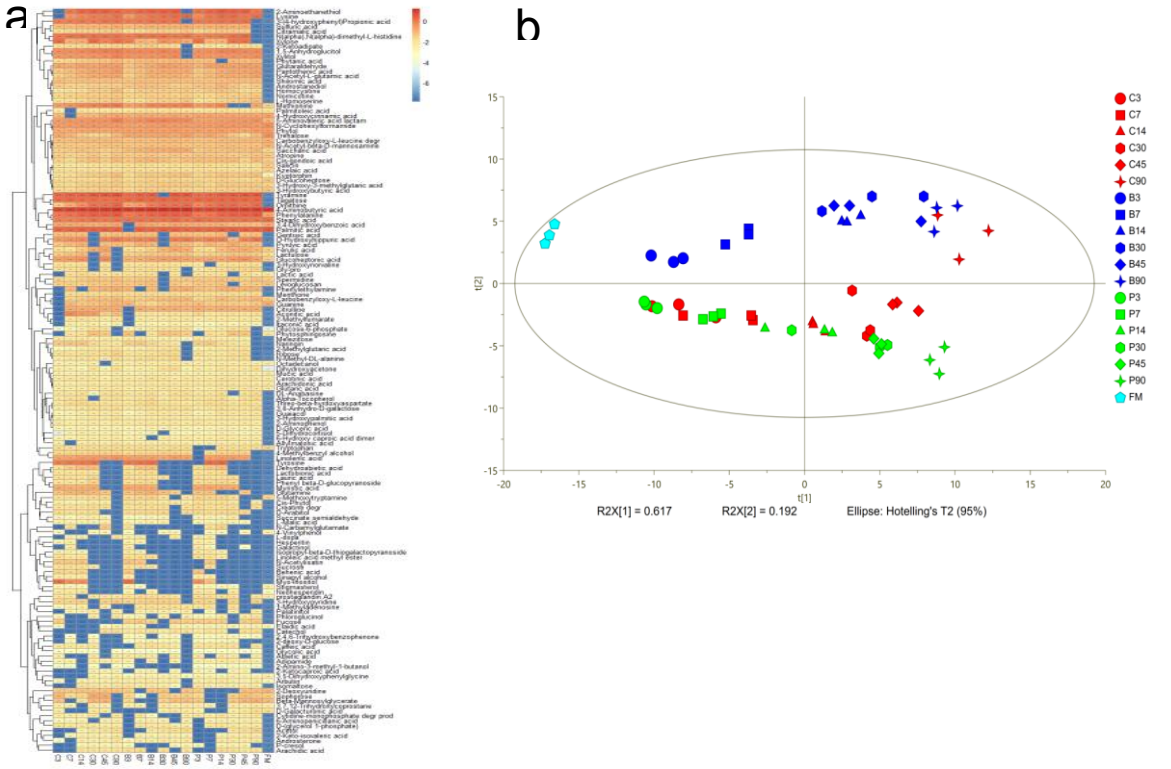

Fig. 3 Untargeted metabolomics of the whole crop corn silages metabolome. (**a**) A heatmap of the relative concentrations of sum of differentially expressed metabolites. (**b**) Principal component analysis (PCA) metabolic profiles in whole crop corn silage inoculated without (control) or with *L. plantarum* or *L. buchneri* (n = 3) for different fermentation time. Input data were the total mass of the signal integration area of each sample, and the signal integration area was normalized with method of internal standard normalization for each sample.

Microbial alterations contribute to functional shifts after fermentation. In order to determine if the observed variations in bacterial community succession contribute to community-wide functional shifts, we predicted KEGG specify in full pathway in level 2 with Phylogenetic Investigation of Communities by Reconstruction of Unobserved States （PICRUSt）(Fig. 4a-4c). The inoculants of *L. plantarum* and *L. buchneri* modulated the microbial communities, which resulted in the marked differences on functional shift. The results predicted that the pathways closely related with forage fermentation were *carbohydrate metabolism*, *amino acid metabolism*, *energy metabolism*, *metabolism of cofactors and vitamins*, *metabolism of other amino acids*, *glycan biosynthesis and metabolism*, *biosynthesis of other secondary metabolites*, and *xenobiotics biodegradation and metabolism*. The relative abundances of certain function genes were also dynamics.

Predicted functional shifts were further examined for their association with the relative extinction or blooming of specific phylotypes. All of the differences between every fermentation time couldn't be identified statistically, thus we divided fermentation process into early period (before 7 days for fermentation, aerobic in-silo phase in the early stage), middle period (7 to 45 days for fermentation, anaerobic fermentation) and later period (after 45 days for fermentation, anaerobic storage). We observed driving or attenuating functional shifts of *flavones and flavonol biosynthesis*, *polycyclic aromatic hydrocarbon degradation*, *glycosaminoglycan degradation* and *D-alanine metabolism*, which were focused in corn ensiling (Fig. 4d). Although bacterial taxa contributed to reducing functional shifts, the statistical data showed that marked upregulation of these pathways

in middle period of fermentation that are largely dependent on *Lactobacillus* reactions. The pathway of *glycosaminoglycan degradation* and *D-alanine metabolism* were just showed difference in control group and samples inoculated with *L. buchneri*, respectively. As for *flavones and flavonol biosynthesis*, samples inoculated *L. buchneri* increased the upregulation degree and the opposite result was observed in samples inoculated with *L. plantarum* compared with control group. Compared with control group, the inoculation of *L. plantarum* also decreased the upregulation degree of *polycyclic aromatic hydrocarbon degradation* pathway. The pathways that predicted significantly various in the later period compared with the middle stage of fermentation were not closely correlated with ensiling.

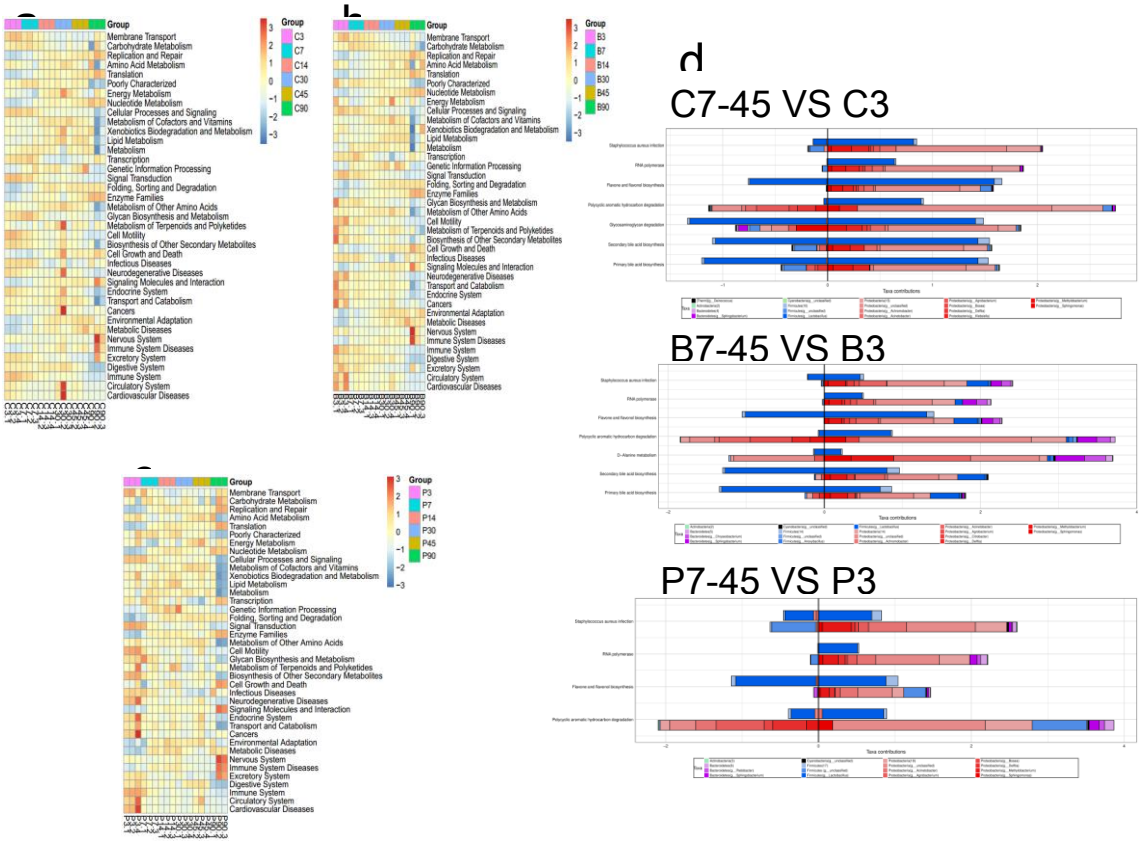

Fig. 4 Microbial alterations contribute to functional shifts after fermentation with or without inoculants. Summary of significant functional shifts predicted by the PICRUSt (**a-c**). For each KEGG pathway, shown is the second level of the predicted functional shift with respect to fermentation process and treatments. (**a**) The corn silage without inoculants. (**b**) The corn silage inoculated with *L. buchneri*. (**c**) The corn silage inoculated with *L. plantarum*. Comparing taxon-level contribution profiles of functional shifts in fermentation process **(d).** An illustration of a FishTaco-based taxon-level contribution profile, decomposing functional shifts into taxon-level contribution scores, and of the four different contribution modes identified by FishTaco. For each KEGG pathway, shown is the third level of the predicted functional shift with respect to fermentation process and treatments. Comparison of middle stage (7-45 days for fermentation) and early stage (3 days for fermentation) of fermentation of corn silage with or without inoculants.

Correlations between the microbiome and metabolome in corn silage. The widespread associations between the microbiome and metabolome composition across the silages with or without inoculants were presented. The correlations between bacteria species and well-predicted metabolites with biofunctions in silages with different treatments were clearly exhibited in Fig. 5a-5c (the absolute value of correlation coefficients > 0.6: between LAB and metabolites; absolute value of correlation coefficients > 0.9: between other bacteria and metabolites). In control group, essential amino-acids (lysine, methionine and phenylalanine) were distinct positively correlated with *L. buchneri*, *L. silagei* and *L. parafarraginis*. Inoculants especially *L. plantarum* decreased the positive correlation between essential amino-acids and LAB. The correlations between metabolites with bacteriostatic activity (naringin and 3,4-dihydroxybenzoic acid) and LAB were markedly negative. Inoculants increased positive correlation between metabolites with bacteriostatic activity with LAB. Samples inoculated with *L. plantarum* improved the positive correlations with *L. pentosus* and samples inoculated with *L. buchneri* improved the positive correlations with *L. brevis* and *L. xiangfangensis*. Metabolites with antioxidant activity were found in present study like ferulic acid and catechol (Ggaf, 1992). The correlation between ferulic acid and LAB were markedly positive, seven LAB species positively correlated with ferulic acid ($r > 0.8$), but inoculants decreased the positive correlations. Among this, ferulic acid just positively correlated with *L. silagei*, *L. panis* and *L. kefiri* in samples inoculated with *L. buchneri* and *L. plantarum*, respectively. Lower correlations between catechol and LAB species in corn silage, just *L. acetotolerans* and *L. silagei* positively correlated with catechol

in samples without inoculations and inoculated with *L. plantarum*, respectively. Metabolite 4-aminobutyric acid that is gamma-aminobutyric acid with central nervous system (CNS) inhibitory activity, can decrease blood pressure and insulin secretion (Pouliot-Mathieu et al., 2013)·(Diana et al., 2014) was identified in present study. Lower positive correlations between LAB and 4-aminobutyric acid were observed in corn silage, just *L. acetotolerans* (r = 0.655) positively correlated with it in control group and *L. buchneri* and *L. parafarraginis* positively correlated with it in samples inoculated with *L. plantarum*. Linolenic acid is a kind of essential fatty acid with well-established health benefits was detected (Burdge, 2006). Corn silage inoculated with *L. plantarum* increased the positive correlation between LAB and linolenic acid, there were twelve LAB species positive correlation with linolenic acid but no LAB species positively correlated with it in samples inoculated with *L. buchneri* and control group. Salicin is an alcoholic with anti-inflammatory activity and it will be metabolized into salicylic acid when consumed by animal (Albrecht et al., 1990). The correlations between LAB and salicin were negative in control group and inoculants increased the positive correlation with *L. plantarum* in bacterial community, inoculation *L. plantarum* also increased the positive correlation with other LAB species especially *L. pentosus* (r = 0.943). L-malic acid with antibacterial and antioxidant activity (Gadang et al., 2008;· Tezcan et al., 2009) and it can be carbon source (Mcfeeters, 2003) for LAB and flavour agent also. Corn silage without inoculations and inoculated with *L. buchneri* showed negative correlations between L-malic acid and LAB species, inoculated with *L. plantarum* increased the positive correlations with LAB species mainly occurred of *L. plantarum* and *L. pentosus* (r = 0.714).

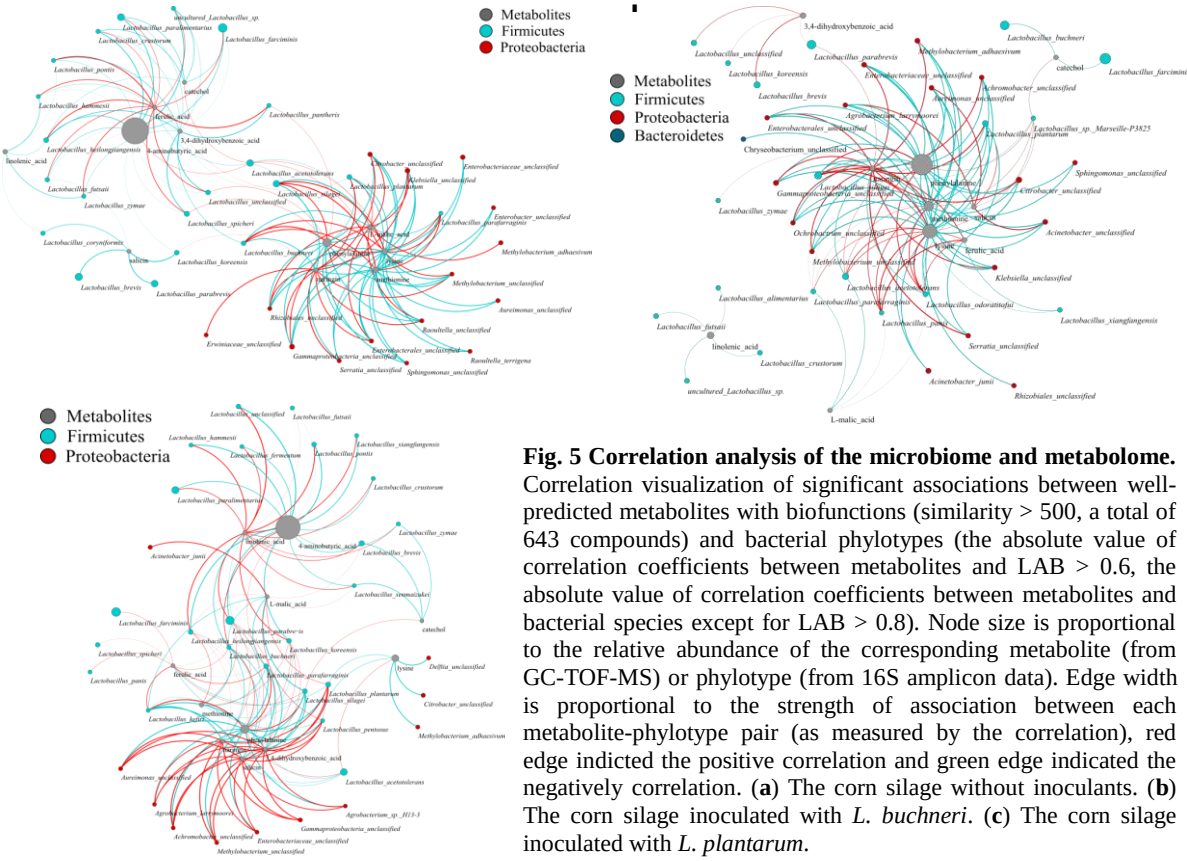

Fig. 5 Correlation analysis of the microbiome and metabolome. Correlation visualization of significant associations between well-predicted metabolites with biofunctions (similarity > 500, a total of 643 compounds) and bacterial phylotypes (the absolute value of correlation coefficients between metabolites and LAB > 0.6, the absolute value of correlation coefficients between metabolites and bacterial species except for LAB > 0.8). Node size is proportional to the relative abundance of the corresponding metabolite (from GC-TOF-MS) or phylotype (from 16S amplicon data). Edge width is proportional to the strength of association between each metabolite-phylotype pair (as measured by the correlation), red edge indicted the positive correlation and green edge indicated the negatively correlation. (**a**) The corn silage without inoculants. (**b**) The corn silage inoculated with *L. buchneri*. (**c**) The corn silage inoculated with *L. plantarum*.

Correlations between the microbiome, metabolome and fermentation quality in corn silage. To further characterize the effects of bacteria species and metabolites on fermentation quality, the correlation analysis between species, fermentation quality and well-predicted metabolites in different treatments have been investigated （Fig. 6a-6c）. The results indicated that the variables of fermentation quality positively correlated with *Lactobacillus* and negatively correlated with *Proteobacteria* (the pH is positively with *Proteobacteria* but the low pH value is good for fermentation quality). The samples inoculated *L. plantarum* increased the positive correlation between acetic acid and LAB species and inoculated with *L. buchneri* decreased the positive correlation between lactic acid and LAB species. The LAB strains positively correlated with fermentation quality mainly tracked to *L. silagei*, *L. buchneri* and *L. parafaraginis* in control group; it can be tracked to *L. silagei*, *L acetotolerans* in control group and *L. odoratitofui* in samples inoculated with *L. buchneri*; and it can be tracked to *L. silagei*, *L. parafaraginis*, *L. kefiri* and *L acetotolerans* in *L. plantarum*-treated samples, but inoculants decreased the positive correlation with *L. buchneri*. The fermentation quality closely correlated with organic acids and amino acids during corn ensiling. Lactic acid and acetic acid were positively correlated with well-

predicted metabolites, meanwhile, propionic acid and pH were negatively correlated with well-predicted metabolites in control group. The samples inoculated with *L. buchneri* decreased the positive correlations between lactic acid, acetic acid and well-predicted metabolites. Samples inoculated with *L. plantarum* increased the positive correlations between variables of fermentation quality and well-predicted metabolites. As for correlations between metabolites and bacteria species, well-predicted metabolites and bacteria species which closely correlated with each other were various in different treatments. In control group, N-methyl-DL-alanine was positively correlated with *L. silagei* and negatively correlated with *L. plantarum*. Phytol was negatively correlated with Proteobacteria and *L. plantarum* but positively correlated with *L. silagei* and *L. parafarraginis*. Levoglucosan and xylitol positively correlated with *L. parafarraginis*. Trehalose was negatively correlated with LAB strains. In samples inoculated with *L. buchneri*, the closely correlation between well-predicted metabolites with bacteria species just showed negatively correlations between tyramine and Proteobacteria. In samples inoculated with *L. plantarum*, 2-methylfumarate, ribose and tyramine were positively correlated with *L. acetotolerans*, *L. farciminis* and *L. silagei*, respectively. Levoglucosan and trehalose were negatively correlates with *L. buchneri* and *L. kefiri*, respectively. Phenylethylamine was negatively correlated with LAB species *L. plantarum* and *L. pentosus* but positively correlated with *L. kefiri*. Xylitol was closely negatively correlated with Proteobacteria.

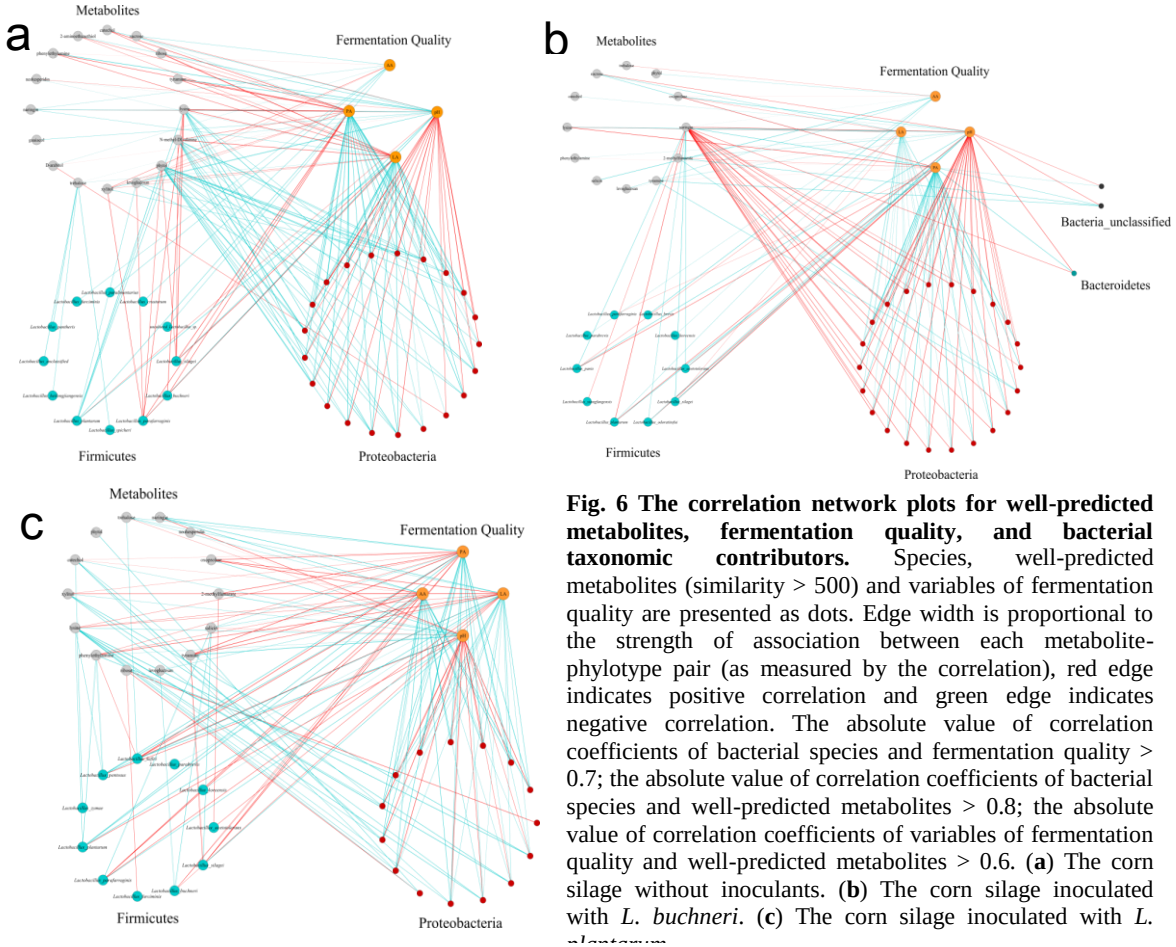

Fig. 6 The correlation network plots for well-predicted metabolites, fermentation quality, and bacterial taxonomic contributors. Species, well-predicted metabolites (similarity > 500) and variables of fermentation quality are presented as dots. Edge width is proportional to the strength of association between each metabolite-phylotype pair (as measured by the correlation), red edge indicates positive correlation and green edge indicates negative correlation. The absolute value of correlation coefficients of bacterial species and fermentation quality > 0.7; the absolute value of correlation coefficients of bacterial species and well-predicted metabolites > 0.8; the absolute value of correlation coefficients of variables of fermentation quality and well-predicted metabolites > 0.6. (**a**) The corn silage without inoculants. (**b**) The corn silage inoculated with *L. buchneri*. (**c**) The corn silage inoculated with *L. plantarum*.

DISCUSSION

To link microbiome community dynamics to function in the modulation of inoculants, we undertook a multi-omics approach to characterize the changes in the microbiom and metabolome after ensiling. Inoculants *L. buchneri* and *L. plantarum* were not the dominant strains during ensiling, but they modulated the various microbial communities and metabolome dynamics in different ways. Although the community structures in three groups had observable differences with regard to the total metabolome, they were indistinguishable with respect to total micrbiome dynamics. It might be due to co-occurrence patterns of microbiota resulted in the various on microbial communites dynamics and end products with or without inoculants. The role of microbial communities in ecosystem functioning is defined (Graham et al., 2016; Fierer, 2017), and correlations of microrganisms are complex in forage fermentation ecosystem. Maybe there are some species whose impact on the community is large, and disproportionately large relative to its abundance (Power et al., 1996). This study is the first to identify keystone taxa with network topological properties in silage. The results indicated that inoculations altered the correlations of microflora and keystone species were identified to certificate their regulation. The end products during fermentation either directly produced by microbial or indirectly as a result of degradation and transformation substances of forages. The plant enzymes in aerobic silo stage (Dwayne et al., 2003) and various

abundances of LAB strains (Ohshimaa and Mcdonald, 1978) in communities dynamics resulted the dynamics of metabolome, which were mainly contributed by variations of many amino acids in whole crop corn silage we ensiled.

Our analysis of predicted functional shift allowed us to evaluate the impact of microbial communities on changes of pathway during ensiling. Of note, most pathways closely related to fermentation (*carbohydrate metabolism*, *amino acid metabolism*, *energy metabolism*, *metabolism of cofactors and vitamins*, *metabolism of other amino acids* and *xenobiotics biodegradation and metabolism*) were predicted to constitutively upregulated in middle stage (7 to 45 days for fermentation) of fermentation. Although the inoculation of *L. buchneri* upregulated the *flavones and flavonol biosynthesis* pathway contributed to *Lactobacillus* phylotypes, no flavonoids have not been detected with GC-TOF-MS. In the majority of silage fermentations, the substrates are fructose and glucose. Once cut, fructose and glucose concentrations can only increased by polysaccharides (Dwayne et al., 2003). The *glycosaminoglycan degradation* pathway was significantly upregulated by *Lactobacillus* at middle stage of fermentation just in corn silage without inoculants. However, fructose and glucose have not been detected with GC-TOF-MS. The glucose-6-phosphate is the key intermediate to understand the glucose metabolism, which was increased with start of fermentation in all treatments. The present study with metabolome approach verified the previous research theories (Dwayne et al., 2003), but this pathway suggested that polysaceharides were hydrolyze until middle stage of corn fermentation. The *D-alanine metabolism* pathway was upregulated by *Lactobacillus* in corn silage inoculated with *L. buchneri*. D-alanine is an amino acid that occurs only in the peptidoglycan of bacterial cell walls (Schleifer and Kandler, 1972), which can be a as a marker of bacterial. Deamination can be induced alanine to product acetate (Li et al., 2018) within amino acids metabolism. Thus, inoculants of *L. buchneri* can improve aerobic stability via upregulation of this pathway to produce much acetate. However, acetic acid in present study produced via other pathways as indicated by the concentration of alanine and acetic acid in corn silages showed the similar trend (increase) with fermentation process. The inconsistent of pathways and metabolites might be because of the same approach for metabolites extraction and oven temperature ramp for testing through GC-TOF-MS, some of end products in silage could not be well-extracted or could not be detected. On the other hand, the gene expression is impacted by much of factors in forage fermentation system like changing pH, substrates for microbiota fermentation, other metabolites regulation and interactions between microbes in this system (Dwayne et al., 2003; Guo et al., 2018; Song and Chan, 2019).

Alternately, this study gives insight into to the biofunctions of silage, combined with metabolic and microbiota network to delineate the association between metabolic output and microbial ecology during ensiling. The close correlations between three essential amino acids and *L. silagei*, *L. parafarraginis* and *L. buchneri* were found in corn silage with or without inoculants. These three LAB species were showed higher relative abundances in after 45 days for fermentation, thus as suggested that we can screen essential amino acids-producing LAB strains from corn ensiled longer than 45 days as alternative strains to make silage good for animal health and welfare. Metabolites naringin and 3,4- dihydroxybenzoic acid with bacteriostatic activity can inhibit undesirable microorganisms to reduce nutritional loss and inhibit mycotoxin biosynthesis of silage (Haskard et al., 2001). The two substances showed higher relative concentrations in 3 and 7 days-fermented corn silage. Previous study reported that species *L. plantarum* with antifungal property as produced a phenolic-related antibiotic or 3-hydroxy fatty acids (Sjögren et al., 2003; Valan Arasu et al., 2013). In the other hand, the species *L. plantarum* positively correlated with the two metabolites in silage with or without inoculants. Thus, *L. plantarum* could be a consider species for screening inoculants to making high quality silage. Substances ferulic acid and catechol with antioxidant activity indicated various dynamics in corn silages with or without inoculants. Ferulic acid is a hydroxycinnamic acid, inoculants decreased the closely positive correlation with LAB strains. It might be because the increased ferulic acid inhibited the growth and viability (Rodríguez et al., 2009) of some species in inoculants-treated corn silage. Catechol is a member of flavonoids, which can be metabolized from protocatechuic acid by *Lactobacillus* spp. (Filannino et al., 2015). However, LAB species indicated lower positive correlations with catechol in this study. It might be because lack of LAB species and protocatechuic acid to produce catechol in the corn silage fermentation ecosystem. 4-Aminobutyric acid with central nervous system (CNS) inhibitory activity blood pressure regulation and insulin secretion was increased with fermentation process in corn silage, especially *L. buchneri*-treated corn silage. which is consistent with previous study on alfalfa silage (Guo et al., 2018). However, there were no correlations between 4-aminobutyric acid and LAB species in *L. buchneri*-treated corn silage. A number of bacteria and fungus have been reported to produce 4-aminobutyric acid (Kono and Himeno, 2000; Lu et al., 2008). The most practical microorganisms for 4-aminobutyric acid production is LAB, which produce high levels of 4-aminobutyric acid. In addition, different fermentation factors affect the 4-aminobutyric acid production by microorganisms, the most important factors are pH, temperature and nutrition of culture (Dhakal et al., 2012). Therefore, we speculated that many of microorganisms and their dynamics, as well as fermentation factors in corn ensiling system co-effected the end production of 4-aminobutyric acid rather than certain species dominated the 4-aminobutyric acid production. The linolenic acid may be used for the promotion of animal health and well-being, which can be produced by LAB (Salsinha et al., 2018). There were no positive correlations between linolenic acid and LAB species were detected in corn silages without inoculants and inoculated with *L. buchneri*, but there were twelve LAB species positively correlated with linolenic acid in *L. plantarum*-treated corn silage. Linked to the dynamics of linolenic

acid and microbial community, we found that the changes of relative abundances of *L. heilongjiangensis* and *L. pontis* were accordant with concentration of linolenic acid. Thus, the linolenic acid could be produced by *L. heilongjiangensis* and *L. pontis.* Salicin with anti-inflammatory activity indicated decrease of relative concentration after fermentation, although inoculation *L. buchneri* remised the decrease. It might be because some bacteria species utilized salicin(Wang et al., 2009; Cai et al., 2012) and the community structure and dynamics resulted lower positive correlations between salicin and LAB strains. In addition, salicylic acid, the metabolites from salicin and salicin with antipyretic activity (Mackowiak, 2000). If the silage contains the two substances, the functional silage can be used to treat inflammatory conditions in periparturient or heat stressed dairy cow (Trevisi, 2008). With many benefit activities, malic acid also as a key intermediated in the citric acid cycle of biological tissues, which had been used as feed additive for ruminants to improve performance and efficiency (Ke et al., 2018). However, malic acid is metabolized to lactic acid, that is malic acid can be consumed during forage fermentation (Ke et al., 2017). L-malic acid has not been detected in fresh corn and the trace amount during ensiling in this study. It might because the extraction and test conditions of GC-TOF-MS were not optimal for precisely detect some substances, especially trace metabolites. In this regard, targeted metabolomic analysis should be profiled in further study.

This study also indicated that multiple community structures of microbiome and metabolome can affect fermentation quality, as suggested that complex correlations between fermentation quality and metabolites and microbiota. Inoculants modulated the microbial community and succession, as well as dynamics of metabolites during ensiling results in the fermentation quality. The results of multi-omics analysis and network approaches underscore the importance of looking beyond microbiome community structure and measuring functional aspects when determining the relationship between the microbiome and silage biofunctions. On the other hand, we gained much scientific information on screening LAB strains to making high quality as well as with functional silage to enhance animal health and welfare. We can gain insight into the metabolic functions of the microbiome during ensiling, using metabolomic approaches as we have done in this study, or metagenomic, or transcriptomics, or metaproteomic. Characterization should lead to directive modality targeting the LAB additives to making functional silage for various animal growth or production period to guarantee quality and safety of animal products.

CONCLUSIONS

Profiling of metabolome and microbiome dynamics in ensiled whole crop corn improved our current understanding of the biological process underlying silage formation. Inoculants *L. buchneri* and *L. plantarum* modulated the various microbial communities and metabolome dynamics in different ways. They also altered the correlations of microflora and keystone species were identified to certificate their regulation. Our analysis of predicted functional shift allowed us to evaluate the impact of microbial communities on changes of pathway during ensiling. The metabolic and microbiota network delineated the association between metabolic output and microbial ecology during ensiling, which provided a scientific direction for screening some LAB strains as additives to making functional silage that good for animal health and welfare.

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SILAGE UNDER A CHANGING CLIMATE

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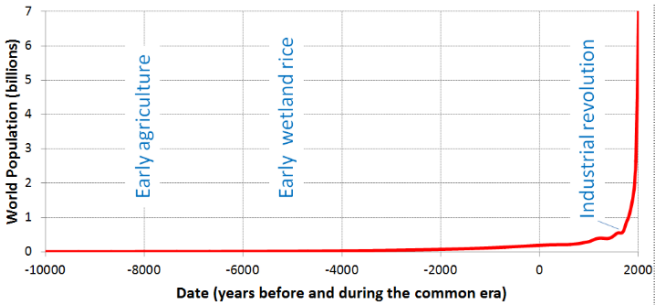
SUMMARY

Global climate historically has shown natural, gradual, cyclical trends in temperature and rain patterns which has allowed nature to adapt to the changing environment. Anthropogenic factors starting with the industrial revolution and increasing with a growing population and global mechanisation / global adoption of fossil fuel derived consumables have led to a dramatically accelerated change in climate that is faster than nature can adapt to. The nutritional profile and yield of forages used to produce silage are becoming increasingly influenced by their conditions of growth, and the mechanistics of micro-organisms used to drive the fermentation of these forages into silage are equally being impacted by elevated temperatures during the ensiling period that influences not only the profile of the organisms at time of ensiling but also the survivability of the organisms during the ensiling period. Modification to the microflora during the fermentation can shift the efficiency, rate and end points of the fermentation and the climate that is expected during ensiling needs to be considered by the farmer and consultant to optimise fermentation potential.

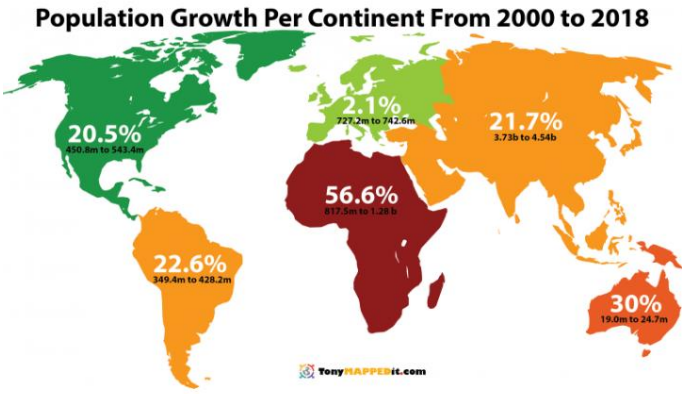
INTRODUCTION

Impact of global warming has been widely acknowledged for the last 40 years, with modelling programmes accurately reproducing the influence of the El Nino Southern Oscillation on drought over land but also the observed global mean aridity trend from 1923 to 2010 (Dai).



The ultimate effect of climate change on the planet remains undefinable, inclusive of the rate and ultimate extent of change, but these changes will be increasingly manifested in ways inclusive of extremes of temperature and precipitation, decreases in seasonal snow and ice extent and sea level rise with anthropogenic climate change likely to continue for many centuries (Karl and Trenberth).

Climate changes cyclically as can be tracked within ice cores over previous millennia, but the atmospheric changes that have occurred within the last 100years fall outside of historical patterns and can be directly related to the dramatic increase in global population observed over the same time period, with the increased industrialisation that has occurred in parallel with population expansion.



Populations have continued to dramatically rise since the turn of the current millennia, with demand for food predicted to rise by 50% by 2050 (Flies, et al), and, with increased anthropogenesis through human action and concomitant demand for food the increased production of food will lead to further clearance of forestation with resultant localised eutrophication compounding the issues. Equally, increasing demand for silage for bioenergy is impacted by climatic impact on crop growth and indirectly impacts the usage of carbon fuels, supporting their maintained usage.

Changing weather patterns bring national and local changes to wind, temperature, rain fall patterns (both in volume and timing of rain patterns) and snow / sleet patterns that directly influence the timing of land work, seeding, germination, insecticide and fertiliser application, crop growth, weed growth, fertiliser utilisation, yield and timing of harvest before the farm starts to even practically address the production of silage.

DROUGHT

Lower rainfall (than historically predicted) is an observed effect of the changing climate in many countries (and a change to the seasonality of the rainfall pattern). Emerson et al compared the 2010 and 2012 crop characteristics in Iowa, Georgia and Nebraska as below using ANOVA. (Table 1), where 2010 was a typical year for rainfall and 2012 was a drought year.

Table 1 – Selected Forage Characteristics in Drought and Non-Drought years

Feedstock	Year	n	Extractive	Cellulose	Total Glucan	Xylan	Lignin
Corn Stover	2010	11	13.8[a]	35.5[a]	na	20.3[a]	15.1[a]
Corn Stover	2012	11	23.3[b]	32.6[b]	na	17.6[b]	12.3[b]
Mix grass	2010	24	22.7[a]	23.1	23.5	17.6[a]	12.3[a]
Mix grass	2012	24	29.1[b]	22.9	23.0	18.5[b]	10.6[b]
Miscanthus	2010	12	18.6[a]	37.7[a]	36.9[a]	20.3[a]	20.0[a]
Miscanthus	2012	12	26.4[b]	28.9[b]	29.0[b]	19.7[b]	14.7[b]

Water stress / depravation has various impacts on the growth of plants depending on the time(s) of the season that the stress occurs. Total yield is negatively impacted, as can be the formation of ears / grain in cereal and corn crops, with a parallel increase in the extractable fraction of the forage and reduction in the structural fraction of the plant. Additionally, nitrogen uptake and utilisation by the plant is dramatically impacted by ongoing water availability to the plant which can directly impact the forage fermentation through residual nitrogen in the ensiled plant buffering the fermentation and leading to the production and potential evolution of poisonous silo gases during the first days of ensiling, both leading to a slower fermentation, greater dry matter loss and parallel losses in digestibility and energetic value. Combining drought with additional plant environmental stressors such as parasite, temperature, hail etc.. also influences the plant epiphytic microbiology, directly leading to increased fungal levels which act as increased challenge to the ease of forage fermentation at ensiling.

Fermentation pattern and ultimate dry matter intake (or energetic potential to biomass fermenters) can be dramatically impacted by the climate (Bernardes). Marley highlighted the thermal death temperatures of inoculant bacteria are potentially being reached during the first days of ensiling, but, in combination with additional bacterial stressors such as drought and parallel decrease in water potential, mesophilic lactic acid bacteria are increasingly challenged leading to reduced growth rates of the desired epiphytic / applied lactic acid bacteria. The undesirable epiphytic organisms (enterobacteria, bacillus, yeast and mould) are generally less negatively impacted by lower water potential of the forage and their fermentations become more pronounced, leading to less efficient fermentations, greater dry matter and digestibility losses and the production of less desirable fermentation products (Table 2. Rooke and Hatfield).

Table 2 – Bacterial Fermentation Pathway efficiency

Organisms	Pathway	Substrate	Product	Loss DM (%)	Gross Energy Loss (%)
LAB	Ho	Glucose	2 lactate	0	0.7
LAB	He	Glucose	1 lactate, 1 ethanol, 1 CO_2	24	1.7
LAB	He	3 Fructose	1 lactate, 1 acetate, 2 mannitol, 1 CO_2	4.8	1.0
LAB	Ho/He	2 Citrate	1 lactate, 3 acetate, $3CO_2$	29.7	-1.5
LAB	Ho/He	Malate	1 lactate, 1 CO_2	32.8	-1.8
Enterobac.		2 Glucose	2 lactate, 1 acetate, 1 ethanol, 1 CO_2	17	11.1
Clostridia		2 Lactate	1 butyrate, $2CO_2$, $2H_2$	51.1	18.4
Yeast		Glucose	2 ethanol, $2CO_2$	48.9	0.2

The influence of drought directly reduces the volume of forage produced (Meisser), but also increases production of volatile acids and ethanol which directly negatively impacts palatability and the intake potential of the silage. Less silage. Less value silage. Less dry matter intake.

The microbiology of the soil : plant ecosystem is significantly impacted by extremes of drought and flooding, leading to long term ‘legacy’ impact on the soil populations (Nguyen). Prolonged drought produced a

log reduction in the abundance of bacteria with an increase in the dominance of spore forming bacteria – unfortunately no enumeration was made of yeast and mould counts in the study.

FLOOD

Climate change has adjusted the timing and volume of rainfall patterns. de Perez speaks of 'compared observed rainfall and temperature with food security classifications, we quantify how much the chance of food insecurity increases when rainfall is low' with respect to food security modelling in East Africa, highlighting the historic influence of rainfall patterns and interaction of rainfall with temperature to influence yield and quality that the African continent has historically struggled with, and that the rest of the world is now facing (to a lesser degree).

Timing, volume and frequency of rainfall has significantly changed across the European continent bringing drought, but also periods of significant flooding in many countries. Little work has been undertaken on the impact of flooding on the plant microbiota. Cui elucidated the microbiota of non flooded and flooded rice culms, finding that with flooding there was a significant decrease in the abundant (most common) phylogenetic diversity with *Bacillus* becoming significantly more dominant in the flooded crop, accounting for 52.6% of the taxonomic units.

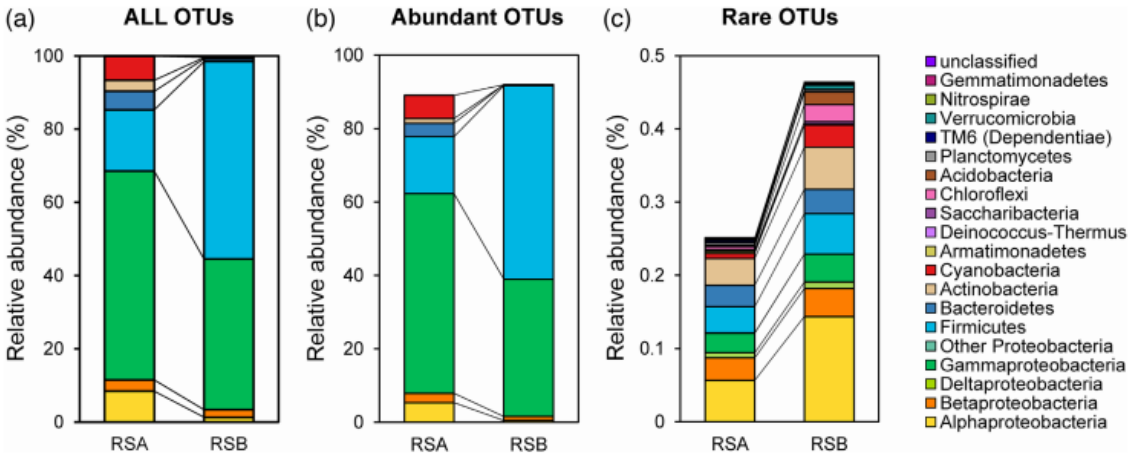


Figure 3. Bacterial compositions on non-flooded (RSA) and flooded (RSB) rice culms. **(a)** The average relative abundances of all OTUs at the phylum level (Proteobacteria divided into classes) on RSA and RSB. **(c)** and **(d)** only showed the relative abundances of the abundant and rare OTUs, respectively.

Cui elucidated microbial diversity of RSA and RSB (non-flooded and flooded rice culms respectively into operational taxonomic units (OTUs) for diversity assessment. Silage was not made from this forage.

An increased level of *Bacillus* on forage with prolonged flooding is to be expected on any forage (flooding leads to de-oxygenation of the soil and the flooded plant area promoting the level of spore forming organisms), with a parallel increase in Enterobacteria and yeast numbers. Bacillus and Enterobacteria fermentations are relatively similar in efficiency and endpoints:

$$\text{Glucose} \longrightarrow 2\ \text{Ethanol} + \text{Lactic Acid} + \text{Acetate} + 2,3\ \text{Butane-diol}$$

$$\text{Glucose} \longrightarrow \text{Lactic Acid} + \text{Succinate} + \text{Ethanol} + CO_2 + \text{Acetate} + 2,3\ \text{Butane-diol}$$

with both fermentations leading to significant loss of dry matter, digestibility and the formation of undesirable end points which negatively impact intake and, if dry matter is sufficiently high at ensiling, will negatively challenge aerobic stability of resultant silage. Legacy effects of flooding dictate that the epiphytic profile of forage directly impacted by flood water will move away from Firmicute dominated profiles to less fermentationally and stability desirable profiles. Research on the impact of flooding on the epiphytic profile of forages should consider the timing, duration and stage of growth to elucidate legacy impacts on changing microbiota and the impact on fermentation efficiency of the ultimately ensiled forages.

TEMPERATURE

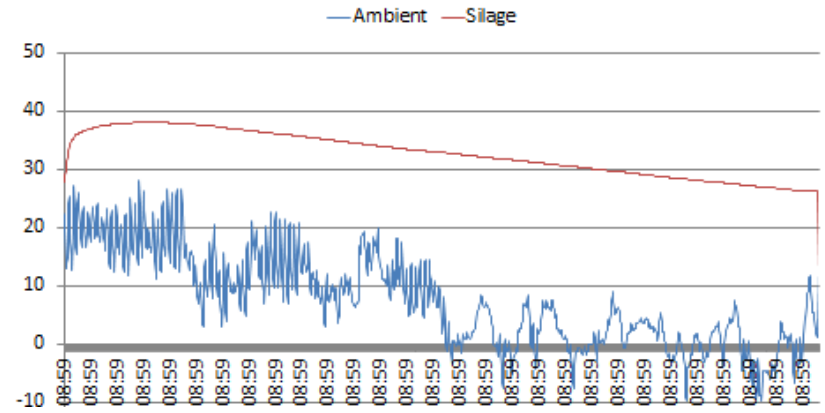
Temperature Increase 2000 to 2100 (°C)			
Model	**Total**	**Land**	**Ocean**
CCSR/NIES	4.7	7.0	3.8
CCCma	4.0	5.0	3.6
CSIRO	3.8	4.9	3.4
Hadley Centre	3.7	5.5	3.0
GFDL	3.3	4.2	3.0
MPI-M	3.0	4.6	2.4
NCAR PCM	2.3	3.1	2.0
NCAR CSM	2.2	2.7	2.0

The main parameter that is globally recognised within climate change is an increase in temperatures. All micro-organisms have an optimal temperature at which they grow, and a range of temperatures over which they are capable of growing at sub-optimal growth rates. Marley highlighted the thermal death points of common inoculant fermentation bacteria, which, once this temperature is achieved, bacteria lose viability and have no further impact on the ensiling fermentation.

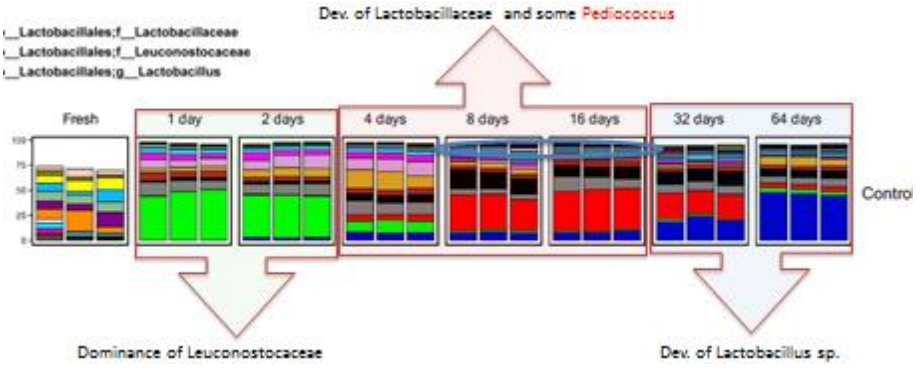
Various models have been produced to predict the rise in temperatures under the auspices of the InterGovernmental Panel on Climate Change, with the key indicator models listed in the

accompanying table as part of the Special Report on Emission Scenarios. The models show varying degrees of temperature rise based upon adoption of international adoption of varying levels of environmental policies, with all scenarios predicting between a 2.7 – 7.0°C rise in land temperature over the coming century which will be directly reflected in the ensiling temperature and resultant temperatures that the ensiled forage reaches during the first days of ensiling.

Forage temperature increases during the first days of ensiling as entrapped air is converted to carbon dioxide. The degree of temperature rise being related to the pore space within the ensiled forage, the dry matter, the microbial profile of the forage, the compaction regime and the sealing system in use, with the ultimate maximum temperature also being related to the ambient temperature at time of ensiling. Typically a 10 - 15°C rise in temperature is observed in the silage compared to ambient temperature (the associated chart relates to 2018 Czech corn silage from ensiling 02 September 2018 to feed out in February 2019). During initial ensiling the forage rapidly increased in temperature to 35°C ., peaking at 38°C, which is generally above the thermal death point for *Leuconostoc* spp (*Leuconostoc* spp optimally grow between 20-30°C, with growth occurring between 5-30°C. Bergeys Manual of Systematic Bacteriology).

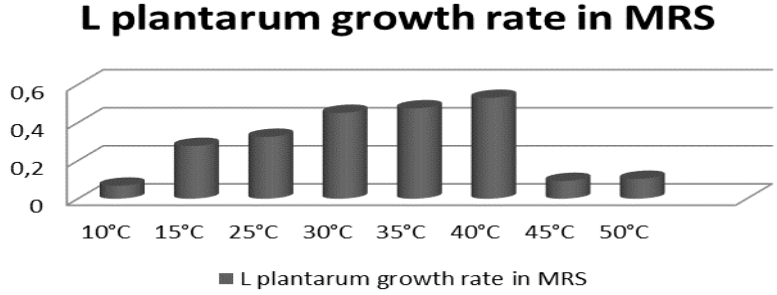


Being facultatively anaerobic *Leuconostoc* are typically present during ensiling forage (unpublished genomic data) when the environment changes from aerobic to anaerobic. Ensiling temperatures of corn in Czech are in excess of the thermal death point of many species of *Leuconostoc*, which shifts the fermentation profile away to more heat stable, less efficient bacteria resulting in slower fermentations and greater losses of dry matter, digestibility and milk potential of the silage. The impact of global warming is to further adjust the *normal* microbial fermentation profile to one that is less rapid and less efficient in protecting dry matter recovery.



High temperature increases fibre / lignin deposition in the plant. Schlenker and Roberts reported that corn yields dropped when growing temperature was above 29°C. When yields are challenged during the season there is pressure to maximise yield by lowering cut height, which in turn increases the high fibre fraction in the silage, increases the difficulty to compact which concomitantly leads to more entrapped air and a compounded higher temperature in the silage with greater difficulty for desired bacteria to survive / efficiently grow within the forage, all leading to a greater loss in dry matter, digestibility and feed value.

Mechanistic studies of lactic acid bacteria show highly variable laboratory growth rates under different temperatures. Outside of laboratory conditions the impact of temperature is more dramatic when additional stressors are encountered (competition, changing environmental temperature, pH , [O_2/CO_2], a_w etc.



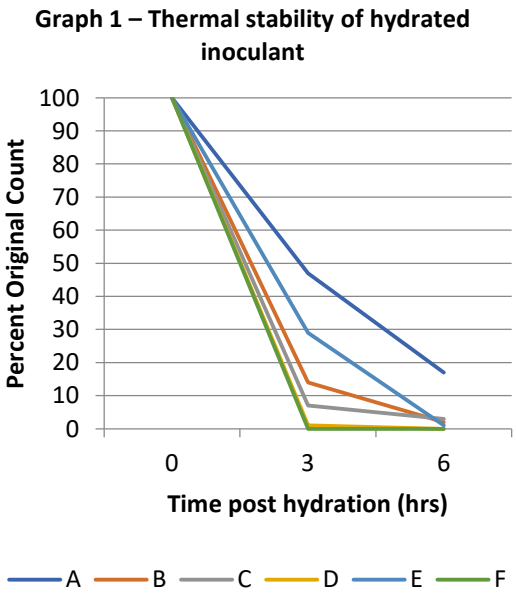
To highlight the impact of minor temperature changes, increasing the temperature from 40°C to 45°C under optimal media conditions sees a 5.7 fold decrease in the growth rate of one of the most common organism found in silage inoculants. Some common laboratory thermal death temperatures of micro-organisms are shown in Table 3. Many desirable homolactic and heterolactic bacteria have thermal death points that are below 45C, meaning that if the ensiled forage reaches these temperatures prior to their point of action within the ensiling pH fall, they will die before they are active.

Table 3 – Thermal Death Points of Selected Silage Relevant Bacteria

Thermal Death Temperature (°C)					
Inoculant		Epiphyte		Yeast and Mould	
Lactobacillus buchneri[1]	45°C	*Escherichia*[1]	70°C+	*Aspergillus spp*[7,8]	60°C+
Lactobacillus plantarum[3]	42°C	*Klebsiella*[4]	60°C+	*Candida spp*[9]	60°C+
Lactobacillus paraplantarum[2]	43°C	*Salmnonella*[5]	55°C+	*Fusarium spp*[7]	60°C+
Lactococcus spp (typical)[2]	45°C	*Shigella*[5, 6]	50°C+	*Penicillium spp*[7]	60°C+
Lactobacillus brevis[1]	43°C				

([1] Bergeys; [2] Milic; [3] Lahtinen; [4] de Veen; [5]Jones; [6]Pepper; [7]Bollen; [8]Dornsch; [9]Dumalisile

Epiphytic bacteria and the fungal species found on forage and in silage are more thermally stable, with their mechanics having adapted in order to re-cycle nutrients under both aerobic and anaerobic conditions, and adapted to the higher temperatures encountered within these recycling roles. These organisms, when lactic acid bacteria (LAB) stability challenging temperatures are reached, start to play a greater role in the initial fermentation role, adversely impacting the fermentation profile and DM recovery, as well as reaching higher levels in the silage which in turn leads to greater aerobic challenge to the silage at feed out (Kleinschmidt and Kung showed a direct correlation to silage yeast levels and aerobic stability).



The impact of temperature on inoculant bacteria was highlighted by in work at the University of Delaware. Mulrooney and Kung (2008) hydrated commercial inoculants at various temperatures and noted significant drop in viability of some commercial inoculants even at 35 °C, but when the products were hydrated at 45 °C (Graph 1) the viability of the commercial inoculants on the market at that time very rapidly dropped, with some commercial products being completely dead after 3hours of 45 °C temperature under laboratory conditions. Under field conditions of increased stress (gas environment, fluctuation in temperature, water activity stress, pH change) the reduction and rapidity in reduction of viability will be even greater.

Considering only the significant silage producing areas of Central and Eastern European countries, Austria, Bulgaria, Croatia Czech, Greece and Hungary all recorded ambient air temperatures peaking in excess of 40 °C in 2018, meaning that some silages in these countries will likely reach in excess of 50 °C during the initial period of ensiling, with many forages in Oceania and across North America being ensiled at significantly higher temperatures.

Fermentation organisms and hence the resultant fermentation of ensiled forage is impacted increasingly by the effects of global warming, with the impacts being felt sometimes regionally, sometimes nationally, but increasingly frequently. It is crucial to farmers' profitability that farmers, advisers and the industry as a whole recognise the issues that a changing climate brings to all facets of farming as a whole, and that the silage industry adapts to meet the demands of managing forages that need to be fed rapidly after ensiling due to lack of feed, but also silage that is harder to ensile and less stable once feeding commences.

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USING A SILAGE INOCULANT CONTAINING *LACTOBACILLUS PLANTARUM* MTD/1 IN A MULTI-CUT SYSTEM FOR GRASS SILAGE

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ABSTRACT

Using laboratory scale silos (1.8 L), we followed the fermentation of grass from south west Wales, cut and ensiled as part of a multi-cut system every four weeks from early May 2018. Forage from the first four cuts, with DM ranging from 28 – 50%, was either untreated (U) or treated with Ecosyl 100 (ES) applying *Lactobacillus plantarum* MTD/1 at 1×10^6 cfu/g. The U silage from all four cuts fermented poorly with recorded pH after three months ranging from 4.49 – 5.88, whereas ES silage consistently achieved significantly lower pH ranging from 3.74 – 4.07 ($p < 0.0001$). Consequently, the early stages of fermentation in U silages were dominated by enterobacteria, which were inhibited significantly ($p < 0.001$) at the low pH of the ES silage. Untreated silage from cuts 1, 2 and 4 also accumulated butyric acid (BA) to levels more than 10 times higher than in ES silage ($p < 0.0001$). Overall, the improved fermentation in the ES silage resulted in DM losses 30 – 55% lower than those of the U silage ($p < 0.0001$). This study highlights some of the challenges of a multi-cut system and clearly demonstrates the benefits of using a homofermentative silage inoculant.

INTRODUCTION

A multi-cut system for making grass silage, where younger grass is harvested at more frequent intervals during a growing season, can allow production of higher quality silage whilst maintaining similar yields to a conventional cutting system. However, the reduced time between cuts can lead to problems with fermentation owing to increased crop buffering capacity due to residual nitrogen fertiliser and/or higher crude protein (CP) levels (Binnie and Harrington, 1972), as well as slurry contamination. In this study, we tested the ability of the Ecosyl 100 silage inoculant to influence the fermentation process in forage cut as part of a multi-cut approach aiming to cut every four weeks from early May 2018.

MATERIALS AND METHODS

The crop, harvested at farm in south west Wales, was a four-year-old perennial ryegrass ley which received nitrogen at 111 units/ha and slurry at 34 m^3/ha six weeks prior to 1st cut. The crop also received nitrogen at 86 units/ha and slurry at 17 m^3/ha after each cut. Slurry was applied using a trailing shoe to minimise contamination of the grass crop. A multi-cut approach was used aiming to harvest six cuts, every four weeks, from early May 2018.

We ensiled material from the first four cuts of grass and measured the fermentability coefficient (FC) of the forage as ensiled using the method of Weissbach et al. (1974). Forage was either untreated (U) or treated with Ecosyl 100 (ES; Volac International Ltd, UK), applying *Lactobacillus plantarum* MTD/1 at 1×10^6 cfu/g, and ensiled for up to three months in laboratory scale silos (1.8 L). Silos were destructively sampled after 2-3, 6-9 or 90-95 days. Fermentation acids and alcohols were assayed by HPLC (Muck and Dickerson, 1988). Ammonia was measured using a YSI 2950D Biochemistry Analyzer and microbiology counts were performed using the method of Miles and Misra (1938) on MRS, VRBGA and RCA agar (Oxoid). DM was determined by drying in an oven at 60°C for 48 h. Samples of fresh grass were analysed approximately every 2 weeks during April to September 2018 using NIR (Sciantec Analytical, UK) and for microbiology counts using the methods above. All outcomes were modelled using a linear model in R (v3.5.1).

RESULTS AND DISCUSSION

Analysis of the fresh crop from late April to early September 2018 indicated that the grass was highly digestible (average D value of 75) and high in protein (average crude protein (CP) of 19.2%) as expected of young grass harvested as part of a multi-cut system. The FC of forages from cuts 1 and 2 were 40 and 56 respectively. No assessment was made for cut 3, and cut 4 had an FC of 30. Weissbach et al. (1974) considered forages with and FC of > 35 to be relatively easy to ensile in most circumstances. Counts of epiphytic lactic acid bacteria (LAB) and enterobacteria were within previously reported ranges (Pahlow *et al.*, 2003), but enterobacteria were at higher levels than LAB (average 4.62 and 3.74 $\log_{10}$cfu/g respectively).

After three months of ensiling, the pH of ES silage was significantly lower than U silage in all four cuts ($p < 0.0001$) due to a homofermentative lactic acid fermentation resulting in a significantly higher ($p < 0.0001$) proportion of lactic acid as a percentage of the total fermentation acids (LA % TFA; Table 1). Anaerobically stable silage is dependent on a low pH which inhibits the activity of undesirable microorganisms such as enterobacteria and clostridia (Pahlow *et al.*, 2003). Enterobacterial counts were significantly higher in U (5.60 to 9.18 $\log_{10}$ cfu/g), relative to ES (2.92 to 6.50 $\log_{10}$ cfu/g), silage from all four cuts during the first 6-9 days ($p < 0.001$). The presence of enterobacteria in silage is undesirable as their facultatively anaerobic nature means they can compete with lactic acid bacteria for available nutrients. Further, enterobacterial fermentations generally result in high DM and energy losses (McDonald *et al.*, 1991) and are also commonly responsible for producing

ammonia (NH_3) in silage (Spoelstra, 1987). DM losses and NH_3 levels were consistently lower in ES, relative to U, silage from all four cuts ($p < 0.0001$, Table 1).

Table 1. Fermentation and microbiology analysis of grass silages after three months ensilage.

Cut	Treatment	pH	DM loss (%)	BA (g/kgDM)	NH_3 (g/kg DM)	LA % TFA
Cut 1	U	4.88	9.97	33.89	4.33	20.18
	ES	3.82	7.01	2.19	1.85	91.51
Cut 2	U	4.49	10.10	27.67	3.71	42.52
	ES	3.74	4.54	ND*	1.00	95.48
Cut 3	U	4.99	4.87	ND*	1.33	80.81
	ES	4.07	3.41	ND*	0.74	95.84
Cut 4	U	5.88	13.53	46.96	10.47	2.27
	ES	4.06	6.42	3.05	4.22	84.34
Treatment		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Cut		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Treatment × Cut		< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

*None detected

Untreated silage from cuts 1, 2 and 4 accumulated BA to levels more than 10 times higher than in ES silage ($p < 0.0001$, Table 1). BA in silage is usually associated with clostridial activity resulting from soil and/or slurry contamination. Isolates sharing high sequence identity (> 99%) with the 16S rRNA gene of *Clostridium tyrobutyricum* ATCC 25755 were present at between 6.1 – 6.5 $\log_{10}$cfu/g of U silage after three months in cuts 1 and 2 (putative clostridial isolates form cut 4 were not identified by DNA sequencing). This clostridium was likely responsible for the BA fermentation in these silages. Like the enterobacterial fermentations outlined above, clostridial fermentations resulting in BA also cause high DM and energy losses (McDonald *et al.*, 1991). It is probable that the low pH of the ES silage in cuts 1, 2 and 4 prevented the accumulation of BA as clostridial activity is generally highly restricted by pH lower than 4.5. However, clostridia may also be inhibited at a higher pH at higher silage DM (Pahlow *et al.*, 2003). As such, the high DM (50%) of the forage in cut 3 likely contributed to the absence of BA in both U and ES silages at this time.

Both treatment and cut significantly influenced all the fermentation parameters examined in this study. However, there was also a significant interaction between cut and treatment (Table 1); on average, the treatment effect was different across the four cuts. Whilst many factors may have contributed to the differences observed by cut, the wide range of DM of the forage as ensiled (37%, 32%, 50% and 28% for cuts 1 to 4 respectively) was likely a significant influencing factor as crop DM, and associated differences in water activity, is known to influence bacterial activity and therefore the fermentation process (Pahlow *et al.*, 2003).

CONCLUSIONS

In the multi-cut system used during this trial, treating with Ecosyl 100 consistently improved silage quality across all four cuts, significantly reducing BA, preserving more of the DM and reducing NH_3 levels.

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HETEROFERMENTATIVE LAB AND 1,2-PROPANEDIOL PRODUCTION IN MAIZE SILAGES WITH LOWER DRY MATTER CONTENT

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ABSTRACT

The aim of this work was to evaluate and compare the effects of two combinations of homofermentative and heterofermentative lactic acid bacteria on the production of propane-1,2-diol in maize silages produced under laboratory conditions from low dry matter that were harvested at different stages of vegetation development (21/08, 28/08, 05/09, and 19/09/2017). Each sample consisted of 10 whole plants that were collected from 5 randomly selected silage maize hybrids (FAO group: 130, 170, 220, 230 and 240) on the experimental field (650 m.a.s.l.). We used two different types of fermentation additives which both contained homofermentative and heterofermentative lactic acid bacteria. The control group was silaged without the additive used. When using additive A, the concentration of propane-1,2-diol (DM basis) in silages reached clearly and significantly the highest concentrations. The differences in propane-1,2-diol concentration when using additive A compared to additive B and to the control group were statistically significant ($p<0.001$), despite relatively short fermentation (31-41 days, average: 36.75 days). The maximum average concentration of propane-1,2-diol was reached in the third sample collection: 24,10 ± 6,86 g/kg DM.

Key words: *maize silage, low dry matter content, Lactobacillus buchneri, 1,2-propanediol*

INTRODUCTION

The development and course of silage fermentation is fundamentally influenced by the fermentation microflora, which is primarily composed of epiphytic microorganisms. The course of fermentation is achieved by a targeted addition of various species and phyla of lactic acid bacteria (LAB) that are isolated from nature. Acetic acid is a dominant factor enhancing aerobic stability of silages (Driehuis F. et al., 1999 - b; Danner et al., 2003). In anaerobic conditions, the acetic acid is increasingly produced by heterofermentative lactic acid bacteria, not only from water-soluble sugars, but also by fermentation of lactic acid (Driehuis, F. et al., 1999 - a). Propane-1,2-diol and traces of ethanol are also formed during the transformation of lactic acid into acetic acid (Oude Elferink et al., 2001, Danner et al., 2003). Propane-1,2-diol does not affect the increase of aerobic stability (Danner et al., 2003), but, at the same time, it also represents an important glucoplastic agent in the diet of high-producing cows.

The aim of our work was to evaluate and compare the effect of two combinations of homofermentative and heterofermentative lactic acid bacteria on the production of propane-1,2-diol in maize silages from lower dry matter harvested at different stages of vegetation development.

MATERIALS AND METHODS

Each sample consisted of 10 whole plants that were collected from five selected maize silage hybrids (FAO group: 130, 170, 220, 230, and 240) on the experimental field in PD Nová Ľubovňa (650 m.a.s.l.). Green plant samples were collected in four stages of vegetation development (21/08, 28/08, 05/09, 19/09/2017). The average weight of ten green maize plants for the first sample collection was 5.92 kg; 5.97 kg for the second; 6.56 kg for the third; and 6.44 kg for the fourth. The dry matter content of green plants did not exceed 28%, which was caused by the weather conditions in the given year.

Table 1: **Calibration models - FT-NIR Analyzer**

nutrient parameter	RMSEC	$R^2_{calibration}$	RMSEP	$R^2_{prediction}$
NL	3,29	0,9527	3,07	0,9471
Crude fat	3,84	0,9905	4,87	0,9917
ash	5,97	0,9712	5,06	0,9618
NDV	19,1	0,9329	19,7	0,9283
ADV	12,0	0,9378	10,8	0,9322
starch	19,1	0,9823	15,7	0,9919
Water-soluble sugars	2,97	0,9451	2,45	0,9302

Green samples of whole plants were mechanically cut into particles smaller than 3 cm in the laboratory to achieve the best homogeneity and representativeness. Three silage alternatives were preserved from each green cut sample: control group (no preservatives added), additive A (*BONSILAGE FIT M: 1k2075 - Lactobacillus buchneri, 1k20711 - Lactobacillus rhamnosus, 1k2079 - Lactobacillus plantarum*, number of *BMK*: min 3.0 x 1011 in 1 gr; dose: 1 gr / 1 t) and additive B (*BONSILAGE SPEED M: 1k2075 - Lactobacillus buchneri, 1k20711 - Lactobacillus rhamnosus, 1k20752 - Lactobacillus diolivorans*; number of BMK: 2.5 x 10 11 in 1 gr; 1 gr / 1 t. CO_2 gas was applied for more consistent displacement of oxygen into the samples stored in polyethylene bags (1000 gr of inoculated matter). Consequently, the samples were vacuum-sealed and stored in a dark room at the temperature of 17 ° C during the fermentation period, which lasted from 31 to 41 days.

Table 2: Nutritional composition of green maize

date	dry matter	NL	fat	ash	starch	sugars	NDV	ADV
	%	gr/kg dry matter						
21.8.2017	17,75	82,40	24,33	58,67	77,68	259,51	471,27	287,01
28.8.2017	19,36	80,10	23,97	57,14	111,45	244,47	470,72	287,47
5.9.2017	19,79	77,78	25,50	56,30	139,69	252,13	456,75	277,65
19.9.2017	23,75	73,37	26,92	52,93	234,21	188,46	408,45	250,00

Table 3: Nutritional composition of silages

Ensilaging date	additive	dry matter	NL	fat	ash	starch	sugars	NDV
		%	gr/kg dry matter					
21.8.2017	A	16,11	86,35	30,41	66,58	35,17	19,43	520,63
	B	16,21	82,82	27,63	66,40	42,86	14,67	517,90
	K	16,24	86,70	27,17	59,85	33,19	29,99	517,08
28.8.2017	A	17,69	78,99	25,86	59,48	72,77	25,14	501,47
	B	17,85	79,58	26,99	59,64	77,49	18,99	482,56
	K	18,00	85,78	26,01	60,66	81,93	31,67	486,17
5.9.2017	A	18,05	75,85	26,80	63,39	110,51	22,90	476,80
	B	17,77	78,52	27,13	63,15	124,12	14,73	495,82
	K	18,96	83,57	24,39	64,35	121,30	34,92	469,99
19.9.2017	A	23,17	72,01	28,40	51,06	232,26	21,49	398,28
	B	22,28	72,95	26,21	54,86	224,95	14,62	425,60
	K	23,86	75,70	26,45	54,97	236,56	32,53	412,84

Note: * Control

Nutritional parameters (nitrogenous substances, crude fat, ash, NDV, ADV, starch and water-soluble sugars) were analyzed from a dry ground sample using Antaris II FT-NIR Analyzer, using own calibration models (Table 1).

Fermentation parameters: **pH** from aqueous extract using HANNA HI 221 instrument; **lactic acid and volatile fatty acids** were tested using Dionex UltiMate 3000 Series UHPLC system - Thermo Fisher Scientific Inc .; ACCLAIM OA 5µm 4 x 250mm Column, UV detector; propane-1,2-diol was tested using a Hewlett Packard 5890 series II; Restek stabilwax Column, length 30m, ID 0.53mm, df 1µm; carrier gas: N2; FID detector.

Statistical analysis was conducted using the NCSS 12 (64bit) Program and Microsoft Office 365 - Excel.

Table 4: **Fermentation composition of silages**

Ensilaging date	**additive**	**pH**	**Lactic acid**	**Acetic acid**	**Butyric acid**	**propan-1,2-diol**	**Fermentation length**
		%	gr/kg dry matter				days
21/08/2017	A	3,78 ± 0,03	81,13 ± 11,16	34,37 ± 4,65	0,00 ± 0,00	10,91 ± 3,15	36
	B	3,80 ± 0,04	67,93 ± 7,81	63,03 ± 8,66	0,00 ± 0,00	0,05 ± 0,10	37
	K	3,74 ± 0,03	97,80 ± 9,48	25,68 ± 5,62	0,00 ± 0,00	0,66 ± 0,54	38
28/08/2017	A	3,72 ± 0,07	90,69 ± 11,75	37,94 ± 10,16	0,00 ± 0,00	9,02 ± 3,15	39
	B	3,69 ± 0,03	88,91 ± 8,29	60,39 ± 11,28	0,00 ± 0,00	0,04 ± 0,07	42
	K	3,78 ± 0,08	77,45 ± 12,73	42,56 ± 15,43	0,00 ± 0,00	0,40 ± 0,38	42
05/09/2017	A	3,73 ± 0,04	73,08 ± 9,00	38,71 ± 6,76	0,00 ± 0,00	24,10 ± 6,86	41
	B	3,74 ± 0,04	61,48 ± 9,16	52,22 ± 11,00	0,00 ± 0,00	0,00 ± 0,00	41
	K	3,73 ± 0,02	81,35 ± 7,71	29,06 ± 2,04	0,00 ± 0,00	0,37 ± 0,25	41
19/09/2017	A	3,80 ± 0,04	62,16 ± 4,36	27,38 ± 3,42	0,00 ± 0,00	19,98 ± 5,68	31
	B	3,74 ± 0,05	54,54 ± 4,48	45,00 ± 6,73	0,00 ± 0,00	0,04 ± 0,05	34
	K	3,74 ± 0,03	64,60 ± 5,40	16,75 ± 1,44	0,00 ± 0,00	0,32 ± 0,30	34

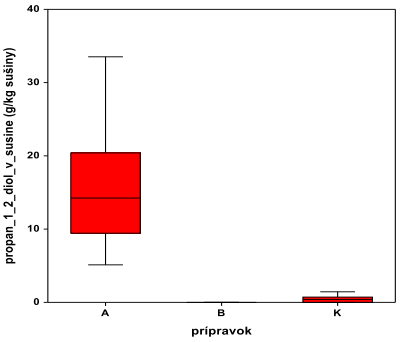
RESULTS

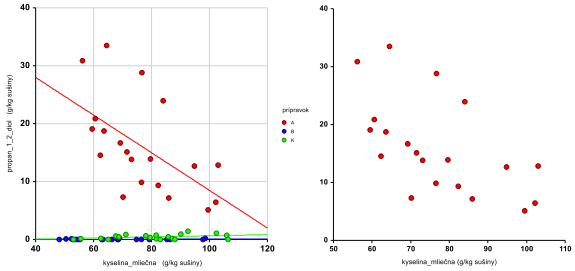
The average dry matter content during an individual collection of green samples ranged from 17.75 to 23.75%. The average starch concentration in the dry samples of the green samples increased by 7.77% to 23.4%, and the concentration of water-soluble sugars reversed inversely from 25.95% to 18.85% (Table 2).

The dry matter content of the ensilaged material ranged from 16.11% to 23.86% and copied the initial dry matter content. The average starch concentration in the silage dry matter ranged from 3.32% to 23.66% and the concentration of water-soluble sugars in the dry matter ranged from 1.46% to 3.49% (Table 3).

The pH of the silage ranged from 3.64 to 3.93. The lactic acid concentration in the dry matter ranged from 48.14 to 103.42 gr/kg of dry matter and a concentration of acetic acid ranged from 15.00 to 80.72 gr/kg of dry matter. Butyric acid was not detected in experimental silages. The propane-1,2-diol concentration ranged from 0 to 33.49 gr/kg of dry matter. The concentration of propane-1,2-diol in silage dry matter (Graph 1) reached clearly and significantly the highest concentrations when using additive A. Differences in propane-1,2-diol concentration using additive A when compared to additive B and to the control group were statistically significant (<.001).

Graph 1 **Propan-1,2-diol in dry matter silages**





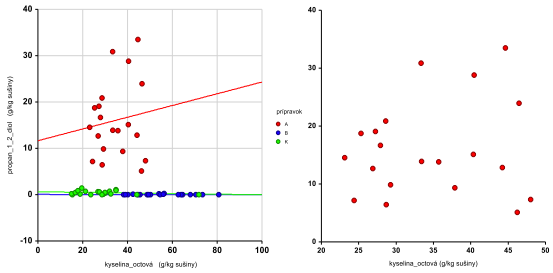
Graph 2 **Relationship between propane-1,2-diol and lactic acid concentrations in the dry matter of experimental silages**

Graph 3 **Relationship between propane-1,2-diol and acetic acid concentrations in the dry matter of experimental silages**

The relationship between the concentration of propane-1,2-diol and the concentration of lactic acid in the dry matter when using additive A (Graph 2) is statistically significant (p= 0.0078, r= -0.5763). The relationship between the concentration of propane-1,2-diol and the concentration of acetic acid in the dry matter when using additive A (Graph 3) is statistically insignificant (p= 0.5891, r= 0.1289).

DISCUSSION

By combining one phylum of Lactobacillus buchneri with two homofermentative phyla in additive A, a relatively pronounced production of propane-1,2-diol was achieved, despite relatively short fermentation (31-41 days, mean: 36.75 days).



In fermentation of maize silages inoculated with homofermentative BMK and treated with acidic preparations which lasted from 90 to 141 days, Weiss et al. (2005) reached an average of 6.1 gr of propane-1,2-diol in 1 kg of dry matter (range: 0.6 to 12.6 gr/kg of dry matter). These results are consistent with our findings of low concentrations of propane-1,2-diol in the control silages in which the epiphytic microflora was applied.

On the contrary, Kleinschmit and L. Kung, Jr. (2006), found no production of propane-1,2-diol by day 42 of the fermentation when using a combination of L. buchneri and Pediococcus pentosaceus. However, when applying additive A, the production of propane-1,2-diol proceeded relatively early and rapidly (average fermentation time: 36.75 days).

The highest production of propane-1,2-diol (average: 24,20; min.: 13,90; max.: 33,49 gr/kg of dry matter) was found in the silage samples from 05/09/2017 with the longest fermentation period. These findings are in line with Nishino et al. (2003), who achieved a concentration of propane-1,2-diol at the level of 30.1 gr/kg of dry matter and 49.4 gr of propane-1,2- diol in 1 kg of dry matter on the 60th and 120th day of fermentation in maize silages inoculated with L. buchneri, respectively.

The decrease in lactic acid concentration and the simultaneous increase in propane-1,2-diol concentration when using additive A together indicate that L. buchneri ferments lactic acid to propane-1,2-diol. However, we cannot exclude the fact that the same L. buchneri in the additive B also produced propane-1,2-diol, which, could have been further transformed to propanol and propionic acid by the used L. diolivorans that were not observed in this experiment.

A positive effect on the metabolism of production cows during their transition period was found by Butler et al. (2006) when providing them with a daily dose of 500 gr of pro-1,2-diol. If we assume a feeing consumption of 15 kg of maize silages dry matter treated with the additive A, it opens up a potential for production cows' daily intake of 250 to 500 gr of propane-1,2-diol. That already represents a significant positive effect on the metabolism and health of cows.

CONCLUSIONS

Even in the fermentation of maize silages with a low dry matter, a significant level of propane-1,2-diol production was achieved by means of using additive A already during a 38-day-lasting silage fermentation. That confirms the declared characteristics and properties of additive A.

In the silages preserved by additive B, a very rapid production of acetic acid was obtained. That lays the foundations of an effective aerobic stability and confirms the declared features and properties of additive B.

The results and differences between additive A and B indicate that the performance of the same L. buchneri phylum in propane-1,2-diol production is highly likely to be dependent on its use and action in combination with other phyla of Lactobacillus species. Proportional representation of this phylum may also play a role when compared to other BMK in preparations.

The results achieved in this study are a challenge for further systematic monitoring of a wider range of parameters (propanol, propionic acid, etc.) during the fermentation of maize silages. It should, however, be taken into account that the composition and number of germs of the epiphytic microflora can significantly affect the results.

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THE FERMENTATION CHARACTERISTICS, AEROBIC STABILITY AND MICROBIAL POPULATION OF MAIZE ENSILED IN LABORATORY AND BIG BALES

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INTRODUCTION

Silage quality and nutritive value depend on the production of sufficient amount of organic acids in the right ratio to inhibit the activity of undesirable microorganisms under anaerobic conditions (Muck, 2013). Fermentation products may also contribute the silage aerobic stability under certain conditions (Hafner et al., 2013). A high temperature of the silage can accelerate the number of aerobic microorganisms that further accelerate the process of silage deterioration. (Auerbac, 2018; Borreani et al, 2018). The aim of the study was to compare the differences in fermentation, microbial population and aerobic stability for inoculated whole crop maize ensiled in big bales and laboratory-scale silos.

MATERIALS AND METHODS

Whole crop maize (*Zea mays*) was harvested at dough stage physiological maturity of grain adjusted to achieve a 10 mm theoretical cut length and was immediately ensiled in big bales (1.2 m diameter × 1.2 m height) either untreated or treated with the bacterial silage inoculant containing *Lactococcus lactis* DSM11037 and *Lactobacillus buchneri* DSM22501 50:50 (SiloSolve® FC) at 1.5 × 10^5 colony forming units g^{-1} (cfu g^{-1}) of herbage. The 20 big bales (10 from each treatment) were marked with individual tracking numbers for later identification and were stored outside without stacking and remained undisturbed for 120 days. For laboratory scale experiment the same chopped maize forage as for big bales was ensiled in laboratory silos of 3.0 L content with the same inoculants and concentration as for big bales. The 20 laboratory silos (10 from each treatment) were made (5 for chemical and microbial analyses and 5 for aerobic stability measurement). In order to determine dry matter (fermentation) losses, the laboratory scale silos (5 from each treatment) respectively the bales (5 from each treatment) were weighed before and after the storage period. At day 120 of storage all the silages were sampled for the chemical analysis and microbial counting. Aerobic stability was determined by the amount of time it takes to exceed the ambient temperature more than 3 ^oC. Data were statistically analysed as a randomized complete block by using the GLM procedure of SAS.

RESULTS AND DISCUSSION

At silo opening (day 120 of storage) the values of chemical composition of the big bale and laboratory silage were within the range for maize silage values (Table1).

Table 1. Chemical composition, DM loss, fermentation pattern and microbial counts of the big bale and laboratory maize silages after 120 days storage period and after exposure to air

	Big bale silage			Laboratory silage		
Treatment	T1B	T2B	SE	T1L	T2L	SE
	After 120 days fermentation period					
DMc g kg^{-1}	315.6	323.4*	1.722	315.9	320.7**	0.587
DM loss g kg^{-1}	85.9	54.7**	3.142	67.7	49.4**	1.536
NH_3-N g kg^{-1} N	72.15	55.65**	2.154	81.43	61.54**	3.412
Alcohols g kg^{-1} DM	16.86	11.21**	0.949	20.63	11.45**	0.747
Lactic acid g kg^{-1} DM	22.92	39.98**	1.202	37.75	65.69**	2.403
Acetic acid g kg^{-1} DM	8.54	17.47**	0.456	19.01	33.73**	1.063
Butyric acid g kg^{-1} DM	2.20	0.37**	0.241	1.19	0.15**	0.119
Propionic acid g kg^{-1} DM	0.56	0.70**	0.080	0.82	1.27**	0.076
pH	4.07	3.91**	0.009	3.91	3.79**	0.013
LAB log_{10}cfu g^{-1} FM	5.60	7.27**	0.162	6.69	8.14**	0.174
Yeast log_{10}cfu g^{-1} FM	4.55	2.36**	0.169	3.79	1.56**	0.097
Mould log_{10}cfu g^{-1} FM	2.56	1.22**	0.091	2.05	1.06**	0.051
	After exposure to air					
DM g kg^{-1}	288.1	301.3*	3.360	285.2	294.7 ^{ns}	3.587
DM loss g kg^{-1}	24.2	12.4**	1.523	29.5	20.5**	0.980
pH	4.93	4.31**	0.028	7.27	4.05**	0.121
LAB log_{10}cfug^{-1} FM	5.35	7.16**	0.184	6.27	8.02**	0.099
Yeast log_{10}cfu g^{-1} FM	8.09	3.76**	0.111	8.52	4.47**	0.124
Moulds log_{10}cfu g^{-1} FM	6.11	3.68**	0.233	7.80	4.34**	0.102
Aerobic stability h	406.8	715.2**	33.669	86.40	338.40**	5.724

DM - dry matter, DMc - dry matter corrected for volatiles, NH_3 – N - ammonia nitrogen, LAB - lactic acid bacteria, cfu - colony forming units, FM - fresh matter, T1B - control big bale, T2B - inoculated (SiloSolve® FC) big bale, T1L - control laboratory silos, T2L - inoculated (SiloSolve® FC) laboratory silos; SE - standard error, * and ** - statistically significant difference at $P<0.05$ and $P<0.01$.

A significant reduction in dry matter loss was found in the inoculated silage compared to the uninoculated silage. Viable lactic acid bacteria treatment accelerated fermentation, as identified by the decreased pH ($P<$ 0.01) at silo opening compared with the untreated control. Despite the silo type, when *Lactobacillus buchneri* DSM22501 was combined with *Lactococcus lactis* DSM11037 (SiloSolve® FC), lactic acid and acetic acid content was increased ($P<$ 0.01). Hu et al. (2009) also found increases in the lactate concentration of corn silage inoculated with *Lactobacillus buchneri* and *Lactobacillus plantarum* and increases in the lactic acid and acetic acid concentrations of corn silage inoculated with *Lactobacillus buchneri* alone or combined with *Lactobacillus plantarum*. Butyric acid content, alcohols and ammonia N concentrations were reduced ($P<$ 0.01) by inoculation when compared with the control silages. Low pH values inhibit protein degradation in silages and, therefore, ammonia N concentration was lowered in the inoculated silage. When added to maize forage, viable lactic acid bacteria (LAB) dominates the resulting fermentation because the counts of residual LAB after a 120-day ensiling period were significantly higher in inoculant treated silages when compared with control silage. In the inoculated big bale silage yeast and fungi counts were significantly lower than in the untreated silages. In the treated laboratory silage count of yeasts and moulds were more than two time ($P<0.01$) lower compared to control silage.

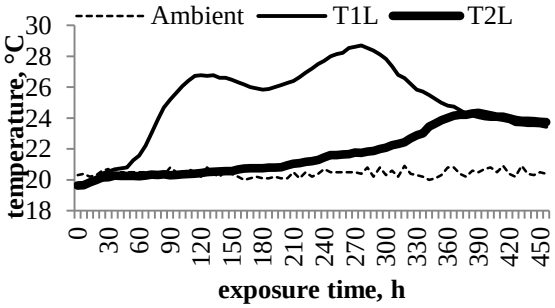

Figure 1. Temperature changes inside laboratory silo control (T1L) and inoculated (T2L)

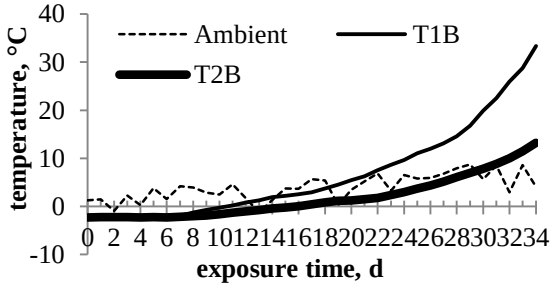

Figure 2. Temperature changes inside big bales control (T1B) and inoculated (T2B)

The parameters used to measure aerobic deterioration: temperature, pH rise, yeast and moulds growth inside big bales and laboratory silos and the growth of fungi on the surface of the big bales were changed (Table 1). The application of the silage inoculants gave a significant temperature response to treatment and improved the aerobic stability in the laboratory silos and the round bales. The silage without additives heated up earlier, and temperature increase was stronger in comparison to the bales or laboratory silos with inoculant (Figs. 1 and 2). Results indicated that the tested LAB inoculant was efficient to prevent temperature increment, yeast and fungal growth during aerobic storage of maize silage. All the parameters used to measure aerobic deterioration: pH rise, maximum temperature above ambient and sum of temperature rise, yeast and mould population showed a good correlation with dry matter loss. The final bale and laboratory silos weight, concentration of dry matter were higher, and dry matter loss and pH value were lower for inoculated silages over exposure to air period. This is in line with the higher aerobic stability as aerobic deterioration is associated with dry matter losses

CONCLUSIONS

Overall, it can be concluded that application of viable homo and hetero LAB *Lactobacillus buchneri* DSM22501 combined with *Lactococcus lactis* DSM11037 (SiloSolve® FC) did succeed in altering the silage fermentation profile, microbial characteristics and aerobic stability of maize ensiled in big bales and laboratory silos. The similarities observed between the big bale and laboratory silage showed that laboratory silage can serve as a model for big bale silage and small scale silage can be used to test the efficacy of silage additives.

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THE EFFECTS OF DOSAGE OF MOLASSES ON THE FERMENTATION QUALITY OF PAPER MULBERRY SILAGE

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INTRODUCTION

With the development of dairy industry in China, high crude protein feed is deficient. On the other hand, leaves of paper mulberry are rich in crude protein and good palatability. Because of its high value of nutrition, it has become a new kind of fodder[1]. So reasonable development and efficient utilization of woody fodder are very necessary. The downside of paper mulberry is they are lack of water soluble carbohydrate. Adding some carbohydrate in paper mulberry silage can increase the content of lactic acid and acetic acid[2]. Molasses is a kind of fermentation accelerator in silage, adding molasses can provide fermentation substrate for lactic acid bacteria and cover the deficiency of carbohydrate.

In this experiment, paper mulberry was chosen to be a represent of new developed high-crude silage material to evaluate the different dosage of molasses on the fermentation quality and nutrient content of silage. Based on the result, we may infer the best dosage of molasses in paper mulberry[3].

MARETIALS AND METHODS

Plant material is paper mulberry(*Broussonetia papyrifera*) which is collected from an plantation from Lankao, China. Plant material was harvested and cut into 4-5 cm in length by hand chopper before wilted under the sun for 3 hours. We added three different dosage of molasses in silage (1%, 2% and 3%). A blank control group was set up with addition of same volume of water. Each treatment has three replications and was sealed in polyethylene bags (25*35cm) for fermentation, about 200g plant material was put in a plastic bag. Silage bags were kept in room temperature for 180 days. After fermentation, plant material were gotten out of bags for examination.

All statistical analysis were performed by SPSS one-way ANOVA (IBM statistics 21).

Table 1. Chemical compositions of paper mulberry before fermentation.

	DM (g/kg FM)	CP (g/kg DM)	NDF (g/kg DM)	ADF (g/kg DM)	ADS (g/kg DM)	WSC (g/kg DM)
Paper Mulberry	341.8	222.8	374.3	201.9	172.4	71.5

DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADS, hemicellulase; WSC, water soluble carbohydrates.

RESULTS AND DISCUSSION

The basic nutrient value of paper mulberry is presented in Table 1, the dry matter is 34.18% FM, which is higher than 30% indicating that this kind of plant material could be made into high quality silage easier. The water soluble carbohydrate is 7.15% DM, which is a little higher than alfalfa.

As shown in Table 2, the pH value of Paper Mulberry added 2% and 3% molasses is significantly lower than that of control and the treatment of adding 1% molasses ($P<0.05$). There is no significant change in the amount of lactic acid bacteria(LAB) and the content of NH_3-N. No mold(M) or yeast(Y) was detected in each treatment.

The chemical composition of all treatments is shown in Table 3. The content of crude protein(CP) of adding 3% molasses treatment is significantly lower than any other treatment and control ($P<0.05$). But other nutrition values have no significant change, including dry matter(DM), neutral detergent fiber(NDF), acid detergent fiber(ADF), hemicellulase(ADS) and water soluble carbohydrate(WSC).

Table 2. Fermentation quality of each treatment.

	Treatment	pH	LAB (cfu/g)	M&Y (cfu/g)	NH_3-N %
Paper Mulberry	CK	4.79a	7.86	ND	8.04
	1% molasses	4.52ab	7.34	ND	7.40
	2% molasses	4.24b	7.52	ND	6.68
	3% molasses	4.30b	7.09	ND	5.96

M&Y,Mould and Yeast; ND, None Detected.

CONCLUSIONS

The nutrient composition and fermentation quality of different addition of molasses have been improved in varying degrees. After fermentation, the DM content, ADF content and ADS content of all treatment have increased while the content of CP, NDF and WSC have dropped. The data shows that those materials added 2% molasses have the lowest pH value and WSC content, the content of NH_3-N is relatively low. In conclusion, adding 2% molasses in Paper Mulberry silage can produce high-quality silage.

Table 3. Chemical compositions and protein fractions of paper mulberry silage.

	Treatment	DM (g/kg FM)	CP (g/kg DM)	NDF (g/kg DM)	ADF (g/kg DM)	ADS (g/kg DM)	WSC (g/kg DM)
Paper Mulberry	CK	344.2	203.8a	451.2	275.1	179.0	9.0
	1% molasses	345.9	183.2ab	449.0	280.2	168.8	9.2
	2% molasses	360.0	188.2ab	453.1	266.7	186.4	7.2
	3% molasses	361.9	181.7b	447.5	274.1	173.4	12.0

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EFFECTS OF DIFFERENT ADDITIVES ON THE QUALITY OF PAPER MULBERRY SILAGE FERMENTATION

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INTRODUCTION

Paper mulberry(*Broussonetia papyrifera*) belongs to the Moraceae genus (Broussonetia), a perennial arbor, which is a multi-functional tree with important value. The leaf is a high-quality protein feed. It has long been used in rural China to feed the pigs, cattle and sheep (B.W.Si etl,2008). Because of the complex molecular structure of the leaves, the bio-fermented leaves of the livestock and poultry are digested and absorbed after consumption. The rate is not high. This paper mainly discusses the effects of different additives on the fermentation quality of hybrid mulberry silage, and provides a theoretical reference for promoting large-scale planting and comprehensive utilization of mulberry.

MATERIALS AND METHODS

The material was harvested in August 2018 from the Zhuozhou District of Hebei Province (E115° 44′ - 116° 15′ ,N39° 21′ -39° 36′ , 69.4 m above sea level). About 1.2m, the height of the stubble is 30~40cm, the sample is artificial cut 1~2cm, and the silage additive were immediately added (2% sucrose fresh weight (T), 2×105 cfu / g lactobacillus fresh.Added 1kg 2 × 105 cfu / g Lactobacillus + 2% sucrose fresh weight (J + T)) into the raw material, mixed uniformly, and the control group was added with an equal volume of distilled water. Each 200g of raw material was packed into a polyethylene bag (24cm × 40cm), each process is repeated 3 times, after 53d is sealed, the sample is opened, and the silage sample is subjected to dry matter (DM), pH and organic acid concentration, protein and sugar.analysis.

RESULTS AND DISCUSSION

Table 1 shows the dry matter and fermentation quality of paper mulberry silage. The pH of the mulberry silage treated with all additives was significantly lower than that of the control group ($P < 0.05$). The lactic acid content of the mulberry stock with J was higher than that of CK, T and T+J ($P < 0.05$). Compared with CK, T and J in silage silage, J + T treatment significantly increased the acetic acid content ($P < 0.05$). There was no significant difference in the content of propionic acid between CK, T and T+J. Some of the treated butyric acid content was not detected and was detected to be close to zero. The addition of T+J can increase the number of lactic acid bacteria on the silage surface, and the mold and yeast are not detected, indicating that the silage fermentation is very good.

CONCLUSION

Silage additives are related to the fermentation quality of paper mulberry. Adding T can improve the quality of mulberry silage. J+T can be used as an effective additive to improve the fermentation quality of mulberry silage.

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Table1. Fermentation quality, chemical and microbial composition of paper mulberry silage

Items	Treatment				SEM
	CK	J+T	J	T	
Dry matter (g/kg)	330ab	340a	360a	360a	0.02
pH	5.06a	4.33c	4.15d	4.74b	1.96
NH3-N (g/kgTN)	65.6a	19.5cd	22.1c	46.6b	2.02
Lactic acid (g/kg DM)	11.7c	91.5a	114.4a	43.5b	0.51
Acetic acid (g/kg DM)	43.6c	53.3a	25.3ab	24.8a	2.00
Putyric acid (g/kg DM)	6.7cd	5.7d	8.7b	20.5a	0.02
Bropionic acid (g/kg DM)	ND	ND	ND	ND	0.03
Acid Detergent Fiber (g/kg DM)	547.5a	534.1a	446.7a	582.5a	7.95
Neutral Detergent Fiber (g/kgDM)	408.4a	408.9a	339.24a	452.7a	6.49
CP(g/kg DM)	97.0a	129.5a	109.1a	89.5a	1.96
Water-Soluble Carbohydrate (g/kg DM)	2.4c	4.0a	2.9bc	2.5cd	0.13
Lactic Acid Bacteria (log cfu g^{-1}FM)	6.10b	7.06a	6.89a	6.29b	0.43
Moulds(log cfu g^{-1}FM)	ND	ND	ND	ND	0
Yeasts(log cfu g^{-1}FM)	ND	ND	ND	ND	0

Means in the same row ($^{a-d}$) with different superscript letters differ significantly ($P< 0.05$).

EFFECT OF PROMPT AND DELAYED PACKAGING ON FERMENTATION AND AEROBIC STABILITY OF SOYBEAN CURD RESIDUE SILAGE STORED WITH AND WITHOUT BEET PULP OR RICE STRAW

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INTRODUCTION

Production of tofu and soy milk generates considerable amounts of soybean curd residue (SCR) during the manufacturing process (Hatvani, 2001). About 0.7 million tons of SCR is disposed in Japan annually, but some of them are yet incinerated and landfilled, which costs around 16 billion yen per annum for disposal (Yokoi et al., 2002). SCR is rich in highly digestible protein, fiber, and other nutrients, and thus is a potential source for lowering the cost of animal production. On a dry weight basis, SCR contains 36–38% protein, 9.3–10.9% fat, 3.8–5.3% sugars, and 52.8–58.1% dietary fiber (Nakornpanom et al., 2010). It is reported that the quality of SCR protein is better than that of other soy products (Liu, 1997). However, high moisture content (70–80%) is a great obstacle to retain the quality, whereas drying by conventional means is expensive for wide use (Radendo et al., 2008). Ensiling is thus a most feasible way to preserve and use SCR as a feed. Regardless of ensiling materials, prompt packaging is recommended to suppress activity of aerobic microorganisms and nutrients loss. In summer SCR would start spoilage and heating even within a day after production, and thus quick packaging is not necessarily secured in practice. In this study, we examined how prompt and delayed packaging can influence on fermentation and aerobic stability of SCR silage.

MATERIALS AND METHODS

Two sets of SCR were obtained from two tofu factories (SCR1 and SCR2 respectively), and then each was further divided into two subsets. The first subset of SCR was ensiled soon after production (prompt packaging), and the second subset was ensiled two days after production (delayed packaging). A 300 g of wet SCR was packaged in a plastic bag in triplicate, with and without 60 g of beet pulp or 60 g of rice straw. Air was removed using a vacuum sealer and bags were stored at room temperature. Silages were opened after 14 and 90 days to examine fermentation products and aerobic stability. A 80 g of silage was placed in a separate polyethylene bottle with a loose compaction, and a digital thermometer was placed in the center of the silage. Aerobic stability was tested for 7 days; silage was judged to have spoiled when the temperature reached 2°C above the ambient temperature (Nishino et al., 2004). For microbiota analysis, 5 g of silage sample was added to a 95 ml of sterilized phosphate-buffered saline (pH 7.4), and DNA extraction was performed as described by Yu et al. (2004). Polymerase chain reaction (PCR) was used to amplify fragments of the bacterial 16S rRNA and the fungal 18S rRNA genes; amplicons were separated via DGGE as described by Wu et al. (2014).

Table 1. Fermentation products content and microbial composition of soybean curd residue (SCR) silages prepared with and without beet pulp (BP) and rice straw (RS)

	Item	Prompt packaging			SE	Delayed packaging			SE
		Control	BP	RS		Control	BP	RS	
SCR1	DM g/kg	204 ^{b}	324 ^{a}	333 ^{a}	4.20	180 ^{b}	318 ^{a}	326 ^{a}	1.80
	pH	4.17 ^{ab}	4.03 ^{b}	4.26 ^{a}	0.04	4.03 ^{ab}	3.87 ^{b}	4.16 ^{a}	0.03
	Lactic acid (g/kgDM)	42.1	34.0	38.7	3.10	35.0	29.3	34.0	1.80
	Acetic Acid (g/kgDM)	2.99	3.98	4.26	0.40	2.70 ^{b}	3.74 ^{ab}	4.22 ^{a}	0.30
	Ethanol (g/kgDM)	2.62	3.24	3.40	0.30	1.16	2.08	0.63	0.10
	Total bacteria (log cfu/g)	6.31 ^{a}	4.11 ^{b}	3.98 ^{b}	0.20	7.31	7.23	7.07	0.10
	Lactic acid bacteria (log cfu/g)	6.14 ^{b}	6.87 ^{a}	6.19 ^{b}	0.10	7.27	7.15	7.00	0.20
	Yeasts & Molds (log cfu/g)	3.24	3.46	3.30	0.03	4.23 ^{a}	3.60 ^{b}	4.02 ^{a}	0.90
SCR2	DM g/kg	237 ^{b}	355 ^{a}	366 ^{a}	6.10	234 ^{b}	348 ^{a}	356 ^{a}	6.10
	pH	4.30	4.16	4.32	0.02	3.92 ^{b}	3.75 ^{c}	4.08 ^{a}	0.03
	Lactic acid (g/kgDM)	66.4 ^{a}	41.2 ^{c}	49.4 ^{b}	0.84	78.7 ^{a}	54.4 ^{c}	65.7 ^{b}	1.90
	Acetic Acid (g/kgDM)	8.42 ^{a}	6.94 ^{b}	8.90 ^{a}	0.23	16.4 ^{a}	7.67 ^{c}	13.7 ^{b}	1.70
	Ethanol (g/kgDM)	4.83 ^{a}	3.67 ^{b}	1.98 ^{c}	0.25	2.20 ^{a}	0.98 ^{b}	0.88 ^{b}	0.20
	Total bacteria (log cfu/g)	6.55 ^{b}	7.14 ^{ab}	7.68 ^{a}	0.10	7.25 ^{ab}	7.10 ^{b}	7.43 ^{a}	0.04
	Lactic acid bacteria (log cfu/g)	6.32 ^{a}	6.32 ^{b}	7.11 ^{a}	0.20	7.99	7.92	7.95	0.05
	Yeasts & Molds (log cfu/g)	4.21	4.21	4.17	0.30	3.80	3.71	3.86	0.10

Means for triplicate silages. Values in the same row with different superscript letters are significantly different ($P<0.05$).

RESULTS AND DISCUSSION

Regardless of factories, lactic acid predominated over the fermentation of SCR silage even without any additions. The lactic acid (LA) content was higher with SCR2 than SCR1. Addition of BP did not greatly affect the fermentation of SCR1 silage, but addition of BP and RS lowered the lactic acid and ethanol content of SCR2 silage. Addition of BP also decreased the acetic acid content of SCR2 silage. The total bacteria and lactic acid bacteria (LAB) count was significant higher by treatments with prompt packing silage, however the bacterial count prompt packed silages was lower than delayed picked silages. There was no significant difference of yeast and mold count between treatments of SCR silages, remarkably lower fungal count was detected with factory #2 of delayed packed silage.

Spoilage was observed within 7 days after aerobic exposure for promptly packaged silage. Addition of RS improved the stability but this benefit was not seen with addition of BP. Interestingly, delayed packaged silages did not spoil regardless of factory and addition of BP and RS. Looking at cluster analysis of bacterial DGGE profiles; delayed packaged silage of SCR2 were grouped separately from other silages. Fungal DGGE profiles were similar for all silages, suggesting that fungi may have not been involved in variations of aerobic stability in this study.

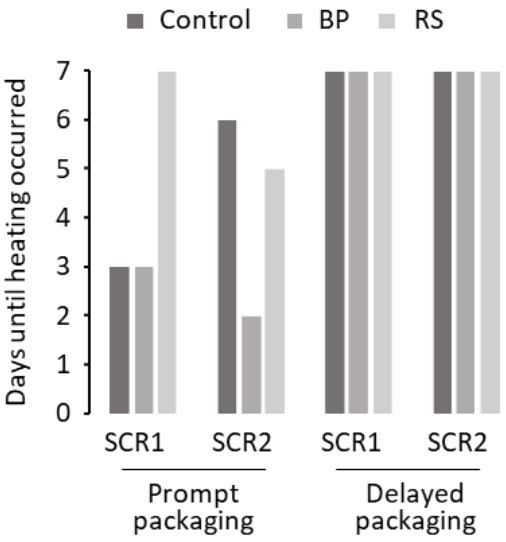

Fig. 1. Aerobic stability of soybean curd residue (SCR) silages prepared with and without beet pulp (BP) and rice straw (RS)

CONCLUSION

Lactic acid was predominated over the fermentation of all SCR silages even without any additions. Effect of mixing with BP and RS was different according to the factories producing SCR. Although silage was well preserved both for promptly packaged and delayed packaged silages, spoilage was observed only for promptly packaged silages. We need further investigation to specify what type of microbiota could account for the differences of aerobic stability between promptly packaged and delayed packaged silages.

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SILAGE QUALITY IN FINLAND

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INTRODUCTION

Grass silage consisting of timothy, meadow fescue and sometimes red clover typically covers about 50 % of dairy cow ratio in Finland. While Finnish climate is suitable for grass growth, it is not optimal for prewilting, additionally timothy and red clover are considered being crops with low sugar concentration. Despite challenges top herds achieve more than 13 000 kg ECM per lactation.

MATERIAL AND METHODS

Dairy cooperative Valio Ltd is analyzing 25 000 – 30 000 silage samples yearly, which is more than five samples per farm. Silage fermentation products are analyzed by titration method, chemical composition by NIRS and DM by owen drying. Background information is given by the farmers in the cover letter of each sample. The silage fermentation quality results from 2017 were classified by silo type, used silage additive and DM concentration. The results of two most common silo types (clamp silo and round bale) and two most common silage additive types (biological additive and formic acid based additives) were selected.

RESULTS

The silages in round bales or clamp silos cover 80% of all the silage samples. The used silage additive type was strongly linked to the used harvesting method. Silages ensiled in clamp silos were mainly (78%) ensiled using formic acid based additives, while 53% of round bales were made with biological additives (Figure 1). The bale silages included also plenty of haylages having DM above 40 %. The fermentation quality of the silages was strongly affected by the silage DM concentration, but as well silage additive and silo type had effect on the fermentation parameters (Figures 2-4).

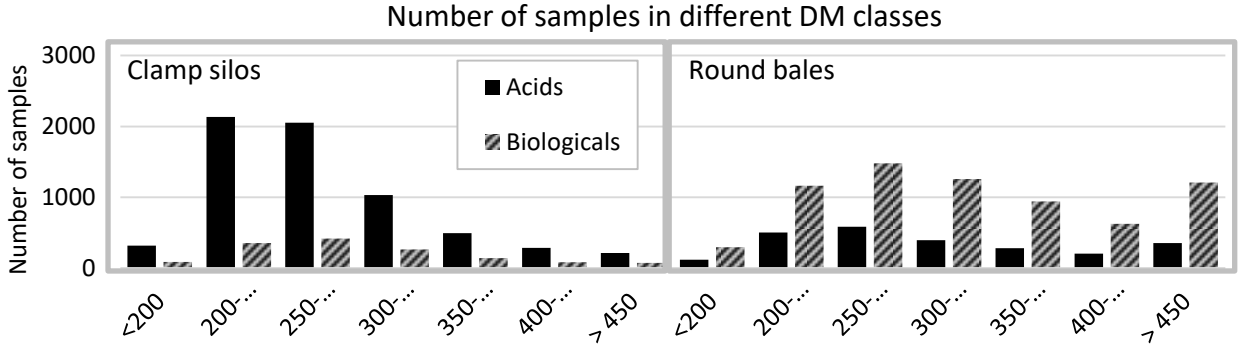

Figure **1**. Number of silage samples in DM classes separately for silo type and additive type.

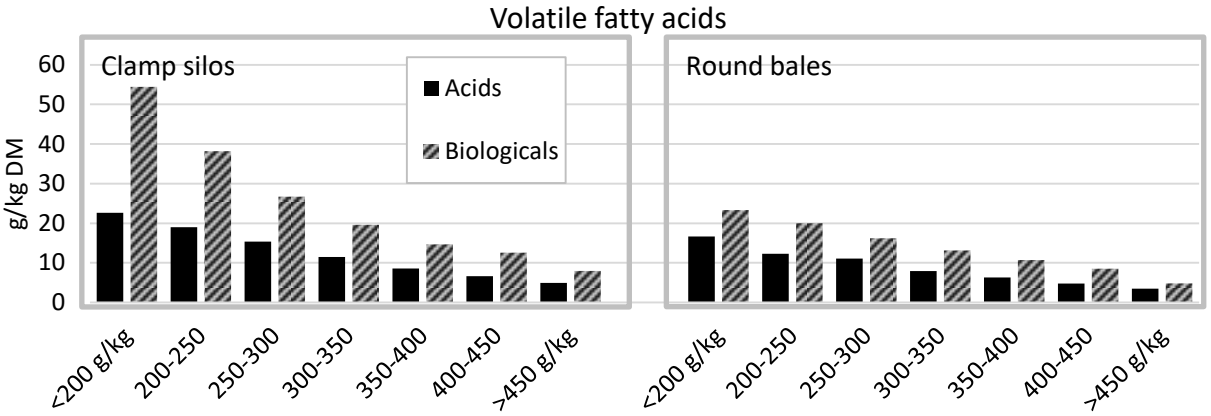

Figure **2**: Volatile fatty acid (VFA) concentration in silage samples in DM classes separately for silo type and additive type. VFA lower than 10 g/kg DM was achieved by combination of prewilting and use of additive, where formic acid based additives were more efficient than biological additives.

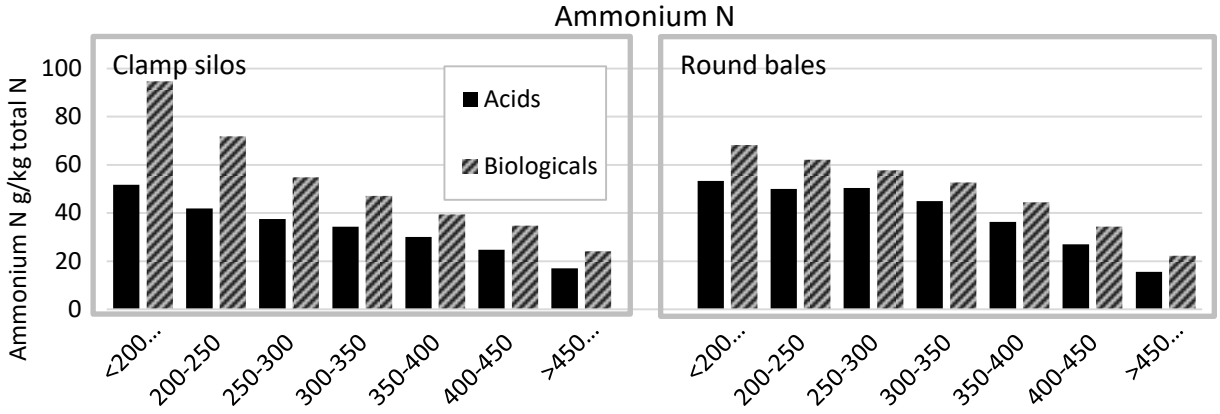

Figure **3**: Ammonium concentration in silage samples in DM classes separately for silo type and additive type. Maximum limit for ammonium N concentration in high quality silage is 40 g/kg total N. Formic acid based additives were clearly more efficient in preventing proteolysis than biological additives.

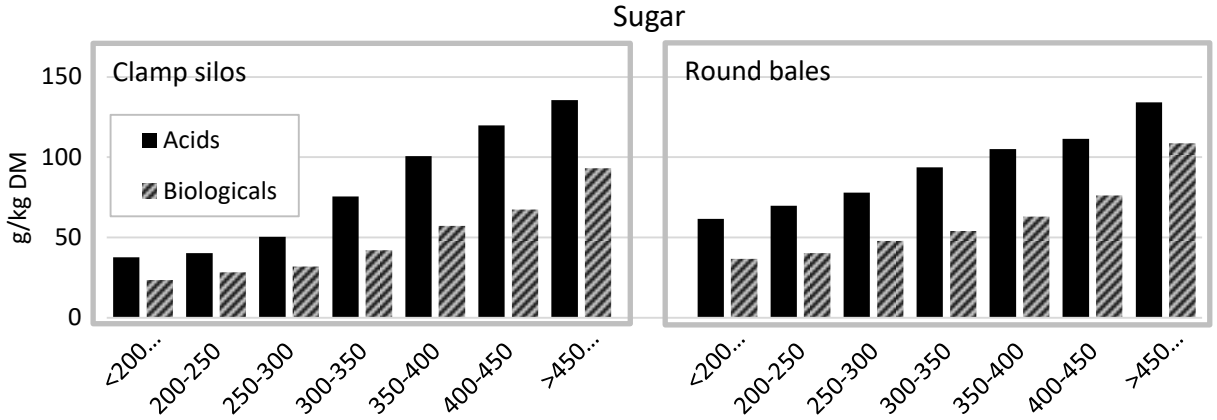

Figure **4**. Sugar concentration in silage samples in DM classes separately for silo type and additive type. Formic acid based additives were able to restrict silage fermentation as proven by the effect in sugar concentration. The effect was visible also in higher DM concentrations.

DISCUSSION

When targeting to restricted fermentation process the recommended application level of formic acid is about 4.5 kg/t. Results suggest that farmers are following the recommendation. Benefits of the restricted silage fermentation include higher silage intake, higher milk production and higher fat and protein content in milk (Huhtanen *et al.* 2003, 2007).

These results suggest, that very wet silage is more prone to spoil in clamp silo than in round bale. The fermentation in round bales may be less extensive because grass material is not precision chopped. Maybe also, airtight conditions are quicker achieved in bales. It is also possible that the raw material differs between the silo types. When interpreting the results, it is notable, that there is no information available, how big silage volume each sample represents.

CONCLUSIONS

The farm silage samples reveal, that Finnish dairy farmers are eager to use silage additives to improve their silage quality. Differences in fermentation quality between formic acid based additive and biological silage additive are clearly demonstrated in the farm silage analysis results. Seems, that farmers tend to select acid based additives in the situation when the biggest benefit is seen in the fermentation quality, namely in low DM silages ensiled in clamp silos.

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DEVELOPMENT OF AN EVALUATION SCHEME FOR AEROBIC DETERIORATION OF TOTAL MIXED RATIONS

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INTRODUCTION

Aerobic deterioration is a major source of feed losses. It is caused by aerobic respiration of microorganisms once oxygen is available again in the ensiled feed. Main end products of the metabolization of carbon sources such as lactic acid and residual sugars are carbon dioxide and water, and the release of heat. Thus, a temperature increase of 2 or 3 °C above ambient is generally assumed as indicator of the onset of aerobic instability of silages. However, an adequate description of the loss of feed quality is not inherent. Thus, the authors intend to develop an evaluation scheme which combines several simple indicators for describing aerobic deterioration in a more complex approach to address the diverse microbial processes which take place in a variety of ensiled feeds. A first step presented here is the evaluation of aerobic changes in total mixed rations with silages. We asked: Which are meaningful indicators of aerobic deterioration? Can they be combined in an evaluation scheme? Which conditions are suitable to test the efficacy of TMR stabilizers?

MATERIALS AND METHODS

From 2017 to 2019 several test runs on aerobic stability of total mixed rations (TMR) were performed. TMR containing one third grass silage, one third maize silage and one third concentrate (barley plus rapeseed meal) on DM base and TMR from the experimental farm mixed for high performing dairy cows were evaluated for their aerobic behavior, both without (CON) and with TMR stabilizers (STAB) based on potassium sorbate, sodium propionate, formate or mixtures of organic acids and their salts. In one trial, also yeasts (YEA) were applied (*Wickerhamomyces anomalus* MUCL 20294) at 10 ml/kg FM from a 24 h culture to provoke aerobic deterioration. DM concentration varied either by the native content of the ingredients or by adding defined amounts of tap water. Samples of TMR were weighed (~250 g FM) into containers made of PVC sewage tubes, closed with PVC caps (Ø 11cm) with a hole (Ø 5 mm) in the center, in triplicates to quintuplicates. A single layer of gauze was placed on the ground to avoid losses of small particles. During filling, a data logger for temperature, programmed to half-hour saving, packed in a small single-use bag (PE-HD) was included. The container was stored in a polystyrene cylinder (EPS, 6 cm thick) and covered with a lid both at the bottom and on the top as described by Honig, 1990. A constant ambient temperature was maintained. Different temperature regimes between 21 °C and 28 °C were tested. Length of storage time varied starting with 8 d and then reduced to maximum 6 d. Dry matter content, pH, lactic, acetic, propionic, butyric acid and ethanol were determined from the original mixture as well as nutrients and in some cases the initial amount of yeasts. After terminating the aerobic stability test (AST), the gross weight of the samples in their untapped container was determined, the degree of condensation on tube walls and cap was notified (0 to 4 points, dry to water is running together in the cap), evaluated for visual mould and yeast infestation (0 to 4 points, no colony to completely contaminated)(DLG TestService GmbH, 2018, modified). The pH was measured again and the DM determined at 105 °C for 24h.

Different Pearson correlations were tested in a linear regression analysis. A variance analysis on the effect of the factors DM (3 categories), ambient temperature (3 levels), addition of TMR-stabilizers (yes/no) was performed by using the GLM procedure Univariate (IBM SPSS Statistics, V19, IBM Company).

RESULTS AND DISCUSSION

The pH before the AST was 4.53 on average (±0.28). TMR contained around 42.6 g/kg DM lactic acid (±10.5), around 9.4 g acetic acid (±2.7) and a maximum of 0.4 g butyric acid. Yeasts counted between 5.6 to 6.9 log cfu/g at DM <500g/kg and <4.4 log cfu/g when DM was higher. Table 1 describes the FM losses, difference in pH, visible mould and yeast infestation, humidity development and sample temperature increase at different ambient temperatures and initial DM concentrations. TMR after 8 d of aerobic storage was completely spoiled and extremely wet. Thus yeast colonies could hardly be identified and treatments differentiated. Therefore, the time of AST was shortened.

When relating the DM concentration before the AST to the maximum temperature difference (MTD) for all samples evaluated after 3-6 d AST (n=110), R^2 was 0.50 (*P<0.001*). This correlation is both a result of the reduced heat capacity and conductivity with decreasing water content, and of limited microbial activity in a drier environment, corresponding to the findings of Rinne et al. (2018). Remaining factors are e.g. the use of TMR stabilizers and initial yeast infestation. Visible yeast occurrence (in 97 out of 110 cases) was stronger correlated with pH difference (R^2=0.53, *P<0.001*) than visible mould occurrence (mould in 25 of 110 cases, R^2=0.36, P<0.001), the same applied for yeast and MTD (R^2=0.75, P<0.001), and mould (R^2=0.17, P<0.001). However, mould occurrence was always coupled with yeast occurrence here and the effect thus could not be evaluated separately. MTD and humidity score were closely related (R^2=0.79, P<0.001), same applied for MTD and the days until temperature was 3°C > ambient (R^2=0.78, P<0.001). The correlation between FM and DM losses was weak (R^2=0.245, P<0.001), and between these losses and MTD or days until 3 °C > ambient even weaker (R^2<0.2, P<0.001). An explanation could be the shortcoming in determining the net weight because of

condensation. With regard to DM losses, for the determination of DM by oven drying there is no correction factor for fermented feeds having undergone aerobic changes. Thus, it poses a challenge to determine the real loss of mass.

Table 1 Development of TMRs under different aerobic conditions

Treatment	DM[1] (g/kg)	AST (d)	Ambient (°C)	FM losses[2]	DM losses[2]	pH increase	Y	M	H	MTD (°C)	d	n	
CON	377	3	27.5	3.91	8.79	3.21	2.5	0.6	3.4	14.3	0.8	5	<45%DM/>25°C
YEA	355	3	27.5	3.87	-0.74	3.34	2.7	0.5	3.6	13.6	0.7	5	
CON	438	3	27.5	3.53	6.25	1.90	2.8	0.0	3.8	13.9	1.4	5	
YEA	400	3	27.5	3.81		2.21	3.4	0.4	3.4	13.5	0.9	5	
CON	387	4	25.0	6.21	4.62	3.35	3.3	2.0	4.0	14.7	0.7	3	
CON	387	4	28.8	19.5	12.8	3.80	3.7	1.7	3.3	12.2	0.7	3	
CON	433	8	24.0	8.92	20.7	4.32	0.8	1.8	2.7	18.5	0.8	12	<45%DM/23-25°C
STAB	433	8	24.0	8.82	20.9	4.38	0.9	1.7	2.8	17.3	1.0	12	
CON	402	3	24.3	1.94	-1.09	0.99	2.5	0.0	3.3	12.2	1.7	6	
YEA	409	3	24.5	2.49	4.99	1.49	3.0	0.0	4.0	13.6	1.7	5	
STAB	407	3	24.3	0.69	-1.39	0.14	0.8	0.0	0.3	3.13	2.8	6	
YEA_STAB	387	3	24.5	0.56	-1.78	0.07	1.1	0.0	0.4	2.89	2.9	5	
45-50%DM/23-25 °C													
CON	454	2-3	23.5	3.36	1.67	1.55	3.0	0.0	3.0	2.33	1.2	2	
STAB	466	2-3	23.5	1.63	0.96	0.78	1.7	0.0	2.2	2.64	1.8	2	
45-50%DM/<23°C													
CON	450	3	21.1	2.62	-2.31	1.56	4.0	0.0	3.9	14.1	1.4	4	
STAB	484	3	21.1	1.06	3.42	0.42	2.8	0.0	2.0	9.34	2.4	4	
>50%DM/23-25°C													
CON	586	6	23.7	0.99	-8.45	-0.02	0.3	0.0	0.0	-0.02	>6.0	4	
STAB	579	6	23.7	0.90	-0.44	-0.02	0.1	0.0	0.0	-0.51	>6.0	12	
>50%DM/<23 °C													
CON	531	6	21.2	1.56	1.05	0.48	2.6	0.4	0.9	6.95	4.1	9	
STAB	536	6	21.2	0.68	-0.33	0.12	0.9	0.0	0.0	2.48	5.9	21	
>50%DM/>25°C													
CON	501	4	25.0	4.63	3.14	3.70	4.0	0.5	4.0	14.9	1.2	3	
DM				*0.294*	*0.066*	*<0.001*	*<0.001*	*0.788*	*<0.001*	*<0.001*	*<0.001*		*P*
Ambient temperature				*0.010*	*0.006*	*<0.001*	*<0.001*	*0.017*	*<0.001*	*<0.001*	*<0.001*		
Stabilizer				*0.078*	*0.495*	*<0.001*	*<0.001*	*0.396*	*<0.001*	*<0.001*	*<0.001*		

[1]Initial DM; [2]in %; AST aerobic stability test; Y yeasts, M moulds, H humidity (Y, M, H: points 0-4); d until >3°C>ambient

CONCLUSIONS

For testing stabilizers challenging conditions such as DM<450 g/kg and a temperature around 25 °C proved suitable. Several used parameters indicate changes of feed quality and losses. Some of them are correlated in this study. To develop a comprehensive evaluation scheme many more materials (silages and other fermented feeds) have to be tested.

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MODULATION OF BACTERIAL COMMUNITIES OF GRASS SILAGE BY ADDITIVES, COMPACTION AND SOIL CONTAMINATION

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INTRODUCTION

Identifying the bacterial community diversity of silages treated with different additives and/or untreated provides new insight into understanding the process of silage fermentation. Each specific bacterial community can shift the nutritional composition and hygienic quality of the feed and also influence ensiling losses. Optimally silage fermentation is dominated by *Lactobacillus*, but there are numerous different bacteria present, some of which are detrimental causing spoilage and fermentation losses. The objective of the present study was to trace the profile of bacterial communities of grass silages treated with different additives at different compaction levels and with or without soil contamination.

MATERIALS AND METHODS

Information of the ensiling process is described in Franco et al. (2019). Four additive treatments were used including control without additive, formic acid based additive (FA), homofermentative strains of lactic acid bacteria (LAB) and salt based additive (SALT). Silos were stored at room temperature with protection from light and opened after an ensiling period of 93 days. Samples were taken for DNA extraction, the PCR amplification of bacterial 16S rRNA gene V4 region was performed using universal primers. Sequencing was done on Illumina MiSeq and sequence data was processed using Qime v 1.9.1 pipeline. Data was analysed using a CORR and MIXED procedures (SAS Inc. 2002-2012, Release 9.4; SAS Inst., Inc., Cary, NC) of SAS at 5% of probability.

RESULTS AND DISCUSSION

Epiphytic bacteria species in fresh matter were rather low, with *Sphingomonas* and *Stenotrophomonas* genera being the most abundant. After fermentation both *Lactobacillaceae* family and as part of it *Lactobacillus* genus were dominant with *Sphingomonas* genus in most of the treatments. FA decreased the abundance of *Lactobacillaceae* family whereas LAB naturally increased it. Soil contamination reduced the amount of other *Lactobacillaceae* family ($P<0.05$), but boosted the growth of *Lactobacillus* genus ($P<0.05$), especially relative abundances in control (83.1 %) and SALT (83.3 %) silages, which may be signs of uncontrolled fermentation in them. There was a significant difference between compaction levels for *Pediococcus* genus, which was more predominant in tight than loose compaction. Pearson's correlation was performed to identify the relationships within bacterial populations as well as between bacterial communities and fermentation quality of the silages. *Devosia* genus was positively correlated with *Agrobacterium* ($r=0.98$; $P<0.01$), *Rhizobium* ($r=0.96$; $P<0.01$) and *Sphingomonas* ($r=0.92$; $P<0.01$). *Lactobacillus* genus presented a strong a negative correlation with *Mycoplana* ($r=0.63$; $P<0.01$), *Devosia* ($r=0.57$; $P<0.01$) and *Sphingomonas* ($r=0.66$; $P<0.01$), indicating that *Lactobacillus* compete for the same substrate and the ideal development conditions for them are different. Lactic acid production was negatively correlated to the presence of *Devosia* ($r=0.88$; $P<0.01$), *Agrobacterium* ($r=0.91$; $P<0.01$) and *Sphingomonas* ($r=0.86$; $P<0.01$) communities in the silage, and those same genera were positively correlated to the residual amount of water soluble carbohydrates in the final silage ($r=0.74$, 0.83 and 0.88, respectively), indicating that the fermentation was restricted. The increase in aerobic stability was well reflected by a positive correlation with *Lactobacillus* genus, but negatively correlated to *Devosia* ($r=0.70$; $P<0.01$) and *Agrobacterium* ($r=0.69$; $P<0.01$) genera and *Comamonadaceae* ($r=0.78$; $P<0.01$) family. *Sphingobacteriaceae* ($r=0.39$; $P=0.02$) and *Lactobacillaceae* ($r=0.46$; $P<0.01$) families were negatively correlated with the ammonia production and low ammonia N in total N is generally considered as a good indicator of silage fermentation quality.

CONCLUSIONS

Different types of additives resulted in varied bacterial profiles. Great shift was observed in bacterial profiles from fresh material towards silage. *Lactobacillaceae* family and *Lactobacillus* genus were below 1% of relative abundance in fresh grass, however they became predominant in the final silage together with *Sphingomonas* genus. Strong correlations between bacterial communities and fermentation quality parameters provided clear insight of the role of the most abundant populations on the fermentation process of grass silage.

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Table 1. Abundance of bacterial populations of grass silage treated with additives under different compaction (Comp) and soil contamination (Cont) levels.

Contamination		Non-contaminated					Soil contaminated							SEM[2]	P-value[3]	
Compaction		Loose				Tight				Tight						
Additive	FM[1]	Control	FA	LAB	Salt	Control	FA	LAB	Salt	Control	FA	LAB	Salt		Comp	Cont
g_Cryocola	0.47	2.45^{b}	3.84^{a}	1.27^{c}	2.63^{b}	2.80^{b}	3.82^{a}	1.33^{c}	2.26^{b}	0.42^{d}	4.07^{a}	1.09^{cd}	0.49^{cd}	0.164	0.96	<0.01
f_Sphingobacteriaceae	0.38	0.99^{bc}	2.14^{a}	0.95^{bc}	1.03^{bc}	1.24^{b}	2.09^{a}	1.11^{bc}	0.96^{bc}	0.38^{c}	2.70^{a}	0.87^{bc}	0.41^{c}	0.155	0.53	0.10
g_Pedobacter	0.67	1.34^{bcd}	2.17^{ab}	1.34^{bcd}	1.28^{bcde}	1.54^{bc}	2.22^{ab}	1.54^{bc}	1.11^{cde}	0.36^{e}	2.87^{a}	1.07^{cde}	0.47^{de}	0.184	0.60	0.02
g_Enterococcus	0.00	1.55^{b}	0.08^{c}	0.01^{c}	2.85^{a}	2.25^{ab}	0.12^{c}	0.01^{c}	2.76^{a}	2.18^{ab}	0.07^{c}	0.23^{c}	1.98^{ab}	0.205	0.28	0.95
f_Lactobacillaceae	0.03	11.66^{bcd}	2.76^{cd}	57.81^{a}	18.93^{b}	15.63^{bc}	9.78^{bcd}	59.66^{a}	14.81^{bcd}	0.19^{d}	0.83^{cd}	61.61^{a}	0.16^{d}	2.945	0.31	<0.01
g_Lactobacillus	0.01	7.24^{c}	2.24^{c}	0.27^{c}	4.89^{c}	4.62^{c}	0.94^{c}	0.26^{c}	18.80^{b}	83.12^{a}	4.89^{c}	2.49^{c}	83.30^{a}	1.501	0.03	<0.01
g_Pediococcus	0.01	2.16^{bcd}	1.17^{dc}	6.03^{a}	4.54^{abc}	4.93^{ab}	1.49^{bcd}	6.42	7.30^{a}	0.28^{d}	1.30^{dc}	6.62^{a}	0.38^{d}	0.702	<0.01	0.01
f_Leuconostocaceae	0.00	2.08^{ab}	0.58^{ab}	0.00^{b}	1.98^{ab}	0.82^{ab}	0.12^{b}	0.01^{b}	2.88^{ab}	2.77^{ab}	0.06^{b}	1.32^{ab}	3.34^{a}	0.592	0.63	0.10
g_Mycoplana	0.14	1.71^{bc}	2.12^{ab}	0.98^{de}	1.70^{bc}	1.91^{bc}	2.10^{ab}	0.92^{de}	1.43^{cd}	0.33^{f}	2.61^{a}	0.65^{ef}	0.31^{f}	0.106	0.64	<0.01
f_Aurantimonadacea	0.14	1.88^{ab}	1.13^{cd}	0.33^{e}	1.76^{ab}	2.06^{a}	0.96^{d}	0.39^{e}	1.49^{bc}	0.18^{e}	1.20^{cd}	0.28^{e}	0.18^{e}	0.099	0.47	<0.01
g_Devosia	0.19	2.28^{a}	1.75^{a}	0.69^{b}	2.06^{a}	2.33^{a}	1.75^{a}	0.64^{b}	1.85^{a}	0.23^{b}	2.09^{a}	0.51^{b}	0.21^{b}	0.118	0.55	<0.01
g_Agrobacterium	0.17	2.18^{a}	1.45^{c}	0.64	2.09^{ab}	2.17^{a}	1.43^{c}	0.55^{de}	1.59^{c}	0.18^{e}	1.68^{bc}	0.48^{de}	0.19^{de}	0.089	0.02	<0.01
g_Rhizobium	0.14	1.33^{a}	1.38^{a}	0.33^{b}	1.50^{a}	1.56^{a}	1.45^{a}	0.33^{b}	1.25^{a}	0.12^{b}	1.57^{a}	0.29^{b}	0.12^{b}	0.102	0.85	<0.01
g_Sphingomonas	1.89	16.61^{ab}	19.46^{a}	9.02^{cd}	16.16^{ab}	18.05^{ab}	17.93^{ab}	9.41^{cd}	13.57^{bc}	3.67^{e}	20.27^{a}	6.83^{de}	3.71^{e}	0.954	0.40	<0.01
f_Comamonadaceae	0.26	1.33^{a}	1.20^{a}	0.66^{b}	1.47^{a}	1.53^{a}	1.14^{a}	0.62^{b}	1.21^{a}	0.17^{c}	1.23^{a}	0.59^{b}	0.18^{c}	0.079	0.52	<0.01
g_Stenotrophomonas	1.31	3.11^{bcde}	6.74^{a}	0.67^{de}	3.66^{abcd}	2.54^{cde}	6.27^{ab}	0.68^{de}	1.05^{de}	0.35^{de}	5.51^{abc}	0.51^{de}	0.24^{e}	0.664	0.07	<0.01

Additive treatments: control without additive; formic acid (FA) based additive (AIV Ässä Na, Eastman Chemical Company, Oulu, Finland at 5 l/t); homofermentative strains of lactic acid bacteria (LAB) *Lactobacillus plantarum* (KOFASIL® LAC, ADDCON, Bitterfeld-Wolfen, Germany at 1 g/t); and salt based additive (Safesil Challenge, Salinity AB, Göteborg, Sweden at 2 l/t).

Values with same letter in a row are not significantly different at 5% Tukey test.

Taxa with overall average abundance of less than 1% were excluded from the data set.

[1]FM: fresh matter was excluded from the comparisons. [2]Standard error of the mean. [3]Effect of compactions (583 vs. 424 kg/m^3 for tight and loose compactions, respectively) and soil contamination. Tight compaction was done manually by dropping a lead plummet ten times after adding a handful of grass into the cylindrical silo, while loose compaction was done by dropping the lead plummet two times.

DYNAMIC OF FERMENTATION AND AEROBIC STABILITY OF GRASS/LEGUME MIXTURE ENSILED IN BIG BALES WITH OR WITHOUT INOCULATION

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INTRODUCTION

Numerous studies have evaluated the impact of single viable lactic acid bacteria (LAB) strains and/or the combining specific homo- and hetero-lactic bacteria strains as a silage inoculant on fermentation of the silage, the efficacy to control yeast and mould growth and to enhance silage aerobic stability (Muck et al., 2018). The objective of this study was to evaluate the effectiveness of using a silage inoculant on fermentation variables, microbial composition and aerobic stability of grass/legume silage after 8, 32 and 120 days of fermentation.

MATERIALS AND METHODS

A homogenous plot of a grass/legume crop (red clover, alfalfa and timothy 50:20:30) was divided into two blocks and was mown with disk mower-conditioner, set to place windrows and wilted up to 35.9 % DM. Wilted crop was baled into 1.2 m wide and 1.2 m diameter cylindrical bales, wrapped and then labelled. Big bales of silages were prepared without inoculant (control – C) or with blend of LAB strains *Lactococcus lactis* DSM 11037 and *Lactobacillus buchneri* DSM22501 (SiloSolve® FC) targeting a dosage of $1.5x10^5$ colony forming units (CFU) per g of fresh forage. All 60 big bales were individually weighed after wrapping and again after 8, 32 and 120 days of storage for measuring dry matter (DM) losses. Five big bales from each treatment chosen at random were sampled at day 8, 32 and 120 of storage and were tested for DM, pH, crude protein, NDF, ADF, WSC, VFA, lactate, acetate, butyrate, propionate, ammonia-N, DM losses, yeasts and moulds and total lactic acid bacteria. Temperature changes was measured inside five replicates of big bale silages by inserting 70 cm long temperature sensors into the opened bales at 5 different points. The aerobic stability was defined as the number of hours the silage remained stable before rising more than a 3°C above the ambient temperature. Visual scoring of fungal growth, area of fungal growth on the surface of bales were performed. Data were statistically analysed as a randomized complete block by using the GLM procedure of SAS.

RESULTS AND DISCUSSION

Treatment with SiloSolve® FC (SSFC) increased the fermentation rate of legume/grass silage, resulting in a lower pH at 8 days, 32 days and 120 days after ensiling. The LAB mixture led to significantly higher amount of lactic acid and acetic acid and in the significant decrease of ammonia-N, alcohols and butyric acid (Table 1). The results indicate a special fermentation pattern of the LAB mixture containing *Lactococcus lactis* DSM 11037 and *Lactobacillus buchneri* DSM22501 as a significantly higher concentration of propionic acid was observed after 32 and 120 of storage in comparison with control silage.

Table 1. Fermentation characteristics and microbiological parameters of ensiled in big bales grass/legume crop without or with inoculant SiloSolve® FC after different storage periods and aerobic stability test

	Fermentation period					
	8 days		32 days		120 days	
	C	SSFC	C	SSFC	C	SSFC
	After fermentation					
DMc, g/kg	347.0	349.5	363.3	347.2**	328.5	344.2**
DM loss, g/kg	56.3	48.4	94.8	58.6**	120.9	70.1**
NH_3-N, g/kgN	30.8	30.6	43.6	33.7**	60.1	39.3**
Alcohols, g/kg DM	6.0	5.3	9.0	6.8**	10.3	7.0**
Lactic acid, g/kg DM	19.9	26.6	33.4	57.2**	51.6	70.2**
Acetic acid, g/kg DM	10.9	13.5*	14.9	23.3**	21.1	32.9**
Butyric acid, g/kg DM	0.7	0.1*	1.4	0,3**	1.1	0.2**
Propionic acid, g/kg DM	0.2	0.2	0.2	0.4*	0.2	0.6**
pH	5.3	5.0	4.6	4.50**	4.4	4.2**
LAB, log_{10}cfu/ g FM	7.1	8.9 **	8.2	9.9**	8.0	9.4**
Yeast, log_{10}cfu/g FM	3.5	2.3**	2.9	1.1**	2.3	1.0**
Mould, log_{10}cfu/g FM	3.2	1.9**	2.6	1.6**	2.4	1.2**
	After exposure to air					
DM, g/kg	318.6	327.2 *	309.3	321.7**	302.9	317.6**
Weight loss, %	3.7	2.7 *	4.6	2.6**	3.7	1.6**
pH	7.2	5.9**	8.9	6.7**	5.0	4.3**
Yeast, log_{10}cfu/g FM	7.7	3.2**	6.6	1.9**	5.6	1.1**
Moulds, log_{10}cfu/g FM	7.0	3.6**	6.0	3.7**	6.9	1.4**
Visible mould growth, score	0.6	0,2	1.4	0,2	2.0	0.2
Aerobic stability, h	0.0	0.0	57	198**	346	720**

DM - dry matter, DMc - dry matter corrected for volatiles, NH_3 – N - ammonia nitrogen, LAB - lactic acid bacteria, cfu - colony forming units, FM - fresh matter, C - control big bale, SSFC - inoculated big bale, SSFC – SiloSolve® FC,* and ** - statistically significant difference at $P<0.05$ and $P<0.01$.

Despite the increased lactic acid and acetic acid production the significant reduction in DM loss of the inoculated silages was observed after 32 and 120 days of fermentation. Application of the inoculant significantly reduced weight loss during silage exposure to air period, resulted in an increase of lactic acid bacteria presence in the treated silages and significantly reduced the growth of yeast and mould after all three periods of fermentation and after exposure to air (Table 1). During aerobic exposure period, pH value increased in both control and inoculant treated silages, however, the pH increase of the inoculated silage was significantly lower.

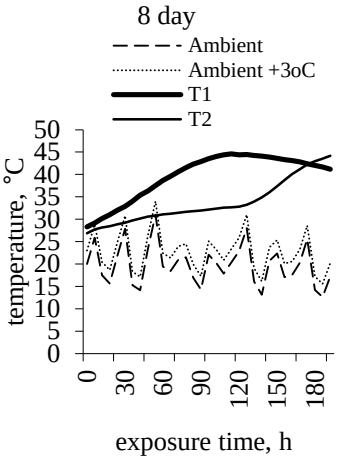

Figure 1

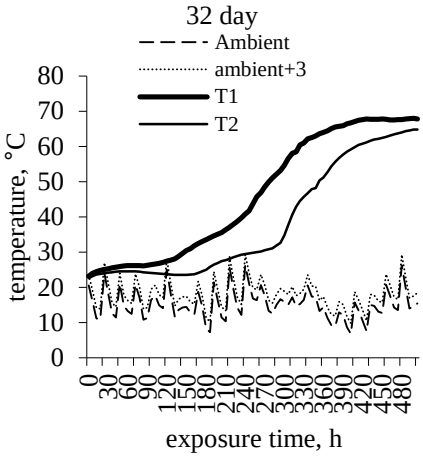

Figure 2

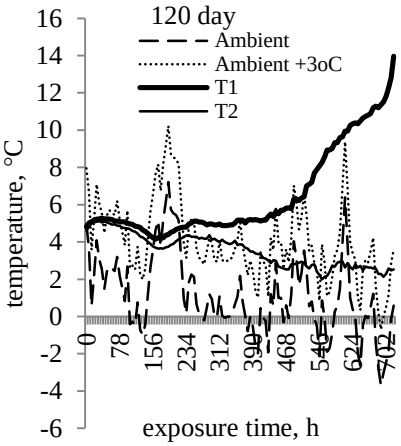

Figure 3

Figures 1, 2, 3. Temperature changes inside bales after 8, 32, and 120 days fermentation

The results clearly indicate that hetero-fermentative *Lactobacillus buchneri* DSM22501 in combination with homo-fermentative *Lactococcus lactis* DSM 11037 were able to control yeast and mould growth inside silage and to decrease the growth of fungi on the surface of the big bales. Yeasts are generally the initiators of aerobic deterioration, consuming sugars and fermentation acids and raising silage temperature and pH. Mould completes this process leading to rising temperature of the unstable silage (Pahlow et al., 2003).

The big bale silage without additive heated up earlier and temperature increase was stronger in comparison to baled silage with inoculant (Figures 1, 2, 3). Reduction in yeast and mould population during fermentation and anaerobic phase of silage conservation and during aerobic exposure appears to be the main reason for the aerobic stability improvement of the inoculated silage. The present results are in agreement with several previous studies that reported improvement in aerobic stability of different silages treated with the same inoculant (Witt et al., 2015; Copani et al., 2017).

CONCLUSIONS

The results of the present study indicate the potential of LAB mixture of *Lactococcus lactis* DSM 11037 and *Lactobacillus buchneri* DSM22501 to change the fermentation profile of big bale grass/legume silage, to decline pH, to reduce weight loss and dry matter loss and to improve aerobic stability along short and long storage periods. Inoculant was superior in to reduce ammonia, ethanol and butyric acid production and to control yeast and mould growth.

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EFFECT OF COMPACTION, SOIL CONTAMINATION AND ADDITIVE TREATMENTS ON GRASS SILAGE QUALITY

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INTRODUCTION

Silage quality in practice is still a concern and work to improve it is continuously needed. General good management practices in silage making include tight compaction to ensure anaerobic conditions and avoiding soil contamination to prevent inoculation with spoilage microbes. Additives are commonly used to improve the fermentation quality of the forage (Muck and Kung, 1997). The objective of this experiment was to evaluate how different types of silage additives are able to manipulate the ensiling process under varying management conditions represented by two levels of compaction and soil contamination.

MATERIALS AND METHODS

Mixed timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*) grass was harvested on June 4th 2018 at Luke in Jokioinen, Finland (60°48'N, 23°29'E), precision chopped using farm scale machinery and transported to laboratory without any additive. Silages were prepared using two compaction levels (Table 1). The tightly compacted grass was also inoculated with soil and dairy cow faeces. Four additive treatments were used including control without additive, formic acid (FA) based additive, homofermentative strains of lactic acid bacteria (LAB) and salt (SALT) based additive. The grass was ensiled into cylindrical pilot scale silos with 12 litre capacity using three replicates per treatment. Silos were stored at room temperature with protection from light and opened after an ensiling period of 93 days. Deteriorated parts were discarded and silage was carefully mixed and samples were taken and analysed for chemical composition and fermentation quality. Aerobic stability was evaluated by measuring the temperature with thermocouple wires automatically at 10-minute intervals from silage samples stored in polystyrene boxes. Data was analysed using a MIXED procedure (SAS Inc. 2002-2012, Release 9.4; SAS Inst., Inc., Cary, NC) of SAS at 5% of probability with additive, compaction and soil contamination as fixed effects and replicates as random effect.

RESULTS AND DISCUSSION

Grass dry matter (DM) was 346 g/kg, metabolizable energy content 11.7 MJ/kg DM, in vitro organic matter (OM) digestibility 796 g/kg OM and its chemical composition was representative for a typical grass used in Northern Europe (Huhtanen et al., 2006) with ash, crude protein (CP), sugars and neutral detergent fibre of 79, 156, 137 and 503 g/kg DM, respectively. There were no effects ($P>0.05$) of compaction nor soil contamination on DM, ash and CP concentrations of the silages (Table 1). There were effects ($P<0.05$) of compaction and soil contamination on pH, which was higher for loose than tight compaction and higher for non-contaminated than for contaminated material. Control resulted in highest pH among treatments ($P<0.05$), followed by SALT and then FA. Lowest values for pH ($P<0.05$) were found for LAB treated silages. Non-contaminated silages resulted in higher concentration of ammonia ($P<0.05$) and additive treated silages showed lower ($P<0.05$) concentration of ammonia than control treatment. Tight compaction resulted in more extensive fermentation ($P<0.05$) with higher lactic acid concentration than loose compaction. The sugar content of the current material was relatively high and use of LAB increased ($P<0.05$) the conversion of sugars into lactic acid, which may have a positive effect on silage hygienic quality. On the other hand, FA restricted fermentation resulting in silages with higher sugar and reduced concentration of total fermentation products, which would be beneficial if sugar content is low and which may promote higher intake of silage. Aerobic stability was higher ($P<0.05$) for soil contaminated silages than non-contaminated probably due to greater concentration of acetic acid but in general, uncontrolled pathways of fermentation that produces acetic acid are less desirable (Kung, 2010).

CONCLUSIONS

Use of formic acid, lactic acid bacteria strains and salt based additives improved fermentation quality of grass ensiled under different management conditions. Tight compaction resulted in well preserved silages and should be aimed in farm scale. Soil contamination stimulated wild-type fermentation that somehow improved some parameter of silage, but is not recommended as an ideal pathway to preserve silage under farm conditions, because it could cause losses in nutritive value, detrimental effect for animals and hygienic risks in the food chain.

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Table 1. Chemical composition, fermentation quality, aerobic stability, ensiling losses and microbial quality of grass silage treated with additives under different compaction (Comp) and soil contamination (Cont) levels.

Contamination	Non-contaminated								Soil contaminated				SEM[1]	P-value[2]	
Compaction	Loose				Tight				Tight						
Additive	Control	FA	LAB	Salt	Control	FA	LAB	Salt	Control	FA	LAB	Salt		Comp	Cont
Dry matter (DM), g/kg	331^{b}	345^{ab}	348^{ab}	337^{ab}	332^{ab}	345^{ab}	344^{ab}	335^{ab}	341^{ab}	348^{a}	339^{ab}	341^{ab}	3.2	0.67	0.20
pH	5.78^{a}	4.85^{c}	4.02^{ef}	5.48^{b}	5.53^{b}	4.78^{c}	4.00^{f}	5.36^{b}	4.26^{d}	4.83^{c}	4.01^{f}	4.21^{de}	0.039	<0.01	<0.01
Ammonia N, g/kg N	64^{a}	26^{c}	21^{c}	42^{b}	59^{a}	25^{c}	21^{c}	43^{b}	43^{b}	25^{c}	22^{c}	43^{b}	2.2	0.46	0.03
Chemical composition, g/kg DM															
Ash	88^{a}	82^{dc}	85^{abcd}	88^{a}	86^{ab}	82^{d}	85^{abcd}	86^{ab}	86^{ab}	83^{bcd}	84^{bcd}	86^{abc}	0.8	0.11	0.80
Crude protein	177^{a}	164^{c}	172^{abc}	177^{a}	172^{abc}	166^{bc}	171^{abc}	175^{ab}	170^{abc}	169^{abc}	170^{abc}	171^{abc}	1.9	0.27	0.35
Sugars	87^{cd}	187^{a}	73^{d}	120^{bc}	120^{bc}	195^{a}	76^{d}	135^{b}	5^{e}	181^{a}	66^{d}	6^{e}	8.2	0.02	<0.01
Ethanol	29.9^{ab}	7.8^{de}	3.6^{e}	31.8^{a}	16.5^{c}	4.7^{e}	3.2^{e}	22.0^{bc}	16.4^{c}	2.5^{e}	3.9^{e}	15.0^{cd}	1.56	<0.01	0.06
Acids, g/kg DM															
Formic[3]	0^{d}	0.9^{c}	0^{d}	0^{d}	0^{d}	1.7^{b}	0^{d}	0^{d}	0^{d}	2.9^{a}	0^{d}	0^{d}	0.14	0.07	0.01
Lactic (LA)	12.7^{d}	1.2^{e}	113.1^{a}	12.4^{d}	21.8^{c}	1.6^{e}	114.5^{a}	17.7^{c}	86.2^{b}	0.7^{e}	115.5^{a}	87.8^{b}	0.93	<0.01	<0.01
Acetic	7.5^{c}	7.5^{c}	12.8^{b}	9.0^{bc}	8.3^{bc}	7.7^{c}	12^{bc}	9.9^{bc}	30.4^{a}	7.4^{c}	9.4^{bc}	25.9^{a}	0.95	0.72	<0.01
Propionic[3]	0.15	0	0.08	0.11	0.17	0	0.10	0.09	0.24	0.34	0.11	0.23	0.100	0.94	0.06
Butyric	0.91	0.28	0.03	0.19	0.29	0.37	0.05	0.16	0.03	0.85	0.03	0.03	0.288	0.51	0.94
Total volatile fatty acids	8.64^{bc}	7.82^{c}	12.90^{b}	9.39^{bc}	8.76^{bc}	8.12^{bc}	12.12^{bc}	10.19^{bc}	30.76^{a}	8.82^{bc}	9.55^{bc}	26.18^{a}	0.964	0.87	<0.01
Total fermentation acids	21.3^{d}	9.0^{e}	126.0^{a}	21.8^{d}	30.5^{c}	9.7^{e}	126.6^{a}	27.9^{c}	116.9^{b}	9.5^{e}	125.0^{a}	114.0^{b}	1.16	<0.01	<0.01
LA/total fermentation acids	0.59^{d}	0.12^{e}	0.90^{a}	0.57^{d}	0.71^{bc}	0.15^{e}	0.91^{a}	0.63^{cd}	0.74^{bc}	0.07^{e}	0.92^{a}	0.77^{b}	0.022	<0.01	0.12
Total fermentation products	51^{b}	17^{c}	130^{a}	54^{b}	47^{b}	14^{c}	130^{a}	50^{b}	133^{a}	12^{c}	129^{a}	129^{a}	1.7	0.05	<0.01
Aerobic stability[4]	41^{d}	109^{bcd}	118^{bc}	46^{cd}	73^{bcd}	98^{bcd}	133^{b}	48^{cd}	469^{a}	127^{b}	90^{bcd}	480^{a5}	14.8	0.37	<0.01
Ensiling losses, g/kg of initial DM	89^{a}	13^{fg}	3^{i}	60^{d}	79^{b}	10^{gh}	17^{f}	68^{c}	44^{e}	4^{hi}	41^{e}	43^{e}	1.2	0.01	<0.01
Yeasts, cfu/g	4.7×10^{5}	2.9×10^{3}	1.6×10^{3}	1.4×10^{4}	1.4×10^{4}	4.3×10^{2}	3.0×10^{2}	1.3×10^{3}	1.0×10^{2}	9.6×10^{2}	4.0×10^{4}	1.0×10^{2}	9.4×10^{4}	0.09	0.93
Moulds, cfu/g	3.1×10^{3b}	2.2×10^{3b}	3.2×10^{2b}	1.4×10^{4a}	5.2×10^{3b}	4.1×10^{2b}	3.1×10^{2b}	1.4×10^{4a}	1.0×10^{2b}	3.1×10^{3b}	4.6×10^{2b}	3.0×10^{2b}	1.6×10^{3}	0.94	<0.01
Clostridia, spore/g	-	-	-	-	42	34	3	7	3	13	3	14	16.3	-	0.28
Zearalenone, ppb	403	371	-	-	234	221	-	-	1598	313	-	-	-	-	-
Deoxynivalenol, ppb	299	297	-	-	322	385	-	-	558	252	-	-	-	-	-

Additive treatments: control without additive; formic acid (FA) based additive (AIV Ässä Na, Eastman Chemical Company, Oulu, Finland at 5 l/t); homofermentative strains of lactic acid bacteria (LAB) *Lactobacillus plantarum* (KOFASIL® LAC, ADDCON, Bitterfeld-Wolfen, Germany at 1 g/t); and salt based additive (Safesil Challenge, Salinity AB, Göteborg, Sweden at 2 l/t). Values with same letter in a row are not significantly different at 5% Tukey test.

[1]Standard error of the mean. [2]Effect of compactions (583 vs. 424 kg/m^3 for tight and loose compactions, respectively) and soil contamination. Tight compaction was done manually by dropping a lead plummet ten times after adding a handful of grass into the cylindrical silo, while loose compaction was done by dropping the lead plummet two times. [3]Corrected for its amount in the FA based additive. [4]Time taken to increase the temperature of samples by 2 °C above the ambient temperature (22 °C). Data collection lasted for 480 h. [5]Treatment did not reach the threshold during the evaluation period.

ADDITIVES IMPROVE FERMENTATION QUALITY AND AEROBIC STABILITY OF RED CLOVER SILAGE UNDER CHALLENGING CONDITION

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INTRODUCTION

Red clover is the most important forage legume in Northern Europe due to its nitrogen (N) fixation capacity, high nutritional quality and good overwintering capability. However, it is rather challenging to ensile due to typically high buffering capacity and low dry matter (DM) and water soluble carbohydrate (WSC) concentration (McDonad et al., 1991). Soil contamination during harvesting can further challenge the fermentation conditions. Silage quality in practice is still a concern and work to improve it is continuously needed. Additives are commonly used to improve the fermentation quality of the forage. The objective of the present study was to evaluate the fermentation quality, aerobic stability and microbial quality of silages produced from a second cut red clover dominated sward treated with different additives including a challenge in the form of soil contamination.

MATERIALS AND METHODS

Mixed red clover (*Trifolium pratense*) and timothy (*Phleum pratense*) grass with a botanical proportion of 0.76 and 0.24, respectively, was cut from the first regrowth on 1st of August 2018 in Tammela, Finland (60°83'N, 23°76'E), wilted overnight, precision chopped using farm scale machinery and transported to laboratory without any additive. Six additive treatments were used including control without additive, formic acid based additive (FA; at 5 l/t), salt acid based additives (Salt 1, Salt 2 and Salt 3; at 3 l/t) and an inoculant (LAB; at 1 g/t). Additional silages were prepared with soil and faeces contaminated raw material to test the efficiency of the additives under challenging conditions using four of the additive treatments: control, FA, Salt 1 and LAB. The grass was ensiled into cylindrical pilot scale silos (5.5 litres, density 622 kg/m^3) using three replicates per treatment, stored at room temperature with protection from light and opened after an ensiling period of 3 months. Deteriorated parts were discarded and silage was carefully mixed, samples taken and analysed for chemical composition and fermentation quality. Aerobic stability was evaluated by measuring the temperature with thermocouple wires automatically at 10-minute intervals from silage samples stored in polystyrene boxes. Data was analysed using a MIXED procedure (SAS Inc. 2002-2012, Release 9.4; SAS Inst., Inc., Cary, NC) of SAS at 5% probability.

RESULTS AND DISCUSSION

Grass composition of ash (100 g/kg), crude protein (162 g/kg), neutral detergent fibre (460 g/kg) and in vitro organic matter digestibility (670 g/kg organic matter) were representative for this type of sward, but the DM (261 g/kg) and WSC (37 g/kg DM) concentrations were rather low. The results of the fermentation quality presented in Table 1 reveal that this was the case as e.g. acetic acid concentration was high in all silages (mean 40 g/kg DM) although proportion of ammonia N in total N which usually is a quite sensitive indicator of silage fermentation quality was not particularly high (mean 61 g/kg total N). Treatment had an effect on ammonia N concentration, which was lower ($P<0.05$) for FA treated silages. Fermentation was restricted by FA silages with higher ($P<0.05$) residual amount of WSC and lower ($P<0.05$) concentrations of acetic acid, total volatile fatty acids, total fermentation acids and total fermentation products. There was a contamination effect ($P<0.05$) for ethanol and propionic acid with higher concentrations in contaminated silages. Some of the silages contained very high butyric acid concentrations but high dispersion in the data resulted in no statistically significant differences. There still seemed to be a clear pattern as butyric acid was extremely high in one control and two LAB treated silages non-contaminated and also one in control contaminated silage. The individual values of butyric acid for control for the three replicates were 48.48, 0.78 and 0.04, while for LAB 38.03, 29.07 and 1.05 g/kg DM in non-contaminated silages, while for control contaminated silages were 32.79, 5.59 and 0.65 g/kg DM. It is noteworthy that in the same treatment, the replicates could differ so dramatically from each other. This emphasizes the nature of fermentation, which may lead to quite different directions based on minute differences in the starting situation. Salt 1 and Salt 2 resulted in greater ($P<0.05$) aerobic stability than control and FA for non-contaminated raw material. In contaminated silages, FA and Salt 1 improved ($P<0.05$) stability during aerobic phase.

CONCLUSIONS

Overall, tested additives were effective in improving fermentation quality and aerobic stability of mixed timothy and red clover silage, which in general proved to be difficult to ensile. Avoidance of soil and faeces contamination improved fermentation quality by reducing ethanol and propionic acid concentrations.

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Table 1. Chemical composition, fermentation quality, aerobic stability, ensiling losses and microbial quality of grass silage treated with additives and challenged conditions under soil and faeces contamination (Cont).

	Non-contaminated						Soil contaminated				SEM[1]	P-value
	Control	FA	Salt 1	Salt 2	Salt 3	LAB	Control	FA	Salt 1	LAB		Cont[2]
Dry matter (DM), g/kg	263	276	261	269	260	260	271	274	269	273	5.5	0.10
pH	4.64^{bc}	4.38^{c}	4.68^{bc}	4.74^{abc}	4.66^{bc}	5.25^{a}	4.91^{ab}	4.34^{c}	4.75^{abc}	4.79^{abc}	0.103	0.59
Ammonia N, g/kg N	65^{a}	36^{b}	64^{a}	65^{a}	76^{a}	71^{a}	67^{a}	40^{b}	65^{a}	62^{a}	2.9	0.75
Chemical composition, g/kg DM												
Ash	110	108	109	109	106	111	113	108	112	111	1.7	0.33
Crude protein	179	175	181	181	179	179	177	175	182	178	2.1	0.76
Water soluble carbohydrates	3.2^{b}	24.3^{a}	4.4^{b}	4.6^{b}	3.3^{b}	2.5^{b}	2.8^{b}	24.1^{a}	4.1^{b}	2.3^{b}	1.78	0.83
Ethanol	4.7^{bc}	1.6^{e}	2.6^{de}	2.6^{de}	3.2^{cd}	5.4^{b}	5.0^{b}	2.3^{de}	2.8^{de}	7.1^{a}	0.29	<0.01
Acids, g/kg DM												
Lactic (LA)	48.7	41.8	46.5	23.6	59.5	20.2	41.7	42.2	46.6	39.0	12.00	0.72
Acetic	32.3^{bc}	20.6^{c}	47.4^{ab}	62.9^{a}	41.9^{abc}	35.7^{bc}	37.6^{bc}	20.0^{c}	47.3^{ab}	50.4^{ab}	4.95	0.19
Propionic[3]	0.96^{bc}	0.68^{c}	0.50^{c}	0.41^{c}	0.30^{c}	2.45^{b}	0.96^{bc}	0.80^{bc}	0.76^{c}	5.19^{a}	0.329	<0.01
Butyric	16.43	0.33	0.04	0.04	0.04	22.72	13.01	0.23	0.04	0.08	7.288	0.22
Total volatile fatty acids	50.03^{abc}	21.67^{bc}	48.08^{abc}	63.55^{a}	42.43^{abc}	61.21^{a}	51.92^{ab}	21.11^{c}	48.35^{abc}	55.98^{a}	6.075	0.83
Total fermentation acids	98.7^{ab}	66.8^{b}	94.6^{ab}	87.1^{ab}	101.9^{a}	81.5^{ab}	93.6^{ab}	66.8^{b}	95.0^{ab}	95.0^{ab}	6.83	0.66
LA/total fermentation acids	0.45	0.63	0.48	0.27	0.57	0.23	0.42	0.63	0.48	0.41	0.104	0.59
Total fermentation products	103^{ab}	68^{b}	97^{ab}	90^{ab}	105^{a}	87^{ab}	99^{ab}	69^{b}	98^{ab}	102^{ab}	7.0	0.56
Aerobic stability, 2 °C[4]	207^{e}	268de	406^{bc}	484^{ab}	311^{cde}	351^{bcd}	184^{e}	596^{a}	449^{b}	291^{cde}	26.6	<0.01
Ensiling losses, g/kg initial DM	3.3^{ab}	1.4^{b}	2.9^{ab}	2.0^{ab}	2.4^{ab}	3.7^{a}	3.6^{a}	1.6^{ab}	1.7^{ab}	3.4^{ab}	0.45	0.51
Microbial quality												
Yeast, cfu/g	<2.0×10^{2}	<1.0×10^{2}	<1.0×10^{2}	<1.0×10^{2}	<1.0×10^{2}	<1.0×10^{2}	<2.0×10^{2}	<1.0×10^{2}	<1.0×10^{2}	1.8×10^{3}		
Mould, cfu/g	1.7×10^{4}	1.4×10^{3}	<1.0×10^{2}	<1.0×10^{2}	<3.5×10^{2}	1.3×10^{5}	1.0×10^{5}	1.2×10^{4}	<2.3×10^{2}	6.4×10^{2}		

Additive treatments: control without additive; formic acid based additive (FA; formic acid, propionic acid, potassium sorbate, sodium formate; AIV Ässä Na at 5 l/t); salt based additive (Salt 1; sodium benzoate, sodium nitrite, hexamethylenetetramine and sodium propionate; Xtrasil Ultra at 3 l/t), salt based additive (Salt 2; sodium nitrite, hexamethylenetetramine and potassium sorbate; Xtrasil Ultra HD at 3 l/t), salt based additive (Salt 3; sodium nitrite, hexamethylenetetramine, sodium benzoate and ammonium propionate; Xtrasil LP at 3 l/t) and LAB (*Lactobacillus plantarum*, *Lactobacillus paracasei* and *Lactobacillus buchneri*; Xtrasil BioUltra at 1 g/t). The FA additive was provided by Eastman Chemical Company, Oulu, Finland, while salt based additives and LAB were provided by Konsil Scandinavia, Tvååker, Sweden. Values with same superscript letter in a row are not significantly different at 5% Tukey test. [1]Standard error of the mean. [2]Effect of soil and faeces contamination. [3]Corrected for its amount in the FA based additive; before corrections, concentrations of propionic acid in FA treatments were 4.05 and 4.19 g/kg for non-contaminated and soil contaminated, respectively. [4]Time taken (in hours) to increase the temperature of samples by 2 °C above the ambient temperature (22 °C).

DYNAMICS OF QUALITY ANALYSIS DURING ENSILING ON PAPER MULBERRY SILAGE WITH DIFFERENT MOISTURE CONTENT

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INTRODUCTION

Nowadays, the shortage of feed resources is one of the important factors that restrict the development of animal husbandry. However, China is rich in woody forage resources. Among them, paper mulberry (*Broussonetia Papyrifera* (Linn.) L'Hér. ex Vent) belongs to Moraceae genu, it was wildly planted in our country because of the rich nutrition and remarkable feeding effects. Research showed that paper mulberry silage might enhance the immune and antioxidant function of dairy cows and increase the production performance (Si et al., 2018). In 2015, it was listed as one of China's ten targeted poverty alleviation projects. As a kind of emerging forage material, paper mulberry may be potentially valuable to relieve feed shortage. Ensiling is a traditional preservation method of fresh forage, the moisture contents of raw material may affect the silage quality during ensiling (Wang et al., 2018). This study aimed to investigate the dynamics of quality analysis during ensiling on paper mulberry silage with different moisture contents and to provide more-detailed reference for application about paper mulberry silage.

MATERIAL AND METHODS

Paper mulberry materials were harvested in Lankao County, Henan, China on October, 2018. The experiment was divided into two treatments. The unwilted material had a moisture content of 69% (M1), while the wilted material was wilted for about 2 h to obtain a target a moisture content of 60% (M2). Samples were cut into 2cm in length and then were packed into plastic bags with 400g per bag (n=3). The dynamics fermentation quality and chemical composition were tested after 3, 7, 14 and 55 days ensiling. The level of significance was set to $P < 0.05$.

RESULTS AND DISCUSSION

The dynamics fermentation quality and chemical composition of paper mulberry silage were shown in Table 1. Significant effects on contents of CP, WSC, LA and pH value was observed on ensiling time ($P < 0.05$), while the effect of moisture treatment on contents of DM, CP, WSC, LA and pH value was significant ($P < 0.05$). The combination of two factors highly affected the contents of CP, NDF, LA and pH value ($P < 0.05$). The decrease of pH and the increase of LA were considered to be the key indicators of high silage quality (Ni et al., 2015). It indicates that ensiling time and moisture treatment would impact on the production of high quality paper mulberry silage.

With prolonged ensiling time, the pH value and WSC content of all samples reduced, and their LA conents increased, while the BA contents below the detectable levels during ensiling. It was shown that the lactic acid bacteria presented on silage surface convert WSC into organic acid (LA) to reduce pH, might inhibit the growth of harmful bacteria in the ensiling process and thereby contributing to the improvement of paper mulberry silage quality. Compared with M2 treatment, lower pH value ($P < 0.05$) and higher LA content ($P < 0.05$) in M1 treatment were detected in our present research. Besides, NDF content revealed a decreasing trend and CP content displayed an increase trend with the extended ensiling time in M1 treatment. However, M2 treatment showed higher WSC content than M1 ($P < 0.05$), which probably because after wilting, low moisture environment in M2 treatment was not conducive to microbial activity. In fermentation, lactic acid bacteria mainly use WSC to produce lactic acid, low moisture content might limit the number of lactic acid bacteria with highly efficient fermentation ability, then slowed down the fermentation process, thus retaining more available WSC substrate.

CONCLUSION

In this research, the dynamics of quality analysis during ensiling on paper mulberry silage with different moisture contents were studied, and found that ensiling time and moisture treatment would impact on the quality of paper mulberry silage. Silage with 69% moisture content significantly improved the quality than that with 60% moisture content, and better effects on silage were shown after 55 days ensiling.

Table 1 Chemical compositions and fermentation characteristics of different moisture content paper mulberry silage ensiling for 3, 7,14 and 55 days.

Items	Treatment	Ensiling time				SEM	Significant		
		3 days	7 days	14 days	55 days		T	M	T×M
DM (g/kg)	M1	295.4B	294.5B	290.9B	282.1B	5.051	0.091	<0.001	0.437
	M2	383.2A	372.2A	384.7A	372.1A				
CP（g/kg DM）	M1	134.8b	150.12b	160.47Aa	161.49a	6.640	0.007	0.004	0.042
	M2	148.49	158.33	133.48B	157.99				
NDF（g/kg DM）	M1	493.5a	434.9ab	442.3ab	416.9b	15.560	0.447	0.798	0.011
	M2	426.2	472.1	443.7	456.1				
ADF（g/kg DM）	M1	297.0	274.3	290.1	274.7	15.962	0.633	0.519	0.101
	M2	265.6ab	303.9a	246.8b	290.0a				
WSC（g/kg DM）	M1	37.53Ba	36.68Ba	17.39Bb	10.52Bc	3.217	<0.001	<0.001	0.054
	M2	61.27Aa	47.09Ab	39.67Ab	19.73Ac				
pH	M1	6.75a	6.29Bb	5.94Bc	5.40Bd	0.066	<0.001	<0.001	<0.001
	M2	6.77a	6.70Aa	6.70Aa	5.99Ab				
LA（g/kg DM）	M1	7.90b	19.50a	25.68Aa	25.96Aa	1.876	<0.001	<0.001	0.020
	M2	7.07c	15.67ab	11.93Bb	19.37Ba				
AA（g/kg DM）	M1	0.83	1.21	1.34	2.70	0.563	0.104	0.136	0.401
	M2	NDb	0.74ab	0.97a	1.12ab				
PA（g/kg DM）	M1	2.03	2.76	2.21	2.81	0.949	0.383	0.084	0.358
	M2	2.47	4.16	5.41	2.73				
BA（g/kg DM）	M1	ND	ND	ND	ND	-	-	-	-
	M2	ND	ND	ND	ND				

M1, moisture content of 69% treatment; M2, moisture content of 60% treatment; DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; WSC, water-soluble carbohydrates; LA, lactic acid; AA, acetic acid; PA, propionic acid; BA, butyric acid; ND, not detected; T, ensiling time; M, moisture; T×M, the interaction between ensiling time and moisture; a–d means in the same items and moisture content followed by different ensiling time differ ($P < 0.05$); A-B means in the same items and ensiling time followed by different moisture content differ ($P < 0.05$).

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EFFECTS OF DIFFERENT ADDITIVES ON FERMENTATION QUALITY AND NUTRIENT COMPOSITION OF BROUSSONETIA PAPYRIFERA L. (PAPER MULBERRY)

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INTRODUCTION

Broussonetia papyrifera L. (B. papyrifera, paper mulberry) is a deciduous tree or shrub in the family Moraceae that is native to eastern Asia, and widespread in China.(Si *et al.* 2018)Broussonetia papyrifera is a green and efficient feed source with strong adaptability, heat and humidity resistance, developed root system, large biomass and high protein content. *Lactobacillus buchneri* belongs to heteromorphic fermentation lactic acid bacteria, which can effectively maintain the nutritional components of silage and improve the aerobic stability. Corn silage with LB reduced the concentrations of lactic acid and ethanol and increased silage pH and concentrations of acetic acid, propionic acid, and 1,2-propanediol(da Silva *et al.* 2018).Glucose is the most direct substrate for lactic acid bacteria fermentation. Adding glucose to silage raw materials can significantly improve the quality of silage fermentation. Wood vinegar has strong acidity and can be used as feed additive to improve meat quality of livestock, but there are few studies on it as silage additive.The purpose of this experiment was to study the effects of *Lactobacillus buchneri* and different additives on the fermentation quality of paper mulberry.

MATERIALS AND METHODS

Paper mulberry was cultivated in Lankao, Henan, China, harvested at the third-cut grass on 4 June 2018. The fresh harvested materials were cut into 3-4 cm long by a feed cutter. After 3 hours in the sunshine, it is divided into five treatments and sealed in polyethylene bags after adding different additives. Chopped grasses were treated with (1) equal amount of sterilized water（CK）; (2) 10^6 cfu/g *lactobacillus buchneri* (B); (3)10^6 cfu/g *lactobacillus buchneri* and 20g/kg glucose (B+T) ; (4) 10^6 cfu/g *lactobacillus buchneri* and 6ml/kg wood vinegar(B+S); (5)10^6cfu/g *lactobacillus buchneri*, 20g/kg glucose and 6ml/kg wood vinegar acid(B+T+S). After 180 days of ensiling, the triplicate samples from each treatment were opened for analysis of chemical composition, fermentation quality. Datas were processed using SAS 9.2 software with a multiple comparison test (Tukey/Kramer) used for comparisons at 5% significant level.

RESULTS AND DISCUSSION

As can be seen from Table 1, the pH of the control group and the B+T group was significantly lower than that of the B group, and there was no significant difference between the other groups. Among the additives, the B+T had the lowest PH value. The lactic acid content of the control group was higher than that of the other groups. Compared with the control group, the acetic acid and propionic acid contents of each group were significantly increased. Butyric acid was not detected in the silages, except in the B+T and B+S silage. The NH3-N/TN in the control group and the B+T group was significantly lower than that in the B group. The number of lactic acid bacteria in group B was the highest, and no yeast or mold was detected in each treatment group. As can be seen from Table 2, the dry matter content of each group was significantly lowered as compared with the control group. The LAB-treated silages had lower fiber content than the control silages, singly or as mixed silage. Among them, the fiber contents in the B+T group was the lowest. The content of CP in B and B+T group was higher than that in the control group.

CONCLUSIONS

The results showed that the addition of *lactobacillus buchneri* reduced the content of lactic acid, increased the ratio of acetic acid and propionic acid, inhibited the growth of harmful microorganisms. The addition of *lactobacillus buchneri* and glucose increased the crude protein content and decreased PH and the fiber content, indicating that the addition of *lactobacillus buchneri* and glucose could improve the fermentation quality. The fermentation quality of adding wood vinegar and glucose was better than adding wood vinegar alone.

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Table 1. Fermentation quality and microbial population of silages after 180 days of ensiling

Items	CK	B	B+T	B+S	B+T+S	P-value
PH	4.79b	5.56a	4.81b	5.22ab	5.14ab	0.03
Lactic Acid（g/kg DM）	63.97a	21.53b	27.50b	23.20b	22.87b	<0.01
Acetic Acid （g/kg DM）	20.50b	60.03a	50.03a	55.17a	46.97a	<0.01
Propionic Acid （g/kg DM）	22.27d	37.33c	63.37a	50.50b	68.87a	<0.01
Butyric Acid （g/kg DM）	ND	ND	0.77a	0.46ab	ND	0.03
NH3-N(%TN)	8.36b	14.40a	8.97b	9.62ab	10.85ab	0.09
LAB(log cfu/g of FM)	7.86	8.21	7.38	7.65	8.03	0.74
Colibacillus(log cfu/g of FM)	ND	ND	ND	ND	ND	-
Mould and Yeast(log cfu/g of FM)	ND	ND	ND	ND	ND	-

Means in the same row ($^{a-d}$) with different superscript letters differ significantly ($P< 0.05$)

Table 2.Chemical composition of silages after 180 days of ensiling

Items	CK	B	B+T	B+S	B+T+S	P-value
DM（g/kg）	340.86a	245.96b	250.84b	268.59b	256.53b	<0.01
Neutral Detergent Fiber (g/kg DM）	459.70	408.67	389.70	391.40	438.63	0.40
Acid Detergent Fiber (g/kg DM)	274.20	262.30	247.90	277.57	273.13	0.77
CP（g/kg DM）	203.8	210.33	205.80	185.83	190.37	0.27
Water-soluble Carbohydrate(g/kg DM）	9.00	6.47	7.37	7.73	6.77	0.30

Means in the same row ($^{a-d}$) with different superscript letters differ significantly ($P< 0.05$)

EFFECTS OF DIFFERENT PROCESSING METHODS ON FERMENTATION QUALITY AND NUTRITIONAL COMPONENTS OF PAPER MULBERRY SILAGE

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INTRODUCTION

Paper mulberry (*Broussonetia papyrifera*) with abundant of protein has been a promising source of green fodder for animal feed (Si et al., 2018). The harvest of paper mulberry is seasonal with high moisture, which needs suitable storage technique. Ensiling is a principle way to preserve wet biomass with low fermentation loss. Although paper mulberry contains more protein content than many other types of forage, the natural fermentation of paper mulberry usually leads to poor silage quality. The aim of this study was to evaluate the effects of chopping and rubbing filament machine on the fermentation quality and nutritional components of paper mulberry silage.

MATERIALS AND METHODS

The raw material is the first stubble of paper mulberry harvested in Zhuozhou, Hebei Province, China. The whole fresh-harvested paper mulberry was divided into two different treatment methods: chopping(chopped with lengths of 1 to 1.5 cm by a hand forage chopper) and rubbing filament machine. Then placed in plastic film bags and each treatment was repeated three times. Silages stored indoors at ambient temperature around 29 °C. After 60 d bags were opened for later examination of all conventional index and protein fractions (Zhang et al. 2017; Tao et al. 2017).

Table1. Chemical composition of paper mulberry leaves before fermentation.

Items	Paper Mulberry
Ca（mg/g）	16.05813
P(g/kg DM)	0.271625
HT（mg/g）	7.782092
CT（mg/g）	25.26134
WSC(g/kg DM)	7.339869
NDF(g/kg DM)	44.38745
ADF(g/kg DM)	35.18854
EE(g/kg DM)	7.229521
CP(g/kg DM)	15.05833

NDF, neutral detergent fiber; ADF, acid detergent fiber; WSC, water soluble carbohydrates; EE, ether extract; CP, crude protein; HT, hydrolyzable tannins; CT, condensed tannins. Means within the same row with different superscripts differ significantly from each other ($P < 0.05$).

RESULTS AND DISCUSSION

As presented in Table 2, compared with chopping, after kneading silk, the drying of the raw materials of paper mulberry was accelerated, and the dry matter content in silage would be increased. At the same time, NDF content was significantly reduced, ADF content was relatively reduced, hydrolyzed tannin and condensated tannin were fully released. However, a large number of WSC was released and CP would decompose rapidly in the process of treatment. Table 3 showed that compared with the chopping treatment, the pH value of the whole silage decreased significantly ($P<0.05$), the LA content increased about 2.5 times, and the AA, PA and BA contents decreased significantly, which effectively improved the fermentation quality of the whole silage.

Table2. Nutritional Components of paper mulberry in Different Processing Ways.

Items	chopping	kneading silk
DM(g/kg FW)	32b	42a
NDF(g/kg DM)	48.34a	42.75b
ADF(g/kg DM)	38.39	36.40
WSC(g/kg DM)	0.59	1.69
EE(g/kg DM)	7.63a	6.24b
CP(g/kg DM)	16.45a	14.11b
Ca（mg/g）	18.48	16.84
P(g/kg DM)	0.28	0.27
HT（mg/g）	10.80a	8.11b
CT（mg/g）	36.78a	25.68b

Table 3. Fermentation quality of silage paper mulberry with different processing methods.

Items	chopping	kneading silk
pH	5.98a	4.44b
N-NH_3(g/kg TN)	8.14a	3.02b
LA(g/kg DM)	2.19b	7.63a
AA(g/kg DM)	1.99a	1.24b
PA(g/kg DM)	1.31a	0.87b
BA(g/kg DM)	0.09	0.00

pH, pH value; LA, lactic acid; AA, acetic acid; PA, propionic acid; BA, butyric acid.Means within the same row with different superscripts differ significantly from each other ($P<0.05$).

CONCLUSION

Compared with chopping, the pre-treatment of rubbing filament machine can effectively improve the fermentation quality and nutritional components of whole paper mulberry silage.

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IMPACT OF MATURITY STAGES FROM DIFFERENT SORGHUM VARIETIES ON FERMENTATION CHARACTERISTICS AND LEACHATE LOSSES

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Keywords sorghum, volatile organic compounds (VOC), ethanol, leachate

INTRODUCTION

Apart from tropical regions, sorghum becomes more and more important in many temperate areas (Bolsen et al. 2003). Besides draught stress, pests like western corn rootworm (*Diabrotica virgifera*) menace maize more often. Concerning yield and quality safety of home grown forage, alternative crops will become more important for cattle nutrition in the future. There is only little experience regarding sorghum cultivars and their nutritive value for cattle in Central Europe. Apart from that, sorghum varieties with different properties (biomass [bm], silage [si], grain [gr]) are existing. However, it´s not clear which type of sorghum varieties is the optimal choice for cattle farmers. The European Innovation Parntership (EIP) funds projects focusing on agricultural productivity and sustainability. The project "Nutritive value and fermentation of whole plant silages from different sorghum varieties in cattle feeding" is part of an EIP project and was started in 2016 by different partners to get answers regarding plant production, forage conservation and animal nutrition.

MATERIALS AND METHODS

Six different sorghum cultivars (i Aristos[bm], ii ES Harmattan[si], iii RGT Vegga[si], iv Nutrigrain[si/gr], v RGT Primsilo[gr], vi RGT Ggaby[gr],) were cultivated in Hafendorf (R 15°18'40.7"; H 47°27'19.3") and compared with maize silage (cultivar Angelo) in three years (2016 to 2018). Crop management (cultivation, fertilisation, maintenance) was executed with regard to recommendations of good practice. Sorghum harvest was carried out at three different maturity stages (grain ripeness: i early = soft dough, ii middle = dough, iii late = physiological maturity). Yield of total green mass and also of separated panicles (heads) and residual plants (stems and leaves) was measured from each sorghum cultivar. Samples of separated plant material were collected and prepared for chemical analysis (oven drying 48 h, 50°C). Material of chopped whole plants was compacted into plastic barrels (60 litre) and sealed hermetically via cover plates. All barrels were quickly transported to Gumpenstein (R 14°06'13.0"; H 47°29'36.9") for storage. Approximately 70 kg dry matter (DM) of sorghum silage were needed for different experiments (fermentation, leachate production, in vivo and in vitro digestibility, in situ degradability). After four months, barrels were weighed and opened to get samples of silage and leachate. Chemical analyses (DM, nutrients, fiber components, minerals, pH, NH_3-N, volatile organic compounds [VOC]) were carried out at AREC Raumberg-Gumpenstein via standardised wet chemical methods (VDLUFA 1976).

RESULTS

Sorghum varieties showed lower DM content (194.9 to 332.8 g/kg FM) than maize silage (334.3 g/kg). DM content was significantly affected by factors cultivar ($P<0.01$), maturity ($P<0.01$) and year ($P<0.05$). Due to low DM content (below 280 g/kg DM) at early and middle grain maturity, leachate was produced (up to -12.4% of total FM) during fermentation, predominantly in silage sorghum varieties. Cultivar Aristos contained a spongy marrow inside the stems and therefore, leachate was bonded very effectively under low DM conditions in early maturity. Except for variety Aristos (64.6 g/kg DM), all sorghum cultivars contained higher crude protein content (XP) than maize silage (67.3 g/kg DM) – NutriGrain had the highest XP content (85.0 g/kg DM). Content of non-fiber carbohydrates (NFC) showed a high variance (237 to 425 g/kg DM). Compared to maize (425 g/kg DM), silage type of sorghum had markedly lower NFC content (below 300 g/kg DM), grain types had a NFC content between 303 and 368 g/kg DM. Content of structured carbohydrates (NDF, ADF, ADL) was reverse to NFC. Maize silage showed lowest NDF content (424 g/kg DM), cultivars of silage type 515 to 589 g and biomass type Aristos 606 g NDF/kg DM – RGT Ggaby was the sorghum cultivar lowest in NDF (452 g/kg DM).

In early grain ripeness, natural acidification of some sorghum cultivars was suboptimal, because of pH above recommendation level (DLG 2012). On average, a higher total VOC content was observed in silage sorghum varieties compared to biomass and grain type or maize. Acetic acid production was nearly optimal in every variety – content was between 10 and 23 g/kg DM. No problems with *clostridia tyrobutyricum* occurred in whole plant sorghum silages as average content of butyric acid was less than 1.0 g/kg DM. Only one sample of cultivar Primsilo contained 18.2 g/kg DM butyric acid in 2018. That´s the reason why the cultivar average raised up to 9.2 g/kg DM. Vendramini et al. (2018) found similar fermentation characteristics in sweet sorghum silage. In general, content and especially percentage of ethanol in total VOC was high in sorghum silages (average 32.8%) with a strong influence of the year (22.7% in 2018, 45.9% in 2016). Other fermentation parameters (pH, VOC, NH_3) were also most affected by weather conditions (factor year). Biomass and silage type showed proteolysis above 8% ammonia content of total nitrogen. The increase of grain maturity caused decreasing content of some VOC and ammonia in sorghum silages (table 1).

Table 1: DM content, fermentation parameters and leachate production of silage from various sorghum cultivars at different grain maturities in comparison with maize silage

	parameter	Dry matter		pH		lactic acid		acetic acid		propionic acid		butyric acid		ethanol		ammonia (NH_3)		leachate (FM-loss)	
	statistics	avg	sd	avg	sd	avg	sd	avg	sd	avg	sd	avg	sd	avg	sd	avg	sd	avg	sd
cultivar	maturity	g/kg FM				g/kg DM		g/kg DM		g/kg DM		g/kg DM		g/kg DM		% of N_{total}		% of FM	
Aristos[bm]	early	249	35	4.8	1.5	31	29	15	13	0.8	0.3	0.6	0.3	20	17	10	5.1	0	-
	middle	281	24	4.2	0.3	24	17	21	8	0.7	0.1	0.5	0.3	34	15	9	4.7	0	-
	late	290	31	4.1	0.1	24	10	20	5	0.6	0.1	0.2	0.1	40	14	10	3.9	0	-
Harmattan[si]	early	195	28	4.3	0.8	36	33	19	7	1.0	0.3	1.7	2.1	21	7	10	4.2	4.4	4.7
	middle	225	24	3.9	0.1	45	13	20	4	0.9	0.1	0.2	0.2	18	9	11	4.3	0.5	0.8
	late	229	15	4.1	0.1	41	19	21	5	0.9	0.2	0.1	0.1	28	19	9	2.9	0.3	0.7
Vegga[si]	early	212	10	3.9	0.2	51	31	23	6	1.3	0.2	0.8	0.6	29	24	9	4.0	2.9	3.3
	middle	227	25	3.8	0.1	52	16	23	5	0.9	0.2	0.3	0.4	24	13	9	3.7	1.6	1.7
	late	245	18	4.0	0.1	35	8	21	7	0.8	0.3	0.2	0.1	47	28	7	2.3	0.6	1.0
NutriGrain[si/gr]	early	227	2	4.1	0.3	46	37	17	0	0.9	0.5	0.4	0.1	23	13	9	1.5	2.2	1.6
	middle	242	4	4.0	0.0	38	3	11	1	1.0	0.2	0.1	0.1	13	2	9	1.5	1.0	0.7
	late	254	16	4.2	0.1	30	1	13	4	0.8	0.4	0.1	0.0	13	0	7	1.3	0.5	0.7
Primsilo[gr]	early	298	5	4.5	0.4	19	23	10	8	1.2	0.6	9.2	12.8	15	5	7	5.3	0	-
	middle	319	17	4.0	0.1	35	3	13	7	0.6	0.0	0.2	0.1	10	1	6	4.0	0	-
	late	328	33	4.1	0.1	29	3	12	5	0.6	0.3	0.1	0.1	20	12	4	2.3	0	-
Ggaby[gr]	early	275	21	4.5	0.9	25	20	14	7	0.9	0.3	0.8	0.6	20	6	8	3.9	0.1	0.4
	middle	333	63	4.3	0.5	23	15	12	7	0.7	0.1	0.1	0.2	13	5	8	4.6	0	-
	late	305	29	4.3	0.2	25	9	12	7	0.7	0.1	0.1	0.0	12	2	6	1.4	0	-
Maize-silage Angelo (reference)	middle	334	22	4.0	0.2	29	15	15	6	1.0	0.6	0.2	0.1	16	5	9	4.1	0	-

statistics: avg = average, sd = standard deviation; sorghum type: bm - biomass, si - silage, gr - grain

CONCLUSIONS

In comparison with maize silage, fermentation characteristics of most tested sorghum varieties were similar. However, cultivars of silage type were tending to have lower DM content and higher production of leachate. These silage sorghum cultivars showed loss of leachate especially at early and middle grain maturity. Biomass sorghum had low content of valuable nutrients (XP, NFC). Therefore, this type will be dropped from the list of alternative crops for cattle farmers. Concerning nutrient composition, fermentation quality and leachate losses, cultivars of grain sorghum type could be an alternative to maize silage, especially in temperate regions with draught stress in summer.

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PELLETED FORAGE BY NON CONVENTIONAL METHOD

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INTRODUCTION

The most commonly used forage conservation techniques are hay and silage, but they present some disadvantages such as high production cost, or transportation and storage difficulties for commercialization, and suggest the search for techniques capable of minimizing these disadvantages (REIS and MOREIRA, 2017). The pelletizing of forage plants is a technique that can be used with the objective of conserving this food for commercialization or supplementation in critical periods of forage production, and it features some advantages: a) the form of pellets storage, which does not require special temperature or anaerobic conditions; b) their acceptance by the animals; and c) ease of logistics for the transport and marketing of preserved material (TABIL, 1996). Despite all the advantages of the conventional method of pelletizing, it presents drawbacks related to the production cost, due to high investment necessary for acquisition and operation of the conventional pelletizer, which presents high consumption of electric energy. The objective of this study was to evaluate the viability and stability of the mixture of gliricidia (*Gliricidia sepium*) with milled corn (*Zea mays*) or *algaroba* (*Prosopis juliflora)* pod flour in different proportions in the form of pellets, produced by an alternative method to conventional pelletizing.

MATERIALS AND METHODS

The initial tests were conducted in Laboratories of Bromatology and Animal Nutrition in Federal Universities of *Vale do São Francisco* and *Recôncavo da Bahia,* Brazil. For the preparation of the pellets, gliricidia, *algaroba* pod flour and milled corn were used as ingredients by means of an adaptation of the methodology described by Tabil et al. (1997), without the use of pelletizing machine. After collection and drying process gliricidia was carried out, the material was processed in the "Willey" type mill with a 2.0 mm mesh screen. The *algaroba* pod flour used was obtained in the appropriate granulometry (1 mm), as well as the corn (2mm). The mixture of pellet ingredients was made in plastic buckets in the following proportions: 40:60, 50:50 and 60:40, based on the air-dried sample, with gliricidia being a fixed ingredient for the 6 mixtures, changing only the concentrated food. To the proportion of the mixtures water was added between 1.0 liter and 1.35 liter, with a temperature between 90 and 100 ° C per kg of mixture, gradually, until it was possible to form a lump in one hand, which did not crumble, nor let water run through your fingers. Afterwards, the blends were processed in a 10 mm matrix BRAESI BMC-10/1 meat grinder for the formation of pellets, which were distributed in aluminum trays and taken to forced ventilation drying at 55-60 ° C per 36 hours.

RESULTS AND DISCUSSION

The pelletizing by alternative method with using the BRAESI BMC-10/1 meat grinder was effective, resulting a stable and commercially viable product. The ingredients, agglomerated by mechanical action, formed cylindrical structures, with an average length of 2 to 4 cm, called pellets (Figure 1). Considering that the alternative pelletizing used in the present study does not require high consumption of electric energy and machinery of high cost, it is understood that these are advantages of the method described here, compared to the process carried out in conventional pelletizers, whose applicability may be limited to animal feed factories.

Figure 1. Pellets produced by non-conventional method (ANICETO, 2018)

CONCLUSIONS

The alternative method of pelletizing proved promising for *G sepium* as a possible technique for forage conservation. However, it is recommended to investigate the effects of pelleting on nutritional characteristics of the food, as well as the application of microbiological tests to evaluate the shelf life of the pellets to better support this forage conservation recommendation.

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EFFECT OF CHEMICAL AND BIOLOGICAL ADDITIVES ON THE FERMENTATION PROCESS OF *AVENA STRIGOSA* UNDER UNCERTAIN ENSILING CONDITIONS

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INTRODUCTION

Droughts during summer force the farmers to harvest roughage in late autumn to avoid shortage of feed material. A warming climate might be the reason for draughts but gives the opportunity of late autumn cuts also. Climatic conditions at late autumn cuts are often inappropriate for wilting, but wilting is an important factor to improve the ensiling conditions of grass cut's (Kalzendorf and Milimonka, 2018). The fermentation coefficient (FC) (Schmidt, 1971) describes the ensilability of crops by the DM content, and the sugar/buffer capacity quotient These parameters are quite often insufficient for ensiling in autumn. With increasing soil moisture in autumn the risk of a higher crude ash content in the material for ensiling rises and thus the number of clostridia.

Forage, harvested at unsuited conditions for ensiling, is subject to a high risk of undesired fermentation. These conditions were provoked at the present lab trial e.g. by harvesting in the mid of November and chopping wet material at early morning.

MATERIAL AND METHODS

A late cut was taken at 15th of November 2018 from a Black Oat (*Avena strigosa*) field in the district Jessen (Elster), Germany. The crop traits for the cutted Black Oat were (g/kg DM): dry matter (DM) 166, crude protein (CP) 109, crude ash (CA) 83, sugar content (S) 204, nitrate 0.33, buffer capacity (BC) 47.7 g lactic acid/kg DM, S/BC 4.3 and the fermentation coefficient was 51. The number of epiphytical lactic acid bacteria was 5.5 * 10^6 CFU/g FM and there were 3.9* 10^3 CFU/g FM clostridia found in the crop material. Except for the number of clostridia the fermentation conditions were good considering the fermentation coefficient.

The crop material was ensiled in 1.5 litre jars. Each treatment was prepared from 5 kg fresh crop and treated with: 1) nitrite, HMTA, (KL) (3 litres/t FM), KOFASIL LIQUID, ADDCON Europe GmbH, 2) *Lactobacillus plantarum*, (LAC) (1 * 10^5 CFU/g forage) KOFASIL LAC, ADDCON Europe GmbH, 3) Control. The additives were applied according to the dosage mentioned above but for better distribution the liquid additives were filled up to 50 ml with water. The control 50 ml of water was sprayed. The glasses were stored at 25 °C temperature.

The silages were opened after 90 days. Crop parameters from fresh crop as well as for the silage were evaluated based on VDLUFA (2011). The setup of the experiment was a random block design 3 times replicated. Statistical analyses were done using the ANOVA procedure, program "R". When the overall P-value was significant at 5% level, pair wise comparisons between LSMEANS of treatments were done using Tukey´s test.

RESULTS AND DISCUSSION

After 90 days of fermentation there were big differences between the treatments. The use of sugar differed widely. The untreated control and the silage treated with *L. plantarum* showed a high sugar consumption. There was a maximum of 0.9% sugar left in one of the silage treated with lactic acid bacteria. All untreated silages and inoculated silages showed lower sugar contents left in the silage. The chemical treated silage showed a sugar consumption of approximately 25% of the base sugar content of the substrate. Similar results were described by Milimonka et al. (2019). All treatments with high sugar consumption also showed higher total amounts of lactic acid and acetic acid (Table 1). High sugar decrease can be explained by a high epiphytical lactic acid bacteria content of 5.5 * 10^6 CFU/g FM of the substrate. High acid contents led to extremely low pH values up to a minimum of pH 2.96. The correlation between sugar content and pH was r= 0.91 (Figure 1). This result can be explained by the effect of DM, sugar content and buffer capacity on fermentation intensity and content of fermented acids. Grass silages with a low DM content showed a more intensive fermentation characterized by higher contents of lactic and acetic acid compared to wilted silages with a higher DM (Hoedke & Zeyner, 2010). The silage with a pH of 2.96 had a sugar consumption of 100% of the base sugar content of 204 g/kg DM. The low crude ash and crude protein content did not impair the fermentation conditions. The lactic acid content of 234.4 g/kg of DM is extremely high. These really high values might come about the interaction of low DM, high temperature, high sugar content, low buffer capacity and a high number of lactic acid bacteria. Microbial processes found perfect conditions for a high fermentation intensity. Because of this, the control of the microbiological processes by silage additives might be helpful.

The KL treated silage was controlling a smooth fermentation. The average of pH values was 4.21 (Table 1) with a minimum of 4.13 and a maximum of 4.28. This is a suited pH for the given DM in case of use of a clostridia controlling additive. The lactic acid content between 90.8 and 106.5 g/kg of DM is near to the upper limit for optimum of 50 to 100 g/kg DM lactic acid at a required silage (Wyss, 2005). The ethanol content of the KL treatment is high significantly lower compared to the control (p=0.00005) and to the LAC inoculated variant (p= 00003). The fermentation losses were corrected after Weißbach (2005). The KL treated silage showed significant lower DM losses compared to the Control (p= 0.00380) and compared to the LAB treated silage (p= 0.00797). The fermentation pattern of the chemical treated silage is well-balanced compared to the other

variants. A balanced organic acid content - in this trial guaranteed by a chemical additive - is the basis for a high feed intake (Eisner, et al., 2006).

Though there was a high content of clostridia (3.9* 10^3 CFU/g FM), there was no butyric acid found in the silages. This could be explained by a fast production of lactic acid and thus a fast pH drop.

Table 1: Fermentation parameters in a wet silage (16.6 % DM) affected by different silage additives. Different letters show sign. differences between treatments, P<0.05.

	sugar [g/kg DM]	pH	lactic acid [g/kg DM]	acetic acid [g/kg DM]	ethanol [g/kg DM]	fermentation loss [g/kg DM]
Control	0,00[a]	3,30[a]	187,5[a]	51,9[a]	46,2[a]	87,3[a]
KL	133,53[b]	4,21[b]	98,7[a]	18,8[a]	2,7[b]	37,5[b]
LAC	7,76[a]	3,18[a]	219,1[a]	26,1[a]	51,3[a]	80,3[a]

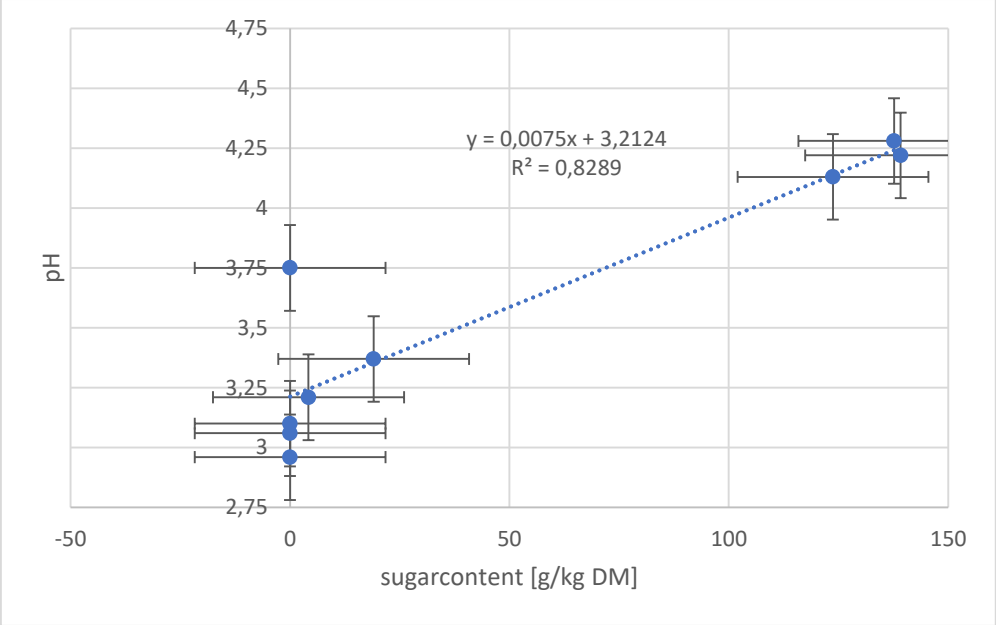

Figure1: Correlation of sugar content and pH of the silage after 90 days of storage

CONCLUSION

Chemical based additives can not only improve fermentation of substrates which are difficult to ensile shown by a low FC, but can also ensure the ensiling of substrates characterized by a FC higher than 45 but low DM.

The chemical treatment was controlling a smooth fermentation, whereas the fermentation in the Control and the LAB treatment was quite intensive and showed lactic acid contents up to 200 g/kg in the DM and a pH-decrease to 3.0. A balanced organic acid content- in this trial guaranteed by a chemical additive- is the basis for a high feed intake.

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STUDIES REGARDING THE EFFECT OF MICROBIAL INOCULA ON THE FERMENTATIVE CHARACTERISTICS AND AEROBIC STABILITY OF MAIZE ENSILED IN A TROPICAL ENVIRONMENT

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INTRODUCTION

Maize silage is one of the most used feeds in ruminant livestock production in temperate climates. Likewise, microbial inoculants containing bacteria producing lactic acid (LAB) either homo (IBHO), hetero (IBHT) fermentative or their mixtures (IBH) are one of the most used additives to improve the fermentative characteristics and aerobic stability of the silage (Muck, et al., 2018). Due to differences in the chemical composition and epiphytic microorganisms of the vegetative material before ensiling, fermentation characteristics and aerobic stability and the effectiveness of microbial additives forages fermented in tropical climates cannot be extrapolated from studies conducted in temperate climates (Rodriguez, 1996). Additionally, previous research has shown a lower lactic acid content and higher acetic acid content in forage sorghum fermented in a tropical environment as compared with the same crop ensiled in a temperature climate (Rodriguez, 1996). However, the higher acetic acid content reported in the forage sorghum ensiled in the tropical climate did not prevent the aerobic instability of the resulting silage. Thus, these results could indicate that a higher acetic acid content and a low lactic acid:acetic acid (LA:AA) ratio (<3) does not prevent silage aerobic deterioration.

MATERIALS AND METHODS

A descriptive summary of three experiments was carried out to evaluate the effect of the addition of IBH on the fermentative characteristics: pH and contents of lactic acid (LA) and acetic acid (AA) and aerobic stability of tropical maize silage var. Mayorbella (TMM) ensiled under tropical climates conditions. For all experiments, TMM was harvested at the University of Puerto Rico, Lajas Agricultural Experimental Station (18°27'46"N 67°02'05"W). Tropical maize was ensiled in laboraroty scale micro-silos (1.5 kg capacity) and treated or not with microbial inocula contaning different combinations of IBHO and IBHT. Microbial additives were applied at different inoculation rates. Silages were fermented during different lenghts of fermentation (45 to 90 days) and exposed to different aerobic conditions periods (Table 1).

Table 1. Ensiling charactersitics of tropical maize var. Mayorbella fermented in a tropical climate and exposed to aerobic conditions

Trial	Microorganisms	Inoculation Rate cfu/g	Fermentation Length (d)	Aerobic Exposure (h)
1	*Lactobacillus plantarum* *Enterococcus faecium* *Lactobacillus brevis*	10^{10}	45	96
2	*Lactobacillus plantarum* *Enterococcus faecium* *Lactobacillus buchneri*	10^{5}	58	120
3	*Lactobacillus plantarum,* *Enterococcus faecium* *Lactobacillus buchneri*	10^{6}	90	168

In all experiments, silages were analysed to determine pH, LA and AA content and LA:AA ratio. Time (hours) of the fermented material exposed to aerobic conditions to reach ambient temperature plus 3°C was utilized as criteria to determine silage aerobic deterioration.

RESULTS AND DISCUSSION

In experiment 1, pH was lower (P<0.05) in TMM fermented with IBH (4.07) than in the control (4.67), but the content of AA (14.5 vs 10.1 g/kg DM) and LA (21.3 vs 13.0 g/kg DM) was higher (P<0.05) (Table 2). Results from experiment 2, indicated that treating TMM with IBH compared with control silages had similar (P>0.05) pH (4.72 vs 4.67), and AA content (13.42 vs 15.61 g/kg DM), but higher (P<0.05) LA (22.8 vs. 16.4). In experiment 3, the pH (4.00 vs 4.02), AA (11.2 vs 13.7) content and LA (45.9 vs 44.6) content was similar (P>0.05) for both treatments. For all experiments LA: AA ratio was higher (P<0.05) in TMM treated with the IBH than control. The pH values and organic acid concentrations of TMM silages treated or not with microbial inocula found in these experiments are consistent with the fermentation parameters reported for maize and other crops ensiled in tropical climates.

It is documented that silages with high AA content might be more stable to aerobic conditions than fermented vegetative material with low AA content. However, the results of this descriptive study indicate that independent of the AA content, silages with LA:AA ratio higher than 3.25 were more stable compared with silages that had lower LA:AA ratio values. On average, silages that have an LA:AA ratio higher than 3.25 lasted

43 hours prior to aerobic deterioration while silages with a LA:AA ratio less than 1.75 lasted 11.5 h prior to deterioration upon exposure to air (the time of the TMM exposed to aerobic conditions in reach ambient temperature plus 3°C).

Inoculating TMM with IBH enhanced the aerobic stability of the resulting silages by delaying the time to reach ambient temperature plus 3°C. On average, time of the tropical maize ensiled with the microbial additive and exposed to aerobic conditions to reach ambient temperature plus 3°C was 30 h, compared with 13 h in untreated silages.

Table 2. Fermentation parameters and aerobic stability of tropical maize silage treated with inoculants containing mixtures of homo and hetero fermentative bacteria producing lactic acid

	Treatment		
Experiment	**No additive**	**IBH**	**P**
	pH		
1	4.67	4.07	0.05
2	4.72	4.67	0.14
3	4.02	4.00	0.73
	LA[1]		
1	13.0	21.3	0.01
2	16.4	22.8	0.05
3	44.6	45.8	0.40
	AA[1]		
1	10.1	14.5	0.05
2	15.61	13.41	0.15
3	13.7	11.2	0.52
	LA:AA		
1	1.28	1.46	0.05
2	1.05	1.70	0.01
3	3.25	4.08	0.05
	Hours to reach ambient temperature + 3°C		
1	2	14	
2	6	24	
3	32	54	

[1] g/kg DM

CONCLUSION

Our three experiments indicate that the aerobic stability of maize silage ensiled under tropical conditions is short lived (ranging from 2 to 32 h). Ensiling maize in a tropical climate with microbial inocula improved the LA:AA ratio and enhanced the aerobic stability of the resulting silage. In experiment 1, higher acetic acid content resulted in maize silage more stable to aerobic conditions. However, the AA values determined for experiments 2 and 3 are too similar between treatments to explain the improved aerobic stability observed. We hypothesize that an undetermined compound different from AA, and not AA, could have been the key factor in improving aerobic stability of maize silage ensiled under tropical conditions. Our results also suggest that increasing the lenght of fermentation from 45 to 90 d may play a more important role than the content of AA per se in improving aerobic stability. Upon exposure to air, silages with LA:AA ratio greater than 3.25 were more stable than those with lower values.

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INFLUENCE OF ENSILAGING ADDITIVES ON FERMENTATION QUALITY AND AEROBIC STABILITY OF MAIZE SILAGE

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INTRODUCTION

Maize is generally considered to be easily ensilageable, provided that adequate conditions are available for ensilaging. The said conditions include, additionally to technical ones, also the contents of dry matter and nutrients. Khan et al. (2015) state that the dry matter intake (DMI), milk production and the contents of dairy proteins in the milk increased with increasing ripeness of the maize and achieved an optimum level at maize silage ensilaged with dry matter contents of 30-35 %, slightly decreasing when the dry matter was increased above 35 %. According to Peyrat et al. (2016), when ensilaging or harvesting maize with dry matter above 35 %, the digestibility of starch decreases and the amount of starch passing the digestive tract of the animals without benefit increases. That results in higher amount of starch in excrements.

The experiment was aimed at comparing the results, verifying the efficiency of selected ensilaging additives at ensilaging the maize harvested with almost optimum dry matter, or in optimum vegetation stage, respectively.

MATERIAL AND METHODS

The maize was ensilaged on 6. 9. 2017. Additionally to the control sample without additive (marked with K0), we used *Lactobacillus plantarum* MTD/1 s $1{,}54 \times 10^{11}$ CFU/g as ensilaging inocculant, in a ratio of 3 g/t (internally marked with E); we also used a preservative consisting of sodium benzoate, potassium sorbate and sodium nitrite, which was experimentally applied in a ratio of 1 l/t fodder (marked with SAF), as well as an ensilaging additive based on poly-beta-hydroxy-buryric acid in a ratio of 40 ml/t (marked with ALB), which is approved as utility design (CZ 032516 U1). Additionally, we tested sodium nitrite in a ratio of 1 l/t (D1), in a ratio of 3 l/t (D3) and D1 combined with E (ED1). The last additive was a new one (marked with AFS). It is based on the autolysate of biomass of non-pathogenic soil fungus, *Cephaliophora tropica*, grown in an environment enriched with trace elements. It was applied in a ratio of 40 ml/t. We filled 24 bags in total. We put them into a bunker silo. The silage was duly tamped down and covered. The bags (utility design CZ 031429 U1) with the silages were opened on 28. 2. - 5. 3. 2018. The temperature measurement interval for the silages in the bags was set at 15 minutes. We used battery thermomenters, Thermochron iButton Device DS 1921G-FS# (Maxim Integrated, USA), calibrated for measurement with an accuracy of 0,065 °C. The aerobic stability was determined in the laboratory, using the device according to patent CZ 303098 U1 (2012) and the method according to Ranjit and Kung (2000), respectively by the number of hours when the silage density increased by 3 °C. The ambient temperature in the laboratory oscillated between 19 and 20 °C. The temperature measurement interval for the silages with respect to aerobic stability in the laboratory was each 10 minutes.

RESULTS AND DISCUSSION

Fermentation development. Immediately after putting the bags into the bunker silo, the silage temperatures of the eight variants started quickly increasing. From the initial 18 °C, the temperature increased to 29,5 °C during 14 days, i.e. by almost one °C per day; then it gradually decreased, but considerably more slowly (10x), by about one tenth of °C per day. As compared to the control silage (K0), the temperature development of E differed, as its temperature increased more than that of K0 and it decreased more slowly. The E and D1 combination had the opposite effect; the temperature of ED1 did not achieve the level of K0 and it subsequently decreased more quickly. As compared to the control silage (K0), the temperature development of SAF differed, as its temperature increased more than that of K0 and it decreased more slowly. The temperature development of AFS was opposite; the temperature did not achieve the level of K0 and then it decreased more quickly. Interestingly, the temperatures increased and decreased relatively evenly, but the temperature increased by several tenths of °C for about 8 hours in irregular intervals. During the initial 14-day fermentation stage, the temperature increase was shorter; later, the time and intensity of temperature increase rose. We registered the said phenomenon only when using very sensitive temperature sensors for measurement, registering temperature changes from intervals of 0.065 °C. The explanation consists in mere speculation for the time being. It could be a physical phenomenon, but it is more probable that some microorganisms reproduce for a short time and consume the saccharides which are still free. Each microorganism activity is related to a chemical reaction which generates heat. It should be noted that when measuring aerobic stability, the said temperature changes did not occur.

Fermentation result. Table 1 shows the nutritional values and indicators of fermentation of maise silages, preserved by different ensilaging additives and without additives (K0). The average dry matter was 34.8 %, which can be considered almost ideal, according to Khan et al. (2015) and Peyrat et al. (2016). The differences in dry matter between variants were not conclusive; therefore the individual treatments can be compared among each other. However, the differences between variants were not conclusive for individual nutrients either.

Variant E showed a higher contents of saccharides than control variant K0. The other silages with the other tested ensilaging additives showed lower contents of saccharides. It could be confirmed that when microbial additive E is used, the fermentation development accelerates and therefore free saccharides are not consumed in such amount. When assessing the fermentation products, no significant differences could be found between the variants. The only exception consists in the proportion between the contents of lactic acid (LA) to volatile fatty acids (VFA), which was significantly higher at the variant with combined E and D1 (ED1), when compared with the other variants. The proportion of LA/VFA was the lowest at K0 (which proves a worse fermentation result), but the result was not conclusive.

Table 1 Nutritional values of maize silages preserved by different ensilaging additives

Indicator	K0	E	ED1	D1	D3	SAF	ALB	AFS	SEM
Dry matter (%)	36.0	34.6	35.2	34.8	35.1	34.9	33.6	34.5	0.785
Proteins (% of dry matter)	8.21	8.18	8.07	8.31	7.99	8.08	8.13	8.08	0.122
ADF (% of dry matter)	22.1	21.9	22.7	13.3	20.8	21.3	21.2	22.0	2.464
Saccharides (% of dry matter)	0.87	0.96	0.68	0.54	0.66	0.65	0.63	0.66	0.144
pH	3.73	3.74	3.73	3.73	3.74	3.73	3.76	3.75	0.007
LA/VFA	5.10^{a}	5.18^{a}	5.77^{b}	5.26^{a}	5.19^{a}	5.27^{a}	5.21^{a}	5.18^{a}	0.042
$N\text{-}NH_3$	19.8	19.8	19.8	19.8	19.7	19.8	19.8	19.9	0.026

The differences between values were conclusive (P>0.05) for LA/VFA (lactic acid to volatile fatty acids).

Aerobic stability. The biochemical processes occurring after the contact of silage with air and accompanied by temperature increase cause not only losses of energy and nutritional value of silages, but have also negative impact on their hygienic quality due to incrreased risk of spreading of potentially pathogenic or otherwise undesirable microorganisms. A secondary negative effect consists in decreasing and fluctuating intake of the silage by the animals. The use of different ensilaging additives may reduce the losses and aerobic degradation but it often cannot make up the primary fermentation losses (Ranjit and Kung Jr., 2000). The type of ensilaging additive should be therefore well considered. None of them is so universal to have simultaneous positive impact on nutritional values, fermentation result, aerobic stability, palatability and yield of the animals. For the above stated reasons, aerobic stability should be intensively researched.

The indicators of fermentation quality were assessed after three and seven days of leaving the silages in the open air at laboratory temperature of about 19 to 20 °C. Significant differences between the variants were measured only on the third day, for pH. Variants D1, D3, SAF and ALB differed significantly from K0 that had the lowest pH from among all variants.

While pH increased to the highest level on the third day, the acidity of water extract decreased. That can be explained by the fact that the contents of lactic acid decreased on the third day and increased on the seventh day. All differences between the first, third and seventh day of aerobic stability measurement were significant (P<0,05). The differences had identical development for all variants.

The aerobic stability and losses can be very positively evaluated. The silages with the said dry matter showed relatively good aerobic stability.

The temperature of K0 increased the most quickly and was the first to achieve its peak. All ensilaging additives were able to reduce the silage temperature at least a little or to delay its culmination. During the measurement, the temperatures fluctuated considerably, but we could not see any system in it and the fluctuations between measurements were not very high (0.1 to 0.3 °C). It can be stated that, surprisingly, the aerobic stability of silage E was significantly better (142 hours) hand in hand with lower losses (4.6 % of weight), as compared to silage K0 (72 hours and 4.9% weight loss). Surprisingly as well, silages SAF showed a stability of "only" 127 hours and 4.9% weight loss.

CONCLUSION

The results show that at a given dry matter of about 35 %, which, according to literature, is almost ideal, the differences of nutrient contents and fermentation quality between silages with different preservatives and silages without preservatives are not significant. The use of a preservative in this case is suggested only as a safeguard to reduce any risk. However, when assessing the aerobic stability according to temperature increase after exposing the silage to air, all tested silages treated with ensilaging additives had better aerobic stability than the control silage without preservative. The point is that all tested ensilaging additives were able to reduce the silage temperature at least a little or to delay its culmination.

Dedication: MZE-RO0718

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PRESERVATION OF ALFALFA WITH NEW-TYPE ENSILAGING ADDITIVES

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INTRODUCTION

The experiment was aimed at assessing the fermentation and aerobic stability of silages of alfalfa (*Medicago sativa*), harvested with about 32 % dry matter, with different chop lengths, under use of new-type ensilaging additives. There are substances which can influence the course and result of the fermentation process or the aerobic degradation of silages either by supporting the existing microflora through a nutrient (e.g. molasses), or by reducing an undesirable microflora (tannins). The said alternative approaches were described e.g. by Greatheadem (2003), or Broderick et al. (2017).

Biopolymers are the new alternative additives which could potentially improve the silage quality or delay the aerobic degradation of silages; such biopolymers are generated e.g. as secondary products of food processing or pharmaceutical industry. They include also the additives we have marked as ALB and AFS and tested their efficiency with the alfalfa ensilaging experiment.

MATERIAL AND METHODS

We silaged alfalfa from the first harvest, with 32 % dry matter and with longer chops, i.e. about 4 cm (D), and shorter chops, i.e. about 2 cm (K). The alfalfa chops were pressed into special bags which were subsequently stored in a bunker silo. The control variant was without any preservative (A0). The additives marked as ALB and AFS were tested. The ALB additive is based on the hydrolysate of *Bacillus megaterium*, whose active substance consists in poly-beta-hydroxy-butyric acid. It also produces the poly-γ-glutamic acid. Additionally, it contains a specially chosen mix of mineral substances. It is distributed in liquid form with a density of 1300 kg/m^3. The AFS additive is based on the autolysate of biomass of non-pathogenic soil fungus, *Cephaliophora tropica* D3, grown in an environment enriched with trace elements. Both additives have similar consistency to molasses. ALB was experimentally complemented with chemical preservative A3, containing a combination of organic acids and their salts, i.e. formic acid, propionic acid, sodium formate and sodium benzoate. It was applied in a ratio of 4 l/t. Both ALB and AFS were applied in a ratio of 40 ml/t of chops. Except for the variant with preservative A3, each variant was complemented with 40 ml distilled water per 10 kg chops to facilitate the application.

RESULTS AND DISCUSSION

Course and result of fermentation

The harvested alfalfa contained nitrogen substances in an average of 21,8 % dry matter and water-soluble saccharides (WSC) in an average of 5,5 % dry matter. The physically effective fibre (peNDF) amounted to 34,2 % in longer chops, i.e. it was provably higher (P<0,05) than in shorter chops (28,7 %). It can be stated that the fermentation temperatures had a characteristic development, as compared to our preceding experiments between 2016 and 2018 and that the individual variants did not significantly differ from each other. The differences between the control sample and the other preservatives were higher (by about 1°C) at silages with longer chops than at silages with shorter chops (about 0.5°C).

When comparing the results by nutritional values of the silages (dry matter, protein, ADF, NDF, WSC), no significant differences between the control sample without preservatives and the other tested variants or differences between the variants were found. The situation is similar when comparing experimental silages by pH, lactic acid (LA), volatile fatty acids (VFA), ammoniac nitrogen). No significant differences were found between the control sample without preservative and the other rested variants or differences between the variants. ALB resulted in the highest titrable acidity (TA) of water extract, but the values were not statistically higher than at silages with the other additives.

The aerobic stability was evaluated by the chemically analyzed products on the third and seventh day after removing the bags from the bunker silo and leaving them in the laboratory at a temperature of about 20 °C and subsequently by temperatures measured with special sensors each 15 minutes. The values of pH, LA, LA/VFA ratio, ammoniac nitrogen or TA did not significantly differ between the variants of the additives used or between the chop lengths used at silaging. A significant difference was found only at silages with long chops for acetic acid which, on the seventh day, had the lowest values at silages preserved by chemical preservative (A3) and the highest values for control silage without additive (K0). While during the seven days of measurement of aerobic degradation, the pH and ammoniac nitrogen increased, the other indicators, i.e. the content of LA, acetic acid, LA/VFA ratio and KVV decreased.

Higher aerobic stability (by almost 2 days in some cases) was found at silages with shorter chops, as compared with longer chops. As for silages with lohger chops, the worst stability was found at silage K0 without preservative (2 days). The best aerobic stability could be found at silages preserved with ALB, both at silages with lohger chops and at silages with shorter chops.

It turns out that reducing the length of chops is more efficient than applying additives; reducing the length of chops creates better preconditions for technological processing, primarily for displacing air from the silaged mass.

Evaluation of new additives

ALB was tested several times between 2016 and 2018 by our laboratory when silaging alfalfa. In the first experiment, we compared it with the bacterial and chemical additive and also tested the application ratio of ALB and addition of saccharide. Alfalfa chops with dry matter of about 45 % constituted the silaging material. The difference between the ratios of 40 ml/t and 80 ml/t was not significant; therefore the lower ratio was recommended for further experiments, also with respect to the price of the additive. The addition of saccharide did not improve the fermentation result. The differences in quality of silages between the control sample without preservative, with baterial inocculant and chemical preservative were not significant. At the control silage without additive, the temperature increased from the initial 25 ^{o}C (which was also the laboratory temperature) to 34 ^{o}C, while at the silage with ALB, it increased to 29 ^{o}C. ALB had higher aerobic stability by about 46 hours as compared to the control silage without additive.

In the subsequent year, the experiment was aimed at assessing the efficiency of ALB in alfalfa silage with high dry matter (about 60 %) and different lengths of chops. The applied preservative, the chop length and the lower content of dry matter did not affect the differences between the variants in the nutrient contents or in the indicators of fermentation quality to make them statistically significant. The losses of dry matter were usually lower at silages with shorter chops, but the differences in losses were not significant. The temperatures of silages after opening the silo increased from 19 ^{o}C to less than 24 ^{o}C, first at the control sample and only after 4 days (the laboratory temperature was about 20 °C). In silages with longer chops, the aerobic stability was 33 hours shorter in average (48 hours in silages with longer chops, against 81 hours in silages with shorter chops). The lowest aerobic stability was found at silages with longer chops without additives (26 hours) and with formic acid (30 hours), while the highest stability was found at silages with longer chops, preserved by ALB (81 hours). When silages were made with shorter chops, the temperature differences between silages with different additives were minimal.

Another experiment was made with alfalfa withered to about 35 %. Even in case of such dry matter, the added ensilaging additives did not break through in assessing the fermentation quality. Only the aerobic stability can be positively assessed; it was better at all variants (i.e. including ALB) of silages with longer chops than at control silages without preservative. In case of silages with shorter chops, the silage with ALB had significantly longer aerobic stability than all other variants, the temperature of the silage with ALB did not increase by 2 °C (i.e. did not exceed 21 °C) even after 7 days of measurement.

AFS was tested by our laboratory at alfalfa silaging for the first time. The silage with AFS did not show any significant difference in results of fermentation quality or aerobic stability, neither when compared to control silage without additive nor when compared with silages preserved by well established and tested additives. It is probably caused also by the fact that the alfalfa was ensilaged with a dry matter with which the fermentation has almost ideal development, and therefore the use of preservative constitutes rather a safeguard than a really improving action.

CONCLUSION

The use of new ensilaging additives for ensilaging of alfalfa showed that the indicators of nutritional values or of fermentation quality were not significantly different when compared with control silages without preservatives or with silages with different ensilaging additives commonly used in practice. ALB showed higher aerobic stability, primarily at silages with shorter chops (about 2 cm). It turns out that reducing the length of chops is more efficient than applying additives; reducing the length of chops creates better preconditions for technological processing, primarily for displacing air from the silaged mass.

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ANTIOXIDANT PROFILES OF *LACTOBACILLUS PLANTARUM* 24-7 AND ITS APPLICATIONS ON ALFALFA SILAGE: FERMENTATION CHARACTERISTICS, FATTY ACID COMPOSITION AND ANTIOXIDANT POTENTIAL

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INTRODUCTION

Excessive free radicals have been proven that processed negative effects on tissues of organisms. Lactic acid bacteria (LAB) and its metabolites have been reported to exist powerful antioxidant activity (Kullisaar et al., 2002), and may remove excessive production of free radicals to alleviate oxidative stress (OS) in ruminants when the animal's antioxidant system balance is disrupted during metabolic disorder (Zhou et al., 2018). However, studies of antioxidant LAB and the application of it in silage has not been previous discussed. Therefore, the objectives of this study were to investigate the effects of *Lactobacillus plantarum* 24-7 with antioxidant activity on fermentation characteristics and antioxidant properties of alfalfa silage.

MATERIALS AND METHODS

The fresh chopped alfalfa was wilted to the different dry matter (DM) contents of approximately 300 (30% DM) and 400 (40% DM) g/kg fresh weight, and then ensiled with treatments of distilled water (control) and antioxidant *Lactobacillus plantarum* 24-7. Then vacuum-sealed entirely, with 3 replicates for each treatment. The bag silos were deposited at an environmental-temperature on day 60 d for fermentation characteristics, fatty acid compositions and antioxidant properties.

RESULTS AND DISCUSSION

The application of the strain 24-7 led to lower silage PH, aNDF and ADF and greater WSC especially compared with the control (Table 1). At different DM contents the application of 24-7 strain resulted in lower percent of saturated fatty acid (SFA) and higher percent of monounsaturated fatty acids (MUFA), and increased the percentage of polyunsaturated fatty acids (PUFA) at 30% DM content after ensiling (Table 2).

Table 1. Chemical composition of alfalfa silages ensiled at different forage DM for 60 d (DM basis)

Item[1]	Inoculant treatment			Dry Matter (30%)		Dry Matter (40%)			ANOVA (*P*-value)		
	Control	24-7	SEM[2]	Control	24-7	Control	24-7	SEM	I	DM	I×DM
PH	5.38	5.14	0.01	5.58^{a}	5.34^{b}	5.18^{c}	4.95^{d}	0.01	<0.001	<0.001	0.533
aNDF, g/kg DM	327.3	306.2	0.22	312.4^{b}	294.3^{c}	342.2^{a}	318.2^{b}	0.16	<0.001	<0.001	0.375
ADF, g/kg DM	242.7	225.5	0.17	229.7^{b}	216.8^{c}	255.6^{a}	234.2^{b}	0.12	<0.001	<0.001	0.115
WSC, g/kg DM	5.2	5.6	0.00	5.12^{c}	5.36^{b}	5.44^{b}	5.77^{a}	0.00	<0.001	0.002	0.972
NH_3-N, g/kg TN	105.6	88.6	0.13	139.4^{a}	107.5^{b}	71.9^{c}	69.7^{c}	0.09	0.001	0.001	0.001

[a-d]Means of inoculation treatment within a row with different superscripts differ ($P < 0.05$).
[1]aNDF, neutral detergent fiber assayed with a heat-stable amylase; ADF, acid detergent fiber; WSC, water soluble carbohydrate; NH_3-N, ammonia nitrogen.
[2]SEM, standard error mean.

As shown in Table 3, the T-AOC, GSH-Px and CAT activity increased significantly ($P < 0.05$) with 24-7 treated group compared to those of the control, whereas T-SOD activity decreased ($P < 0.05$). When antioxidant 24-7 strain added to forage, it improved T-AOC, GSH-Px and CAT activity because of the high antioxidant activity in strain. However, T-SOD activity decreased with the application of 24-7 strain. One of the possible explanations was silage was an anaerobic fermentation process and oxygen pathway will be close, meanwhile, result in PH rapid declined with existence of LAB.

CONCLUSION

Application of antioxidant Lactobacillus plantarum 24-7 could effectively improve alfalfa silage fermentation quality, antioxidant activity and reduce proportion of SFA compared to the control, which has the potential to be an ideal silage additive.

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Table 2. Total FA content (g/kg of DM) and FA compositions (g/100g of Total FA) of alfalfa silage ensiled at different forage DM for 60

Item[1]	Dry Matter (30%)		Dry Matter (40%)		SEM[2]	ANOVA (*P*-value)		
	Control	24-7	Control	24-7		I	DM	I×DM
TFA, g/kg DM	12.90^{ab}	11.54^{b}	12.78^{ab}	13.34^{a}	0.17	0.285	0.042	0.025
SFA	50.17^{a}	47.49^{b}	45.86^{c}	44.65^{d}	0.13	<0.001	<0.001	0.021
MUFA	4.31^{c}	5.20^{a}	4.05^{d}	4.91^{b}	0.03	<0.001	0.001	0.793
PUFA	45.52^{c}	47.31^{b}	50.09^{a}	50.44^{a}	0.21	0.010	<0.001	0.412

$^{a-d}$Means of inoculation treatment within a row with different superscripts differ ($P < 0.05$).
[1]TFA, total fatty acids; SFA, saturated fatty acid; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
[2]SEM, standard error mean.

Table 3. Antioxidant activity of alfalfa silages ensiled at different forage DM for d 60.

	Inoculation treatment		SEM[2]	Dry Matter (30%)		Dry Matter (40%)		SEM	ANOVA (*P*-value)		
Item[1]	Control	24-7		Control	24-7	Control	24-7		I	DM	I×DM
T-AOC (U/mg)	21.91	27.12	0.23	20.50^{c}	23.03^{b}	23.31^{b}	31.20^{a}	0.32	<0.001	<0.001	<0.001
T-SOD (U/mg)	51.97	45.99	0.22	54.12^{a}	49.94^{b}	49.81^{b}	42.04^{c}	0.16	<0.001	<0.001	<0.001
GSH-PX (U/mg)	175.09	212.13	1.27	145.90^{c}	201.68^{b}	204.29^{b}	222.57^{a}	0.90	<0.001	<0.001	<0.001
CAT (U/mg)	0.52	2.61	0.06	0.86^{c}	2.89^{a}	0.18^{d}	2.33^{b}	0.04	<0.001	<0.001	0.503

$^{a-c}$Means of inoculation treatment within a row with different superscripts differ ($P < 0.05$).
[1]T-AOC, total anti-oxidation capacity; T-SOD, total superoxide dismutase; GSH-PX, glutathione peroxidase; CAT, catalase.
[2]SEM, standard error mean.

EFFECTS OF FOUR ORGANIC ACIDS KNOWN AS KEY INTERMEDIATES IN CITRIC ACID CYCLE ON FERMENTATION, BIOCHEMICAL COMPOSITION, AEROBIC STABILITY AND DIGESTIBILITY OF ALFALFA SILAGE

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Keywords: alfalfa silage, fermentation quality, proteolysis, aerobic stability

INTRODUCTION

As important intermediates in citric acid cycle, fumaric, malic, citric, and succinic acids play crucial roles in microorganisms. Previous studies have confirmed that these four acids could stimulate the growth of microbial in rumen and ultimately improve animal performance. It was supposed that addition of these four acids may not only have effects on silage fermentation but also indirectly provide animals with feed additives after ingestion of organic acid–treated silages. In addition, different dry matter contents also have effects on the fermentation quality of silage. Thus, this study aimed to investigate the effects of fumaric, malic, citric, and succinic acids on fermentation quality, proteolysis, aerobic stability and digestibility of alfalfa silage ensiled at two different DM contents, and to evaluate the utilization efficiency of these four organic acids in ensiled alfalfa after fermentation.

MATERIAL AND METHODS

Alfalfa from the first-cut was harvested in April 2018 by using a field mower. After wilted to approximately DM contents of 330 (normal DM) and 400 (high DM) g/kg of fresh weight, the forage was chopped into lengths of 1-2 cm. The Chopped alfalfa was ensiled with treatments of distilled water alone (control); (2) 0.5% malic acid (MA); (3) 0.5% citric acid (CA); (4) 0.5% succinic acid (SA); (5) 0.5% fumaric acid (FA). The chopped forages were mixed thoroughly and randomly divided into 20 sub-samples and four of each were treated with each additive and ensiled singly. Each treatment was ensiled in four replicates and kept at ambient temperature for 60 d. The effects of these four acids on fermentation quality and proteolysis of alfalfa silage were investigated after 60 d of ensiling. Data from silages of each sampling time were analyzed with the general linear model procedure of the Statistical Package for the Social Science (SPSS 21.0, SPSS, Inc., Chicago, IL). The differences among treatment means were tested by using the Tukey's multiple range test, and significance was declared at $P < 0.05$.

RESULTS AND DISCUSSION

The effects of four organic acids on the fermentation quality of alfalfa after 60 d are shown in Table 1. Compared with the control group, MA and CA-treated silages had lower pH at two different DM contents. It may due to the acid properties of MA and CA. However, no difference was observed in SS or SF-treated silages on the pH value relative to the control. In this study, succinic acid and fumaric acid were applied in the form of sodium salt because these two acids have poor solubility in the water. However, sodium succinate and sodium fumarate are both alkaline compounds, application of these two additives could result a rise in pH in treated groups. This may be the reason for comparable pH in SS and SF treatments. As expected, greater pH was observed in MA and CA-treated silages with a high DM versus a normal DM. It was probably due to the lack of moisture in silages ensiled at a high DM. Because of additional fermentation substrates provided by these four additives, greater contents of WSC were observed in treated silages when compared to the control group. In general, fermentation is closely related to the moisture contents of forage, and reducing moisture content could result in a lower WSC consumption during ensiling. In this study, the WSC were greater in silages with a high versus a normal DM. In the present research, treatments of MA and CA reduced the concentrations of NPN and NH_3-N relative to the control silage. It was probably due to the lower pH in MA and CA treated silages as plant enzymes are quickly inactivated with a decrease in pH (Kung and Bedrosian, 2010). Compared with silages ensiled at a normal DM, silages ensiled at a high DM had lower concentrations of NPN and NH_3-N. This is consistent with the observation from Cavallarin et al. (2005) where a significant decrease in proteolysis was found in alfalfa with increasing DM levels. In addition, lower NDF and ADF were observed in MA and CA-treated when compared to the control silage at two different DM contents. It was probably due to the lower pH in MA and CA-treated silages. The application of SS and SF reduced the contents of NDF in treated silages with a high DM, but it may due to the randomness of samples. Previous studies also suggested that malic and citric acids could be utilized as carbon sources by some yeast strains (Zhao et al., 2004; Seo et al., 2007) and it may be the reason for the poor aerobic stability in MA and CA-treated silages.

CONCLUSION

Application of these four acids could effectively improve silage fermentation quality with greater WSC and limited proteolysis during ensiling. Addition of MA and CA reduced the pH value and the contents of NDF and ADF at two different DM while the effects of SS and SF were quite small. Malic and citric acids are suitable additives in alfalfa silage fermentation. However, the negative effects of MA and CA should be pay attention in practice.

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Table 1 The effects of four acids on the fermentation quality of alfalfa silage after 60 d

Variable	Forage DM	Treatments (T)					SEM	*P*-value		
		Control	MA	CA	SS	SF		DM	T	DM × T
pH	Normal	4.92	4.58	4.67	4.89	4.99	0.013	<0.001	<0.001	0.005
	High	4.95	4.73	4.79	5.00	4.99				
WSC, g/kg DM	Normal	10.8	18.1	18.9	22.0	19.0	0.055	<0.001	<0.001	<0.001
	High	12.6	20.3	23.7	25.1	19.2				
NPN, g/kg TN	Normal	793	670	670	747	740	1.087	<0.001	<0.001	<0.001
	High	704	599	578	639	621				
NH_3-N, g/kg TN	Normal	161	69.8	80.4	94.2	107	0.536	<0.001	<0.001	<0.001
	High	91.8	28.0	32.2	36.7	38.8				
NDF, g/kgDM	Normal	32.0	27.5	29.6	32.5	32.0	1.191	0.500	<0.001	<0.001
	High	33.7	28.5	30.2	30.3	29.8				
ADF, g/kg DM	Normal	23.1	21.1	21.6	23.2	23.6	0.972	0.002	<0.001	0.042
	High	23.7	22.3	23.2	23.8	23.2				
Aerobic stability, h	Normal	239	231	223	221	233	0.597	<0.001	<0.001	<0.001
	High	200	128	214	196	224				

MA, malic acid; CA, citric acid; SS, sodium succinate ; SF, sodium fumarate .

SEM, Standard error of the mean; DM, dry matter; WSC, water soluble carbohydrates; TN, total nitrogen; NPN, non-protein N; NH_3-N, ammonia N; NDF, neutral detergent fiber; ADF, acid detergent fiber.

EFFECTS OF CLASS IIA BACTERIOCIN-PRODUCING *LACTOBACILLUS* SPECIES ON FERMENTATION QUALITY AND AEROBIC STABILITY OF ALFALFA SILAGE

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Keywords: alfalfa silage, bacteriocin, lactic acid bacteria, aerobic stability

INTRODUCTION

Extensive research in recent years has revealed the potential of bacteriocin produced by lactic acid bacteria as alternatives to feed antibiotics for improving the preservation of forages. Previous study has shown that inoculating forage with bacteriocin-producing bacteria was more effective than bacteriocin-free controls at inhibiting the growth of *L. monocytogenes* (Amado et al., 2012). By application of the pure bacteriocin produced by *P. acidilactici*, Amado et al. (2016) further confirmed that the applied bacteriocin could effectively inhibit the growth of *L. monocytogenes*. As a new bacteriocin, however, little information is available on the application of class IIa bacteriocin-producing LAB in ensiled forages. Thus, the objective of this study was to examine effects of class IIa bacteriocin-producing LAB on fermentation quality, microbial counts, proteolysis and aerobic stability of alfalfa silage.

MATERIALS AND METHODS

The chopped forage was ensiled with or without two strains of class IIa bacteriocin-producing lactic acid bacteria, *Lactobacillus delbrueckii* F17 (**F17**) and *Lactobacillus plantarum* (BNCC336943) (**LPB**), and a proven bacteriocin-free inoculant *Lactobacillus plantarum* MTD-1 (NCIMB40027) (**LPN**) in the laboratory silo, each at an application rate of 1×10^6 cfu/g. The mini-silos were then stored in an air-conditioned room (25 ± 2°C) and ensiled for 3, 7, 14, 30 and 60 d. Three replicates were prepared for each treatment at each ensiling duration.

Table 1. Effects of additives and ensiling time on pH and microbial counts of alfalfa silage

Items	Treatment[1]	Ensiling time (d)[3]					Mean	SEM	Significance[2]		
		3	7	14	30	60			T	D	T×D
pH	C	5.88^{aA}	5.84^{bAB}	5.87^{aA}	5.73^{aB}	5.60^{aC}	5.79^{a}	0.023	0.001	<0.001	<0.001
	F17	5.76^{aB}	5.96^{aA}	5.72^{bBC}	5.63^{bC}	5.34^{cD}	5.68^{ab}				
	LPB	5.56^{bB}	5.78^{bA}	5.56^{cB}	5.47^{cB}	5.29^{dC}	5.53^{c}				
	LPN	5.72^{aA}	5.54^{cB}	5.73^{bA}	5.66^{bA}	5.48^{bB}	5.62^{bc}				
Yeasts, $\log_{10}$ cfu/g	C	8.05^{aA}	2.74^{aC}	3.66^{aB}	ND^{cD}	ND^{D}	2.88^{b}	0.027	< 0.001	< 0.001	< 0.001
	F17	6.57^{cA}	ND^{bC}	3.06^{bB}	2.40^{bC}	ND^{C}	2.41^{c}				
	LPB	7.36^{bA}	ND^{bC}	2.77^{bB}	2.70^{abB}	ND^{C}	2.57^{c}				
	LPN	7.77^{abA}	2.70^{aB}	< 2.00^{cC}	3.05^{aB}	ND^{D}	3.10^{a}				
Molds, $\log_{10}$ cfu/g	C	4.70^{bA}	ND^{bD}	2.13^{aC}	ND^{cD}	3.59^{aB}	2.08^{b}	0.021	< 0.001	< 0.001	< 0.001
	F17	ND^{cC}	ND^{bC}	2.09^{aB}	ND^{cC}	2.30^{bA}	0.88^{c}				
	LPB	ND^{cB}	ND^{bB}	< 2.00^{bA}	< 2.00^{aA}	ND^{cB}	0.73^{d}				
	LPN	5.59^{aA}	3.00^{aB}	< 2.00^{bC}	< 2.00^{bC}	ND^{cD}	2.43^{a}				

Means in the same column with different lowercase letters differed ($P < 0.05$) and means in the same row with different uppercase letters differed ($P < 0.05$).

[1]C, control, no additive; F17, *Lactobacillus delbrueckii* F17; LPB, *Lactobacillus plantarum* (BNCC 336943); LPN, *Lactobacillus plantarum* MTD-1 (NCIMB 40027).

[2]T, treatment; D, ensiling time; T × D, the interaction between treatment and ensiling time.

[3]< 2.00, below the detection limit; ND, not detected.

RESULTS AND DISCUSSION

As shown in Table 1. On d 3 of ensiling, LPB-treated silage had the lowest pH but by d 7, LPN-treated silage had the lowest pH followed by LPB and the control silages. Yet after ensiling for 14, 30 and 60 d, F17, LPB and LPN had reduced ($P = 0.001$) silage pH compared to the control and the lowest pH was consistently in the LPB-treated silages. In addition, application of F17 and LPB decreased the number of yeasts and molds

relative to control and LPN-treated silages. Large numbers of yeasts and molds (> 10^5 $\log_{10}$ cfu/g) adversely affect the preservation of silage and may lead to rapid spoilage when the silage is exposed to air (Gerlach et al., 2013). Compared to the control silage, inoculant-treated silages had greater aerobic stability (Fig 1), water-soluble carbohydrate and crude protein concentrations, and lower neutral detergent fiber, amino acid nitrogen, and ammonia nitrogen concentrations (Table 2).

Table 2. Dry matter and chemical composition of alfalfa silages ensiled for 60 d

Item	Treatment (T)[1]				SEM	*P*-value
	C	F17	LPB	LPN		
DM, g/kg of FM	306.57^{c}	326.33^{a}	314.93^{ab}	310.46^{b}	2.519	0.005
DM loss, g/kg DM	84.88^{a}	56.39^{b}	42.37^{b}	51.59^{b}	5.641	0.013
WSC, g/kg of DM	3.86^{c}	4.35^{b}	4.54^{a}	4.65^{a}	0.092	< 0.001
CP, g/kg of DM	202.15^{c}	215.40^{b}	212.38^{b}	222.78^{a}	2.309	< 0.001
NPN, g/kg of total N	621.43^{ab}	632.81^{a}	499.01^{c}	607.88^{b}	16.230	< 0.001
AA-N, g/kg of total N	239.59^{a}	142.08^{b}	147.57^{b}	143.20^{b}	12.582	< 0.001
NH_3-N, g/kg of total N	132.49^{a}	111.11^{b}	84.56^{c}	61.93^{d}	8.095	< 0.001
aNDF, g/kg of DM	345.24^{a}	336.14^{b}	331.98^{b}	334.87^{b}	1.859	0.032
ADF, g/kg of DM	257.55^{a}	252.24^{ab}	247.97^{b}	249.79^{ab}	1.512	0.106

Means in the same row with different lowercase letters differed ($P < 0.05$).
[1]C, control, no additive; F17, *Lactobacillus delbrueckii* F17; LPB, *Lactobacillus plantarum* (BNCC 336943); LPN, *Lactobacillus plantarum* MTD-1 (NCIMB 40027).

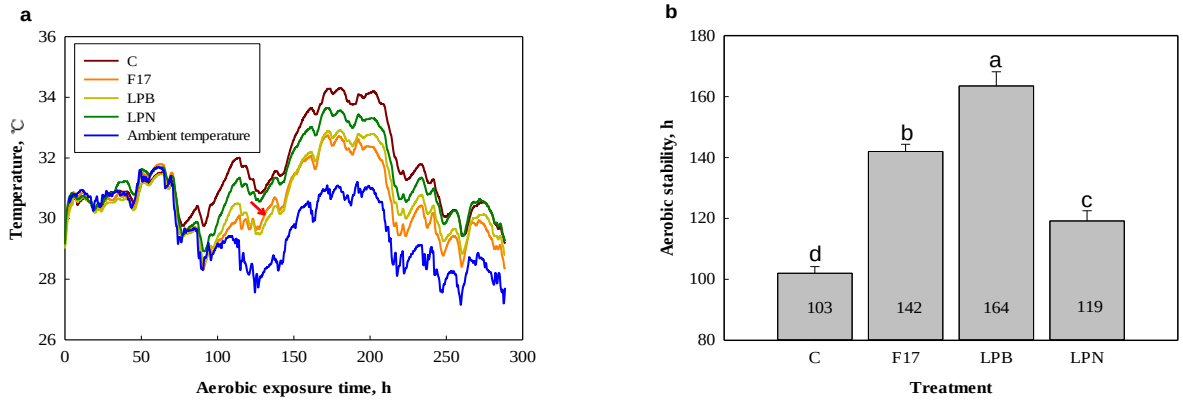

Fig 1. Effects of bacterial inoculants on aerobic stability of alfalfa silage. a, Temperature change during the aerobic phase; b, Aerobic stability of alfalfa silage treated without or with different inoculants.

CONCLUSIONS

Inoculation with the class IIa bacteriocin-producing LAB strains, *Lactobacillus delbrueckii* F17 and *Lactobacillus plantarum* (BNCC 336943), at ensiling of alfalfa improved silage fermentation quality, reduced counts of molds and yeasts at early fermentation stage and improved aerobic stability compared with the *Lactobacillus plantarum* MTD-1, which does not produce bacteriocin.

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Section 3: Nutrition value of silages and their effect on production and health of animal

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PROTEIN QUALITY OF GRASS SILAGE AFFECTED BY MANAGEMENT FACTORS

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INTRODUCTION

The emphasis on silage protein evaluation is justified as protein - or more correctly amino acids (AA) - are essential nutrients to both ruminants and humans. Shortage of AA will greatly impair the live functions including production of ruminant livestock. Protein supply is in many cases limiting in the diet and protein supplements are used to promote production. The term requirement is however partly misleading as for lactating dairy cows, the milk output is rather a response to the supply of nutrients rather than a fixed requirement *per se* (Huhtanen & Nousiainen, 2012).

Digestible crude protein concept was replaced several decades ago with more developed protein evaluation methods which take into account the crude protein (CP) degradation in the rumen, the need of a nitrogen (N) source of rumen microbes, and the fact that AA available for the host ruminant consist mainly from microbial protein synthesized in the rumen and to a lesser extent, from undegraded feed protein (see e.g. Weisbjerg et al., 2010). The more complicated protein evaluation systems also require detailed accurate and precise analyses of the feeds, which may in some cases be a limiting factor in their use.

Grasses are typically relatively high in protein, but the protein quality is not considered very good from animal nutrition point of view, and further, it is decreased during the preservation to a varying extent. The aim of the current review is to discuss the effects of grass silage protein quality from the point of view of ruminant nutrition. We emphasize the animal production responses, which give the most realistic picture of the true protein value, and take into account the complicated and interdependent processes within the ruminant animal that finally dictate the amount of AA they are able to derive from different diets. It is important to keep in mind that individual feed components form jointly the whole diet and judging individual feed components is not very fruitful. For example, high amount of rumen degradable nitrogenous compounds in silage leads to low N use efficiency (NUE) in a diet that has high CP content, but if it supplements an otherwise low CP diet, the rumen degradable N is more efficiently used in producing microbial protein in the rumen.

PROTEIN QUALITY - HOW TO DEFINE IT?

We need to start by defining what we understand with protein quality. Crude protein can be analytically characterized as true protein (TP) and non-protein nitrogenous compounds (NPN). Further, the NPN can be divided into peptides, amino acids, amides, ammonia-N etc., and the profile of the AA can be analysed. These characteristics are of great importance if silage protein would be used for monogastric farm animals such as pigs (Rinne et al., 2018). However, they do not tell much about the amount of AA available to the host ruminant, because of the massive effect of the rumen on the nitrogenous compounds of the feed (see e.g. Van Soest, 1994).

To face the challenge of protein evaluation for ruminants, sophisticated methods have been developed to take into account the complicated digestive system of the ruminant animal. Protein concentration and characteristics, intake, digestibility, efficiency of use of ingested nutrients, concentration of fibre (soluble in acid and neutral detergents) among others are used in different protein evaluation systems based on several statistical models to predict the silage quality. Themethods to charactreize silage protein include approaches such as the *in situ* nylon bag based ruminal protein degradability approach developed originally by Ørskov et al. (1980), the Cornell Net Carbohydrate and Protein System (CNCPS; Fox et al., 2004) and various *in vitro* analyses (e.g. Stefanski et al., 2013, Gidlund et al., 2018, Südekum & Böttger, 2019).

The ultimate measure of protein quality is however the production responses of the animals and this emphasizes the need to verify changes in feed quality using animal experiments. In an *in vivo* situation, factors such as effects on voluntary feed intake, rates of ruminal digestion and passage, and links between energy and protein supply can be taken into account, which is very challenging based on *in vitro* data even if combined with sophisticated models. Huhtanen & Broderick (2016) presented very clearly that our attempts to characterize analytically forage protein quality have not been very successful, and not even for concentrate feeds (Huhtanen, 2019).

ANALYTICAL METHODS TO EVALUATE PROTEIN QUALITY OF SILAGES

There are several parameters to evaluate the protein of the grass silage. The CP concentration of a particular feed can be estimated chemically from the concentration of N and the analysis routinely used for its determination was developed by Johan Kjeldahl in 1883 (AOAC, 1990; method 984.13), while nowadays also the Dumas method is widely used (AOAC, 1990; method 968.06). The concentration of N is actually analysed and it is converted to CP by multiplying the N concentration with a factor of 6.25. This process is based on two assumptions:

1. All N in the feed is in the form of true protein;
2. All proteins contain 16% N.

It is well known that both assumptions are not entirely correct for silages, because:

1. Many other NPN compounds are present in silage including amides, amines, free amino acids, ammonium salts, glucosides, alkaloids, pigments etc.

2. Feed proteins have different levels of N and it would be more correct to use different conversion factors for different types of protein which vary from 13% to 18%. The use of factor 6.25 (16%) is a simplification but, although erroneous, it is justified in practice, except when feeds with clearly deviating N concentrations are used as major components of the diet.

Routinely laboratory analyses of silage nitrogenous components include only CP and proportion of ammonia N in total N (NH_3-N). The protein contained in ruminant feeds is composed of rumen degradable protein (RDP) and a fraction that escapes ruminal degradation, called undegraded dietary protein (UDP) or by-pass protein. In the ensiling process after proteolysis the amount of UDP is reduced and NPN compounds are produced, including not only NH_3-N but also peptides, free amino acids and biogenic amines. The degradation of proteins in the rumen occurs through the action of enzymes secreted by the ruminal microorganisms (Ørskov & McDonald, 1979). The metabolizable protein requirements of ruminants are met by rumen-synthesized microbial protein and dietary protein that escapes ruminal fermentation, which makes protein nutrition of ruminants quite complex.

In order to determine in detail the various types of protein fractions in feeds depending on theirs rate and extent of degradation in the rumen, the CNCPS has been developed (Fox et al., 2004). Knowing the protein fractions, equations can be used to determine the digestion and the passage of the fractions, considering ruminal dynamics (Sniffen et al., 1992). According to CNCPS, feeds are subdivided based on their chemical and physical characteristics, ruminal degradation and post-rumen digestibility, generating information that can be used to estimate nutritional value, intake and animal performance (Nocek & Russell, 1988, Fox et al., 2004).

The CNCPS model uses chemical reagents to determine the protein fractions that are divided into five components: A, B1, B2, B3 and C. The fraction A represents nitrogenous components of NPN that are solubilized and assumed to be degraded instantly. The chemical determination of Fraction A is performed as the ratio of soluble protein in borate-phosphate buffer solution that does not precipitate into trichloroacetic acid. On the other hand, fraction C represents the protein which is bound to the acid detergent fibre and is not degraded in the rumen (nor later in the digestive tract). Fraction C contains lignin-associated proteins, tannins and Maillard products known as acid detergent insoluble protein (Licitra et al., 1996).

Fraction B represents the potentially degradable fraction and is divided into three subfractions according to their degradation rates, subject to the effects of passage. Fraction B1 represents the fraction of soluble protein in borate-phosphate buffer, but precipitates in trichloroacetic acid (rapidly degraded in the rumen). Fraction B3 is calculated as the difference between the fraction of the protein recovered in the neutral detergent insoluble residue and that recovered in the acid detergent insoluble residue. This fraction represents the potentially degradable protein in the cell wall of plants, is slowly degraded in the rumen and has a digestion rate of 0.02 to 1.0% per hour. This fraction presents N associated with the constituents of the cell wall and proteins belonging to the extensin classes, abundant proteins in proline and glycine, and proteins associated with arabinose and galactose (Showalter, 1993). Fraction B2 represents the fraction of insoluble protein in borate-buffer present in the cell contents, being obtained by the difference between the total value of the feed protein and the sum of the fractions A, B1, B3 and C and shows an intermediate ruminal degradation rate.

The CNCPS, through works by Sniffen et al. (1992) and Russell et al. (1992), emphasizes the need for synchronization in the degradation of nitrogen compounds and carbohydrates in the rumen in order to obtain maximum efficiency of microbial protein synthesis, as well as reduction in energy and nitrogen losses due to ruminal fermentation. Through mechanistic models it is possible to estimate the amount of microbial protein synthesized, ruminal nutrient escape and metabolizable protein, based on data about carbohydrate and protein fractions and their respective rumen degradation rates.

PROTEIN DIGESTIBILITY

It is important to distinguish between true and apparent CP digestibility of feeds as they pass through the digestive tract. Apparent digestibility is calculated as the difference between CP intake and faecal CP excretion divided by CP intake. Faeces contains metabolic (microbial) and endogeneous (secretions from the digestive tract) nitrogenous compounds which actually have been digested and this results in lower apparent than true digestibility of CP (Van Soest, 1994). The metabolic and endogenous excretion of CP is mainly related to dry matter (DM) intake of the animals. Although true digestibility of dietary CP is almost complete, the constant metabolic and endogenous excretion of CP on DM intake basis results in an artefact that the CP digestibility is the lower, the lower the CP concentration of the diet (Figure 1). This makes apparent CP digestibility a practically useless measure of dietary CP quality, which is merely an indicator of diet CP concentration.

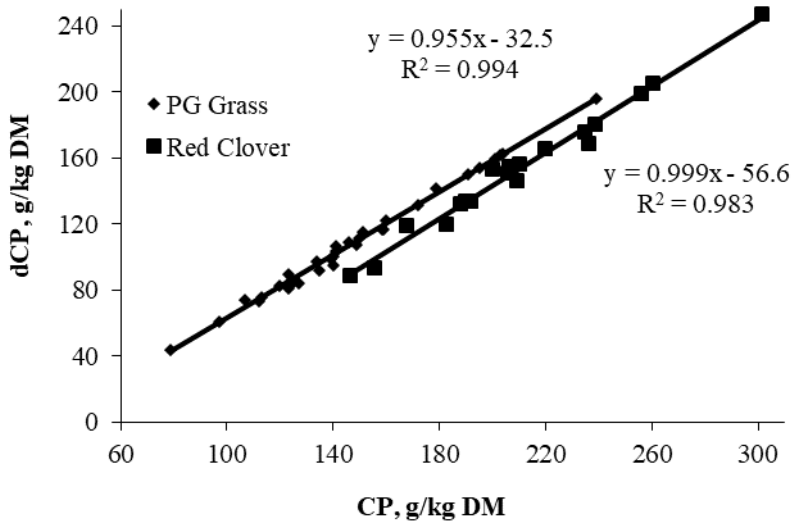

Figure 1. The "Lucas equation" reveals that the true digestibility (slope of the regression equation) of primary growth (PG) grass and red clover protein is close to complete when the concentration of dietary crude protein (CP) is plotted against the concentration of digested CP (dCP). The intercept of the equation represents the metabolic and endogenous faecal CP excretion (modified based on Huhtanen & Broderick, 2016).

THE RUMINANT ANIMAL IS A PROTEIN SYNTHESIZER

Virtanen (1966) demonstrated that dairy cows can be maintained on purified diets containing no AA. The study showed that lactating cows fed ammonium salts and urea as the sole source of N could fulfil their maintenance requirements and also produced reasonable amounts of milk over extended periods (several lactations). This study proved more than half a century ago the potential of the rumen to synthesize AA from simple nitrogenous compounds and purified carbohydrates which were used as non-AA containing energy sources. Even the current dairy cow obtains majority of her AA from microbial protein synthesized in the rumen.

Under commercial situations, dairy cows are typically fed diets consisting of forage, cereal grains and by-product feeds so that the amount of human edible feeds can be very low in their diet. In Figure 2, three different scenarios are presented with different ratios between milk protein production and the amount of human-edible and non-human-edible protein in the diet of the cow. This indicates the high potential of dairy cows in upgrading the dietary CP, which could in many cases be utilized to an even greater extent with benefits in environmental and ethical quality of ruminant based production.

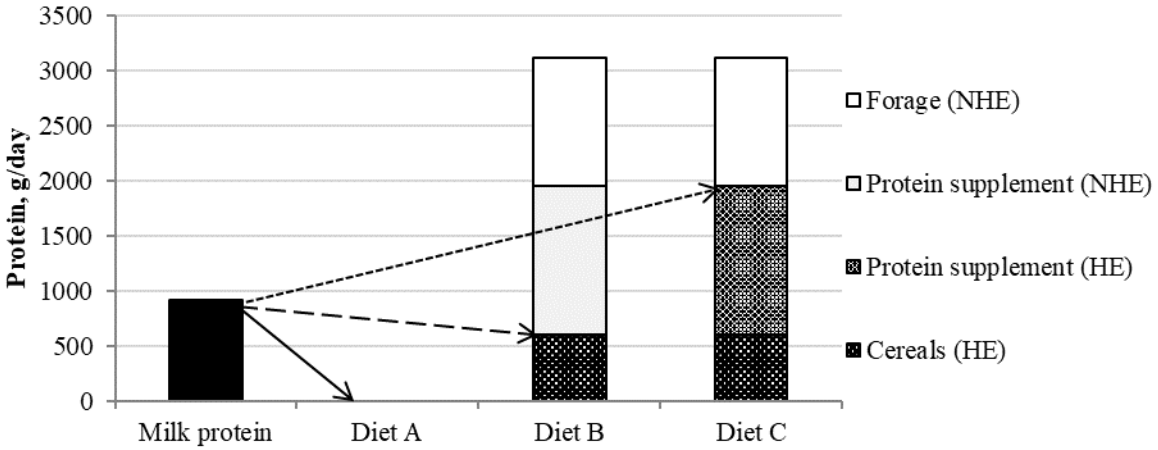

Figure 2. A schematic comparison of the amount of milk protein production (solid black bar) compared to the amount of dietary consumption of human-edible (HE) and non-human-edible (NHE) protein. Diet A is a hypothetical diet in the spirit of Virtanen (1966) containing only urea or ammonia N; Diet B consists of forage, cereal grains and a non-human edible protein supplement such as rapeseed meal; Diet C is otherwise similar to B but the protein supplement is human-edible such as soybean meal. The arrows highlight that the intake of HE protein by cows can be clearly (solid arrow) or moderately (long dashed arrow) lower than that produced as milk protein, but also clearly higher (short dashed arrow) depending on ration components used.

HIGH NITROGEN USE EFFICIENCY IS AN IMPORTANT TARGET

It is essential that we develop livestock production systems to be environmentally more sustainable. This also applies to protein feeding as N contributes to eutrophication, ground water nitrification, and ammonia and nitrous oxide emissions. The NUE is commonly used term and it is calculated as the ratio of N in the products (typically milk) and the dietary N intake. In milk production, the value is typically around 250 - 300 g/kg N, but it varies quite much and is dominated by the concentration of CP in the diet (Figure 3; Huhtanen et al., 2008a). In line with decreasing NUE, the proportion of N excreted in urine rather than in faeces increases with increasing CP in the diet, which may affect the environmental impacts of the manure.

The simplest tool to improve NUE and subsequently decrease the environmental load from ruminant livestock operations is to decrease the CP concentration of the diets used for the livestock. There seems to be little to be gained from manipulating the quality of forage protein in this respect (Huhtanen at al., 2008a). Even using different types of protein supplements and treatments of them resulting in clear differences in e.g. *in situ* ruminal CP degradation have shown only very limited if any *in vivo* benefits in milk production and subsequently in NUE (Huhtanen, 2019).

Options to decrease dairy cow CP intake in order to improve NUE include actions such as:

- Dilute high CP diets with low CP feeds. Use of maize or whole crop small grain cereal silages to dilute the CP concentration of grass silage based diets provides potential additional benefits in manure spreading and grass renewal possibilities under cover crop which may also result in improvements in total farm performance.
- Use less protein supplements - the use of protein supplements is mainly optimized economically taking into account the feed cost vs. additional milk income and currently indirect costs related to low NUE are not considered.
- Decrease N concentration of grass by avoiding unnecessarily early timing of harvest and over-fertilization of N, and by providing good growing conditions so that other growth factors such as drought or flooding, other nutrients, soil structure, weeds etc. are not limiting grass DM accumulation.

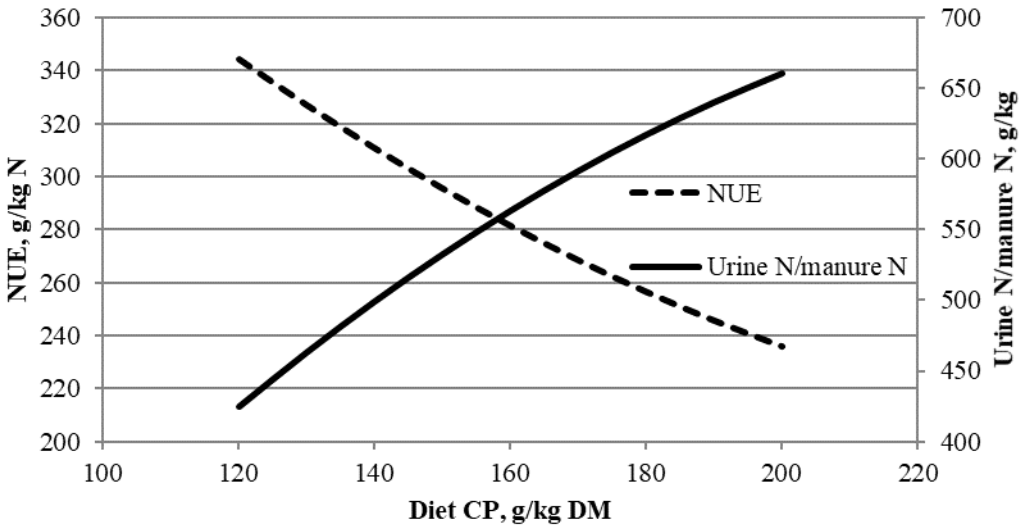

Figure 3. Increasing dairy cow diet crude protein concentration leads to decreased nitrogen use efficiency (NUE, g N excreted in milk per kg dietary N intake) and increases the proportion of N excreted in urine compared to that excreted in manure (equations from Huhtanen et al., 2008a).

KEY SILAGE MANAGEMENT FACTORS AFFECTING CRUDE PROTEIN

Basically all management factors related to grass silage production also have an impact on protein including plant species and variety, plant maturity, growth cycle, fertilization and soil type, wilting, weather conditions (temperature, water availability), additive treatment, silo type, chop length, length of preservation period and aerobic deterioration during feed out. Some of the most relevant management options are discussed here. It is however important to notice that a change in one management factor typically has several effects and it may sometimes be difficult to identify exactly which change is causing the effects. If we for example look at plant in a more advanced stage of maturity, it results in decreased CP concentration and solubility, but at the same time, typically sward DM and cell wall concentrations increase while the extent of silage fermentation, diet digestibility and voluntary feed intake decrease. In many cases, it is difficult to separate the effects of different simultaneously changing factors from one another.

PLANT NITROGEN AVAILABILITY

When considering grasses, the very important factor affecting the grass and subsequent silage CP concentration is the amount of plant available N (nitrate or ammonia N) in the soil, which is mainly affected by the amount of fertilization used. The grasses quickly pick up N from the soil and with progressing growth and accumulating biomass DM, that N is diluted resulting in a typical decreasing concentration of CP in forages with progressing growth time (Figure 4). The extent of CP dilution can be affected by other growth factors limiting the DM accumulation, e.g. water scarcity. For forage legumes, this phenomenon is not as clear because N fixation takes place as growth progresses.

Grasses typically respond to increasing N fertilization by both increasing the DM yield and CP concentration of the biomass. In a data set from Finland consisting of N fertilization studies (n=336), one kg additional N fertilization per hectare increased grass DM yield by 21 kg per ha and grass CP concentration by 0.24 g/kg DM (Korhonen et al., 2005).

Increased soil N availability may result in accumulation of nitrate N in the biomass, which may in extreme cases cause ammonia poisoning in cattle. Nitrate N is however quickly reduced to NH_3-N in the silage (McDonald et al., 1991). Nitrate N in the herbage or nitrite based additives inhibit Clostridia growth in silage and may help prevent the formation of butyric acid into silage (König et al., 2019).

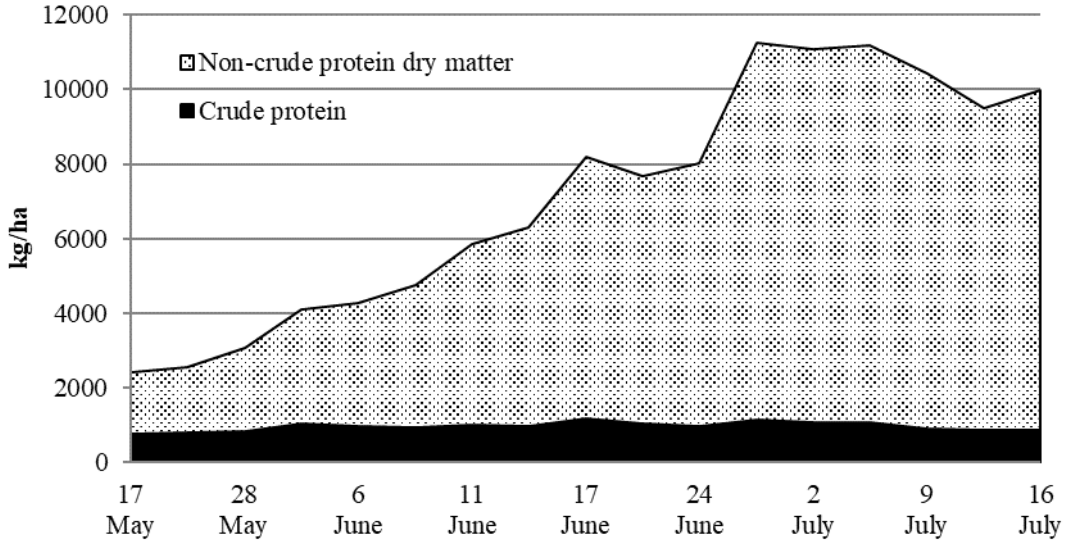

Figure 4. Amounts of crude protein (CP) and non-CP dry matter (DM) of a timothy-meadow fescue sward with progressing primary growth in Finland (Rinne et al., unpublished). The CP concentration was <300 g/kg DM in the beginning of sampling period and declined to >100 g/kg DM in the end.

PLANT MATURITY

The changes in the grass plant development during progressing growth are very large as the young leafy plant starts to develop stem and inflorescence. The differences between early and late maturity stage in grass composition are typically larger than those between different forage plant species, which must be kept in mind when making species comparisons. The stage of plant maturity leads to some changes in the form of CP present in the plants simultaneously as CP is diluted into the increasing biomass (Figure 4). Rinne et al. (1997) sampled timothy-meadow fescue grass in primary growth at one-week intervals and noted that as CP concentration decreased from 172 to 113 g/kg DM, the CNCPS fractions in grass did not vary systematically except for increased concentration of fraction C. However, in subsequent silages, the solubility of N was clearly decreased with progressing maturity (Figure 5).

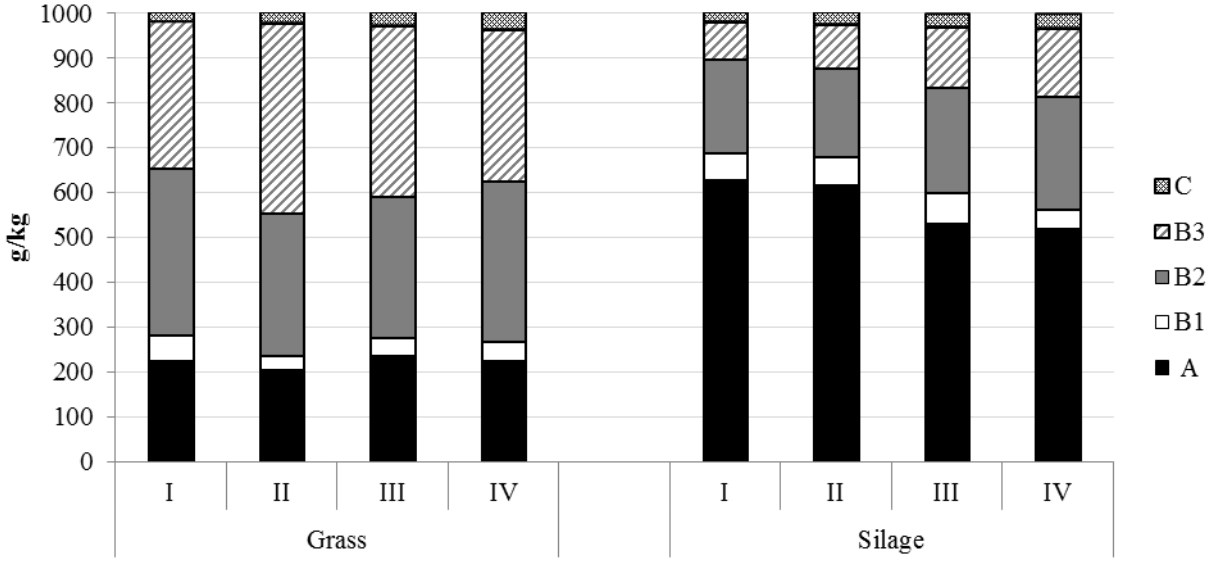

Figure 5. The CNCPS protein fractions of grasses and subsequent silages harvested from primary growth at one week intervals (from I to IV; Rinne et al., 1997).

Wilting

Wilting is an efficient method to restrict the extent of microbial fermentation during the ensiling process as water activity decreases (McDonald et al., 1991). According to Huhtanen et al. (2013), risks of clostridial fermentation in the silage and effluent losses decrease after wilting the grass. However, increasing DM concentration may also increase nutrient losses during drying, impair the microbiological quality of crop and expose the silage to aerobic deterioration. Wilting makes the nutrients more concentrated which helps in achieving conditions for successful fermentation particularly regarding water soluble carbohydrates, which are readily fermented. According to Papadopoulos and McKersie (1983), wilting resulted in silages with lower concentration of NH_3-N. Nadeau et al. (2019) concluded that wilting under good conditions resulted in decreased solubility (increase in B3 fraction of CNCPS) which increased the calculated amount of rumen undegradable protein.

Wilting has become a mainstream activity in grass silage production, which has several benefits such as improved silage fermentation quality, easier logistics and prevention of silage freezing. However, benefits in milk production responses from increased DM concentration and decreased protein solubility of silage are not evident unless the fermentation quality of the unwilted silage has been clearly inferior compared to the wilted silage. It is also noteworthy that sometimes the weather conditions prevent efficient wilting. Extended wilting under poor conditions may lead to substantial DM losses and elevated NH_3-N (McDonald et al., 1991). Under poor conditions, ensiling the grass at lower than targeted DM concentration may be a better option than extending the unsuccessful wilting period.

Modifications to protein due to fermentation and possibilities to modify them by the use of additives

The ensiling process as such does not typically affect the amount of CP if the preservation is successfully conducted (Huhtanen et al., 2005), but during ensilage, the solubility of CP increases clearly resulting in a decrease in TP concentration (Figure 5). The single most common protein of green crops is Rubisco (Ribulose-1,5-bisphosphate carboxylase/oxygenase), which is the main photosynthetic enzyme. This leads to the fact that green crop AA profile is relatively stable. Rubisco as such is nutritionally of high quality with a good AA profile and high digestibility. It is however quickly degraded into peptides and AA after ensiling. It seems that the majority of the protein solubilization is due to plant derived exo- and endopeptidases (McDonald et al., 1991, Tao et al., 2012). In a large (n > 100 000) Finnish on-farm silage data set, water soluble N was 413 (SD = 129.9) and that of NH_3-N 44 (SD = 24.8) g/kg N when the mean CP concentration was 147 (SD = 26.6) g/kg DM. The values for both soluble N and NH_3-N are relatively low, which is explained by the wide use of formic acid based silage additives in Finland.

The numbers show that a relatively small proportion of silage CP is degraded to the final step to NH_3-N. For ruminants, even NH_3-N can be utilized by rumen microbes. High NH_3-N does however indicate poor fermentation quality which is likely to be linked with other negative impacts such as high nutrient losses during ensiling. High NH_3-N has also been linked to decreased voluntary silage intake although NH_3-N did not appear as a statistically significant factor in the silage DM intake index presented by Huhtanen et al. (2007). It is noteworthy that in the earlier version of the index, NH_3-N had a negative impact on DM intake (Huhtanen et al., 2002). The reason for its absence in the later version may be the lack of high NH_3-N silages in the updated data set.

The NH_3-N is considered a sensitive indicator of silage hygienic quality (Wilkinson, 1990) and it is the final degradation product of AA resulting mainly from Enterobacteria and Clostridia activity in the silage (McDonald et al., 1991). Although NH_3-N was emphasized in this discussion, also other measures (pH, residual water soluble carbohydrates, volatile fatty acid concentrations, extent of fermentation, microbial quality, aerobic stability etc.) of silage quality are important and no single parameter should be looked at in isolation when silage quality is evaluated.

Silage additives provide a practical tool to positively manipulate silage quality. There is plenty of experimental results available from comparisons conducted using different types of additives e.g. in the Proceedings of the current Symposium and in the series of International Silage Conferences. Various inoculants which are mainly selected strains of lactic acid bacteria (LAB) are very popular additives although in some markets organic acids or salt based additives are also used. Formic acid based additives are typically able to effectively restrict silage fermentation including protein breakdown, which is typically reflected as a lower NH_3-N compared to untreated or LAB treated controls (Jaakkola et al., 2006, Seppälä et al., 2016). Franco et al. (2019a) working with mixed red clover and timothy found that silages treated with formic acid resulted in the lowest pH values and also lower concentrations of NH_3-N, lactic and acetic acids and ethanol than untreated control, LAB or salt treated silages. Further, contaminating the silage with soil and faeces tended to increase NH_3-N emphasizing the importance of good management practises in silage making (Franco et al. 2019a).

PROTECTION OF FORAGE PROTEIN FROM RUMEN DEGRADATION

A substantial amount of research has been directed to decrease the protein degradability of feeds including both forages and concentrate feeds. Although protecting the feed AA from rumen degradation is theoretically a tempting idea which can be demonstrated *in vitro*, it has not been very successful when evaluated based on dairy cow production responses (Huhtanen, 2019). The reasons behind it include decreased microbial protein synthesis due to reduced rumen degradable protein supply, overprotection of protein leading to decreased digestibility and poorer AA profile and digestibility of by-pass protein compared to the microbial protein.

Choice of forage plant species affects protein degradability to some extent. Red clover is known for the lower protein degradability in the silo (Sullivan & Hatfield, 2006) and in the rumen (Vanhatalo et al., 2009). Papadopoulos & McKersie (1983) reported that the proteolysis during wilting was faster in lucerne than in red clover. These effects have been attributed to the presence of polyphenoloxidase (PPO) in red clover. PPO forms complexes between phenols and proteins which will protect the plant proteins from degradation, but it may also lead to lower total tract digestibility of red clover protein (Huhtanen & Broderick, 2016).

Tannins have been suggested to be used to protect protein in forages. Certain forage species such as sainfoin (*Onobrychis viciifolia*) naturally contain tannins and have lower *in vitro* rumen protein degradability (Lorenz et al., 2010). Marita et al. (2010) demonstrated that designing mixtures of different plant species so that in the combination both PPO and quinones are present may result in protein protection in silo. Extracted tannins may also be added at the time of ensiling and resulted in decreased proteolysis based on *in vitro* analysis (Herremans et al., 2018). It would be important to study these effects in animal experiments to be sure that the benefits can be realised also under practical conditions and e.g. feed intake, rumen microbial protein synthesis or total tract digestibility are not negatively affected. Also the agronomic performance of the crops with these special characteristics must be considered so that the whole farm performance is not compromised.

ANIMAL RESPONSES

When assessing silage quality, we should keep in mind what the silage is used for and what are the relevant quality criteria in that particular use. The main use of grass silage is as the main feed component of ruminant animal diets. E.g., in Finland 45 % of diet DM of dairy cows is grass silage (Huhtamäki, 2019). If the silage is used for some other purpose, e.g. as a feedstock for a green biorefinery (see e.g. Franco et al., 2019b), the quality criteria may be different regarding the amount and form of nitrogenous compounds. However, even within the use as ruminant feed, the requirements of "good" silage vary greatly depending on the types of animals it is used for, and the amount and quality of the other components of the diet.

Feeding experiments as a tool to estimate silage protein value

Dairy cows are an excellent model for evaluating overall production potential of feeds due to their high nutrient requirements and quick responses to changes in nutrient supply in terms of feed intake and milk production, which can be analysed statistically using efficient change-over designs. However, treatments must be carefully designed so that several simultaneously changing effects are not confounded which may mask the true effects.

Dairy cow experiments have revealed that the responses to protein supplements (mainly rapeseed or soybean based feeds) are not dependent on basal forage protein concentration (Huhtanen & Broderick, 2016). To illustrate this, examples from two experiments are shown in Figure 6. The use of rapeseed meal increased milk protein production irrespective of silage harvest time (Rinne et al., 1999) or N fertilization (Shingfield et al., 2001).

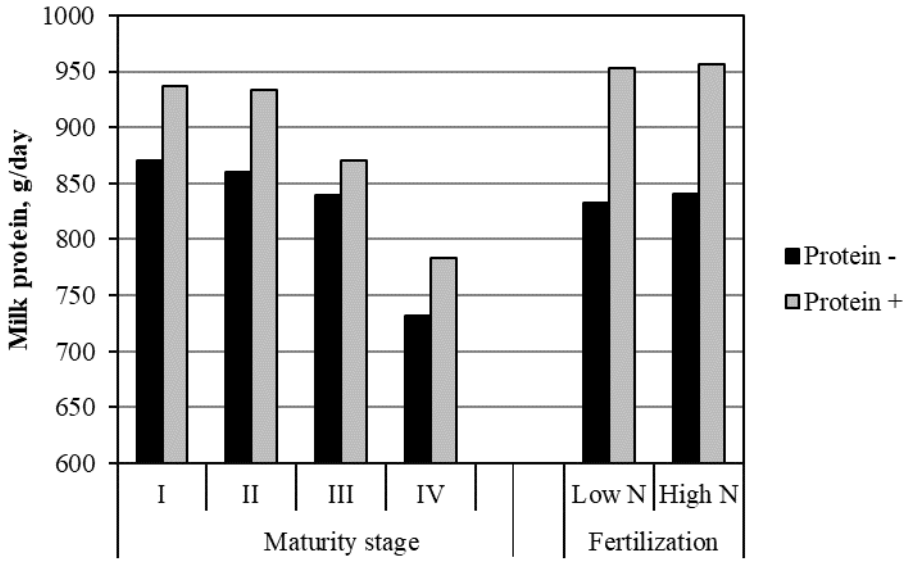

Figure 6. Milk protein production responses of dairy cows to additional dietary rapeseed feed supplementation were similar irrespective of silage CP concentration manipulated by stage of maturity at harvest (bars on the left; Rinne et al., 1999) or N fertilization of the sward (bars on the right, Shingfield et al., 2001).

Milk production responses to silage protein characteristics

Based on the previous chapter, silage protein does not affect responses to additional protein supplementation. The dairy cows also seem not to be responsive to the changes in silage protein quality based on meta-analysis approach. A lack of milk production responses to concentration of silage soluble non-ammonia N suggested that the partition of silage N into soluble and insoluble N (excluding NH_3-N) did not markedly influence silage metabolizable protein concentration (Huhtanen et al., 2008b). However, silage NH_3-N had a negative impact on milk production (Huhtanen et al., 2008b). In further analysis (Rinne et al., 2009), using constant rather than variable silage effective protein degradability value in rumen was a better predictor of milk yield.

Grass silage as part of the diet

The question of target grass silage quality is often presented, but should always be looked at within the context the feed is to be used in. It is obvious that different animal groups have different needs if we e.g. compare high yielding dairy cows or dry beef cows in mid-pregnancy. But even beyond that, grass silage typically only comprises part of the diet. There is plenty of scope to balance the whole diet by choosing suitable concentrate feed components and other forage feeds to balance the whole ration.

The key to balance the ration is to separately estimate the N requirements of the rumen microbes and the AA requirements of the host ruminant as is done in modern ruminant protein evaluation systems the examples from Northern Europe being NorFor (Volden, 2011) and the Finnish system (Luke, 2019). Such systems were an enormous improvement compared to the previous protein evaluations systems such as that of digestible CP used earlier in the Nordic countries (for historical review of feed evaluation systems, see Weisbjerg et al., 2010).

Usually the energy content of the diet is the first limiting nutritional parameter in forage-based diets for ruminants, but in some cases inadequate protein level can limit animal production. Such diets are rare in intensive dairy and beef systems, but may become more common if more emphasis is put to improving NUE in ruminant production. It should be the aim in all cases to supply the rumen microbes sufficient amount of N, and even simple N sources such as ammonium salts or urea can be useful in those cases (Virtanen, 1966, Ahvenjärvi & Huhtanen, 2018). The high CP of grass silages and particularly those containing forage legumes will also be beneficial in those cases. Forage legumes have the additional benefit of fixing atmospheric N into the farm system (Järvenranta et al., 2016).

According to the Finnish protein evaluation system (Luke, 2019), the protein balance in the rumen (PBV) should not be negative in dairy cow rations to ensure adequate N supply to the rumen microbes. The values for typical silages are clearly positive giving scope to include feeds with negative PBV values such as cereal grains or whole crop cereal silages in the diet. Growing cattle have even lower ruminal N requirements and according to Luke (2019), the PBV values of growing cattle rations can be -10 g/kg DM consumed. Huuskonen et al. (2014) in their meta-analysis estimated that the PBV could be even -20 g/kg DM intake without harmful effects on growth performance, which will greatly improve the NUE of beef production.

A key to sensible ration planning is to evaluate carefully the feed stocks available at the farm by analysing them. This can be done efficiently already at the time of ensiling when sampling is easier and the

information is available well before the time of feeding. The chemical composition and digestibility of the grass material will not change much during ensiling if preservation is successful (Huhtanen et al., 2005).

NOVEL USES OF GRASS MAY REQUIRE HIGHER PROTEIN QUALITY

Fractionation of green crop protein has been on the agenda already for a long time (see eg. Wilkins, 1977), but due to good availability and low price of imported soybean protein into EU, has not been competitive. A renewed interest in green biorefineries where grass biomass is processed to various novel products has now arisen due to environmental, social and political concerns related to dependence on soya. Green biorefineries provide an alternative use of grass, which is otherwise limited mainly by the size of the ruminant livestock. Grass cultivation has the potential to provide multiple benefits to the society and is an efficient way of capturing solar energy into chemical form so new ways of utilizing it should help resolve some of the environmental problems we are facing currently. In the biorefinery process, soluble grass protein and other nutrients are liberated from the fibre matrix and will thus be available e.g. as a feed material for pigs or even directly for human consumption.

Typically the first step of a green biorefinery is the separation of the liquid and solid fractions using screw pressing, when soluble protein is concentrated into the liquid fraction (Damborg et al., 2018, Stefanski et al., 2018, Franco et al., 2019b). In this kind of applications, the protein quality may play a more important role than in ruminant feeding and e.g. use of fresh grass instead of ensiled grass has benefits. However, the well-known efficiency of ensiling in preserving the easily perishable fresh grass may justify applications where also ensiled grass is used as the raw material for biorefining. As pointed out earlier, although large part of CP in silage is NPN, it is highly soluble which may help in separation. Further, in good quality grass silages only less than 5 % of CP is in the form of NH_3-N, which has no value in the nutrition of monogastrics.

One of the options to use the solid fibrous pulp from the biorefinery process is to feed it to ruminants (Savonen et al., 2018, Damborg et al., 2019). The milk production potential of it is only marginally reduced compared to the original silage and it could serve in improving the NUE due to reduced CP concentration.

CONCLUSIONS

Grass silage must be considered preferably as an energy feed, but it also contributes to the protein supply of the ruminant animal indirectly via the organic matter fermented in the rumen and directly via providing N source to rumen microbes and to a lesser extent via by-pass protein constituting of the ruminally undegraded grass protein. It is important to verify the theoretical hypotheses and results derived from laboratory and *in vitro* analyses using *in vivo* experiments which may in some cases result in conflicting recommendations. In general, good management practises in forage production and subsequent preservation as silage serve many purposes and are not in conflict with good protein quality. In evaluating individual steps of silage production and utilization chain, the whole system should be kept in mind to prevent partial optimization of single steps. The protein quality of silage should not be considered in isolation of the whole diet effects on nutrient supply to the ruminant animals, the excretion of N, and effects of silage and other feed production impacts at farm scale and up to global effects in terms of food supply and environment. The quality of grass silage protein seems to be one of those factors that may get overemphasized in some cases.

The following suggestions may be useful to many grass silage based ruminant feeding systems when targeting for optimal performance regarding the whole chain:

- High digestibility of silage providing fermentable organic material into rumen thus promoting microbial protein synthesis. High digestibility also allows using less concentrate feeds in the diet so that the ability of ruminants as fibre digesters can be utilized to a full extent.
- Low CP concentration to result in high N use efficiency - but enough to provide N source for the rumen microbes.
- High forage yield per area of land to secure economic and environmental efficiency.
- Small DM and nutrient losses during ensiling and high hygienic quality achieved by good silage management practises.

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SILAGE SAFETY PRACTICES THAT SAVE LIVES

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INTRODUCTION

In 2018 approximately 140 million metric tonnes of silage was produced in the USA. Few farming operations invite as many different opportunities for a serious injury or fatality as a silage program. Beginning with harvesting the forage in the field, followed by transporting the chopped material to the farm and placing it into storage, and subsequent feeding of the silage, employees and bystanders are exposed to numerous serious risks in every silage program. Silage-related tragedy knows no age boundary as persons of all ages have been injured or killed during silage harvest and feed-out.

Safety is the control of recognized hazards to reach an acceptable level of risk. A hazard does not always affect the person who caused it - the hazard can affect anyone. Accidents are caused by unsafe behavior or conditions due to the actions of people: stepping over or standing too close to a rotating PTO shaft, working too close to the feed-out face of an over-filled bunker silo, or moving a forage harvester without checking all sides and honking three times. Countless victims have learned the hard way that there is no such thing as a safe bunker silo or silage pile! Every serious injury or fatality silage-related accident could have been prevented. We are not going to create a safety bubble for our employees or a silage program that is hazard-free, but following the guidelines will reduce the risk of someone being injured or killed on the farm. It is important to discuss safe silage management practices several times a year with a silage team because injury-related statistics suggest that many employees do not consistently follow the recommended safety guidelines.

MATERIALS AND METHODS

There are seven major hazards encountered when managing silage in bunker silos and drive-over piles that could endanger lives. These include: 1) complacency and fatigue, 2) exposed to silage gases. 3) tractor or truck rollover, 4) run-over by machinery or equipment, 5) entangled in machinery or equipment, 6) fall from height, and 7) crushed by an avalanche or collapsing silage. A detailed account of case studies involving the last three hazards and guidelines for reducing the risk of a serious injury or death from each of the three hazards are presented here.

RESULTS AND DISCUSSION

Entangled in machinery or equipment: a case study and prevention guidelines

The accident happened on June 21, 1976 on the family farm in Minnesota when Doug Sawatzke was 13 years old. The apron had broken on the silage wagon, and Doug took it upon himself to pull the wagon up to the tower silo and fork out the forage. It was an old Decker box wagon with two rows of front beaters, and no safety mechanism to stop the machine. When the wagon was nearly empty, Doug and Jim Hessel, a friend his age, were cleaning up the floor with the beaters and cross apron still running. Doug's fork got entangled in the beaters, and when he grabbed for it, he ended up in the beaters with the fork. Jim saved Doug's life by jumping over the side of the wagon and turning off the PTO. Doug remembers his dad coming from the field and holding him until the ambulance arrived. Doug's injuries included a broken femur and multiple lacerations. He spent 5 weeks in traction and 3 months in a body cast. Doug said, "It was the only time that I ever saw my dad cry, and I am lucky that I did not get conveyed into the silo blower." Doug said, "It was my decision to pull the wagon up to the silo, and my dad had no knowledge that I was doing that. He was out in the field chopping. My dad was not responsible for my accident." Doug fully recovered from his injuries and is a dairy nutritionist in Minnesota.

Guidelines that will reduce the risk of entanglement accidents include: 1) keep machine guards and shields in place to protect the operator from an assortment of rotating shaft, chain and v-belt drives, gears and pulleys, and rotating knives on forage harvesters, wagons, and silage feeding equipment; 2) when inspection or service work is needed, shut down the engine and remove and pocket the keys to prevent accidental starting by another person; 3) stop the machine before lubricating, adjusting, inspecting, or unplugging; and 4) wait for the cutter head to come to a complete stop before adjusting or unplugging; and 5) never approach the blades of a silage defacer while the machine is in operation.

Fall from height: a case study and prevention guidelines

On January 26, 2013, Alisdair Davidson was working with his father, William Davidson, at Poldean Farm, Moffat, Scotland. At about 2:00 p.m. they accessed the silage shed via the rear door and walked along the top of the silage to the front of it. They began to remove the sandbags and peel back the plastic sheets. Alisdair was about 3 meters from the edge of the silage completing his work with the sandbags. William had taken hold of the top black sheets and was engaged in pulling them back. Alisdair was busy with his own task and did not directly see what led his father to lose his balance and fall. When he heard his father shout and looked, it appeared likely that William's feet become entangled in the sheets, and he toppled over the edge of the silage pile. He fell about 5 meters and struck his head on the concrete floor below. Alisdair rushed to his father's aid,

dialed 999, and was given instructions on resuscitation over the phone before the arrival of paramedics who carried out CPR but found Mr. Davidson had died.

Guidelines that will reduce the chance of a fall from height accident include: 1) installing guardrails on above ground level walls; 2) when removing plastic or oxygen-barrier film, tires, tire sidewalls or gravel bags, use caution and wear a safety harness tethered with a heavy rope or cable; 3) never standing closer to the feed-out edge than the height of the silage face and 4) use equipment operating from ground level to remove surface-spoiled silage; 5) never allow a person to ride in a front-end loader bucket; and 6) there should be no horseplay when working on top of the silage in a bunker or pile.

Crushed by an avalanche or collapsing silage: a case study and prevention guidelines

In the fall of 2000, Ted Gramm's family cattle operation in west-central Minnesota used a custom operator for the first time and over-filled a bunker silo with corn silage. The feed-out face was 4 to 4.5 meters high. Ted was doing chores and calving cows in the late afternoon on a Sunday in early March 2001. As Ted recalls, "It hadn't been a good day; it was gloomy, I didn't get to go to church, and I was feeling down about a lot of things. My family was in the house and knew that I probably wouldn't make it home for supper." When Ted was ready to feed silage, he noticed that a tire had come down off the pile. He walked up and grabbed the tire, and that was the last thing he knew … the avalanche hit him. In Ted's words, "Only by the Powers from Above did my brother drive in the yard, see me get buried, and knew where I was. He managed to find me and pull me out." Ted suffered displaced ankles and had surgeries on both knees. Ted continued, "These types of things are real, they happen. During the minute, or so, that I was buried; I could see my kids around the table. A lot of things flashed through my mind." Ted fully recovered from his injuries and is a feed sales manager in Minnesota.

Guidelines that will reduce the risk of a serious accident or fatality caused by a silage avalanche include: 1) do not fill bunkers and build piles more than 3 to 3.5 m maximum height; 2) when filling bunker silos, do not exceed the height of the safety rail on the walls or the maximum height at which unloading equipment can remove silage from the feed-out face safely; 3) never allow people to approach the feed-out face; 4) never stand closer to the feed-out face than three times its height; 5) suffocation is a primary concern and a likely cause of death in many silage avalanches, so follow the 'buddy rule' and never work alone in a bunker or pile; 6) the top edge of the feed-out face is highly unstable so use caution when removing plastic or oxygen-barrier film, tires, tire sidewalls or gravel bags; 7) never ride in a front-end loader bucket; 8) never park vehicles or equipment near the feed-out face; 9) never drive the unloader parallel to and in close proximity of the feed-out face in an over-filled bunker or pile; 10) do not walk close to the top edge of the feed-out face; 11) post warning signs around the perimeter of bunkers and piles saying, 'Danger! Silage Face Might Collapse'; and 12) avoid being complacent and never think that an avalanche cannot happen to you.

CONCLUSIONS

A silage accident can occur anywhere, any time, in any bunker or pile, in a fraction of a second, and without warning. We cannot totally prevent accidents from happening, but we can prevent people from being under them. Every farm, feedlot, dairy, digester plant, and silage-harvesting contractor should have silage safety policies and procedures for their silage team members, and they should schedule regular meetings with all their employees to discuss safety. If a silage program is not safe, nothing else about it really matters.

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COMPARISON OF NDF DEGRADABILITY AND DEGRADATION RATE OF DIFFERENT SILAGES IN HUNGARY (2013-2018). THE 'HEAT STRESS DIET' ASPECT.

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INTRODUCTION

Forage production and feeding strategies has to be adapted to the climate change in dry continental regions of Europe and consider the elevated nutrient requirements of the high producing lactating cows (2000-2016 Hungary = +2,765 kg milk/305 days lactation). The various types of whole-crop cereals are excellent source of fermented forage (silage and haylage), for ruminants. Digestion characteristics of NDF influence feeding and rumination behaviour, dry matter intake, and efficiency of milk component output (Grant and Cotanch, 2012). High NDF digestibility forages are associated with: more fast-pool NDF, less slow-pool NDF, and less $uNDF_{240}$. Feeding strategy during summer time can be modified according to these parameters and forage NDF- characteristics in order to optimize dry matter intake even on heat stress days.

The author investigated the NDF rumen degradability (aNDFomd48 – amylase treated ash corrected NDF 48 hours *in vitro* degradability), the NDF degradation rate (NDF *in vitro* incubation 12, 24, 30, 48, 120 and 240 hours) by NIR of routine samples derived from maize silage (2018 n=370), alfalfa silage/haylage (n=1811), ryegrass silage (n=462), rye silage harvested in boot stage (n=789), triticale silage, barley silage and wheat silage (in heading and in early dough stage).

MATERIALS AND METHODS

Routine laboratory samples derived from large scale farms in Hungary (n= 3633) between 2013-2018. Results based on routine laboratory NIR analyses (Livestock Performance Testing Ltd, Hungary). Dry matter yield, crude nutrient content, fibre fractions (aNDFom, ADF, ADL), organic matter digestibility detected during 48 hours incubation time *in vitro* (OMd_{48}), amylase treated and ash corrected NDF rumen degradability with 48 hours incubation time *in vitro* ($aNDFomd_{48}$) were determined by Q-Interline Quant FT-NIR analyser. Degradable aNDFom ($daNDFom_{48}$) was calculated as multiplication of aNDFom and $aNDFomd_{48}$. Samples were dried at 70°C and ground according to the Guidelines of Samplinq® system (Eurofines Agro, Wageningen, The Netherlands). The EG guideline L54 2009/152 was applied for determination of moisture content (dry matter determination). Spectra were determined according to the guidelines of NEN-EN-ISO 12099 (Q-Interline Quant FT-NIR analyser, ISO 12099:2010 guidelines for the application of near infrared spectrometry).

RESULTS AND DISCUSSION

Nutrient content, fiber degradability ($aNDFomd_{48}$) and organic matter digestibility OMd_{48}) of different ensiled forages in Hungary (2013-2018) are shown in Table 1.

Table 1 Nutrient content, fiber degradability ($aNDFomd_{48}$) and organic matter digestibility OMd_{48}) of different ensiled forages in Hungary (2013-2018).

	Sample no.	Dry matter g/kg	Crude protein g/kg DM	Total starch g/kgDM	NDF[1] g/kg DM	ADF g/kg DM	ADL g/kg DM	$NDFd_{48}$[2] g/kg DM	$dNDF_{48}$[3] g/kg DM	OMd_{48}[4] g/kg DM
Maize silage 2018.	370	381	69	337	388	215	19	53.7	209	76
Alfalfa silage/haylage	1811	408	193	-	426	327	62	40.5	171	66
Grass silage/haylage (intensive)	462	343	141	-	502	305	26	65.1	323	73
Rye silage (in boot-early heading)	789	294	136	-	554	330	27	66.2	363	71
Triticale silage (in heading)	24	316	106	-	579	348	28	59.4	341	67
Triticale silage (early dough stage)	59	362	81	124	516	320	34	47.4	249	65
Barley silage (in heading)	17	318	132	-	552	329	30	60.4	332	67
Barley silage (early dough stage)	59	337	93	134	494	289	31	47.9	232	67
Wheat silage (in heading)	10	293	118	-	571	337	34	57.9	322	66
Wheat silage (early dough stage)	32	365	92	122	499	301	34	46.6	237	66

[1]aNDFom - amylase treated ash corrected NDF, [2]$aNDFomd_{48}$ – degrdabilty of amylase treated ash corrected NDF, 48 hours *in vitro* incubation,
[3]$daNDFomd_{48}$ - degradable aNDFom, 48 hours *in vitro* incubation, [4]organic matter digestibility *in vitro* 48 hours incubation

Ryegrass silage and rye silage had higher $NDFd_{48}$ values (66.2% and 65.1%, respectively, $p<0.05$) compared to the maize silage (2018: 53.7%) and alfalfa silage (40.5%). The $NDFd_{48}$ values of the early dough stage were lower with 11.3% (wheat silage), 12.5% (barley silage) and 12.0% (triticale silage) compared to the boot stage. These results confirm the importance of the whole crop cereal silages (harvested in boot stage) in the diet fed summer time to maintain the dry matter intake.

The boot stage and the milky-early dough stage of different whole crop silages (triticale, barley and wheat) have been compared from the NDF profile point of view (NDFd 12, 24, 30, 48, 120 and 240 hours). It can be concluded that the early cut whole crop cereal silages (rye, triticale, barley and wheat in boot or in heading) and the intensive grass silage have significantly ($p< 0.05$) higher NDF degradation rate compared to the maize silage or the alfalfa silage/haylage (at 12, 24, 30,48, 120 hours *in vitro* incubation time). The whole crop cereal silages (rye, triticale, barley and wheat) cut in boot or in heading has significanly ($p< 0.05$) higher degradation rate than that harvested in early dough stage (at 12, 24, 30,48, 120 and 240 hours *in vitro* incubation time). The highest undegradable NDF content was found in the alfalfa silage/haylage samples (+87% compared to the maize silage) and it was 2,5 times higher than in rye silage (cut in boot stage).

Table 2 NDF rumen degradation rate of different ensiled forages in Hungary (2013-2018).

	Sample no.	$NDFd_{12}$	$NDFd_{24}$	$NDFd_{30}$	$NDFd_{48}$	$NDFd_{120}$	$NDFd_{240}$	$uNDF_{240}$[1]
		%NDF	%NDF	%NDF	%NDF	%NDF	%NDF	%NDF
Maize silage 2018.	370	20.6	35.4	41.1	53.7	70.9	73.8	26.1
Alfalfa silage/haylage	1811	17.8	28.7	32.6	40.5	47.3	49.6	48.9
Grass silage/haylage (intensive)	462	29.2	46.8	52.9	65.1	74.8	75.7	24.2
Rye silage (in boot)	789	28.9	47.1	53.6	66.2	79.3	80.7	19.3
Triticale silage (in heading)	24	24.3	41.1	47.4	59.4	78.0	80.6	19.3
Triticale silage (milky-dough stage)	59	16.6	29.1	34.2	47.4	65.6	71.3	28.1
Barley silage (in heading)	17	37.5	57.6	63.8	60.4	80.6	80.7	19.3
Barley silage (milky-dough stage)	59	16.1	28.4	33.5	47.9	64.8	69.8	29.8
Wheat silage (in heading)	10	21.9	37.9	44.2	57.9	77.4	80.6	19.3
Wheat silage (milky-dough stage)	32	19.1	32.8	38.1	46.6	68.3	73.0	26.3

[1]uNDF240 - undegradable NDF, 240 hours *in vitro* incubation

CONCLUSIONS

The higher passage rate (*kp*) resulted by 'fast' fibre makes it possible to empty the rumen quicker achieving higher dry matter intake (Raffrenato and Van Amburgh, 2010). This is why the optimization of forages for high producing cows shall be highlighted, and the advantage of their lower lignin content and greater extent of NDF digestion shall be utilized (Grant and Cotanch, 2012). The highest (early) NDF degradation rate ($NDFd_{12}$, $NDFd_{24}$) was found in the case of grass silage (intensive type), rye silage (cut in boot stage) and barley silage (cut in heading). NDF degradation rate decreased significantly ($p<0,05$) between boot-heading stage and milky–dough stage. Edmisten et al (1998b) found that the IVDMD (*in vitro* dry matter disappearance) of 4 cereal species in the majority of cases decreased from the vegetative (765–854 g kg^{-1}) through the milk stage (505–662 g kg^{-1}). We can conclude that the degradable NDF content (g/kg DM) of the whole-crop winter rye, triticale, barley and wheat cut at boot stage or in heading was higher significantly ($p<0.05$) as compared to the maize silage and alfalfa silage/haylage, respectively. It can be summarized that the intensive grass silage and the early-cut whole-crop cereal silage can be determined as an important source of rumen degradable fibre (according to the degradation rate and degradation efficiency) for the high lactating dairy cows, especially during the summer heat stress period having potential effect on rumen passage and dry matter intake.

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RESULTS OF FEEDING EXPERIMENT WITH SILAGE PROCESSED BY TECHNOLOGY OF SHREDLAGE

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INTRODUCTION

Any improvement in usage of maize silage is of a considerable significance because this feed is basic roughage with a high concentration of energy in the form of starch, and digestible neutral detergent fibre (NDF) for feeding rations in cows. When using the technology "Shredlage", thanks to patent rollers in a cutter, the long chopped forage (26-30 mm) is crushed more intensively, corn grains are ground better and in the same time particles of chopped forage are lengthwise broken – they are unravelled, corn cobs are ground and bark of stems is separated from core. Due to longer chopped forage, content of physically effective fibre is enhanced and therefore, a number of its donators (hay, straw) can be reduced, and die to it, a portion of maize silage may be increased. Simultaneously, better availability of starch and the extended surface of particles should lead to better digestibility of forage chopped processed in the way above mentioned. All these facts should be resulting in higher dry matter intake, better working of the rumen, better health state and subsequently, in better milk performance for about 1 lit/day per cow. Beintmann et al. (2016) did not find differences in milk performance in a similar experiment, however, the maize "shredlage" silage improved supplementation of dairy cows by energy, and negative energy balance (NEB) at the beginning of the lactation period was lower.

MATERIAL AND METHOD

In the Research Institute of Animal Science, we realized a periodic-group experiment with two well-balanced groups of high-yielding dairy cows (together 35 animals – 31 Holstein and 4 Czech Pied breeds, respectively). Maize silages were proceeded using the *Walterino KWS* (FAO 280) hybrid. Silages were filled into long plastic bags, and the preparative *Bonsilage* was used as a preservative agent.

Group	DIM	Average day milk for DIM (kg)	Average day milk 6 days before starting (kg)	Average number of lactations
I.	76,94	40,70	43,10	2,94
II.	77,59	40,10	42,82	2,88

Group	**Diet (period)**		
I.	S	K	S
II.	K	S	K

The trial continued for 3 months (three 30days' periods) when each of the periods was compound of 20days' stage for preparation of digestive apparatus in cows and 10days' experimental stage during which all measurements and samplings were carried out.

Content of nutrients in harvested matter

In the control diet (K) prepared in the form of TMR, the main item was the classical maize silage (IL 10mm) whereas during the experimental periods (S) cows were given the same diet (also in the form of TMR) but with maize "shredlage" silage (IL 26mm). Both TMRs were prepared in a vertical mixing wagon "*Czernin*" and the maize silages were picked from the silage bags using a manipulator. Cows were given TMRs *ad libitum*.

Nutrients	Ideal length of chopped forage (IL)			
	10 mm (K)		26 mm (S)	
	in original matter	in absolute DM	in original matter	in absolute DM
Dry matter	34.98	100	34.26	100
Nitrogen substances	2.02	5.78	1.88	5.47
Fat	1.15	3.30	1.05	2.08
Fibre	7.61	21.76	7.21	21.03
ADF	7.96	22.76	7.70	22.47
NDF	18.00	51.46	17.60	51.38
Starch	10.74	30.72	10.50	30.66
Ash	1.29	3.68	1.30	3.80

The way of processing of two maize silages (classical vs. shredlage) was just the sole difference between the feeding rations. Consumption was recorded individually by means of troughs standing on tenzometric scales connected with a PC outfitted with the appropriate software.

RESULTS AND DISCUSSION

An average milk performance (41.22 kg) in periods with shredlage silage (S) was higher for 0.89 kg ($P < 0.05$) in comparison with the periods with control silage (K; 40.33 kg). The concentration of milk fat was statistically inconclusively lower in S-periods and contents of proteins and lactose were on the same level in both periods. Milk yield after conversion to FCM was higher in S-periods, namely for 0.26 kg. It appeared similarly in ECM which was higher for 0.48 kg in S-periods as well. The milk performance above mentioned was reached with almost the same TMR consumption in both

periods S and K (48.46 and 48.37 kg/cow/day, resp.). Within the context of a different harvest technology, the time of chewing was recorded as well.

Even though we didn't succeed (according to the shaking of single sieves of a separator) in a substantial increase of the long chopped maize forage portion on the upper sieve of the separator, the time of chewing became longer for 21.76 min/day when using the technology of shredlage.

According to the portion of chopped maize on sieves of the separator, it may be said the increase of milk yield in S-periods when feeding shredlage was in a decisive rate caused by the higher portion of better ground maize grains. This fact is supported by a statistically higher rate (17.75%) of maize on the bottom sieve of the separator. The difference on the bottom of the separator between both treatments was 6.09%. Any metabolic disorders were not found within the experiment.

Average time of chewing within periods (min/day)

Period	
K	S
401.92	423.68

CONCLUSION

When feeding maize silage processed by the technology of "shredlage" statistically conclusively higher ($P < 0.05$) daily milk performance was reached namely for 0.89 kg. Despite of lower concentration of milk fat was found (for 0.06%), the total daily fat yield was higher, similarly as in next indicators (FCM, ECM). Any metabolic disorders weren't found when feeding shredlage maize silage.

REFERENCES BY AUTHORS

This experiment was carried out under the support of The Ministry of Agriculture of The Czech Republic No. RO 0718.

Feeds	kg
Maize silage	17.00
Lucerne silage	12.00
Wet maize grain	4.20
Wheat meal	3.37
Barley meal	1.08
Soya extracted meal	1.35
Rapeseed extracted meal	1.89
MOLA feed – KMG	1.70
Brewery draff	4.00
C 16	0.45
Premin DO 1	0.72
Sodium bicarbonate	0.13
ProMel	0.50

Feeding ration (kg per cow and day)

Content of nutrients in diets (g per cow and day)

Dry matter	23 370.60
Nitrogen substances	4 277.25
PDIA	991.49
PDIN	2 484.32
PDIE	2 123.29
Fat	670.80
Fibre	3 314.87
Starch	5 113.96
NEL	169.9
Ca	207.65
P	94.17
Na	102.25
K	259.55
Mg	82.00
S	17.56
NEL in DM	7.27
% Nitrogen subs. in DM	18.30
% Fibre in DM (%)	14.18
% Starch in DM	21.88
Ca/P	2.22
K/Na	2.48

Average values of milk performance in control (K) and experimental (S) periods (cow/day)

Indicator	Units	Period	
		K	S
Milk performance	kg	40.33	41.22
Fat content	%	3.49	3.43
Proteins content	%	3.15	3.14
Lactose content	%	5.01	5.04
Fat yield	kg	1.41	1.44
Protein yield	kg	1.27	1.29
FCM yield	kg	37.28	37.64
Urea content	mg/100ml	31.83	32.09
ECM yield	kg	37.04	37.52

Portion of chopped maize in fresh state on single sieves of separator (%)

Diameter of sieve mesh	Shredlage chopped forage (TL 26 mm)	Classically chopped forage (TL 10 mm)
19 mm	3.94	3.22
8 mm	56.45_a	66.67_a
4.75 mm	21.86_a	18.44_b
Bottom	17.75_a	11.66_b

EFFICIENCY OF CP FROM FORAGE SILAGES DETERMINES THE REQUIREMENT OF RUP

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ABSTRACT

Forage silages are currently major components of dairy cows' diets. The role of these silages is not only to ensure the rumen is functioning properly but also to produce the main products of rumen fermentation, i.e. VTA and microbial protein. These ones meet the majority requirement of ME and digestible AA in intestine required for milk production, milk protein synthesis and tissue formation. However, microbial protein is not sufficient to meet the AA requirements for high performance dairy cows and diet must be enriched with RUP.

Based on the working test we show the practical way how to feed high performance dairy cows without SBM and how to reduce amount of RSM in diet without losing performance.

The most important result of the test is reducing cost of purchased proteins feedstuffs.

INTRODUCTION

Proportion of RDP and RUP in forage silage. The most of CP in silages are degradable in the rumen and serve as the source of nitrogen for rumen microorganisms. We can say that effective CP utilization from silages consists mainly in microbial protein production. Of course, the synchronization of N and energy availability in the rumen is necessary during fermentation process.

Unlike the other protein feeds - like SBM or RSM - forage silage contains the part of N in the form of NPN. The remain of CP is true protein. From NPN point of view, just only ammonia, free amino acids and peptides are important for microbial protein production. The proportion of NPN and true protein is various in silages. In addition, the true protein has various degradability and rate of degradation in rumen. NPN content and TP properties determine the proportion of RDP and RUP in silage.

RDP and RUP belong to the basic values that nutritionists work with. However, it should be stressed that values do not always correspond to reality.

The ratio between RDP and RUP is variable and depends on many factors. 1) Type of silage crop, 2) Phenophase of the crop at harvest time, 3) Duration and conditions of the wilting, 4) Type of silage additive etc. From a dairy cow of view, it is very important lactation phase and DMI as well as degree of pregnancy.

Despite the facts mentioned above, the proportion of RDP is always significantly higher than RUP in forage silages. That means the main role of CP from silages is to support production of microbial protein and just only a minor part of CP flows from rumen to intestine as RUP. Although it is only a small part of silage CP, this small part can play a major role in the final AA composition in intestine especially in alfalfa or clover silages because these ones have a wider Lys-Met ration than it is recommended. In the case that SBM is used as the main source of RUP the Lys-Met ration will increase even further.

RUP as a supplement to microbial protein. It is generally stated that the ideal RUP is one that resembles microbial protein as much as possible in its AA profile.

It is considered that microbial protein has an excellent AA composition for lactating dairy cows. The main focus is primarily on Lysine and Methionine as the first limiting AA and Lys–Met ratio. The others AA are more or less at the beginning of the investigation. It is well known that the first limiting AA of microbial protein is Histidine.

MATERIALS AND METHODS

The tested product was RSM treated with LinaropAgri®. LinaropAgri® is complementary feed intended for treatment of all rape products (meal, cake, seed). The treatment is very easy because the only thing necessary is to add LinaropAgri® into RSM during the production of the mixture. In our case we tested LinaropAgri® with RSM. We termed this final product R-LRA.

After our practical experience with replacement of SBM with R-LRA in the ratio 1:1 we were looking for a breeder who feed only RSM to the dairy cows without any SBM. Because LinaropAgri® increases digestibility of CP as well as fibre we decided to replace RSM with R-LRA in the ratio 1:0,75.

In our working test we started co-operating with one of the best breeders of dairy cows in Czech Republic. This agricultural company breeds 1 000 Holstein dairy cows with the performance 11 000 l milk per year per cow.

The cows are housed on two farms. There are 650 cows in the farm „A" and 350 cows in the farm „B".

The feedstuffs are the same for both of the farms. The milking cows are divided into two groups. The basic diet consists of maize silage, alfa silage, rye silage, barley straw, molasses, brewers grain and soybeans. The production mixtures consisted of wheat, barley, corn, RSM, bypass RSM and mineral-vitamin additives for both of the farms.

We started the trial test on the farm „B" in October 2018 and we finished it in April 2019 because of reconstruction of stable on farm „B". But the feeding with R-LRA is still going on there.

We did not make any changes in the basic diets on either farm. We made just only change in the production mixture for farm „B". We replaced the bypass RSM with R-LRA in the ratio 1:1 and we put the R-

LRA instead of RSM in the ratio 0,75:1. In this way we reduced the total amount of rape products and even the daily dose of production mixture per cow in farm „B“ (TAB 1). There was no problem during change and reduction of production mixture.

TAB 1: *Daily dose of feeds for cows groups on farm „B“ (kg/cow)*

Feed per cow per day	Before test Gr.I	Gr-II	During test Gr.I	Gr.II
Total production mixture	**8,5**	**9,7**	**7,9**	**8,9**
Total rape products	**3,43**	**3,9**	**2,65**	**3**
RSM	3,0	3,4	-	-
RSM – high bypass	0,43	0,5	-	-
R-LRA			2,65	3

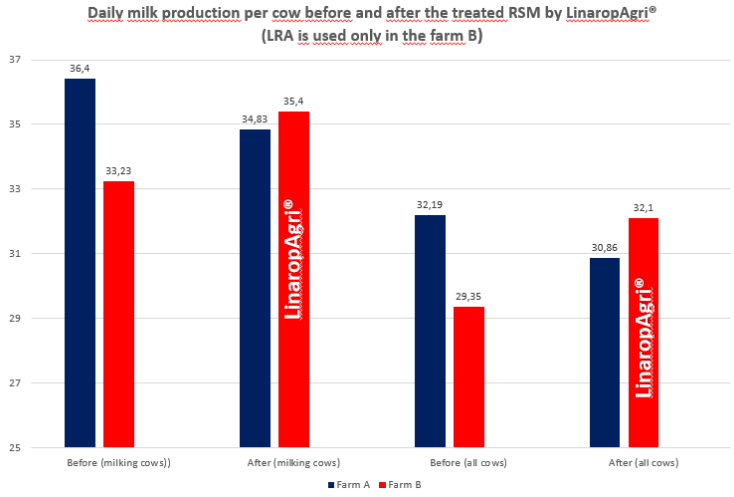
RESULTS AND ECONOMICA

After the change the daily milk production on the farm „B“ began to rise. Farm „B“ gradually equalled farm „A“ and eventually overtaken it in daily milk production. It was historically the first time when the farm „B“ achieved the better results than farm „A“. It ought to be mentioned that the cows on the farm „B“ are fed only once a day unlike the farm „A“ where the cows are fed twice a day.

The data comparison of daily milk yield of milking cows and all cows in the herds between both of the farms is in TAB 2. These data from TAB 2 are shown on the graph below too. The results „before“ are the data from the performance control in September 2018 and the results „after“ are the data from performance control in April 2019.

TAB 2: Daily milk yield (l/cow)

	Farm „A“	Farm „B“
Milking cows September 2018	**36,4**	**33,23**
Milking cows April 2019	**34,83**	**35,40**
All cows in the herd September 2018	**32,19**	**29,35**



All cows in the herd April 2019	**30,86**	**32,10**

Despite the increase in daily milk production there was no negative effect on the milk fat and the milk protein content in the test. On the contrary, the milk fat content increased during the short time after beginning the test as well as the milk protein content as is shown in TAB 3. The milk fat and milk protein content were higher on the tested farm „B“ than on the control farm „A“ almost throughout the test.

TAB 3: The milk fat, and milk protein content and their ration in the test

	Milk fat Test	Contr	Milk protein Test	Contr	Fat:Prot ration Test	Contr
September 2018 (before the test)	3,70	3,90	3,55	3,46	1,04	1,13
October 2018 (after begennig the test)	4,02	4,02	3,70	3,56	1,09	1,13
April 2019 (the end od test)	4,00	4,06	3,58	3,51	1,12	1,16

Economical production of milk is the most important aim of every dairy cows breeder. The aim of this test was to reduce the costs of purchased proteins feeds while maintaining current performance on the tested

farm. We achieved much better results than we expected. The average monthly savings on rape feeds was 645 EUR during the test. There are included costs of LinaropAgri® in these savings. In addition, the average milk sales increased by 3 700 EUR per month throughout the test. So the whole average benefit was 4 345 EUR per month on the tested farm „B“ since the beginning of the test. It represents the average monthly benefit 16 EUR per milking cow on the tested farm. According to calculations, the feeding of R-LRA instead of the other rape products will bring annual benefit about 43 000 EUR, i.e. 124 EUR per every cow in the herd on the tested farm „B“ (TAB 4).

TAB 4: Benefit

	Per month during test (EUR)	Per milking cow per month (EUR)	Per every cow per month (EUR)	Annual calculation per farm “B” (EUR)
Savings	645	2,4	1,8	6 450
Increased sales	3 700	13,7	10,6	37 000
Total benefit	4 345	16	12,7	43 450

DISCUSSION AND CONCLUSION

Forage silages CP serves mainly as the source for rumen bacteria and consequently for microbial protein production. Microbial protein is the basic source of AA in intestine but is insufficient as the sole source for high performance dairy cows. Besides that, its AA composition is not ideal because its first limiting AA is Histidine. On top of that the Met contain is the most variable in microbial protein together with His.

Considering the AA composition of silages RUP it can be assumed the first limiting AA of diets in European conditions are Met and His. Based on these assumptions RSM seems to be the best solution how to improve AA profile in intestine. However the limiting factor of RSM is lower digestibility of CP. This factor is possible to eliminate if we treat RSM with LinaropAgri®.

R-LRA feeding:

- allows to reduce proportion of RSM as well as concentrate feeds in TMR. It is very important because we can feed reduce-protein diets to high performance dairy cow.
- reduces the risk of acidosis which we demonstrated in this test because the ratio between milk fat and milk protein increased by 0,08.
- reduces the risk of milk fat depression syndrome which confirms the recommendation for maximum possible amount of USFA in rumen and the results of our test proved it.
- In economic term and subsequently with respect to nutrition requirements of dairy cows is R-LRA the best purchased protein feed as the supplement to forage silages in European region.

DIGESTA- AND MUCOSA-ASSOCIATED MICROBIOTA IN VARIOUS GUT SEGMENTS OF GOATS FED CORN SILAGE

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INTRODUCTION

Gastrointestinal tract of animal is the natural habitat for a dense and dynamic microbiota and the population is divided into two subpopulations, *i.e.* digesta-associated and mucosa-associated microbiota. Mucosa-associated microbiota may have principal roles in immunological regulation, and digesta-associated microbiota can be essential for nutrient digestion. Although a number of studies have been performed on gut microbiota of silage-fed animals, most of them examined fecal and digesta-associated microbiota, mainly because of the ease and convenience of the sampling. Likewise, despite the fact that gut microbiota varies across the gastrointestinal tract both in abundance and in composition, most studies have concentrated on the microbiota of rumen and rectum (feces). In this study, we examined microbiota of various gut segments (rumen, abomasum, small intestine, cecum, and rectum) of goats fed corn silage and concentrates. Digesta- and mucosa-associated microbiota was separately examined, because we speculated that consumption of *Lactobacillus*-rich silage could confer probiotic effect in ruminants, and if *Lactobacillus* spp. in silage could demonstrate a satisfactory survival in the gut.

MATERIALS AND METHODS

Four female goats (35.6 ± 3.3 kg) were fed a diet containing whole crop corn silage, alfalfa hay, and concentrates at 40, 10, and 50% on a dry matter basis. Goats were kept in stalls and fed *ad libitum* to assure 10% orts, with free access to fresh water during the trial for 14 days. When goats were sacrificed, digesta and mucosa samples were collected from the rumen (dorsal and ventral), abomasum, small intestine, cecum, and rectum. Mucosa sample was obtained by scraping the surface of gut tissue using a sterilized glass slide.

Bacterial DNA was extracted using repeated bead beating plus column method. The PCR amplification using primers targeting the V4 region of the 16S rRNA genes was employed according to Wu et al. (2019). The PCR amplicons were subjected to 250 bp paired-end sequencing performed on an Illumina MiSeq platform at FASMAC Co., Ltd. (Kanagawa, Japan). Raw sequences were processed using QIIME (version 1.9.0) running the virtual box microbial ecology pipeline and paired-end sequences were joined using fastq-join with more than 20 bp overlap. Chimeric sequences were identified with USEARCH and removed. Data analyses were performed using Primer version 7 with Permanova+ add-on software (Primer-E, Plymouth Marine Laboratory, Plymouth, UK).

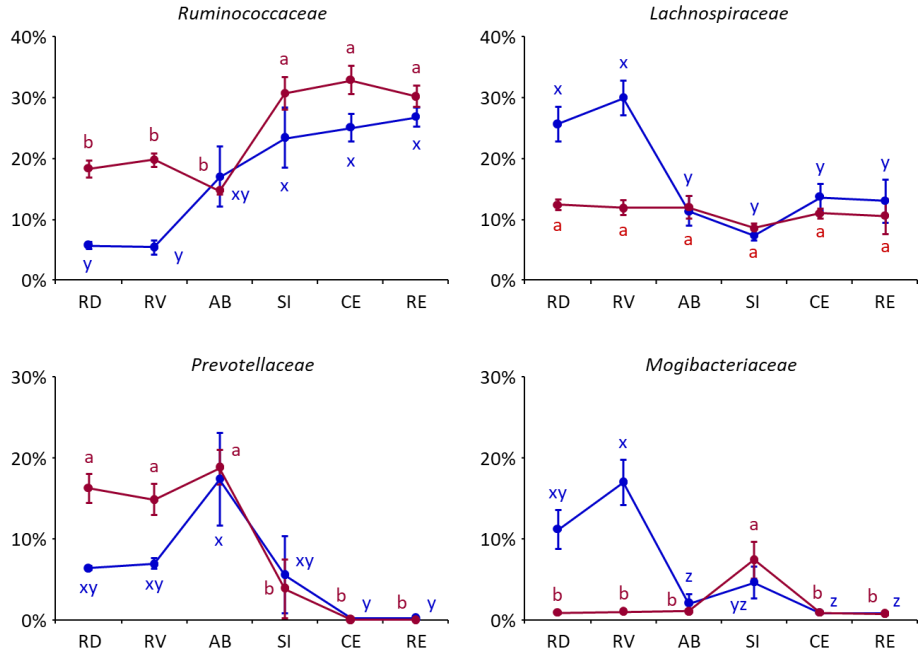

Fig. 1. Relative abundances of prevalent gut microbiota across the gastrointestinal tract of goats fed a diet containing whole crop corn silage, alfalfa hay, and concentrates at 40, 10, and 50% on a dry matter basis.

RESULTS AND DISCUSSION

Lactobacillaceae accounted for about 80% of corn silage microbiota, and *Enterobacteriaceae* and *Acetobacteriaceae* were found at much less relative abundance.

In the rumen, *Ruminococcaceae* and *Prevotellaceae* were prevalent in digesta-associated microbiota and *Lachnospiraceae* and *Mogibacteriaceae* were abundant in mucosa-associated microbiota. Differences between

digesta- and mucosa-associated samples were seen for *Ruminococcaceae*, *Prevotellaceae*, *Lachnospiraceae*, *Mogibacteriaceae*, *Erysipelotrichaceae*, and *Veillonellaceae*, with few differences between dorsal and ventral parts. Unlike in the rumen, relative abundances of major families (detected at >5% in any gut segments) between digesta- and mucosa-associated samples were similar in the abomasum, small intestine, cecum, and rectum. From upper to lower gastrointestinal tract, relative abundances of *Ruminococcaceae*, *Bacteroidaceae*, and *Rikenellaceae* increased and those of *Prevotellaceae* and *Veillonellaceae* decreased in digesta-associated samples. In mucosa-associated samples, relative abundances of *Ruminococcaceae*, *Bacteroidaceae*, and *Rikenellaceae* increased and those of *Lachnospiraceae*, *Mogibacteriaceae*, and *Erysipelotrichaceae* decreased over the gastrointestinal tract.

Although *Lactobacillaceae* was the most in corn silage, the proportion was close to undetectable level (<0.01%) in the rumen and remained at <0.4% throughout the digestive tract, indicating that it may be tough for silage lactic acid bacteria to survive the digestive process and inhabit the gut epithelium.

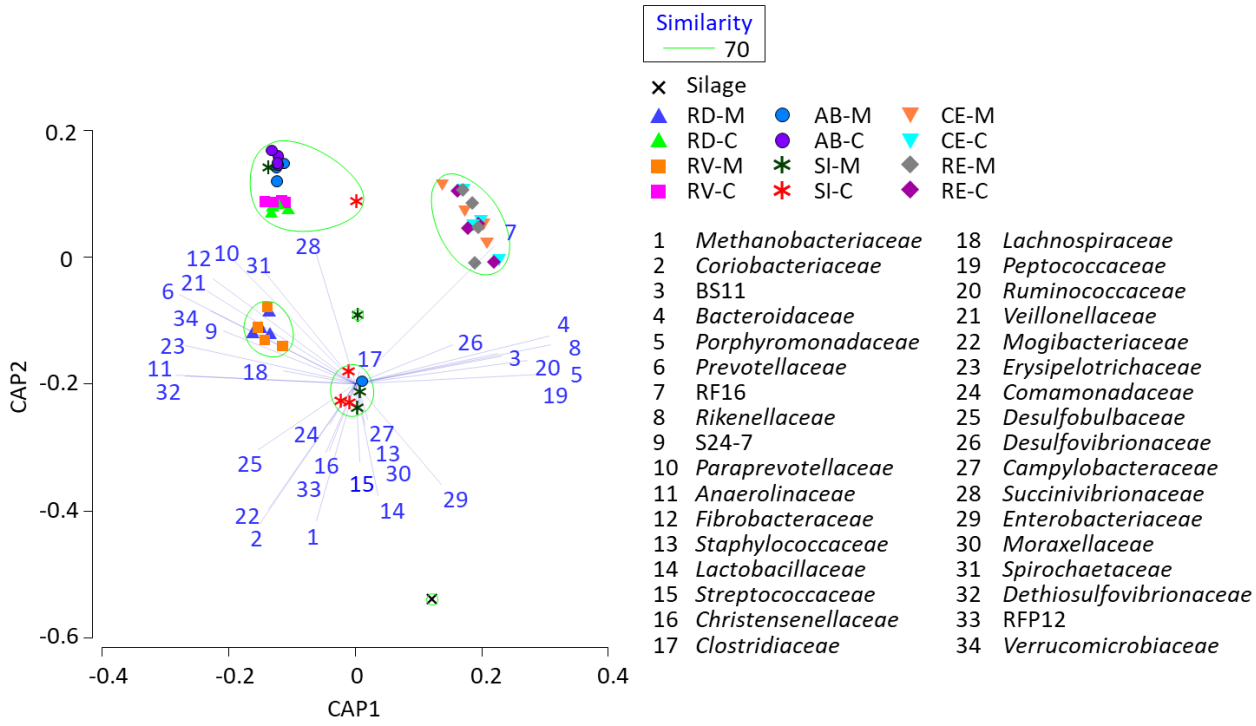

Fig. 2. Canonical analysis of principal coordinates plot characterizing gut microbiota across the gastrointestinal tract of goats fed a diet containing whole crop corn silage, alfalfa hay, and concentrates at 40, 10, and 50% on a dry matter basis. RD, rumen dorsal; RV, rumen ventral; AB, abomasum; SI, small intestine; CE, cecum; RE, rectum; M, mucosa-associated microbiota; D, digesta-associated microbiota. Samples enclosed in a green circle are regarded to be in the same group at a 70% similarity level.

HOW TO MINIMIZE CLOSTRIDIA IN SILAGE AND MITIGATE HEALTH RISKS TO DAIRY COWS

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Nutrition is considered to be the most important external factor which allows to reach full genetic potential of the cow and the herd. Nutrition determines milk yield, milk quality, cow health and fertility.

Silage is an essential component of ruminant diets. Silage quality defines the nutritional value and palatability of the diet and dry matter intake. The quality of silage is influenced by management of ensiling, storage, feed out, ration mixing and feeding to cows.

Preserved forages can also pose risks to the cow health. Even a good silage that meets high quality requirements can quickly turn into spoiled and contaminated one. Every spring and summer we see aerobic spoilage, silage rotting and fungi growth on many farms. Aerobic spoilage is caused by yeast, bacteria and fungi. Yeast plays a major role in this process. Due to lactic acid breakdown silage pH is increased, creating conditions favourable for undesirable bacteria and fungi. Thus, decay products, mycotoxins and biogenic amines such as cadaverine, tyramine, putrescine, spermidine, tryptamine and histamine accumulate in the silage. Counts of Escherichia coli, Clostridium, Listeria and other bacteria increase in silage. Consequently, silage has a low nutritional value and becomes insanitary. This may have a negative impact on animal health, performance and fertility. Feeding preserved forages of poor hygienic quality reduces TMR intake, impairs rumen fermentation, causes absorption of undesirable substances, harms liver and kidney function, and negatively effects ovarian activity and parenchyma of the mammary gland. Feeding of poor forage predisposes cows to mastitis, laminitis and is an important cause of embryonic mortality.

Increased clostridia counts in silage have a strong negative effect on the health of cattle. Naturally, there are some clostridial spores in every silage and in the digestive tract. If forage is contaminated with soil, sewage water or slurry, clostridium counts are increased considerably, causing altered silage fermentation and production of large amounts of ketogenic butyric acid, free ammonia and amines in the silage. Clostridia and their spores are increasing in numbers and posing a great health risk to the animals and a major hygiene problem. Clostridial spores can contaminate milk, affect its quality and hamper cheese production. Massive presence of clostridia in the digestive tract can induce inflammatory reactions on the mucous membranes, produce toxins that are absorbed and disrupt the liver and kidneys. Clostridia can penetrate through the inflammatory mucosa into the body, causing inflammatory processes in the peritoneum, liver, kidneys, myocardium, lungs and muscle. Clostridia produce gas that accumulates in the subcutaneous tissue, and toxins that impair the general health of the animal. Massive infection causes fever and may lead to sepsis and death. Every year we see many cases of the disease caused by clostridia from preserved forage.

In the herd A, clostridial infection was diagnosed in dairy cows and young cattle fed grass silage of low dry matter harvested in bad weather and contaminated with soil. In the herd B, the source of infection was clover-grass silage contaminated with sewage water and insanitary water from a stream flowing through the pasture. Dairy cows were grazed and fed TMR with a high silage inclusion rate once a day. Both the herds suffered of massive diarrhea and a drop in milk yield. In the herd A, daily milk yield decreased by 3 to 5 liters per head, in the herd B by 8 to 12 liters. At the same time there was a significant increase in milk somatic cell count and incidence of subclinical and clinical mastitis. In the herd A, 8 cows of 240 died, in the herd B, 24 cows of 320 died. In 40% of cows in the herd A, subcutaneous emphysema was found. In the herd B, the incidence of subcutaneous emphysema was rare, but swelling of bottom jaw and dewlap was noted. Cows significantly reduced their feed intake. The cows were lethargic, some developed tachycardia or even dyspnoea. Rectal temperature ranged from 39.6°C to 41°C. Metabolic tests performed in some animals (10 and 12 in the herds A and B, respectively) showed dehydration and impaired liver function. Rumen fluid analyses revealed rumen alkalosis. Cl. perfringens was detected in intestinal contents and exudates. A high number of Cl. perfringens spores was also found in faeces.

Autopsy was performed on selected dead cows (2 and 4 in the herds A and B, respectively) and 2 dead heifers and the post-mortem findings were evaluated. The findings included smelly ruminal contents, rumen mucosa erosions, catarrhal to hemorrhagic inflammation of abomasal and intestinal mucosa, marked intestinal balooning, liver and heart dystrophy, increased amount of exudate in the abdominal and thoracic cavities. In some corpses croupous pneumonia was found.

After the exclusion of spoiled silage and other dietary measures and symptomatic therapy the health of the cows gradually improved, but diarrhea persisted in some cows for over 10 days. Milk production increased very slowly and did not reach the previous level even a month later. The increased bulk tank somatic cell counts lasted for 3 months.

In order to minimize the risk of clostridial infections in dairy cows, attention must be paid to silage production and storage:

- Avoid contamination with soil
- Implement the best possible silage management practices
- Use silage inoculants that rapidly decrease silage pH
- Ensure good compaction and sealing of silage
- Protect silage from contamination with slurry and waste water
- Ensure safety of drinking water
- Don´t use untested water sources at pasture

Adherence to proper silage management practices during the harvest, ensiling and storage using suitable silage inoculants are fundamental to the production of good and safe preserved forage. Attention must be paid to the feed-out of silage and presentation to the cows. Silage contamination with soil is always a major risk and may cause the development of clostridial infections.

EFFECT OF BRUSHES IN RUMEN OF DAIRY COWS ON THEIR PERFORMANCE

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INTRODUCTION

Structural fibre has a significant effect on the motor activity of the rumen. This activity depends on physically effective neutral detergent fibre (peNDF) in TMR. The peNDF is determined by multiplying the content of the NDF in DM and the % residue on the 8 and 19 mm sieves. Beauchemin & Yang (2005) confirmed that particle length is a reliable indicator of the rumination period, but it is not necessarily an indicator of rumen acidosis. Acidosis is defined as a state of high pathological acidity of the blood, and the incidence is increasing in ruminants. The duration at which pH remained below the threshold value of 5.8 in a 24-h period (Valente *et al.*, 2017) was used as an indicator to characterize of subacute ruminal acidosis (SARA). Plaizier (2004) determined that a peNDF of 12.5% DM or lower resulted in a rumen pH indicative of SARA. Rumen acidosis could be supposedly reduced by using ruminal mechanical stimulating (RMS) brushes, European patent EP0609045A2 (Meiwa-Sangyo Co. Ltd, Kyoto, Japan), commercially known as Rumenfibe (RF). This device administered as three pieces per dairy cow could be used for stimulating the physical function of the rumen mucosa. Golder *et al.* (2017) examined the effects of RMS brushes on rumen fermentation and subsequent milk production in early lactation dairy cows from a commercial pasture-based herd in the Australian spring and summer. Consequently, rumen fermentation properties and milk production were not affected by RMS brushes administration. The present paper evaluates the beneficial effect of RMS brushes administration on the performances of high-yielding dairy cows in early lactation fed on TMR with minimal structural fibre, *i.e.*, low peNDF (10.9% DM).

MATERIALS AND METHODS

The feeding experiment was carried out in accordance with the practices outlined in the Act No. 183/2017 Sb. Protection of animals against cruelty. The experimental methodology (project MZe NAZV QJ1510391) was approved by the Czech Ministry of Agriculture (13320/2015-MZE-17214). A total of 22 Holstein dairy cows were included in the experiment. The cows were divided into experimental (RF) and control (C) groups by the pairing method. Each period lasted 3 weeks. At the beginning of the second (P2) period, 3 pieces of artificial RMS brushes were administered *per os* using a special applicator to the rumen of cows in the RF group. Each RMS brush consisted of synthetic polymer bristles held in place with a metal component and was enclosed in a paper capsule that dissolved after insertion (Golder *et al.*, 2017). The cows were stabled in an experimental barn equipped with tensometric feeding troughs (Insentec, Marknesse, NL) connected to a computer system. The intake of feed was continuously monitored during the experiment, individually for each cow. The lying down boxes contained wood sawdust on the floor, to avoid the eating of straw by animals.

The chemical composition in each TMR was repeatedly analysed 3 times in each experimental period and determined according to the methods of the Association of Official Analytical Chemists (AOAC, 2005). The yield and quality of milk and the LW of cows were monitored daily. Milk quality was analysed in the accredited (ČSN EN ISO/IEC 17025:2005) laboratory (MILCOM a.s., Prague, CR) using the following methods: Fat in % by the butyrometric method (ČSN ISO 2446), protein in % by the Kjeldahl method (ČSN 57 0530) and urea in mg/L milk by infrared analyser. Rumen fermentation properties were analysed using an inoLab level 1 pH meter for pH, using ITP/CZE analyser IONOSEP 2003 for volatile fatty acids (mmol/L of rumen fluid) and a Biochrom Libra s22 spectrophotometer for ammonia nitrogen (mg N/100 g rumen fluid). Statistical data were analysed using the General Linear Model (GLM) procedure of SAS (SAS Institute, Cary, NC, USA, 1999) using regression to the values in the each initial period (1).

RESULTS

Results are shown in Table 1. The TMR intake was similar in P1 and P2, although at the beginning of this period, the RMS brushes were applied to the cows. After application of the RMS brushes in P2, the difference in milk production and milk quality between the RF and C group was not recorded ($P>0.05$). The limit of urea in milk (300 mg/L) was exceeded in the period P1. Since the protein content in the milk was lower than 3.2%, it can be suggested that the TMR in P1 contained less energy and excess protein. In P2 TMR was deficient in energy but sufficient in protein. These results cannot be directly compared with those reported by Golder *et al.* (2017), as their RMS brushes experiment took place with dairy cows on pasture. In a feeding experiment using 6 lactating dairy cows that had ruminal cannulas and were fed maize diets that varied in the peNDF content by altering the particle length of the corn silage. In addition, neither beneficial nor detrimental effects of RMS brushes administration on the rumen fermentation parameters were noted, which was also consistent with the results in the study of Golder *et al.* (2017).

No differences ($P >0.05$) between groups were also observed in any of the rumen fermentation parameters. Only acetic acid in the rumen fluid had a tendency ($P<0.1$) to be significantly higher in the RF group

than in control group without RMS. For the mitigating effect of RMS brushes administration on the risk of SARA, the most important key factor is a pH decline associated with an increase in organic acid production, particularly lactic acid. According to Valente *et al.* (2017) the threshold value to characterize SARA is a pH below 5.8 for a duration of 24 hours. The average pH of rumen fluid was not below 6.31 in any period and group. Although the composition and dilution of the TMR tested was at the limit of the peNDF critical value (12.5), SARA was not detected. Therefore, a mitigating effect of RMS brush administration on the risk of SARA was not demonstrated.

Table 1 Feed intake, milk production and milk quality and rumen fluid fermentation metabolites content

		Period 1*			Period 2			
		RF	C	s.e.	RF	C	s.e.	*P*-value
Feed intake	kg/cow.day	55.9	55.9	0	52.5	52.2	0.93	0.79
Milk production	kg/cow.day	43.9	43.9	0	42.2	41.8	0.68	0.64
Milk fat	%	4.20	4.20	0	4.05	3.93	0.17	0.66
Milk protein	%	3.06	3.06	0	3.09	3.14	0.04	0.43
Milk urea	mg/L	347	347	0	258	235	12.4	0.20
Rumen fluid fermentation metabolites								
pH		6.44	6.44	0	6.31	6.33	0.11	0.91
Ammonia nitrogen	mg N/100 g	14.6	14.6	0	14.6	13.3	0.42	0.57
Acetic acid	mmol/L	76.4	76.4	0	82.8	72.8	1.87	0.10
Propionic acid	mmol/L	22.5	22.5	0	24.5	23.0	0.96	0.26
Butyric acid	mmol/L	16.1	16.1	0	18.1	17.9	0.64	0.77

*Period 1 with regression (s.e. = 0); s.e., standard error of mean; RF, experimental group; C, control group

CONCLUSIONS

Feed intake, milk production, milk quality or fermentation properties in rumen fluid were not affected by the group or the interaction between groups. . Only acetic acid in the rumen fluid had a tendency ($P<0.1$) to be significantly higher in the RMS group than in control group without RMS.

There appeared to be no effect of RMS brushes on these indicators. Thus, in the present study, negative impacts of the RMS brushes in the rumen of the high yielding dairy cows were not observed, although a positive effect has also not been demonstrated. In the present study, minimal peNDF in all experimental TMR did not increase the risk of SARA, contributing to the unclear efficacy of RMF brushes administration. Thus, RMS brushes administration may fulfil its potential as a prophylactic device for peNDF in high grain feeding environments such as feedlots or in high-concentrate feeding for high-performance lactation cows

The most important role of rumination in a ruminant is regurgitation of fibrous components and the buffering action of saliva flow into the rumen fluid, neutralizing the acidic state and activating fibrolytic microbes. It seems that the role of RMS brushes for rumination is dependent on the peNDF content in the feed.

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AFFECTING OF CORN WHOLE-PLANT CHOPPED MATERIAL BY DIFFERENT PROCESSING

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ABSTRACT

The aim of this study was to evaluate the effect of different technologies used for corn silage harvest on qualitative parameters of whole-plant chopped material. Harvest technologies vary namely using different corn cracker concept (classical, MCC MAX and Shredlage). Chemical composition, proportion of chop length, kernel processing, physically effective fiber (peNDF) and rumen degradability were determined and compared. The best-processed kernel and also the highest content of peNDF in corn silages produced by technology Shredlage were detected. Compared with the classical corn cracker concept, the chopped material, produced by MAX and Shredlage technologies, had the higher rumen degradability of dry matter, neutral-detergent fiber and starch.

INTRODUCTION

Well processed corn chopped material during harvest caused better punning and fermentation process (McDonald, 1981). Bal et al. (2000) described that good corn processing leads to increasing of starch digestibility. However, high amount of low particles can negatively affect fibre digestibility and rumen pH (Mertens, 1997). Physicall effect of feeds is evaluated using peNDF, which is based on easy measuring using Pen State Separator (Lammers et al., 1996).

The aim of this study was to evaluate the effect of different technologies for corn silage harvest on qualitative parameters of whole-plant chopped material.

MATERIALS AND METHODS

Experimental harvesting proceed in year 2018. Corn was harvested from one field (one hybrid) in two dry matter (DM) levels (approx. 35 and 40 %). Control whole-plant chopped corn material was prepared by harvester Claas Jaguar 860 (equipped with a conventional type processing rolls; TLOC 14 mm, lower DM). Harvester Claas Jaguar 870 was used for harvest of corn by shredlage (SHR; equipped with a shredlage processing rolls; TLOC 27 and 19 mm for lower and higher DM, respectively) and MAX (MAX; equipped with a MAX processing rolls; TLOC 14 and 12 mm for lower and higher DM, respectively) technologies.

Three subsamples from each chopped corn material were taken. All samples were analysed on DM, ash, crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF) and starch (AOAC, 2005) content. Quality of processing of all types of chopped material was done using Penn State Particle Separator (PSPS; sieves 19; 8 and 4 mm). Physically effective NDF (peNDF) was calculated by multiplication of NDF content and part of particles up to 8 mm. Quality of kernel processing was evaluated by % starch passing a 4.75 mm sieve (Ferreira and Mertens; 2005), where values lower than 50 % is bad processing, 50 to 70 % is sufficient and more than 70 % is perfect processing.

Rumen degradability of tested whole-plant chopped material was measured using in situ method, where two Holstein cows were used. Material was incubated in fresh and incubated in nylon bags (10 x 20 cm; 15 g/bag) in three repetitions per cow. Samples were incubated for 24 and 48 hours.

All results were evaluated using GLM procedure of SAS (SAS 9.4; 2012), where type of processing was used as fixed effect and repetition and animal as random effect.

RESULTS AND DISCUSSION

Parameters of chemical composition, processing and rumen degradability of whole-plant chopped material harvested by different methods are presented in Table 1. Parameters of chemical composition did not differ among harvest technologies with the exception of DM and starch contents, which were caused by targeted harvests in two DM levels.

The highest differences among tested technologies were in processing score which describe quality of kernel processing. Shredlage had the highest values of processing score, following by MAX and the lowest values were detected for control. Better processing could leads to increasing of starch (Hoffman et al., 2011).

Evaluation of particle sizes using PSPS showed the highest amount of particles above 19 mm for SHR. Also, the amount of particles above 8 mm and peNDF content were higher for SHR. There were not differences between MAX and control in these parameters.

Rumen degradability of DM, NDF and starch was higher for SHR and MAX in comparison with control. Couderc et al. (2006) declared that TLOC did not affect NDF degradability. In our study better degradability is caused by higher disruption of corn material when new technologies are used.

CONCLUSIONS

New technologies used for corn silage harvesting had positive effect on kernel processing, which was the highest for SHR. Harvesting with SHR or MAX technology leads to higher rumen degradability of DM, NDF and starch in comparison with control. The SHR technology increased also the peNDF values.

Table 1. The effect of corn harvest technologies on chemical composition, processing and degradability of whole-plant chopped material

		Lower DM		Higher DM		sem	*P*
	Control	**SHR**	**MAX**	**SHR**	**MAX**		
Chemical composition							
Dry matter, g/kg	335^{c}	372^{b}	334^{c}	393^{b}	431^{a}	8.8	**
OM, g/kg DM	959	958	961	959	959	1.7	N
CP, g/kg DM	88	85	90	88	87	0.9	N
EE, g/kg DM	18	17	20	16	20	0.4	N
CF, g/kg DM	245	254	233	242	244	6.6	N
NDF, g/kg DM	527	535	507	527	502	77.5	N
ADF, g/kg DM	281	292	266	275	283	6.6	N
Starch, g/kg DM	201^{b}	202^{b}	209^{ab}	216^{ab}	247^{a}	15.9	*
Processing score, %	59.4^{d}	79.2^{a}	75.6^{b}	77.1^{b}	64.8^{c}	2.7	**
Penn State separator sieves, % as fed retained							
19 mm	3.6^{b}	21.3^{a}	4.7^{b}	19.4^{a}	1.6^{b}	12.0	**
8 mm	59.4^{a}	48.4^{c}	61.3^{a}	52.9^{bc}	55.9^{ab}	2.91	**
4 mm	25.2^{a}	15.4^{b}	22.1^{a}	15.1^{b}	24.9^{a}	1.35	**
Bottom pan	11.9^{b}	14.9^{ab}	11.9^{b}	12.6^{b}	17.6^{a}	1.92	**
Parts up 8 mm, %	62.9^{bc}	69.7^{ab}	66.0^{ab}	72.3^{a}	57.5^{c}	5.68	**
peNDF, g/kg DM	332^{b}	372^{a}	334^{b}	381^{a}	288^{b}	22.0	**
Rumen degradability, %							
DM, 24 h	63.5^{b}	68.2^{ab}	70.6^{a}	68.1^{ab}	69.6^{ab}	7.96	**
DM, 48 h	74.4^{b}	77.2^{ab}	80.2^{a}	79.7^{a}	79.7^{a}	3.23	**
NDF, 24 h	49.5^{b}	53.6^{ab}	56.4^{a}	54.8^{ab}	53.8^{ab}	10.2	*
Starch, 24 h	91.6^{b}	95.4^{a}	95.3^{a}	94.9^{a}	95.1^{a}	1.93	**

**P < 0.01; *P < 0.05; N=nonsignificant; $^{a, b, c}$ Means within rows with different superscript letters indicate a significant difference at P < 0.05.

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QUALITY OF ALFALFA SILAGES PRODUCED IN SLOVAKIA BETWEEN 2015 – 2018

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INTRODUCTION

Alfalfa silages are important protein feed in our feed rations. Growing alfalfa in Western Slovakia has been problematic in the recent years due to the climate change, which causes frequent long dry periods during the vegetation period. High temperatures as well as insufficient rainfall negatively affect the quality and quantity of the produced fodder feed.

The objective of this study was to monitor in the period from 2015 to 2018 select parameters of nutritional value and the fermentation quality of alfalfa silages produced in Western Slovakia, which is affected the most by the negative influences of the climate change.

MATERIALS AND METHODS

In the years 2015 – 2018 we analysed 400 alfalfa silages a year in the laboratory of Research Institute for Animal Production. Silages were from various agricultural enterprises of Western Slovakia. After statistic evaluation of the data set, we excluded the outliers and calculated the arithmetic mean.

The parameters of organic analysis were determined: dry matter content (gravimetric analysis), crude protein content according to Kjeldahl, content of crude fibre according to Commission Regulation (EC) No 152/2009 (2009), acidodetergent and neutral fibre according to Van Soest, parameters of fermentation process were determined: pH by electrometric method, acids by gas chromatography and content of ammonia nitrogen by microdiffusion method according to Conway.

RESULTS AND DISCUSSION

According to our results, the content of dry matter in the silages in the studied period was between 37.2 and 39.2 %. Concentration of crude protein was between 190 and 207 g/kg of dry matter, which is for alfalfa considered an average or slightly lower value. The analysis showed that the fiber complex (CP, ADF, NDF) was at slightly higher level compared to regions which do not suffer from excessive high temperatures or insufficient rainfall during the vegetation period.

Table 1. Content of selected nutrients in alfalfa silages produced in Western Slovakia.

		Year			
		2015	**2016**	**2017**	**2018**
Dry matter	mean	392.24	384.19	383.14	379.03
	Min.	213.36	207.11	241.76	241.22
	Max.	590.76	615.08	532.13	533.87
*Crude protein	mean	191.86	202.58	202.05	206.84
	Min.	139.11	152.41	155.40	143.53
	Max.	234.44	260.26	238.22	259.86
*Crude fibre	mean	304.30	273.10	265.98	274.49
	Min.	232.78	196.53	199.24	191.19
	Max.	395.32	328.14	336.67	325.78
*ADF	mean	349.21	314.98	323.42	323.60
	Min.	244.74	214.56	256.72	223.35
	Max.	512.40	464.04	439.28	411.83
*NDF	mean	395.70	371.43	376.19	383.74
	Min.	258.00	245.34	290.29	294.48
	Max.	552.66	529.40	485.54	528.16

* in g/kg DM,

Average parameters of fermentation process induce good quality of the produced alfalfa silages even though the maximum butyric acid confirms that in some cases, the technology of ensilaging was not mastered.

CONCLUSIONS

Our analysis confirmed that in Western Slovakia, which is during the main vegetation period regularly affected by hot and dry weather, the climate changes have negative impact on some nutrition parameters of alfalfa silages compared to other regions of Slovakia. These are mainly lower crude protein concentration and higher fibre complex content. Influence of fermentation process was not determined.

ACKNOWLEDGMENT

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Table 2. Parameters of fermentation process in alfalfa silages produced in Western Slovakia.

		Year			
		2015	**2016**	**2017**	**2018**
pH	mean	4.68	4.60	4.59	4.65
	Min.	4.17	3.89	4.19	3.88
	Max.	5.65	5.42	5.05	5.35
*Lactic acid	mean	61.83	73.83	82.46	71.72
	Min.	25.45	35.63	46.44	20.20
	Max.	118.12	117.24	123.76	119.51
*Acetic acid	mean	17.26	17.84	15.60	21.47
	Min.	4.37	4.33	6.18	5.42
	Max.	58.49	36.11	24.56	45.81
*Butyric acid	mean	1.32	1.00	0.25	1.54
	Min.	0.00	0.03	0.03	0.00
	Max.	8.69	10.03	0.82	13.17
**NH_3 - N	mean	8.65	7.62	7.51	8.04
	Min.	4.23	4.34	4.82	4.09
	Max.	22.42	9.44	9.30	13.61

* in g/kg DM, ** in mg N/100 g

HYGIENIC QUALITY OF TRADITIONAL TMR AND COMPACT TMR

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ABSTRACT

A clover-grass silage from a second harvest at 60:40 proportion to concentrate was used in the study to compare a microbial composition of traditional total mixed ration (TMR) with compact total mixed ration (CTMR) mixture during 24 hours feeding regime. The CTMR is a wetter and more finely mixed version of traditional TMR. The average dry matter (DM) content of CTMR was 37.0 % whereas the traditional TMR contained on averaged 51.8 % of DM. There was no single effect of ration in yeast and mould growth. The effect of ration was pronounced in interaction with the effect of time. The CTMR displayed significantly higher yeast and mould count than at 0 and 12 hours after silo removal whereas similar differences in traditional TMR were not significant. Results indicate the CTMR to be more susceptible to spoilage than traditional TMR when feeding time exceed more than 12 hours.

INTRODUCTION

Total mixed ration (TMR) is worldwide common feeding system of dairy. A new feeding practice has recently been elaborated in Denmark (Kristensen, 2015). So called "compact total mixed ration" (CTMR) is based on the already established concept TMR. The CTMR is a wetter and more finely mixed version of TMR. The dry matter (DM) content of CTMR targets 37 % and the feedstuffs should be close to indistinguishable from one another. TMR's are commonly a mixture of wet anaerobically stored (silage) feed with dry aerobically stored feed. This combination often causes a good precondition for undesirable microbial growth in the mixture. The occurrence of increased undesirable growth of yeasts and molds in TMR than in silage was early observed by Kung (2010) and Cogan *et al*. (2017). The question arises, how more intensive mixing procedure and a water addition affect a hygienic quality of CTMR mixture. Therefore, the aim of the study was to compare a microbial composition of a traditional TMR with a CTMR mixture during 24 hours feeding regime.

MATERIALS AND METHODS

The study was performed at SLU experimental station in Lövsta during winter 2017. Both mixtures hade the same composition as well as silage to concentrate proportion at 60:40 on a DM basis. A clover-grass silage from a second harvest ensiled in bunker silo was used. Hygienic quality of the feed was assessed by the sampling of both rations at 0, 12 and 24 hours after removing from the silo. Samples were collected from four feeding mangers within each ratio type, placed in a plastic box and thoroughly mixed at each sampling time. An approximately 300 g sample was extracted from the plastic box into a plastic bag for further microbial analyses. The sampling procedure was repeated sex days creating 18 samples for each ratio type. Microbial analyses included determination of yeasts and moulds using plate method where yeasts and moulds were cultured aerobically at 25 ºC on Malt Extract Agar (Merck) supplemented with 0.12 *M* lactic acid (50mL/L).

RESULTS AND DISCUSSION

The average DM content of CTMR was 37.0 % whereas the traditional TMR contained on averaged 51.8 % of DM. There was no effect of ration on yeast growth ($p=0.6$) indicating that more intensive mixing and lower DM content of the CTMR did not affect yeast counts compared to traditional TMR. The yeast count in both rations were significantly affected by time ($p<0.001$) displaying a trend of increasing numbers of yeasts with longer exposure time. Differences were particularly obvious at 24 hours after silo removal where CTMR displayed significantly ($p<0.001$) higher yeasts count than at 0 and 12 hours after silo removal. Similar differences in traditional TMR were not significant. There were no differences between 0 and 12 hours in both rations. The mould growth in both rations followed the same pattern as in yeasts growth. No effect of ration type on mould growth was detected ($p=0.5$). However, effect of time was pronounced in CTMR having remarkably more moulds after 24 hours of exposure than after 0 and 12 and at same time not significant similar differences in TMR. It can be concluded that significantly higher counts of yeasts and moulds in CTMR at 24 hours after removal from silo indicate this ration to be more susceptible to spoilage than traditional TMR when feeding time exceed more than 12 hours.

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Table 1. Microbiological composition of experimental feed rations CTMR and TMR at 0, 12 and 24 hours after removal from the silo (n=6). Different superscripts within a column differ (p=<0.05)

	Yeasts, log cfu g^{-1} FM		Moulds, log cfu g^{-1} FM	
	Mean	SD	Mean	SD
CTMR0	4.43^{a}	0.292	1.94^{a}	0.708
CTMR12	4.48^{a}	0.049	2.39^{a}	0.156
CTMR24	5.95^{d}	0.885	3.41^{b}	0.800
TMR0	4.81^{abc}	0.788	2.01^{a}	0.627
TMR12	5.05^{abc}	0.702	2.37^{a}	0.922
TMR24	5.38^{cd}	0.660	2.78^{ab}	0.922
Standard Error	0.259		0.307	
$P_{treatment}$	0.6		0.5	
P_{time}	<0.001		0.004	
$P_{treatment*time}$	0.08		0.5	

HETEROGENEITY OF CORN SILAGE ACROSS THE FACE OF THE BUNKER AND THROUGH THE BUNKER

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Keywords: Variability, Heterogeneity, Dry Matter, Fermentation

ABSTRACT

Heterogeneity of corn silage was assessed and quantified through the height of the bunker and with time. Both time and height of sampling showed silage to be non-homogenous in feed value.

INTRODUCTION

Dry matter and overall nutritional value of silage is inherently variable within a bunker. Multiple factors impact the nutritional value (different fields, delays in harvest, different cuts, air penetration, variable compaction etc), and this is reflected in the variable nutritional value of the silage at a single time point of feeding and as the bunker is being fed. This assessment aimed to define the variability in DM and fermentation profile across a corn bunker face at a single point of time, and across the face at different time points.

MATERIAL AND METHODS

An open bunker of corn silage that was consistently fed using a defacer from time of opening, crossing the face on a daily basis by a minimum of 20 cm was identified (Hanácká zemědělská společnost Jevíčko a.s., farm Jaroměřice, Czech Republic, 2018 harvest, treated with a commercial inoculant). Corn had been ensiled in a concrete bunker using train wheel compaction, with standard vacuum plastic sealing. Corn had been ensiled for 5 months at the time of the initial sampling, and 8 months at time of the second sampling. Silage was cored using the Dairy One Master Forage Probe from 6 locations across the open face of the bunker as identified, at 2 time points, with the core samples (C) being used for determination of silage density (corrected for DM, using the Dairy One spreadsheet) and of nutritional profiling via dry NIR. Cores were taken 1m in from the wall at a height of 1.2m and 2m on both sides of the bunker, and also centrally. Cores were taken in February 2019 and then again in May 2019. Cores were vacuum sealed prior to analysis.

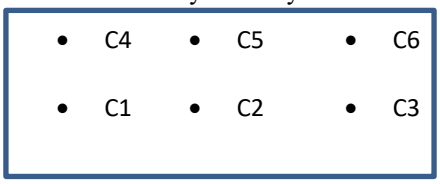

Table 1 – Density of Silage Cores

Core	Density Kg DM m^{-3}
C1	261
C2	283
C3	252
C4	249
C5	243
C6	249

RESULTS AND DISCUSSION

Density of the corn silage was recorded at the sample point C1-C6 as shown in Table 1.

Density of the silage was lower at the base of the bunker than at the top of the bunker as cumulative packing time is greater the further down in the bunker and the weight of the silage pressing down is also greater the further down the bunker travelled. The density of the silage at the extreme left and right of the bunker is lower than in the centre of the bunker. This is generally due to slightly uneven spreading of the forage at time of ensiling, but, when train wheels are used, is also a facet of the extreme left and right getting less 'run time' of the compactor compared to the centre area of the bunker as the train wheel compactor moves across the surface of the forage. At the top of the bunker there is no difference in the density of the forage as the impact of the cumulative packing time of the forage becomes lesser the closer to the top of the bunker.

Cores were analysed in all instances for DM, starch and NDF, with the initially taken samples also analysed for fermentation profile. The second set of samples was not analysed for fermentation profile. Results are shown in Table 2.

Table 2 – Nutritional profile of silage cores at Time T=1 and T=2

	DM		Starch		NDF		LA	AA	TVFA
	T1	T2	T1	T2	T1	T2			
C1	32.0	34.0	24.4	23.7	43.3	37.5	7.79	2.91	10.7
C2	30.0	33.2	23.9	22.6	44.7	41.0	7.16	3.63	10.79
C3	30.9	34.2	24.7	22.6	45.4	39.5	7.02	3.8	10.82
C4	39.7	43.3	19.3	27.6	52.7	41.5	4.64	2.69	7.33
C5	40.5	37.6	26	26.9	46	42.4	4.46	2.69	7.15
C6	41.1	45.3	25.2	25.7	45.9	45.6	4.77	2.69	7.46

Table 3 – Summary of dry matter analysis

	Mean		SD		CV	
	T1	T2	T1	T2	T1	T2
C1-C3	31.0	33.8	1.00	0.53	3.23	1.55
C4-C6	40.4	42.1	0.70	3.97	1.73	9.44
C1-C6	35.7	37.9	5.24	5.19	14.70	13.67

Table 3 analyses the variability of dry matter within the samples at the two sample times. A 9% variance in tested DM is observed between the base of the bunker and the top of the bunker. This is accounted for by the increased compaction within the bunker and adsorption of moisture in the lower area of the bunker. The CV of the top samples and bottom samples is similar, but between top and bottom samples is highly different indicating a 14% variation between the DM of at the two sample heights.

Table 4 – Summary of Starch analysis.

	Mean		SD		CV	
	T1	T2	T1	T2	T1	T2
C1-C3	24.3	23.0	0.30	0.61	1.67	2.67
C4-C6	23.5	26.8	3.66	0.97	15.57	3.61
C1-C6	23.9	24.9	2.37	2.21	9.92	8.87

Table 4 analyses the variability of starch content within the samples at the two sample times. Starch levels remain relatively consistent, with the CV of the sample grouping being variable between 1.67 and 15.67% at different sample heights, the CV across the entire sample points at each time of sampling remains below 10%. Variability in starch analysis cannot be attributed to variability in silage as this degree of analytical variability can easily be accounted for through sampling and analytical error.

Table 5 – Summary of NDF analysis

	Mean		SD		CV	
	T1	T2	T1	T2	T1	T2
C1-C3	44.5	39.3	1.07	1.75	2.40	4.45
C4-C6	48.2	43.2	3.90	2.15	8.09	4.97
C1-C6	46.3	41.2	3.27	2.74	7.06	6.65

Table 5 analyses the NDF of the different silage sample cores and shows that the fibre content of the samples to be relatively consistent throughout the bunker. This can be attributed to the corn plants across all fields being harvested at a comparable development / maturity stage and is not necessarily indicative of homogenous silage.

Table 6 – Summary of Fermentation parameters

	C1 – C3			C4 – C6			C1 – C6		
	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV
Lactic Acid	7.32	0.41	5.60	4.62	0.16	3.37	5.97	1.50	25.19
Acetic Acid	3.45	0.47	13.71	2.69	0.00	0.00	3.07	0.51	16.65
TVFA	10.77	0.06	0.58	7.31	0.16	2.13	9.04	1.90	20.97

Table 6 presents the fermentation profile of the different areas of the bunker at the initial sampling time. Fermentations at a single height within the bunker are homogenous, with the lactic acid levels being consistent at a sampling height with low CV values, but between sampling heights the lactic acid levels are very different and the CV increases from less than 6 to over 25. The same pattern can be seen with the acetic acid profiles at the 2 sampling heights and the total volatile acid profile.

CONCLUSIONS

Samples taken at a single level within the corn bunker showed the silage to be homogenous both in dry matter but also fermentation profile. Variability is observed in DM and fermentation profile between different levels of the bunker at a single sample time which has potential negative ramifications for feeding a known, balanced ration to the dairy animal unless a defacer is used which crosses the face of the bunker fully each time feed / TMR is produced (eg block cutter TMR will likely introduce significantly more feed variation and rumen instability). Samples taken at different times showed the same profile across a height level but varied considerably both between sampling heights and sampling times. The variance in silage dry matter and fermentation highlights the need for regular, good sampling of the silage and consideration of the feeding variability that is introduced inherently with differing silage removal systems.

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VARIABILITY OF THE MAIZE SILAGE YIELD AND QUALITY DURING THE LAST 6 YEARS ACCORDING THE WEATHER CHANGES IN THE DRY-CONTINENTAL REGION OF EUROPE

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INTRODUCTION

Difficulties can be expected of maize cultivation in many European countries, especially in dry continental and Mediterranean regions (greater occurrence of weather extremes: increasing number of heat stress days, groundwater shortages, mycotoxin contamination). Weather extremes affected the corn silage in the north-eastern United States in 2012 and 2013 (Ketterings et al, 2015), also.

It is highly important to understand the role of NDF digestibility in order to be able to predict cow response, especially during the summer time. Grasses, legumes, and grain-containing forages show a different behaviour in the rumen (Grant and Cotanch, 2012). The digestibility of NDF influences various factors, such as rumen fill, time budgeting and feeding management, chewing responses and ruminal pH, and efficiency of milk production (Grant and Cotanch, 2012). The higher passage rate (*kp*) resulted by 'fast' fibre makes it possible to empty the rumen quicker achieving higher dry matter intake (Raffrenato and Van Amburgh, 2010), what can be essential during the heatstress period. This is why the optimization of forages for high producing cows shall be highlighted, and the advantage of their lower lignin content and greater extent of NDF digestion shall be utilized (Grant and Cotanch, 2012). Therefore the rumen degradable NDF ($daNDFom_{48}$) content has important role in high performance dairy cow nutrition.

The objective of this study was to show the current situation in Central-Europe. The author reported the yield, the nutrient content (n= 3131) and the digestibility ($aNDFomd_{48}$ – amylase treated ash corrected NDF 48 hours *in vitro* degradability, OMd_{48} – organic matter digestibility *in vitro* 48 hours incubation) of maize silage. Correlations between the above mentioned parameters were determined int he period of 2013-2018.

MATERIALS AND METHODS

Routine laboratory samples derived from large scale farms in Hungary (n= 3131) between 2013-2018. The annual silage samples covered the period of 1st September – 30th May of the harvest year, respectively. Results based on routine laboratory NIR analyses (Livestock Performance Testing Ltd, Hungary). Dry matter yield, crude nutrient content, fibre fractions (aNDFom, ADF, ADL), organic matter digestibility detected during 48 hours incubation time *in vitro* (OMd_{48}), amylase treated and ash corrected NDF rumen degradability with 48 hours incubation time *in vitro* ($aNDFomd_{48}$) were determined by Q-Interline Quant FT-NIR analyser. Degradable aNDFom ($daNDFom_{48}$) was calculated as multiplication of aNDFom and $aNDFomd_{48}$. Samples were dried at 70°C and ground according to the Guidelines of Samplinq® system (Eurofines Agro, Wageningen, The Netherlands). The EG guideline L54 2009/152 was applied for determination of moisture content (dry matter determination). Spectra were determined according to the guidelines of NEN-EN-ISO 12099 (Q-Interline Quant FT-NIR analyser, ISO 12099:2010 guidelines for the application of near infrared spectrometry).

Yield data derived from the national database (Research Institute of Agricultural Economics, 2014-2018).

RESULTS AND DISCUSSION

The forage yield range was found 22.5-31.1 ton fresh matter/ha for Hungary in the period of 2013-2018 (Table 1).

Table 1 The green and dry matter yield of maize, as whole crop in Hungary 2013-2018.

		2013	2014	2015	2016	2017	2018
Green yield	t AF/ha	22,5	31,1	24,3	30,6	26,7	30,7
DM yield	t DM/ha	7,4	11,1	8,6	11,0	9,8	11,7

Dry matter content varied between 328-381 g/kg (Table 2) and strong positive correlation was found (Table 3) between green yield and dry matter content (r= 0.72). During the extreme hot summer (2012: 21.5 mm precipitation in August, 46 heat stress days in the June-August period when $T_{max} \geq 30.0$ °C), the maize plant was harvested with lower dry matter content compared to the normal years (DM 328±58 g/kg, n=724). So the maize plant injured by the heatstress in milky stage may cause lower dry matter content than the optimal. Starch content ranged between 257-360 g/kg DM during 2012-2018. Correlation between starch concentration and dry matter content (r=0.69) was found strong and positive. It was found strong and positive correlation between the starch content and green yield (r=0.96), also. However, strong negative correlation was found between the $NDFd_{48}$ value (2012-2018: 45.2-50.3%) and starch content (r=-0.73). So, as the starch concentration is increasing, the NDF rumen degradability is decreasing with the maturation, while there is reduction in the aNDFom- (r=-0,99) and degradable $aNDFom_{48}$-content (r=-0,96). Declinig aNDFom- and $dNDFom_{48}$ content during the seed maturation is typical process for this type of forages. It was found negative and strong correlation between $NDFd_{48}$ value and green yield results (r=-0.62). Degradable NDF (daNDFom48) range was 180-242 g/kg DM and strong negative correlation was found with green yield (r=-0.88).

Table 2 Nutrient content (n= 3131), fiber degradability(aNDFomd$_{48}$) and organic matter digestibility OMd$_{48}$) of maize silage 2013-2018.

		Dry matter	Crue protein	Crude fiber	Total sugar	Total starch	NDF[1]	ADF	ADL	NDFd$_{48}$[2]	dNDF$_{48}$[3]	OMd$_{48}$[4]
		g/kg	g/kg DM	g/kg DM	g/kg DM	g/kg DM	g/kg DM	g/kg DM	g/kg DM	g/kg DM	g/kg DM	g/kg DM
2013.	Mean	328	75	216	22	257	444	250	18	54	242	73
n=724	St. dev.	58	11	28	15	72	55	31	3	4	43	2
2014.	Mean	357	73	168	17	360	356	198	17	50	180	75
n=526	St. dev.	52	8	22	6	55	42	25	2	4	31	2
2015.	Mean	352	75	195	21	299	411	229	18	53	220	74
n=617	St. dev.	56	10	28	11	72	56	32	3	4	44	2
2016.	Mean	359	70	172	18	357	367	206	18	49	180	75
n=441	St. dev.	49	8	23	7	51	42	26	4	4	31	2
2017.	Mean	367	74	184	20	319	393	217	18	53	210	75
n=453	St. dev.	52	9	24	7	60	45	27	3	4	34	2
2018.	Mean	381	69	185	19	337	388	215	19	54	209	76
n=370	St. dev.	53	8	23	6	57	43	26	3	4	35	2

[1]aNDFom - amylase treated ash corrected NDF
[2]aNDFomd$_{48}$ - degrdabilty of amylase treated ash corrected NDF, 48 hours *in vitro* incubation
[3]daNDFomd$_{48}$ - degradable aNDFom, 48 hours *in vitro* incubation
[4]organic matter digestibility *in vitro* 48 hours incubation

Table 3 The correlation coefficients between different parameters of maize silage in Hungary 2013-2018 (n=3131).

	Green yield	DM yield	DM	Starch	aNDFom	NDFd$_{48}$	dNDF$_{48}$
Green yield							
DM yield	0,98						
DM	0,72	0,84					
Starch	0,96	0,93	0,69				
aNDFom	-0,93	-0,89	-0,63	-0,99			
NDFd$_{48}$	-0,62	-0,48	-0,06	-0,73	0,76		
dNDF$_{48}$	-0,88	-0,82	-0,49	-0,96	0,98	0,88	

CONCLUSIONS

Weather extremes in 2012-2018 impacted corn silage yields for many dairies in Hungary. Variability of the maize yield and risk of feed bunk shortage have been proven between 2013-2018 in the Cenrtal European dry continental region. We can conclude that the degradable aNDFom$_{48}$ content of the maize silage was lower with approximately 40-50% as compared to the the whole-crop winter rye cut at boot stage (BBCH 49-51: 376-394 g kg$^{-1,}$ unpbulished), therefore the maize silage can be determined as not an optimal source of degradable aNDFom compared to the early-cut whole crop cereals. So, there are two imporant reasons of the growing interest in double cropping of winter cereals for harvest as high quality forage in the spring, just before the maize seed sowing into the same field. This technology strengthens the corn position in Europe.

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LABOUR INPUT FOR LOOSE BARN DRIED HAY PRODUCTION ON BAVARIAN DAIRY FARMS.

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INTRODUCTION

Improvements on barn hay drying technologies and better marketing possibilities for hay milk lead to increasing interest of dairy farmers in barn hay drying systems.

The barn hay drying can be performed in loose or baled form. The production of loose barn dried hay is the system of interest to be studied in this investigation. By loose barn dried hay production and using up-to-date technology, the hay is harvested with a loader wagon. The drying takes place in drying boxes which filling is performed by a hay crane. For loose barn hay drying, a dry matter content of 60 % is considered as ideal for the hay harvesting from field (Wirleitner et al., 2014). This enables to reduce the time of field hay drying and thereby the weather influence and field losses finally to produce hay of high quality. However, only rare information is available on the labour requirement/input for barn dried hay production with up-to-date barn hay drying technology. Due to many influence factors, labour requirement/input values for production of grass silage, field dried hay and barn dried hay vary widely in available publications (Eichhorn, 1999; Schick and Stark, 2002; Ammann, 2007, BW agrar online, n.d.). From the available publications, it can be supposed that a higher labour requirement can be expected for barn dried hay production in comparison to grass silage production in silos and even in comparison to the field dried hay production. The aim of the study was to investigate the labour input for loose barn dried hay production by recently used technology and to compare it with the labour input needed for grass silage production in silos on Bavarian dairy farms.

MATERIAL AND METHODS

Up to ten Bavarian dairy farms should have been included in the study for each conservation method. All farmers or other farm operators included in the studied processes should record their time needed for all tasks performed by production of loose barn dried hay or silage in silos (i.e. for tasks from mowing until final storage of forage). Work diaries were used to document the recorded labour input. As in common practice, the mowing and harvesting were performed in one batch (or with one day difference) by silage production and in several batches by barn dried hay production. The data recording was performed in year 2016 and 2017.

By the data evaluation, mean values per cut and farm were calculated at first. They were used to calculate mean values per cut and also mean values per farm over all cuts. Finally, mean values per farm over all cuts were included in statistical data evaluation. For statistical data evaluation, an unpaired t-test or a Mann Whitney rank sum test was used. The significance level was set at $\alpha<0.05$.

RESULTS AND DISCUSSION

Unfortunately, not all farms delivered data or data for all performed cuts. Therefore, the data could be analysed only from 5 farms producing barn dried hay and 8 farms producing grass silage (6 in bunker silos and 2 in tower silos (built underground), and the number of farms per individual cut differed.

The labour input for barn dried hay production (Figure 1) was slightly lower than observed in the study of Ammann and Wyss (2007) testing also recently used systems. Values published in the study of Schick and Stark (2002) were even higher than in the study of Amman and Wyss (2007). However, blowers (i.e. not up-to-date technology anymore) for the filling of boxes were tested in that study and not a hay crane. Thereby, using a hay crane seems to be more effective. The labour input for silage production was between the values observed in the study in BW agrar online (n.d.) and in the study of Eichhorn (1999).

The labour input for barn dried hay production was significantly ($P<0.05$) higher than for silage production (Figure 1). However, the higher labour input was not caused only by additional tasks performed by the drying process and after it (labour input for these tasks tended to be higher ($P=0.065$) than labour input for covering of silos) but also due to higher labour input for some field tasks (Figure 2). By tedding and turning, this was expected because the frequency of performing these tasks is higher for barn dried hay production than for silage production. If and to what extent the labour input for loading of forage in drying boxes or silage silos will differ was not clear. On the examined farms, the labour input for loading of forage in drying boxes was about twice higher than for the loading in silage bunkers/silos.

CONCLUSIONS

The labour needed for dried hay production using modern available technology seems to be lower than by using of previously applied systems. However, still the farmers producing barn dried hay have to reckon with higher labour requirement than by silage production in silos.

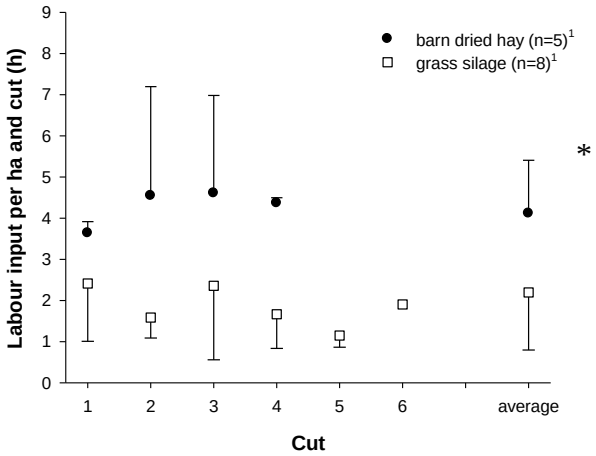

Figure 1. Mean labour input in h (± SD) according to the cut and on average for all cuts for barn dried hay and grass silage production on examined Bavarian dairy farms ([1]the number of farms per individual cut can differ). *Significant difference between conservation method at P<0.05.

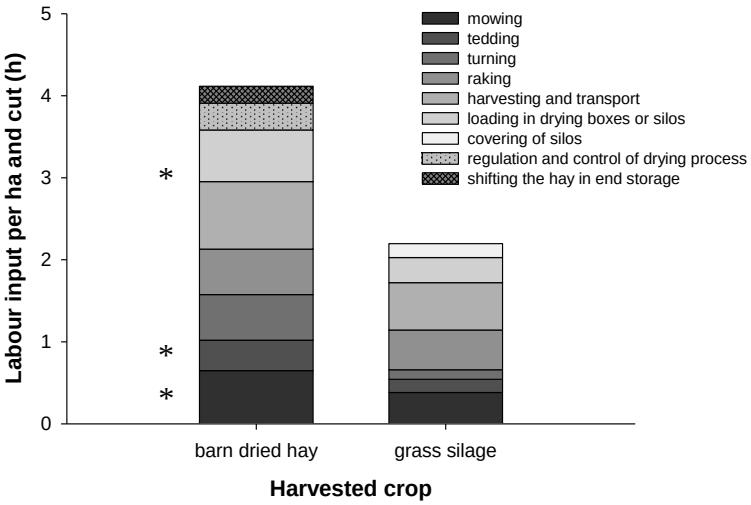

Figure 2. Mean labour input in h for tasks performed during the barn dried hay and silage production on examined Bavarian dairy farms. *Tasks with significant difference between conservation method at P<0.05.

ACKNOWLEDGEMENT

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ASSESSMENT OF THE PARAMETERS OF NUTRITIONAL VALUE AND NITROGEN RETENTION IN SILAGE OF THE SELECTED TYPE OF LEGUME-CEREAL INTERCROP HARVESTED IN TWO PHENOLOGICAL STAGES

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ABSTRACT

The silage was made from the selected legume-cereal intercrops (LCI-B: peas with barley, LCI-W: peas with wheat) harvested at two different phenological stages. Four produced silages were tested within balance experiments with heifers. The data obtained from the classical balance experiments were used for objective assessment of parameters of nutritional value in four tested silages. It was possible to characterize the trends in changes of nutritional values of selected feed in different maturity levels (phenophases) of LCI. Values for nitrogen intake retention were 18.01 ± 1.19 % and 21.88 ± 2.62 % in LCI-B1 and LCI-B2, respectively ($p<0.05$). The same significant increasing was found between LCI-W1 (13.73 ± 2.25 %) and LCI-W2 (19.64 ± 0.77). Values for nitrogen digested retention were 28.63 ± 2.33 % and 35.70 ± 4.51 % in LCI-B1 and LCI-B2, respectively ($p<0,05$). Also, for this parameter, the significant increasing was found between LCI-W1 (20.57 ± 2.99 %) and LCI-W2 (30.09 ± 1.73).

INTRODUCTION

The interest in utilizing legume-cereal intercrops (LCI) is increasing, especially because of their positive effect on soil, ability to increase and stabilize yields (Awal et al., 2006), to reduce weed growth (Jensen et.al., 2006), to promote the occurrence of useful insects predators (Hauggaard-Nielsen and Andersen, 2000) and improve the health of the stands. LCI for feed production is appreciated for the high content and quality of crude protein (CP) in dry matter. Also favorable is the content and qualitative composition of fiber and the ratio of nitrogenous substances to net energy. Not only the content of crude protein is important but also its qualitative parameters is essential for the ruminant nutrition. We can get a feed that could be appropriately incorporated in the feed ration of cattle to achieve better yield, reproduction and a positive health response in farmed animals. By using modern varieties, with the right choice of components and their appropriate representation in the seed rate and especially during harvesting, we can purposefully direct the ratio between CP and energy in the dry matter of the harvested material for feeding purposes. Objective assessment of nutrient concentrations, their ratios and dietary value of produced LCI feeds is necessary to exploit their potential. The nutritional value of CP in ruminant feed is characterized not only by the digestibility but also by the rate of degradation in the rumen. In the PDI system, deg (degradability of nitrogenous compounds) and dsi (intestinal digestion in the rumen of non-degraded nitrogenous compounds) are used, and are connected with the application of sophisticated methodologies or tabular values. For assessment the quality of feed nitrogen compounds can be used the fractionation-based *in vitro* chemistry method comprising the determination of CP solubility in buffer, neutral and acidic detergent solutions (Licitra et al., 1996; Chrenkova et al., 2014).

The aim of the work was to determine the nutritional value and nitrogen retention parameters in balance experiments in two variants of LCI, that were harvested each in two phenological stages. Four balance experiments were carried out, each with five heifers. The experimental design allowed to obtain the precise results of the studied parameters.

MATERIALS AND METHODS

Selected LCI variants (LCI-B and LCI-W) were grown in 2017 on two separate adjacent areas of one soil block. Individual variants were harvested (mowed, cut after pre-wilting and ensilaged in trial silage pits) in two growth phases (Meier 2001) of peas BBCH 75 (about 50% of pods reached species-specific or varietal-specific sizes) and BBCH 79 (pods reached typical size, green ripe and peas are fully formed). The time interval between the two harvests was 10 days.

Balance trials were organized as classical balances with heifers according to methodological recommendations Vencl (1985) and Pozdisek (1999). During a preparatory period, the animals were subjected to the test feeds. The feed intake and the animals' adaptation to the conditions in the experimental balance stable were monitored. The groups of animals for balances were balanced in terms of live weight and dry matter intake per unit of live weight. Feeds (LCI silages tested) were weighed for individual animals prior to feeding into containers (10 g accuracy). A sample of feed was taken daily. In the main period (7 days), feed intake was monitored and faeces (separately faeces and urine) collected from individual animals by a permanent service. After weighing the faeces from 24 hours period and homogenizing them, a portion of the sample was weighed and preserved with 5 ml of chloroform / kg of faeces. From the conserved amount, an aliquot sample was weighed in a specified amount of 2-5% of faeces weight per day (individually for each animal throughout the main period). A sample was collected for analytical determinations in the laboratory from the collected and mixed amounts of faeces from the balance period (from individual partial sampling). Relative urine samples were preserved with 20% HCl at 3.0 mL / 100 mL to decline the pH below 6 and stored in the cold. The next procedure was analogous to faeces. The obtained samples from the main period of the balancing experiments were analyzed by the laboratory of Agrovýzkum Rapotín, accredited by ČIA, o.p.s. under No. 1340 according to

the Czech norm: EN ISO/IEC 17025, using appropriate procedures. A total of four balances were carried out, each with five animals. In the main period from 17th March to 23rd March 2018 with silages LCI-B and LCI-W, which were harvested in the first term and from 07th April to 13th April 2018 with the same LCIs that were harvested in the second term. Data were analyzed by ANOVA with Levene's test for homoscedasticity and Tukey HSD post hoc test for multiple comparisons.

RESULTS AND DISCUSSION

In 2017, the climatic conditions at the time of vegetation, in particular the distribution and amount of precipitation, and subsequent changes in the proportion of components in cultivated LCIs did not manifest as in the previous years 2015, 2016, which Pozdisek and Hunady (2017) pointed out. The quality of the harvested LCI for silage for the balance experiments in 2018 could be considered as standard. The analytical results of the silage, after correcting for fermentation products (fatty acids and alcohol) are shown in Table 1.

Table 1. Mean values of chemical composition of LCI silage.

Harvest		LCI-Barley		LCI-Wheat	
		BBCH 75	BBCH 79	BBCH 75	BBCH 79
DM	*g/kg*	378.0	544.7	329.3	494.4
Crude protein	*g/kg DM*	145.1	135.6	144.6	144.8
Fat	*g/kg DM*	25.0	21.4	26.5	20.4
Crude fiber	*g/kg DM*	217.0	231.0	235.4	238.9
NFE	*g/kg DM*	528.4	530.5	531.0	511.6
Ash	*g/kg DM*	84.2	81.5	62.5	84.3
Organic matter	*g/kg DM*	915.8	918.5	937.5	915.7
NDF	*g/kg DM*	421.9	520.2	455.1	487.5
ADF	*g/kg DM*	262.3	288.3	258.0	267.3

NFE – nitrogen-free extract, NDF – neutral detergent fiber, ADF – acido detergent fiber

When comparing the crude protein content in the dry matter of LCI-B silage, a decrease in the crude protein concentration between the first and the second harvest period can be seen. In contrast, in LCI-W, the nitrogen concentration remains at the same level. The fiber, NDF and ADF content can be seen to increase their dry matter content for all silages tested, but with more pronounced differences in barley silages.

Table 2. Coefficients of digestibility for LCI silage [%].

Harvest	LCI-Barley		LCI-Wheat	
	BBCH 75	BBCH 79	BBCH 75	BBCH 79
Crude protein	63.0	61.4	66.6	65.3
Fat	59.4	57.5	65.5	58.7
Crude fiber	48.9	48.4	47.9	50.1
NFE	76.6	72.5	76.9	75.3
Ash	25.6	22.1	35.4	17.9
Organic matter	67.4	64.5	67.7	66.8
NDF	53.1	56.7	55.0	57.5
ADF	46.6	48.7	38.7	44.7

Table 2 shows mean values of balance digestibility in performed balance experiments (each on five animals) in four tested silages. In the case of nitrogenous substances, there was a slight decrease in balance digestibility in the later harvesting period for both types of LCI. By comparing the digestibility between LCI-B and LCI-W in the second harvest term, the difference is more pronounced (LCI-B 61.38% and LCI-W 65.32%), a difference of 3.94% greater in LCI-W. This fact is related to the increasing proportion of crude protein in wheat grain. In the digestibility of organic matter, as an important parameter for feed energy prediction, comparable silage values were recorded in the first term of harvest. On the other hand, in the second harvest term, the difference in organic matter digestibility was 2.29% - higher for LCI-W. This fact is related to the increase in NDF concentration in the second harvesting period for LCI-B.

In order to assess LCI silages, as a bulk feed and its inclusion in bovine feed rations, in particular dairy cows, it could be stated that the necessary energy concentrations (NEL) and nutrients are achieved while ensuring satisfactory cultivation and conservation conditions. Table 3 shows, in an analogous arrangement to the

table with the values of balance digestibility, the found nutrient values and energy contents in the feed evaluation system NEL - PDI.

In the Table 3, there are also reported the values of nitrogen retention in % nitrogen retention from the intake and digested nitrogen of the tested two variants of LCI silages (LCI-B and LCI-W), which were harvested each in two terms, according to the pea development in the stands.

Table 3. Means and standard deviations for nutrient value and nitrogen retention.

		LCI-Barley		LCI-Wheat	
Harvest		BBCH 75	BBCH 79	BBCH 75	BBCH 79
ME	Mean	9.41[a]	9.02[b]	9.68[a]	9.33[ab]
MJ/kg DM	S.d.	0.10	0.25	0.17	0.23
NEL	Mean	5.51[a]	5.24[b]	5.68[a]	5.45[ab]
MJ/kg DM	S.d.	0.07	0.18	0.12	0.16
NEF	Mean	5.30[a]	4.96[b]	5.47[a]	5.23[ab]
MJ/kg DM	S.d.	0.09	0.22	0.15	0.20
Ret.N int.	Mean	18.01[a]	21.88[c]	13.73[b]	19.64[ac]
%	S.d.	1.19	2.62	2.25	0.77
Ret.N dig.	Mean	28.63[a]	35.70[c]	20.57[b]	30.09[a]
%	S.d.	2.33	4.51	2.99	1.73
PDIA	Mean	31.35	27.97	29.21	28.90
g/kg DM	S.d.	-	-	-	-
PDIN	Mean	88.13	80.49	86.62	85.47
g/kg DM	S.d.	-	-	-	-
PDIE	Mean	75.28[ab]	73.30[b]	73.46[b]	75.82[a]
g/kg DM	S.d.	0.39	1.55	1.01	1.40
PDIN:PDIE	Mean	1.17[a]	1.10[b]	1.18[a]	1.13[b]
-	S.d.	0.01	0.02	0.02	0.02

[a, b, c]within a row, means without a common superscript differ ($p < 0.05$)

The performance of balance tests, as described in the methodology, enabled not only to detect the total urine that was eliminated in 24 hours, but also to take samples that were not contaminated. Under these assumptions, it was possible to determine the level of nitrogen retention which, in the form of percentages of nitrogen ingested and digested, is shown for each from the balances in Table 3. When comparing LCI types, values for LCI-barley are more favorable, in both phenophases. For these parameters, harvesting in the BBCH 79 stage is preferred. Differences between mean values with 0.95 confidence intervals for both nitrogen retentions, harvest and LCI are presented in Fig. 1 and 2.

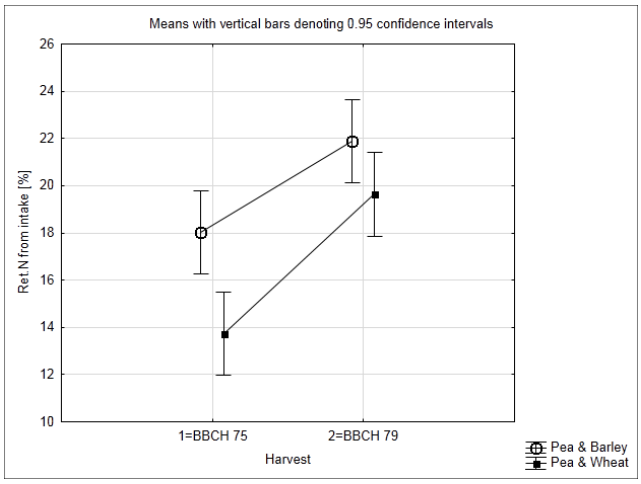

Figure 1. Nitrogen intake (ingested) retention [%] at phenophases BBCH 75, LCI 1 and BBCH 79, LCI 2.

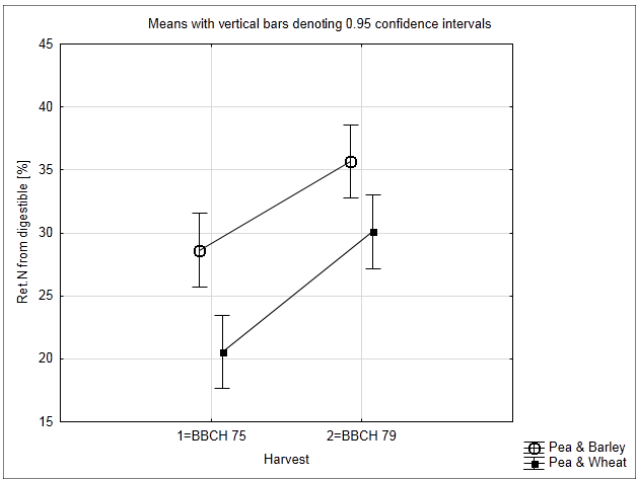

Figure 2. Nitrogen digested retention [%] at phenophases BBCH 75, LCI 1 and BBCH 79, LCI 2.

CONCLUSIONS

Legume-cereal intercropping and the preserving fodder (silage) made from them have a potential to increase the feed value of the basic ration in ruminant nutrition. Nutrient concentrations and their ratios vary according to the date of harvest in relation to the development phase of the components and their presence in the vegetation. The nutritional value can be significantly influenced by the year, especially the amount and distribution of precipitation during the growing season. The findings confirm the possible benefits of using legumes, which can also replace some of the more expensive protein feeds for cattle nutrition.

ACKNOWLEDGEMENT

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EFFECT OF ENSILING AND TOASTING OF EARLY HARVESTED FIELD PEA GRAINS ON FORMATION OF MAILLARD POLYMERS FROM LYSINE AND ARGININE

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Keywords: legume grains, ensiling, heat treatment, Maillard polymers

INTRODUCTION

The cultivation of grain legumes such as field peas has increased in recent years in Germany. In addition to the cultivation benefits, state subsidy programs have contributed to this trend. High protein solubility primarily limits the possible use of unprocessed peas in ruminant diets. The combination of ensiling and hydro-thermic treatment (toasting) might be a promising method for on-farm processing of peas, with limited protein solubility during ensiling due to the high dry matter content of the grains but elevated contents of rumen-protected protein available for digestion and absorption in the small intestine. This, however, might work only if heat-induced protein damage can be avoided. Heat damage is inter alia characterized by the formation of complex Maillard products. The aim of the study was to investigate the impact of ensiling and subsequent toasting of field pea grains on the content of Maillard products.

MATERIAL AND METHODS

Peas of the cultivar *Alvesta* were grown conventionally and harvested with a dry matter (DM) content of 749 g/kg. Peas were squashed with a grinder bagger (Murska 2000 S2x2) and ensiled with lactic acid bacteria (*L. plantarum* LMG 18053) in a silage bag (⌀ 1,65 m). The ensiled grains were toasted with a soybeantoaster (ECOToast; agrel GmbH) at temperatures set between 100 and 200 °C, as well as different throughput rates (50–100 kg/hour). This resulted in grain temperatures of 60–110 °C. Analyses of DM, proximate nutrients, and protein fractions were carried out according to VDLUFA methods (VDLUFA 2012) and Licitra et al. (1966), respectively. Protein solubility was calculated as non-protein N (fraction A) + true protein, which is soluble in borate-phosphate buffer (fraction B1). Lysine-associated carboxymethyllysine (CML) and pyrraline, and arginine-associated methylglyoxal hydroimidazolone (MG-H1) were analyzed by HPLC-ESI-MS. Fructoselysine was calculated from furosine. Furosine, lysine and arginine were determined after acid hydrolysis and ninhydrin post-column derivatization. Aerobic silage stability was analyzed using the method of Honig (1990), organic acids and alcohols by HPLC-RID. Statistical analysis was performed using a model with fixed treatment effect at a $P < 0.05$ significance level.

RESULTS AND DISCUSSION

Peas had 190 g crude protein (CP)/kg DM. Pea silage had 2.3 g lactic acid, 0.3 g ethanoic acid, and 9.4 g ethanol/kg DM, and was aerobically stable for at least 7 days. Despite best practices, pH was not reduced below 6.1. Protein solubility decreased from 73 to 33 % of CP after ensiling and to 11 % of CP after toasting with temperatures of 100 °C and above ($P < 0.001$; Figure 1). Acid detergent insoluble protein (fraction C) started with 0.7 % of CP in the native grain and was rather unaffected until a grain temperature of 90 °C due to toasting, but increased then rapidly 18.8 % of CP with a grain temperature of 110 °C ($P < 0.001$; Figure 1).

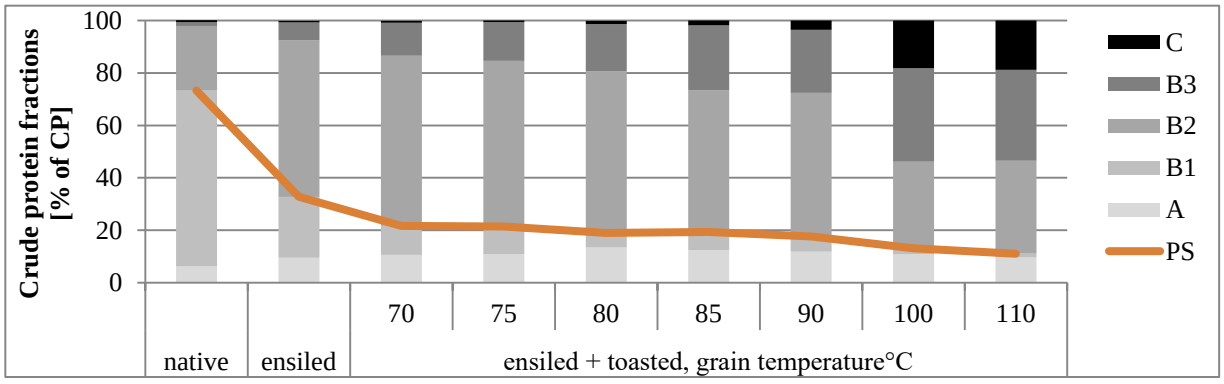

Figure 1: Development of crude protein fractions after ensiling and toasting at different temperatures in early harvested peas; PS, protein solubility.

Fructoselysine increased from 0.141 to 3.65 g/kg DM after ensiling, to 8.19 g/kg DM after toasting at 70°C grain temperature, and decreased to 1.94 g/kg DM with rising temperature ($P < 0.001$; Figure 2). CML, pyrraline, and MG-H1 increased after ensiling from 0.8, 0.4, and 1.2 to 21.8, 2.8, and 3.9 mg/kg DM, respectively ($P < 0.05$ in CML; $P > 0.05$ in pyrraline and MG-H1; Figure 2). Toasting increased CML at 80 °C (59.9 mg/kg DM; $P < 0.001$), and decreased it to 33.8 mg/kg DM ($P < 0.01$). Pyrraline and MG-H1 increased to maximal 648.5 and 91.9 mg/kg DM, respectively ($P < 0.001$). Lysine decreased from 11.6 to 9.6 g/kg DM after ensiling and 4.7 g/kg DM after toasting ($P < 0.01$). Arginine was not affected by ensiling, but decreased during toasting from 7.6 to 4.3 g/kg DM ($P < 0.001$; Figure 2).

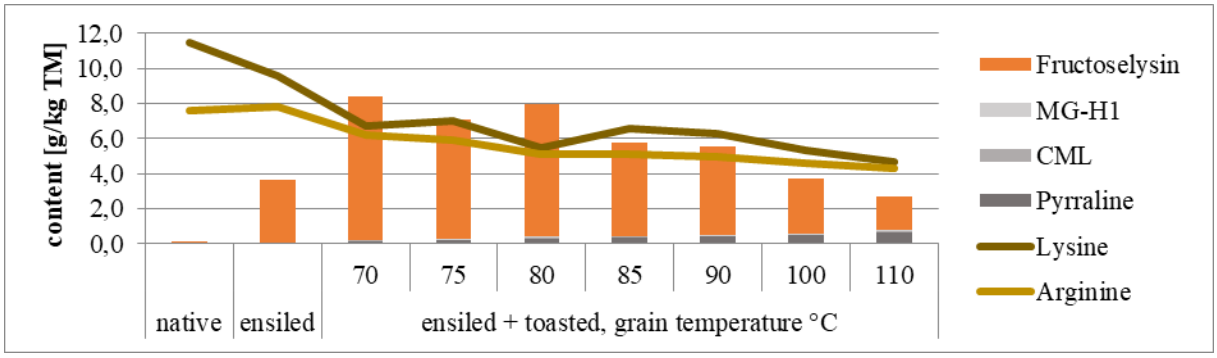

Figure 2: Development of Maillard polymers after ensiling and toasting at different temperatures in early harvested peas.

CONCLUSIONS

The results indicate an increase in ruminally insoluble protein, when ensiled and thermally processed pea grains are used in ruminant diets. With increasing temperature, the content of Maillard products increase, which may reduce the levels of lysine and arginine. The development of protein fraction C on the one hand and diverse Maillard products on the other are not completely going in parallel indicating a highly complex character of heat damage, which needs to be investigated further particularly with respect to its physiological meaning. For the time being we can state that the grain temperature during heat treatment should not exceed 100 °C to avoid protein damage.

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INFLUENCE OF TMR COMPOSITION AND QUALITY ON RUMEN DIGESTION AND DAIRY COW PERFORMANCE

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INTRODUCTION

The beneficial influence of yeasts on the production efficiency of animals can be explained also by the fact that after their application, the animals show increased fodder intake and improved digestibility of nutrients (Williams et al., 1991; Erasmus et al., 1992; Mutsvanga et al., 1992). Regarding the fact that yeasts in the rumen accelerate the decomposition of solids contained in the fodder during the first 6-8 hours after feeding, the animals can take in their digestive tract a larger amount of dry matter and the rate of chyme removal from the rumen may increase. The stimulation of the growth of bacteria and their activities causes a more intensive decomposition of cell walls in higher plants, which finally results in the generally increased fodder intake. Together with the improved digestibility, which is usually observed under these circumstances, the animals receive more nutrients and their production efficiency is increasing. Moreover, yeast has a positive influence on the prevention of acidoses and may stimulate the immunological system of the animal organism (Maekawa et al.,2002). All these factors together serve to explain the positive effects of yeast on ruminants. Many works (Sune, 1998; Alshaikh et al., 2002 and others) document the favourable effects of yeast cultures not only on the rumen environment of dairy cows itself but also on the improved microbial activity because they facilitate digestion of fibre, reduce lactate accumulation, suppress oxygen concentration in the rumen fluid and enhance utilisation of starch in the ration. Thus they effect (slow down) the production of volatile fatty acids (VFA), which results in an improved stability of rumen environment and extent of digestion. Doreau and Jouany (1998) recorded that yeast reduce the daily fluctuation of pH value and mitigate individual differences between various individuals. In one of our earlier studies (Doležal et al., 2005) we observed a similar situation. We found out that with the increasing concentration of yeast in the diet of dairy cows, the rumen fluid shows not only an increasing total content of VFA, an increasing percentage share of propionic and acetic acids, a decreasing amount of ammonia but also an increasing total count of bacteria and infusorians in the rumen. Similar conclusions were reported by Nursoy and Baytok (2003), Strohlein (2003), Enjalbert et al. (1999), Kamra et al. (2002), Sune (1998) and others.

MATERIALS AND METHODS

The experiment included 12 dairy cows of Holstein breed, which were divided by ten into 2 equal groups with respect to productivity, order of lactation and live weight. The experimental period lasted 120 days. The cows were kept at loose and fed 3-times daily the same ration (TMR) with the experimental group of animals receiving in the production feeding ration mixture supplemented by yeast culture Biosaf® (*Saccharomyces cerevisiae* – SC 47). The additive at feed ration of additive on level 5 g per animal and day. The control group of cows did not receive any yeast supplementation. Animals received a diet TMR based of good maize silage with a higher dry matter content (14 kg), 14 kg of lucerne-grass haylage, 5 kg of crushed ears of maize, 5 kg of beet pulp silage, 3 kg of crimped wheat, 2 kg of meadow hay, and 7.0 kg feed mixture. The yeast culture (5 g/day) was added to the mixture. Residual fodder was removed after each feeding. The cows were milked in a milking shed 2-times daily. Samples of rumen fluids were taken from the cows of both groups by using oesophageal probe by a method described by Hofírek and Dvořák (2002) within 3 hours after feeding in the fourth month of the experiment as a response to feeding diet. Rumen fluid was analyzed for the total content of volatile fatty acids (VFA), relative % share of acetic, propionic and butyric acids, pH value, abundance of infusorians and ammonia content. VFAs were measured by the method of gas chromatography and the ammonia content was ascertained by the AOAC method (1980). The total content of infusorians was established according to a method described by Hofírek and Dvořák (2002). The values were compared with reference values according to Vrzgula et al. (1990).

RESULTS AND DISCUSSION

Results of the effect of yeast culture supplementation on the biochemical indicators of rumen fermentation in dairy cows are presented in Tab. 1 and Figure 1 and they are compared with those recorded in cows of the control group and with the reference value. The achieved results show that the pH value of experimental cows is within the reference range and after 30 and 60 days it is lower (6.27±0.3 and 6.3±0.01 resp.) than that recorded in the control group after 30 (6.48±0.02) resp. after 60 days (6.4±0.06). In this experimental survey, we did not succeed to prove the conclusions of other authors (Kamra et al., 2002) about the increased and stabilized rumen fluid pH through the supplementation of yeast. On the opposite, our results rather correspond to findings reported by Kung et al. (1997), Putnam et al. (1997), Garg et al. (2000), who similarly did not prove any profound stabilizing effect on pH and other products of fermentation. The achieved results of yeast culture effect on VFA production showed that the yeast culture supplementation led to increased ($P<0.05$) VFA production (124.98±26.49 mmol/litre after 30 days resp. 149.14±36.64 mmol/litre after 60 days) as compared with cows in the control group (114.36±25.44 mmol/litre after 30 days resp. 145.46±32.47 mmol/litre after 60

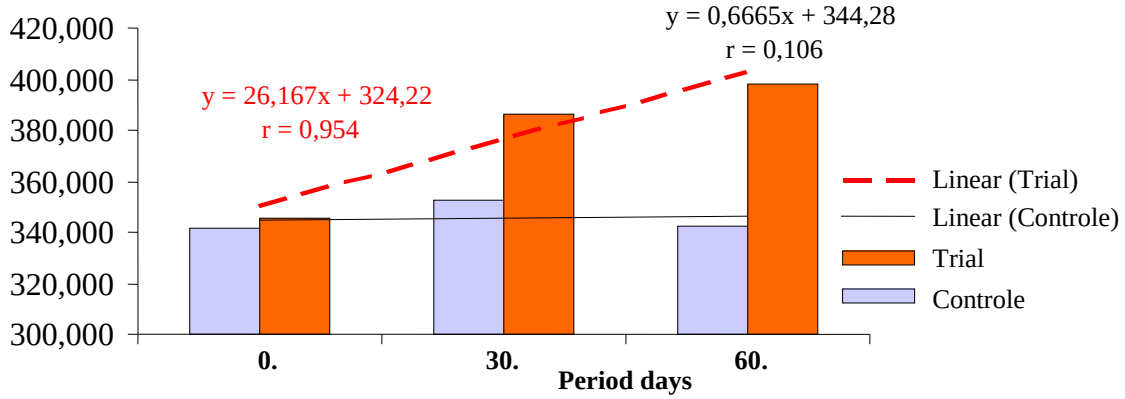
days). A significant increase was detected in both sampling terms. The achieved results agree with the findings of other authors (Pestevsek et al. 1998; Brydl et al., 1995; Kamra et al., 2002 and others).

Table 1: Average biochemical parameters of rumen fluid of cows experimental and control groups

Group	Day of collection	pH	VFA (mmol/L)		NH_3 (mmol/L)		Infusoria (thous/mL)	
Control	0.	6.49±0.04	113±25.38	/	8.95±0.03	/	341±6.53	/
	30.	6.47±0.02	114±25.44	a	8.46±0.32	a	353±6.97	A
	60.	6.4±0.06	146±32.47	b	8.15±0.16	b	343±4.77	B
Experimental	0.	6.62±0.03	114±24.84	/	8.05±0.18	/	346±9.90	/
	30.	6.26±0.03	125±26.49	c	7.92±0.07	c	386±3.91	C
	60.	6.29±0.01	149±36.64	d	7.69±0.13	d	398±6.51	D
Reference value		6.2-6.8	80-120	/	6.00-16.00	/	300-500	/

a, b, c, d – (P< 0.05); A, B, C, D – (P< 0.01)

Figure 1: The effect of yeast culture on the ruminal infusoria content



The milk of cows in the experimental group also showed a higher milk fat content in individual months (3.6±0.71%, resp. 3.45±0.51%, resp. 3.76±0.19%) as compared with the milk of cows in the control group (2.93±0.5%, resp. 3.13±0.59%, resp. 3.34±0.4%). The results indicate that the addition of yeast culture had a positive effect on the milk production efficiency of cows in the experimental group. The effect was demonstrated on the increase of milk fat and protein contents in cows of the experimental group. All differences were statistically significant (P<0.05) and in favour of the experimental group. Similarly, Zhang-ying Lai et al. (2000) reported the increased milk production efficiency, production of milk, protein, lactose and dry matter in cows receiving the Saccharomyces cerevisiae supplement in the diet by 7.1% (2.01 kg), 20.2% (175.6 g), 8.6% (68.4 g), 7.9% (104.3 g) and 11% (361.4 g) as compared with the control groups. Henderson (2004) and Wohlt et al. (1991) confirmed, too, the positive effect of yeast cultures on daily milk production, which increased by up to 2 litres.

CONCLUSION

The aim of this work was to review the influence of the addition of yeast culture *Saccharomyces cerevisiae* (SC-47) in the total mixed ration with higher starch content on rumen fermentation and milk production in the puerperal period dairy cattle. The yeast culture significantly (P < 0.05) influenced the production of VFA in rumen as compared with the control group. The addition of yeast culture decreased the content of ammonia in comparison with the control group. The cows of the experimental group were diagnosed the higher counts of infusorians for all donations as compared with the cows in the control group. The significant difference was high (P < 0.01). Compared with the control group, the cows of experimental group had higher average daily yield (38±4.31 kg) and FCM production (38±3.33 kg).

ACKNOWLEDGEMENT

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REFERENCES BY AUTHORS

DRY MATTER CONTENT AS AN INDICATOR OF THE NUTRITIONAL VALUE OF XERIC AND THERMOPHILIC HERB-RICH GRASSLANDS.

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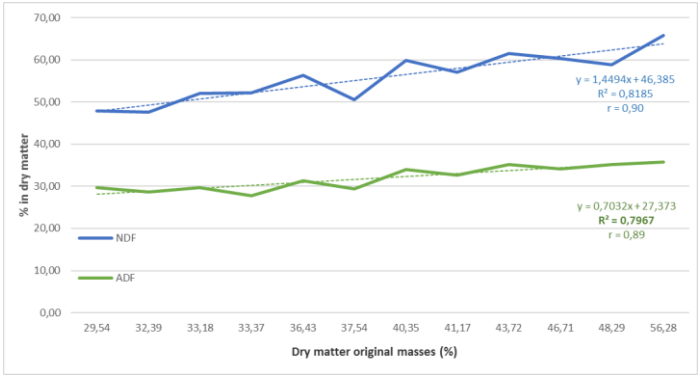
INTRODUCTION

Paying more attention to xeric and thermophilic vegetation is related to climate changes. Increasingly, these changes result, besides other things, in cyclical water shortages, to which the xeric and thermophilic communities have adapted. Another reason why study these communities is the ever-enlarging areas of natural habitats where it is necessary to preserve or improve biodiversity. Agricultural production is not a priority in these areas, however, as a way of maintaining them, they are more and more frequently used to graze ruminants. This implies that it is also necessary to determine the nutritional value of such vegetation. One of its distinctive features is a significantly higher dry matter content in all phenological phases, as compared with vegetation in production agriculture systems (VESELÝ and MICHELE, 2017). This higher dry matter content plays the role of an integrating factor between the developmental phases of the vegetation and, to some extent, even in species composition. Based on the above facts, we focused on the possibility to evaluate potential prediction of the nutritional value of the vegetation, the prediction being based on the dry matter content in its original matter.

MATERIALS AND METHODS

In order to evaluate the potential prediction of the nutritional value of xeric and thermophilic vegetation, on June 2nd and October 11th in 2015 in Mohelno serpentinite steppe nature reserve, samples were taken from six sites, each 2x2 metres in size. The samples were dried and the contents of dry matter, crude fibre, crude protein, crude fat and crude ash were laboratory-determined (ANONYM, 2001, 2009), NDF and ADF (ANONYM, 2011, 2012). Nitrogen-free extracts were calculated by difference. GE, ME, NEL, NEV, PDIN and PDIE was calculated using the regression equations (VESELÝ and ZEMAN, 1995, 1997). Statistical evaluation of the data was done using the statistical software provided as part of the program Microsoft Excel 2000.

Graph l NDF and ADF in the dry matter of the steppe vegetation.



RESULTS AND DISCUSSION

The year 2015 was chosen because there was low total annual precipitation (406,1 mm) and high average annual temperature (7,2 °C). Unlike VESELÝ and MICHELE (2017) study dealing with one site within a longer period of time, six Mohelno steppe plateau sites displaying average nutritional values were subject to evaluation. The crude fibre content, NEL, NEV, PDIN and PDIE fell within the limits for very young to older pastureland as cited by ZEMAN et al. (1995). The dry matter content, however, differed significantly. Unlike the 13.5 to 21% range as cited by Zeman et al. (1995), the values for dry matter in steppe vegetation ranged between 29.54% and 56.28%. Even within this range, a green hue was preserved in the steppe vegetation and the vegetation did not demonstrate any major signs of drying up. As evidenced in the graphs below, the changing dry matter content in the vegetation correlated noticeably with its nutritional value. The increasing dry matter content was reflected in the increasing NDF and ADF content (r = 0,90 and r = 0,89) (graph 1), while by contrast there was some decrease in both NEL and NEV (r = - 0,86 and r = - 0,86) (graph 2) and PDIN and PDIE (r = - 0,86 and r = - 0,88) (graph 3). The data obtained show clearly that in 2015 it was possible to use dry matter content to predict the nutritional value of vegetation.

CONCLUSIONS

The use of dry matter content as an indicator of the nutritional value of vegetation is particularly helpful due to the simplicity and low cost of its determination. Prediction of the nutritional value dynamics in these natural habitats is important, because in order to provide protection, it is often required to graze these habitats at later growing phases. Then the prediction is necessary not only to optimise the nutrition of animals grazed there,

but also to calculate the financial compensation sought by landowners from nature protection authorities. The prediction might also be useful for production areas which in low precipitation years might approximate the development and subsequent nutritional characterization of xeric and thermophilic herb-rich grasslands.

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DEDIKACE

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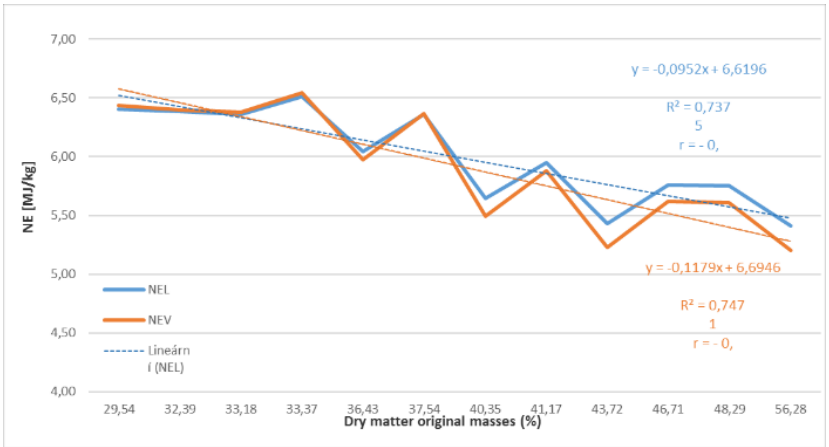
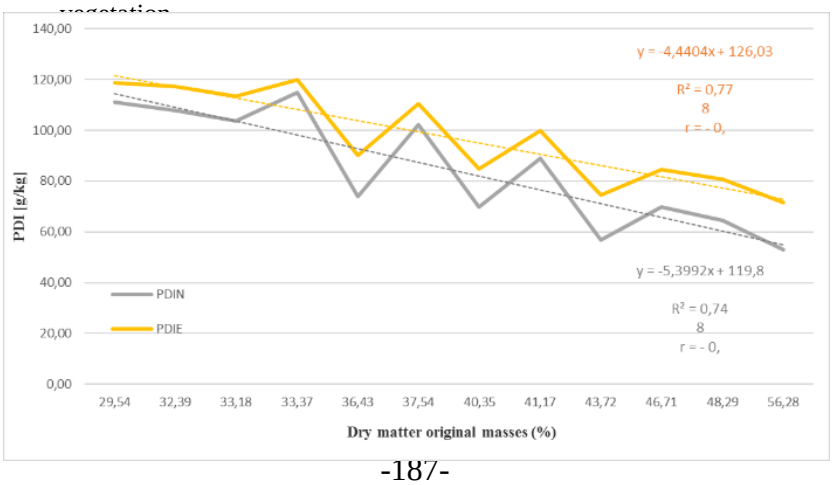
Graph 2 NEL and NEV in the dry matter of the steppe vegetation.



Graph 3 PDIN and PDIE in the dry matter of the steppe vegetation



Section 4: Precision farming – feeding (NIR technology)

HISTORY AND CURRENT USE OF NEAR-INFRARED SPECTROSCOPY (NIRS) FOR THE ANALYSIS OF FORAGE AND PRESERVED FEED AT GRASLAND RESEARCH STATION JEVÍČKO IN THE CZECH REPUBLIC

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INTRODUCTION

One of the challenges of the twenty-first century is to achieve a more productive agriculture, while improving the safety and quality of food (García-Sánchez et al. 2017) and feed (Molano et al. 2016; Hopkins et al. 2019). Agricultural practice in past and present years places high demands on the quality and quality of agricultural crops, raw materials, as well as voluminous feedstuff (Míka and Paul 1989; Givens et al. 1997; Míka 1997; Berzaghi and Riovanto 2009; Deepa et al. 2016; Nerušil et al. 2018, and others). However, these demanding feedstuff quality requirements are difficult to meet only through classical analytical (laboratory) methods (e.g. Tilley and Terry 1963; Orskov and McDonald 1979; Mertens 2002; ISO 16472 2012, and others), which are accurate and sufficiently reproducible, but at the same time very often demanding on staff working in a specialized laboratory, further on time (relatively long time to determine the result), but also on financial means (Kong et al. 2005; Čižmár 2006; Wittkop et al. 2012; Mudřík et al. 2013; Berzaghi and Marchesini 2014; Nerušil et al. 2016; Yang et al. 2017).

The first non-visible region in the absorption spectrum, i.e. near-infrared (NIR), was discovered in 1800 by Frederick William Herschel (Herschel 1800a, 1800b; Allen 1975; Pasquini 2003). The NIR region was, however, not considered significant until 150 years later. During this time, analytical techniques that could provide more unambiguous results were favoured over NIR spectroscopy, especially in terms of the explanation of molecular structures /e.g. mid-infrared spectroscopy / (Manley 2014). The physical, non-destructive technique of Near-Infrared Spectroscopy (NIRS) has been described as 'the most practicable and exciting analytical technique to hit the agricultural and food industries since Johann Kjeldahl introduced the Kjeldahl test' (Williams and Norris 1987; Norris 1992; Deaville and Flinn 2000; Davies 2011). Since its introduction in the 1960s for measuring moisture in grains (Norris and Hart 1965; Surprenant and Michaud 2010), the number of analytical applications of NIR in quality control /analytical technique for the rapid prediction of chemical compositions for screening different cereal crop species and forage grasses/ has expanded dramatically (Dara et al. 1989; Rabotnikof et al. 1995; Deaville and Flinn 2000; Boval et al. 2004; Park et al. 2004; Kong et al. 2005; Decruyenaere et al. 2009; Berzaghi et al. 2010; Andueza et al. 2011; Baloyi et al. 2013; Tellado and Azorit 2015; Keim et al. 2016; Hetta et al. 2017; Parrini et al. 2018, Bell et al. 2018 and others). In addition, NIRS does not need solvents or reagent, avoids environment pollution and is regarded as an eco-friendly method, which is in accordance with the principles of green chemistry (Cayuela and García 2017). Some feed companies and analytical labs in the world, however, have switched from expensive and time-consuming wet chemistry analysis to NIRS machines to evaluate nutrient content, quality of forage etc. (Harris et al. 2018). NIRS is widely utilized for the evaluation of forage quality, including the content of nitrogen, moisture, fibre, structural carbohydrates, amino acids and minerals (Cozzolino et al. 2001; Fontaine et al. 2001; Cozzolino and Moron 2004; Lundberg et al. 2004; Andrés et al. 2005; Čižmár 2006; Font et al. 2006; Campo et al. 2013; Dreccer et al. 2014; Meng et al. 2015; Lundberg et al. 2016; Yang et al. 2017; Hopkins et al. 2019). At present, in modern agricultural practice (Smart Farming, Precision Farming etc.) mobile NIRS machines (portable or directly mounted on harvesting machines) are used to evaluate the quality of forage, resp. feed, e.g. AgriNIR™ /*Dinamica Generale, St. Charles, Italia*/ (Loucka et al. 2014; Old et al. 2016; Weld and Armentano 2018); John Deere HarvestLab ™ 3000 /*Deere & Company, One John Deere Place, Moline IL, USA*/ (Fikejs et al. 2017; Fikejs 2019); CORONA PLUS 45 /*Carl Zeiss, Inc., Thornwood, NY, USA*/ (Montes et al. 2009; Cernoch and Groenbaek 2009; Lohr et al. 2017); SPECNIR /*ITPhotonics, Fara Vicentino, Italia*/ (Marchesini et al. 2017); AURORA NIR /*GraiNit S.r.l., Padua, Italy*/ (Cherney and Cherney 2019) and others. Recent studies deal with the evaluation of forage quality (elements content) using X-ray fluorescence - spectroscopic method of analytical chemistry belonging to electromagnetic spectroscopy methods (Berzaghi et al. 2018).

Infrared spectroscopy is an analytical method designed primarily for the identification and structural characterization of organic compounds and also as a technique useable for the determination of inorganic substances (Norris and Hart 1965; Williams and Norris 1987; Míka and Paul 1989; Burns and Ciurczak 2008). The principle of the method is the absorption or reflection of various wavelengths of incident radiation, which depends on the chemical composition of the analysed sample. NIRS is an analytical technique that uses a source of emitting radiation of known wavelength (usually 800–2500 nm, ie 12500–4000 cm^{-1}). For practical reasons, the far infrared (FIR) range is distinguished by wavefronts, delimiting a wavelength of up to 200 cm^{-1}, midle infrared (MIR) corresponding to 4000-200 cm^{-1} and near infrared (NIR) corresponds to the wavebands range 12821–4000 cm^{-1} (Muselík 2012). It contains most of the vibrations of the -CH, -OH, -SH, and -NH bonds

(Deaville and Baker 1993; Givens et al. 1997). All absorption bands are the result of overtones or a combination of transitions to the basic MIR bands (Siesler et al. 2002). All organic materials absorb electro-magnetic waves at selective wavelengths from 700–2500 nm (infra-red) depending on their molecular building blocks, as energy is absorbed at specific wavelengths at different intensity (Baker and Barnes 1990). The change of slope with respect to wavelength directly reflects the physical composition of the feed (Deaville and Baker 1993). Thus each individual nutrient, defined by its functional chemical group and chemical bonds (-CH, -NH, -OH, -SH), has a specific peak or slope /= qualitative-chemical nature/ along the spectrum at which light will be reflected, at a specific intensity /= quantitative-physical nature/ (Deaville and Baker 1993; Harris et al. 2018). However, each sample has its own inherent factors which affect distribution of individual constituents along the spectra and therefore, NIRS data has to be initially calibrated with traditional methods (De Boever et al. 1993).

The aim of the paper is to present the history and current use of NIRS for analysis of fodder and preserved feed at the Grassland Research Station (GRS) Jevíčko in the Czech Republic.

MATERIAL AND METHODS

NIRS instrumentation - dispersive spectrometer FOSS NIRSystems 6500 instrument (*Company NIRSystems, Inc., Silver Spring, USA*) was acquired at GRS Jevíčko in 1995 with financial support from the projects of the Ministry of Agriculture of the Czech Republic /Project MZe, no. RE 5540 "Study of potential uses of NIRS method for evaluation of chemical composition and nutritional quality of plant biomass"/ and Czech Science Foundation /Project no. 503/95/1457 "Development of expeditive methods of grass biomass quality evaluation"/, the researcher was Dr. Václav Míka. /*Under the leadership of V. Míka, an expert and specialist in the field of forage production, grass breeding and evaluation of feed quality, there was a unique development of the use of NIR technology in Czech agricultural research at the GRS Jevíčko workplace, especially in the field of quality evaluation of plant products and feed* (Míka 1997; Míka et al. 2003; Míka et al. 2008)/. Prior to the purchase of the spectrometer, for the evaluation of grass biomass quality in the early 1990s, an instrument of the same brand was used at the Research Institute of Meadows and Grasslands in Banská Bystrica, where the GRS Jevíčko had organizationally belonged until the Czech and Slovak Federal Republic divided /NIRS method was introduced here by Prof. Vladimir Krajcovic/

Samples are usually measured using small ring cups in two parallel replicates. Sample scanning is set in reflectance mode for the 400–2500 nm range, ie in the visible (UV-VIS) and near infrared (NIR) regions of the spectrum, with a scanning step of 2 nm. WinISI II software (*Infrasoft International, Inc., USA*), version 1.50, is used to develop calibration equations and validate them, including graphical outputs. The statistical method for developing calibration equations is usually the Partial Least Squares (PLS) method, the best and oldest method used for spectral calibration and prediction among multiple linear calibration algorithms (Wold et al. 1983; Næsset et al. 2005; Wang et al. 2006; Huang 2018; Ferragina et al. 2019). Furthermore, modified PLS (Martens and Naes 1992; Cheng and Wu 2006; González-Martín et al. 2015; Olivieri 2018) and the neural network method (Nørgaard et al. 2013) are used. Although the spectrometer allows registration of spectra in transmitance mode (detection of radiation passing through the analysed material), all previous applications were processed in reflectance measurement mode (detection of radiation reflected from the surface of the scanned sample). For the purpose of calibration at the GRS Jevíčko, the most frequently used NIR range was in the range 1100–2500 nm, characterized by favourable detection in absorption bands of the most widespread chemical compounds in organic materials (Siesler et al. 2002; Burns and Ciurczak 2008).

RESULTS AND DISCUSSION

The first analyses to elaborate their own calibration equations for grassland quality determination were started in October 1995. The predicted parameters were: Dry Matter (DM), Crude Protein (CP), CP digestibility, Fat, Crude Fibre (CF), Ash, Water Soluble Carbohydrates (WSC), Potential Protein Feeding Value (PDIN, PDIE), Net Energy for Lactation (NEL), Net Energy of Fattening (NEF), Metabolizable Energy (ME) (Míka 1997). This activity in the evaluation of grassland fodder samples represented the first and for many years a major milestone in the use of the NIRS instrument at the GRS Jevíčko /*In the period of the highest demand of the applicants for analysis, up to 4-5 thousand samples including station's own samples were analysed per year in some harvest years*/.

In 1997, in cooperation with the Federal Agricultural Research Station in Changins, NYON, Switzerland (Dr. J. Scehovic), a calibration was performed to determine the IANP (negative effect index of secondary metabolites) in permanent grassland (PG) with a higher proportion of herbal component (Míka et al. 1998a). Further work on the determination of phenolic substances in meadow stands is given in Míka et al. (2001). In 1998, the team at GRS Jevíčko led by V. Míka performed an evaluation (application) using discriminant analysis to predict the mass proportions of agrobotanical groups (grass, legumes and herbs) as well as the botanical composition of grassland in two- and three-component mixtures (Míka et al. 1998b, 1998c). In addition, calibration experiments were performed to predict foliage in grass and legume breeding programs.

The second important milestone in the use of NIR technology occurred at GRS Jevíčko at the beginning of the millennium. In 1999, work began on calibration equations for analysis of whole rapeseed seeds in the parameters of fat, erucic acid (separately for low and high concentrations) and glucosinolate content (Míka 1997;

Koprna et al. 2002; Míka et al. 2003b). In 2001, the CP content was calibrated and in 2006 the fatty acid content (oleic acid, linoleic acid and linoleic acid) was determined (Koprna et al. 2006; Míka et al. 2008). Furthermore, since 2007, in cooperation with the Association of German Agricultural Analytical and Research Institutes (VDLUFA) Kassel, Germany. Dr. P. Tillmann, calibration equations were processed to analyse small quantities of whole rapeseed seeds in modified measuring cells, equipped with a plastic insert with a small circular opening in its centre. Measurements and analyses of whole rape seed samples were carried out in the years 1999–2012 for almost all rape breeders in the Czech Republic in the range of 4 to 5 thousand samples per year. The parameters of fat and glucosinolates content were recalibrated repeatedly in order to refine the determination. In connection with checking the accuracy of the prediction, the laboratory of the GRS Jevíčko regularly participated in the ring tests organized in 2001–2013 by the Central Institute for Supervising and Testing in Agriculture Brno /*Interlaboratory comparative tests - analysis of oilseeds*/ and ring tests by VDLUFA Kassel (Germany) in 2002–2008. Both cases were comparative analyses of whole oilseed rape seeds for fat and CP content. Especially in the case of fat content prediction, among the thirty participating laboratories the results of the GRS Jevíčko were excellent.

Arable soil analyses have become another distinct area of application. In 2002, calibration equations for determination of carbon content (Cox, Ctot, Chwl), nitrogen (Ntot), sulphur (Stot) were performed using samples from long-term experiments of Crop Research Institute Prague-Ruzyně (Míka et al. 2008).

In 2006, in collaboration with the Höhere Bundeslehr- und Forschungsanstalt für Landwirtschaft (HBLFA) Raumberg-Gumpenstein (Austria) and the Research Institute for Cattle Breeding, Ltd., Rapotin, the calibration of the Organic Matter Digestibility (OMD) parameter was made for grass digestibility, based on the Tilley and Terry (1963) method (Nerušil et al. 2008). Development work on the calibration of parameter VI (voluntary intake of forage by cattle) was less successful, where a satisfactory agreement of the calibration and validation model could not be achieved.

At present (2016–2019 period) applications focused on development of calibration equations for prediction of nutritional parameters of non-fermented samples of maize hybrids intended for production of maize silage, resp. fermented samples of preserved maize silage are successfully tested at the GRS Jevíčko. Predicted parameters: DM, CP, CF, Starch, Neutral Detergent Fiber (NDF), Acid Detergent Fibre (ADF), and OMD (Nerušil et al. 2016; Nerušil et al. 2018). However, the first work on the calibration of nutritional parameters determination in dried samples of fermented corn silage already began much earlier in cooperation with the Institute of Animal Science (IAS) Prague-Uhříněves (Míka et al. 1999). The development of calibration equations for the determination of nutritional value parameters of preserved alfalfa silage is solved in cooperation with IAS (Loučka et al. 2019). Other current activities include work aimed at developing calibrations to determine the digestibility of organic matter (OMD) and fibre fractions (ADF and NDF) in forage samples from extensively used PG - very favourable statistical parameters of accuracy of prediction have been achieved, publication is underway (Menšík et al. - unpublished). Furthermore, within the research activities with NutriVet, Ltd. a Strom Praha, Joint-stock Company, the quality of fresh fodder (corn fodder) is evaluated using a portable feed analyzer AgriNIR ™ / *Dinamica Generale, St. Charles, Italia*/ (Menšík et al. 2018), respectively with a HarvestLab ™ 3000 /*Deere & Company, One John Deere Place, Moline IL, USA*/ located directly on the forage harvester. Recent research at GRS Jevíčko also focuses on the development of calibration equations for the prediction of soil quality parameters / soil organic matter, pH, nutrients and risk elements, etc./(Kunzová et al. 2018).

CONCLUSION

NIR spectroscopy is already clearly established as a standard laboratory analytical system (quality of agricultural raw materials, feed and processed agricultural production, etc.) in agricultural analysis in advanced agricultural countries in the world. However, it is no longer confined to a laboratory, but on-line analysis in agricultural and processing plants is becoming increasingly common (the principle of precision farming, development and miniaturization of on-line analysis equipment in situ). Nevertheless, there is still much room for improvement and use of NIRS applications (development and use of global calibration equations or models, evaluation methods, etc.).

The introduction of the NIRS method at the GRS Jevíčko has clearly contributed to the wider use of precise procedures in agricultural research, breeding and practice in the Czech Republic. Over the past 25 years, a number of applications has been developed at the GRS Jevíčko to assess quality (nutrient content, digestibility, energy, etc.) of plant products (grass fodder, maize; oilseeds) as well as feed (corn and alfalfa silage) or parameters soil quality.

The potential of research in the near future at the GRS Jevíčko will consist in the precision and extension of current calibration equations (digestibility of feed - fibre fraction, Water Soluble Carbohydrates, etc.), as well as development of new calibration models e.g. potential biogas / methane production in agricultural substrates biogas stations; prediction of soil quality parameters (fractionation of humus substances, degradation processes, etc.), but also for research of on-line NIRS analyses directly in the field (fresh matter) for conditions of precise agriculture.

DEDICATION

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TESTING THE QUALITY OF FERMENTED ALFALFA AND RYE FORAGES BY MEANS OF ELECTRONIC NOSE TECHNOLOGY

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INTRODUCTION

Fermented forages are generally used worldwide to feed dairy cows. Not only is milk production influenced by the amount determined in the ration, but the nutrient content and fermentation of the silage can also affect the performance and health of the animals (Fulgueira *et al*., 2007). Since current systems of dairy production demand exact knowledge of the production processes, it is important to monitor feeds during preparation and consumption. Regular monitoring of the quality of fermentation is particularly important in larger dairy farms, where silage silos may contain large quantities of potentially inhomogeneous, fast-consumed feed.

For feed professionals, the first information in the monitoring is provided by the sensory tests (Fulgueira *et al*., 2007). The sensory evaluation may include the examination of the structure of the feed, its tact (moisture content), the quantity and distribution of components, the degree of crushing, the clarity from foreign matter, and the odor. However, this information may be subjective in view of the age, lifestyle, routine of the sampler, or other sampling conditions and environmental impact. The odour is highly informative when it comes to filtering harmful fermentation processes and deterioration (Kung *et al*., 2018), so volatile substances can be critical indicator parameters for assessing the animal health risk of silages. The odor determination should be improved with quick, objective methods that can simultaneously analyze large amounts of samples, helping the daily work of the farm animal feed industry to achieve efficient production and to maintain animal health. Electronic nose technologies provide a quick and objective method for quality control, both during ensiling and exploitation (Masoero *et al*., 2007). The measurements do not require special sample preparation, and the speed and low operational costs may be an important advantage in the daily routine.

The objective of this study was to describe the chemical composition and odor profile of alfalfa and rye silages and to reveal the relationship between the compositional data and the electronically measured odor in order to establish a quality control system for forages based on machine sensing.

MATERIALS AND METHODS

Samples. Alfalfa and rye silage samples (n = 22 and 38, respectively) were collected from dairy farms in ready-to-feed form by Állattenyésztési Teljesítményvizsgáló Kft., Hungary. Fresh samples were analyzed for dry matter, crude protein, crude fat, crude fiber, crude ash content, pH, acetic acid and lactic acid concentrations by means of near-infrared (NIR) spectroscopy using an internationally recognized calibration database. The quality of samples was evaluated based on the pH and the lactic acid / acetic acid ratio. After NIR measurement, each sample was equally distributed into six plastic bags. The prepared sub-samples were stored at -20 °C in the sealed bags until odor measurements.

Electronic nose. The odor measurement was performed on six days when the six sets of sub-samples were analyzed daily (n = 60 per day). The odor was examined with an Alpha MOS FOX4000 electronic nose based on the metal oxide semiconductor sensor array technology (Alpha M.O.S., Toulouse, France). The instrument was equipped with 18 sensors and measured the relative resistance changes ($\Delta R/R0$) of each sensor after the injection of 5 ml of the headspace generated above 2 g of silage sample. The maximal relative resistance changes experienced on the sensors were saved in each measurement cycle, thus, each measured sample was described with 18 variables.

Multivariate data analysis. The recorded data of the electronic nose were analyzed with multivariate classification methods using AlphaSoft v12 (Alpha M.O.S., Toulouse, France). Non-supervised principal component analysis (PCA) was used to select outliers and to describe the multivariate structure of the dataset. Supervised discriminant factor analysis (DFA) was applied to test the possibility of group identification based on the odor properties. Cross-validation was used to test the supervised classification models when sub-samples of a single sample were left out of the modelling iteratively and were used for testing the classification capability of the model.

RESULTS AND DISCUSSION

There were no extremities involved in the sample set, based on chemical composition and overall quality. No distinguishable odor pattern of the different plant materials (alfalfa vs. rye) was detected, but both the alfalfa and rye silages separated according to the different agrotechnologies of ensiling. In the case of rye, the maturity of the crop upon ensiling caused significant odor variations, while in the case of alfalfa, the application of wilting resulted in variations of the odor profile (Table 1). In average, more than 65% of samples were classified correctly in the cross-validations in both types of silages.

Table 1: Cross-validation results of the odor-based classification of silages according to the applied agrotechnologies

Rye silages			Alfalfa silages		
Phenophase groups	(1)	(2)	Processing group	(1)	(2)
(1) Before heading	77.4%	22.6%	(1) Direct-cut silage	66.7%	33.3%
(2) Heading	41.7%	58.3%	(2) Wilted haylage	30.0%	70.0%

Samples were ranked according to their quality based on the pH or the lactic acid / acetic acid ratio. DFA classifications were performed to evaluate the possibility of identifying the different quality groups based on the odor profiles. Tables 2 and 3 show the cross-validation results of the developed models for the pH and acid ratio, respectively. In the case of rye silages, 64.5% of the samples were classified correctly according to the pH groups. The overall average performance of the classification based on the acid ratio was weaker, but the identification of the low-quality group (ratio < 5) was highly successful (75% success rate). Results of alfalfa samples were generally less accurate, but similarly, good identification (75 % success rate) of the good-quality acid ratio group was experienced.

Table 2: Cross-validation results of the odor-based classification of silages according to the quality groups defined by the measured pH values

	Rye silages		Alfalfa silages	
pH groups	< 4.4	≥ 4.4	< 4.4	≥ 4.4
< 4.4	66.0%	34.0%	66.7%	33.3%
≥ 4.4	37.0%	63.0%	46.1%	53.9%

Table 3: Cross-validation results of the odor-based classification of silages according to the quality groups defined by the lactic acid / acetic acid ratios

Acid ratio groups	Rye silages			Alfalfa silages		
	≥ 10	≥ 5; < 10	< 5	≥ 10	≥ 5; < 10	< 5
≥ 10	37.3%	21.4%	41.3%	75.0%	20.8%	4.2%
≥ 5; < 10	33.3%	41.7%	25.0%	25.0%	25.0%	50.0%
< 5	6.7%	16.7%	76.7%	17.9%	32.1%	50.0%

CONCLUSIONS

The time of harvesting (phenophase of the crop) and the applied ensiling procedure (inclusion of wilting) cause significant odor deformation in the case of rye silages and alfalfa silages, respectively. The quality groups defined by pH or acid ratio are distinguishable based on the measured odor patterns. In the case of rye silages, the identification of the low-quality samples is the most successful, while in the case of alfalfa silages the identification of the good-quality samples is suitable. According to our results, the electronic nose technology is proven to be applicable to classify the rye and alfalfa silage samples by their quality, even among conditions when quality groups are defined by NIR predicted values, causing double prediction error. Accuracy may be increased with the expansion of the sample set and application of wet chemical reference data.

ACKNOWLEDGMENTS

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METHODS OF PRECISE AGRICULTURE IN FODDER PLANT AND FODDER INDUSTRY

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INTRODUCTION

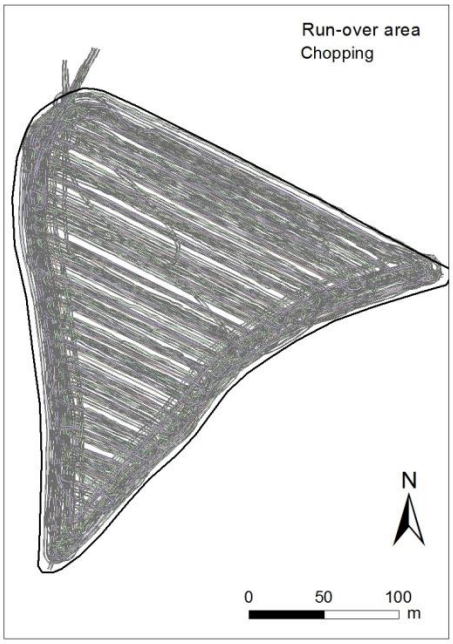
The use of precision farming methods from today's perspective represents a very broad field of activities, which takes up the use of modern technology, large amounts of data processing, fulfilling agri-environmental measures and planning. The definition of precision agriculture continues to evolve as technological advances evolve and our attainable knowledge grows. Over the years, attention has shifted from a variable application, an automatic machine navigation system to a focus on production quality and the environment. Part of the truth is that the techniques and application interventions have focused on the main field crops. However, if we quantify the proportion of permanent grassland in the agricultural land fund of the Czech Republic and add to that the proportion of fodder crops on arable land, including silage maize, this is not a negligible proportion.

The system of controlled traffic farming with GPS support is used for grassland harvesting. Also, with the increase in the area of maize for silage, mainly due to the need for energy purposes, the machinery fleet was significantly changed. This was demonstrated especially in deliveries of high-performance forage harvesters, which are equipped with technique for mapping yield and quality of forage or in purchasing of modern seeders with the possibility of variable sowing and down force pressure on furrow openers. On the other hand, there is a problem with the risk of erosion on these sown areas. Forage is once again brought to the forefront, especially in the form of grasslands, which are established as protective or break-down strips within the standards DZES.

As a result of the reported changes in the approach to land block management, it is necessary to work with new technologies of soil cultivation, plant establishment and planning. This again confirms the application of a wide range of activities from the technology itself to the work with GIS applications. The paper presents the activities of the employees of the Center of Precision Agriculture in the fodder plant.

WORKING PRACTICES FOR REDUCING UNWANTED SOIL COMPACTION AND THE ROOT SYSTEM DAMAGE

The protection of soil against soil erosion or compaction is increasingly important for many farmers today. The technologies based on respecting the slope of the land or reducing the passage on the land are directly dependent on the precise guidance of the machinery and the observance of work discipline. Passes reducing are applied on arable land especially for main crops. However, the problem of intensive and accidental passes also affects other crops, such as arable forage and perennial grassland. Two technologies were compared: self-propelled forage chopper and round baler. The results show that total runs over area and the frequency of repeated passes did not differ between technologies. Figure 1 and 2 shows all machinery passes during harvest of grass for silage. In both cases, the double passes are the most frequent repetitive. In case of self-propelled forage chopper was run over 63.8 % of 1 ha area. In case of round baler it was 63.4 %.



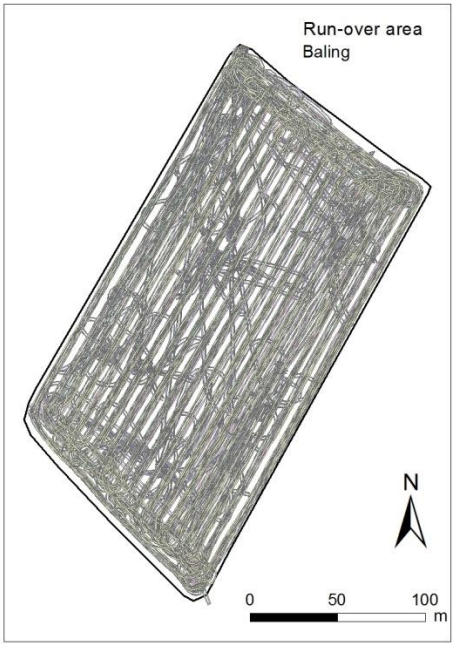


Figure 1: Graphical representation of machinery passes for chopping (left).
Figure 2: Graphical representation of machinery passes for baling (right).

A significant reduction of wheeled allows adoption of fixed track system for machinery traffic (CTF). Better organization and cooperation of transport trailer with chopper would certainly help to reduce the repeated crossings. In presented results, tractor with an empty trailer followed tractor operating with a chopper at all times.

Information about the movement of balers can be used in the collection of bales, where we can easily determine the location of individual bales.

Knowing the position of the bales, it is possible to design a disposal route with the requirement for the shortest distance travelled by means of economic-mathematical modelling methods (Figure 4).

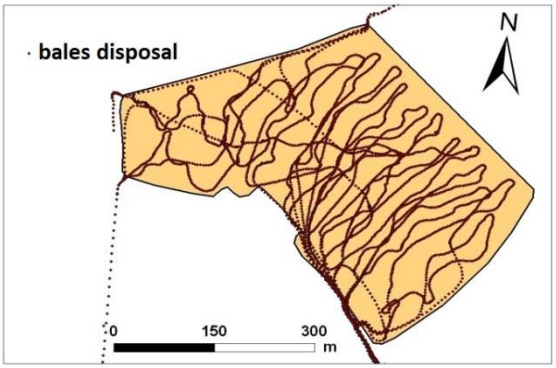


Figure 3: Trajectory of tractor which transported bales.

ZONAL PROCESSING OF SOIL AND MAIZE SOWING.

The organization of passes during soil tillage and sowing is an important factor in reducing soil compaction. With the use of guidance devices targeted zonal tillage, fertilization, protection and sowing are become more popular. Recently, we have been increasingly experiencing lack of rainfall, extreme weather conditions or uneven rainfall distribution. One of the benefits of zonal and targeted soil preparation is the support of rainwater management and its targeted distribution in the soil profile. By differentiated soil tillage for traditional wide-row crops, we can specifically influence the movement of water in the soil and the preference of water infiltration paths (Figure 4). In addition, this measure may be relevant for targeted sowing and fertilization. The figures show the differences in water infiltration in vertical soil profile between plowing and strip-till technology on field with maize. The white color represents water colored with blue color for the purpose of the visualisation. In plowing it is possible to observe individual chunks, between which water unfiltered. The infiltration to deeper profile was limited by compacted subsoil layer at a level of about 0.25 m. The preferential water flow does not respect the future rows of the crop.

Between individual chunks it is often possible observe the presence of plant residues, which also affect the movement of water. The second picture illustrates the preference of water flow to the locally prepared profile. Also the presence of the cracks which were created by the working tool supported water infiltration to the deeper profile. The strip thus treated was intended for fertilizer application and sowing. If we consider the availability of water for plants, it is obvious that water will prefer the conditions which we prepare.

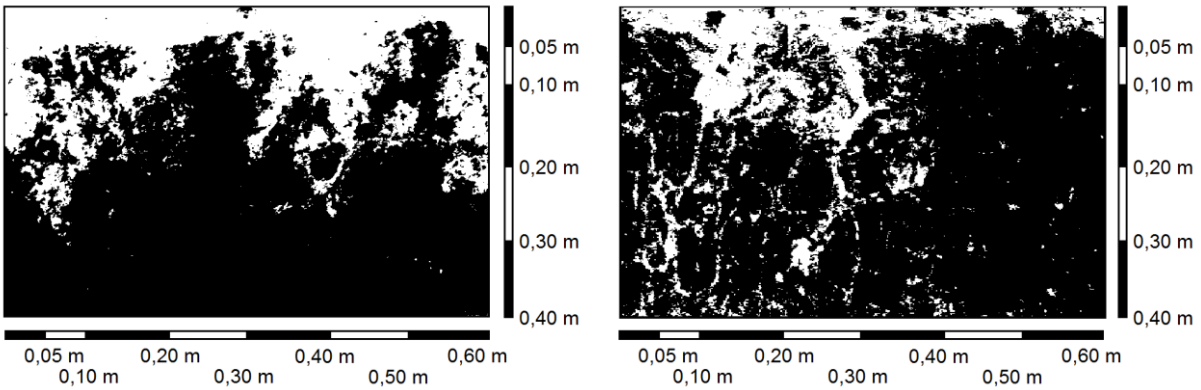


Figure 4: Visualization of water flow in vertical profile, across plant rows. Tillage technology on the left, Strip-till technology on the right.

In connection with the reduction of chemicals substances, support of infiltration of water into the soil, reduction of unproductive vapor, introduction of organic matter into the soil and above all reduction of the risk of soil erosion the utilization of intercrops and auxiliary crops is increasing in importance. The sowing of intercrops in wide-row crops is a technology utilizing the positive effect of so-called biotic effects. These

procedures, which require significant technological discipline, could not be implemented without the support of accurate navigation.

VARIABLE OF DOWN FORCE PRESSURE TECHNOLOGY FOR MAIZE SOWING.

The site specific conditions respecting is an important step into the new growing season when new field crops are established. There are many demands in sowing and it is difficult to set priorities. The requirements for maintaining of the correct placement depth of the seed also increase with the variability of the field. An important parameter is the maintenance of the down force on the individual furrow openers. Figure 5 shows a map of the measured down force acting on the furrow openers support wheel, which is also information for setting a suitable sowing depth. The system was introduced by Ag Leader Company.

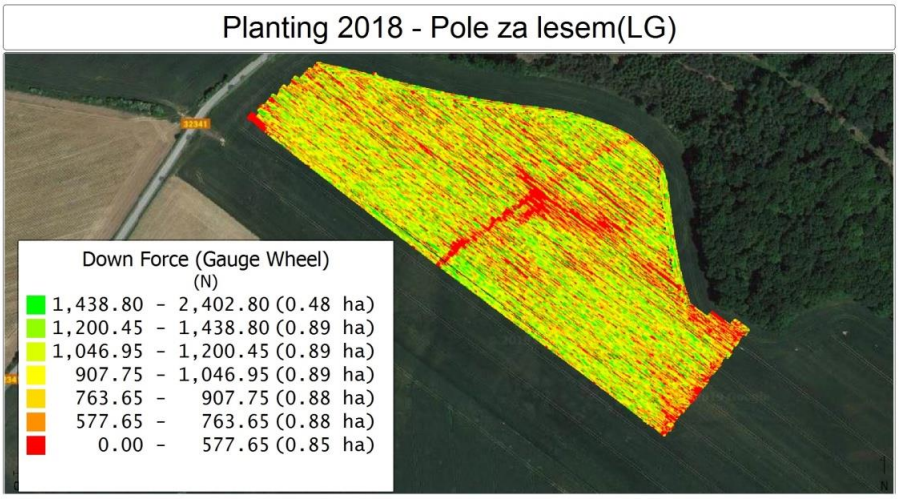


Figure 5: The values of measured down force on the support wheel during maize sowing.

The graph in Figure 6 illustrates the importance of adjusting the down force on the furrow openers. The results were obtained as part of a field trial, where a different level of down force was set when maize sowing.

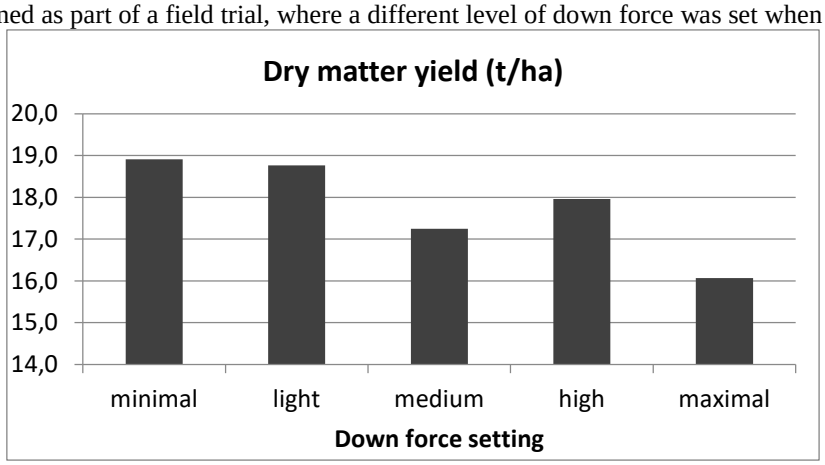
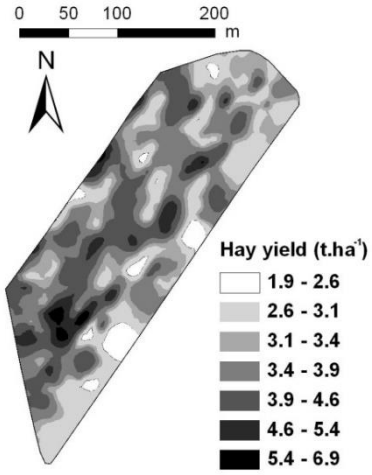


Figure 6: Maize dry matter yield at different down force settings on furrow openers.

EVALUATION OF CROP YIELD AND QUALITY PARAMETERS OF HARVESTED PRODUCTS.

Technical solution for straw and forage yield mapping, when using round baler with variable chamber for harvest, was developed by authors. The principle is based on monitoring of instantaneous position of tension roller in the press chamber. Detailed description brings *Utility model No. CZ 19754 U1*. Yield map of hay in figure 7 was created on the basis of data gained from the sensor.

The quantitative and qualitative variability of silage maize biomass varies considerably due to drought and due to postponement of sowing deadlines in response to the weather in spring in recent years. Optimization of silage maize harvesting dates or chopped treatment on the basis of knowledge of biomass qualitative parameters is based on data collected continuously by optical sensors installed in forage harvesters. Figure 8 illustrates the biomass dry matter variability (%) during harvest based on HarvestLab sensor data.



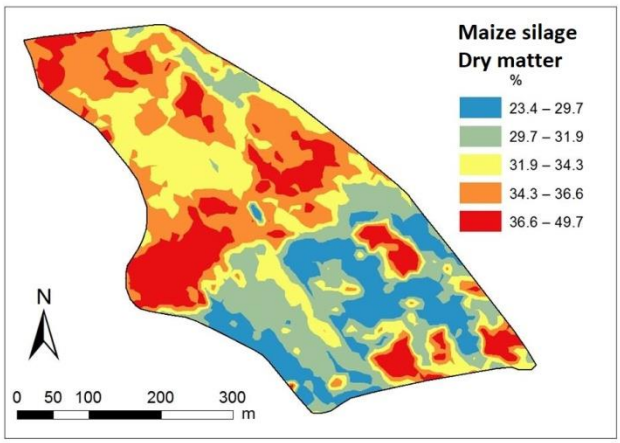


Fig. 7: Yield map of hay, sensor data (t/ha).
Figure 8: Biomass dry matter (%) variability during maize harvest.

CONCLUSION.

This overview represents part of the Center's activities. It documents possibilities of application of elements of precision agriculture across individual activities. There is also space for new technological means, in particular remote sensing and the use of unmanned aerial vehicles (UAV). An interesting area is the support of autonomy and robot platforms.

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MULTISPECTRAL IMAGING APPLICATIONS IN AGRICULTURE AND AGRO-FOOD PRODUCT QUALITY AND SAFETY CONTROL

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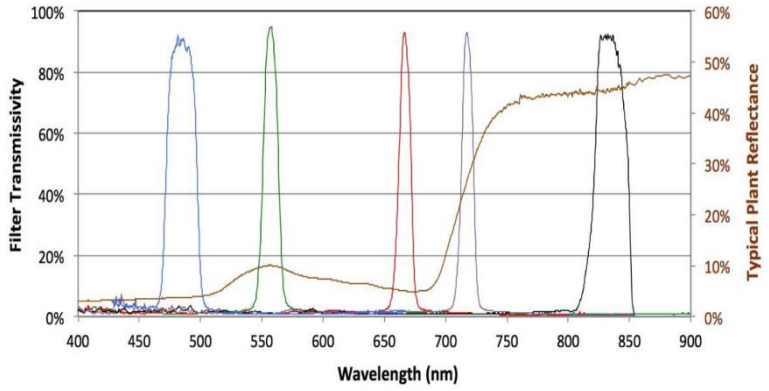
ABSTRACT

The paper deals with multispectral imaging, which is based on capturing image data at specific wavelengths across the electromagnetic spectrum. We show examples of multispectral imaging applications in agriculture and the quality and safety of food products. For this purpose, we use multispectral camera with global closures for distortion-free images, including narrow-band red, green, and blue bands for RGB color images that are aligned to all visible and invisible bands and vegetation indexes during processing. The obtained images were processed by Matlab.

INTRODUCTION

We use multispectral camera captures five panchromatic images from the imaging spectrum displayed in Fig. 2. The sensors in the camera are sensitive in the following narrowband: blue (475 nm), green (560 nm), red (668 nm), red-edge (717 nm) and near-infrared (NIR, 840 nm), see Fig.1.



The Spectral bands of multispectral camera [1].

THE IMAGE COMBINING ALGORITHMS

The Fig. 2 describes creating of combined images. A registration process must be taken after panchromatic images capturing. After that, the combined images can be calculated (RGB – using composing of spectra in Matlab; NDVI using eq. 1 and NDRE using eq. 2).

The RGB composite image were obtained from the panchromatic images captured by a multispectral camera. Another type of combined imaging used in the agriculture and food industry are images weighted by Normalized Difference Vegetation Index (*NDVI*) and Normalized Difference Red Edge (*NDRE*) [2-11].

To calculate *NDVI* a difference formula is used to quantify the density of plant growth on the Earth:

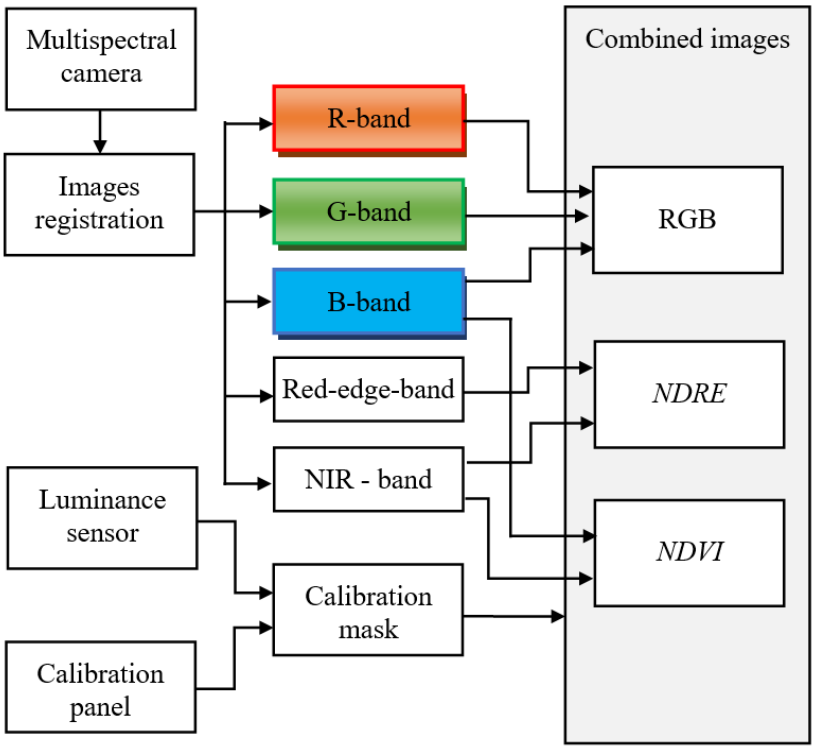
$$NDVI = \left(\frac{NIR - RED}{NIR + RED} \right) \quad (1)$$

As plants become healthier, the intensity of the reflection increases with the NIR and decreases in the RED, which is the physical basis for most vegetation indices. NDVI values can be a maximum value of 1, with lower values indicating lower plant vigor. Therefore, 0.5 typically indicates low vigor whereas 0.9 indicates very high vigor. NDVI is also effective for distinguishing vegetation from soil. NDVI is recommended when looking for differences in above-ground biomass in time or across space. NDVI is most effective portraying variation in canopy density during early and mid-developmental stages but tends to lose sensitivity at high levels of canopy density [6].

NDRE index is similar to *NDVI* but uses the ratio of Near-Infrared and the edge of red as follows:

$$NDRE = \left(\frac{NIR - RE}{NIR + RE} \right) \quad (2)$$

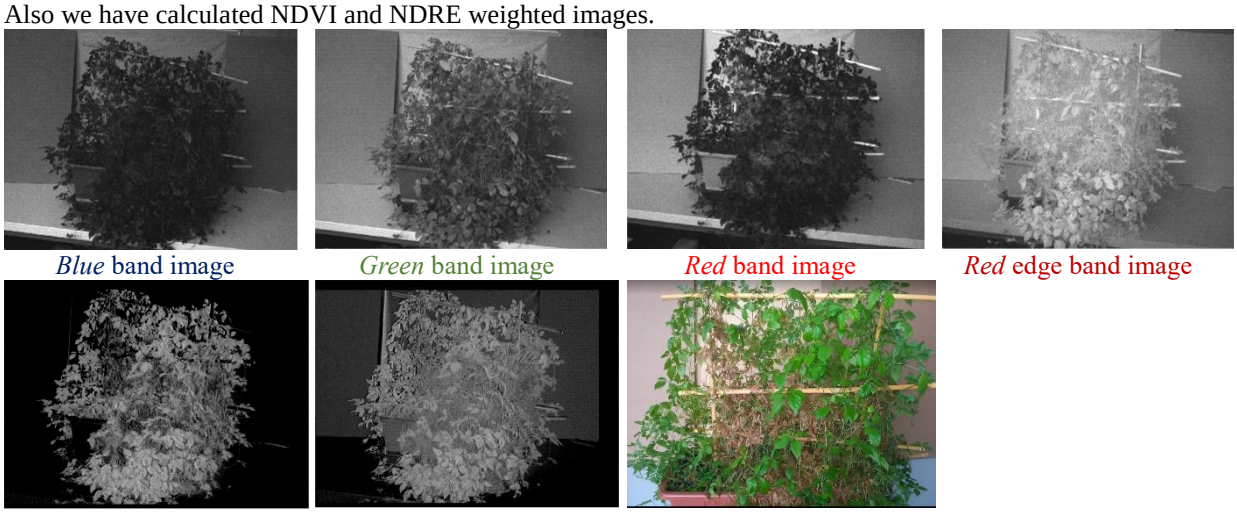
NDRE is an index that can only be formulated when the Red edge band is available in a sensor. It is sensitive to chlorophyll content in leaves (how green a leaf appears), variability in leaf area, and soil background effects. High values of *NDRE* represent higher levels of chlorophyll content in a leaf. Soil typically has the lowest values, unhealthy plants have intermediate values, and healthy plants have the highest values. Consider using *NDRE* if you are interested in mapping variability in fertilizer requirements or foliar Nitrogen, not necessarily Nitrogen availability in the soil [6].



Block diagram of image processing.

EXAMPLES OF MULTISPECTRAL IMAGING APPLICATIONS

Using multispectral camera we obtained the acquisition of panchromatic images of jiaogulan – Fig. 3. Also we have calculated NDVI and NDRE weighted images.

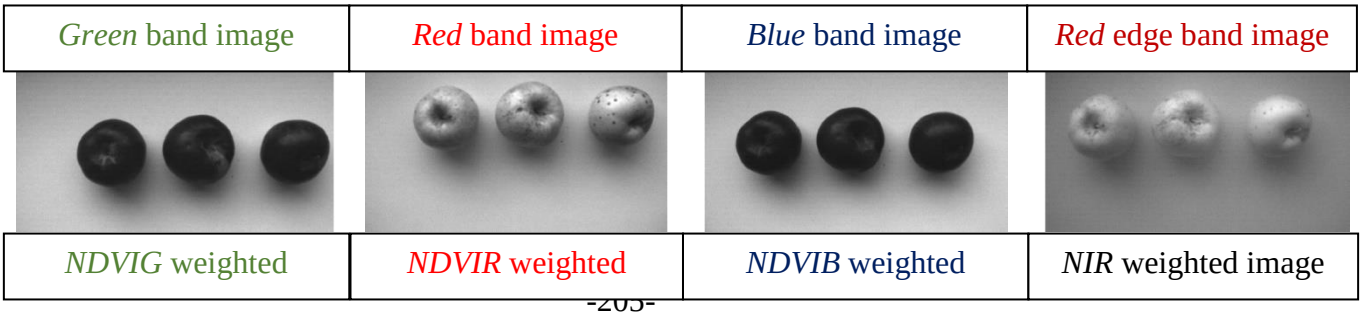


Blue band image | *Green* band image | *Red* band image | *Red* edge band image

NDVI weighted image | *NDRE* weighted image | RGB image

Fig. 1. Different types of jiaogulan images.

Agronomists and growers can accurately identify crop stress, track the success of a nutrient application, discover disease earlier. The Fig. 4 shows diseases of apples and lemons in different weightings.



Green band image | *Red* band image | *Blue* band image | *Red* edge band image

NDVIG weighted | *NDVIR* weighted | *NDVIB* weighted | *NIR* weighted image

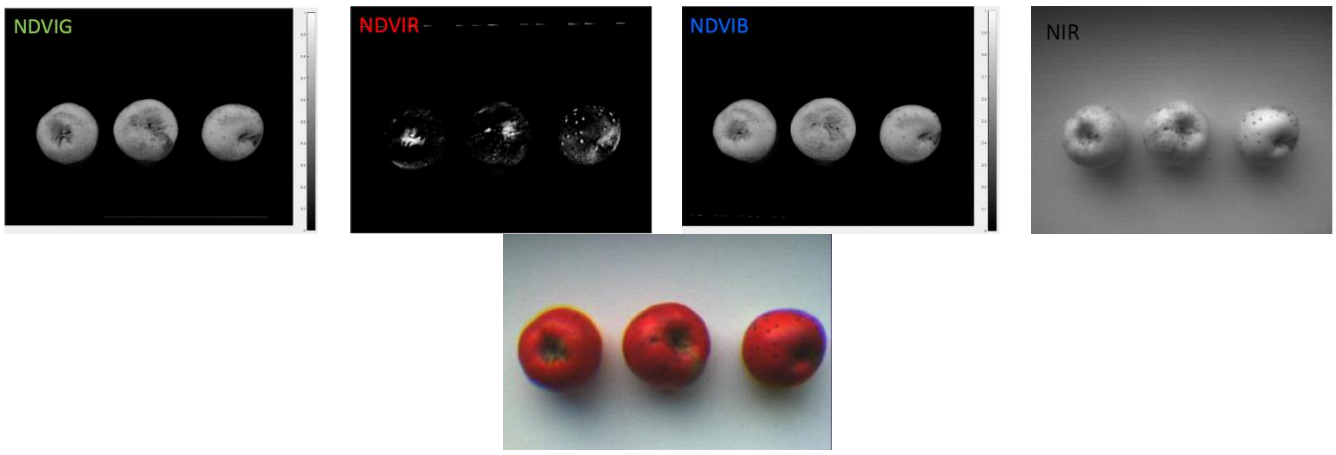


Fig. 2. Different types of apples images.

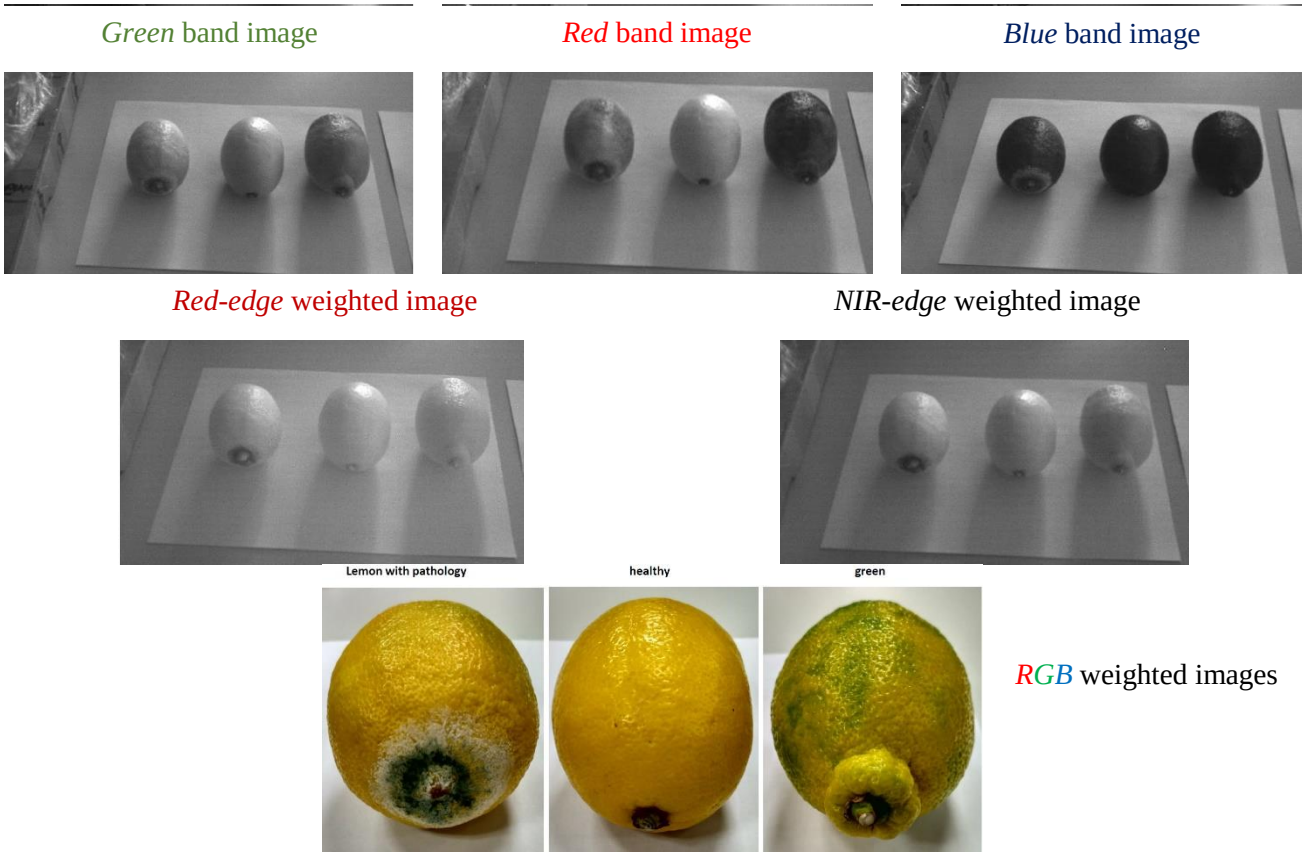


Fig. 3. Different type of lemons images.

Similarly, we can obtain field images and create, for example, a NDVI or chlorophyll maps.

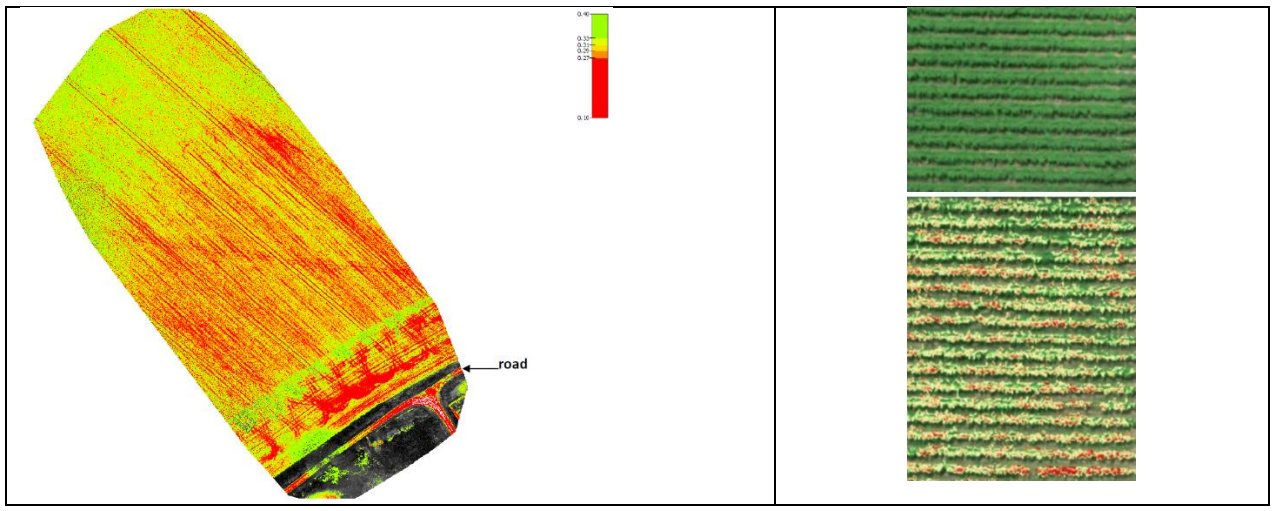


Fig. 4.: Left) NDVI map – Field with small wheat; rigt) RGB and chlorophyll map.

CONCLUSIONS

Multispectral Imaging Applications can be used advantageously in agriculture and agro-food product quality and safety control. This article showed only some of these uses. From the panchromatic images we can calculate in Matlab program different weighted images. For example NDVI, NDRE or Chlorophyll maps. These maps can tehn help to draw attention to the spread of diseases on crops in time.

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USE OF SPECIAL BOLUSES TO MEASURE CHANGES PH IN RUMEN

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INTRODUCTION

Acidosis is defined as a state of highly pathological acidity of the blood, and the incidence is increasing in ruminants. It includes situations of ruminal or systemic acidity and represents the most important nutritional disorder in dairy cattle. The duration of the pH of rumen remaining below the threshold value of 5.8 in a 24-h period (Valente et al., 2017) was used as an indicator to characterize the subacute ruminal acidosis (SARA).

The pH of rumen can be measured since 2008 with the help of the wireless telemetric method using the eCOW bolus (Mottram et al., 2008). The eCOW bolus ranks among boluses with detection function. It is a small device that is able to measure almost continuously (in adjustable, e.g. 15-minute intervals) selected values of the internal environment of the rumen, i.e. pH and temperature. The boluses are perorally applied with the help of a special applicator. The breeder can apply the boluses perorally without the veterinarian. The relatively recent availability of continuously monitored data from the pH bolus has been offering, in the course of time, new opportunities to distinguish normal and abnormal patterns of pH and temperature changes in the rumen thanks to a more sensitive and specific method (Denwood et al., 2018). Philips et al. (2010) recommended this method of monitoring the rumen function for a longer period without the need of invasive sampling; in the first 40 days of continuous recording, they found a significant correlation ($P<0.01$, r=0.982) with values directly measured under use of the rumen fistula. The measurement of the rumen pH constitutes now a common method for detection of dietetic disorders, see e.g. Neubauer et al. (2018).

MATERIAL AND METHODS

The eCOW boluses (eCow Devon, Ltd., Great Britain) were perorally applied into the rumen of 36 dairy cows within 6 experiments made at the farm of the Institute of animal science in Prague-Uhříněves (IAS). In weekly intervals (on Tuesdays, at about 10:00), a special wi-fi handset with an antenna (HathorHBClient v. 1.8.1) was used to download their data into a notebook and to evaluate them using diagrams.

Additionally, during the first experiment, the rumen pH of two dairy cows with a rumen cannula was measured with the help of the WQL probe. Both types of the measuring devices were set to measure the rumen pH with an accuracy of 0.1 degree each 15 minutes. Simultaneously with the pH, both devices recorded also the rumen temperature, which indicates primarily when the dairy cow drank, which may influence the rumen pH for a short time (by diluting its contents).

The methodology of all experiments was set so that the total mix ratio (TMR) was changed in three-week periods for all cows. After the first period, the cows were divided into the experimental and control group with the help of the pairing method so that the initial values were approximately identical. At the beginning of the second period, the cows in the experimental group were perorally applied RMS brushes (in form of a paper boulus) in order to stimulate the rumen motor activity (patent EP0609045A2, Meiwa-Sangyo Co. Ltd, Kyoto, Japan). In each period, rumen liquids and milk were sampled from all cows. The samples were sent to the laboratory for chemical analyses. Additionally, the values of fodder intake and the frequency of rumination were continuously measured. The results were processedby the SAS program, with and without the regression method, and also by the Statistica Komplet program (StatSoft, USA), by the ANOVA procedure and by the subsequent POST-HOC Tukey test.

RESULTS AND DISCUSSION

The information on the eCOW boluses are available at https://ecow.co.uk/, including results. The IAS experiments took place in 2015 to 2018. In this year, 2019, an experiment is planned only for autumn. 36 boluses were used in total; 12 of them, i.e. 33 %, were not functional. The longest time (176 days) of functionality was shown by the boluses used in the experiment from January 17 to July 12, 2017. Some boluses recorded the pH, but with an increasing tendency. It was then difficult to estimate when the boluses recorded realistic values and from which point the values were distorted already.

The results were also compared with the values of the fodder intake and the rumination frequency, with the values of the composition of the rumen liquid sampled with the help of a gullet probe, with other values of production assessment and with ethological measurement of the dairy cows' daily activities.

In total, a huge amount of data was acquired; the data were evaluated in separate papers, usually at seminars where professionals from practice had the opportunity to learn to know the values. Therefore we are offering brief comments only:

the application of the RMS brushes influenced the pH only minimally; the differences between the experimental and the control group usually did not exceed 0.2 pH,

changes of the feeding ratio did not manifest themselves immediately, but usually after several days; however, their effect was usually the contrary to that expected, i.e. the pH did not decrease, but it slightly increased; it decreased after several days only;

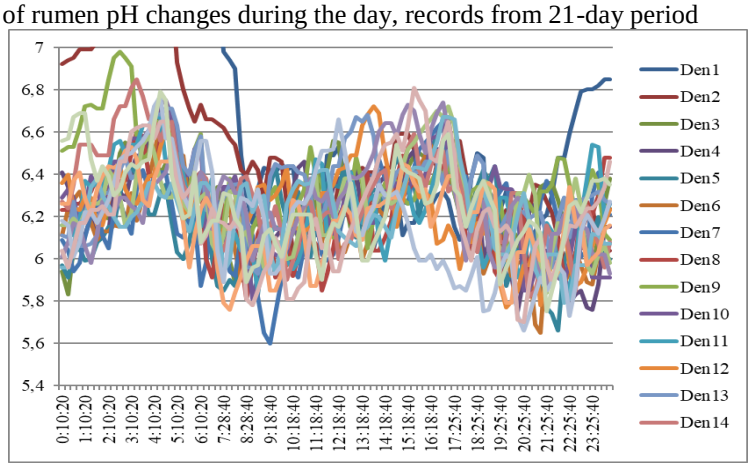
we could observe daily pH changes caused by the daily regime (for an example see the enclosed diagram); the pH decreased when the cows went to the milking parlour and after milking, they stayed in the pen until the shed was cleaned; as soon as the cows had access to fodder, their pH increased,

the pH changes during the day were relatively radical; if the pH dropped below a physiological limit, e.g. below 5.8, it suddenly rose in a dramatic way; the cow probably drank or ate, the rumen contents was mixed, the cow swallowed a greater amount of saliva and the partially digested particles moved to the fourth stomach.

The decrease of the rumen pH usually takes longer, if compared with the increase of pH. The causes may be:

short chops, decreased peNDF,
increased contents of concentrates in TMR,
sudden change of TMR quality,
birth, stress from heat or shortage,
daily and night period.

Diagram 1: Example of rumen pH changes during the day, records from 21-day period



CONCLUSION

It can be seen that continuous measurement of pH helps in zootechnical work to learn to better know the individual needs of the dairy cows and to respond to them by goal-directed measures to improve the situation. Unfortunately, the devices are not yet at such a level to be reliable and to measure for longer time. The downloading of the data from the bolus to the handset is problematic for practical use for the time being. However, from scientific perspective, the eCOW boluses bring a lot of precious knowledge.

Dedication: QK1810137 and MZE-RO0719

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COMPARISON OF RESULTS OF CHEMICAL ANALYSES OF MAIZE SILAGES AND RESULTS FROM SEVERAL NIR DEVICES

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INTRODUCTION

Nowadays there are numerous chemical analytic methods, like spectroscopy in near infrared spectral range (NIRs), which determine the basic parameters of nutritional value (e.g. at fresh maize chops, but also at fermented maize silage) while making use of their physical and physical-chemical properties (Lovett et al., 2004; Jacobi et al., 2011; Kański and Kowalski, 2005; Decruyenaerea et al., 2015; Nerušil et al., 2016, 2018).

NIRs are more and more often used also in agricultural practice, both to assess chops and finished silages. Maize chops and maize silages are probably most frequently focused. The chemical laboratory of NutriVet, s.r.o. company disposes of the AgriNIRs device, which is used also by the representative of VVS, s.r.o. Verměřovice. Other devices, which are slightly different (HarvestLab) but work on the same principle, are now owned by STROM Praha a.s. that sells John Deere harvesting machines. The devices analyze fresh fodder. In order to compare the work of the said devices, the representatives of the above stated companies were invited with their devices to Pohořelice to compare the results on several maize silages. The aim consisted in mutually comparing the work of the devices, as well as in comparing their work with chemical analysis and spectrometer analysis based on dry material.

MATERIAL AND METHODS

The maize silages were sampled in November in several companies (Příkazy and Bratčice), from several places in the respective bunker silos. The samples were taken to the laboratory of NutriVet s.r.o. Pohořelice. The laboratory analyzed the samples with three spectrometric devices (1x AgriNIRs - NutriVet, 1x AgriNIRs - VVS Verměřovice, 1x HarvestLab - STROM Prague) for green mass (chops, or silage, respectively), and it performed a chemical analysis with common methods according to AOAC (2005). The following factors were assessed: dry matter, protein, starch, NDF, ADF, and ashes. After drying, identical samples were measured at dispersion spectrometer FOSS NIRSystems 6500 instrument (Company NIRSystems, Inc., Silver Spring, USA), placed in the centre of The Crop Research Institute (CRI) Prague, research centre (VS) Jevíčko (J). The following programs were used for statistics: STATISTICA 10, multifactorial ANOVA and Tukey HSD test for significance level (*P*, 0,05).

RESULTS AND DISCUSSION

Table 1: Comparison of work of HarvestLab (STROM) and AgriNIRs (NutriVet, VVS) by localities

Localities	Příkazy			Bratčice		
Device	HarvestLab	NutriVet	VVS	HarvestLab	NutriVet	VVS
Dry matter (DM) %	37.1^{ab}	41.7^{cd}	43.5^{d}	35.1^{a}	37.1^{ab}	39.3^{bc}
Proteins % in DM	7.55^{a}	8.44^{cd}	8.07^{b}	8.15^{bc}	9.28^{e}	8.79^{d}
Starch % in DM	34.4^{c}	31.6^{bc}	32.0^{bc}	27.6^{ab}	28.8^{ab}	25.7^{a}
NDF % in DM	39.3^{a}	40.2^{a}	41.1^{ab}	45.2^{cd}	43.1^{bc}	47.0^{d}
ADF % in DM	25.4^{b}	22.6^{a}	26.0^{b}	28.2^{c}	24.5^{b}	30.4^{d}
Ashes % DM	N	3.34^{a}	3.99^{cd}	N	4.25^{d}	4.82^{e}

Different letters in the number indexes stand for significant difference (P<0.05); SEM=average mean error; N = not determined

Table 2: Comparison of analyses at NIR devices (NutriVet, VVS) intended for measurement from fresh silage with chemical analyses (CHEM)

Localities	Příkazy			Bratčice		
Analyses	NIRs	CHEM	SEM	NIRs	CHEM	SEM
Dry matter (DM) %	40.8^{c}	39.8^{bc}	0.49	37.1^{ab}	34.9^{a}	0.85
Proteins % in DM	8.0^{b}	8.9^{a}	0.08	8.7^{a}	9.3^{a}	0.14
Starch % in DM	32.6^{c}	29.4^{c}	0.64	27.4^{ab}	22.8^{a}	1.11
NDF % in DM	45.1^{b}	45.5^{b}	0.38	40.2^{a}	44.2^{b}	0.65
ADF % in DM	24.7^{a}	24.1^{a}	0.38	27.7^{b}	25.3^{ab}	0.66

Different letters in the number indexes stand for significant difference (P<0.05); SEM=average mean error

Table 3: Comparison of chemical analyses (CHEM) with analyses at FOSS spectrometer in VS Jevíčko (NIR-J), intended for measurement from dry matter

Localities	Příkazy			Bratčice		
Analyses	CHEM	NIR-J	SEM	CHEM	NIR-J	SEM
Dry matter (DM) %	39.8[b]	35.6[a]	0.75	35.0[a]	33.7[a]	1.06
Proteins % in DM	8.91[b]	7.98[a]	0.18	9.43[b]	9.33[b]	0.25
Starch % in DM	29.4[ab]	32.4[b]	1.70	22.8[a]	26.9[ab]	2.41
NDF % in DM	44.2	44.9	0.98	45.5	47.4	1.38
ADF % in DM	24.1	24.8	0.71	25.3	27.2	1.01
Ashes % in DM	3.67[a]	3.33[a]	0.26	5.46[b]	4.52[ab]	0.37

Different letters in the number indexes stand for significant difference (P<0.05); SEM=average mean error

Table 1 shows the differences in results both among the devices of different types which measured by the NIR principle and among two identical AgriNIRs devices (NutriVet, VVS). The differences are significant also at the level of localities. The HarvestLab device did not determine ashes.

Table 2 shows that in case of the parameters of dry matter and starch contents, no significant differences were found among the methods used to determine the quality of the maize silage. As for the nitrogen substance contents, a difference was found in the locality of Příkazy; the NIR method determined a lower contents. The NDF and ADF parameters showed differences in the locality of Bratčice.

When comparing the chemical analyses with the analyses made with NIR-J (Table 3), differences in the applied methods were found only in case of dry matter and nitrogen substances in the locality of Příkazy. The NIR method determined lower values in both cases. No significant differences were found in the locality of Bratčice.

CONCLUSION

The mutual differences in the results of the analyses of the silage samples among the devices which analyze fresh silage, as well as the differences between the devices and the chemical analysis according to AOAC (2005) were significant for most nutritional values, but not for the dry matter contents. Dry matter constitutes the most fequently measured parameter, and therefore the measurement of dry matter at harvesting can be recommended based on the results. The differences in the other parameters measured in one locality were significant, but usually were not confirmed in other localities. The best coincidence was found at starch, dry matter and NDF. The differences between the chemical analyses according to AOAC (2005) and the dispersion spectrometer FOSS analysis (made at samples after drying) were insignificant in most cases. So in most cases, the chemical analysis can be replaced by the analysis of dry sample in VS Jevíčko. In future, the calibration curves must be made more accurate both for fresh and for dry samples.

Dedication: QK1810137; MZE-RO0418

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PRECISE FARMING LEADING TO REDUCE THE FODDER VARIABILITY

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INTRODUCTION

In 2015 through 2018, the Czech Republic experienced strong droughts, and therefore the fodder stocks in agricultural companies were significantly reduced. Several farmers went bankrupt, many of them had to reduce the animal stocks and actually all of them had to substantially change their approach to fodder management. That included production of higher-quality fodder, reduction of losses and more accurate estimates of stocks.

Our research was aimed at setting a system providing for efficient use of spectrometry with near infrared radiation (NIR) primarily to reduce the fodder variability. The digestive system of ruminants which digests the fodder primarily in the rumen with the help of microorganisms is set for long lasting stable supply of mixed feeding ratio of identical composition of fodders and their quality.

The technology of NIR analyzers at cutters allows analyzing the fodder immediately at the harvesting machine during the harvest. The data measured are then automatically sent to the central server and they are available at any time to the company management. The measurement runs automatically, without interventions of the operator. The variability of the silage quality or of the mixed feeding ratio (TMR) is ascertained in a more complicated manner, but nowadays it can be managed too. As an example, we could refer to the study by Hetta at al. (2017) which assessed 132 maize samples for nutritional and morphological properties by using a high-quality NIR spectrometer. They got significant results in prediction of the contents of protein, starch and water-soluble carbohydrates (WSC).

Accurate measurement, correct evaluation of status and possible accurate application of additives can help to significantly reduce the relatively high variability of fodder quality indicators, which contributes to considerably saving the fodder costs, increasing production, improving the production quality and, in many cases, improving the environment or the animal health. NIR spectrometry can be used to measure in a fast and cheap manner the dry matter and nutrients in the harvested fodder and in silages. The calibration models derived from oven-dried green fodders cannot be applied to silage samples; the calibration curves must be processed separately for green (fresh) fodder and for preserved fodder (Andueza at al., 2016).

Another option consists in measuring the temperature of ensilaged fodder and the aerobic stability of silages in order to reduce the losses which are always related to increased fodder temperature. Therefore the temperature measurement can help to prevent or at least significantly reduce losses.

The measurement is done with bar or battery thermometers which work with high accuracy and record the temperatures with the help of data loggers, connected by remote transmission to the computer of the person who uses the acquired data to evaluate the energy losses which are always related to increased temperature of the fodder.

The use of drons is examined as well, not only to map the vegetation, which has become common practice, but also to assess the stocks. The dron cameras may have different focuses and functions; they can for example monitor the condition of chlorophyll or the incidence of weeds, which helps to purposefully plan interventions into the vegetation. A camera can be also used to develop a 3D record of filling of the storage spaces. The dron movement can be exactly programmed; therefore it does not need the constant presence of an operator. After evaluating the records, purposeful interventions into the growth and harvest can be planned or the fodder stocks can be estimated with relative accuracy in order to take adequate measures for the animals to get the necessary feed. Some actions can be automated already; therefore a decision of the responsible person need not be awaited.

The study was aimed at experimentally verifying the above stated suggestions.

MATERIAL AND METHODS

To verify the use of spectrometry, primarily to reduce the variability of fodders, we have chosen the records from the HarvestLab device which was situated on the John Deere cutter during harvest. We processed 5160 measurements; each measurement included the values of dry matter, ADF, NDF, starch and proteins.

We measured the temperatures directly in the silage with the help of special battery thermo-sensors with the size of a watch battery (Thermochron iButton Device DS 1921G-FS# Maxim Integrated, USA). Their advantage consists in high accuracy, 0.0625 °C, and in the opportunity to get a huge number of measurements, programmable for intervals from 1 minute to 255 minutes. Their disadvantage consists in the fact that the temperatures cannot be read in the course of the measurement, but they can be read only after the sensors have been removed from the silage. This disadvantage is compensated in case of more expensive systems which have thermo-sensors placed at the end of a bar and the date are transferred to the mobile phone or to the computer with the help of the data logger.

The fodder stocks of the selected company were estimated with the help of a dron which mapped them by 3D-projection camera.

RESULTS AND DISCUSSION

Table 1 shows the average values measured and their correlations. The dry matter contents of the measured samples (5160 pcs) was 36.2 %, but with a higher variation coefficient (v_x = 0,12). A high correlation was found between the contents of ADF and NDF, and low correlation was found between the contents of starch and proteins. The contents of dry matter showed a medium-high and positive correlation with the contents of NDF and ADF, while the contents of proteins and starch showed a negative correlation. Table 2 shows that the samples with dry matter contents under 28 %, which were not numerous (3.6 %) as compared to the other ones, had significantly ($P<0.01$) lower values of ADF and NDF contents, but significantly higher values of starch and protein contents, as compared to the other groups of samples, divided by the dry matter contents. Significant differences were found among the other groups (in view of the high number of measurements), but they were not so significant. We can see also a trend of increasing ADF and NDF contents and of reducing starch and protein contents depending on increasing dry matter contents. The evaluation of standard deviations is interesting as well: the group with dry matter under 28 % showed higher standard deviations than the other groups, while the situation was opposite in case of proteins. That leads to the conclusion that younger plants have a relatively stable contents of proteins, while the differences between plants rise with increasing dry matter contents.

Table 1: Correlation coefficients of dry matter and main nutrients

Indicator	Averages	Standard deviation	Variab. coeff.	DM	ADF	NDF	Starch	Proteins
Dry matter (DM)	36.2	4.37	0.12	1.00	0.43	0.50	-0.44	-0.56
ADF	26.1	1.86	0.07	*	1.00	0.98	-0.46	-0.33
NDF	39.1	3.69	0.09	*	**	1.00	-0.42	-0.46
Starch	35.3	1.57	0.04	*	*	*	1.00	-0.09
Proteins	6.12	0.41	0.07	*	*	*	N	1.00

**, high correlation; *, medium correlation; N, low correlation

Table 2: Nutrient contents depending on dry matter contents

Group of	Frequency		ADF		NDF		Starch		Proteins	
dry matter (DM)	n	%	% DM	sd	% DM	sd	% DM	sd	% DM	sd
up to 28 %	184	3.6	20.2^{a}	0.10	26.4^{a}	0.19	37.3^{e}	0.10	7.2^{d}	0.23
28-32 %	856	16.6	26.1^{c}	0.05	38.7^{c}	0.09	35.3^{c}	0.05	6.3^{c}	0.31
32-36 %	1168	22.6	25.7^{b}	0.04	38.3^{b}	0.08	36.4^{d}	0.04	6.1^{b}	0.32
36-40 %	1872	36.3	26.5^{d}	0.03	39.9^{d}	0.06	35.1^{b}	0.03	6.1^{b}	0.34
above 40 %	1080	20.9	26.9^{e}	0.04	40.9^{e}	0.08	34.2^{a}	0.04	5.9^{a}	0.32

sd, standard deviation; Different letters in the indexes of the numbers stand for significant difference (P<0,05)

After evaluating the records from the battery thermo-sensors, we found that in the course of the fermentation, the silage temperature rises by about 0.5 °C in a specific period (usually not longer than 8 hours). We can only speculate about the reason for the time being. The explanation will require further measurements.

CONCLUSION

The use of the system of accurate feeding results not only in increasing amount of milk and improved quality of milk but particularly in saving costs for fodders, in better health of the animals and in reducing negative environment impacts (lower emissions of methane, phosphorus and nitrogen in the animal excrements). Our study was aimed at evaluating the records of measurement of the contents of dry matter and of the contents of selected nutrients acquired during the harvest of maize, under use of the HarvestLab device situated on the John Deere cutter. The records serve to derive the dependence of nutrient contents on dry matter of the chops.

Dedication: QK1810137

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SELECTION OF MAIZE HYBRIDS WITH THE USE OF HARVESTLAB ON JOHN DEERE FORAGE HARVESTER

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Keywords: DNDF, Nutritive value, potencial milk production, software

INTRODUCTION

Selection of maize hybrids according nutritive value is very important factor for production of milk. High variability of maize hybrid according genetic potential is very wide. According Oba and Allen (1999) is very high variability (40 to 70 %) of digestibility of NDF (DNDF) and that have very high effect for determination of NEL (Nett Energy of Lactation) of maize silage. Concentration of energy and level of DNDF have very high effect on intake of dry matter (DM) and potential production of milk. In the last time during harvesting of maize for silage was very popular to use NIR technology, which determines nutritive value of chopped maize. According content of nutritive value determinate by HarvestLab and determination of DNDF for different maize hybrid we evaluate special software for calculation NEL and production of milk per ha and kg of milk per 1 t of DM maize hybrids. New software is a good tool for the selection of maize hybrids for production of maize silage which is the main foodstuff for high-yield cows.

MATERIALS AND METHODS

On the spring 2017 we have planted 9 different maize hybrids (FAO 280 to 290) at agriculture farm named Agrospol a.s. Kninice near of Boskovice town for plots. All hybrids we have harvested at the same day 15.9.2017 by harvester John Deere with HarvestLab device. HarvestLab is NIR sensor developed by the John Deere Co. in conjunction with Carl Zeiss AG Co. already before more than 10 flights. John Deere developer verify the technology AutoLOC. AutoLOC system automatically adjusts longitude chopped forage depending on DM plus according to preset parameters attendance. John Deere its HarvestLab revamped about possibility scan of parameters like DM, ADF, NDF, Starch, Crude Protein (CP) or DNDF. HarvestLab with measurement frequency 17x per second will ensure so lump of dates that the designating high accurate average appreciate given to materials. The dates consumer from field chopper John Deere will relay either reinforcing USB flash disk or are automatically shipped on portal MyJohnDeere.com. For the calculation of concentration of NEL is important parameter of digestibility of fibre. During harvesting is important to take 3 times of samples of each hybrid a determinate at laboratory for chemical analyses (AOAC, 2005) and DNDF by *in sacco* method (Ørskov and McDonald , 1979).

For determination of nutritive value each maize hybrid was developed by new software which calculate all parameters which we need for the calculation of NEL. Software was prepared for HarvestLab as a new tool, which will to calculate ascertained nutritive funds chopped maize hybrids.

RESULTS

We added yield of green matter of chopped maize hybrids in tone per hectare, Content of DM and nutritive value we determined by the new program and we calculated nutritive value for 9 maize hybrids and prepare table 1 and graph 1 where you can see order all of maize hybrids according their quality and yields. According results which you can see at graph 1 we can say that above - average values for milk yield per ha and milk per 1 t of DM was for 2 hybrids, e.g. ES Gallery and AgroVitalo. According the results which we take by HarvestLab we can advise both hybrids for next year to produce maize silage. For next year we advise two best hybrids and for next selection we advise to choose from offer next seeds companies next 4 or 6 hybrids.

CONCLUSIONS

According the yield of tested maize biomass, nutritive value and DNDF, determined by HarvestLab, we can calculate at the new program other indicators of nutritive value quality and potential of milk production per ha and per 1 tone of DM. According results we can choose 2 of the best maize hybrids for our conditions and production of maize silage and production of milk.

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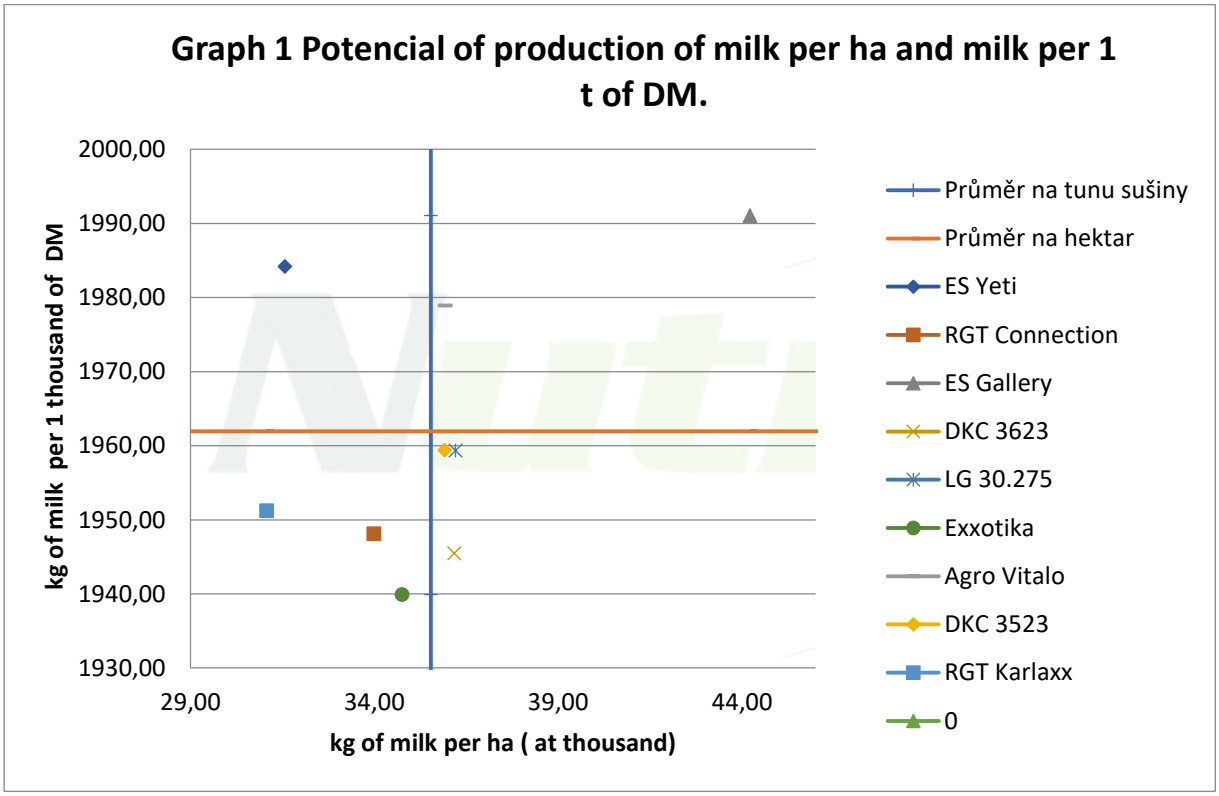
Table: The basic quality indicators of the tested hybrids

Hybrid	DM	DM yield	ADF	NDF	Starch	DNDF	NEL	Methane	Milk Production	
	%	t/ha	% DM	% DM	% DM	% DM	MJ/kg	l/kg DM	kg/ha in thousand	kg/t DM
ES Yeti	30.0	15.9	21.9	32.8	33.0	48.7	6.29	341	31.6	1984
RGT Connection	28.4	17.4	24.9	38.9	33.1	47.6	6.18	352	34.0	1948
ES Gallery	35.6	22.2	22.1	34.4	29.2	48.0	6.31	336	44.2	1991
DKC 3623	28.8	18.6	24.8	38.7	33.6	47.5	6.17	352	36.2	1945
LG 30.275	29.0	18.5	24.1	37.3	33.4	48.7	6.21	349	36.2	1959
Exxotika	29.9	17.9	24.3	38.0	32.5	45.8	6.15	348	34.8	1939
Agro Vitalo	35.3	18.2	22.0	33.9	30.7	46.8	6.27	336	35.9	1979
DKC 3523	30.9	18.3	23.6	36.7	33.3	47.0	6.21	346	35.9	1959
RGT Karlaxx	28.9	15.9	24.6	39.2	32.9	47.9	6.19	351	31.1	1951



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UTILIZATION OF COWSHED TECHNOLOGY OF CONTINUOUS MEASUREMENT FOR CONTROL OF NUTRITION OF DAIRY COWS

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INTRODUCTION

A number of scientific papers, e.g. that by Valente et al. (2017), lead to the conclusion that the utilization of new technologies of so called precise agriculture (detailed analyses based on automatic continuous measurement of different quantities) allows, additionally to more detailed determination of the culmination of rut, also the detection of the onset of illnesses, metabolic disorders, the effect of stressors and other causes of restricted wellbeing of the animals and, thanks to it, taking steps to rectification in real time. The experiment was aimed at finding out how the monitored quantities change during continuous measurement, if the work of the rumen is influenced by an external intervention (brush for stimulation of rumination motor activity, RMS) or by the change of physically efficient fibre (peNDF) of the mixed feeding ratio (TMR), including a higher risk of acidosis at dairy cows.

MATERIAL AND METHODS

The more detailed monitoring was focused in 4 holstein dairy cows with average milk yield in preceding 305-day lactation of 9184 kg and in 66 days after calving of 47 kg/day. At start of the first experimental period (P1), the said dairy cows were perorally applied the eCOW bolus which measured the rumen pH with an accuracy of 0.1 degrees each 15 minutes. Each period took 3 weeks. At start of period P2, a half of the dairy cows were perorally applied the RMS brushes in order to stimulate their rumen motor activity (patent EP0609045A2, Meiwa-Sangyo Co. Ltd, Kyoto, Japan). At start of period P3, the dairy cows were changed the TMRs. The main differences between the TMRs consisted in the structure (the TMR in P1 and P2 had peNDF of 11,4, the TMR in P3 had peNDF of 14,5). The peNDF values were determined under use of Penn State Particle Separator (PSPS) with 19 and 8 mm sieves according to Beauchemin and Yang methodology (2005). The consumption of fodders was continuously measured under use of automatic feeding boxes. To prevent the dairy cows from "enriching themselves" with structural fodder, their bedding consisted of wood shavings only. The cows' feeding activities, frequency of rumination, fodder consumption, amount and quality of drawn milk was continuously recorded every day. The statistical values were calculated under use of the STATISTICA 10 program (StatSoft, USA).

RESULTS AND DISCUSSION

Although the component composition of both TMRs was different, their chemical composition was comparable. A significant difference consisted particularly in the peNDF. While TMR1 had a peNDF of 11.4 %, i.e., according to Plaizier (2004), with higher risk of acidosis (zy (threshold: 12.5 %), TMR2 had a peNDF of 14.5 %, i.e., according to Plaizier (2004), with lower risk of acidosis (threshold: 14 %). A detailed daily monitoring of the activities of dairy cows together with the indicators of yield, consumption of TMR and pH in the rumen (Table 1) offers the opportunity of a detailed analysis of the said factors for the individual dairy cows. Although the cows were offered the same feeding ratio for a period of three weeks at the minimum, the daily fluctuation of the individual indicators was relatively strong. The application of the RMS brushes to the dairy cows in the experimental group (transition from P1 to P2) did not have any marked effect on the monitored indicators, which corresponds with the results of Golder et al. (2017).

Table 1 Average values of continuous measurement of indicators of yield and activities of dairy cows

Indicator	Group	P1 avg	sd	P2 avg	sd	P3 avg	sd
TMR consumption	RF	46.5	4.1	48.3	3.6	46.8	3.1
kg/head/day	C	47.7	6.1	48.7	4.5	46.4	4.1
Eating time	RF	311	51	349	38	307	54
minutes / day	C	251	48	271	33	225	33
Rumination	RF	499	87	523	43	497	80
number / day	C	525	94	569	48	511	50
Drawn milk	RF	45.4	2.5	43.8	2.1	42.5	2.5
kg/head/day	C	42.9	2.8	42.0	1.7	40.2	2.2
rumen pH	RF	5.86	0.28	5.86	0.28	6.00	0.28
average / day	C	5.91	0.30	5.77	0.30	5.81	0.30

The consumption of TMR did not significantly change after the application of the RMS brushes and after the change of the feeding ratio either. After the application of the RMS brushes, the time of eating increased in the experimental RF group, as compared to the control group. The pH values of rumen of the control group decreased from 5.91 to 5.77 (which can be considered critical values) and the decrease survived even the change of the feeding ratio (5.81), while the experimental group with the RMS brushes preserved its pH (5.86) and increased it to 6.0 when the TMRs were changed. The standard deviations of the pH values measured were also higher than those of the experimental group in all periods. As for the other indicators monitored, including the quality of milk (the values are not stated in this paper due to lack of space), no significant influence of the application of the RMS brushes or effect of change of TMR was found.

CONCLUSION

The application of the RMS brushes in the experimental group of dairy cows had no marked effect on the monitored indicators, even in case of change of the feeding ratio. It must be added that both TMRs were deliberately put together so that their parameters were at the threshold of the risk of acidosis. We consider significant only the fact that while the experimental group preserved its rumen pH after the application of RMS brushes at the same level, increasing it slightly after the change of the feeding ratio (by 0.14 pH), the control group without the RMS brushes decreased its pH from the original 5.91 to 5.81. That means that, although the pH of the experimental group was 5.86 and that of the control group was 5.91 in the initial period, the third period experienced the opposite situation: the experimental group had a pH of 6.0 and the control group had a pH of 5.81.

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