Mendelova univerzita v Brně

19th International Symposium FORAGE CONSERVATION

Brno, Czech Republic, April 25-27, 2023

Ing. Václav Jambor, CSc., Ing. Soňa Malá (eds.)

2023

ISBN 978-80-7509-919-8

Mendelova univerzita v Brně
Conference Proceedings

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

19TH INTERNATIONAL SYMPOSIUM

FORAGE

CONSERVATION

Ing. Václav Jambor, CSc., Ing. Soňa Malá (eds.)

25-27TH APRIL, 2023
Brno, Czech Republic

2023

Scientific committee:

Further copies available from:

NutriVet Ltd.
Vídeňská 1023
691 23 Pohořelice
Czech Republic

E-mail: nutrivet@nutrivet.cz
Tel./fax.: +420 519 424 247, mobil: + 420 606 764 260

ISBN 978-80-7509-919-8

Published for the 19th International Symposium of Forage Conservation, 25-27th April, 2023 in Brno by MU Brno (Mendel University in Brno, CZ)
Editors: Ing. Václav Jambor, CSc., Ing. Soňa Malá

Diamant sponzor

Golden sponzors

WWW.VVS.CZ

WWW.LALLEMANDANIMALNUTRITION.COM

WWW.CORTEVA.COM

WWW.PASSIONAG.COM

WWW.ALLTECH.COM

WWW.SCHAUMANN.CZ

WWW.AGRALL.CZ

Silver sponsors

WWW.MRAZAGRO.CZ

WWW.CHR-HANSEN.COM

WWW.NOURIVIT.COM

WWW.NOURIVIT.COM

WWW.BAYER.COM

WWW.BAYER.COM

WWW.STROMPRAHA.CZ

WWW.CLASS.CZ

Bronz sponsors

WWW.SALINITY.COM

WWW.DINAMICAGENERALE.COM

WWW.ATCZ.CZ

WWW.LGSEEDS.CZ

WWW.OSEVA.COM

WWW.AGROTEC.CZ

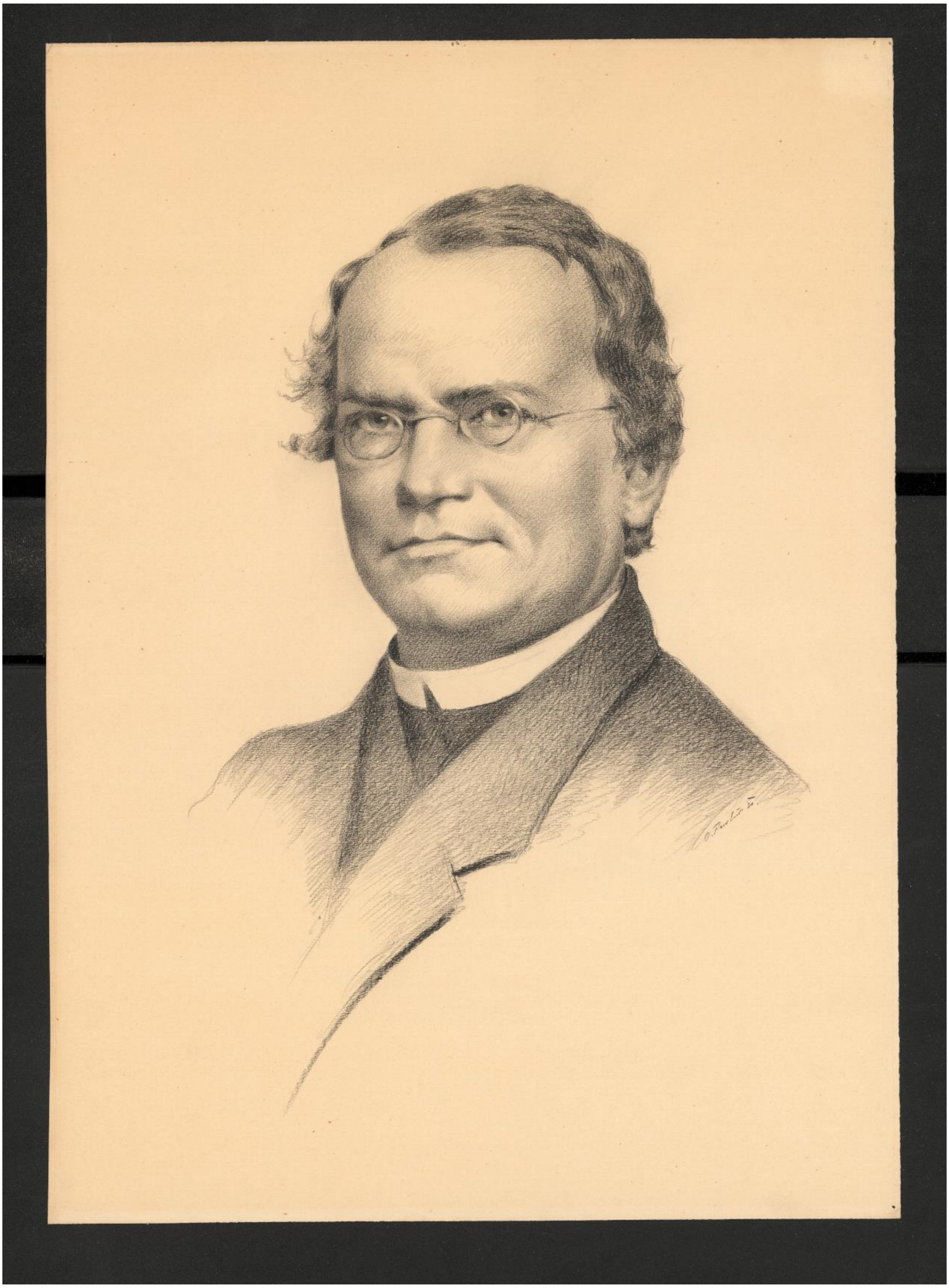
WWW.BIOFERM.CZ

WWW.DEERE.CZ

TABLE OF CONTENTS

SECTION 2: FERMENTATION PROCESS OF SILAGES – HARVESTING, ADDITIVES, CONSERVATION, STABILITY AND STORAGE

POSTERS



Gregor Johann Mendel (1822-1884). Source: Mendel Museum Archive

GREGOR JOHANN MENDEL – A MAN OF MANY TALENTS, PROFESSIONS AND VOCATIONS

KŘÍŽOVÁ, B.
Mendel Museum of Masaryk University, Brno, The Czech Republic

INTRODUCTION

Most people know Mendel as the founder of genetics and for his experiments with pea plants, which he used to explain the Laws of Inheritance. Personally, I am fascinated by the breadth of Mendel's multidisciplinary scope.

Besides his exacting experiments with plant hybrids, he also conducted research in meteorology, beekeeping, or horticulture, while serving as a priest and abbot of the Old Brno Abbey and even the director of a bank.

The Augustinian Abbey, which houses the Mendel Museum of Masaryk University and which I am honoured to have as a workplace, is an immensely inspirational location. In these halls, Faith and Science continue to meet and mingle even today. Items from Mendel's estate are a lasting, and remarkable, testament to this.

FROM THE CHILDHOOD OF A HUMBLE GENIUS

Johann was born in July 2022 in Hynčice, Silesia, into the family of German-speaking small farmers Anton and Rosina Mendel. The exact date of his birth has been disputed as either July 20 or 22. Records in the church mention his birthday and baptism as July 20, along with birth and baptismal records for several other infants born in 1822. However, Mendel himself consistently claimed July 22 as his birth date, the day when he and his family celebrated it. Three of Rosina and Anton's children lived to adulthood. Johann had an older sister Veronika and a younger sister Theresia. Two daughters (both named Rosina) died early in life.

From an early age, the boy was very curious. He found many stimuli in his father's orchard and apiary. Already at elementary school, his teacher noticed his unusual interest in learning. From this time, his life took a course different from that which one might expect from his family background...

THE PATH TO EDUCATION

On recommendation of his teacher Makitta and the parish priest, Father Schreiber, Mendel transferred from the village school to the Piarist school in Lipník nad Bečvou. From 12 years of age, he continued at the gymnasium in Opava. Due to the very poor harvest and his father's accident in the forest, his parents could not fully support their son's studies, and therefore he earned extra money by tutoring his less successful classmates from wealthier families. Out of personal interest, Johann took a course for private tutors, which entitled him to teach privately. In autumn 1840, the Department of Philosophy in Olomouc accepted him as a student. There he came into closer contact with the Czech language, and had to learn it. Funds from his parents and his tutoring were insuficient; he felt ill due to the stress, and spent a year at home. He completed his studies thanks to his sister Tereza, who gave up a part of her dowry. In 1843, Johann Mendel wasrecommended to Abbot C. F. Napp as the most suitable student for the Augustinian Order at the St. Thomas monastery in Brno. Here, he accepted his religious name Gregor (Řehoř in Czech), which, according to tradition, is written before one's first name. He also used the signature Gregor Mendel.

MENDEL'S TIME IN BRNO

Between 1845 and 1848, Mendel studied at the Department of Theology in Brno. He was interested in agriculture, fruit-growing, and viticulture. In 1847 he was ordained a priest. His university studies in physics, mathematics, and natural sciences in Vienna in 1851–1853 were a great contribution to his knowledge. He temporarily taught in Znojmo, and between 1854 and 1868 at the imperial-royal state secondary school (realschule) in Brno. Although he was a respected and popular teacher, he did not manage to pass the teacher's university exams. This, however, did not prevent him from persistent self-study, and openness towards new information. In 1868 he became the Augustinians' abbot, and gradually he became a highly esteemed church figure. The dignity of the abbot's office brought him important positions. For instance, in 1881 he was appointed the director of the Mortgage Bank of Moravia (Hypoteční banka), which also brought him a better income. Mendel also became a member of the Meteorological Society, the Pomological Society, the Imperial-Royal Moravian-Silesian Society for the Improvement of Ploughing, Natural Science and Homeland Studies, the Natural Scientific Association in Brno, and the Zoological-Botanical Society.

THE ORIGINS OF THE NEW SCIENCE

The knowledge gained in Vienna greatly influenced Mendel's future scientific work. With the contribution of the famous physicist Christian Doppler, Mendel deepened his knowledge of mathematics, and mastered the basics of mathematical analysis, which was to become a fixture of his own scientific method. An experimental garden, a heated greenhouse, and an orangery in the abbey garden made Mendel's long-term experiments with pea plants possible in the middle of the 19th century. He also crossbred other plant species, collaborated with breeders, and was happy to share his newly acquired experiences with them.

During his experiments, the scientist cultivated about 27,000 pea plants. He published his results in 1866 in his seminal work Experiments on Plant Hybrids (Versuche über Pflanzen-Hybriden). Nine years of research yielded three laws; misunderstood at that time – but today known world-wide as Mendel's laws of inheritance.

After being appointed as the Augustinian abbot, Mendel did not have much time for experiments. He devoted more time to beekeeping and meteorology. He had an apiary including a small study built in the abbey garden, and three times a day he carried out meteorological measurements, meticulously recording them. Gregor Mendel, seriously ill, died on 6 January 1884, and he was buried at Brno Central Cemetery. The music at the Requiem Mass was most likely conducted by Leoš Janáček. However, Mendel's life and scientific story did not end here – it lives on, and continues to fascinate and inspire.

OFFICIAL RECOGNITION AFTER DEATH

Mendel was the first to discover that genetic endowments (genes), not traits, are inherited. His ingenious idea consists in the observation that traits are pair-based. The legendary natural scientist explained the origin of genotype and phenotype splitting conditions as a result of the pair-based foundation of traits. With great care, he statistically processed the observed phenomena, and compared them to the assumed outcomes.

Mendel is also behind the basic methodology of the study of heredity, which is still used in the crossbreeding of plants and animals. Unfortunately, his presentation of his results in 1865 received only a minimal response. Although his work was sent to about 130 scientists and institutions in Europe as well as overseas, it remained misunderstood and largely unanswered.

Mendel's work received official recognition only posthumously, in 1900. The rediscovery of Mendel's work, which laid the foundation of genetics, was made by Carl Correns, Hugo de Vries, and Erich von Tschermak. Independently of each other, they reached similar results as Mendel. However, when they found out that Mendel had preceded them by about 40 years, they honestly acknowledged his pioneering achievement, and named the newly formulated laws after him.

Even 200 years after the birth of the humble genius, the power of his spirit and his immense effort to push the boundaries of our knowledge continues to fascinate us. Let us set out to discover new horizons, just as Gregor Johann Mendel did in his time!

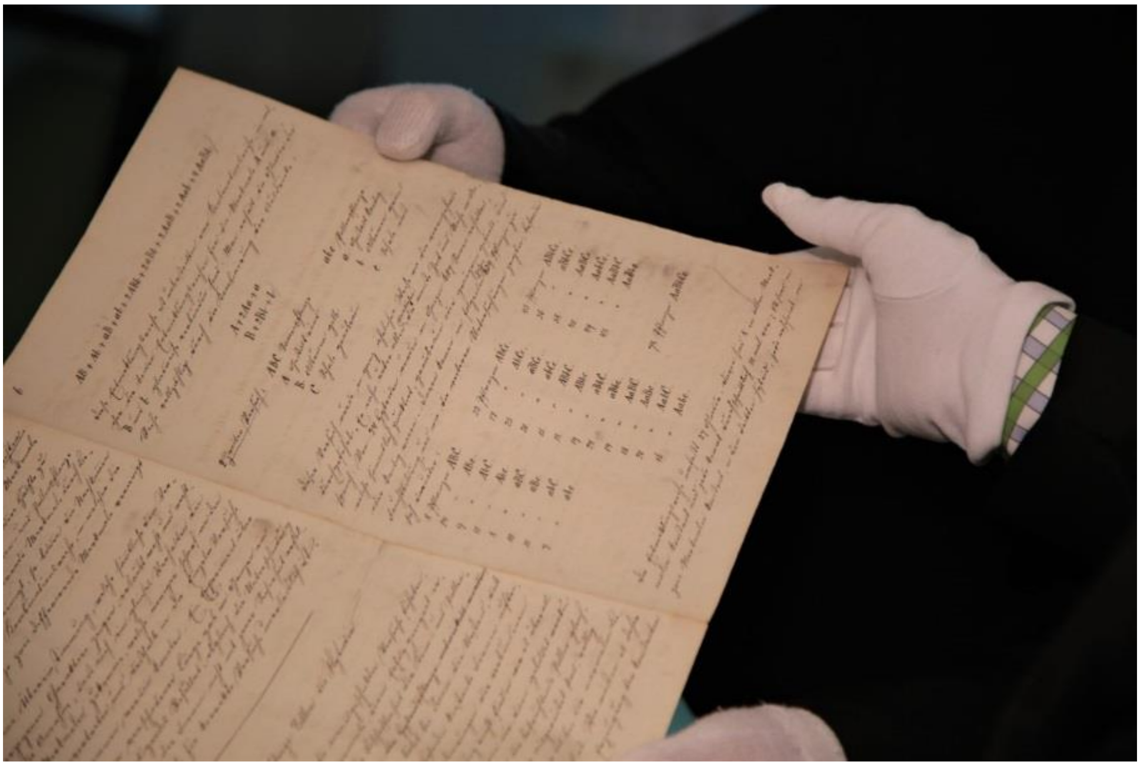


Figure 1: The manuscript of Mendel's seminal work Versuche über Pflanzen-Hybriden (Experiments on Plant Hybrids), published in 1866. Source: Mendel Museum Archive

MENDEL THE GENETICIST

Gregor Johann Mendel is primarily a renowned name in contemporary genetics. He introduced mathematical assessment and symbols, which allowed him to systematically plan experiments, better evaluate the data, and draw general conclusions, which was revolutionary in his time.

According to Mendel, endowments (today we would say genes) are received from both parents – not only from one, as many expected. The fact that we do not simply receive individual traits from our parents, which would only be averaged, but that we inherit endowments that manifest as particular features, was in contradiction to the theory of blending inheritance, which was widely accepted at that time.

The researcher found that hereditary units (genes) must be material, and they transfer to the offspring through gametes. Apart from pea plants, he also crossbred other plant species to obtain new varieties. He was ahead of his time when he formulated three principles, later known as Mendel's laws of inheritance. In 1866 he published a world-famous publication on the principles of inheritance called Experiments on Plant Hybrids (Versuche über Pflanzen-Hybriden). Although the work did not immediately receive due attention, he knew that his time would come…

Mendel realised exactly which scientific topics were crucial for society, and he wanted to contribute to developing knowledge of them. He was not interested in fame, titles, or public recognition. He did science exclusively for science, in an extraordinarily systematic way, with great diligence, but also humility, which allowed him to reach the very essence in his research.

Mendel's good reputation as the founder of genetics became widespread only due to those who rediscovered his work at the beginning of the 20th century. The British biologist William Bateson, who was the first to use the term "genetics" to refer to the science of heredity, deserves particular credit. Today, this dynamic scientific discipline influences our lives more than ever…

MENDEL THE ABBOT

Entering the monastery meant new opportunities for Mendel's education and scientific work. He graduated from his studies in Vienna, which had a decisive influence on his later experiments on pea plants and other plant species. In his study of mathematics and physics, he learned how to make statistical inferences, how to plan experiments, and in general he mastered the methodology of scientific work very well.

Mendel's societal role was greatly supported by his appointment as abbot in 1868. He assumed the position after the deceased Abbot Napp, and became a highly respected church figure. Abbot Mendel's contributions to the overall flourishing of society were appreciated in 1872 by the emperor, who awarded him the Commander's Cross of the Order of Franz Joseph.

Although his research activities had to yield to his duties as abbot, he did not neglect his pastimes, which were often investigative. The monastery garden provided him with enough space for beekeeping, meteorology, and growing fruit trees, and so he was able to combine his hobbies.

Abbot Mendel made a great effort to fight the increasing taxation of monasteries in the 1870s. He also worked as the director of the Mortgage Bank of Moravia (Hypoteční banka), and took on other responsibilities. Despite the important position that he achieved in the community, he always remained a modest man who generously supported his family as well as his socially disadvantaged students.

MENDEL THE METEOROLOGIST

Two-thirds of Mendel's work was concerned with meteorology, which represented an exciting way for him to try to predict the weather, and the possibility of helping farmers plan their work in the fields. Mendel apparently considered himself to be more of a physicist than a biologist. Many of his measuring devices have been preserved. What drew him to meteorology was undoubtedly his study of physics, but also his close relationship to nature, and his peasant background. He started making his daily meteorological measurements after entering the Augustinian monastery, specifically at the local Saint Anne's Hospital.

Thanks to Mendel, Brno has the second longest record of meteorological measurements in the Czech lands (after Prague's Klementinum). They were meticulously kept according to the regulations of the time, and in addition, they contain detailed notes, which demonstrate the researcher's sense of order.

A unique feature is Mendel's written description of a tornado (the first in the world), which hit a part of Brno on 13 October 1870. He called it a "vicious wind", and described the whole event as a "thunderous drama". The scientist's exact description of the tornado includes a number of details, including the specification of damages.

As a profoundly faithful man, Mendel humbly catalogued the divine order with his research. In honour of his meteorological activities, Czech researchers from Masaryk University gave his name to an Antarctic research which was constructed between 2004 and 2006. It is called the Mendel Polar Station.

MENDEL THE FARMER

His family background as well as his first teacher aroused in Mendel an interest in nature and farming, in particular beekeeping and fruit growing. Brno provided the ideal environment for research, as it was called the Austrian or Moravian Manchester, due to its sheep breeding and advanced industry and science. In 1870 Mendel was elected a member of the Moravian-Silesian Society for the Improvement of Agriculture. He was significantly involved in the Commerce Society (Hospodářská společnost). He regularly travelled across Moravia, in particular visiting Augustinian farms. His most distant travel was to visit the 1862 International Exhibition in London. • The advantage of Mendel's agricultural studies was their immediate practical application.

He always tried to improve the agricultural conditions in Moravia. For instance, he was respected for his cultivation of fruit trees. He was also the first to scientifically cultivate a better tasting variety of pea which retained this property in subsequent generations.

MENDEL THE BEEKEEPER

The fundamentals of beekeeping were revealed to the young Johann Mendel by his father Anton in Hynčice. The abbey garden provided ideal conditions for this great hobby, while the technical framework was facilitated from 1870 by the Moravian Beekeeping Association. An apiary designed by Mendel has been preserved to this day.

Mendel was attempting a controlled cross-breeding of bees in order to gain better properties for his bee colonies. However, he was not successful in this effort due to his lack of knowledge of the precise way bees reproduce. Still, he described various ways of wintering bee colonies, and of simplifying apiaries.

Figure 2: Mendel's apiary (photo from 2021). Source: Mendel Museum Archive

MENDEL THE POMOLOGIST

Mendel also has merits in the field of pomology – the description and study of fruit varieties. To the end of his life, he cultivated fruit trees, trying to improve the production and flavour characteristics by combining selected traits of the parent forms.

He was active in organising fruit exhibitions, he set up competitions for cultivating new varieties, and also presented new varieties himself. Thanks to his study of the latest pomological literature and his knowledge of the inheritance of the traits which he could manipulate, he stood out among other gardeners of the time.

MENDEL THE ASTRONOMER

Gregor Johann Mendel also left his mark in astronomy, for instance by his study of the effects of sun spots on local climate. Craters on the moon and on Mars were named after Mendel, but above all, also one minor planet in our solar system, which is by coincidence about the same size as the area of Brno. The Mendel Museum keeps books about the universe which Mendel used to inform his astronomical observations.

REFERENCES

Orel, V. (2003). Gregor Mendel a počátky genetiky. Praha: Academia
Klein J., Klein N. (2016), Osamělost skromného génia, díl 1. Brno: Moravské zemské muzeum
Relichová J., (2022), Johann Gregor Mendel. Brno: Společně, o.p.s.
Fairbanks D. J., (2022), Gregor Mendel His Life and Legacy. Prometheus Books

FORAGE FARMING FOR THE 21ST CENTURY

NEDĚLNÍK, J.
Research Institute for Fodder Crops, Ltd., Troubsko

This post was written at a time when Europe and the whole world are facing challenges that we could not have imagined a few years ago. The Russian invasion of Ukraine shows that, unfortunately, peace and stability cannot be considered the norm in Europe either European value, interests, security, independence and integrity are neither self-evident not free. Europe must be ready and prepared to defend them and actively contribute to preserving peace, preventing conflict and strengthening international security. The issue of security must be conceived in a broader sense; it is not 'only' about the defence of populations and territories, but also about the security of food, raw materials and energy supplies. In the immediate aftermath of the unprecedented COVID-19 pandemic and social isolation, Europe is thus facing new crises to which it must respond.

At the same time, the implementation of the new common EU agricultural policy 2023+ enters into practical life. Fodder has always played, and I believe will continue to play, an important role in all aspects of the sustainable development of Czech and global agriculture. Both on arable land and on permanent meadow pastures. In the Czech Republic, fodder farming has a long and rich tradition and a very high level of cultivation. Both in fodder production and in seed production. This is primarily due to the erudition of the farmers, but also to the excellent level of Czech breeding. Modern varieties of the main forages alfalfa and red clover, varieties of minor forages or the entire range of varieties of grass species belong to the world's top.

The spectrum of perennial fodder crops grown on arable land in the Czech Republic is varied in terms of species, but alfalfa and red clover are still the main clovers used to produce fodder for cattle. As with all other agricultural commodities, the issue of fodder on arable land must also be placed in the context of the long-term development of Czech agriculture. The term non-market crop still persists, at least for fodder for feeding, based on the fact that economic appreciation will only occur when livestock production is carried out. Fodder crops, perhaps most of the crops on arable land, were affected by structural changes in Czech agriculture during the 20th century. It mainly concerns animal production. Cattle numbers remained at three million head for almost the entire 20th century, with a few annual fluctuations. In 2010, the number of cattle reached the threshold of 1.4 million. Thus, there were less than 0.4 cattle per hectare of registered land. At the same time, however, it must be noted that the intensity of milk production has increased significantly and this needs to be remedied by increasing the quality of roughage. In Czech agriculture, the average annual feed ration for cattle is composed of one quarter of perennial fodder on arable land, a slightly smaller proportion of forage from grasslands, a significant component of the feed ration is corn silage. It is clear from this ratio that, despite the declining numbers of livestock, which had a negative effect on the declining areas of forage, forage retains its importance. Another indisputable benefit of fodder is their function of improving the soil structure and microbial composition of the soil. Their pre-crop value is indisputable.

The production importance of clovers lies in relatively constant yields independent due to the ability of symbiotic fixation on nitrogen nutrition. This is especially important in today's high nitrogen fertilizer prices. Clover fodder has a high content of N-substances, but also mineral elements and vitamins, and it is possible to ensure a continuous increase in fodder during the majority of the growing season by combining the cultivation of separate stands and mixtures. The extra-productive importance of clovers is primarily in increasing and stabilizing soil fertility, in a significant melioration effect, their anti-erosion function and weeding effect are also significant.

If we follow the annual development of areas and yields of the main clover crops in the Czech Republic after 1990, there is a noticeable tendency of their permanent decrease with interannual fluctuations. A positive factor is a certain stabilization of income after the slump at the end of the nineties and after 2000. After 1990, the proportion of main clovers grown in the Czech Republic also changed significantly. While in 1990 the acreage of meadow clover was over 192,000 hectares, in 2009 46 thousand. There is a similar trend with alfalfa. In 1990, there were 155 thousand ha, in 2009 68 thousand ha.

It is the desire of every forage farmer to maintain a certain degree of cultivation of these crops on arable land for the reasons mentioned above. On the other hand, realistically, it is necessary to find ways to supply feed of the highest quality for intensive breeding. In addition to the innovative inputs, there is a whole area of breeding, the output of which should be qualitatively new materials. Breeding goals are primarily oriented towards improving the quality of green fodder while maintaining high and stable yields. The orientation towards the search for new genotypes with improved parameters of tolerance to abiotic stresses, including drought, is clear. In the absence of suitable preparations, even chemical or biological ones, for plant protection, the creation of genotypes with resistance to biotic agents is still relevant.

The fact that in 2007 the Czech Republic was the largest European producer of certified red clover seed (1,549 t) and also among the top five in Europe for alfalfa is a testament to the high level of Czech forage production, but also forage seed production.

The spectrum of cultivated clovers is significantly poorer as spectrum of grasses. The dominant species are *Trifolium pratense* L., *Trifolium incarnatum* and *sativa* L. In 2021, other clovers were grown on less than 1 % of the areas only. Among them, the largest growing areas were those of *Onobrychis viciifolia* Scop. and *Lotus corniculatus* L. A positive element is the introduction of other non-traditional species of the *Fabaceae* family in order to use them for agricultural and environmental purposes.

Figure 1: *Medicago sativa*

The number of cultivated varieties (mainly the three main leguminoses) counts approximately one hundred. These are varieties registered both in the Czech Republic and in the European Catalogue of Varieties. The long-term average yields of the seeds of three main clovers are not high. Average seed yield for clover is about 220 kg/ha, alfalfa about 150 kg/ha and at *T. incarnatum* more than 450 kg/ha. The total production of leguminous seeds fluctuates, depending on many factors (species composition, harvest year, etc.). In recent years, the lack of some preparations (desiccants) used for pre-harvest treatment of *Trifolium pratense* L. and *Trifolium incarnatum* L. has been another significant factor.

Figure 2: *Trifolium incarnatum*

Czech clover seeds are an excellent and concurrently traditional export commodity. The data for the period from 2015-2020 shows that overall exports significantly outweighed imports. However, there are significant differences in particular species. *Trifolium pratense* remains a prominent export commodity, as well as species classified as "other clovers", dominated by *Trifolium*. However, *Medicago sativa* L. has become an imported item in recent years. In the last two years (2020-2022) we can see an increase in imports of, and a slight decrease in exports of Czech clover seeds. Czech certified clover seeds are valued abroad mainly for their quality and prices. The Czech Republic holds a good position among the leading European clover producers (5th-6th place within EU countries).

Figure 3: Clover – variety PRAMEDI

So what should forage farming be like in the 21st century? It should be intensive and landscape-forming. It should use modern varieties adapted to changing soil and climate conditions. It should be based on modern technologies for establishing stands as well as modern harvesting technologies enabling maximum use of fodder potential on the one hand and seed performance on the other. Let's hope that fodder crops will gradually return to crop rotations in the Czech Republic and will create a factor of soil-improving crops, which will also leave a non-negligible amount of nitrogen on the site. One of the main problems of Czech agriculture is stagnant or deteriorating soil quality, and fodder crops are ideal crops that could reverse this trend. In the new rules of the common agricultural policy, fodder is included in a wide range of agro-envi measures as a stabilizing element. The Czech Republic has the advantage that, thanks to modern breeding, new varieties of a wide range of forage usable species are created. Their pure cultures or especially mixtures are not only an important landscape-forming element, but also an important environmental factor.

STRATEGIES TO IMPROVE SILAGE FIBER DIGESTIBILITY

FERRARETTO, L.F.
Assistant Professor and Ruminal Nutrition Extension Specialis, Department of Animal and Dairy Sciences, University of Wisconsin - Madison

INTRODUCTION

Whole-plant corn silage (**WPCS**) is the predominant forage used in dairy cattle diets worldwide. On average, approximately 110 million Mg of fresh corn forage per year is harvested in the United States. Besides providing energy for maintenance and lactation, coarser WPCS particles stimulate chewing and salivation, rumination, gut motility and health, regulate feed consumption and are the structural basis of the ruminal mat, which is crucial for ruminal digestion. Starch and fiber are the main sources of energy for dairy cows fed corn silage-based diets and therefore improvements in digestibility of these nutrients may increase milk production or reduce feed costs through enhanced feed efficiency. Greater digestibility of WPCS fiber is desired for productivity, profitability, and environmental reasons. The purpose of this paper is to review selected recent developments and strategies that may influence the nutritive value of WPCS with a focus on fiber digestibility. Furthermore, this manuscript discusses the efficacy, mode of action, benefits, and disadvantages of different technologies for improving fiber digestion.

FIBER DIGESTIBILITY

Incomplete fiber digestion reduces the profitability of dairy production by limiting intake and hence, animal productivity. A 1%-unit increase in in vitro or in situ forage NDF digestibility (**NDFD**) is associated with 0.17 and 0.25 kg/d increases in DMI and 4% fat-corrected milk production, respectively (Oba and Allen, 1999). Lignin is the key obstacle to fiber digestion as it obstructs the enzyme access to the digestible fiber fractions, cellulose, and hemicellulose. In addition, rumen microorganisms cannot breakdown lignin. Due to its importance to animal performance, this association between lignin and other fibrous fractions (i.e. cellulose and hemicellulose) is considered in many diet formulation models. This undigested or indigestible NDF fraction is estimated using either lignin or quantified as the proportion of NDF remaining after in vitro or in situ ruminal incubations (i.e. 240 h uNDF). Thus, the reduction of lignin or undigested NDF fractions in forages improves fiber digestibility.

Genetic improvement

Improvements to fiber digestibility of forages are often accomplished by reducing lignin or undigested NDF concentrations (Grant and Ferraretto, 2018). Brown midrib mutant forages (e.g., corn and sorghum) consistently have lower lignin concentrations compared to conventional forages (Sattler et al., 2010) resulting in greater milk production when the BMR forages are fed. In this context, several studies have reported greater DMI, passage rate, and rate of NDF digestion in cows fed BMR compared to conventional corn silage. In a meta-analysis of published studies, Ferraretto and Shaver (2015) reported increases in total tract NDFD (44.8 vs. 42.3% of intake), DMI (24.9 vs. 24.0 kg/d), yields of milk (38.7 vs. 37.2 kg/d) and protein (1.18 vs. 1.13) for cows fed BMR diets instead of conventional corn silage diets. A summary of studies evaluating BMR corn silage conducted more recently is in Table 1. These benefits are associated with lower rumen gut fill as conventional forage-based diets may have lower rates of passage and digestion, causing physical constraints in the rumen that limit intake.

Table 1. Effect of BMR hybrids on lactation performance by dairy cows[1]

Study	DM intake, kg/d	Milk Yield, kg/d	ECM Yield, kg/d	Milk fat, %
Lim et al., 2015	NS	+2.2	+2.1	NS
Cook et al., 2016	NS	+3.9	+2.9	NS
Hassanat et al., 2017	+1.6	+3.2	+2.9	-0.11
Coons et al., 2019	+1.2	+3.5	+3.1	-0.15
Miller et al., 2020	+0.6	+2.3	+1.4	NS
Miller et al., 2021	+1.5	+2.9	+2.8	-0.07

[1]Data presented as BMR difference to control.

Genetic improvement resulted in BMR hybrids that are higher yielding than earlier hybrids. Nevertheless, it is important to account for lower yields of certain BMR hybrids than conventional hybrids when deciding on which hybrid to grow (Adesogan et al., 2019). UW Hybrid trials reports suggest yield drags from 10-15% and a reduction in starch concentration. Such lower yields may be outweighed by the improved animal performance from BMR hybrids, but the magnitude of the improvements may vary from farm to farm based on the prevailing conditions. Producers should consider establishing guidelines for using BMR hybrids such as feeding them to high-producing cows in early lactation while feeding less digestible conventional hybrids to cows in mid-to-late lactation. Such guidelines should be based on discussions with dairy nutritionists and crop consultants who are acquainted with the specific conditions of the dairy farm.

Chopping and shredding

Despite the undeniable benefits of coarser forage particles on ruminal mat formation, chewing activity, digestion, and milk fat content (Allen, 1997; Mertens, 1997), long forage particles may limit intake through reduced ruminal passage rate and increased fill (Mertens, 1987). Furthermore, they promote dietary sorting (Leonardi and Armentano, 2003), and enhance the time spent consuming a meal (Grant and Ferraretto, 2018). Although particle size can be manipulated to enhance fiber digestibility, research findings have been inconsistent, and the outcome is not related to alterations in the chemical composition of forage fiber. A meta-analysis of published studies (Ferraretto and Shaver, 2012b) reported that digestibility of dietary NDF, DMI, and milk production were not altered by chop length of corn silage. This should not be surprising as fiber digestion is influenced by many factors and the combination of the benefits of long or short forage particles may be countered by the disadvantages. For instance, short forage particles have greater surface area for bacterial attachment, which may enhance forage digestibility despite their faster passage rate (Johnson et al., 1999). In contrast, coarse particles are retained for longer periods in the rumen and require more chewing leading to greater ruminal pH (Allen, 1997), which is conducive for cellulolytic bacteria and forage digestion in general.

Shredding ensiled whole-plant corn at harvest is an effective method to alter the physical characteristics of the silage. A recently developed form of silage, called corn shredlage, is produced when corn forage is harvested with a self-propelled forage harvester fitted with cross-grooved crop-processing rolls set at approximately 20% greater roll speed differential and chopped at a greater theoretical length of cut (22 to 26 mm) than the norm. Despite the longer chop length used, the shredding process causes greater damage to coarse stover particles and kernels than conventional harvesting. Compared to conventionally processed silage, yields of 3.5% FCM and actual milk were increased by 1.0 and 1.5 kg/cow per day when whole-plant corn was harvested as corn shredlage using either conventional (Ferraretto and Shaver, 2012a) or brown midrib (**BMR;** Vanderwerff et al., 2015) hybrids, respectively. These results were attributed to the greater kernel breakage obtained by shredding whole-plant corn and the corresponding improvements in ruminal in situ and total tract in vivo starch digestibility (Ferraretto and Shaver, 2012a; Vanderwerff et al., 2015). Surprisingly, despite what appeared to be more thorough damage to the fibrous portion of the forage, Vanderwerff et al. (2015) reported that total tract NDFD was 2 percentage units lower when cows were fed corn shredlage instead of conventionally processed corn silage. These authors associated this response with the negative effects of the greater digestibility of shredlage starch on total tract NDFD. This premise was supported by the fact that ruminal in situ NDF digestibility of undried and unground corn silage samples did not differ among treatments. Finally, near-infrared reflectance spectroscopy-predicted 30-h NDFD was lower in corn shredlage (55.0 vs. 53.4% of NDF) compared to conventionally processed corn silage in an assessment of 3,900 commercial samples (Ferraretto et al., 2018). Although the benefits of harvesting corn silage with a shredlage processor are undeniable, some factors must be considered when evaluating the cost effectiveness. In addition to the costs associated with acquiring the processor (or a new self-propelled forage harvester), other factors such as changes in fuel usage and roll wear must be considered as they may differ from those involved in conventional processing. To our knowledge, this information is unavailable in the literature and should be the focus of future research.

Chop height and toplage

A harvesting management option to reduce lignin concentration is chop height. With enhanced chop height more lignin is left with the portion that remains in the field, and thus, digestibility of the harvested material is greater. Results from a recent industry-university collaborative study from our group is in Table 1 (Ferraretto et al., 2017). Although our study compared 15 vs. 60 cm, these results are similar to other trials comparing 15 vs. 45 cm of chop height. Briefly, DM yield is reduced as the row-crop head is raised. This is consistent across several studies conducted across the United States. However, decreased DM yields are offset by an increase in the milk per ton estimates at the higher chop height. Greater milk estimate is a response to the greater fiber digestibility and starch concentration of the harvested material. In addition, most studies reported that estimated milk per acre is reduced by only 1 to 3% with high-chop. Also, increased quantities of high-chop silage could be included in the diet, rather than corn grain being added to the diet, providing an economic benefit to implementing increased chop heights.

Nigon et al. (2016) modified a conventional ear-snapping corn header with cut-off knives in an effort to collect more of the upper stalk and leaf portion of the corn plant. The goal was to produce a silage product that fell between high-cut WPCS and snaplage and was referred to as toplage. Yields of DM were reduced by 23 to 28% compared to WPCS. Furthermore, this approach allows a second operation to collect the remaining stalks, and this fractionation was suggested as a tool in ration formulation (Cook et al., 2016); for example, by separating and feeding the less digestible fraction, the stalks, to animals of lower energetic demand (i.e. heifers, far-off dry cows). To our knowledge, experiments evaluating the effects of replacing WPCS or high-cut WPCS with toplage in dairy cattle diets are unavailable in the literature. However, a recent study by Cook et al. (2016) fractionated corn plants at harvest, as toplage and stalklage, and treated the lower plant fraction with calcium oxide to enhance fiber digestibility. Alkali treatments are effective at breaking hemicellulose-lignin and lignocellulose bonds, hydrolyzing uronic and acetic acid esters, and disrupting cellulose crystallinity by inducing cellulose swelling (Jung and Deetz, 1993). These processes increase cell wall degradability and enable ruminal microorganisms to attack the structural carbohydrates and increase degradation of hemicellulose and cellulose (Jung and Deetz, 1993). Additionally, alkali treatment has potential to degrade lignin, thereby increasing its water solubility and allowing it to be removed from the cell wall (Chesson, 1988). Cook et al. (2016) evaluated: 1) the effects of alkali-treated stalklage in combination with toplage on lactation performance by dairy cows in relation to negative (conventional WPCS hybrid) and positive (BMR WPCS hybrid) controls, and 2) the effects of alkali-treating WPCS. Overall, alkali-treatment of WPCS and fractionated WPCS were successful at improving intake and milk production by dairy cows similarly to the well-established effects of BMR WPCS.

Recently, Paula et.al (2019) conducted a meta-analysis to evaluate the effects of chop height on nutrient composition and yield of WPCS. The yield of DM was reduced by 0.05 Mg/ha for each cm of increased chop height. However, for each cm of increase in chop height there was an increase of 0.09, 0.08, and 0.08%-units in DM, starch, and ruminal in vitro NDF digestibility, respectively. A negative linear effect was observed for NDF, with a 0.10%-unit decrease per cm of increase in chop height. Table 2 summarizes this effect for a 25-cm increase.

Table 2. Effects of cutting height on nutritive value of whole-plant corn silage. [1,2,3]

Parameter	n	Effect
Dry matter, % of as fed	62	+2.18
NDF, % of DM	64	-2.48
Lignin, % of DM	25	-0.29
NDFD, % of NDF	49	+2.02
Starch, % of DM	55	+2.08
DM yield, ton/acre	52	-0.52

[1] Adapted from Paula et al. (2019).
[2] Data expressed as expected response per each 25-cm of increased cutting height.
[3] NDFD – ruminal in vitro or in situ NDF digestibility at 30 or 48 h.

Using these responses, we calculated the effect of increasing chop height from 15 to 60 cm, these results are reported in Table 3. Briefly, we used Ferraretto et al. (2017) 15 cm treatments as baseline and simulated what the response would be for 60 cm. Overall, responses were similar to the observed. Our next goal is to validate these equations. Perhaps in the future these equations could be used in team discussions among farmers, nutritionists and crop consultants to determine individual farm priorities for maximum yield versus higher quality prior to the establishment of new chop height guidelines.

Table 3. Predicted effects of chop height on whole-plant corn silage nutrient composition, digestibility, and yield.

Item	Normal chop height[1]	Simulation[2]	Simulation[2]
Cutting height, inches	6	16	26
NDF, % of DM	37.7	35.2	32.7
Starch, % of DM	37.5	39.6	41.6
ivNDFD[3], % of NDF	49.6	51.6	53.6
Yield, Mg/ha	22.0	20.0	19.8

[1]Data from Ferraretto et al. (2017).
[2]Predicted using equations from Paula et al. (2019).
[3]Ruminal in vitro NDF digestibility at 30 h.

Plant population

Increasing plant density is a useful strategy to improve yield and reach required forage inventories. Plant densities have been increasing over the last 30 years with innovations in technology, management practices, and plant genetics. But what is the ideal plant density for silage production? This answer undoubtedly changes for each farm and likely for separate fields within a farm. However, a summary of multiple studies conducted at UW-Madison (Lauer, 2019) may help us navigate through this question. This analysis revealed plant densities currently used commercially are lower than the predicted for maximum silage or grain yield, but similar to the economic optimum, which for this study was approximately 86,000 plants/hectare. Also, milk per ton, a well-known metric for silage nutritive value, decreases as plant population increases indicating worst forage nutritive value. But all tested densities had nutritive value within 95% of the maximum.

However, this effect of plant density on nutritive value is variable in the literature with a reduction in nutritive value reported in some studies but with no effects in others. Plant population could be used to increase yield per area without compromising the nutritive value of WPCS. Thus, the combination of greater plant population with increased chop height could be of interest to maintain yield while increasing silage of WPCS. In our study (Ferraretto et al., 2017), we compared 2 chop heights (15 vs. 60 cm) and 4 plant populations (64,000, 79,000, 94,000 and 109,000 plants per hectare). This experiment was conducted in Wisconsin. As aforementioned, increased chop height improved the nutritive value of whole-plant corn forage at the expense of yield. In contrast, plant population affected yield but not quality of whole-plant corn forage. No interactions were observed. However, the effects of plant population may be affected by location and season. Diepersloot et al. (2021) aimed to evaluate the effect of plant population on yield, nutrient composition and ruminal in vitro NDF digestibility at 30 of whole-plant corn forage grown in Florida during the summer of 2016 and spring of 2017. Plant population effects on nutrient composition were inconsistent across seasons. In the spring, increasing plant population from 65,000 to 75,000 plants per hectare increases DM yield. However, DM yield of the summer crop was not affected by the same change in plant population.

Because each farm has unique challenges for forage production, testing plant density under their conditions is a nice first step. Planting two or three rounds of a chosen field with 10% greater plant density than the current density allows for assessment of plant characteristics (i.e. tillering, ear size and fill, lodging) throughout the growing season and yield and nutrient analysis at harvest. A brainstorming meeting between farmer, crop consultant, agronomist, and nutritionist to streamline this test and implementation of new plant density is advised.

Storage length

Although allowing extended storage may be beneficial for increasing starch digestibility (as reviewed by Kung et al., 2018), research does not support the same fate for NDF digestibility. Overall, data from several sites across the U.S. demonstrate that extended storage does not change or slightly reduces NDF digestibility in corn silage (Kung et al., 2018). In addition, a recent study by Gerlach et al. (2018) evaluated in vitro gas production of the NDF fraction in WPCS stored from 0 to 120 d. Fiber digestibility was unaffected by prolonged storage.

Bacterial inoculants

Applying exogenous fibrolytic enzyme (**EFE**) with microbial inoculants to WPCS at ensiling is beneficial as they may hydrolyze plant cell walls into sugars that serve as fermentation substrates, thus improving silage fermentation, nutrient preservation, and utilization of the silage by animals (Muck and Bolsen, 1991). Consequently, some silage inoculant preparations contain fibrolytic enzymes, mainly cellulases or xylanases, and some studies have reported increased silage NDFD due to application of such products (Queiroz et al., 2012).

Certain inoculants contain bacteria that secrete fibrolytic enzymes including cellulases, xylanases, and ferulic acid esterase (FAE) that may contribute to increased fiber digestion. Addah et al. (2011) reported that using a mixed bacterial culture containing *L. buchneri* LN4017 that produces FAE, and contains *L. plantarum* and L. casei, increased in situ NDF disappearance after 24 and 48 h of incubation by 40.5 and 14.5 %, respectively. Unfortunately, the enzyme activities or enzyme-secreting ability of inoculant bacteria are rarely declared on inoculant labels. Nevertheless, in recent meta-analyses, although no effects on NDFD were observed when bacterial homofermentative and facultative heterofermentative inoculants were applied to forages, milk yield was improved (Oliveira et al., 2017). More information is needed on the enzyme activities produced by inoculant bacteria, as this may lead to development of inoculants that are more potent at increasing fiber digestion.

IMPLICATIONS

Using brown midrib hybrids has been among the most consistent, cost effective and adopted strategies to increase forage fiber digestion and milk production by dairy cows. In this context, more research is needed to examine and validate the efficacy and cost effectiveness of other genetic technologies like transgenic fibrolytic-enzyme secreting forages. Mechanical treatment methods that reduce WPCS particle size vary in effects on fiber digestibility depending on the particle size achieved. A balance between maintaining physical effectiveness of the fiber and reducing the particle size is critical for such approaches even when they increase intake and facilitate handling and transport of feeds. Chemical treatment methods of improving fiber digestibility are consistent and effective, but their widespread adoption has been limited by their caustic nature and cost. Among the biological treatment techniques, some (enzymes and inoculants) have increased fiber digestion and milk production by dairy cows in recent meta-analyses though responses in individual studies have varied.

LITERATURE CITED

Adesogan, A. T., K. G. Arriola, Y. Jiang, A. Oyeabade, E. M. Paula, J. J. Romero, L. F. Ferraretto, and D. Vyas. 2019. *Symposium review*: Technologies for improving fiber utilization. J. Dairy Sci. 102:5726-5755.

Allen, M. S. 1997. Relationship between fermentation acid production in the rumen and the requirement for physically effective fiber. J. Dairy Sci. 80:1447–1462.

Chesson, A. 1988. Lignin-polysaccharide complexes of the plant cell wall and their effect on microbial degradation in the rumen. Anim. Feed Sci. Technol. 21:219–228.

Cook, D. E., R. W. Bender, K. J. Shinners, and D. K. Combs. 2016. The effects of calcium hyrdroxide-treated whole-plant and fractionated corn silage on intake, digestion, and lactation performance in dairy cows. J. Dairy Sci. 99:5385-5393.

Coons, E. M., S. M. Fredin, K. W. Cotanch, H. M. Dann, C. S. Ballard, J. P. Brouillette, and R. J. Grant. 2019. Influence of a novel bm3 corn silage hybrid with floury kernel genetics on lactational performance and feed efficiency of Holstein cows. J. Dairy Sci. 102:9814–9826.

Diepersloot, E. C., E. M. Paula, H. Sultana, E. van Santen, M. O. Wallau, and L. F. Ferraretto. 2021. Effect of plant density and hybrid relative maturity on yield, nutrient composition, ruminal in vitro neutral detergent fiber digestibility and predicted milk production of dairy cattle in whole-plant corn silage in subtropical conditions. Applied Anim. Sci. 37:106-114.

Ferraretto, L. F., and R. D. Shaver. 2012a. Effect of corn shredlage on lactation performance and total tract starch digestibility by dairy cows. The Prof. Anim. Sci. 28:639–647.

Ferraretto, L. F., and R. D. Shaver. 2012b. Meta-analysis: Impact of corn silage harvest practices on intake, digestion and milk production by dairy cows. The Prof. Anim. Sci. 28:141–149.

Ferraretto, L. F., and R. D. Shaver. 2015. Effects of whole-plant corn silage hybrid type on intake, digestion, ruminal fermentation, and lactation performance by dairy cows through a meta-analysis. J. Dairy Sci. 98: 2662–2675.

Ferraretto, L. F., R. D. Shaver, J. G. Lauer, L. H. Brown, J. P. Kennicker, and D. M. Taysom. 2017. Influence of plant population, hybrid relative maturity and cutting height on yield, nutrient content and digestibility in whole-plant corn forage. J. Dairy Sci. 100(Suppl. 2):(Abstr.). Page 150.

Ferraretto, L. F., R. D. Shaver, and B. D. Luck. 2018. *Silage Review*: Recent advances and future technologies for whole-plant and fractionated corn silage harvesting: A contemporary review. J. Dairy Sci. 101:3937-3951.

Gerlach, K., F. Pfau, K. Hunting, K. Weib, W. Richardt, and K.-H. Sudekum. 2018. Effects of length of ensiling and maturity group on chemical composition and in vitro ruminal degradability of whole-crop maize. Grass Forage Sci. 73:599-609.

Grant, R. J., and L. F. Ferraretto. 2018. Silage review: Silage feeding management: silage characteristics and dairy cow feeding behavior. J. Dairy Sci. 101:4111–4121.

Hassanat, F., R. Gervais, and C. Benchaar. 2017. Methane production, ruminal fermentation characteristics, nutrient digestibility, nitrogen excretion, and milk production of dairy cows fed conventional or brown midrib corn silage. J. Dairy Sci. 100:2625–2636.

Johnson, L., J. H. Harrison, C. Hunt, K. Shinners, C. G. Doggett, and D. Sapienza. 1999. Nutritive value of corn silage as affected by maturity and mechanical processing: A contemporary review. J. Dairy Sci. 82:2813–2825.

Jung, H., and D. Deetz. 1993. Cell wall lignification and degradability. Pages 315–340 in Forage Cell Wall Structure and Digestibility. H. G. Jung, D. R. Buxton, R. D. Hatfield, J. Ralph, ed. ASA, CSSA and SSSA. Madison, WI.

Kung, L., Jr., R. D. Shaver, R. J. Grant, and R. J. Schmidt. 2018. Silage review: Interpretation of chemical, microbial, and organoleptic components of silages. J. Dairy Sci. 101:4020–4033.

Leonardi, C., and L. E. Armentano. 2003. Effect of quantity, quality and length of alfalfa hay on selective consumption by dairy cows. J. Dairy Sci. 86:557–564.

Mertens, D. R. 1997. Creating a system for meeting the fiber requirements of dairy cows. J. Dairy Sci. 80:1463-1481.

Mertens. D. R. 1987. Predicting intake and digestibility using mathematical models of ruminal function. J. Anim. Sci. 64:1548–1558.

Miller, M. D., C. Kokko, C. S. Ballard, H. M. Dann, M. Fustini, A. Palmonari, A. Formigoni, K. W. Cotanch, and R. J. Grant. 2021. Influence of fiber degradability of corn silage in diets with lower and higher fiber content on lactational performance, nutrient digestibility, and ruminal characteristics in lactating Holstein cows. J. Dairy Sci. 104:1728-1743.

Miller, M. D., J. S. Lanier, S. K. Kvidera, H. M. Dann, C. S. Ballard, and R. J. Grant. 2020. Evaluation of source of corn silage and trace minerals on lactational performance and total tract nutrient digestibility of Holstein cows. J. Dairy Sci. 103:3147–3160.

Muck, R. E., and K. K. Bolsen. 1991. Silage preservation and silage additive products. Page 105 in Field Guide for Hay and Silage Management in North America. K. K. Bolsen, J. E. Baylor, and M. E. McCullough, ed. Natl. Feed Ingred. Assoc., West Des Moines, IA.

Nigon, B. J., K. J. Shinners, and D. E. Cook. 2016. Harvester modifications to alter composition and dry matter of corn-silage. Appl. Eng. Agric., 32:157-167.

Oba, M., and M. S. Allen. 1999. Evaluation of the importance of the digestibility of neutral detergent fiber from forage: Effects on dry matter intake and milk yield of dairy cows. J. Dairy Sci. 82:589–596.

Oliveira, A. S., Z. G. Weinberg, I. M. Ogunade, A. A.P. Cervantes, K. G. Arriola, Y. Jiang, D.H. Kim, X. Li, M. C. M. Goncalves, D. Vyas, and A. T. Adesogan. 2017. Meta-analysis of effects of inoculation with homofermentative and facultative heterofermentative lactic acid bacteria on silage fermentation, aerobic stability, and the performance of dairy cows. J. Dairy Sci. 100:4587-4603.

Paula, E. M., B. A. Saylor, J. Goeser, and L. F. Ferraretto. 2019. Influence of cutting height on nutrient composition and yield of whole-plant cutting height through a meta-analysis. J. Dairy Sci. 102(Suppl. 1):(Abstr.). Page 104.

Queiroz, O. C. M., S. C. Kim, and A. T. Adesogan. 2012. Effect of treatment with a mixture of bacteria and fibrolytic enzymes on the quality and safety of corn silage infested with different levels of rust. J. Dairy Sci. 95:5285–5291.Sattler, S. E., D. L. Funnell-Harris, and J. F. Pedersen. 2010. Brown midrib mutations and their importance to the utilization of maize, sorghum, and pearl millet lignocellulosic tissues. Plant Sci. 178:229–238.

Vanderwerff, L. M., L. F. Ferraretto, and R. D. Shaver. 2015. Brown midrib corn shredlage in diets for high-producing dairy cows. J. Dairy Sci. 98: 5642-5652.

LALLEMAND

LALLEMAND ANIMAL NUTRITION

THE ROLE OF SILAGE ALCOHOLS AND ACETIC ACID IN DAIRY COW NUTRITION

DANIEL, J. L. P, BRAGATTO, J. M.
Department of Animal Science, State University of Maringa, Maringa, 87020-900, Brazil.
E-mail: jlpdaniel@uem.br

INTRODUCTION

Alcohols are common fermentation end-products in silages. Although ethanol is the main alcohol formed during silage fermentation, other alcohols, such as 1,2-propanediol, 2,3-butanediol, and 1-propanol, are frequently found at significant concentrations in silages. Additionally, traces of methanol, 2-propanol (i.e., isopropanol), 1-butanol, 2-butanol, 2-methyl-propanol (i.e., tert-butanol), 2-pentanol, 3-pentanol, 2-methyl-butanol, 3-methyl-butanol, pentanol, and hexanol have been reported at trace concentrations (ppm) (Mo et al., 2001; Weiss et al., 2009; Kalac, 2011; Hafner et al., 2013).

When diets containing silages are fed to dairy cows, alcohols contribute to total mixed ration (TMR) odor, and upon ingestion, they can be metabolized in the rumen fluid, portal drained viscera (PDV), and liver, with a relatively small proportion of alcohols passing to the peripheral blood (Kristensen et al., 2007; Raun and Kristensen, 2009, 2011, 2012). However, trace amounts of silage alcohols can be secreted in milk (Shipe et al., 1962; Randby, 2007; Kalac, 2011; Bragatto et al., 2022). Consequently, alcohols may affect animal performance (directly and indirectly) and milk quality. Silage alcohols have also been associated with environmental issues (Hafner et al., 2013), but this is out of scope for this manuscript.

Acetic acid is a major fermentation end-product in silage (and in the rumen), and a certain amount of acetic acid is desirable to protect the silage and TMR against aerobic deterioration (Pahlow and Muck, 2009; Wilkinson and Davies, 2013; Kung et al., 2018). However, excessively high concentrations of acetic acid in TMR may negatively affect feed intake regardless of poor fermentation (Gerlach et al., 2021). Upon ingestion, acetic acid from silage is integrated with the ruminal pool of acetic acid.

FORMATION OF ALCOHOLS AND ACETIC ACID IN SILAGES

The course of silage fermentation is mainly determined by moisture content, chemical composition (e.g., soluble sugars, buffering capacity, and nitrate), and microorganisms present in the ensiled crop (Pahlow et al., 2003). However, management factors, such as particle size, application of additives, silo filling readiness, packing/silage porosity, sealing strategies, room temperature, and unloading management, may also affect the profile of fermentation end-products in silage.

High concentrations of ethanol in silage [>20 g/kg dry matter (DM)] are often associated with yeast development (Kung et al., 2018). However, other microorganisms, such as enterobacteria, bacilli, clostridia, and heterofermentative lactic acid bacteria (LAB), may contribute to the pool of ethanol in silages (McDonald et al., 1991). Despite conserving most of the gross energy (~99%), the ethanolic fermentation pathway results in high DM loss (~49%) via carbon dioxide ($1\ C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2$). Furthermore, as ethanol may be partially lost by volatilization and be converted to acetic acid and methane in the rumen, net energy loss is substantial in silages with a high content of ethanol (~35%; Daniel and Nussio, 2011). Even if well managed, crops with high concentrations of soluble carbohydrates (e.g., sugarcane, sweet sorghum, and beet root) are highly susceptible to ethanolic fermentation.

Most 1,2-propanediol in silages is derived from heterofermentative LAB (e.g., *Lentilactobacillus buchneri*) as a coproduct of lactic acid conversion to acetic acid (Oude Elferink et al., 2001), but other silage microorganisms, such as clostridia and yeasts, also produce 1,2-propanediol (Suzuki and Onishi, 1968; Sanchez et al., 1987). Some silage specialists have speculated that 1,2-propanediol contributes to improving the aerobic stability in silages inoculated with *L. buchneri*, but it is unlikely to occur (Zhang et al., 2006). The conversion of 1,2-propanediol to 1-propanol and propionic acid often occurs in silages treated with *L. buchneri* due to indigenous or inoculated strains of *Lentilactobacillus dilivorans* (Krooneman et al., 2002). Additionally, 1-propanol can be produced by yeasts and clostridia via fermentation of amino acids (threonine or methionine) (Giudici et al., 1993; Janssen, 2004).

In most silages 2,3-butanediol is a fermentation product of enterobacteria (Nishino and Shinde, 2007). However, clostridia and bacilli produce 2,3-butanediol in silage (Nishino et al., 2007; Siemerink et al., 2011; Fernandes, 2014). Depending on the ensiling management, grass silages tend to have significant concentrations of 2,3-butanediol. However, the production of 2,3-butanediol in silage is undesirable due to its association with DM loss (Rooke and Hatfield, 2003). Quantitative analysis of 2,3-butanediol by gas (GC) or liquid (HPLC) chromatography without a mass detector is somewhat challenging due to the 2,3-butanediol molecules [which are a mixture of three stereoisomers; i.e., two enantiomers, namely (2R, 3R) and (2S, 3S), and one meso compound, namely (2R, 3S) or (2S, 3R)] split into two distinct peaks during their passage through the column. In most chromatography columns, the first peak coelutes or appears close to propionic acid, and the second peak coelutes or appears close to isobutyric acid. Hence, analysis of 2,3-butanediol, propionic acid, and isobutyric acid requires extra care.

The origin of other alcohols in silage is still debatable. Methanol is likely a product of pectin demethylation by plant enzymes and, perhaps, microorganisms (Fall and Benson, 1996; Steidlova and Kalac, 2002). Some strains of *Clostridium* (e.g., *C. beijerinckii* syn. *C. butylicum*) produce isopropanol, acetone, and butanol (Chen and Hiu, 1986; Xin et al., 2017), but it is unclear whether this occurs in silage. In corn silage, Driehuis et al. (2016) described the occurrence of spores of lactate-fermenting clostridia, and they noted that *C. beijerinckii* represents most of the *Clostridium* population in poorly preserved silage with high counts of butyric acid bacteria (≥4 log MPN/g). Rooke and Hatfield (2003) suggested a pathway for isopropanol formation via glucose or lactic acid fermentation in silage. The final step is the reduction of acetone to isopropanol, but other end-products, such as butanol and butyric acid, are associated with the pathway. Moreover, 2-butanol can be formed from 2,3-butanediol by the action of a dehydratase and a secondary alcohol dehydrogenase (Radler and Zorg, 1986).

Acetic acid is produced by different silage microorganisms, including heterofermentative LAB, enterobacteria, clostridia, bacilli, and propionic acid bacteria, under anaerobic conditions as well as by *Acetobacter* species and homofermentative LAB under the presence of oxygen (McDonald et al., 1991). Crop characteristics, such as high moisture and high buffering capacity, as well as management problems, such as delayed sealing, high silage porosity, and high temperature, often stimulate acetic acid formation (Wang and Nishino, 2013; Kung et al., 2018). Consequently, the final concentration of acetic acid is hardly predictable in silages (Mogodiniyai Kasmaei et al., 2013).

RELATIONSHIP BETWEEN ALCOHOLS AND OTHER FERMENTATION PRODUCTS IN SILAGES

Ethyl lactate, ethyl acetate, and propyl acetate are quantitatively the main esters found in silage. Although esters can be formed in silages by biochemical routes (e.g., microorganisms; Weiss et al., 2020), the esterification of carboxylic acids and alcohols at acidic pH (i.e., chemical reaction) is an important pathway of ester formation. Weiss (2017) described a positive correlation between esters and their respective alcohols in silages. As the concentrations of ethanol and 1-propanol are often lower than those of lactic acid and acetic acid (an exception would be sugarcane silage), alcohols often limit the abiotic formation of esters.

Acetoin is an intermediate of the 2,3-butanediol production pathway (McDonald et al., 1991). Isopropanol and acetone are easily interconverted by a secondary alcohol dehydrogenase (Bruss and Lopez, 2000).

Aldehydes can be formed as intermediates in the catabolism of sugars (e.g., acetaldehyde) and amino acids (e.g., branched-chain aldehydes). Additionally, aldehydes are produced by the oxidation of alcohols and unsaturated fatty acids by the action of lipoxygenases (Kalac, 2011).

EFFECTS OF ALCOHOLS AND ACETIC ACID ON FEED INTAKE, METABOLISM, AND PERFORMANCE OF DAIRY COWS

Although part of the fermentation products may be lost by volatilization from the silo panel and TMR (during mixing, delivering, and exposure at the feed bunk), most fermentation products remain in the consumed TMR under good management (Daniel and Nussio, 2011; Robinson et al., 2016; Bragatto et al., 2022), directly or indirectly changing its feeding value (Daniel et al., 2013a, 2013b). Several studies have reported multivariate analysis and correlations among fermentation products and dry matter intake (DMI). A pioneering study was conducted by Wilkins et al. (1971), in which 70 silages from temperate forages (ryegrass, barley, alfalfa, clover, etc.) were used in several experiments with sheep. The DMI was positively correlated with DM content and lactic acid (proportion of total acids) but negatively correlated with acetic acid and NH_3-N. Rook and Gill (1990) reported a negative association between silage NH_3-N and butyric acid with DMI in beef steers. Thenceforth, other research groups have published studies correlating silage fermentation products and DMI in ruminants (Offer et al., 1998; Steen et al., 1998; Huhtanen et al., 2002; Eisner et al., 2006; Hetta et al., 2007; Krizsan et al., 2007). In a more recent analysis, Huhtanen et al. (2007) reported that total acid concentration showed the highest negative correlation with DMI in dairy cows, but the effect was confounded with the extent of silage fermentation.

The above-mentioned studies suggest that lactic acid and ethanol have little or no effect on DMI, while VFA and NH_3-N may have negative effects on DMI. A major concern with these studies is the occurrence of collinearity among variables, which prevents the establishment of cause and effect. Therefore, several studies have been conducted to demonstrate the effects of sole fermentation end-products.

Ethanol

Interest in ethanol and its effects on the DMI, metabolism, and performance of ruminants is not new (Emery et al., 1959; Frederiksen and Ochia, 1970). Initially, ethanol was studied as a feed additive in poor diets, but research attention moved to the dietary effects of ethanol from fermented feeds (i.e., silages and byproducts derived from fermentation processes).

Early studies have shown that dietary ethanol is metabolized by ruminal microorganisms and partially absorbed in the rumen (Moomaw and Hungate, 1963; Orskov et al., 1967). Durix et al. (1991) infused ^{14}C-labeled ethanol in a semicontinuous flow rumen simulator (Rusitec) and observed that the production of VFA was increased by up to 40% with acetate representing 80% of VFA formed from ethanol, but the microbial protein synthesis was decreased with ethanol supplementation. Most of the reducing equivalents (electrons) derived from ethanol oxidation were used by methanogenic microorganisms to reduce CO_2 to CH_4.

Although some species of rumen microorganisms are susceptible to ethanol (Caldwell and Murray, 1986), this alcohol would (hypothetically) trigger favorable effects on the rumen environment. Chalupa et al. (1964) reported a 6% increase in the in vitro digestibility of cellulose by the addition of ethanol at 20 g/kg DM, suggesting that ethanol was a readily available energy source for ruminal microorganisms, then increasing their capacity for fiber digestion. Furthermore, the supply of electrons by the oxidation of ethanol to acetate may decrease the redox potential (E_h) in the rumen fluid, shortening the lag phase of microbial growth. However, a subsequent trial with rumen cannulated cows has demonstrated that the ruminal ammonia concentration reached a higher peak in animals supplemented with ethanol, suggesting that ethanol decreased microbial activity and, consequently, the use of ammonia for protein synthesis. Otsuki et al. (1991) investigated the effects of high doses of ethanol (56.5 g/kg DM) in high-concentrate diets for cannulated steers; they did not observe any changes in rumen pH and E_h, but they reported that the concentrations of acetate and ammonia were higher in cattle receiving ethanol. In addition, they reported that the ruminal protozoa population was markedly reduced by ethanol supplementation.

Randby et al. (1999) supplemented 600 g/d ethanol to lactating dairy cows, and they reported that cows fed ethanol had greater DMI, energy-corrected milk yield, and milk fat and protein concentrations compared to the control. They also observed higher proportions of C14:0 and C16:0 fatty acids but lower proportions of C14:1, C15:0, C18:1, C18:2, and C20:1 in milk fat from cows fed ethanol. Earlier experiments have also indicated that ethanol supplementation may increase milk fat content and slightly change its fatty acid profile. Orskov et al. (1967) reported greater fat content with higher proportions of C12:0, C14:0 and C16:0 fatty acids but lower proportions of C18:1 and C18:2 in the milk of cows receiving ethanol. Pradhan and Hemken (1970) reported greater fat content with a higher proportion of C16:0 and a lower proportion of C18:1 in the milk of cows supplemented with ethanol. Gould (2000) supplemented 20 g/kg ethanol in a high-forage diet for sheep and observed a lower duodenal flux of fatty acids originating from ruminal biohydrogenation (e.g., CLA cis9, trans11, and C18:1 trans11). The additional supply of electrons by the oxidation of ethanol to acetate may have stimulated ruminal biohydrogenation in the trials where ethanol increased milk fat content. Plascencia et al. (1999) suggested that ruminal biohydrogenation is directly proportional to methane production, which is also frequently observed in experiments with ethanol supplementation (Czerkawski and Breckenridge, 1972; Yoshii et al., 2005).

Daniel et al. (2013a) compared the performance of high-yielding dairy cows supplemented with ethanol (32.0 g/kg DM) or acetic acid (42.6 g/kg DM) for seven weeks. Less than 10% of the compounds present in the offered TMR were lost by volatilization. Acetic acid reduced DMI, but cows tended to adapt to acetic acid across the trial (after three weeks of supplementation). However, ethanol supplementation increased milk, protein, and lactose production. No alteration was observed in milk fat content, suggesting that the portal flow of acetate (and ethanol) likely did not limit fat synthesis in the mammary gland and/or that ruminal biohydrogenation of fatty acids was not changed significantly in their cows.

Kristensen et al. (2007) evaluated the metabolism of silage alcohols in dairy cows. They observed that dietary alcohols were metabolized by rumen microorganisms and/or rumen epithelium, and they reported that most of the absorbed fraction was metabolized in the liver. In a following study, these researchers evaluated ethanol metabolism in fresh cows. As lactation progressed, DMI and ethanol intake increased, but portal recovery of ethanol decreased, which may have been due to ruminal adaptation (microorganisms and epithelium) (Raun and Kristensen, 2009). Pradham and Hemken (1970) also found higher rates of ethanol metabolism in the rumen fluid of ethanol-adapted cows and in cows fed a high-concentrate rather high-forage diet. The phenomenon of adaptation may occur due to an increase in the microbial population and/or enzymatic activity in the ethanol-consuming units (microbial cells and ruminal epithelium cells). Together, rumen microorganisms, rumen epithelium, and liver cells are efficient in metabolizing dietary ethanol, resulting in low arterial concentrations. In both Danish trials cited above, there was no evidence of metabolism saturation given the low arterial concentrations of this compound (Table 1). Considering the parameters of Michaelis–Menten kinetics (V_{max} and K_m) obtained in sheep and the dose of ethanol potentially consumed via silage, Jean-Blain et al. (1992) concluded that, under normal conditions, the enzymatic systems related to ethanol metabolism (microorganisms, ruminal epithelium, and liver) would not be saturated and the arterial concentration of ethanol would always be low in ruminants. In hepatocytes, three pathways of ethanol metabolism operate. In the cytoplasm, ethanol is oxidized to acetaldehyde by alcohol dehydrogenase, which requires the nicotinamide adenine dinucleotide coenzyme in its oxidized form (NAD^+) to carry electrons. In peroxisomes, ethanol is oxidized by catalase, while hydrogen peroxide is the electron acceptor. Cytochrome P450 2E1 (CYP2E1) present in microsomes plays an important role in metabolism when ethanol is present in high concentrations. In mitochondria, acetaldehyde generated by the three pathways is metabolized to acetate by acetaldehyde dehydrogenase with the NAD^+ coenzyme being the electron acceptor (Lieber and Abittan, 1999). As a result, the hepatic oxidation of ethanol generates excess reducing equivalents, and hypothetically, the oxidation of alcohols in the liver could be compromised in animals with ketosis (high $NADH:NAD^+$ ratio). However, the ability of dairy cows to metabolize alcohol has been clearly demonstrated in the Danish experiments, including cows in the transition period (Raun and Kristensen, 2011).

Although some technicians have claimed a negative association between ethanol and DMI, there is no evidence in the literature to support such a premise (Randby et al., 1999; Daniel et al., 2013a), whereas mammals have a good preference for ethanol (Richter and Campbell, 1940). Regardless of the lack of negative effects on animal performance, ethanolic fermentation is undesirable in silage. Considering that most ethanol is converted to acetate in the rumen and assuming that approximately one-fourth of dietary ethanol is recovered in the portal blood of ethanol-adapted cows (Raun and Kristensen, 2009), one may expect that the net energy for lactation (NE_L) value of ethanol is approximately 2.9 Mcal/kg. In silage, 1 kg of ethanol is theoretically produced at the expense of approximately 2 kg of sucrose (McDonald et al., 1991), which contains approximately 4.5 Mcal of NE_L (2×2.26 Mcal/kg; NRC, 2001). Therefore, ethanolic fermentation leads to approximately 35% NE_L loss [(4.5–2.9)/4.5] (Daniel and Nussio, 2011). This proportion may be even higher if ethanol is lost by volatilization, depending on silage management and weather conditions.

Table 1. Ruminal and splanchnic metabolism of ethanol in dairy cows

Reference	Kristensen et al. (2007)	Raun e Kristensen (2009)		
Days in milk	257	4	15	29
DMI (kg/d)	15.8	14.7	16.5	19.7
Dietary ethanol (g/kg DM)	14.2	19	19	19
Ruminal ethanol (mM)	2.86	3.2	3.3	2.8
Portal recovery (%)	-	39%	28%	24%
Net portal flux (mmol/h)	113	71	97	114
Net hepatic flux (mmol/h)	-131	-	-	-
Net splanchnic flux (mmol/h)	-18	-	-	-
Arterial concentration (mM)	0.165	0.046	0.073	0.074

1-Propanol

The effects of 1-propanol on the metabolism of dairy cows have been examined in a sequence of experiments in Denmark (Kristensen et al., 2007; Raun and Kristensen, 2011; Raun and Kristensen, 2012). In all experiments, 1-propanol did not affect DMI and milk yield, but in two experiments, 1-propanol reduced milk fat content. In the rumen fluid, 1-propanol can be metabolized to propionate (via propanal by alcohol dehydrogenase and aldehyde dehydrogenase) and propyl acetate (esterification reaction), decreasing the acetate:propionate ratio (Raun and Kristensen, 2011; Raun et al. Kristensen, 2012). In one experiment, the concentration of butyrate was negatively correlated with the concentration of propyl acetate in the rumen fluid, indicating the potential of this ester to modulate microbial activity in the rumen (Kristensen et al., 2007). The fraction of 1-propanol absorbed can be metabolized in the liver. Hepatocytes have a high affinity and ability to convert 1-propanol to glucose (Raun and Kristensen, 2012). In all experiments, the arterial concentrations of 1-propanol were low even with a high supplementary dose. In those trials, there were no clinical problems or indications of metabolism saturation.

Recently, Silva et al. (2017) examined the effects of 1-propanol (10 g/kg DM) on the performance of dairy cows. Cows supplemented with 1-propanol had higher concentrations of 1-propanol in rumen fluid, higher glycemia, and lower concentrations of nonesterified fatty acids in blood. However, 1-propanol did not change the DMI, apparent digestibility of nutrients, milk yield, or milk composition. The increase in glucogenic status induced by 1-propanol, however, was not sufficient to depress milk fat content in their study.

Isopropanol

When ingested accidentally by nonruminant animals, isopropanol is approximately twofold more toxic than ethanol and about as toxic as methanol, resulting in central nervous system depression, hypotension, vomiting, and abdominal pain (Stice et al., 2018). Yet, coingestion of ethanol has been reported to increase the half-life of isopropanol in humans (Pappas et al., 1991) as isopropanol and ethanol compete for the same metabolic pathway in the liver (i.e., alcohol dehydrogenase enzyme system) (Lieber and Abittan, 1999; Bruss and Lopes, 2000; Jones and Holmgren, 2015). However, at concentrations found in silages (<5 g/kg DM, most frequently <50 mg/kg DM), isopropanol was not able to affect the performance or health of dairy cows. Bragatto et al. (2022) compared diets containing 450 g/kg concentrates and 550 g/kg corn silage either added (DM basis) with 15 g/kg isopropanol, 15 g/kg ethanol, 15 g/kg isopropanol + 15 g/kg ethanol, or water (control). Immediately before feeding, corn silage was sprayed with alcohols, mixed with concentrates, and fed as TMR twice daily. Isopropanol and ethanol supplementation did not alter diet preference or DMI despite the pungent odor and bitter taste of isopropanol to the human sense of smell. Likewise, total tract digestibility, ruminal fermentation pattern (pH, volatile fatty acids, and NH_3), urinary purine derivatives, milk yield, solids composition, and oxidative capacity were not affected by the treatments. However, gamma-glutamyl transferase activity increased in the blood of cows supplemented with alcohols, indicating that isopropanol and ethanol were at least partially absorbed and likely metabolized in the liver (Tennant and Center, 2008; Sato, 2009). Isopropanol entering the PDV can be metabolized in the liver and oxidized to acetone (Lieber and Abittan, 1999; Bruss and Lopes, 2000; Jones and Holmgren, 2015). Acetone is released into the peripheral blood and partially recycled into the rumen fluid. In the rumen, acetone is reduced to isopropanol again. Isopropanol is absorbed, and it enters the liver where it is oxidized to acetone (Bruss and Lopes, 2000). The carbon chain of a single acetone/isopropanol molecule can cycle several times before being excreted in milk, urine, expiration, or, in small amounts, converted to glucose (Black et al., 1972). Therefore, the actual concentration of isopropanol in body fluids may be greater than expected from the daily intake and its first-order pharmacokinetics. Consequently, milk concentrations of isopropanol and acetone increase in cows receiving isopropanol but not ethanol (Bragatto et al., 2022). Due to the liver/rumen cycling of isopropanol/acetone, even at small concentrations in the TMR, isopropanol can be imparted to milk.

2,3-Butanediol

In the 1980s, Mathison et al. (1981) evaluated the effect of 2,3-butanediol supplementation for sheep (0, 10, 30, or 50 g/kg DM), and they reported that 2,3-butanediol did not alter DMI, nutrient digestibility, or weight gain. A small proportion of the ingested 2,3-butanediol was lost by excretion (4 to 12%) with the majority being excreted in urine. In addition, they reported that there was a steep increase in 2,3-butanediol excretion when intake exceeded 2.5 g/kg body weight. To the best of our knowledge, studies on the effects of 2,3-butanediol in dairy cows are not available in the literature.

1,2-Propanediol

Nielsen and Ingvartsen (2004) compiled experiments on the effects of 1,2-propanediol supplementation in dairy cows. The influence of 1,2-propanediol on DMI was not consistent, but 1,2-propanediol reduced DMI in some studies (Dhiman et al.1993; Miyoshi et al., 2001), probably because of the low palatability of this compound (Johnson, 1954). 1,2-Propanediol tended to increase milk yield and reduce milk fat content without affecting milk protein content in early lactation cows (Nielsen and Ingvartsen, 2004), perhaps due to the glucogenic effect of this alcohol and its metabolites produced in the rumen (propanal, 1-propanol, and propionate).

Kristensen et al. (2002) and Kristensen and Raun (2007) examined the metabolism of 1,2-propanediol in dairy cows, and they concluded that most of the 1,2-propanediol ingested was metabolized in the rumen. In addition, these researchers reported that a small part (<10%) of the 1,2-propanediol ingested was lost in urine, and the remainder was slowly metabolized by the animal's tissues. In the rumen fluid, 1,2-propanediol can be degraded to equimolar amounts of 1-propanol and propionate with propanal being an intermediate (Czerkawski and Breckenridge, 1973). In the liver, 1,2-propanediol is mainly converted to L-lactate via alcohol dehydrogenase and aldehyde dehydrogenase. In both Danish experiments, ruminal infusion of 1,2-propanediol increased insulin resistance and decreased the ketogenic:glucogenic metabolite ratio in plasma, decreasing lipolysis and glucose demand by peripheral tissues (Kristensen et al., 2002; Kristensen and Raun, 2007).

At concentrations found in silages, 1,2-propanediol by itself is unlikely to have any effect on DMI and metabolism, particularly considering the fractional consumption of this alcohol throughout the day (contrary to pulse doses of 1,2-propanediol used for treating ketosis). However, 1,2-propanediol and acetic acid are positively correlated in silages (Figure 1), and high concentrations of 1,2-propanediol are frequently found in silages with high concentrations of acetic acid, which may reduce DMI depending on the proportion of silage in the TMR (Gerlach et al., 2021).

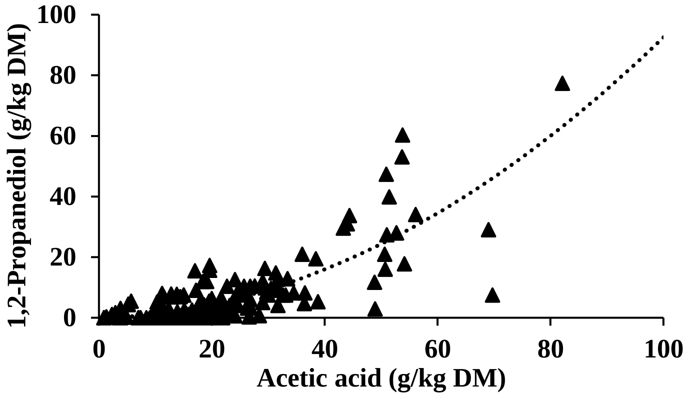


Figure 1. Relationship between 1,2-propanediol and acetic acid in silages ($P<0.01$; $R^2 = 0.65$). Data set adapted from Arriola et al. (2021).

Acetic acid

The effect of acetic acid on feed intake has been debated for almost half a century. Several but not all studies have found that high concentrations of acetic acid in ruminant diets have been negatively associated with DMI (e.g., Demarquilly et al., 1973; Huhtanen et al., 2002; Krizsan et al., 2007). However, different trials have reported a collinearity between acetic acid and other fermentation products derived from poor fermentation. For instance, a meta-analysis conducted by Eisner et al. (2006) showed that the acetic acid concentration was negatively related to silage intake, but in many cases, the negative effect of acetic acid on feed intake was confounded with undesirable compounds with hypophagic effects (e.g., proteolysis end-products). Consequently, when investigating the effect of acetic acid on intake by ruminants, one may focus on forages where the formation of acetic acid occurs without remarkable changes in the concentration of other fermentation products or proximal constituents.

Gerlach et al. (2021) evaluated the effect of the dietary content of acetic acid on DMI in dairy cattle fed rations containing silages treated with *L. buchneri* or silage/TMR supplemented with pure acetic acid. In this meta-analysis, acetic acid concentration was not confounded with mal fermentation, and the relationship between acetic acid and DMI was better described by a broken-line regression. The DMI was almost unchanged when the acetic acid concentration varied from 0 to 17.3 g/kg DM (slope 1 = -0.0012), but the DMI was significantly impaired with 17.3 to 60 g of acetic acid in a kg of diet DM (slope 2 = -0.0044). Although the meta-analysis did not examine the effects of acetic acid on animal performance, reductions in milk yield must be proportional to the reduction of DMI at high concentrations of acetic acid in TMR. Therefore, dairy nutritionists should consider the content of acetic acid in fermented ingredients upon balancing dairy diets.

INFLUENCE OF SILAGE ALCOHOLS ON MILK QUALITY

The transmission of odors from silage/TMR to milk can occur by inhalation (air → lungs → blood → mammary gland → milk) or ingestion (gastrointestinal tract → blood → mammary gland → milk) (Morgan and Pereira, 1962). Shipe et al. (1962) investigated the transfer of silage volatile compounds to milk and their effects on milk aroma; they demonstrated that silage fermentation products can be transferred to milk either by inhalation or ingestion. Milk from cows receiving esters and alcohols had more pleasant and sweeter notes, while ketones (acetone and 2-butanone) transmitted unpleasant notes. Butyric and propionic acids, often present in poorly fermented silages, did not induce off-flavor when they were introduced into the cow's lungs, likely because they are the main end-products in ruminal fermentation.

Randby et al. (1999) supplemented ethanol to lactating cows and observed that no more than 0.2-0.3% of the supplemented ethanol was recovered in the milk. In their trial, treatments were a factorial arrangement of ethanol supplementation and barley grain source (i.e., ensiled or treated with propionic acid). Although milk samples from ethanol-supplemented cows scored lower in a sensory test, indicating off-flavor, the concentration of ethanol in the milk did not explain the worse sensory quality. Furthermore, the effect of ethanol on the milk organoleptic quality was lower for cows receiving barley treated with propionic acid. In parallel, direct addition of ethanol to control milk samples did not result in off-flavor. The transmission of other compounds (derived or not derived from ethanol metabolism) may have contributed to altering milk flavor in cows supplemented with this alcohol. Milk from cows fed ethanol had a greater content of acetone. In contrast, Daniel et al. (2013a) carried out a sensory panel with 56 nontrained persons blinded to treatments and found that the appearance, aroma, and taste of cow’s milk were not negatively affected by ethanol supplementation.

In a follow-up study, Randby (2007) evaluated the influence of supplementation with 1-propanol and dimethyl sulfide (DMS; a methionine degradation product) on the organoleptic quality of milk, and they reported that supplementation with 1-propanol or 1-propanol+DMS reduced the organoleptic quality of evening milk, increasing the frequency of notes referring to malt, feed, rancidity, and mixed flavor. However, they reported that the treatments did not influence the flavor of morning milk, suggesting that the compounds are metabolized within a few hours after intake.

There is a consensus in the literature that normal milk may contain the same aromatic compounds found in milk samples resembling off-flavor. However, rather than changes in individual compounds, alterations in relative concentrations result in off-flavor (Shipe et al., 1962; Mounchili et al., 2005; Huhtanen et al., 2010), but the association between silage fermentation products and the organoleptic quality of milk is still not completely understood.

Beyond the effects on organoleptic traits, the presence of ethanol in raw milk is considered fraud by law. Alcohol may be found in raw milk tampered with extraneous water accompanied by ethanol to reestablish milk cryoscopy and mask such adulteration (Wanjala et al., 2018). Recently, we surveyed Brazilian dairy farms where bulk milk samples tested positive using the official method for detecting ethyl alcohol in raw milk. Screening alcohol-positive bulk milk samples by head-space GC–MS unveiled a higher concentration of isopropanol but not ethanol in comparison to negative milk samples from the same farms. Moreover, the analysis of fermentation end-products in corn silage from positive farms (the sole fermented feed in those herds) revealed a 100-fold increase in isopropanol concentration (~4 g/kg DM) compared to values frequently reported in the literature. Afterwards, we examined the effects of dietary isopropanol on cow’s milk quality (Bragatto et al., 2022). A TMR based on corn silage supplemented with water (control), isopropanol, ethanol, or isopropanol + ethanol was offered to crossbred Jersey × Holstein. As a result, the milk concentrations of isopropanol and acetone increased when cows received isopropanol but not ethanol (Table 2). Surprisingly, dietary isopropanol induced 100% of milk samples to be positive in the official test to detect ethyl alcohol in raw milk [the chromium reducing test, which is based on the reduction of Cr^{6+} (orange color) to Cr^{3+} (greenish color)]. These findings indicated that the Brazilian official test to detect ethyl alcohol in raw milk may result in false-positives and, consequently, should not be adopted as the only method to identify tampered milk. In the meantime, further studies are warranted to identify the origin and management practices capable of triggering the formation of isopropanol in silages.

Table 2. Alcohols and acetone in milk of cows supplemented with isopropanol (ISO) and ethanol (ETH)

	Treatment			
Item	CTRL	ETH	ISO	ETH+ISO
Acetone, µg/mL	21.9^b	20.6^b	101^a	104^a
Isopropanol, µg/mL	4.61^b	3.96^b	97.3^a	98.0^a
Ethanol, µg/mL	1.63	1.78	0.951	2.87
2-Butanol, µg/mL	0.085	0.091	0.091	0.090
Reactivity to ethyl alcohol test	0^b	0^b	100^a	100^a

Source: Adapted from Bragatto et al. (2022)

MITIGATING ETHANOL IN SILAGES – THE CASE OF SUGARCANE SILAGE

Ethanol is the main alcohol found in silages. Even if well managed, crops with high concentrations of soluble carbohydrates are susceptible to ethanolic fermentation. Regardless of the lack of negative effects on animal performance, ethanolic fermentation is undesirable in silage due to the loss of net energy (see discussion above). Sugarcane is perhaps the most susceptible crop to ethanolic fermentation.

The high content of soluble sugars associated with a high population of epiphytic yeasts often induces high fermentation loss in sugarcane (up to 300 g/kg of ensiled DM) due to the formation of ethanol and CO_2 by yeast metabolism (Kung and Stanley, 1982; Pedroso et al., 2005; Avila et al., 2010). Ethanol concentrations above 200 g/kg (DM basis) can be found in sugarcane silage conserved by natural fermentation (Rabelo et al., 2016). Additionally, high yeast counts in sugarcane silages contribute to aerobic deterioration after feedout, increasing total nutrient losses (Avila et al., 2012).

A feasible strategy to reduce yeast activity in sugarcane silage, both during storage and feedout phases, is the application of chemical or microbial additives (Pedroso et al., 2011). In general, obligate heterofermentative LAB (Gomes et al., 2021) and chemical additives with antifungal capacity (Daniel et al., 2015) are effective in attenuating the detrimental effects of yeasts (Table 3). Although calcium oxide can improve DM recovery and aerobic stability, it worsens the hygienic quality of sugarcane silage (Custodio et al., 2016; Jacovaci et al., 2017). Moreover, homofermentative LAB inoculants often stimulate ethanol formation in crops susceptible to ethanolic fermentation, so they should not be recommended for ensiling sugarcane (Zopollatto et al., 2009; Rabelo et al., 2016).

Table 3. Fermentation traits and aerobic stability of sugarcane silage treated with *L. buchneri* or sodium benzoate

	Treatment[1]		
Item	Control	*L. buchneri*	Benzoate
DM[2], g/kg as fed	339[b]	341[b]	353[a]
Soluble carbohydrates, g/kg DM	117[c]	182[b]	250[a]
pH	3.72[a]	3.64[b]	3.67[b]
Ethanol, g/kg DM	111[a]	57[b]	3.1[c]
Lactic acid, g/kg DM	38[a]	38[a]	42[a]
Acetic acid, g/kg DM	14[c]	30[a]	26[b]
1,2-Propanediol, g/kg DM	3.4[b]	11.7[a]	1.4[c]
Ethyl lactate, mg/kg DM	1001[a]	709[b]	47[c]
Ethyl acetate, mg/kg DM	581[a]	600[a]	30[b]
DM loss[2], g/kg DM	102[a]	83[b]	28[c]
DM_{oven} loss[3], g/kg DM	210[a]	148[b]	12[c]
Aerobic stability, h	153[c]	201[b]	>240[a]

[1]Control: without additive, *L. buchneri* applied at 5×10^5 cfu/g FM, and sodium benzoate applied at 2 g/kg FM.
[2]Dry matter corrected for the loss of volatile compounds during oven drying.
[3]Dry matter uncorrected for the loss of volatile compounds during oven drying.
Source: Adapted from Daniel et al. (2015).

FINAL REMARKS

Overall, silage alcohols did not impair the feed intake, performance, or health of dairy cows. However, isopropanol can be imparted to milk and cause negative impacts on the dairy chain in countries where the chromium reducing test is used to detect tampered milk (due to false-positive results).

High concentrations of acetic acid in TMR (>17 g/kg DM) may negatively affect feed intake regardless of poor fermentation in silage. Therefore, dairy nutritionists should consider the content of acetic acid in fermented ingredients upon balancing dairy diets.

REFERENCES

Arriola, K. G., Oliveira, A. S., Jiang, Y., Kim, D., Silva, H. M., Kim, S. C., Amaro, F. X., Ogunade, I. M., Sultana, H., Pech Cervantes, A. A., Ferraretto, L. F., Vyas, D., Adesogan, A. T. 2021. Meta-analysis of effects of inoculation with *Lactobacillus buchneri*, with or without other bacteria, on silage fermentation, aerobic stability, and performance of dairy cows. Journal of Dairy Science, 104:7653–7670. https://doi.org/10.3168/jds.2020-19647.

Avila, C. L. S., Pinto, J. C., Oliveira, D. P., Schwan, R. F. 2012. Aerobic stability of sugar cane silages with a novel strain of Lactobacillus sp. isolated from sugar cane. Revista Brasileira de Zootecnia. 41:249–255. https://doi.org/10.1590/S1516-35982012000200003.

Avila, C. L. S., Bravo Martin, C. E. C., Schwan, R. F. 2010. Identification and characterization of yeasts in sugarcane silages. Journal of Applied Microbiology, 109:1677–1686. https://doi.org/10.1111/j.1365-2672.2010.04796.x.

Black, A. L., Luick, J. R., Lee, S. L., Knox, K. 1972. Glucogenic pathway for acetone metabolism in the lactating cow. American Journal Physiology, 222:1575–1580. https://doi.org/10.1152/ajplegacy.1972.222.6.1575.

Bragatto, J. M., Parra, C. S., Piran Filho, F. A., Silva, S. M. S., Osorio, J. A. C., Buttow, S. C., Santos, G. T., Jobim, C. C., Nussio, L.G., Daniel, J. L. P. 2022. Effect of dietary isopropanol on the performance and milk quality of dairy cows. Animal Feed Science and Technology, 286:115254. https://doi.org/10.1016/j.anifeedsci.2022.115254.

Bruss M. L., Lopez, M. J. 2000. Mixed ruminal microbes of cattle produce isopropanol in the presence of acetone but not 3-D-hydroxybutyrate. Journal of Dairy Science, 83:2580–2584. https://doi.org/10.3168/jds.S0022-0302(00)75151-4.

Caldwell, D. R., Murray, K. 1986. Some effects of ethyl alcohol on the growth of rumen bacteria. Current Microbiology, 14:193–197. https://doi.org/10.1007/BF01568517.

Chalupa, W., Evans, J. L., Stillions, M. C. 1964. Influence of ethanol on rumen fermentation and nitrogen metabolism. Journal Animal Science, 23:802–807. https://doi.org/10.2527/jas1964.233802x.

Chen, J. S., Hiu, S. F. 1986. Acetone-butanol-isopropanol production by *Clostridium beijerinckii* (synonym, *Clostridium butylicum*). Biotechnology Letters, 8:371–376. https://doi.org/10.1007/BF01040869.

Custodio, L., Morais, G., Daniel, J. L. P., Pauly, T., Nussio, L. G. 2016. Effects of chemical and microbial additives on clostridium development in sugarcane (*Saccharum officinarum L.*) ensiled with lime. Grassland Science, 62:135–143. https://doi.org/10.1111/grs.12124.

Czerkawski, J. W., Breckenridge, G. 1973. Dissimilation of 1,2-propanediol by rumen micro-organisms. British Journal of Nutrition, 29:317–330. https://doi.org/10.1079/BJN19730106.

Czerkawski, J. W., Breckenridge, G. 1972. Fermentation of various glycolytic intermediates and other compounds by rumen micro-organisms, with particular reference to methane production. British Journal of Nutrition, 27:131–146. https://doi.org/10.1079/BJN19720077.

Daniel, J. L. P., Nussio, L. G. 2011. Contribution of silage volatile compounds for the animal nutrition. In J. L. P. Daniel, M. Zopollatto & L. G. Nussio (Eds.), Proceedings of the II International Symposium on Forage Quality and Conservation (p. 279–306), São Pedro, Brazil.

Daniel, J. L. P., Amaral, R. C., Neto, A. S., Cabezas-Garcia, E. H., Bispo, A. W., Zopollatto, M., Cardoso, T. L., Spoto, M. H. F., Santos, F. A. P., Nussio, L. G. 2013a. Performance of dairy cows fed high levels of acetic acid or ethanol. Journal of Dairy Science, 96:398–406. https://doi.org/10.3168/jds.2012-5451.

Daniel, J. L. P., Amaral, R. C., Goulart, R. S., Zopollatto, M., Santos, V. P., Toledo Filho, S. G., Cabezas-Garcia, E. H., Lima, J. R., Santos, M. C., Nussio, L. G. 2013b. Short-term effects of silage volatile compounds on feed intake and digestion in beef cattle. Journal Animal Science, 91:2321–2331. https://doi.org/10.2527/jas.2012-5657.

Daniel, J. L. P., Junges, D., Nussio, L. G. 2015. Effect of chemical and microbial additives on the dynamics of gas production during the fermentation of sugarcane silage. In J. L. P. Daniel, G. Morais, D. Junges & L. G. Nussio (Eds.), Proceedings of the XVII International Silage Conference (p. 446–447). Piracicaba, Brazil.

Demarquilly, C., Boissau, J.-M., Bousquet, H., Cuylle, G., Jailler, M., Jamot, J., l'Hotelier, L.1973. Chemical composition, fermentation characteristics, digestibility and voluntary intake of forage silages: changes compared to the initial green forage. Annales de Zootechnie, 22:1–35. https://doi.org/10.1051/animres:19730101. (In French)

Dhiman, T. R., Cadorniga, C., Satter, L. D. 1993. Protein and energy supplementation of high alfalfa silage diets during early lactation. Journal of Dairy Science, 76:1945–1959. https://doi.org/10.3168/jds.S0022-0302(93)77528-1.

Driehuis, F., Hoolwerf, J., Rademaker, J. L. W. 2016. Concurrence of spores of *Clostridium tyrobutyricum*, *Clostridium beijerinckii* and *Paenibacillus polymyxa* in silage, dairy cow faeces and raw milk. International Dairy Journal, 63:70–77. https://doi.org/10.1016/j.idairyj.2016.08.004.

Durix, A., Jean-Blain, C., Sallmann, H. P., Jouany, J. P. 1991. Use of a semicontinuous culture system (RUSITEC) to study the metabolism of ethanol in the rumen and its effects on ruminal digestion. Canadian Journal of Animal Science, 71:115–123. https://doi.org/10.4141/cjas91-013.

Eisner, I., Sudekum, K. H., Kirchhof, S. 2006. Relationships between silage fermentation characteristics and feed intake by dairy cows. Ubersichten zur Tierernahrung, 34:197–221. (In German)

Emery, R. S., Lewis, T. R., Everett, Jr. J. P., Lassiter, C. A. 1959. Effect of ethanol on rumen fermentation. Journal of Dairy Science, 42:1182–1186. https://doi.org/10.3168/jds.S0022-0302(59)90710-6.

Fall, R., Benson, A. A. 1996. Leaf methanol - the simplest natural product from plants. Trends in Plant Science, 1:296–301. https://doi.org/10.1016/S1360-1385(96)88175-0.

Fernandes, J. 2014. Influence of genotype, maturity and storage time on the quality of high moisture corn grain silages. Master Thesis, University of Sao Paulo, ESALQ, Piracicaba, Brazil, 97 p. (In Portuguese)

Frederiksen, J. H., Ochia, B. A. 1970. The effect of ethanol and acetic acid on milk yield and milk composition of cows given rations high in concentrates. Acta Agriculturae Scandinavica, 20:17–24. https://doi.org/10.1080/00015127009433197.

Gerlach, K., Daniel, J. L. P., Jobim, C. C., Nussio, L. G. 2021. A data analysis on the effect of acetic acid on dry matter intake in dairy cattle. Animal Feed Science and Technology, 272:114782. https://doi.org/10.1016/j.anifeedsci.2020.114782.

Giudici, P., Zambonelli, C., Kunkee, R. E. 1993. Increased production of n-propanol in wine by yeast strains having an impaired ability to form hydrogen sulfide. American Journal of Enology and Viticulture, 44:17–22.

Gomes, A. L. M., Bueno, A. V. I., Osmari, M. P., Machado J., Nussio, L. G., Jobim, C. C., Daniel, J. L. P. 2021. Effects of obligate heterofermentative lactic acid bacteria alone or in combination on the conservation of sugarcane silage. Frontiers in Microbiology, 12:643879. https://doi.org/10.3389/fmicb.2021.643879.

Gould, J. 2000. Supplemental ethanol for ruminants consuming forage-based diets. Master Thesis, University of Wyoming, p.83.

Hafner, D. H., Howard, C., Muck, R. E., Franco, R. B., Montes, F., Green, P. G., Mitloehner, F., Trabue, S. L., Rotz, C. A. 2013. Emission of volatile organic compounds from silages: Compounds, sources, and implications. Atmospheric Environment, 77:827–839.https://doi.org/10.1016/j.atmosenv.2013.04.076.

Hetta, M., Cone, J. W., Bernes, G., Gustavsson, A. M., Martinsson, K. 2007. Voluntary intake of silages in dairy cows depending on chemical composition and in vitro gas production characteristics. Livestock Science, 106:47–56. https://doi.org/10.1016/j.livsci.2006.06.013.

Huhtanen, P., Khalili, H., Nousiainen, J. I., Rinne, M., Jaakkola, S., Heikkila, T., Nousiainen, J. 2002. Prediction of the relative intake potential of grass silage by dairy cows. Livestock Production Science, 73:111–130. https://doi.org/10.1016/S0301-6226(01)00279-2.

Huhtanen, P., Rinne, M., Nousiainen, J. 2007. Evaluation of the factors affecting silage intake of dairy cows: A revision of the relative silage dry-matter intake index. Animal, 1:758–770. https://doi.org/10.1017/S175173110773673X.

Huhtanen, P., Sudekum, K. H., Nousiainen, J., Shingfield, K. J. 2010. Forage conservation, feeding value and milk quality. In Schnyder, H. et al. (eds.). Proceedings of the 23th General Meeting of the European Grassland Federation (p. 379–400), Kiel, Germany.

Jacovaci, F. A., Jobim, C. C., Schmidt, P., Nussio, L. G., Daniel, J. L. P. 2017. A data-analysis on the conservation and nutritive value of sugarcane silage treated with calcium oxide. Animal Feed Science and Technology, 225:1–7. https://doi.org/10.1016/j.anifeedsci.2017.01.005

Janssen, P. 2004. Propanol as an end product of threonine fermentation. Archives of Microbiology, 182:482–486. https://doi.org/10.1007/s00203-004-0732-y.

Jean-Blain, C., Durix, A., Tranchant, B. 1992. Kinetics of ethanol metabolism in sheep. Reproduction Nutrition Development, 32:83–90. https://doi.org/10.1051/rnd:19920109.

Johnson, R. B. 1954. The treatment of ketosis with glycerol and propylene glycol. Cornell Veterinarian, 44: 6–21.

Jones, A. W., Holmgren, A. 2015. Occurrence of acetone concentrations > 0.10 g/L in post-mortem femoral blood and association with ethanol and isopropanol concentrations. Toxicologie analytique et Clinique, 27:226–232. https://doi.org/10.1016/j.toxac.2015.10.003.

Kalac, P. 2011. The effects of silage feeding on some sensory and health atributes of cow's milk: A review. Food Chemistry, 125:307–317. https://doi.org/10.1016/j.foodchem.2010.08.077.

Kristensen, N. B., Danfaer, A., Rojen, B. A., Raun, B. M. L., Weisbjerg, M. R., Hvelplund, T. 2002. Metabolism of propionate and 1,2-propanediol absorbed from the washed reticulorumen of lactating cows. Journal Animal Science, 80:2168–2175. https://doi.org/10.2527/2002.8082168x.

Kristensen, N. B., Raun, B. M. L. 2007. Ruminal and intermediary metabolism of propylene glycol in lactating Holstein cows. Journal of Dairy Science, 90:4707–4717. https://doi.org/10.3168/jds.2007-0295.

Kristensen, N. B., Storm, A., Raun, R. B. L., Rojen, B. A., Harmon, D. L. 2007. Metabolism of silage alcohols in lactating dairy cows. Journal of Dairy Science, 90:1364–1377. https://doi.org/10.3168/jds.S0022-0302(07)71623-5.

Krizsan, S. J., Westad, F., Adnoy, T., Odden, E., Aakre, S. E., Randby, A. T. 2007. Effect of volatile compounds in grass silage on voluntary intake by growing cattle. Animal, 1:283. https://doi.org/10.1017/s1751731107683773.

Krooneman, J., Faber, F., Alderkamp, A. C., Oude Elferink, S. J. H. W., Driehuis, F., Cleenwerck, I., Swings, J., Gottschal, J. C., Vancanneyt, M. 2002. *Lactobacilllus diolivorans* sp. nov., a 1,2-propanediol-degrading bacterium isolated from aerobically stable maize silage. International Journal of Systematic and Evolutionary Microbiology, 52:639–646. https://doi.org/10.1099/00207713-52-2-639.

Kung, L. Jr., Shaver, R. D., Grant, R. J., Schmidt, R. J. 2018. Silage review: Interpretation of chemical, microbial, and organoleptic components of silages. Journal of Dairy Science, 101:4020–4033. https://doi.org/10.3168/jds.2017-13909.

Kung, L. Jr., Stanley, R. W. 1982. Effect of stage of maturity on nutritive value of whole-plant sugarcane preserved as silage. Journal Animal Science, 54:689–696. https://doi.org/10.2527/jas1982.544689x.

Lieber, C. S., Abittan, C. S. 1999. Pharmacology and metabolism of alcohol, including its metabolic effects and interactions with other drugs. Clinics in Dermatology, 17:365–379. https://doi.org/10.1016/s0738-081x(99)00020-6.

Mathison G. W., Fenton, M., Milligan, L. P. 1981. Utilization of 2,3-butanediol by sheep. Canadian Journal of Animal Science, 61:649–656. https://doi.org/10.4141/cjas81-078.

McDonald, P., Henderson, N., Heron, S.1991. The biochemistry of silage. 2nd rev. ed. Chalcombe Publ., Marlow, UK.

Miyoshi, S., Pate, J. L., Palmquist, D. L. 2001. Effects of propylene glycol drenching on energy balance, plasma glucose, plasma insulin, ovarian function and conception in dairy cows. Animal Reproduction Science, 68:29–43. https://doi.org/10.1016/s0378-4320(01)00137-3.

Mo, M., Selmer-Olsen, I., Randby, A. T., Aakre, S. E., Asmyhr, A. 2001. New fermentation products in grass silage and their effects on feed intake and milk taste. In V. Jambor, P. Dolezal, L. Zeman, R. Loucka, S. Rudolfova & P. Prochazka (Eds.), Proceedings of the 10th International Symposium on Forage Conservation (p.98–99), Brno, Czech Republic.

Mogodiniyai Kasmaei, K., Rustas, B.-O., Sporndly, R., Uden, P. 2013. Prediction models of silage fermentation products on crop composition under strict anaerobic conditions: A meta-analysis. Journal of Dairy Science, 96:6644–6649. https://doi.org/10.3168/jds.2013-6858.

Moomaw, C., Hungate, R. E. 1963. Ethanol conversion in the bovine rumen. Journal of Bacteriology, 85:721–7222. https://doi.org/10.1128/jb.85.3.721-722.1963.

Morgan, M. E., Pereira, R. L. 1962. Volatile constituents of grass and corn silage. II. Gas-entering aroma. Journal of Dairy Science, 45:467–471. https://doi.org/10.3168/jds.S0022-0302(62)89428-4

Mounchili, A., Wichtel, J. J., Bosset, J. O., Dohoo, I. R., Imhof, M., Altieri, D., Mallia, S., Stryhn, H. 2005. HS-SPME gas chromatographic characterization of volatile compounds in milk tainted with off-flavour. International Dairy Journal, 15:1203–1215. https://doi.org/10.1016/j.idairyj.2004.11.018.

Nielsen, N. I., Ingvartsen, K. L. 2004. Propylene glycol for dairy cows. A review of the metabolism of propylene glycol and its effects on physiological parameters, feed intake, milk production and risk of ketosis. Animal Feed Science and Technology, 115:191–213. https://doi.org/10.1016/j.anifeedsci.2004.03.008.

Nishino, N., Shinde, S. 2007. Ethanol and 2,3-butanediol production in whole-crop rice silage. Grassland Science, 53:196–198. https://doi.org/10.1111/j.1744-697X.2007.00089.x.

Nishino, N., Hattori, H., Kishida, Y. 2007. Alcoholic fermentation and its prevention by Lactobacillus buchneri in whole crop rice silage. Letters in Applied Microbiology, 44:538–543. https://doi.org/10.1111/j.1472-765X.2006.02105.x.

Offer, N. W., Percival, D. S., Dewhurst, R. J., Thomas, C. 1998. Prediction of the voluntary intake potential of grass silage by sheep and dairy cows from laboratory silage measurements. Animal Science, 66:357–367. https://doi.org/10.1017/S1357729800009486.

Orskov, E. R., Hemken, R. W., Moore, L. A. 1967. Effect of ethanol infusion on milk fat content and composition and on volatile fatty acids in the rumen liquor. Journal of Dairy Science, 50:692–695. https://doi.org/10.3168/jds.S0022-0302(67)87494-0.

Otsuki, K., Kobayashy, T., Matsumoto, M., Itabashi, H. 1991. Effects of ethanol on rumen fermentation pattern in steers fed a diet high in concentrate. Bulletin of National Institute of Animal Industry, 51:9–15. (In Japanese)

Oude Elferink, S. J. W. H., Krooneman, J., Gottschal, J. C., Spoelstra, S. F., Faber, F., Driehuis, F. 2001. Anaerobic conversion of lactic acid to acetic acid and 1,2-propanediol by *Lactobacillus buchneri*. Applied and Environmental Microbiology, 67:125–132. https://doi.org/10.1128/AEM.67.1.125-132.2001.

Pahlow G., Muck, R. E. 2009. Managing for improved aerobic stability. In Broderick G.A. (Ed.), Proceedings of the XVth International Silage Conference (p. 77–90), Madison, USA.

Pahlow, G., Muck, R. E., Driehuis, F., Elferink, S. J., Spoelstra, S. F. 2003. Microbiology of ensiling. In Silage Science and Technology. Buxton, D. R., Muck, R. E., Harrison, J. H. (ed.) American Society of Agronomy, Madison, WI, 2003, p. 31–94. https://doi.org/10.2134/agronmonogr42.c2.

Pappas, A. A., Ackerman, B. H., Olsen, K. M., Taylor, E. H.1991. Isopropanol ingestion: a report of six episodes with isopropanol and acetone serum concentration time data. Journal of Clinical Toxicology, 29:11–21. https://doi.org/10.3109/15563659109038593.

Pedroso, A. D. F., Rodrigues, A. D. A., Barioni, W. Jr., Souza, G. B. D. 2011. Fermentation parameters, quality and losses in sugarcane silages treated with chemical additives and a bacterial inoculant. Revista Brasileira de Zootecnia, 40:2318–2322. https://doi.org/10.1590/S1516-35982011001100006

Pedroso, A. F., Nussio, L. G., Paziani, S. F., Loures, D. R. S., Igarasi, M. S., Coelho, R. M., Packer, I. U., Horii, J., Gomes, L. H. 2005. Fermentation and epiphytic microflora dynamics in sugar cane silage. Scientia Agricola, 62:427–432. https://doi.org/10.1590/S0103-90162005000500003.

Plascencia, A., Estrada, M., Zinn, R. A. 1999. Influence of free fatty acids content on feeding value of yellow grease in finishing diets for beef cattle. Journal Animal Science, 77:2603–2609. https://doi.org/10.2527/1999.77102603x.

Pradhan, K., Hemken, R. W. 1970. Utilization of ethanol and its effect on fatty acid patterns in ruminants. Journal of Dairy Science, 53:1739–1746. https://doi.org/10.3168/jds.S0022-0302(70)86472-4.

Rabelo, C. H. S., Mari, L. J., Reis, R. A. 2016. Survey about the use of bacterial inoculants in Brazil: Effects on silage quality and animal performance. In da Silva C. & Santos, E. M. T. Advances in silage production and utilization. IntechOpen, p. 3–37. http://dx.doi.org/10.5772/64472.

Radler, F., Zorg, J. 1986. Characterization of the enzyme involved in formation of 2-butanol from meso-2,3-butanediol by lactic acid bacteria. American Journal of Enology and Viticulture, 37:206–210.

Randby, A. T. 2007. Effect of propanol and dimethylsulphide in grass silage on organoleptic milk quality. Journal Animal Feed Science, 16:102–107. https://doi.org/10.22358/jafs/74122/2007.

Randby, A. T., Selmer-Olsen, I., Baevre, L. 1999. Effect of ethanol in feed on milk flavor and chemical composition. Journal of Dairy Science, 82:420–428. https://doi.org/10.3168/jds.S0022-0302(99)75248-3.

Raun, B. M. L., Kristensen, N. B., 2009. Portal absorption of ethanol and propanol in early lactating dairy cows. In Chilliard, Y., Glasser, F., Faulconnier, Y., Bocquier, F., Veissier, I. (Eds.), Ruminal Physiology: Digestion, Metabolism and Effects of Nutrition. Wageningen Academic Publishers, Wageningen, p. 338–339.

Raun, B. M. L., Kristensen, N. B. 2011. Metabolic effects of feeding ethanol or propanol to postpartum transition Holstein cows. Journal of Dairy Science, 94:2566–2580. https://doi.org/10.3168/jds.2010-3999.

Raun, B. M. L., Kristensen, N. B. 2012. Metabolic effects of feeding high doses of propanol and propylacetate to lactating Holstein cows. Livestock Production Science, 144:37–47. https://doi.org/10.1016/j.livsci.2011.10.011.

Richter, C. P., Campbell, K. H. 1940. Alcohol taste thresholds and concentrations of solution preferred by rats. Science 91:507–508. https://doi.org/10.1126/science.91.2369.507.

Robinson, P. H., Swanepoel, N., Heguy, J. M., Price, T., Meyer, D. M. 2016. ‘Shrink’ losses in commercially sized corn silage piles: Quantifying total losses and where they occur. Science of the Total Environment, 542:530–539. https://doi.org/10.1016/j.scitotenv.2015.10.090.

Rook, A. J., Gill, M. 1990. Prediction of the voluntary intake of grass silages by beef cattle.1. Linear regression analyses. Animal Production, 50:425–438. https://doi.org/10.1017/S0003356100004918.

Rooke, J. A., Hatfield, R. D. 2003. Biochemistry of ensiling. In: Buxton, D. R., Muck, R. E., Harrison, R. E. (Eds.), Silage Science and Technology. American Society of Agronomy, Madison, USA, p. 95–139.

Sanchez, R. F., Cameron, D. C., Cooney, C. L. 1987. Influence of environmental factors in the production of (R)-1,2 propanediol and acetol by *Clostridium thermosaccharolyticum*. Biotechnology Letters, 9:449–454. https://doi.org/10.1007/BF01027450.

Sato, H. 2009. Increased blood concentration of isopropanol in ketotic dairy cows and isopropanol production from acetone in the rumen. Journal Animal Science, 80:381–386. https://doi.org/10.1111/j.1740-0929.2009.00649.x.

Shipe, W. F., Ledford, R. A., Peterson, R. D., Scanlan, R. A., Geerken, H. F. 1962. Physiological mechanisms involved in transmitting flavors and odors to milk. II. Transmission of some flavor components of silage. Journal of Dairy Science, 45:477–480. https://doi.org/10.3168/jds.S0022-0302(62)89430-2.

Siemerink, M. A., Kuit, W., López Contreras, A. M., Eggink, G., Van der Oost, J., Kengen, S. W. 2011. D-2,3-butanediol production due to heterologous expression of an acetoin reductase in *Clostridium acetobutylicum*. Applied and Environmental Microbiology, 77:2582–2588. https://doi.org/10.1128/AEM.01616-10.

Silva, J., Winckler, J. P. P., Pasetti, M. H. O., Salvo, P. A. R., Kristensen, N. B., Daniel, J. L. P., Nussio, L. G. 2017. Effects of *Lactobacillus buchneri* inoculation or 1-propanol supplementation to corn silage on the performance of lactating Holstein cows. Revista Brasileira de Zootecnia, 46:591–598. https://doi.org/10.1590/s1806-92902017000700006.

Steen, R. W. J., Gordon, F. J., Dawson, L. E. R., Park, R. S., Mayne, C. S., Agnew, R. E., Kilpatrick, D. J., Porter, M. G.1998. Factors affecting the intake of grass silage by cattle and prediction of silage intake. Animal Science, 66:115–127. https://doi.org/10.1017/S1357729800008894.

Steidlova, S., Kalac, P. 2002. Levels of biogenic amines in maize silages. Animal Feed Science and Technology, 102:197–205. https://doi.org/10.1016/S0377-8401(02)00217-1.

Stice, S., Thrall, M. A., Hamar, D. W. 2018. Alcohols and glycol. In: Gupta, R.C. (Ed.), Veterinary Toxicology: Basic and Clinical Principles, Third ed., Elsevier Inc. Amsterdam, p. 647–657. https://doi.org/10.1016/B978-0-12-811410-0.00049-0.

Suzuki, T., Onishi, H. 1968. Aerobic dissimilation of α-rhamnose and the production of α-rhamnnoic acid and 1,2 propanediol by yeast. Agricultural and Biological Chemistry, 32:888–893. https://doi.org/10.1080/00021369.1968.10859143.

Tennant, B. C., Center, S. A. 2008. Hepatic function. In Kaneko, J. J., Harvey, J. W., Bruss, M. L. (Eds.), Clinical Biochemistry of Domestic Animals. Elsevier Academic Press, Burlington, p. 379–403.

Wang, C., Nishino, N. 2013. Effects of storage temperature and ensiling period on fermentation products, aerobic stability and microbial communities of total mixed ration silage. Journal of Applied Microbiology, 114:1687–1695. https://doi.org/10.1111/jam.12200.

Wanjala, G. W., Mathooko, F. M., Kutima, P. M., Mathara, J. M. 2018. Prevalence of adulteration and inhibitory substances in raw and pasteurized milk marketed in Nairobi region. Asian Food Science Journal, 3:1–8. https://doi.org/10.9734/AFSJ/2018/42382.

Weiss, K., Kalzendorf, C., Zittlau, J., Auerbach, H. 2009. Novel results on the occurrence of volatile compounds in maize silages. In Broderick G.A. (Ed.), Proceedings of the XVth International Silage Conference (p. 33–34), Madison, USA.

Weiss, K. 2017. Volatile organic compounds in silages - Effects of management factors on their formation: A review. Slovak Journal of Animal Science, 50:55–67.

Weiss, K., Kroschewski, B., Auerbach, A. 2020. Formation of volatile organic compounds during the fermentation of maize as affected by sealing time and silage additive use. Archives of Animal Nutrition, 74:150-163. https://doi.org/10.1080/1745039X.2019.1694357

Wilkins, R. J., Hutchinson, K. J., Wilson, R. F., Harris, C. E. 1971. The voluntary intake of silage by sheep. I. Interrelationships between silage composition and intake. Journal of Agricultural Science, 77:531–537. https://doi.org/10.1017/S0021859600064613.

Wilkinson, J. M., Davies, D. R. 2013. The aerobic stability of silage: key findings and recent developments. Grass and Forage Science, 68:1–19. https://doi.org/10.1111/j.1365-2494.2012.00891.x.

Xin, F., Chen, T., Jiang, Y., Dong, W., Zhang, W., Zhang, M., Wu, H., Ma, J., Jiang, M. 2017. Strategies for improved isopropanol-butanol production by a Clostridium strain from glucose and hemicellulose through consolidated bioprocessing. Biotechnology for Biofuels and Bioproducts, 10:118. https://doi.org/10.1186/s13068-017-0805-1.

Yoshii, T., Asanuma, N., Hino, T. 2005. Effect of ethanol on nitrate and nitrite reduction and methanogenesis in the ruminal microbiota. Animal Science Journal, 76:37–42. https://doi.org/10.1111/j.1740-0929.2005.00235.x.

Zhang, J., Yamada, A., Cai, Y., Kawamoto, H., Aoki, Y., Kamo, M. 2006. Effects of addition of acetic acid, 1,2-propanediol and lactic acid bacteria on the aerobic stability of Italian ryegrass silage. In: Proc. 2nd China-Japan-Korea Grassland Conference, p. 93–95.

Zopollatto, M., Daniel, J. L. P., Nussio, L. G. 2009. Microbial silage additives in Brazil: review of aspects of ensilage and animal performance. Revista Brasileira de Zootecnia, 38:170–189. https://doi.org/10.1590/S1516-35982009001300018.

SILAGE FERMENTATION PROCESSES AND THEIR IMPLICATIONS FOR THE RUMEN FERMENTATION

DAVIES, D.R.
Silage Solutions Ltd, Bwlch Y Blaen, Pontrhydygroes, Ystrad Meurig, SY25 6DP Wales, UK. dave.silage@gmail.com

INTRODUCTION

Silage is produced as the result of a fermentation process, be that restricted by the use of chemical additives or natural through the use of no additive or possibly even enhanced when using inoculants. However, often we forget that the products of the silage fermentation processes are the feed for a second fermentation process in the rumen. Therefore, considering the silage fermentation in isolation of the rumen fermentation could have unexpected consequences for animal production, the environment and the economic sustainability of silage fed ruminant agriculture, be reducing the efficacy of the additive approaches currently being promoted. With this in mind it is worth reminding ourselves of the opening statement in the text book Silage Fermentation (A.J.G. Barnett 1954) 'Any narrow treatment of the matter from the point of view of one particular branch of pure or applied science is impossible and a dissertation on the preparation, characteristics and use of silage is bound to be somewhat digressionary in nature'. The author was, in his opening remarks, reminding us of the countless interactions occurring within the silo that could affect the outcome of the silage as a feed for livestock and that too much focus on one aspect could be detrimental to the overall process and most importantly the end-result, the feed value and all that encompasses of the silage.

It is my profound belief this is where we are now with silage inoculants and our overwhelming focus on the aerobic stability of silages and as a result we have forgotten the rumen, the animal and the detailed assessment of the silage quality. From the 1980's to early 2000's the focus of silage research was on preserving true protein and sugars to improve rumen utilisation of these key nutrients (Davies *et al.* 1998; Fairbairn *et al.* 1988; Heron *et al.*, 1986; Merry *et al.* 1995). Weinberg and Muck (1996) highlighted the benefits of the use of homofermentative lactic acid bacteria as inoculants on improved preservation of nutrients, reduced proteolysis, silage intake and animal performance. However, these authors also highlighted the problems of the approach with the increased problem of aerobic spoilage at feed-out and introduced the idea of heterofermentative inoculants to improve aerobic stability. Since this date the pendulum has swung to the other extreme with the major focus on aerobic stability and the question of detailed silage quality for feeding has to a greater or lesser extent been overlooked (Arriola *et al.,* 2021a; Driehuis *et al.* 2001; Kung and Ranjit, 2001; Muck 2004).

This paper will set out the silo fermentation pathways and consider how they impact on the initial acidification of the silage in terms of producing anaerobically stable silage and retention of nutrients. The paper will examine the effect silage fermentation dry matter losses have on nutrient quality and finally the paper will examine the fermentation pathways for the silage fermentation end-products in the rumen fermentation and how that could impact rumen microbial available energy and energy available for the ruminant.

Silage Lactic Acid Bacterial Fermentation Pathways

Primary fermentation Pathways

Homofermentative and Facultative Heterofermentative fermentation of Hexose sugars

Equation 1 (McDonald, P, Henderson A, R. and Ralton, I. 1973)
Hexose → 2 Lactate
(DM recovery 100% Gross Energy 99.3%)

Obligately Heterofermentative fermentation of Hexose sugars

Equation 2a (McDonald, P, Henderson A, R. and Ralton, I. 1973)
Hexose → Lactate + Ethanol + CO_2
(Recovery DM 75% GE 98.3 %)

Equation 2b (Mcdonald P, Henderson, A.R. and Heron, S.J.E. 1991 P. 92)
Hexose → Lactate + Acetate + CO_2 + H_2O
(Recovery DM 83% GE 79.6%)

Equation 3 (McDonald, P, Henderson A, R. and Ralton, I. 1973)
3 Fructose + H_2O → Lactate + Acetate + 2 Mannitol + CO_2
(Recovery DM 95.2% GE 99%)

Equation 4 (Wisselink, H.W., Weusthuis, R.A., Eggink, G., Hugenholtz, J. and Grobben, G.J. 2002).
1 glucose + 2 Fructose + H_2O → Lactate + Acetate + 2 Mannitol + CO_2
(Recovery DM 95.2% GE 99%)

Secondary Fermentation Pathway by obligately heterofermentative lactic acid bacteria where the starting point is the product of a primary fermentation pathway.
Equation 5 (Oude Elferink, S.J.W.H., Krooneman, J., Gottschal, J.C., Spoelstra, S.F., Faber, F., and Driehuis, F., 2001)
2 Lactic $\longrightarrow$ Acetic + Propan 1,2-diol+ CO_2
(Recovery DM 75% GE 98.7%)

Tertiary fermentation pathway by the obligately heterofermentative lactic acid bacterium *L. diolivorans* where the starting point is the product of a secondary fermentation.
Equation 6 (Krooneman, J., Faber, F., Alderkamp, A.C., Oude Elferink, S.J.W.H, Driehuis, F., Cleenwerck, I., Swings, J., Gottschal, J.C., and Vancanneyt, M. 2002)
2 Propan-1,2-diol $\longrightarrow$ Propionate + Propan-1-ol + H_2O
(Recovery DM 88.2% GE 99.7%)

It is worthy of note that the energy recovery data is based on gross energy and makes no reference to utilisable energy once silage is consumed. It also only represents the energy recover of each mol/g of hexose sugar consumed and not the silage mass. The utilisable energy loss/recovery of which will also be impacted by the dry matter loss or more correctly the digestible organic matter loss associated with each fermentation profile. Finally, the energy and DM recovery data associated with secondary and tertiary fermentation profiles only represent the loss associated with the starting point chemical being fermented and do not take into consideration the losses associated with the primary and secondary fermentation pathways where that product was initially produced. Thus, for equation 5 the accumulative recovery of DM and GE from the original hexose sugar would be 75% and 98% respectively if the lactic acid was produced by the fermentation pathway shown in equation 1 and 58% and 78.3% respectively if the lactic acid was produced by the fermentation pathway shown in equation 2b. Likewise for equation 6 and following the same primary fermentation pathways the DM and GE recoveries would be 63.2% and 97.7% from primary fermentation equation 1 and 46.2% and 78% from primary fermentation equation 2b.

These accumulative losses must be considered when examining the overall effect of one chemical moiety such as propan-1, 2-diol (propylene glycol) on the energy content of silage, because whether we like it or not the First Law of Thermodynamics or more specifically the 'Law of conservation of energy' states that 'energy can be transformed from one form to another, but can be neither created nor destroyed.'

Put simply: - Energy In = Energy Out

Thus, when energy is lost from the silage by the production of water, CO_2 or heat it has to be considered.

Lactic acid bacterial fermentation profiles and their effect on the initial acidification of forages.

It is well accepted that the first most important contribution to preserving nutrients during ensilage is the speed at which the pH in the silage mass drops to a pH low enough to inhibit degradation of proteins, water soluble carbohydrates and lipids by both plant and microbial activity (McDonald *et al.,* 1991; Lee *et al.,* 2008). The pH at which anaerobically stable silage is acquired is crop dry matter dependant but an arbitrary pH of 4 is often assumed. With this in mind the specific lactic acid bacterial fermentation pathways will have a major contribution to the rate of acidification of the silage and the inhibition of both plant enzymes and undesirable microbial proliferation and activity.

Table 1 Indicating the relative acidity of the end-products of the silage fermentation pathways shown in Eqn 1-6 above

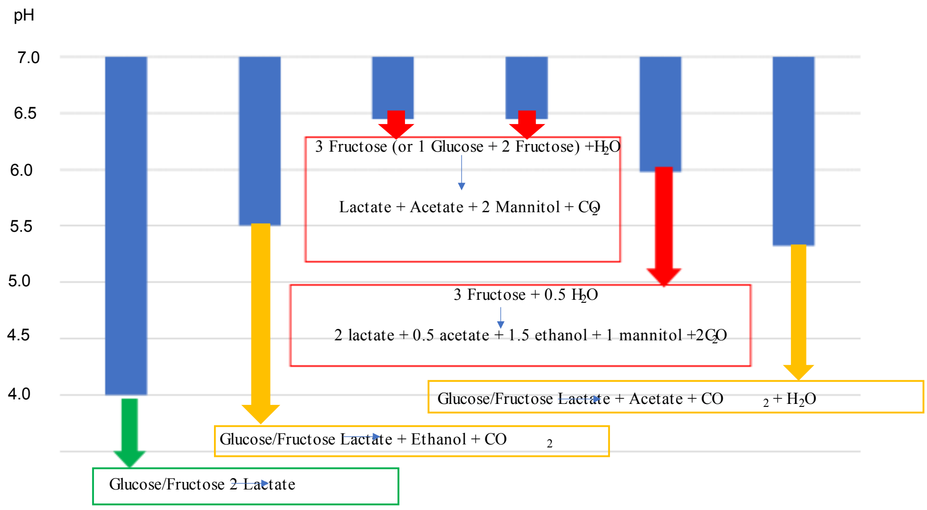
Biochemical	Formula	pKa
Lactic Acid	$C_3H_6O_3$	3.86
Acetic Acid	$C_2H_4O_2$	4.75
Propionic Acid	$C_3H_6O_2$	4.88
Ethanol	C_2H_5OH	NA
Propan-1, 2-diol	$C_3H_8O_2$	NA
Propan-1-ol	C_3H_7OH	NA
Mannitol	$C_6H_{14}O_6$	NA

NA = Not Acidic thus pKa is not applicable.

As the pKa is on a log scale this data can be used alongside the fermentation pathways to calculate the relative acidification effect of each fermentation pathway from the original hexose sugar fermented. Comparing the relative strengths of lactic and acetic acid it can be calculated that lactic acid is 7.76 times stronger than acetic acid, or to put it another way to obtain the same level of pH decline 5.6 g of acetic acid is required for every g of lactic acid.

Using just the primary fermentation pathways as these would be taking place at the start of the fermentation the relative acidification can be established. Figure 1 shows these calculated values, based on the amount of sugar required to drop the pH to 4 by the most efficient fermentation, the homofermentation of hexose to just lactic acid. The pH drop produced by the other fermentation pathways are based on the same level of hexose being fermented by each of the other indicated fermentations pathways.

Figure 1 Theoretical - Acidogenic potential of each primary lactic acid bacterial fermentation pathway using the same hexose equivalent



The figure above clearly shows the negative effect the less efficient obligately heterofermentative fermentation pathways have on the pH decline in the silo. Whilst many silage inoculants have combinations of homo and heterofermentative lactic acid bacteria in their inoculant mix knowing which bacterial fermentation will dominate and which other fermentation pathways will also have an influence then the overall speed of pH decline is difficult to predict. The effect the slower pH decline has on the silage quality is multifactorial but includes:

1. Prolonged activity of the Enterobacteria and Clostridia. Infact Östling and Lindgren (1995) reported positive effects of inoculation with certain Enterobacterial species showing a positive effect on aerobic stability of silages. Is this why inoculation with obligately heterofermentative lactic acid bacteria improve aerobic stability, by allowing more activity from the enterobacterial population rather than their own products of fermentation?
2. Increased use of water soluble carbohydrate thus reducing silage nutritive value and/or increasing the risks of anaerobically unstable silage under low dry matter conditions. Using 2 commercially available silage inoculants one being a mixed facultatively heterofermentative and obligately heterofermentative inoculant and the other being facultatively heterofermentative inoculant Davies *et al.* (2005) showed on average a significant 4.5 fold increase in WSC content over a wide range of %DM contents, with the facultative heterofermentative inoculant alone compared to the mixed species inoculant. Therefore, is it the reduced concentration of WSC, a food source for yeasts, that improves the aerobic stability of silages inoculated with obligately heterofermentative lactic acid bacteria not the antimicrobial effects of acetic acid and propan-1,2-diol?

The effect of fermentation pathway on fermentation DM losses and their consequences

When the silage fermentation produces CO2 and/or water dry matter is lost. However, the scale and the effect of these losses are rarely assessed to the level they should be as the argument put forward is that a 1-2% increased loss of DM in the fermentation phase is acceptable if it saves a 5% DM loss during aerobic spoilage. However, does this scientific argument stand interrogation? The problem is that on farm, aerobically spoiling silage is easy to detect whereas the effect an inoculant may have on reducing silage quality is much more difficult to pinpoint. Meanwhile, if the same level of scrutiny was placed on the reasons for aerobic spoilage as is placed on timing of harvest to improve nutritive value, then the use of inoculants containing obligately heterofermentative would not be required.

Let us interrogate the DM loss argument more closely. Firstly what does dry matter loss in terms of fermentation losses as result of a hetero-lactic fermentation actually represent? It represents a DM loss from the WSC. Thus it represents a loss of digestible energy not just DM. Taking the theoretical argument one step further, if a forage at harvest is 75% digestible and as a result of a hetero-lactic fermentation has a 4% DM loss the 75% digestible forage at harvest is now a 72% digestible forage. If that is converted to metabolizable energy that represents a loss of 0.48 MJ/Kg DM and a milk yield loss of 0.09 kg milk/kg DM. Thus, for a cow consuming just

12 kg DM forage that is a loss of 1.08kg Milk/cow/d. The loss in digestibility results in a relative increase in the undigestible portion, such as ash and lignin (and by association ADF and NDF). Arriola *et al*. (2021a) showed in Sorghum silage that the untreated sample had a significantly lower NDF and a significantly higher *in vitro* digestibility than any other the hetero-lactic inoculant treatments tested. Likewise Navarro-Villa et al 2012 showed lower *in vitro* digestibility with the combination homo-lactic plus hetero-lactic inoculant treated silages compared to all other treatments in grass silage. Alongside the reduced digestibility comes the accepted link between higher NDF content and intake. Thus, when measuring DM losses due to hetero-lactic driven inoculated lactic acid bacterial fermentation, considerations must not only be given to the loss of quantity of silage but also the digestibility, nutritive value and the fibre effect on intake.

Silage fermentation products and their fermentation in the rumen

Lactic + $2H^+$ → Propionic
(Recovery GE 112.7% - due to saving effect of reduced methane by removal of hydrogen into useful end product propionic acid)
Acetic → Acetic (Recovery 100%)
Ethanol → Acetic + H_2O (Recovery 63.8%)
Propan-1, 2- diol → Propionic + $2H^+$ (recovery 87.3%)

Mannitol there is evidence to suggest that mannitol is fermented slowly in the rumen (Ahmed *et* al. 2013) and as it is highly soluble is likely to flow with the liquid phase rapidly from the rumen. Mannitol is used in human nutrition as an artificial sweetener in humans for weight loss as it is very poorly absorbed form the gut. Therefore, further research is required to assess the extent of use of mannitol in ruminants if it is produced in silage as a result of hetero-lactic fermentation because its perceived excellent gross energy retention in the silo could actually result in a very high loss of energy once consumed by the ruminant.

In addition to the equations showing the dominant rumen fermentation pathways it is worthy of note that the requirement of H+ ions in the conversion of lactic to propionic has the effects of reducing rumen methane emissions, capturing more energy and increasing rumen pH due to the fact that pH is a measure of H+ the latter point being supported by the findings of Jaakkola and Huhtanen, 1989. Whereas the release of H+ ions during the conversion of Propan-1,2-diol has the opposite effect, it increases methane emissions by ruminants with its concomitant loss of energy and it has the potential to lower rumen pH.

Fermentation pathways from hexose in the pre-ensiled through the silo fermentation and on into the rumen fermentation

Combining selected silage fermentation pathways (equation 1, 2a, 2b, and 5) with the rumen fermentation pathways give the following results

Obligately Homofermentative and facultatively heterofermentation Lactic Acid Bacterial Fermentation in the Silo
Frome equation 1
2 Hexose $+8H^+$ → 4 Propionic acid + $4H_2O$
(Recovery GE 109%)

Obligately Heterofermentative Lactic Acid Bacterial Fermentations in the silo
From equation 2a
2 Hexose $+4H^+$ → 2 propionic + 2 Acetic + 2 CO_2 + $6H_2O$
(Recovery GE 91.2%)

From equation 2b
2 Hexose → 2 propionic + 2 Acetic + 2 CO_2 + $6H_2O$
(Recovery GE 91.2%)

From Equation 3 and 4
3 Hexose $+2H^+$ + H_2O → propionate + Acetate + 2 Mannitol + CO_2
(Retained GE if Mannitol is available 100% if not 31%)
From Equation 5
2 Hexose → 3 Acetic + Propionic + 2 CO_2 $+2H_2O$ + $2H^+$ (retained GE 74%)

The biochemical pathways pulled together above indicates that examining the GE changes in the silo fermentation will be incorrect when examining the full fermentation process from fresh forage through the silo into the rumen and when the end-point of the silage fermentation is the rumen fermentation all aspects need to be considered.

The most efficient overall fermentation is that of the homo-lactic fermentation which retains more energy for the cow and has the potential to reduce methane emissions compared to any other silage fermentation pathway.

It is also worthy of note that the more efficient homo-lactic fermentation will also result in higher levels of retained WSC which in is not considered in the above calculations. Thus, statements about the energy content of one silage fermentation end-product can not and should not be looked at in isolation as is often the case in the commercial world especially with propan-1, 2-diol (propylene glycol).

CONCLUSIONS

This paper has focused on the theories of known biochemical pathways in the silo and rumen with references to research that has indicated similar findings under more real scenarios of silage and rumen experimentation. The review clearly shows that the most appropriate silage fermentation pathways for energy retention would be a homo-lactic fermentation. Aerobic spoilage can and must be controlled by other means than the hetero-lactic fermentation approach. Whilst this paper has focused heavily on the two extremes, be that homo-lactic fermentation or solely hetero-lactic fermentation recent review papers have indicated that the levels of acetic acid in silages are having a negative impact on intake especially when above 15g/kg DM in the total mixed ration (Gerlach *et al.* 2021). Taking this figure alongside the figure of 30 g/kg DM acetic acid required to improve silage aerobic stability (Kleinschmidt and Kung, 2006) raises the question what is the goal of a silage inoculant, improve animal intake in order to improve production or improve aerobic stability through hetero-lactic fermentation? This statement is supported by the meta-analysis review of animal performance from both mixed homo-lactic/hetero lactic inoculants and hetero-lactic only inoculants (Arriola *et al.* 2021b) showed no benefit to animal performance of using either approach compared to no treatment on the crops examined. However, reading the small print stated there was a trend with maize silages of a negative impact on milk production of such inoculants. These publications are in direct contrast to the met-analysis focusing on silages inoculated with homo-lactic acid bacteria where positive responses were found over a range of crops for fermentation quality and animal performance (Oliveira *et al*., 2017)

Given the increased pressure on ruminant agriculture to reduce its emissions, silage scientists need to ensure the best advice is getting to farmers and educating silage additive companies about their additives and what they can and cannot do must be a starting point.

REFERENCES

Ahmed, S., Minuti, A. and Bani, P. (2013) In Vitro Rumen Fermentation Characteristics of Some Naturally Occurring and Synthetic Sugars. Italian Journal of Animal Science 12: 359-365.

Arriola, K.G. Vyas, D., Kim., D., Agarussi, M.C.N., Silva, V.P., Flores, M., Jiang, Y., Yanlin, X., Pech-Cervantes, A.A., Ferraretto, L.F. and Adesogan A.T., (2021a) Effect of Lactobacillus hilgardii, Lactobacillus buchneri, or their combination on the fermentation and nutritive value of sorghum silage and corn silage. Journal of Dairy Science 104: 9664-9675.

Arriola K.G., Oliveira, A.S., Jiang, Y., Ferraretto, L.F., Vyas, D. and Adesogan, A.T., (2021b) Meta-analysis of effects of inoculation with *Lactobacillus buchneri*, with or without other bacteria, on silage fermentation, aerobic stability, and performance of dairy cows. Journal of Dairy Science 104; 7653-7670.

Barnett, A.J.G. (1954) Silage Fermentation. Publ. Butterworths Scientific Publications, London Pp.206

Davies *et al.* 1998; Davies, D. R., Merry, R. J., Williams, A. P., Bakewell, E. L., Leemans, D. K., Tweed, J. K. S. (1998). Nutrition, feeding and calves. Proteolysis during ensilage of forages varying in soluble sugar content *Journal of Dairy Science*, 81, (2), 444-453

Davies, D.R., Leemans, D.K., Bakewell, E.L. and Merry, R.J., (2005) The effect of dry matter content and inoculation with lactic acid bacteria on the residual water soluble carbohydrate content of silages prepared from a high sugar grass cultivar. XIVth International Silage Conference. *Silage production and utilisation. IGC 2005 Satellite Workshop, Belfast, 3-6 July 2005*, Park, R. S.Stronge, M. D. Wageningen Academic Publishers, P 211

Driehuis, F., S. W. H. Oude Elferink, and P. G. Van Wikselaar. 2001. Fermentation characteristics and aerobic stability of grass silage inoculated with Lactobacillus buchneri, with or without homofermentative lactic acid bacteria. Grass Forage Sci. 56:330–34

Fairbairn, R., L. Alli, and B. E. Baker. 1988. Proteolysis associated with the ensiling of chopped alfalfa. J. Dairy Sci. 71: 152–158.

Gerlach K., Daniel, J. L-P., Jobim, C.C and Nussio, L.G. (2021). A data analysis on the effect of acetic acid on dry matter intake in dairy cattle. Animal Feed Science and Technology Vol 272:

Heron, S.J.E., R. A. Edwards, and P. McDonald. 1986. Changes in the nitrogenous components of gamma-irradiated and inoculated ensiled ryegrass. J. Sci. Food Agric. 37:979–985.

Jaakkola, S. and Huhtanen, P. 1989 The Effect of Lactic Acid on Microbial Protein Synthesis in the Rumen of Cattle. Australasian Journal of Animal Science 2: (No. 3) 398-399

Kleinschmit, D. H., and L. Kung Jr. 2006. A meta-analysis of the effects of Lactobacillus buchneri on the fermentation and aerobic stability of corn and grass and small-grain silages. J. Dairy Sci. 89:4005–4013.

Krooneman, J., Faber, F., Alderkamp, A.C., Oude Elferink, S.J.W.H, Driehuis, F., Cleenwerck, I., Swings, J., Gottschal, J.C., and Vancanneyt, M. (2002) *Lactobacillus dioloverans* sp nov., a 1,2-propanediol-degrading bacterium isolated from aerobically stable maize silage. Int. J. Systematic and evolutionary microbiology 52: 639-645.

Kung, Jr., L., and N. K. Ranjit. 2001. The effect of *Lactobacillus buchneri* and other additives on the fermentation and aerobic stability of barley silage. *J. Dairy Sci.* 84(5): 1149−1155.

Lee, M. R. F., Scott, M. B., Tweed, J. K. S., Minchin, F. R., Davies, D. R. (2008). Effects of polyphenol oxidase on lipolysis and proteolysis of red clover silage with and without a silage inoculant (Lactobacillus plantarum L54). *Animal Feed Science and Technology*, 144, (1-2), 125-136

McDonald, P, Henderson A, R. and Ralton, I. (1973) Energy Changes during ensilage. J.Sci. Fd. Ag. 24: 827 -834.

Mcdonald P, Henderson, A.R. and Heron, S.J.E. (1991). The biochemistry of silage. Second edition, Chalcombe Publications, Marlow, Bucks. P 92.

Merry, R. J., M. S. Dhanoa, and M. K. Theodorou. (1995). Use of freshly cultured lactic acid bacteria as silage inoculants. Grass Forage Sci. 50:112–123.

Muck, R.E. (2004). Effect of corn silage inoculants on aerobic stability. Transactions of the American Society of Agricultural Engineers Vol. 47(4): 1011−1016.

Navarro-Villa, A., O'Brien, M., Lopez, S., Boland, T.M. and O'Kiely, P. 2012 In vitro rumen methane output of grasses and grass silages differing in fermentation characteristics using the gas-production technique (GPT) Grass Forage Sci. 68:228-244

Oliveira, A. S., Z. G. Weinberg, I. M. Ogunade, A. A. P. Cervantes, K. G. Arriola, Y. Jiang, D. Kim, X. Li, M. C. M. Gonçalves, D. Vyas, and A. T. Adesogan. 2017. Meta-analysis of effects of inoculation with homofermentative and facultative heterofermentative lactic acid bacteria on silage fermentation, aerobic stability, and the performance of dairy cows. J. Dairy Sci. 100:4587–4603.

Östling, C. and Lindgren, S. (1995) Influences of enterobacteria on the fermentation and aerobic stability of grass silages. Grass and Forage Science 50: 41-47.

Oude Elferink, S.J.W.H., Krooneman, J., Gottschal, J.C., Spoelstra, S.F., Faber, F., and Driehuis, F., (2001). Anerobic conversion of lactic acid to acetic acid and 1,2,-propanediol by *Lactobacillus buchneri*. Applied and Environmental Microbiology 67: 125-132.

Weinberg, Z.G. and Muck R.E. (1996). New trends and opportunities in the development and use of inoculants for silage FEMS Microbiology Reviews 19: 53-68

Wisselink, H.W., Weusthuis, R.A., Eggink, G., Hugenholtz, J. and Grobben, G.J. (2002). Mannitol production by lactic acid bacteria: a review. International Dairy Journal 12: 151–161.

THE QUALITY MONITORING OF AGRICULTURAL CROPS USING MULTISPECTRAL DRONE IMAGING.

JANOUŠEK, J., MARCOŇ, P., JAMBOR, V.
Faculty of Electrical Engineering and Communication, Brno University of Technology, 61600 Brno, Czech Republic
NutriVet s.r.o., Vídeňská 1023, 69123 Pohořelice, Czech Republic

ABSTRACT

Estimating the optimum harvest time and yield is important for food security. Vegetation indices are proving to be an effective tool for mapping plant health. Using a multispectral camera on a drone, areal information on the plant condition of a heterogeneous field can be obtained. In this study, the relationships between vegetation idnexes and nutritional values determined by chemical analysis of plant samples collected in the field are investigated. Attention is paid to the normalized difference red edge index (*NDRE*), the normalized difference vegetation index (*NDVI*) and the green normalized difference vegetation index (*GNDVI*) in particular, and nutritional values such as dry matter. Dependencies between variables were correlated and described using regression methods. The result is equations that can be applied to calculate dry matter estimation and thus to determine the optimal time window for harvesting maize. The obtained equations were verified on five different types of maize hybrids in fields in the South Moravian Region of the Czech Republic.

Keywords: multispectral imaging; vegetation indices; nutritional analysis; correlation; photogrammetry; optimal harvest time; UAV

INTRODUCTION

The role of remote sensing of the earth has played an important role in precision agriculture for several years. Satellite imagery or hyperspectral or multispectral cameras can be used to image agricultural areas [1]. These are used not only for remote sensing, but also, for example, to detect dying trees infested with pests [2], rotten or mechanically damaged fruits and vegetables [3], detection of fecal contamination [4], inspection of cold damage to cucumbers [5], measurement of fruit ripening [6] or, for example, to classify wheat kernels infected with fungi [7] and many other applications [8], [9]. Recently, the above mentioned problems have been developing very much.

This paper is based on a case study described in publication [1] and builds on the described fact, which is the existing correlation between plant nutritional values and vegetation indices. In the mentioned literature, the results obtained during one harvesting season are processed. This work is based on data measured over three years (seasonal harvests) and aims to verify and extend the results described in the literature [1].

Whole maize plants make up the total biomass of the harvested crop. The basic indicator of plant phenophase is the determination of the dry matter content of the plant. This increases significantly as the crop matures. In maize, dry matter is an indicator of the stage of vegetative maturity of the crop and its quantity influences the quality of the storage process in the form of ensiling [10]. The chemical composition of maize plants changes during the growing season. In the period when the plant has not set ears, the energy in the plant is mainly in the form of fibre. The content varies from plant to plant depending on the harvest time chosen. In order to ensure that the maize silage subsequently processed contains not only fibre but also starch, it is harvested at the milk-wax maturity stage, when the dry matter of the whole plant is between 280 and 330 g/kg. In this case, the milk line stage is 2/3 of the maize grain. Another indicator of vegetative maturity of maize is the ability to make silage or to produce fermentation acids that preserve the silage.

The harvesting time and the amount of total biomass have a very important influence on the quality of the silage fermentation process. The quality of maize cultivation and subsequent ensiling is highly variable from year to year due to weather conditions, the choice of a suitable hybrid with the appropriate FAO (Food and Agriculture Organisation) maize earliness number and the effect of treatment, including the quality of the maize sowing.

Maize samples are routinely taken in the field from various locations in the field to assess the condition and phenophase of the maize. When the milk line stage is reached, samples are taken to the laboratory for chemical analysis to determine the dry matter content of the grain and the whole plant. Depending on the development of the plant and the dry matter, the harvesting time is then pre-determined, which varies according to the purpose of use for milk or methane production in the biogas plant (BPS).

Since the dry matter content is an important parameter for determining the optimum harvest time, the question is whether more accurate information can be obtained on the average dry matter value of the whole harvested field, which is inhomogeneous. Our paper provides an answer to this question by describing the effective integration of different methods and technologies, namely a drone with a multispectral camera, image data analysis to obtain vegetation indices, and chemical analysis of samples collected in the field.

Full field image data capture

Two basic technologies allow us to obtain full-field information on the field condition and thus on the average dry matter content, namely satellite imagery or photogrammetry based on the use of a multispectral or hyperspectral camera mounted on an aircraft or drone. Both technologies can be applied to predict the yield of agricultural crops.

Different types of multispectral or hyperspectral cameras are used for photogrammetric imaging with unmanned aerial vehicles (UAVs). In our experiment, we used a RedEdge multispectral camera that captures 5 different bands, see Table 1. The topic of crop yield prediction from photogrammetric data captured by UAVs has been addressed in the literature [11]. Our paper extends this described issue and presents new results on the correlation between dry matter yield and vegetation indices.

Tab. 1: Parameters of bands captured by the RedEdge Micasense camera

Band Number	Band Color	Wavelength [nm]	Band Width [nm]	Calibration Panel Reflectance
1	Blue (B)	475	20	0.56
2	Green (G)	560	20	0.56
3	Red (R)	668	10	0.55
4	Near infrared (NIR)	840	40	0.54
5	Rededge (RE)	717	10	0.50

A different approach to photogrammetric imaging using drones is the use of artificial satellites or satellites. Compared to UAVs, satellite imagery differs primarily in the distance of the sensor from the area of interest. Satellites move at a constant altitude of 400 km or more in geostationary orbits that orbit the Earth at the equator or in orbits. It is the satellites in orbits that orbit the earth in a north-south direction over the entire earth's surface.

MATERIALS AND METHODS

The flow chart in Figure 1 describes the data collection procedure and mathematical processing. The individual blocks are described in more detail in the following chapters.

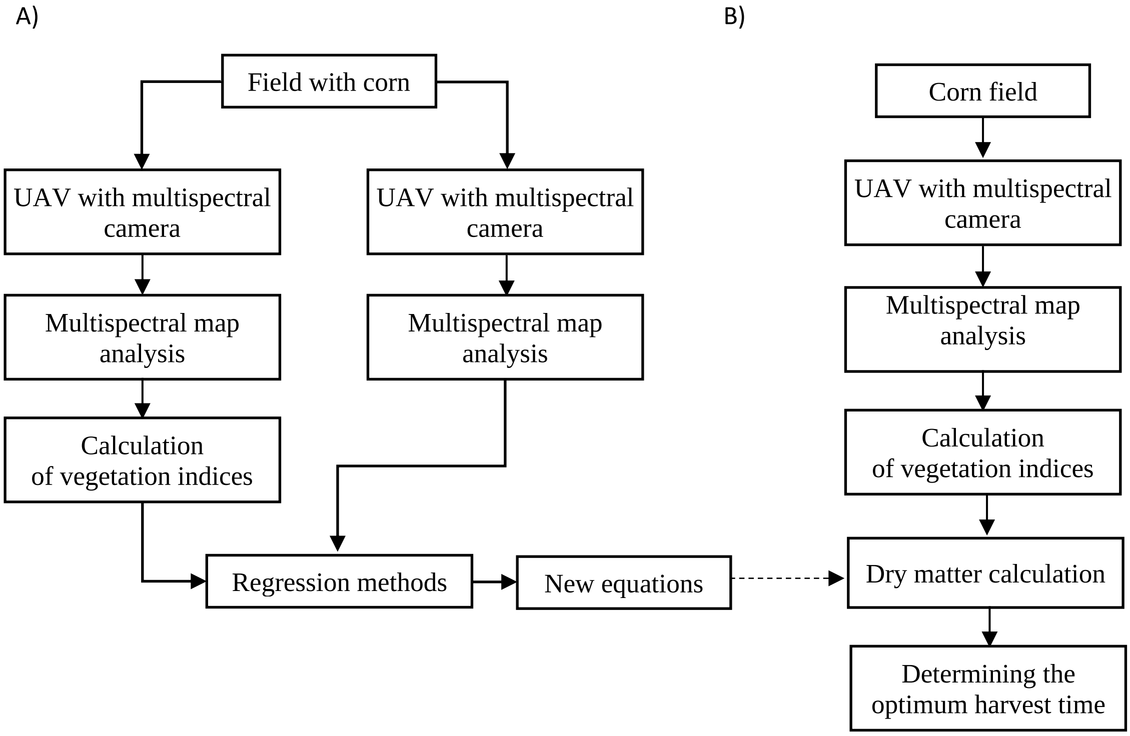


Figure 1: Block diagram presenting the determination (A) and verification (B) of the relationships between vegetation indices and nutritional values of different types of maize hybrids.

Sensing period and location

Photogrammetric imaging with a multispectral camera and manual sampling were carried out in maize stands at different locations in the South Moravian Region in the Czech Republic at different intervals between sampling, with the sampling locations for chemical analysis recorded in multispectral images, see Figure 2. The different locations were chosen because of sufficient heterogeneity of the stand, soil composition or effects of climatic conditions, see [1]. In year 1, sampling was carried out near the village of Troubsko from 23 July 2019 to 4 September 2019, where four different time intervals of plant phenovision were taken for the hybrid intended for ensiling and 5 sampling for the grain hybrid.

Between 12.8.2020 and 7.10.2020, sampling was carried out between the municipalities of Šlapanice and Prace. In order to optimize and compare samples from the first year of the study, a higher frequency of sampling was chosen, on 8 different dates. The same amount of sampling was carried out in the next year, when a site near the village of Velké Pavlovice was allocated for experiments. The sampling took place for two maize hybrids from 2 August 2021 to 21 September 2021.

In order to test the validity of the correlation dependencies and to confirm the hypothesis that changes in the calculated vegetation indices are proportional to changes in the nutritional analysis, an experimental trial was conducted completely separately from the previous experiments for 5 maize hybrids on the day of their actual harvest. These field trials were carried out on plots near the village of Knínice in the Blansko district.

Photogrammetry

In particular, a DJI Matrice 600 pro drone with a Micasence RedEdge multispectral camera was used to capture the photogrammetric data, see Figure 3.

The first step before evaluating the images is to acquire a sufficient number of heterogeneous data, on which a comparison of the methods suitable for achieving the highest possible degree of correlation with the nutritional values obtained from the plant samples is then performed.

Figure 2: Demonstration of capturing image data over a corn field with a RedEdge camera drone.

In the framework of multispectral imaging, vegetation indices are obtained that tell about different reflectance values of the electromagnetic spectrum related to the biological properties of plants.

Each vegetation index tracks different vegetation characteristics and is suitable for specific applications. Indices that do not use the spectral band of the near-infrared region have limiting properties and are therefore not suitable for practical use in monitoring vegetation changes. For the analysis, the ratio indices *NDVI*, *NDRE* and *GNDVI* were chosen, which are calculated in a similar way, but contain different spectral bands and thus comprehensively form a cross-section of important wavelengths. By appropriate combination, it is then possible to use additional vegetation indices that are modifications of existing indices and provide different information compared to the selected indices [35].

Vegetation indices are not constant depending on short-term changes in weather and amount of solar radiation. Calibration is necessary to refine the multispectral sensing results. This is done in different growing seasons under different weather conditions at the same phases of the day. It is important to create a database of data from several measurements from which to select the values that would be achieved under optimum conditions. Calibration in plant imaging will remove inaccuracies that are caused by single samples of imaged crops. By creating additional images of the plants, the indices will be compared with each other.

- **NDVI (Normalized difference vegetation index)**

The NDVI is a numerical indicator of plant health that provides data on vegetation changes. It also provides information on the amount of water stress and the amount of chlorophyll contained in the plant. It evaluates the monitored vegetation surface by the ratio of the reflectance of the red and near-infrared parts of the spectrum [36].

Due to the reflectance of the near infrared spectrum, it can easily distinguish minor differences in vegetation. Shadows and the influence of the atmosphere are non-negligible elements in sensing. Their effect causes reflectance changes in different bands. Elimination of the influence of the atmosphere can be achieved by correcting from the comparison of images taken at different time periods [35].

$$NDVI = \frac{\rho_{NIR} - \rho_{Red}}{\rho_{NIR} + \rho_{Red}}, \quad (1)$$

,where ρ_{NIR} is the reflectance of the near-infrared wavelength band and ρ_{Red} is the reflectance of the red wavelength band.

- **NDRE**

Like the *NDVI* index, the *NDRE* differential index uses the near-infrared spectrum and the frequency band that lies in the transition region between the visually visible spectrum and the infrared spectrum, i.e., the edge of the red $\rho_{RedEdge}$.

$$NDRE = \frac{\rho_{NIR} - \rho_{RedEdge}}{\rho_{NIR} + \rho_{RedEdge}}. \qquad (2)$$

NDRE provides a calculation that assumes that the frequency band is not as strongly absorbed by only the uppermost layers of the plant as in the case of *NDVI*, but will allow better penetration into perennial or later crops. *NDRE* is also less susceptible to saturation in the presence of dense vegetation. Therefore, it may provide a better measure of variability in a region where NDVI takes on values close to +1.0 [38].

- **GNDVI**

The green normalized difference vegetation index uses wavelengths in the green spectral range as opposed to the red spectrum, where ρNIR represents reflectance values in the near-infrared band and ρGreen represents reflectance values from the green band [39].

$$GNDVI = \frac{\rho_{NIR} - \rho_{Green}}{\rho_{NIR} + \rho_{Green}}, \qquad (3)$$

The index also has the potential to remove the lack of sensitivity of *NDVI* due to the green component of the spectrum [40].

CHEMICAL ANALYSIS

Samples of the cultivated maize crops were subjected to chemical analysis in the laboratory to obtain their nutritional parameters. The vegetation indices and the results of the chemical analysis are correlated at different time sequences of the phenological growth phase of the crop under study in order to find the ideal harvest time in terms of yield of the maize grown for the production of maize silage for animal feed or for methane production in biogas plants (BPS).

The sampling was invariably performed at identical time intervals, together with the multispectral imaging. To monitor the quality of the corn hybrid, we opted for sampling according to the methodology recommended by the Central Institute for Supervising and Testing in Agriculture, Brno, Moravia, the Czech Republic [41]. Three different sampling sites of the monitored stand were chosen to achieve representative samples of stand homogeneity. From each site, 10 consecutive whole maize plants were sampled and at the same time the sampling site area was marked on multispectral maps.

The adjusted samples were analysed to obtain the following quantities: *FM* - Fresh matter, fresh weight; later also *EW* - Ear weight. In addition, the following nutritional values are determined by chemical analysis: *DM* - dry matter, the amount of dry matter, from which the values *CP* Crude protein - nitrogenous matter, *CF* Crude fibre - crude fibre, Starch - starch content, *Ash* - ash, *NDF* - (neutral detergent fibre), *DNDF* - (digestibility *NDF*) digestibility, *DOM* - (digestibility organic matter) digestibility. From the analysed data it is possible to calculate the yield per hectare: *YFM* - (yield of fresh matter), *YDM* - (yield of dry matter), where 80 000 maize plants/ha are assumed.

Data correlation

Correlation coefficients were sought to establish correlations between Nut analysis results and Veg vegetation index values $r_{Nut,Veg}$ according to the Pearson correlation coefficient. The degree of correlation is determined by the calculated correlation coefficient, which can take values from -1 to +1. The resulting values of the correlation coefficient +1 determine a completely direct dependence and the first variable tends to increase. Correlation coefficient values of -1 indicate a completely inverse relationship and the first variable tends to decrease. If the correlation coefficient is zero, this means that there is no linear relationship between the parameter of interest and the reflectance or vegetation index.

$$r_{Nut,Veg} = \frac{\frac{1}{n}\sum_{i=1}^{n}(Nut_i - \overline{Veg})(Veg_i - \overline{Nut})}{S_{Nut} \cdot S_{Veg}} \qquad (4)$$

, where Nut is the nutritional analysis value, Veg is the vegetation index value,
$\overline{Veg}$ and $\overline{Nut}$ are the sample means and S_{Veg} a S_{Nut} are the standard deviations.

Second power R^2 coefficient of determination refers to the coefficient of determination, taking values from 0 to 1, which was calculated as a representation of the joint variability of the variables and indicates the quality of the regression model. A value of 1 indicates perfect prediction of the values of the dependent variable and, conversely, a value of 0 indicates minimal information about the knowledge of the dependent variable. The coefficient of determination was calculated according to the following relationships.

The most general definition of the coefficient of determination:

$$R^2 = 1 - \frac{SS_{res}}{SS_{tot}}. \tag{5}$$

In the best case, the modeled values exactly match the observed values, which results in $SS_{res} = 0$ and $R^2 = 1$. A baseline model, which always predicts $\bar{y}$, will have $R^2 = 0$. Models that have worse predictions than this baseline will have a negative R^2 [42].

As a second parameter to determine whether the correlation coefficients take on a value such that we can conclude that there is indeed a relationship between them, their statistical significance was calculated. Statistical significance was determined using a continuous probability distribution according to Student's t-distribution:

$$t_{score} = (r_{Nut,Veg} \cdot \frac{\sqrt{n-2)}}{\sqrt{(1 - r_{Nut,Veg}{}^2)}}), \tag{6}$$

where *n* is the number of observed correlation phases.

To find a statistically significant value, a significance level of 2% was chosen, which corresponds to a quartile of 99%. The t-distribution value for 7 degrees of freedom is 2.998 and for 4 the critical value is 3.747 and for 3 the critical value is 4.541. If the correlation coefficient is greater than the critical value, we can consider the correlation to be statistically significant.

Method of verification of the obtained equations - independent corn field

Validation of the obtained equations for the linear dependence of dry matter values on the correlated vegetation indices *NDVI*, *NDRE* and *GNDVI* was carried out on the results of a single experiment for 5 hybrids (ES Joker, ES Wellington, KTG Karlaxx, Absolutissimo, Rudolfinio) on the day of their actual harvest. Predictions of dry matter content for hybrids intended for grain processing for which the ideal harvest window is shifted to values between 380 g/kg and 420 g/kg

For each of these hybrids, the relationship of the obtained dependencies (11), (12) and (13) was verified. Using nutritional analysis, the dry matter of whole plants was determined, which will be considered as conventional true value dry matter DM_{CTV}. The predicted dry matter value was then calculated from the vegetation indices DM_{PV} Absolute deviations have been determined Δ_{DM} and relative δ_{DM} the deviation between the conventionally true dry matter value and the predicted value:

$$\Delta_{DM\,[g/kg]} = DM_{PV} - DM_{CTV}. \tag{7}$$

$$\delta_{DM\,[\%]} = \frac{\Delta_{DM\,[g/kg]}}{DM_{CTV}} \cdot 100 = \frac{DM_{PV} - DM_{CTV}}{DM_{CTV}}. \tag{8}$$

RESULTS

Data correlation results

Table 8 shows the statistical significance values calculated according to (8) from the correlated data between vegetation indices and nutritional values of the maize hybrids studied over the three-year study. Statistically significant values are highlighted in the table.

Table 8: Statistical significance of correlation data obtained from multispectral scanning and chemical analysis

Sample	Vegetation index	*Nutritional analysis*						*Yield characteristics*	
		DM [g/kg]	*CF* [g/kg *DM*]	*Starch* [g/kg *DM*]	*DNDF* [%]	*DOM* [%]	*FM* [kg/ 10 p]	*YFM* [kg]	*YDM* [kg]
2019 silage hybrid	*NDVI*	2.99	2.87	10.49	NA	NA	NA	NA	NA
	NDRE	5.16	4.74	36.40	NA	NA	NA	NA	NA
	GNDVI	4.05	3.65	2.18	NA	NA	NA	NA	NA
2019 grain hybrid	*NDVI*	8.51	0.13	4.04	NA	NA	NA	NA	NA
	NDRE	11.93	0.14	4.54	NA	NA	NA	NA	NA
	GNDVI	12.97	0.36	7.40	NA	NA	NA	NA	NA
2020 silage hybrid	*NDVI*	5.74	3.24	4.62	1.67	4.19	0.99	0.99	2.87
	NDRE	5.90	3.03	4.26	1.01	3.30	1.13	1.13	2.95
	GNDVI	5.08	2.12	2.79	1.26	2.10	1.53	1.53	2.07
2021 DKC 3568	*NDVI*	5.42	5.51	5.51	5.45	5.21	5.69	5.69	3.00
	NDRE	4.03	2.22	2.80	2.38	2.01	2.45	2.45	3.41
	GNDVI	4.25	3.13	3.14	3.21	2.98	3.18	3.18	3.31
2021 DKC 4279	*NDVI*	5.63	6.36	5.23	3.00	4.60	4.70	4.70	2.76
	NDRE	3.32	3.84	3.34	2.55	2.94	3.44	3.44	2.00
	GNDVI	7.49	4.39	5.59	3.12	3.79	5.90	5.90	3.15

Regression model results

Validation of the equations obtained for the linear dependence of dry matter values on correlated vegetation. Figure 4 presents the results of the dependence of the analyzed vegetation indices on the dry matter value. Using A linear regression model, the following dependencies were found:

$$NDVI = -\,0{,}0007 \cdot DM + 1.0712, \tag{9}$$

$$NDRE = -\,0{,}0007 \cdot DM + 0.7031, \tag{10}$$

$$GNDVI = -\,0{,}0005 \cdot DM + 0.9277. \tag{11}$$

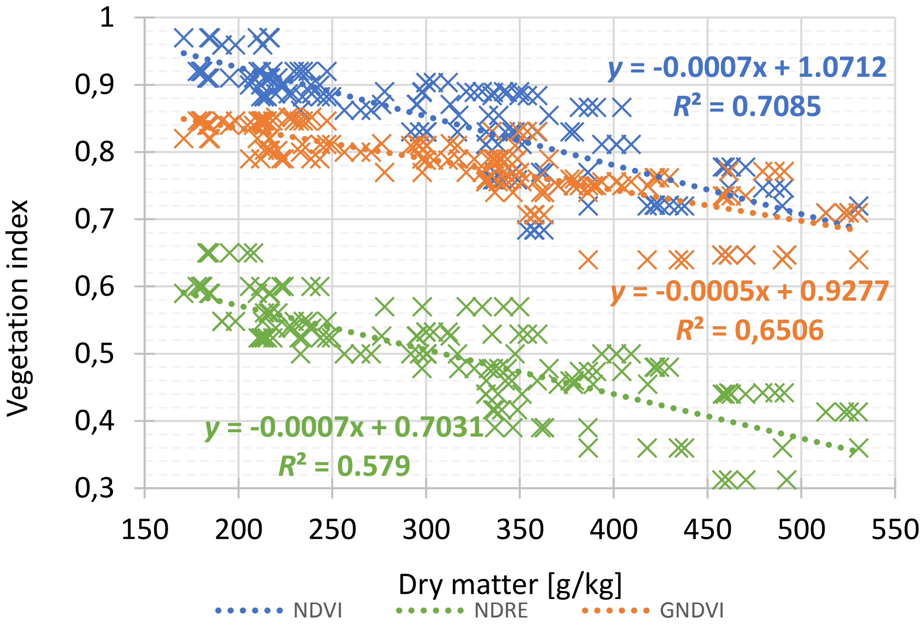


Figure 3: Dry matter dependence of vegetation indices *NDVI*, *NDRE*, *GNDVI.*

Validation of regression models

Table 10 presents the results of the analysis of the relationship between the conventionally true dry matter value determined by chemical analysis of maize samples and the predicted dry matter value determined from vegetation indices according to relations (11)-(13).

Table 10: Conventionally true and predicted dry matter values for the different hybrids studied

Corn hybrid		ES Joker	ES Welington	KTG Karlaxx	Absolutissimo	Rudolfinio
Nutritional analysis DM_{CTV} [g/kg]		459.11	470.83	383.53	423.17	398.67
NDVI	DM_{PV} [g/kg]	458.86	464.53	404.74	429.58	409.41
	Δ_{DM} [g/kg]	-0.26	-6.31	-21.21	-6.41	-10.74
	δ_{DM} [%]	-0.06	-1.36	-5.24	-1.49	-2.62
NDRE	DM_{PV} [g/kg]	457.69	443.85	402.02	429.29	387.14
	Δ_{DM} [g/kg]	-1.42	-26.98	18.49	6.12	-11.52
	δ_{DM} [%]	-0.31	-6.08	4.60	1.43	-2.98
GNDVI	DM_{PV} [g/kg]	461.46	470.78	384.81	426.83	392.71
	Δ_{DM} [g/kg]	2.35	-0.05	1.28	3.66	-5.95
	δ_{DM} [%]	0.51	-0.01	0.33	0.86	-1.52

RESULTS DISCUSSION

Discussion of chemical analysis results

From the nutritional parameters evaluated, it is evident that the dry matter (*DM*) of maize also increases as the phenophase of maize increases. The proportion of starch - Starch in the whole plant also gradually increases as the proportion of grain in maize increases. This is the main source of energy of the harvested plant. In the rest of the plant, there is a gradual decrease in nitrogenous matter (*CP*) and a significant negative decrease in fibre digestibility

(*NDF*) due to lignification. The ideal harvest window for the established plants was around stage 4 of the field trials. This period was determined by dry matter values ranging from 280 to 330 g/kg *DM* during these phases, with an optimum harvest interval. At the same time, the window was determined by an average starch value ranging from 270 g/kg *DM* to 320 g/kg *DM*, where the ideal value of 300 g/kg *DM* corresponds to ⅔ of the milk line of the grain. Other nutritional values monitored such as the digestibility of organic matter *DOM*. which has an increasing character due to the increasing proportion of ears containing starch with high digestibility, also varied throughout the period monitored. At the same time, the remaining *NDF* fibre is gradually lignified and its digestibility decreases in direct proportion. The fresh matter yield *YFM* or dry matter yield *YDM* also increases. which are uneven and highly dependent on the phenophase and maturation stage of the crop.

Discussion of chemical analysis results

This section discusses the statistical significance of the correlation coefficients. The strong correlation can be considered as dry matter e all vegetation indices, where all field trials in all years come out strongly significant. The exception is one hybrid harvested in the first year of harvest for the *NDVI* index, which cannot be considered statistically significant. The correlations of starch with vegetation indices are also statistically significant. Here again we find exceptions in individual years where the correlation came out statistically insignificant. The aforementioned starch content also has a strong influence on the resulting quality of the harvested maize. The values of the *NDVI*, *NDRE* and *GNDVI* indices correlate with the calculated *YDM* values. In some cases, they reach a strong and very strong correlation. Hence, the fact that strong correlations of *NDVI* and *GNDVI* indices can be used not only to determine the appropriate harvest time, but also to predict the amount of resulting organic matter from the yield of the harvested crop, which is very important for determining the yield of organic matter.

Discussion of the verification results of the obtained equations

Absolute differences between the predicted 〖*DM*〗*_PV* and the convexly true values of 〖*DM*〗*_CTV* dry matter content ranged from -0.01 to -26.98 g/kg. The largest difference between the predicted and actual contents was observed for the *NDRE* index.

The average relative deviation δ (*DM*) for the dry matter values calculated from the vegetation index *NDVI* takes the magnitude of 2.154% and 3.078% for the *NDRE* index, and the *GNDVI* index reaches the smallest relative error in dry matter for the 5 different hybrids studied with a value of 0.645%.

Since there is a very strong correlation of *GNDVI* index values with dry matter content values, it can be concluded that *GNDVI* also shows the best prediction results in this linear model. For most of the hybrids studied, the dry matter estimation was less than 1%, namely for ES Joker 0.51%, for ES Wellington -0.01%, for KTG Karlaxx 0.33% and for Absolutissimo 0.86%. For rudolphino, the relative deviation was also small, at -1.52%. The advantage of the *GNDVI* vegetation index is its high correlation with plant biophysical parameters and low sensitivity to other sensed areas. The reflectance at green wavelengths responds better to changes in leaf chlorophyll content and plant health. When using the green band, differences in nutrient deficiencies that correlate with resulting plant production are more likely to be captured.

Based on dry matter values determined in this way, the optimum time for harvesting any maize hybrid can be predicted.

Limits of the proposed solution

There are uncertainties in the proposed method of scanning the field with a multispectral camera on an unmanned aerial vehicle and then calculating the dry weight according to vegetation idnexes. The uncertainties lie in the quality of the multispectral sensor, its correct calibration for the season and the environment, and the unfavourable climatic conditions.

The repeatability of the procedure depends on weather conditions, where UAV operation is affected by rain, wind or the position of the vegetation in a restricted zone for drone use. The disadvantage is the limited range of the UAV. To image a larger area over 200 ha, the imaging process with the method used is more challenging with respect to the operating capabilities of the batteries. Alternatively, fixed wing UAVs provide a longer range per battery (~50 minutes), but up to 90 minutes for the eBee X. The use of a drone is only effective for large vegetation units.

CONCLUSION

The analysis of multispectral images with precise knowledge of the health status of the crop is one of the directions that supports the transition from traditional farming practices to precision agriculture. Increasing the quality of harvested maize and reducing the amount of feed crop consumption by determining the correct harvest time will result in an innovative approach in non-contact analysis of the plant at different stages of its growth with the potential for automated and rapidly scalable application to most cultivated vegetation. For maize, the correct harvest time is determined by the amount of dry matter, depending on whether the cuttings are to be used for silage production for animal feed or as material for methane production for a biogas plant. Therefore, an up-to-date and accurate knowledge of the nutritional values, ideally from the whole crop, is important for deciding on the right time.

This can be achieved by remote sensing using a multispectral camera mounted on a UAV and using the equations presented in Fig. 4. The proposed method eliminates the need to perform chemical analysis on samples collected from only a few locations in a large field with heterogeneous vegetation. The authors attach ecological and economic importance in determining the optimal harvest time also along the whole chain from optimal harvesting of corn according to the determined dry matter to methane production in biogas plants, as well as with respect to the product chain from feed crop to cow milk production.

The vegetation dependency plots in Figure 4 provide equations that can be used to predict the optimal harvest time based on dry matter values. In particular, the information described in the previous subsections suggests that the dependence (13) of the vegetation index *GNDVI* on dry matter will be of greatest importance for prediction. Very good but somewhat worse results than for *GNDVI* were obtained from the dependence of *NDVI* on dry matter (12). Yield characteristics such as *YFM* and *YDM* can also be estimated from *GNDVI* and *NDVI* values.

Since there is a very strong correlation of *GNDVI* values with dry matter content values, it can be concluded that *GNDVI* also shows the best results in prediction in this linear model.

The general validity of the relationships between vegetation indices and dry matter for different maize hybrids is also an important contribution of the described method for determining the optimal harvest time. On the other hand, the climatic conditions where sensing is not possible during rain or high winds are a disadvantage.

REFERENCES

[1] Janoušek, J.; Jambor, V.; Marcoň, P.; Dohnal, P.; Synková, H.; Fiala, P. Using UAV-Based Photogrammetry to Obtain Correlation between the Vegetation Indices and Chemical Analysis of Agricultural Crops. *Remote Sens.* **2021**, *13*, 1878. https://doi.org/10.3390/rs13101878

[2] MINAŘÍK, Robert, Jakub LANGHAMMER a Theodora LENDZIOCH, 2021. Detection of Bark Beetle Disturbance at Tree Level Using UAS Multispectral Imagery and Deep Learning. *Remote Sensing* [online]. **13**(23), 4768. ISSN 2072-4292. Available at: doi:10.3390/rs13234768

[3] Vetrekar, N.T., Gad, R.S., Fernandes, I. *et al.*, 2015. Non-invasive hyperspectral imaging approach for fruit quality control application and classification: case study of apple, chikoo, guava fruits. *J Food Sci Technol* **52**, 6978–6989. Available at doi.org/10.1007/s13197-015-1838-8

[4] PARK, B., LAWRENCE, K.C., WINDHAM, W.R. and SMITH, D.P.2004. Multispectral imaging systém for fecal and igesta detection on poultry carcasses. *Journal of Food Process Engineering*, 27: 311-327. Available at: doi.org/10.1111/j.1745-4530.2004.00464.x

[5] Haiyan Cen, Renfu Lu, Qibing Zhu, Fernando Mendoza, 2016. Nondestructive detection of chilling injury in cucumber fruit using hyperspectral imaging with feature selection and supervised classification.*Postharvest Biology and Technology*, 111, 2016, 352-361. ISSN 0925-5214. Available at https://doi.org/10.1016/j.postharvbio.2015.09.027.

[6] L. Lleó, P. Barreiro, M. Ruiz-Altisent, A. Herrero. 2009. Multispectral images of peach related to firmness and maturity at harvest. *Journal of Food Engineering* [online]. 93(2), 229-235. ISSN 0260-8774. Available at doi.org/10.1016/j.jfoodeng.2009.01.028.

[7] H. Zhang, J. Paliwal, D. S. Jayas, N. D. G. White. 2007. Classification of Fungal Infected Wheat Kernels Using Near-Infrared Reflectance Hyperspectral Imaging and Support Vector Machine. *Transactions of the ASABE (American Society of Agricultural and Biological Engineers)*, 50(5): 1779-1785. Available at doi: 10.13031/2013.23935.

[8] LUKAS, Vojtěch, Igor HUŇADY, Antonín KINTL, Jiří MEZERA, Tereza HAMMERSCHMIEDT, Julie SOBOTKOVÁ, Martin BRTNICKÝ a Jakub ELBL, 2022. Using UAV to Identify the Optimal Vegetation Index for Yield Prediction of Oil Seed Rape (Brassica napus L.) at the Flowering Stage. *Remote Sensing* [online]. **14**(19), 4953. ISSN 2072-4292. Available at: doi:10.3390/rs14194953

[9] Duffková, R.; Poláková, L.; Lukas, V.; Fučík, P. The Effect of Controlled Tile Drainage on Growth and Grain Yield of Spring Barley as Detected by UAV Images, Yield Map and Soil Moisture Content. *Remote Sens.* **2022**, *14*, 4959. https://doi.org/10.3390/rs14194959

[10] GRACIA-ROMERO, Adrian, Omar VERGARA-DÍAZ, Christian THIERFELDER, Jill E. CAIRNS, Shawn C. KEFAUVER a José L. ARAUS. Phenotyping Conservation Agriculture Management Effects on Ground and Aerial Remote Sensing Assessments of Maize Hybrid Performance in Zimbabwe. Proceedings [online]. 2018, 2(7), 7 [cit. 2020-03-22]. DOI: 10.3390/ecrs-2-05181. ISSN 2504-3900. Available at: http://www.mdpi.com/2504-3900/2/7/367

[11] Yang, B.; Zhu, W.; Rezaei, E.E.; Li, J.; Sun, Z.; Zhang, J. The Optimal Phenological Phase of Maize for Yield Prediction with High-Frequency UAV Remote Sensing. *Remote Sens.* **2022**, *14*, 1559. https://doi.org/10.3390/rs14071559

[12] J. Louis et al., "Sentinel-2 Global Surface Reflectance Level-2a Product Generated with Sen2Cor," IGARSS 2019 - 2019 IEEE International Geoscience and Remote Sensing Symposium, Yokohama, Japan, 2019, pp. 8522-8525, doi: 10.1109/IGARSS.2019.8898540.

[13] Drusch, M., Del Bello, U., Carlier, S., Colin, O., Fernandez, V., Gascon, F., Hoersch, B., Isola, C., Laberinti, P., Martimort, P., Meygret, A., Spoto, F., Sy, O., Marchese, F., Bargellini, P., 2012. Sentinel-2: ESA's Optical High-Resolution Mission for GMES Operational Services. Remote Sensing of Environment 120, 25–36.

[14] Islam, M.R. and Garcia, S.C. (2014), Prediction of Dry Matter Yield of Hybrid Forage Corn Grown for Silage. Crop Science, 54: 2362-2372. https://doi.org/10.2135/cropsci2013.10.0710

[15] J. Huang, H. Wang, Q. Dai and D. Han, "Analysis of NDVI Data for Crop Identification and Yield Estimation," in *IEEE Journal of Selected Topics in Applied Earth Observations and Remote Sensing*, vol. 7, no. 11, pp. 4374-4384, Nov. 2014, doi: 10.1109/JSTARS.2014.2334332.

[16] Jue Wang, Paul M. Rich, Kevin P. Price & W. Dean Kettle (2005) Relations between NDVI, Grassland Production, and Crop Yield in the Central Great Plains, Geocarto International, 20:3, 5-11, DOI: 10.1080/10106040508542350

[17] Cabrera-Bosquet, L., Molero, G., Stellacci, A., Bort, J., Nogués, S., & Araus, J. (2011). NDVI as a potential tool for predicting biomass, plant nitrogen content and growth in wheat genotypes subjected to different water and nitrogen conditions, *Cereal Research Communications*, *39*(1), 147-159. doi: https://doi.org/10.1556/crc.39.2011.1.15

[18] Sakamoto, T., Gitelson, A. A., & Arkebauer, T. J. (2014). Near real-time prediction of U.S. corn yields based on time-series MODIS data. *Remote Sensing of Environment*, *147*, 219-231. https://doi.org/10.1016/j.rse.2014.03.008

[19] Ines, A. V., Das, N. N., Hansen, J. W., & Njoku, E. G. (2013). Assimilation of remotely sensed soil moisture and vegetation with a crop simulation model for maize yield prediction. *Remote Sensing of Environment*, *138*, 149-164. https://doi.org/10.1016/j.rse.2013.07.018

[20] Johnson, D. M. (2014). An assessment of pre- and within-season remotely sensed variables for forecasting corn and soybean yields in the United States. *Remote Sensing of Environment*, *141*, 116-128. https://doi.org/10.1016/j.rse.2013.10.027

[21] Ma, Y., Zhang, Z., Kang, Y., & Özdoğan, M. (2021). Corn yield prediction and uncertainty analysis based on remotely sensed variables using a Bayesian neural network approach. *Remote Sensing of Environment*, *259*, 112408. https://doi.org/10.1016/j.rse.2021.112408

[22] Oliveira, M.F.d.; Ortiz, B.V.; Morata, G.T.; Jiménez, A.-F.; Rolim, G.d.S.; Silva, R.P.d. Training Machine Learning Algorithms Using Remote Sensing and Topographic Indices for Corn Yield Prediction. *Remote Sens.* **2022**, *14*, 6171. https://doi.org/10.3390/rs14236171

[23] Tang, Z.; Guo, J.; Xiang, Y.; Lu, X.; Wang, Q.; Wang, H.; Cheng, M.; Wang, H.; Wang, X.; An, J.; Abdelghany, A.; Li, Z.; Zhang, F. Estimation of Leaf Area Index and Above-Ground Biomass of Winter Wheat Based on Optimal Spectral Index. *Agronomy* **2022**, *12*, 1729. https://doi.org/10.3390/agronomy12071729

[24] Wang, R.; Cherkauer, K.; Bowling, L. Corn Response to Climate Stress Detected with Satellite-Based NDVI Time Series. *Remote Sens.* **2016**, *8*, 269. https://doi.org/10.3390/rs8040269

[25] Mkhabela, M. S., Mkhabela, M. S., & Mashinini, N. N. (2005). Early maize yield forecasting in the four agro-ecological regions of Swaziland using NDVI data derived from NOAA's-AVHRR. *Agricultural and Forest Meteorology*, *129*(1-2), 1-9. https://doi.org/10.1016/j.agrformet.2004.12.006

[26] Coelho, A.P., Rosalen, D.L., & Faria, R.T. (2018). Vegetation indices in the prediction of biomass and grain yield of white oat under irrigation levels. *Tropical agricultural research*, *48*, 109-

[27] Bretas, IL, Valente, DSM, Silva, FF, et al. Prediction of aboveground biomass and dry-matter content in *Brachiaria* pastures by combining meteorological data and satellite imagery. *Grass Forage Sci*. 2021; 76: 340– 352. https://doi.org/10.1111/gfs.12517

[28] Ezekia Svotwa, J. Anxious Masuka, Barbara Maasdorp, Amon Murwira, "Spectral Indices: In-Season Dry Mass and Yield Relationship of Flue-Cured Tobacco under Different Planting Dates and Fertiliser

Levels", *International Scholarly Research Notices*, vol. 2013, Article ID 816767, 10 pages, 2013. https://doi.org/10.1155/2013/816767

[29] Angel Maresma, Lindsay Chamberlain, Aristotelis Tagarakis, Tulsi Kharel, Greg Godwin, Karl J. Czymmek, Elson Shields, Quirine M. Ketterings, Accuracy of NDVI-derived corn yield predictions is impacted by time of sensing, Computers and Electronics in Agriculture, 169, 2020, 105236, ISSN 0168-1699, https://doi.org/10.1016/j.compag.2020.105236.

[30] Rahetlah, B. V., Salgado, P., Andrianarisoa, B., Tillard, E., Razafindrazaka, H., Le Mezo, L., & Ramalanjaona, V. L. (2014). Relationship between normalized difference vegetation index (NDVI) and forage biomass yield in the Vakinankaratra region, Madagascar.

[31] Gerighausen H, H Lilienthal, T Jarmer & B Siegmann, 2016. Evaluation of leaf area index and dry matter predictions for crop growth modelling and yield estimation based on field reflectance measurements. *EARSeL eProceedings*, 14(S2): 71-90

[32] JANSE, Pooja Vinod, Ratnadeep R. a Priyanka U. RANDIVE. Vegetation Indices for Crop Management: A Review. IInternational Journal of Research and Analytical Reviews. IJRAR, 2019, 6(1), 1-4. ISSN 2348-1269. Available at: https://www.hindawi.com/journals/js/2017/1353691/

[33] PRICE, J. Leaf area index estimation from visible and near-infrared reflectance data. Remote Sensing of Environment. 1995, 52(1), 55-65. DOI: 10.1016/0034-4257(94)00111-Y. ISSN 00344257. Available at: https://linkinghub.elsevier.com/retrieve/pii/003442579400111Y

[34] PANDA, Sudhanshu Sekhar, Daniel P. AMES a Suranjan PANIGRAHI. Application of Vegetation Indices for Agricultural Crop Yield Prediction Using Neural Network Techniques. Remote Sensing. 2010, 2(3), 673-696. DOI: 10.3390/rs2030673. ISSN 2072-4292. Available at: http://www.mdpi.com/2072-4292/2/3/673

[35] XUE, Jinru a Baofeng SU. Significant Remote Sensing Vegetation Indices: A Review of Developments and Applications. Journal of Sensors. 2017, 2017, 1-17. DOI: 10.1155/2017/1353691. ISSN 1687-725X. Available at: https://www.hindawi.com/journals/js/2017/1353691/

[36] BUMA, Willibroad Gabila a Sang-Il LEE. Multispectral Image-Based Estimation of Drought Patterns and Intensity around Lake Chad, Africa. Remote Sensing [online]. 2019, 11(21), 21 [cit. 2020-03-22]. DOI: 10.3390/rs11212534. ISSN 2072-4292. Available at: https://www.mdpi.com/2072-4292/11/21/2534

[37] CORRIGAN, Fintan. Multispectral Imaging Camera Drones In Farming Yield Big Benefits. DroneZon [online]. DroneZon, 2020, 2019 [cit. 2020-03-22]. Available at: https://www.dronezon.com/learn-about-drones-quadcopters/multispectral-sensor-drones-in-farming-yield-big-benefits/

[38] CAMMARANO, Davide, Glenn FITZGERALD, Bruno BASSO, Garry O'LEARY, Deli CHEN, Peter GRACE a Costanza FIORENTINO. Use of the Canopy Chlorophyl Content Index (CCCI) for Remote Estimation of Wheat Nitrogen Content in Rainfed Environments. Agronomy Journal. 2011, 103(6), 1597-1603. DOI: 10.2134/agronj2011.0124. ISSN 00021962. Available at: http://doi.wiley.com/10.2134/agronj2011.0124

[39] BAUSCH, Walter C., Ardell D. HALVORSON a Jan CIPRA. Quickbird satellite and ground-based multispectral data correlations with agronomic parameters of irrigated maize grown in small plots. Biosystems Engineering [online]. 2008, 101(3), 306-315 [cit. 2020-03-22]. DOI: 10.1016/j.biosystemseng.2008.09.011. ISSN 15375110. Available at: https://linkinghub.elsevier.com/retrieve/pii/S1537511008002742

[40] GITELSON, Anatoly A. a Mark N. MERZLYAK. Remote sensing of chlorophyll concentration in higher plant leaves. Advances in Space Research. 1998, 22(5), 689-692. DOI: 10.1016/S0273-1177(97)01133-2. ISSN 02731177. Available at: https://linkinghub.elsevier.com/retrieve/pii/S0273117797011332

[41] ÚKZÚZ. *Methods of Plant Variety State Tests CISTA, Pursuant to the Valid Wording from the Year 1999*; ÚKZÚZ: Brno, Czech Republic, 1999.

[42] Achen, C. H. (1990). "'What Does "Explained Variance" Explain?: Reply". *Political Analysis*. **2** (1): 173–184. doi:10.1093/pan/2.1.173.

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

FORAGE AND FERMENTATION QUALITY OF RE-ENSILED PRESS CAKES FROM BIOREFINING OF GRASS SILAGES

RESCH, R.[1], WINTER, M.[1], STEINWIDDER, A.[1], MANDL, M. [2], SWEENEY, J. B.[3], MCDONNELL, K.[3]
[1] AREC Raumberg-Gumpenstein, Irdning-Donnersbachtal, Austria
[2] tbw research GesmbH, Wien, Austria
[3] LIFE farm4more, School of Biosystems Engineering, University College Dublin, Dublin, Ireland

Keywords: green biorefinery, screw press, press cake, re-ensiling

INTRODUCTION

The need for more sustainable feed production and protein self-sufficiency are becoming more important (European Parliament, 2011), so green biomass fractionation is also attracting great interest in protein feed production. According to Kromus et al. (2004), biorefinery is a sustainable processing of biomass into a spectrum of marketable products and energy. As part of the international research project Farm4More (LIFE18CCM/IE/001195), three different types of pre-wilted grassland forage (preliminary trial 2020: 1 - grass-rich; main trial 2021: 2 - grass/clover, 3 - red clover) were ensiled into round bales in work package C.5.1 at AREC Raumberg-Gumpenstein and biorefined after fermentation using a practical screw press. This paper does not deal with the protein-rich press juice, but with the fiber-rich press cake, which was ensiled again as residue of the biorefining and can be fed to ruminants. In the following, it will be clarified to what extent the press cake differs from the original grass silage, whether re-silaging is possible and to what extent a renewed fermentation influenced the contents and silage quality of the press cake.

MATERIAL AND METHODS

The used forage came from the 1st growth of grassland at the organic station Lambach (Upper Austria) of AREC Raumberg-Gumpenstein. The round bales transported to Gumpenstein weighed between 900 to over 1,000 kg, were stored for at least 6 weeks, and were mixed in a mixing wagon with a vertical cutter for 30 minutes before baling and chopped to about 5 cm theoretical chop length. DM content was determined from the mixture using the microwave method of Losand and Waldmann (2003), and the amount of water required to dilute to 230 g DM/kg FM was calculated. The required amount of water was added to the silage during the mixing process. After pressing, approximately 45 kg of each fresh press cake was ensiled into 60-liter plastic barrels. The average storage density in the barrels was 275 to 281 kg DM/m³. The containers were hermetically sealed with a plastic lid with a metal clamp. Storage of the filled barrels was at about +20 °C until the silo opening. The storage period was 62 days in the preliminary test (2020) and 52 to 56 days in the trial of 2021. The sample draw on the contents of the opened barrels was performed vertically from top to bottom using stainless steel cylinders (diameter 50 mm) and 2 punctures per container. The composite sample of each variant (4 barrels × 2 punctures) was immediately cooled. Subsequently, further sample preparation was performed according to the analytical method. Chemical analyses were performed according to VDLUFA method book III (1976). The validated data were variance-analyzed using the statistical program Statgraphics Centurion XVII (version 17.1). Mean comparisons were made using the Tukey-HSD method at p-level 95%.

RESULTS AND DISCUSSION

The DM contents of the grass silages differed significantly before watering. Pressing of the watered grass silages resulted in a uniform increase of the DM content in the press cakes to about 370 g/kg FM. The chemical composition of the press cakes changed significantly, compared to the original silage. NDF content increased by about 100 g/kg DM. According to Resch (2016), the high levels of NDF due to biorefining are corresponding to a phenological stage at seed maturity. Almost reductions in protein (-11 to -24%), minerals (-25 to -30%), sugars (-50%), and fermentation products (-55 to -57%) were significant (table 1).

Fermentation products increased significantly with re-ensiling, compared to fresh press cake. Re-ensiling caused pronounced lactic and acetic acid fermentation, which significantly lowered pH values below the critical pH and provided very good fermentation quality. The second fermentation consumed nearly all sugars and also parts of NFC of the press cakes.

CONCLUSIONS

In the international research project Farm4More (LIFE18CCM/IE/001195) we observed that re-ensiling of biorefined press cakes, from grass silage of different forages, worked successfully by triggering a new lactic acid fermentation, even if the fresh press cake was exposed to air for several hours. The feed value of the press cakes was significantly lower than that of grass silage, because much protein, minerals, sugars and fermentation products were added to the press juice by the pressing process. The press cakes contained about 100 g more NDF/kg DM than the origin grass silage.

Table 1: Nutrients, minerals and fermentation quality of grass silage vs. re-ensiled press cake from the biorefinery depending on the used forage

			grass silage			**re-ensiled press cake of biorefinery**	
parameter	unit	grass	grass/clover	red clover	grass	grass/clover	red clover
dry matter	g/kg FM	419.6^{C}	316.3^{B}	249.4^{A}	372.0^{a}	369.2^{a}	372.3^{a}
nutrients, cell wall substances and ammonia							
crude protein	g/kg DM	135.1^{A}	145.8^{A}	158.8^{B}	101.7^{a}	116.2^{b}	126.0^{c}
ammonia	g/kg DM	1.8^{A}	2.3^{AB}	2.7^{B}	1.2^{a}	1.3^{a}	1.2^{a}
NH_4 of N_{total}	%	8.3^{A}	9.8^{A}	10.3^{B}	7.3^{b}	6.7^{ab}	6.0^{a}
NDF	g/kg DM	496^{C}	390^{B}	343^{A}	635^{c}	493^{b}	440^{a}
ADF	g/kg DM	336^{C}	295^{A}	309^{AB}	434^{a}	403^{a}	412^{a}
ADL	g/kg DM	41.0^{A}	32.9^{A}	39.3^{A}	49.3^{b}	40.5^{a}	47.6^{b}
sugars	g/kg DM		86.7^{B}	40.4^{A}		6.5^{a}	5.5^{a}
crude fat	g/kg DM	21.6^{B}	17.5^{A}	22.3^{B}	22.0^{a}	28.1^{b}	27.1^{b}
crude ash	g/kg DM	87.1^{A}	106.7^{B}	110.7^{C}	63.0^{a}	81.2^{b}	88.4^{b}
minerals							
calcium (Ca)	g/kg DM	8.4^{A}	12.3^{B}	14.5^{C}	6.3^{a}	10.2^{b}	12.3^{c}
phosphorus (P)	g/kg DM	3.1^{A}	3.0^{A}	3.0^{A}	1.8^{a}	1.5^{a}	1.5^{a}
potassium (K)	g/kg DM	28.1^{A}	30.2^{AB}	31.7^{B}	13.4^{a}	17.2^{b}	18.1^{b}
iron (Fe)	mg/kg DM	900^{B}	447^{A}	519^{A}	1087^{a}	676^{a}	743^{a}
fermentation quality							
pH-value		4.68^{A}	4.75^{A}	4.58^{A}	4.16^{b}	4.10^{a}	4.22^{c}
lactic acid	g/kg DM	35.8^{A}	36.5^{A}	56.9^{B}	57.1^{a}	75.3^{b}	71.3^{ab}
acetic acid	g/kg DM	11.0^{A}	11.4^{A}	14.2^{B}	11.9^{a}	14.0^{b}	13.4^{b}
propionic acid	g/kg DM	1.5^{AB}	1.5^{A}	2.1^{B}	0.8^{a}	1.0^{a}	1.0^{a}
butyric acid	g/kg DM	2.2^{A}	3.5^{A}	6.0^{B}	1.6^{a}	2.9^{b}	3.9^{b}
ethanol	g/kg DM	10.7^{A}	6.1^{A}	6.7^{A}	5.1^{a}	4.9^{a}	4.2^{a}
VOC total	g/kg DM	61.2^{A}	59.1^{B}	85.9^{C}	76.5^{a}	93.7^{b}	98.0^{b}

indices: capital letters show significant differences between grass silage variants
lower case letters show significant differences between re-ensiled press cake variants

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial contribution of the European Union to the Life project "LIFE Farm4More - Future Agricultural Management for multiple outputs on climate and rural development" with project number LIFE 18 CCM /IE/001195 Farm4More. More information about the project via www.farm4more.eu

REFERENCES

European Parliament (2011): Report A7-0026/2011 4.2.2011. The EU protein deficit: What solution for a long-standing problem? (2010/2011(INI)) Committee on Agriculture and Rural Development, Rapporteur: Marin Häusling.

Kromus S., Wachter B., Koschuh W., Mandl M., Krotschek C., Naradoslawsky M., 2004. The green biorefinery Austria-development of an integrated system for green biomass utilization. Chemical and biochemical engineering quarterly, 18(1): 7-12.

Losand, B., Waldmann, B. (2003). Mit der Mikrowelle auf der richtigen Spur. [On the right track with the microwave] Dlz, Tierhaltung (3) 2003: 126-128.

Resch, R. (2016): Einfluss des Vegetationsstadiums von Grundfutter auf den Gehalt an Strukturkohlenhydraten. [Influence of the vegetation stage of forage on the content of structural carbohydrates]. 43. Viehwirtschaftliche Fachtagung, HBLFA Raumberg-Gumpenstein, Irdning-Donnersbachtal: 11-19.

VDLUFA, 1976: Methodenbuch Band III – Die chemische Untersuchung von Futtermitteln, inkl. Ergänzungsblätter 1983, 1988, 1993, 1997, [Book of Methods Volume III – Chemical Analysis of Feedstuffs, Additional Sheets 1983, 1988, 1993, 1997], VDLUFA-Verlag, Darmstadt.

EFFECT OF PERMANENT GRASSLAND RENOVATION ON FORAGE PRODUCTION AND QUALITY AND BOTANICAL COMPOSITION

HEJDUK, S., BAHOLET, D., KOLÁČKOVÁ, I.
Department of Animal Nutrition and Forage Production, Mendel University in Brno

INTRODUCTION

Permanent grassland renovation is a widely used method for improvement of forage yield and quality when the existing sward is not meeting current objectives (have less than 50% of desired species) and other improvement methods (fertilisation, over-sowing) will not provide the desired results. Desired species used for the renovation should be productive species (cultivated grasses and legumes) or species with specific characteristics as deeper rooting for drought resilience and higher carbon sequestration such as chicory or plantain. Successful use of the selected seed mixture can provide farmers with higher forage production and better forage quality, faster regrowth of the sward, more effective fertilizer utilisation, and, as a result, lower forage costs.

On the other hand, the renovation is costly and not always are the high costs associated with soil tillage and seeds fully covered by higher forage production and quality (especially on low fertile, shallow soils). Furthermore, cultivation of grassland soils leads to rapid mineralisation of soil organic carbon what is linked with lower storing capacity for water and nutrients and it is a significant source of carbon dioxide to the atmosphere (e.g. Wilson, Sousana…). In protected areas, where the main aim of grasslands management is to preserve high biodiversity, swards renovation is usually limited or totally banned to avoid the loss of protected plant and invertebrate species.

The aim of this paper is to present the results of grassland renovation in protected area White Carpathians, Czech Republic. The renovations were initiated by several dry years, that caused a shortage of forage at cattle farms.

MATERIALS AND METHODS

The first experimental site is located in the village Lipov (48.90N, 17.46E, altitude 227 m a.s.l., sum of precipitation 580 mm, average temperature 9.6° C). The half of original, low productive meadow was ploughed down at the beginning of August 2019 and at 25th August new sward was established without nursery crop. The seed mixture consisted of 10 following species (kg/ha): *Trifolium pratense* 4n (1), *T. repens* (0.5), *Lotus corniculatus* (1), *Medicago sativa* (3), *Arrhenatherum elatius* (7), *xFestulolium krasanii* (6), *Phleum pratense* (2), *Festuca rubra* (3), *Dactylis glomerata* (6) and *Festuca pratensis* (3). Sowing rate was 32.5 kg/ha. No fertilisers were applied in the year of establishment; 55 kg N were applied in the both harvest years. The stands were sampled just in the 2nd harvest year at 1st June and 18th August 2021.

The second site is in the municipality Popov (49.07N, 17.95E, altitude 370 m, sum of precipitation 711 mm, average temperature 8.6° C). The original, low productive pasture, managed by organic farm, was tilled in October 2019. Two seed mixtures (standard and species-rich one) were undersown to silage oats at 26th March in four replications and original permanent grassland strips were retained as a control. The composition of standard seed mix was as follows (kg/ha): Trifolium pratense (2), Trifolium repens (1), Lotus corniculatus (1), Onobrychis viciifolia (12), *Arrhenatherum elatius* (7), *Festuca pratensis* (3), *Dactylis glomerata* (6), *Phleum pratense* (2) and *Poa pratensis* (2). Total sowin rate was 40 kg/ha. The species-rich mixture was prepared by the management of protected area of White Carpathians from local high nature value grasslands by brushing (grasses) and cultivation of legumes and forbs. It consisted of (kg/ha) grasses *Festuca rupicola*, *F. rubra*, *Bromus erectus*, *Anthoxantum odoratum*, *Cynosurus cristatus* and some others (17), followed by legumes *Onobrychis viciifolia* (0.4 kg), *Medicago lupulina* (0.4), *Dorycnium herbaceum* (0.2), *Astragalus cicer* (0.4), *Viccia cracca* (0.2) and forbs *Betonica officinalis* (0.2), *Prunella vulgaris* (0.04), *Dianthus cartusiorum* (0.04), Knautia kitaibelii (0.02), Centaurea jacea (0.2), Plantago media (0.04), Leucanthemum ircutianum (0.2), *Agrimonia eupatoria* (0.4), *Galium verum* (0.2), *Salvia pratensis* (0.4), *Filipendula vulgaris* (0.06) and *Veronica teucrium* (0.08). Total sowing rate of this species-rich mixture was 20 kg/ha. No fertilizers were applied on the swards.

Dry matter yield and forage quality (CP, NDF, ADL) were evaluated in two cuts 2021 (2nd harvest year) at exoperiment Lipov and in the 1st cut in Popov, along with botanical composition. For yield and forage quality estimation, four samples from each type of sward were harvested, from the area of 1 m^2 along with evaluation of botanical composition. In Popov site, the botanical composition was evaluated from an area of 25 m^2.

RESULTS AND DISCUSSION

In the Lipov experiment, dry matter yield was increased by the factor 2.08. Despite 10 species sown in the mixture for renovation, in the first harvest only two species (*Dactylis glomerata* and *Arrhenatherum elatius*) formed over 95% of the biomass (Nespěšná, 2022). Legumes were almost absent in renewed sward, despite their weight proportion in the mixture was 17 %. The reason is probably late term of the first cut, N fertilising and released N from soil organic matter mineralisation, which prioritised aggressive grasses.

Concerning the forage quality, renewed swards consist mostly of cultivated grasses, which are adapted to fertile soils, have fast growth rate and produce thin leaves with large specific area and low DM content. Nevertheless, their high digestibility in early stages of growth decline quickly during generative stage (Michaud et al., 2012). In this case, the dominant grasses in renewed sward were in full heading stage, crude protein was too low and the fibre content was too high for productive dairy cattle. In the original sward, there was more dicot plants and grasses with thicker leaves (as *Festuca rubra*) and the maturing of the forage was slower resulting lower fibre content (Table 1).

Table 1: The differences between the original and renewed grasslands (weighed mean from both cuts)

Parameter	Original grassland	Renewed grassland
Dry matter yield ($t.ha^{-1}$)	4.42 ^{a}	9.21 ^{b}
Number of plant species	38	8
Crude protein ($g.kg^{-1}$ of DM)	92 ^{a}	109 ^{b}
NDF ($g.kg^{-1}$ of DM)	491 ^{a}	584 ^{b}
ADL ($g.kg^{-1}$ of DM)	35.3	36.6
Crude fibre ($g.kg^{-1}$ of DM)	269 ^{a}	315 ^{b}

Values with different letters in a column are significantly different (P = 0.05)

In the Popov experiment, we present just the first cut evaluation in 2022. The samples were taken at 21st May using the same methodology as in above described trial. The results are presented in Table 2. Renewal using standard mixture of cultivated grasses and legumes produced the highest yield while when species-rich mixture was used for the renovation, the yield was not increased compared original grassland. The reason for no effect on the forage production was slow initial development of the species used in mixture and also their low production nature. As in previous case, number of plant species declined when standard seed mixture was used, but increased after planting species-rich mixture. Crude protein was higher in forage from original sward due to high proportion od white clover and birdsfoot trefoil. NDF content was higher in renewed grassland due to high proportion of tall grasses, but surprisingly ADL content was the highest at species-rich mixture, probably due to presence of some forbs and also *Rumex obtusifolius* from the seed bank.

Table 2: The differences among the original and renewed grasslands (standard and species-rich mixture), 21. 5. 2021

Parameter	Original grassland	Renewed grassland (standard mixture)	Renewed grassland (species-rich mixture)
Dry matter yield ($t.ha^{-1}$)	3.62 ^{a}	4.95 ^{b}	3.42 ^{a}
Number of plant species	15 - 34	13 - 23	41 - 46
Crude protein ($g.kg^{-1}$ of DM)	140 ^{b}	124 ^{a}	129 ^{ab}
NDF ($g.kg^{-1}$ of DM)	440 ^{a}	551 ^{b}	395 ^{a}
ADL ($g.kg^{-1}$ of DM)	37.6 ^{a}	39.6 ^{a}	53.3 ^{b}

Values with different letters in a column are significantly different (P = 0.05)

CONCLUSIONS

Permanent grassland renovation leads to higher forage production due to introduction of productive grass and legume species and soil organic matter mineralisation after soil tillage. Nevertheless, when harvested at the same time as original, low productive sward, the forage from renewed grasslands contain more fibre and less crude protein due to faster maturing of the cultural species. When using species-rich seed mixture, the biodiversity is higher, but forage production is not increased and forage quality was higher just in some parameters (NDF) but worsen in others (ADL).

ACKNOWLEDGEMENT

This research has received funding from the EU Horizon 2020 Research and Innovation Programme under grant agreement No. 774124 (SUPER-G). Special thanks belong to students of Mendel University in Brno, P. Nespěšná and J. Vysloužil for their help with sampling of the swards and forage preparing for analyses.

REFERENCES

Michaud, A., Andueza, D., Picard, F., Plantureux, S., Baumont, R., 2012, Seasonal dynamics of biomass production and herbage quality of three grasslands with contrasting functional composition. Grass and forage science, 67 (1) 64-76

Nespěšná, P., 2022, Comparison of forage production and quality of grasslands after renovation using commercial and regional seed mixture in the White Carpathians. MSc. thesis, Mendel university in Brno, 68 pp. (in Czech)

OPTIMIZING THE ENSILING OF RED CLOVER BY GRASS INCLUSION, WILTING AND FORMIC ACID-BASED ADDITIVE APPLICATION

RINNE, M., FRANCO, M., STEFAŃSKI, T., MANNI, K. & HUUSKONEN, A.
Natural Resources Institute Finland (Luke), FI-31600 Jokioinen, Finland. Correspondence: marketta.rinne@luke.fi

INTRODUCTION

Red clover (RC; *Trifolium pratense*) has many benefits, such as the ability to fix nitrogen thanks to the symbiotic *Rhizobium* bacteria in its root nodules, it improves biodiversity and it has favourable characteristics as a feed component in ruminant diets. Red clover is typically grown mixed with temperate grasses such as timothy (Tim; *Phleum pratense*), but different N fertilization requirements and optimal harvest timing of plant species result in suboptimal management practises of such mixed swards. This could be overcome by cultivating the different plant species as pure stands and mixing them optimally in the preparation phase of total mixed ration for animals. However, due to the high innate moisture level, low water soluble carbohydrate (WSC) content and high buffering capacity, RC may be challenging to ensile. In this experiment, practically feasible methods of improving the ensiling performance of RC were evaluated: wilting (low and high dry matter(DM)), inclusion of Tim grass (0, 50 and 100% on fresh matter basis) and use of a formic acid-based additive (FA; control vs. FA application).

MATERIALS AND METHODS

Pure RC and Tim swards were harvested separately at Luke research station in Siikajoki, Finland (64°66'N, 25°09'E) from first regrowth of the season on 18 August 2021. The herbages were precision chopped and half of the material was ensiled immediately (low DM), while the other half was artificially dried before ensiling (high DM) for approximately 4 hours in a forced air drier. Both herbages were ensiled as such, and in a 1:1 mixture on fresh matter basis. The silages were preserved without additive (Con), or formic acid-based additive (AIV 2 Plus Na; Eastman, Oulu, Finland) was applied according to commercial guidelines (6 l/ton for low DM RC and 5 l/ton for the other herbages). The experiment was conducted and samples analysed as described by Franco et al. (2022) except that vacuum plastic bags (four replicates per treatment) were used for the 3-month ensiling period. Data was analysed using SAS MIXED procedure (SAS Inc. 2002-2012, Release 9.4; SAS Inst. Inc., Cary, NC, USA) with plant species, DM level and additive as fixed effects and replicate as a random effect.

RESULTS AND DISCUSSION

Humid weather conditions before the harvest resulted in extremely low DM of the direct cut plant materials. The DM contents of the fresh RC and Tim were 110 and 118 g/kg, while after drying they were elevated to 303 and 244 g/kg, which are still moderate. The WSC content (48 and 50 g/kg DM for RC and Tim) and buffering capacity (3.5 and 1.7 g lactic acid per 100 g for RC and Tim) of both species were within the same range, the values being typical for red clover, but WSC content of grass was low. The fermentation coefficients were 30, 36, 33 and 52 for the low and high DM herbages of RC and Tim, respectively. Based on the fermentation coefficient, only high DM Tim was easy to ensile, while all other materials were difficult to ensile. Although statistically significant, the effect of plant species was numerically minor so that benefits of grass inclusion into red clover could not be clearly demonstrated in the current material. The low DM Control silages were poorly preserved with high pH, clearly elevated acetic acid concentrations and proportion of ammonia N in total N, as well as exhausted WSC levels (Table 1). Use of FA efficiently improved the fermentation quality of the silages, the effect being more pronounced in the low than high DM silages. Also, the proportion of ammonia N in total N was high in low DM Con silages, but could be controlled by wilting and FA application. Red clover is known to contain polyphenol oxidase enzyme, which restricts in-silo protein degradation, and it may have been responsible for the linear effect of plant species on proportion of ammonia N in total N in low DM silages. Acetic acid is generally considered to improve the aerobic stability of silages, but in this experiment the very high acetic acid concentrations of low DM Con silages coincided with shorter aerobic stability than in other feeds with lower acetic acid concentration. However, when expressed as undissociated acetic acid per g fresh matter, the low DM Con silages did not differ from the other silages. FA application improved aerobic stability of low DM silages concomitantly with overall improved fermentation quality. In high DM silages, FA did not improve aerobic stability, which could be explained by the reduction in acetic acid concentration in those silages compared to Con. In spite of clear differences in the preservation quality between treatments, the range in ensiling losses was relatively small. The use of vacuum bags restricted the effluent losses, so these values are not directly comparable to farm scale conditions.

CONCLUSIONS

Both increased dry matter content and use of the formic acid-based silage additive improved the fermentation quality of the experimental silages. However, under the conditions of the current experiment, timothy grass inclusion into red clover had only a minor effects on the fermentation quality, and the low water soluble carbohydrate content has probably contributed to it. Under challenging conditions, low dry matter red clover and timothy silages without additive treatments were very poorly preserved, emphasizing the importance of proper silage management practises.

REFERENCES

Franco, M., Pirttiniemi, J., Stefański, T., Jalava, T., Tapio, I., Huuskonen, A. & Rinne, M. 2022. Fermentation quality and bacterial ecology of grass silage modulated by additive treatments, extent of compaction and soil contamination. Fermentation 8: 156. Available at: https://doi.org/10.3390/fermentation8040156.

Table 1. Chemical composition, fermentation quality, aerobic stability, ensiling losses and microbial counts of ensiled red clover mixed in different proportions with timothy grass preserved under different dry matter (DM) contents and treated with a formic acid-based silage additive.

Plant species	Red clover				Mixture				Grass				SEM[1]	Statistical significance[2]					
DM level	Low		High		Low		High		Low		High			1	2	3	4	5	6
Additive treatment[3]	Con	FA	Con	FA	Con	FA	Con	FA	Con	FA	Con	FA							
DM, g/kg	114	121	305	302	118	125	284	283	123	135	252	258	2.6	***	***	***	***	*	**
pH	4.78	4.18	4.42	4.17	4.76	4.08	4.30	4.05	4.77	3.96	4.01	3.99	0.018	***	***	***	***	ns	***
Ammonia N, g/kg N	72	30	62	44	95	59	57	34	126	71	53	31	5.5	***	***	***	***	ns	***
Ethanol, g/kg DM	11	8	2	3	15	10	2	4	15	20	3	7	0.9	***	***	***	ns	***	***
WSC[4], g/kg DM	2	12	3	12	2	8	3	24	2	5	4	44	3.1	***	***	***	***	***	***
Acids, g/kg DM																			
Lactic (LA)	36	54	99	60	22	72	88	47	9	56	83	28	4.4	***	***	*	ns	ns	***
Acetic (AA)	78	19	40	23	75	26	32	17	75	21	20	10	2.1	***	***	***	***	*	***
Propionic	8.6	0.4	0.3	0.1	10.1	0.6	0.4	0.2	10.8	0.5	0.3	0.2	0.17	***	***	***	***	***	***
Butyric	0.1	1.6	0.0	0.0	0.2	1.3	0.0	0.0	0.1	1.1	0.0	0.0	0.40	ns	***	ns	*	ns	***
Total volatile fatty acids	87	22	41	23	85	28	33	17	86	23	21	11	2.2	***	***	***	***	o	***
LA/AA ratio	0.46	2.70	2.45	2.59	0.29	2.80	2.74	2.85	0.13	2.63	4.10	2.65	0.153	***	***	***	***	***	***
Aerobic stability, hours	61	202	220	204	63	188	220	213	55	216	194	158	13.5	o	***	*	***	ns	***
Ensiling losses[5]	29	23	38	34	30	27	40	35	28	33	37	36	2.4	o	***	ns	o	*	ns
Microbial quality, cfu[6]/g																			
Yeasts	1.3×10^2	5.0×10^1	5.0×10^1	8.8×10^1	5.0×10^1	8.8×10^1	5.0×10^1	5.0×10^1	8.8×10^1	5.0×10^1	5.0×10^1	5.0×10^1	3.3×10^2	ns	ns	ns	ns	ns	ns
Moulds	8.8×10^1	2.2×10^3	5.0×10^1	5.0×10^1	1.6×10^2	8.8×10^1	8.8×10^1	8.8×10^1	1.8×10^2	8.8×10^1	5.0×10^1	3.1×10^2	5.6×10^2	ns	ns	ns	ns	ns	ns

[1] SEM = Standard error of the mean
[2] Contrasts: 1 = Linear effect of plant species proportion; 2 = Effect of DM; 3 = 1 × 2; 4 = Con vs FA; 5 = 1 × 4; 6 = 2 × 4. *** = P<0.001; ** = P<0.01; * = P<0.05; o = P<0.1; ns = non-significant
[3] Con = Control without additive; FA = Formic acid-based silage additive (composition 74-78 % formic acid and 4.5-6.5 % sodium formate)
[4] WSC = Water soluble carbohydrates
[5] Ensiling losses as g per kg initial DM calculated as weight loss × 1.44 (taking into account the amount of water formed with CO_2 production)
[6] Cfu = Colony forming units

ASSESSING THE IMPACT OF INOCULANT TREATMENT ON THE FERMENTATION, MICROBIAL CHARACTERISTICS AND AEROBIC STABILITY OF WHOLE-PLANT RYE SILAGE

JATKAUSKAS, J.[1], VROTNIAKIENE, V.[1], EISNER, I.[2], WITT, K.[2], GIUSEPPE, C.[2]
[1]Institute of Animal Science of Lithuanian University of Health Sciences, R.Žebenkos 12, Baisogala, Radviliškis distr., Lithuania. lgipts@gmail.com, [2]Chr-Hansen A/S, Hoersholm, Denmark

INTRODUCTION

Double-cropped winter annuals can increase total dry matter (DM) yield per hectare and can decrease N leaching, reduce phosphorus loss, and increase income over feed cost (Ranck et al. 2020). Like other small-grain winter annuals (wheat, triticale, and barley), whole-crop rye can be a valuable forage for silage production and a good forage source for many ruminants. The aim of the present study was to evaluate the potential of the blends of homo- and heterofermentative lactic acid bacteria (LAB) as an additive for rye silage.

MATERIALS AND METHODS

Rye (*Secale cereale* L.) was harvested at three stages of maturity from the same field and location: (1) the early-cut harvest, slightly wilted (273.6 g/kg) before ensiling) and (2) the whole-crop harvest (soft dough stage of grain) was gathered, when forage contained 457 g kg-1 DM. Prior to ensiling, rye forage was separated into four piles, and four additive treatments for each maturity stage were applied: control (T0) with no additive (only tap water added) and three commercial (Chr. Hansen A/S, Denmark) LAB inoculants: (1) a homofermentative SiloSolve® MC (T1) containing *L. plantarum* (DSM26571), *E. faecium* (DSM22502), and *L. lactis* (NCIMB30117), at the proportion 40:30:30, (2) a hetero- and homofermentative SiloSolve® AS200 (T2) containing *L. plantarum* (DSM26571), *E. faecium* (DSM22502), and *L. buchneri* (DSM22501), at the proportion 20:30:50, and (3) a hetero- and homofermentative SiloSolve® FC (T3) containing *L. buchneri* (DSM22501) and *L. lactis* (DSM11037), at the proportion 50:50. The products were applied at the dose of 150 000 CFU g-1 forage. Ten experimental silages were prepared for each vegetation stage and for each treatment: five for the chemical and microbiological analyses and five for the aerobic stability test after the targeted fermentation period. Silages were stored for 60 days in a dark room at an ambient temperature (20–22 °C). Data were statistically analysed as a randomized complete block by using the GLM procedure of SAS.

RESULTS AND DISCUSSION

Compared to control (T0), homofermentative (T1), and hetero- and homofermentative LAB (T2) treatments resulted in a significantly higher DMc content of the silages. The residual WSC content was the highest for the T1 treatment among both vegetation stages of rye indicating more effective WSC utilisation by homofermentative LAB. Inoculation with L. buchneri in combination with homofermentative LAB (T3) resulted in a lower content of residual WSC in silages. Similar results were reported by Kleinschmit and Kung (2006).

Table 1. Nutrient composition and microbial characteristics of rye silages

TR	DMc g/kg	g kg/ DMc					Log_{10} CFU g/FF		
		CP	CF	WSC	ADF	NDF	LAB	Yeast	Mold
	Early-cut (boot stage)								
T0	252.8[c]	96.3[a]	201.7	42.3[c]	260.5[a]	455.1[a]	5.38[b]	1.88[a]	2.19[a]
T1	261.6[b]	99.9[b]	195.4	100.0[b]	255.5[a]	448.0[a]	7.13[a]	1.37[b]	1.78[b]
T2	259.1[b]	99.9[b]	198.1	89.4[b]	257.5[a]	451.9[a]	7.31[a]	1.12[c]	1.55[c]
T3	261.4[b]	98.8[b]	199.3	50.1[c]	254.4[a]	452.6[a]	7.46[a]	1.06[c]	1.48[c]
SE	*1.300*	*1.061*	*3.543*	*4.871*	*3.009*	*3.502*	*0.22*	*0.06*	*0.06*
	Soft dough stage of grain								
T0	432.9[b]	70.3[b]	291.3[b]	23.2[b]	352.3[b]	504.7[b]	5.67[a]	3.45[a]	2.90[a]
T1	446.7[c]	73.5[c]	285.9[b]	42.5[c]	346.4[a]	493.3[b]	7.16[b]	3.02[b]	2.07[b]
T2	444.1[d]	71.8[d]	287.7[b]	24.9[b]	346.9[a]	502.3[b]	8.21[c]	2.89[b]	1.70[c]
T3	445.9[cd]	70.0[bd]	286.3[b]	17.6[d]	344.4[a]	501.4[b]	8.58[c]	1.18[c]	1.12[d]
SE	*0.853*	*0.999*	*2.867*	*1.933*	*5.196*	*8.179*	*0.18*	*0.06*	*0.04*

TR = treatment; DMc = dry matter corrected for volatiles; CFU = colony forming units; FF = fresh forage; SE = standard error; CP = crude protein; CF = crude fibre; WSC = water-soluble carbohydrates; ADF = acid detergent fibre; NDF = neutral detergent fibre, LAB = lactic acid bacteria. Within a column and stage of maturity mean values followed by different letter differ significantly (p<0.05)

The homofermentative LAB treatment (T1) produced a typical effect on silage quality at both vegetation stages of rye: more lactic acid, less acetic acid, less ethanol, and lower proteolysis (lower ammonium-N content) compared to control (T0). A positive effect of T1 treatment was also manifested by a lower pH value of this silage and a lower content of butyric acid in it. The homofermentative LAB treatment (T1) resulted in the lowest DM losses among all inoculant treatments during the storage period.

Table 2. Fermentation characteristics and dry matter losses of rye silages

TR	pH	Ammonia-N, g/kg N	g/kg DMc				
			LA	AA	Alcohols	BA	DM loss
	Early-cut (boot stage)						
T0	3.91^{a}	50.27^{a}	52.37^{a}	22.28^{a}	25.94^{a}	7.01^{a}	93.7^{a}
T1	3.62^{b}	32.63^{b}	91.50^{b}	8.23^{b}	11.37^{b}	0.70^{b}	53.2^{b}
T2	3.69^{c}	36.45^{c}	79.91^{c}	25.49^{a}	12.66^{bc}	0.74^{b}	63.6^{c}
T3	3.74^{d}	39.03^{c}	73.97^{c}	45.87^{c}	13.74^{c}	1.42^{b}	57.8^{bc}
SE	*0.016*	*1.130*	*2.298*	*1.330*	*0.499*	*0.324*	*2.078*
	Soft dough stage of grain						
T0	4.41^{a}	51.32^{a}	12.58^{a}	5.89^{a}	12.52^{a}	6.93^{a}	75.2^{a}
T1	3.70^{b}	32.73^{b}	34.57^{b}	5.65^{a}	8.32^{b}	0.65^{b}	29.7^{b}
T2	3.77^{c}	34.97^{b}	32.74^{b}	10.69^{b}	5.25^{c}	0.71^{b}	36.1^{c}
T3	3.96^{d}	39.06^{c}	7.78^{c}	29.26^{c}	6.78^{d}	0.73^{b}	36.8^{c}
SE	*0.014*	*1.106*	*1.030*	*0.997*	*0.482*	*0.265*	*1.555*

TR = treatment; DMc = dry matter corrected for volatiles; SE = standard error; LA = lactic acid; AA = acetic acid; BC = butyric acid. Within a column and stage of maturity mean values followed by different letters differ significantly (p<0.05)

The aerobic stability of T1 silages treated with homofermentative LAB was clearly reduced compared to the control (T0) and the other (T2 and T3) treatments (Table 3). Moreover, at the end of the aerobic stability test, T1 silages manifested the fresh weight loss and the number of yeasts and moulds close to the control (T0) silage at all three maturity stages of rye. Auerbach et al. (2020) indicated that the high residual sugar content may stimulate the extent and the rate of yeast survival and growth in the silages exposed to air and reduce the aerobic stability of these silages.

Table 3. Characteristics of rye silage at the end of the aerobic stability test

TR	pH	DM, g/kg	Weight loss, %	AS[1] ,h	Highest temp., °C	Log_{10} CFU g^{-1} of FF	
						Yeast	Mold
	Early-cut (boot stage)						
T0	8.09^{a}	211.4^{b}	10.10^{a}	120.0^{c}	31.5	6.86^{a}	8.36^{a}
T1	8.48^{a}	225.0^{a}	9.88^{a}	98.4^{c}	29.7	6.75^{a}	7.26^{b}
T2	8.05^{a}	221.3^{a}	8.83^{b}	369.6^{b}	25.6	5.73^{b}	5.04^{c}
T3	5.56^{b}	224.0^{a}	3.51^{c}	687.6^{a}	23.0	3.95^{c}	3.22^{d}
SE	*0.159*	*3.373*	*0.362*	*18.969*	-	*0.224*	*0.247*
	Soft dough stage of grain						
T0	6.23^{a}	396.5^{a}	5.29^{a}	176.4^{a}	29.8	7.60^{a}	8.06^{a}
T1	7.17^{b}	407.4^{b}	6.44 ^{b}	64.8^{b}	29.9	8.34^{a}	7.26^{b}
T2	7.63^{c}	409.7^{b}	5.96^{ab}	116.4^{c}	28.2	7.71^{a}	7.05^{b}
T3	4.32^{d}	411.5^{b}	2.51^{c}	339.6^{d}	23.3	4.78^{b}	2.24^{c}
SE	*0.149*	*3.303*	*0.289*	*11.392*	-	*0.255*	*0.214*

TR = treatment; DM = dry matter; AS = aerobic stability; CFU = colony forming units; FF = fresh forage; SE = standard error; [1]Number of hours until the silage reached a sustained temperature greater than 3 °C above ambient. Within a column and stage of maturity mean values followed by different letters differ significantly (p<0.05).

CONCLUSIONS

The homofermentative LAB inoculant treatment improved the fermentation profile and reduced DM losses at both stages of maturity but impaired aerobic stability at soft dought stage. The product containing homofermentative *Enterococcus faecium*, *L. plantarum*, and heterofermentative *L. buchneri* improved fermentative profile, reduced DM losses at all stages of maturity, but aerobic stability was improved only in early cut rye silage. This strain combination can be recommended if rye is harvested at early growth stage in the double-cropping systems. The dual-purpose inoculant containing *L. buchneri* and *L. lactis* supported good fermentation with reduced DM losses and ensured long-lasting protection against aerobic deterioration caused by yeasts and moulds independently from the actual maturity stage of the crop.

REFERENCES

Ranck, E.J., Holden, L.A., Dillon, J.A., Rotz, C.A. & Soder, K.J. (2020). Economic and environmental effects of double cropping winter annuals and corn using the integrated farm system model. Journal of Dairy Science 103: 3804–3815.

Kleinschmit, D.H. & Kung, L.Jr. (2006). A meta-analysis of the effects of Lactobacillus buchneri on the fermentation and aerobic stability of corn and grass and small-grain silages. Journal of Dairy Science 89: 4005–4013.

Auerbach, H., Theobald, P., Kroschewski, B. & Weiss, K. (2020). Effects of various additives on fermentation, aerobic stability and volatile organic compounds in whole-crop rye silage. Agronomy 10: 1873.

EFFECTS OF INOCULANT TREATMENT ON WHOLE CROP MAIZE AND BARLEY FERMENTATION CHARACTERISTICS AND REDUCTION OF AEROBIC DETERIORATION

VROTNIAKIENE, V.[1], JATKAUSKAS J.[1], EISNER, I.[2], WITT, K.[2], GIUSEPPE, C.[2]
[1]Institute of Animal Science of Lithuanian University of Health Sciences, R. Žebenkos 12, Baisogala, Radviliškis distr., Lithuania. lgipts@gmail.com, *[2]Chr-Hansen A/S, Hoersholm, Denmark*

INTRODUCTION

Whole crop maize is the major crop ensiled worldwide. Recently, there has been increasing interest in the use of alternative forage with small grain cereal crops, like barley. Maintaining the nutritive value of harvested forage until it is fed to animals has considerable economic importance in animal production. Silage additives are used to improve fermentation, prolong aerobic stability by inhibiting the growth of undesirable micro-organisms, thus preventing spoilage of the feeds and minimizing nutrient and energy losses (Wilkinson and Muck, 2019). In practice, silage surface spoilage by yeasts and moulds is a problem, particularly in silages that are inadequately sealed and in silages that have a relatively high surface to volume ratio, such as wrapped bales (Driehuis et al.,2001). The objective of these two experiments was to evaluate the efficacy of silage inoculant containing a three lactic acid bacteria strains *Lactobacillus buchneri* (DSM22501), *Lactobacillus plantarum* (DSM26571), *Enterococcus faecium* (DSM22502) on fermentation quality and aerobic stability of whole crop maize and barley forages ensiled in round big bales.

MATERIALS AND METHODS

Big bales of silage were produced either from whole-plant barley (soft dough stage of grain, 35.4 % DM) and from whole plant maize (dough stage of grain, 34.16 % DM). Barley or maize forage before ensiling were untreated (C1 and C2, respectively) or treated with inoculant SILOSOLVE AS containing lactic acid bacteria strains *Lactobacillus buchneri*, *Lactobacillus plantarum*, *Enterococcus faecium* (AS1 and AS2, respectively). The inoculant product was applied during the bailing process and targeted dosage of 1.5×10^5 CFU/g of fresh forage. 5 bales per treatment and per forage were assigned to testing fermentation quality, and 5 bales per treatment and per forage were assigned to aerobic stability testing in the field conditions. The silages in the bales were fermented under outdoor farm conditions for 120 days and after were sampled and analyzed for nutrient composition, fermentation and microbiological parameters. After uncovering the bales, aerobic stability testing was done by exposing the bales to air under ambient conditions, and taking hourly temperature measurements. In parallel aerobic stability testing was done by exposing silage samples from big bales to air under laboratory conditions. The aerobic deterioration of silages was evaluated by observing temperature dynamics inside the big bale and in the laboratory, the pH value, weight loss and the number of yeasts and moulds at the end of the aerobic stability test. Data were statistically analyzed as a randomized complete block by using the GLM procedure of SAS.

RESULTS AND DISCUSSION

Inoculation of whole plant barley (AS1) or whole plant maize (AS2) forages with viable hetero -and homofermentative LAB strains affected the silage nutrient composition and fermentation profile. After 120 days of the fermentation period, inoculant treated relative to untreated whole plant barley (AS1) or whole plant maize (AS2) silages was associated with significantly increased DM content, significantly reduced fresh weight and dry matter loss and was related with improved fermentation indicators of inoculated silages compared with untreated silages (Table 1). Inoculant-treated silages showed significantly improved positive indicators of fermentation, including a reduced pH, increased total acid concentrations, and increased individual concentrations of lactic and acetic acids, significantly lowered negative indicators of fermentation, including reduced concentrations of the ammonia N, alcohols, and butyric acid. Counts of yeasts, moulds and clostridia were significantly lower in inoculant-treated whole-plant barley (AS1) or whole-plant maize (AS2) silages when compare with not inoculated silages (C1 and C2). The number of LAB was significantly increased in AS1 and AS2 silages. After uncovering bales mould spots were detected on the surface of uninoculated silages (C1 and C2) whereas no mold spots were detected on the surface of inoculant-treated silages (AS1 and AS2). Driehuis et al. (2001) indicate that the survival of yeasts during the anaerobic ensilage phase is reduced, resulting in lower yeast counts in silages inoculated with *L. buchneri* than in silages without *L. buchneri* and growth of yeasts during exposure of silage to the air is inhibited too.

During the aerobic stability tests in the field and in the laboratory, inoculant treated whole plant barley (AS1) or whole plant maize (AS2) silages relative to untreated silages (C1 and C2) showed significantly lower fresh weight loss, significantly lower pH-value, significantly higher number of LAB and significantly lower number of molds and yeasts at the end of aerobic stability test (Table 2). Temperature monitoring inside silages showed significantly longer duration of aerobic stability of the AS1 and AS2 silages compared with C1 and C2 silages. At the end of aerobic stability test in the field AS1 and AS2 silages showed significantly lower number of mold spots on the surface of bales than C1 and C2 silages did. The meta-analysis of Kleinschmit and Kung (2006) reported greater aerobic stability in *Lactobacillus buchneri*, treated corn silage grass, and small grain silages than in untreated silages.

Table 1. Nutrient composition and fermentation variables of barley and maize silage ensiled in big bales after 120 days of storage

	Whole crop barley				**Whole crop maize**			
Treatment	**C1**	**AS1**	**SE**	**P**	**C2**	**AS2**	**SE**	**P**
DMc, g/kg	302.0	325.2	2.225	P<0.01	327.3	330.0	0.806	P<0.05
DM loss, g/kg	175.4	102.4	4.006	P<0.01	65.26	50.26	1.151	P<0.01
Crude protein, g/kg DM	114.3	117.0	2.974	ns	78.5	80.7	0.543	P<0.05
WSC, % DM	10.8	13.6	1.660	ns	10.7	12.8	1.232	ns
pH	4.18	4.09	0.026	ns	3.97	3.94	0.006	P<0.05
N-NH_3, g/kg total N	57.34	50.71	2.144	ns	43.89	35.16	0.959	P<0.01
Alcohols, g/kg DM	10.52	7.20	0.338	P<0.01	10.73	7.22	0.335	P<0.01
Lactic acid, g/kg DM	20.64	31.42	1.267	P<0.01	36.18	47.25	1.539	P<0.01
Acetic acid, g/kg DM	7.14	17.23	0.536	P<0.01	7.18	9.83	0.332	P<0.01
Butyric acid, g/kg DM	3.11	0.63	0.191	P<0.01	1.52	0.46	0.105	P<0.01
Propionic acid, g/kg DM	0.32	0.34	0.070	ns	0.05	0.07	0.029	ns
LAB, Log_{10} CFU/g	6.77	8.55	0.063	P<0.01	6.33	7.79	0.166	P<0.01
Yeast, Log_{10} CFU/g	2.55	1.00	0.041	P<0.01	2.69	1.47	0.077	P<0.01
Molds, Log_{10} CFU/g	2.31	1.06	0.080	P<0.01	1.91	1.06	0.047	P<0.01
Clostridia, Log_{10} CFU/g	2.09	1.00	0.031	P<0.01	2.00	1.02	0.031	P<0.01

Table 2. Results at the end of the aerobic stability evaluation in the field and in the laboratory

	Whole crop barley				Whole crop maize			
Treatment	C1	AS1	SE	P	C2	AS2	SE	P
Aerobic stability outdoors (30 days of aerobic exposure)								
Fresh weight loss, %	4.05	2.41	0.186	P<0.01	3.31	1.94	0.092	P<0.01
pH	4.86	4.20	0.045	P<0.01	6.05	4.09	0.031	P<0.01
Yeast, Log10 CFU/g	4.52	2.10	0.071	P<0.01	5.42	2.65	0.190	P<0.01
Molds, Log10 CFU/g	4.69	2.03	0.098	P<0.01	4.89	2.49	0.194	P<0.01
LAB, Log10 CFU/g	6.41	8.47	0.088	P<0.01	6.66	7.94	0.275	P<0.05
Moulds score	1.80	0.20	0.300	P<0.01	1.80	0.20	0.374	P<0.05
Aerobic stability, h	398.4	652.8	6.841	P<0.01	694.5	720	2.635	P<0.01
Aerobic stability in the laboratory (12 days of aerobic exposure)								
Fresh weight loss, %	5.03	2.65	0.353	P<0.01	5.74	2.97	0.158	P<0.01
pH	8.63	4.67	0.119	P<0.01	7.16	5.88	0.142	P<0.01
Yeast, Log10 CFU/g	8.46	3.42	0.108	P<0.01	8.91	3.24	0.081	P<0.01
Molds, Log10 CFU/g	9.00	3.43	0.206	P<0.01	8.55	2.31	0.169	P<0.01
LAB, Log10 CFU/g	5.87	7.99	0.153	P<0.01	7.18	9.56	0.146	P<0.01
Aerobic stability, h	177.6	265.2	5.692	P<0.01	169.2	344.4	6.573	P<0.01

CONCLUSIONS

In conclusion adding inoculant SILOSOLVE AS containing lactic acid bacteria strains *Lactobacillus buchneri*, *Lactobacillus plantarum*, *Enterococcus faecium* to whole plant barley or whole plant corn ensiled in big bales significantly improved fermentation characteristics, reduced fresh matter and DM loss and significantly suppressed growth of mold, yeast and clostridia and ensured long-lasting protection against aerobic deterioration.

REFERENCES

Wilkinson, J. M. & Muck, R.E. (2019). Ensiling in 2050: some challenges and opportunities. Grass and Forage Science 74: 178–187.

Kleinschmit, D. H. & Kung, L. Jr. (2006). A meta-analysis of the effects of Lactobacillus buchneri on the fermentation and aerobic stability of corn and grass and small-grain silages. Journal of Dairy Science 89: 4005–4013.

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

CONSISTENT FEED INTAKE THOUGH HIGH QUALITY FORAGE AND MANAGEMENT IS MANDATORY FOR HEALTHY, HIGH PERFORMING COWS – BUT REALITY OFTEN IS DIFFERENT

LOOFF, F.
Pioneer Hi-Bred Northern Europe Sales Division GmbH, Riedenburger Strasse 7, 81677 Muenchen, Germany

INTRODUCTION

The higher the milk yield the higher the demand of dairy cows for nutritional intake. Thus, high yielding dairy cows frequently are fed diets high in digestibility which is often accomplished by diets high in readily digestible concentrates such as starch and soluble fibre. Drops in ruminal pH represent a major challenge, known as "subactute ruminal acidosis" (SARA), which is related to various costly metabolic disorders (HUMER ET AL., 2018). In their review, the authors summarise current knowledge on various factors that play a role with the appearance of SARA.

This paper will discuss some of aspects of management as well as forage related shortcomings on practical farms on the incidence of SARA and, more importantly, practical approaches to find and mitigate bottlenecks on farm but also strategies to improve especially forage production.

MATERIALS AND METHODS

It is the experience of the author based on numerous farm visits that very frequently inconsistent feed intake during the day represents a major challenge on practical farms, see examples below. From a scientific standpoint, effects of fluctuations of feed intake on ruminal pH is well established: In a challenge trial, DOHME ET AL. (2008) showed the effect of feed restriction and subsequent high grain concentrations on ruminal pH of dairy cows. GASTEINER ET AL. (2012) showed the impact of TMR feeding vs. separate feeding of grass silage, corn silage and compound on practical farms using commercially available boli. DEFRIES AND KEYSERLINGK (2009) demonstrated elevated feed intake ("Slug feeding") of heifers that were able to sort the diet provided. Slug feeding may affect passage rate and thus also the proportion of starch being digested in the rumen from those starch compound feed that is rather slowly being digested in the rumen (i.e. corn grain or sorghum). As discussed by HUMER ET AL. (2018) slug feeding can be linked not only to elevated passage rates but also to more fluctuations in ruminal pH not only because of ruminal fermentation but also due to variations in saliva release. Vice versa, MACMILLAN ET AL. (2017) showed that cows high in risk for SARA spend more time in the eating period 8h after dropping feed in front of the cows vs. cows low in risk for SARA. As another aspect, cows sorting their feed will spend more time feeding while the dry matter intake remains relatively unchanged as outlined by GRETER ET AL. (2010). Several ways exist to overcome sorting and adding water to the diet is one of them as recently showed by DENIßEN ET AL. (2021); but it may come at the expense of more heating of the diet – as shown by the authors as well – which obviously may hamper palatability.

Thus, negative impact of SARA on performance and health is as well established, as the impact of management practices and here among others consistency of feed intake on its prevalence in dairy cows. Nevertheless, on practical farms SARA due to management deficiencies frequently is an issue based on the experience of the author. Advice must focus both on offsetting the most limiting bottleneck in an affordable way as a short-term fix but also on long-term solutions.

CONCLUSIONS

Several tools exist to find bottlenecks on farm and examples will be shown during the presentation. These tools comprise usage of:

- Penn State Particle Separator (PSPS; LAMMERS ET AL., 1996) which is a great tool to monitor and improve consistency of delivery of feed in front of the cows on-farm (OELBERG AND STONE, 2014). For example, bad mixing can easily result in high coefficients of variation between samples examined using the PSPS. Thus, cows do not receive the same diet in different locations at the feed bunk. The PSPS can also help to uncover issued with sorting on farm. All of that may result in SARA as outlined above.
- A time-lapse camera can be used to investigate feeding behaviour (e.g. DEFRIES AND KEYSERLINGK, 2008) but also to monitor management practices like duration of milking as well as feed access before and after milking. For example, feeding in the morning during cows being away in milking parlour can result in cows being off feed for 2-3 h or more (which is then easy to document using a time lapse camera) which may result in slug feeding and SARA.
- A thermometer or an infrared camera is helpful to document both heating of silage in the clamps as well as TMR in front of the cows. For example, ½ m^3 heated corn silage roughly is 300 kg (as fed) – and that weight will increase until push-out of refusals 24 h later; large farms often target feed refusals of 3-5 % in the high string groups which is about 180 – 300 kg (as fed) in a 100 cow group. Thus, cows may stop feeding much earlier, if the TMR is spoiled due to heating. When the new feed is dropped in front of cows, slug feeding and SARA may be expected.

REFERENCES

Denißen, J., Beintmann, S., Hoppe, S., Pries, M., Hummel, J. and Südekum, K.-H. 2021. Influence of the addition of water to total mixed rations on the feeding behaviour, feed intake and milk performance of high-yielding dairy cows. Livest Sci. 254:104743.

DeVries, T.J., von Keyserlingk, M. A. G. 2009. Short communication: Feeding method affects the feeding behavior of growing dairy heifers. J. Dairy Sci. 92:1161–1168.

Dohme, F., DeVries, T. J. and Beauchemin, K. A. 2008. Repeated Ruminal Acidosis Challenges in Lactating Dairy Cows at High and Low Risk for Developing Acidosis: Ruminal pH. J. Dairy Sci. 91:3554–3567.

Gasteiner, J., Boswerger, B. and Guggenberger, T. 2012. Erfahrungen zum praktischen Einsatz des Pansensensors auf Milchviehbetrieben. 5. Tierärztetagung 2012 59 – 70.

Greter, A. M., Leslie, K. E., Mason, G. J., McBride, B. W. and DeVries, T. J. 2009. Effect of feed delivery method on the behavior and growth of dairy heifers. J. Dairy Sci. 93 :1668–1676.

Humer, E., Petri, R. M., Aschenbach, J. R., Bradford, B. J., Penner, G. B., Tafaj, M., Südekum, K.-H. and Zebeli, Q. 2018. Invited review: Practical feeding management recommendations to mitigate the risk of subacute ruminal acidosis in dairy cattle. J. Dairy Sci. 101:872–888.

Lammers, B. P., D. Buckmaster, and A. Heinrichs. 1996. A simple method for the analysis of particle sizes of forage and total mixed rations. J. Dairy Sci. 79:922–928.

Macmillan, K., Gao, X., Oba, M. 2017. Increased feeding frequency increased milk fat yield and may reduce the severity of subacute ruminal acidosis in higher-risk cows. J. Dairy Sci. 100:1045–1054.

Oelberg, T. J., and W. Stone. 2014. Monitoring total mixed rations and feed delivery systems. Vet. Clin. North Am. Food. Anim. Pract. 30:721–744.

HARVESTING TECHNIQUES TO PRODUCE HIGH PROTEIN FEED FROM ALFALFA

MAXA, J.[1], PANTKE, S.[2], LUBER, E.[3], THURNER, S.[1]
[1]Bavarian State Research Centre for Agriculture, Institute of Agricultural Engineering and Animal Husbandry, Vöttingerstr. 36, 85354 Freising, Germany; [2]Technical University of Dresden, Chair of Agricultural Systems and Technology, Bergstr. 120, 01069 Dresden, Germany; [3]Brand Landtechnik GmbH, Im Oehl 4, 92339 Beilngries, Germany

INTRODUCTION

Legume leaves such as from alfalfa (*Medicago sativa*) have a great potential as feed protein due to their high protein as well as their essential amino acid content. In organic feeding of monogastric animals such as pigs, essential amino acids are needed and together with feeding of roughage can generate additional animal welfare benefits. Technical separation of leaf mass and stem in green legumes in order to specifically use the higher concentration of protein and amino acids in the leaves for monogastric nutrition was proposed by Sommer and Sundrum (2015). Until nowadays, however, such technical solutions have existed only in rudimentary form. Recently, Liebhardt et al. (2022) compared two methods of harvesting techniques in alfalfa – a leaf stripping technique with a conventional whole plant harvesting technique. For the tested leaf stripping technique further improvement and optimisation were suggested before practical use. Meanwhile, new technical approaches to harvest alfalfa leaves and plant tops were developed (Maxa et al., 2021).

Therefore, the main aim of this study was to analyse and test two innovative technical approaches to harvest alfalfa leaves and alfalfa plant tops to produce high protein feed.

MATERIAL AND METHODS

The experiments with technical approaches to harvest alfalfa as high protein feed were conducted in two field trials over the 1st cut of the years 2020 and 2021 and included i) the technique for harvesting alfalfa leaves using an adapted combine harvester (T1) and ii) the technique for harvesting alfalfa plant tops using an adapted mower (T2). The sites with alfalfa (variety Verko) and 4-6 large plots/replicates (264 m^2 for T1 and 200 m^2 for T2) per technical approach were located in Pollanten (year 2020) and Matzenhof (year 2021), both near the river Altmühl in Bavaria. The developmental stages of the plants at harvesting dates were at the BBCH scale 51 – 55 (early to late bud stage) according to Meier (2018).

T1 was a combine harvester that was optimised for harvesting wilted alfalfa leaves by the Chair of Agricultural Systems Engineering at the Technical University of Dresden and the company Brand Landtechnik GmbH (Figure 1). The optimization process for the combine harvester focused on emptying the grain tank, the pick-up and tests of different sieves and working speeds. During the field trials alfalfa was cut and placed in a wide swath and dried to the target dry matter (DM) content of approximately 650 g/kg for the harvest with the combine harvester.

For T2 an innovative machine called "Top Cut Collect" from the company Zürn Harvesting GmbH & Co. KG was deployed. This machine was originally developed for mechanical weed control in the case of resistance problems or chemical-free arable farming strategies. The horizontal double-blade mower was well suited for a "high cut", where the plant tops (top 30% of the plants height) and thus the young plant parts with higher protein content can be harvested separately, without separation of leaves and stems (Figure 1). The rest of the plants were mown after the high cut and wilted until the target DM content of approximately 600 g/kg.

The average DM yield was calculated for each technique per hectare. Leaves proportion (LP) in % in harvested material was estimated using Near-Infrared Spectroscopy (NIRS). Furthermore, crude protein (CP) on DM basis was analysed by the laboratory of Thuenen Institute of Organic Farming (Trenthorst, Germany) according to Commission Regulation No 152/2009. The statistical analysis was performed for each year individually due to extreme weather conditions, negatively influencing plant development during the 1st cut of 2020. Analysis of variance was carried out using SAS Software for the harvested material consisting of DM yield and LP.

Figure 1: Adapted combine harvester for harvest of alfalfa leaves (left); Top Cut Collect machine for harvest of alfalfa tops (right). (© J.Maxa)

RESULTS AND DISCUSSION

Table 1 presents the DM yield, CP content and LP for both harvesting techniques and observed years separately. While the alfalfa plants of the 1st cut in 2020 were negatively influenced by severe drought (low stand but good drying conditions in the field), the alfalfa plants during the 1st cut 2021 were densely developed but with a lower proportion of leaves relative to stems (55% leaves in 2020 versus 42% leaves in 2021). Likewise, the drying conditions in the field were relatively inconvenient in the second year. Compared to a previous study of Liebhardt et al. (2022) presenting CP content for harvested alfalfa leaves of 265 g/kg the CP content found in our study for alfalfa plant tops harvested by T2 reached 318 g/kg in the year 2021. The rest of the plant can after the harvest with T2 still be used as a high-quality product for ruminants. Compared to T1, however, lower DM yields from the plant tops and the additional hot or warm drying in order to conservate the harvested material was necessary. With T2, the initially intended, time-consuming separation of the alfalfa leaves from the stems can thus be omitted. Although T1 resulted in a lower CP content in the harvested material, higher DM yields and the direct storability of the harvested material was achieved. Moreover, the CP content harvested with T1 were similar to the values obtained by Liebhardt et al. (2022). The leave material from the grain tank of T1 had under optimal harvesting conditions a DM content of 880 g/kg. Thus, the T1 avoided the costand energy-intensive drying of the harvested leave material. Contrary to harvested leaves by T1, the stems needed a further drying process either on the field or in a processing factory.

For the determination of the LP in the harvested material of T1 and T2, a NIRS calibration model was created. There were no significant differences found in LP of leave material and plant tops harvested by T1 and T2 in 2020, respectively. Contrary in 2021 both mentioned harvested materials differed significantly due to LP decrease in T1. There were no leaves obtained in stems after the harvest with T1 in both years. Further, LP up to 30 % was left on the rest plant after the harvest with T2, which resulted in higher CP content in 2020 compared to the stems of T1 in the same year.

Table 1: Dry matter (DM) yield, crude protein content (CP) and leave proportion (LP) in alfalfa.

Harvesting technique	year 2020			year 2021		
	DM yield (kg/ha)	CP[3] (g/kg DM)	LP (%)	DM yield (kg/ha)	CP (g/kg DM)	LP (%)
T1[1] - leaves	1220[a]	260	81[a]	2000[a]	256	56[a]
T1 - stems	740[b]	152	0[b]	1520[a]	193	0[b]
T2[2] - plant tops	450[b]	292	78[a]	730[b]	318	86[c]
T2 - plant rest	1560[a]	184	30[c]	2510[c]	193	13[d]

Means in the same column with different uppercase letters differed ($P < 0.05$).
[1]Combine harvester; [2]Top Cut Collect; [3]Based on single values (sample from all repetitions within technique and year).

CONCLUSIONS

Both tested harvesting techniques showed potential to produce feed with high protein content from alfalfa leaves and plant tops which can be used for feeding monogastric animals such as pigs. Advantage of T1 was the direct storability of separated leaves harvested under optimal harvesting conditions as well as higher DM yields compared to T2. On the other hand, T1 is until nowadays still in a prototype stage. Deployment of T2 thus eliminated the need for the separation of alfalfa leaves from stems and resulted in higher CP content in the harvested alfalfa plant tops. The rest of the plant left from both harvesting techniques can still be used as a high-quality feed product for ruminants.

Nevertheless, further investigations are needed in order to investigate the post-harvesting processes, conservation and processing the product into a concentrated protein feed for pigs and ruminants.

ACKNOWLEDGEMENTS

This study was supported by funds of the Federal Ministry of Food and Agriculture (BMEL, Germany, grant nr. 2815OE039) and the Bavarian State Ministry of Food, Agriculture and Forestry (StMELF, Germany, grant nr. KL/21/04). The optimization of the combine harvester used in this study was supported by fund of the Federal Ministry of Economic Affairs and Climate Action (BMWK, Germany, grant nr. 16KN043325).

REFERENCES

Liebhardt, P., Maxa, J., Bernhardt, H., Aulrich, K., Thurner, S. 2022. Comparison of a Conventional Harvesting Technique in Alfalfa and Red Clover with a Leaf Stripping Technique Regarding Dry Matter Yield, Total Leaf Mass, Leaf Portion, Crude Protein and Amino Acid Contents. Agronomy, 12, 1408.

Maxa, J., Liebhardt, P., Thurner, S. 2021. Evaluation of harvesting processes for alfalfa with the aim of attaining a high proportion of leaves as a high protein feed for monogastric animals, CIGR, Quebec, Canada.

Meier, U. 2018. Growth Stages of Mono- and Dicotyledonous Plants: BBCH Monograph; Open Agrar Repositorium: Berlin/Braunschweig, Germany.

Sommer, H. and Sundrum, A. 2015. Ganzpflanze und Blattmasse verschiedener Grünleguminosen als Eiweißquelle in der Schweinefütterung. 13. Wissenschaftstagung Ökologischer Landbau, Eberswalde, Germany.

A REVIEW OF THE NOVEL *LENTILACTOBACILLUS HILGARDII* CNCM I-4785 IN COMBINATION WITH *LENTILACTOBACILLUS BUCHNERI* NCIMB 40788 TO IMPROVE FERMENTATION AND AEROBIC STABILITY OF DIFFERENT FORAGES

QUEIROS, L., KUECHENMEISTER, F., ANDRIEU, B., CHEVAUX, E., VILLOT, C., DEMEY, V., BRIGHT, J.

Silage can account for up to 50% of a dairy ration (Grant *et al.* 2018) and with the cost of purchased feeds at historical highs, producing the best quality silage possible is one way producers can offset these external costs. Making quality silage requires a multifactual approach, from harvesting to ensuring the silo management and ensiling processes are conducted as diligently as possible (Borreani *et al.* 2018). However, the everyday realities on farm can be far from the ideal silage practices that are vital when trying to produce the best quality silage.

A large survey across a diverse range of farms, revealed that labour availability, investment strategies, environmental conditions and other parameters can force producers to make compromises that can affect the quality and value of the silage produced (Andrieu *et al.*, 2015). Poor consolidation, poor feed out management practices were notable, resulting in aerobic unstable silage (Andrieu *et al.*, 2015). Aerobic spoilage of silages is manifested as heating during the feeding period and as spoilage of surface layers with yeast and moulds. It generates significant dry matter (DM) losses and reduces the hygienic quality and palatability of silage (Borreani *et al*., 2018). Spoilage is usually initiated by yeasts and thereafter, other undesirable microorganisms such molds and bacilli start to proliferate reducing the quality of the silage even further (Driehuis *et al.*, 1999).

In truth aerobically unstable silage can be seen, even in silages where management practices are followed more precisely. The higher the DM of a silage the more difficult it is to consolidate adequately. (McEniry et al., 2010; Goeser *et al*., 2015) Changes to the environment over the past couple of decades have made it more difficult to predict forage quality and DM. Marley (2019) noted that, ensiled forage is impacted increasingly by the effects of global warming, with the impacts being felt increasingly frequently resulting in silage that needs to be fed quickly after ensiling not just due to lack of feed, but also as the silage is harder to ensile and less stable once feeding commences.

The discovery of the facultative anaerobic *Lactobacillus buchneri* NCIMB 40788 strain (LB) in the late 90's has been a truly breakthrough in forage inoculant technology. Over the proceeding years LB has been proven to work over an even wider variety of forage types and under many different environmental conditions. A recent meta-analysis confirmed the positive effect of *Lentilactobacillus buchneri* on improved aerobic stability and diminished yeast count together with an increase in acetic and propionic acid content (Blajman *et al.*, 2018). *L. buchneri* was shown to reduce the proportion of spoiled corn silage grown in hotter climates by over 50% and reduce the associated energy and nutrient losses from the silage. It also reduced the yeast and mold populations and consequently reduced heating during the aerobic feedout phase (Bernades *et al.*, 2018).

But to fully support future needs and provide the best support possible for famers, Borreani *et al. (*2018) noted that future investigations should consider increasing ensiling efficiency in terms of more effective use of cost-effective inoculants that increase aerobic stability quicker than LB can achieve, thus giving producers more flexibility to use silage as and when needed.

The recent discovery and registration of a new inoculant strain as *Lentilactobacillus hilgardii CNCM I-4785* (LH) precisely came to grant more flexibility to producers to secure aerobic stability of their silage than with LB, without affecting the benefits of LB if applied in conjunction with the new candidate. Resulted from a wide screening process from naturally stable sugarcane silage, LH was the most active isolate to enhance sugarcane silage fermentation and aerobic stability (Avila *et al*., 2014; Carvalho *et al*., 2014). The rationale behind the choice of sugarcane in this selection process laid on the strong fungal epiphytic pressure present on this roughage likely to trigger aerobic instability. A microorganism efficient in such naturally challenging material will likely display a similar effect on more conventional forages like maize and grasses. Maximizing the potential of LB and LH became the most appropriate next step by associating them in a new formulation.

The following selection of papers aims at illustrating the synergistic effect of using LB in conjunction with LH on silage quality stability, and microbiota changes across a varied range of forages and ensiling conditions.

Gomes *et al.*, (2018) reported that sugarcane silage treated with a combination of LB and LH improved fermentation characteristics, lowered silage pH, ethanol and gas losses from 10 days ensiling onwards. Dry matter losses after 70 days ensiling was also numerically lower in the combination treatment compared to the control and of that of LH and LB alone. Aerobic stability was numerically better or similar to that of LH and LB alone. In addition, Ferrero *et al.*. (2018) and Borreani *et al.* (2018) also reported similar findings in whole corn silage. The combination of LH and LB improved aerobic stability characteristics significantly compared to the control and LB only treatments and numerically improved stability compared to LH alone at 15 days ensiling. LH+LB also reduced the levels of undesirable yeast populations numerically through the ensiling period compared to the control, LH and LB alone treatments. The same effect became significant compared to the control from 30 days onwards and compared LB alone at 30 days of ensiling. The combination of LH+LB increased numerical acetic acid levels compared to the control, LH and LB only treatments from 15 days but significantly from 100d, while improving 1,2 propanediol levels compared to all other treatments significantly from 15 days. (Borreani *et al.*, 2018). In other forages such as High Moisture Corn, (Da Silva *et al.*., 2021) reported an enhanced aerobic stability from 15 days and even greater improvements up to 130 days using the combination of LH and LB in conjunction with an homolactic strain *Pediococcus pentosaceus* NCIMB 12455. HMC corn inoculated with this combination significantly lowered the pH compared to the control after 15 days and also had numerically lower yeast population. After 130 days ensiling the pH was still numerically lower with significantly lower population of yeast in the inoculated group compared to the control, as reflected in the greater aerobic stability from 15 days to 130 days. Improving the fermentation and aerobic stability using the combination of LH and LB was further characterized in terms of fungi populations changes in whole corn silage through an aerobic exposure period by Drouin *et al.* (2020). The combination of LH and LB altered the microbial succession by reducing the relative abundance (RA) of lactate assimilating yeast such as *Pichia* and *Issatchenkia* compared to the control from day 3 of an air exposure period to day 10. The combination of LH and LB drove the RA almost exclusively to non lactate utilizing *Saccharomyces*. This resulted in corn silage with significantly improved aerobic stability characteristics by 77 hours compared to the control and maintained pH and lactic acid levels through the 10-day aerobic exposure period. Interestingly as mycotoxins are a recurring issue in livestock feeding and represent silage-related animal and human health concerns, (Grant et al. 2018) the improved aerobic stability also resulted in a significant reduction in mycotoxins Roquefortine C, Fumonisin B1 and numerical reduction in DON (Drouin *et al*., 2020), further illustrating the role of this inoculant in limiting the growth of aerobic fungi.

CONCLUSIONS

The isolation of *L. hilgardii* CNCM I-4785 from sugarcane silage has proven efficacy in various forages, however, its combination with LB has not only been proven to improve ensiling characteristics and aerobic stability after shorter ensiling periods such as 15 days, but the positive effects are also repeatedly reported when ensiled for longer periods, to a greater extent than each strain taken separately. Finally, this strain association has contributed to reduce undesirable bacterial and fungi populations through the feedout period, resulting in cleaner silage with lower risk of mycotoxin contamination. Therefore, this dual-strain inoculant becomes a reliable treatment in optimizing the silage-making process in a wide range of forages.

REFERENCES

Andrieu, B.; Demey, V. On-farm corn silage investigation: Multi-analyses on silage practices, silage quality and its effect on aerobic stability. In Proceedings of the XVII International Silage Conference, Piracicaba, Brazil, 1–3 July 2015; Daniel,J.L.P.,Morais,G.,Junges,D.,Nussio,L.G.,Eds.

Ávila C. L. S., Carvalho B. F., Pinto J. C., Duarte W. F., Schwan R. F. (2014). The use of Lactobacillus species as starter cultures for enhancing the quality of sugar cane silage. Journal Dairy Science, 97: 940–951. 10.3168/jds.2013-6987

Borreani,G. E. Tabacco, R. J. Schmidt,B. J. Holmes, and R. E. Muck (2018). Factors affecting dry matter and quality losses in silagesJournal of Dairy Science. 101:3952–3979

Carvalho, B. F., C. L. SÁvila, J. C. Pinto, J. Neri, R. F. Schwan. 2014. Microbiological and chemical profile of sugar cane silage fermentation inoculated with wild strains of lactic acid bacteria. Animal Feed Science Technology. 195:1–13. doi:10.1016/j.anifeedsci.2014.04.003.

da Silva, E.B., Smith, M.L., Savage, R.M., Polukis, S.A., Drouin, P. and Kung, L., Jr (2021), Effects of Lactobacillus hilgardii 4785 and Lactobacillus buchneri 40788 on the bacterial community, fermentation and aerobic stability of high-moisture corn silage. Journal Applied Microbioly 130: 1481-1493

Driehuis, F., Oude Elferink, S. J. W. H., & van Wikselaar, P. G. (1999). Lactobacillus buchneri improves aerobic stability of laboratory and farm scale whole crop maize silage but does not affect feed intake and milk production of dairy cows. In Proceedings of the 12th international silage conference / Eds. T. Pauly [et al.]. - Uppsala : Swedish University of Agricultural Science, 1999: 264-265

Drouin, P., Tremblay, J., Renaud, J. and Apper, E. (2021), Microbiota succession during aerobic stability of maize silage inoculated with Lentilactobacillus buchneri NCIMB 40788 and Lentilactobacillus hilgardii CNCM-I-4785. MicrobiologyOpen, 10: e1153.

Ferrero, F., S. Prencipe, D. Spadaro, M. L. Gullino, L. Cavallarin, S. Piano, E. Tabacco, G. Borreani, Increase in aflatoxins due to Aspergillus section Flavi multiplication during the aerobic deterioration of corn silage treated with different bacteria inocula. Journal of Dairy Science, 102 (2):1176-1193.

Grant, R. and A.T. Adesogan (2018). Journal of Dairy Science Silage Special Issue: Introduction. J. Dairy Sci. 101:3935–3936. Goeser, J. P.; Heuer, C. R.; Crump, P. M. (2015) Forage fermentation product measures are related to dry matter loss through meta-analysis. In : The Professional Animal Scientist, 31, 2: 137–145.

Kleinschmit, D.H. and L. Kung, Jr.(2006)The Effects of Lactobacillus buchneri 40788 and Pediococcus pentosaceus R1094 on the Fermentation of Corn Silage Journal ofDairy Science, 89:3999–4004. https://doi: 10.3168/jds.S0022-0302(06)72444-4.

McEniry, J.; O'Kiely, P.; Clipson, N. J. W.; Forristal, P. D.; Doyle, E. M. (2010) Assessing the impact of various ensilage factors on the fermentation of grass silage using conventional culture and bacterial community analysis techniques. In : Journal of Applied Microbiology, 108:5:, 1584–1593

Marley, G. (2019). Silage under changing climate. Proceedings of the 18th international symposium on forage conservation,13-16 August 2019, Brno, Czech Republic.

INOCULANT APPLICATION INFLUENCES CHANGES IN ACID CONTENT DURING AERATION OF HIGH-MOISTURE CORN

DUVNJAK, M., KLJAK, K.
University of Zagreb Faculty of Agriculture, Svetošimunska cesta 25, 10000 Zagreb

Keywords: silage aeration, inoculant, lactic acid, volatile fatty acids

INTRODUCTION

Aeration activates undesirable microorganisms that cause silage spoilage, which can be seen by the increase in silage temperature. Aerobic stability of silages is influenced by several factors. One of the most important is the concentration of lactic acid and volatile fatty acids (VFA) in silages. Lyophilized lactic acid bacteria are used as inoculants in the production of silage. This often allows for proper fermentation and acid production, as well as increased aerobic stability of silages. The aim of this study was to investigate the effect of the addition of the inoculant *Lactiplantibacillus plantarum* during silage preparation on the aerobic stability of high-moisture corn.

MATERIALS AND METHODS

During the 7-day aeration, the changes in the concentrations of lactic acid, VFA (acetic, propionic, and butyric acids), ethanol, and methanol in the silages ensiled without and with the inoculant were quantified by the HPLC method. In addition, the temperature in the silages was recorded every 15 minutes, since aerobic stability is defined as the period of time during which the temperature in the silage is not 2 °C above the ambient temperature. The effects of ensiling, the ensiling additive and their interactions on the lactic acid, VFA, ethanol and methanol were analyzed using the PROC MIXED procedure of SAS 9.4.

RESULTS AND DISCUSSION

Although all silages remained aerobically stable and temperatures were no more than 2°C above ambient, changes in acids and alcohols were observed ($P < 0.001$) and they differed between silages (inoculated vs. control silages, $P < 0.01$). In the control samples, the lactic acid content was twice as high at the end of the aeration as at the beginning (11.05 vs. 6.84 g/kg DM), while the values in the inoculated samples remained similar (18.24 vs. 17.96 g/kg DM). Acetic acid content increased in the control samples (1.9 vs. 2.61 g/kg DM), while in the inoculated samples the final value was slightly lower than the initial value (1.3 vs. 0.91 g/kg DM). Propionic acid content was uniform in the inoculated samples, while in the control samples there was a gradual decrease until day 3 (0.15 g/kg DM) and an increase until day 7 (0.29 g/kg DM). The highest methanol content was measured in the control samples on the first day (11.47 g/kg DM), after which there was a decrease, while methanol content was fairly uniform in the inoculated samples. For both groups of samples, ethanol content decreased until day 3, after which there was a slight increase, but values on the last day were lower than on the first day (7.91 vs. 3.86 g/kg DM for the control samples and 4.88 vs. 1.68 g /kg DM for the inoculated samples).

CONCLUSION

The results show that although the silage remained aerobically stable throughout the entire research period, the application of the inoculant modulated the changes in acids and alcohols during aeration.

REFERENCES

Available on request

FERMENTATION QUALITY OF CRIMPED ENSILED GRAIN TREATED WITH DIFFERENT TYPES OF SILAGE ADDITIVES

FRANCO, M.[1], SEPPÄLÄ, A.[2], RINNE, M.[1]
[1]Natural Resources Institute Finland (Luke), FI-31600 Jokioinen, Finland. Correspondence: marketta.rinne@luke.fi
[2]Eastman Chemical Company, FI- 90620 Oulu, Finland.

INTRODUCTION

In humid harvesting conditions, freshly harvested cereal grains need to be artificially dried or spoilage must be prevented by other means. Ensiling crimped grains can bring substantial energy and cost savings compared to drying (Jokiniemi et al., 2014). Additionally, the method reduces weather dependency and widens the harvesting window compared to drying grain (Franco et al., 2022). Compared to dry grain, crimped grain has resulted in equal dairy cow milk production (Rinne et al., 2022) and improved growth performance of growing bulls (Huuskonen et al., 2020). Crimping (also called rolling) breaks and flattens the grain exposing the grain content. This contributes to better compaction and elimination of oxygen. The fermentation process can be managed by using silage additives to prevent malfermentation, reduce losses, and ensure high palatability and good stability after silo opening (Franco et al., 2022). In this trial, the objective was to evaluate the effect of different silage additives on fermentation quality of crimped ensiled barley grains.

MATERIALS AND METHODS

Barley grain was combine-harvested on 10th of August 2021 in Loimaa, Finland. It was crimped using a roller mill and transported to the Natural Resources Institute Finland (Luke) in Jokioinen, Finland. Ten silage additive treatments were applied to 6 kg batches of grain using four replicates per treatment. A control treatment without additive (C) and a treatment with lactic acid bacteria inoculant (Bonsilage CCM, 2 g/t; LAB) were included. In addition, eight treatments with blends of organic acids and their salts (OA) were applied at 5 l/t. The OA treatment abbreviations were coded as follows: total formic acid (%) including formates (F), total propionic acid (%) including propionates (P), and additional small amounts (≤5 %) of sorbates or benzoates were coded by + symbol. All the blends were partially buffered. The OA treatments were: F62P20+; F56P10+; F61P10+; F49P28; F55P15; F34P38and P90. Crimped barley was ensiled into 6 l cylindrical silos for 83 days. For details on the experimental procedures, see Franco et al. (2022). Data was analysed using a MIXED procedure of SAS with additive as fixed effect and replicates as random effect.

RESULTS AND DISCUSSION

Before ensiling, the crimped grain had a moisture content of 340 g/kg. On dry matter (DM) basis, the concentrations of neutral detergent fibre, crude protein and ash were 170, 140 and 29 g/kg, respectively. Yeast counts were 6.8 and mould counts were 6.4 $\log_{10}$ cfu/g prior to ensiling. The grains had substantial microbes capable of fermentation as only minor differences were observed between C and LAB in the extent of fermentation. Despite high microbe numbers prior to ensiling, all the ensiled grains had aerobic stability longer than 220 hours (Table 1), which is in line with the relatively high moisture content of the grains (Franco et al., 2022). Total amount of fermentation products was 45 g/kg DM for C and LAB treatments, dominated by lactic acid. Although C and LAB grains had a low pH (3.8), they contained minor amounts of butyric acid. The OA treatments restricted fermentation, as evidenced by lower amounts of ethanol, lactic, acetic and butyric acids and higher pH of the grains compared to C and LAB. The strongest restriction of fermentation was achieved with treatments that had the highest amount of free formic acid (F62P20+; F56P10+; F61P10+), and the total amount of fermentation products was less than 21 g/kg DM in those treatments. The lower amount of fermentation acids was reflected in a higher pH of those silages, which was 4.0-4.1. Generally, ammonia levels were low in all treatments but even lower for formic acid based treatments than for C or LAB treated grains.

CONCLUSIONS

The results obtained in the current experiment indicate that the use of formic and propionic acid based additives in ensiling crimped barley supports good fermentation of the grains and ensures good nutritional and hygienic quality for animal feeding. Ensiled crimped barley grains treated with organic acid based additives resulted in lower production of ammonia-N, ethanol, lactic, acetic, and butyric acids and lower ensiling losses than preserving without additive or using an inoculant. LAB had only minor effects on the quality of the crimped grain with slightly higher acetic and slightly lower butyric acid concentrations compared with the control.

REFERENCES

Franco, M., Tapio, I., & Rinne, M. 2022. Preservation characteristics and bacterial communities of crimped ensiled barley grains modulated by moisture content and additive application. Frontiers in Microbiology, 13, 1092062.

Huuskonen, A., Rinne, M., & Manni, K. 2020. Effects of different barley grain preservation techniques on intake, growth and carcase traits of finishing dairy bulls fed grass silage-based rations. Journal of Agricultural Science, 158, 748–755.

Jokiniemi, T., Jaakkola, S., Turunen, M., & Ahokas, J. 2014. Energy consumption in different grain preservation methods. Agronomy Research, 12, 81–94.

Rinne, M., Franco, M., Tapio, I., Stefański, T., Bayat, A.-R., & Mäntysaari, P. 2022. Effects of grass silage additive type and barley grain preservation method on rumen fermentation, microbial community and milk production of dairy cows. Agriculture, 12, 266.

Table 1. Chemical composition, fermentation quality, aerobic stability and ensiling losses of crimped barley treated with silage additives.

	Additive treatment									SEM[1]	P-value[2]		
	C	LAB	F62P20+	F56P10+	F61P10+	F49P28	F55P15	F34P38	P90		Treat	C vs O	C+LABvsOA
Dry matter (DM), g/kg	663^{ab}	662^{ab}	657^{b}	655^{b}	655^{b}	662^{ab}	655^{b}	664^{ab}	669^{a}	2.0	<0.001	0.082	0.053
Moisture content, g/kg	337^{ab}	339^{ab}	343^{a}	345^{a}	345^{a}	338^{ab}	345^{a}	336^{ab}	331^{b}	2.0	<0.001	0.082	0.053
pH	3.80^{g}	3.83^{fg}	4.10^{a}	3.97^{bc}	4.03^{b}	3.92^{cde}	3.97^{bc}	3.86^{efg}	3.89^{def}	0.013	<0.001	<0.001	<0.001
Ammonia-N, g/kg N	26.0^{b}	25.9^{b}	13.2^{c}	14.1^{c}	15.0^{c}	14.0^{c}	15.9^{c}	17.4^{c}	27.7^{b}	1.00	<0.001	<0.001	<0.001
Ethanol, g/kg DM	4.65^{a}	4.34^{a}	0.73^{b}	0.82^{b}	0.91^{b}	0.66^{b}	1.05^{b}	1.11^{b}	0.79^{b}	0.144	<0.001	<0.001	<0.001
Acids, g/kg DM													
Lactic (LA)	35.0^{a}	35.3^{a}	9.5^{e}	16.3^{d}	16.2^{d}	19.2^{cd}	20.6^{cd}	29.5^{b}	30.1^{b}	0.96	<0.001	<0.001	<0.001
Acetic (AA)	4.47^{b}	5.06^{a}	1.90^{g}	2.24^{fg}	2.22^{fg}	2.53^{ef}	2.68^{ef}	3.49^{cd}	3.98^{bc}	0.121	<0.001	<0.001	<0.001
Propionic	0.10^{f}	0.11^{f}	1.56^{d}	0.82^{e}	0.85^{e}	2.29^{c}	1.29^{d}	3.16^{b}	6.54^{a}	0.080	<0.001	<0.001	<0.001
Butyric	0.39^{a}	0.25^{b}	0.01^{c}	0^{c}	0.01^{c}	0^{c}	0^{c}	0^{c}	0^{c}	0.017	<0.001	<0.001	<0.001
Total volatile fatty acids	5.0^{cd}	5.4^{c}	3.5^{f}	3.1^{f}	3.1^{f}	4.9^{d}	4.0^{e}	6.7^{b}	10.6^{a}	0.10	<0.001	0.069	0.550
Total fermentation acids	40.0^{a}	40.7^{a}	13.0^{e}	19.4^{cd}	19.3^{d}	24.0^{bc}	24.6^{b}	36.2^{a}	40.6^{a}	0.97	<0.001	<0.001	<0.001
Total fermentation products	44.7^{a}	45.1^{a}	13.7^{e}	20.3^{d}	20.2^{d}	24.7^{cd}	25.6^{c}	37.3^{b}	41.4^{ab}	0.98	<0.001	<0.001	<0.001
LA/AA ratio	7.9^{a}	7.0^{a}	4.9^{b}	7.3^{a}	7.3^{a}	7.6^{a}	7.7^{a}	8.5^{a}	7.7^{a}	0.36	<0.001	0.135	0.692
Aerobic stability 2 °C, hours[3]	>220	>220	>220	>220	>220	>220	>220	>220	>220	-	-	-	-
Density, kg/m^3	739	770	761	756	779	775	762	775	754	13.3	0.532	0.074	0.363
Discarded, % of fresh matter	12.7^{a}	13.1^{a}	10.8^{ab}	10.2^{ab}	9.1^{ab}	12.4^{ab}	8.7^{ab}	9.2^{ab}	6.1^{b}	1.33	0.028	0.064	0.004
Visual mould	1.75^{a}	1.75^{a}	2.00^{a}	1.88^{a}	1.94^{a}	1.94^{a}	1.88^{a}	2.00^{a}	0.63^{b}	0.144	<0.001	0.821	0.734
Ensiling losses, g/kg of initial DM	24.5^{b}	28.3^{a}	17.8^{c}	22.7^{b}	22.2^{b}	12.9^{d}	24.3^{b}	16.9^{c}	13.8^{d}	0.60	<0.001	<0.001	<0.001

For treatment descriptions, see Material and Methods. Commercial names of the existing products: F62P20+ = AIV Ässä Na; F56P10+ = AIV 2000 Plus Na; F49P28 = AIV VIA; F55P15 = Acitra LH2 NC and P90 = Eastman Propcorn NC.

Values with same letter in a row are not significantly different at 5% Tukey test. If there were no differences in Tukey test, letters were removed.

[1]SEM, Standard error of the mean.

[2]P-value: Treat, effect of additive treatment; C vs O, Control versus all other additive treatments; C+LAB vs OA, Control + Bonsilage versus blends of organic acids;

[3]Crimped grain silage samples did not heat after 220h of evaluation period.

EFFECTS OF PARTIAL RECYCLED PLASTIC, DECREASED THICKNESS, NUMBER OF LAYERS AND MANTEL FILM TO BALED GRASS-CLOVER SILAGE

NADEAU, E.[1,2], DAHLSTRÖM, F.[1], WEISS, K.[3], LARSSON, A.[4]
[1] *Swedish University of Agricultural Sciences, Department of Animal Environment and Health, Box 234, 532 23 Skara, Sweden.*
[2]*The Rural Economy and Agricultural Society, 514 05 Länghem, Sweden*
[3]*Humboldt Universität zu Berlin, Faculty of Life Science, Invalidenstrasse 42, 10115 Berlin, Germany*
[4]*Trioworld, Box 143, 333 23 Smålandsstenar, Sweden*

INTRODUCTION

Baling is a common storage method for silage in many European countries, both as a complementary storage method to bunker silo storage on larger farms and as the major storage method on smaller farms. To decrease climate impact from stretch film production, there is a need to decrease the thickness, to include recycled plastic and to evaluate the effect of number of stretch film layers on silage quality. Furthermore, use of mantel film instead of net under the stretch film makes it possible to recycle the films together, which is not possible when using a net. The aim of this project was to study 1) the effect of decreased thickness or including a proportion of recycled plastic in stretch film and 2) to evaluate the number of stretch film layers and mantel film replacement of net under the stretch film on technical traits of the bales and silage fermentation characteristics.

MATERIALS AND METHODS

Grass-clover forage in the first regrowth was mowed, wilted overnight to 32% DM and baled at Lantmännen Research Farm Viken, Falköping, Sweden on July 8, 2021 with a McHale Fusion 3 Plus combined baler/stretch film applier (McHale, Ballinrobe, Ireland) that could handle both net and mantel film. Four, six or eight layers of the stretch films Triowrap plus 19µ (white, 730 mm × 0.019 mm × 2100 m), Triowrap 25µ (white, 750 mm × 0.025 mm × 1500 m) and Triowrap loop 25µ (white, 30% recycled material, 750 mm × 0.025 mm × 1500 m) were used (Trioworld, Smålandsstenar, Sweden). In addition, net or mantel film (TrioBaleCompressor, white, 1400 mm × 0.016 mm × 2200 m; Trioworld, Smålandsstenar, Sweden) were used under Triowrap plus 19µ and Triowrap 25µ, whereas only net was used under Triowrap loop 25µ. A total of 15 treatments were represented in each of 5 blocks evenly distributed over one field. The bale volume, presence of carbon dioxide (CO_2; Biogas 5000 (Geotechnical Instruments, Warwickshire, UK)) and silage density were registered. Fermentation quality, dry matter losses and aerobic stability (temperature method) were analysed using routine analytical procedures. Data were analysed as a randomized block design in PROC GLM of SAS. When the global *F*-test showed significance, pairwise comparisons between means were done with Tukey´s test ($P < 0.05$).

RESULTS AND DISCUSSION

Inclusion of 30% recycled plastic or decreased thickness of the stretch film gave similar volume and proportion of CO_2 in the bale and similar silage density, fermentation quality and aerobic stability as stretch films of 25µ thickness of virgin plastic (Table 1).

Table 1. Effect of stretch film on technical traits and silage quality using net under the film (n = 15).

	Triowrap plus 19µ	Triowrap 25µ	Triowrap loop 25µ	SEM	*P*-value
CO_2, %	34.2	32.6	31.9	2.65	0.815
Volume, m^3	1.75	1.75	1.78	0.010	0.280
Density, kg DM/m^3	134	136	133	1.5	0.331
DM, %	32.8	31.6	33.0	0.61	0.250
pH	4.49	4.53	4.57	0.036	0.316
Lactic acid, % DM	5.58	5.59	5.61	0.294	0.996
Acetic acid, % DM	1.07	1.16	1.22	0.083	0.448
Ethanol, % DM	0.47	0.48	0.54	0.028	0.161
NH_3-N, % total N	11.9	12.6	12.7	0.33	0.182
WSC, % DM	4.94	5.07	5.14	0.341	0.914
DM losses, %	4.8	4.1	4.5	0.29	0.222
Aerob. stability, days	6.7	7.1	6.5	1.57	0.969

No interactions between stretch film type and number of layers of the stretch film were found. Six and eight layers of the stretch film increased the proportion of CO_2 in the bale compared to four layers ($P < 0.001$; Table 2), which shows a greater sealness of the stretch film as there were no differences in DM losses and fermentation products with increased CO_2 production between the number of layers. Bales with eight layers of stretch film had a smaller volume than bales with less layers of stretch film ($P = 0.001$).

Table 2. Effect of number of stretch film layers on technical traits and silage quality using net under the film (n = 15).

	4 layers	6 layers	8 layers	SEM	*P*-value
CO_2, %	22.4[b]	34.8[a]	41.5[a]	2.65	<0.001
Volume, m^3	1.80[a]	1.77[a]	1.71[b]	0.010	0.001
Density, kg DM/m^3	134	133	137	1.5	0.109
DM, %	32.7	32.7	32.1	0.61	0.722
pH	4.54	4.51	4.53	0.036	0.881
Lactic acid, % DM	5.32	5.67	5.80	0.294	0.499
Acetic acid, % DM	1.12	1.19	1.15	0.083	0.853
Ethanol, % DM	0.53	0.50	0.46	0.028	0.200
NH_3-N, % total N	12.4	12.3	12.4	0.33	0.966
WSC, % DM	4.76	5.15	5.24	0.341	0.578
DM losses, %	4.2	4.6	4.6	0.29	0.560
Aerob. stability, days	5.0	7.4	7.9	1.57	0.404

The comparison between mantel film and net under stretch films of virgin origin showed greater proportion of CO2 in the bale using mantel film, which could be related to greater sealness of the mantel film as the DM losses and fermentation pathways producing CO2 were similar between mantel film and net, which is in agreement with results by Spörndly and Nylund (2016). Also, the pH (4.51) and concentrations of water-soluble carbohydrates (5.0% DM), lactic acid (5.65% DM) and NH3-N (12.1% total N) were not affected but the aerobic stability tended to be improved by use of mantel film. Mantel film resulted in bales with smaller volume ($P < 0.001$) and higher density of the silage ($P = 0.015$).

Table 3. Effect of mantel film/net under stretch film of virgin plastic on technical traits and silage quality (n = 30).

	Mantel film	Net	SEM	*P*-value
CO_2, %	41.5	33.4	1.87	0.004
Volume, m^3	1.70	1.75	0.008	<0.001
Density, kg DM/m^3	139	135	1.0	0.015
DM, %	33.0	32.2	0.43	0.227
Acetic acid, % DM	1.05	1.12	0.049	0.322
Ethanol, % DM	0.49	0.47	0.019	0.549
DM losses, %	4.5	4.5	0.20	0.950
Aerob. stability, days	8.7	6.9	0.62	0.064

CONCLUSIONS

The stretch film of 19µ thickness or 30% recycled material resulted in as good sealness of the bales, silage density and fermentation quality as the stretch films of 25µ thickness of virgin material. Furthermore, increased number of stretch film layers and use of mantel film instead of net resulted in tighter bales without any effect on silage fermentation.

ACKNOWLEDGEMETS

This study was financed by Agrovāst, Nötkreatursstiftelsen Skaraborg, Trioworld and Lantmännen.

REFERENCE

Spörndly, R. and Nylund, R. 2016. Net replacement film in round bale ensiling of ley crops. *Grassland Science in Europe* 21, 284-286.

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

DEVELOPMENT OF CALIBRATION EQUATIONS FOR MONITORING THE QUALITY OF SORGHUM FORAGE

MENŠÍK, L.[1], MRKVICOVÁ, E.[2], PAVLATA, L.[2], NERUŠIL, P.[1], PLISKOVÁ, J.[1], SMUTNÝ, V.[2], ŠŤASTNÍK, O.[2], ŘIHÁČEK, M.[2], DOLEŽAL, P.[2], JAMBOR, V.[3]
[1] Crop Research Institute /CRI/ Prague 6 - Ruzyně, Grassland Research Station /GRS/ Jevíčko, K. H. Borovského 461, 569 43 Jevíčko, Czech Republic
[2] Mendel University in Brno, Institute of Animal Nutrition and Forage Production, Department of Agrosystems and Bioclimatology, Zemědělská 1665/1, 613 00 Brno
[3] NutriVet, Ltd., Vídeňská 1023, 691 23 Pohořelice

INTRODUCTION

Sorghum is now a globally important cereal crop, with more than 40 million hectares harvested for grain production in 2021, including 285 thousand hectares in Europe (FAOSTAT 2023). However, in addition, it is also grown as a forage crop for biomass production with feed use in ruminants /milking cows/ (Cattani et al. 2017). Sorghum for forage production is grown as an annual crop with many outstanding characteristics, including high biomass production (Marsalis et al. 2010), high forage quality /ADF, NDF, OMD, etc./ (Mirahki et al. 2023), high drought tolerance (Smith and Frederiksen 2000, OECD 2017) and high nitrogen use efficiency (Rosati et al. 2019). The aforementioned prerequisites make it an ideal crop for use in environments with lower soil fertility or areas with recurrent drought (Fardin et al. 2023). The aim of breeders and farmers is to produce high quality forage sorghum for mixed livestock rations (TMR). The content of crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF) and water-soluble carbohydrates etc., are among the most important forage quality parameters, that determine the intake and digestibility of forage in cattle (Fahey 1994). Determination of basic forage quality parameters by standard laboratory analysis is very laborious, costly and takes an average of 10 days depending on the number of parameters determined (Nerušil et al. 2018). Near-infrared spectroscopy (NIRS) is an efficient analytical technique (Manley 2014) used in a number of research and testing laboratories in the field of quality control of plant and animal products (Muselík 2012). Unlike most laboratory methods, NIRS analysis requires no chemicals, solvents or reagents, does not pollute the natural environment and is considered an environmentally friendly method (Yang et al. 2017). Currently, there is little information in the literature on the use of NIRS calibration models to predict sorghum forage quality and determine its most important parameters (crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), organic matter digestibility (OMD), etc.). The objective of this study is to present the development of calibration equations to predict forage quality parameters (CP, fiber, fat, NDF, ADF, OMD) in dryland green biomass samples using NIRS technique in a one-cutting of grain sorghum (*Sorghum bicolor* L.).

MATERIALS AND METHODS

The basis for the development of calibration equations for the determination of the nutritive value of grain sorghum (*Sorghum bicolor* L.) hybrids were dried and standardly ground (particle size <1 mm) samples of whole sorghum plants of a wide range of silage and grain varieties from the harvest years 2015 - 2022. Sorghum biomass was collected during the harvest at the experimental plots of Mendel University in Brno (Žabčice site). This allowed to collect a comprehensive collection of 445 samples with a high variability of values of forage quality indicators of individual sorghum varieties. The samples were laboratory-determined for the dry weight of green matter at 105 °C (average value of two parallel determinations of one sample). Chemical analyses of the samples in the range of determination of organic nutrient content (crude protein, fibre, fat) were carried out using reference methods according to Decree No. 415/2009 Coll. on the determination of sampling requirements and the publication of methods for laboratory testing of products for animal feed. In addition, according to Mertens (2002) and in accordance with ISO 16472 (2012), ADF and NDF were determined. Organic matter digestibility (OMD) was determined *in vitro* by the pepsin-cellulase ELOS method (Míka et al. 2009) using an ANCOM Daisy Incubator (ANKON Technology, Macedon, NY, USA). Identical soil samples were screened on a FOSS NIRSystems 6500 instrument dispersion spectrometer (Company NIRSystems, Inc, Silver Spring, USA), located at CRI Prague, Research Station Jevíčko. Sample measurements were performed in small ring cups in two parallel repetitions. Scanning of the sample was set in reflectance mode for the 400–2500 nm region, i.e., in the visible and near-infrared region of the spectrum, scanning step 2 nm. WinISI II software (Infrasoft International, Inc., USA), version 1.50, was used to develop the calibration equations and validation equations including graphical outputs. The Partial Least Squares (PLS) method, the best and oldest method used for spectral calibration and prediction among multiple linear calibration algorithms (Wang et al. 2006), was used. Furthermore, the modified PLS /MPLSR/ (González-Martín et al. 2015) was applied.

RESULTS AND DISCUSSION

The sorghum biomass collection with reference values totalling 445 samples was divided into a calibration set of 361 samples and a validation set of 84 samples (by selecting every Ith sample /19/ from the original collection). The validation (independent of the calibration) set was similar to the calibration set in its forage quality parameters and was used to verify the accuracy of the prediction. The contents (concentrations) of each forage quality parameter (CP, fibre, fat, NDF, ADF, OMD) were not significantly different between the calibration and validation sets. The results obtained for the accuracy of the prediction of the content of forage quality parameters (CP, fibre, fat, NDF, ADF, OMD) verified on an independent validation set confirmed the assumption of a high level of agreement of the laboratory methods with the NIRS determination (validation set, R^2 = *0.94–0.72*). The highest values of prediction accuracy, as expressed by the coefficient of determination (R^2) of the calibration set, were achieved for CP content, fibre, ADF, NDF (R^2 = *0.96–0.98*), followed by OMD (R^2 = *0.89*). According to the criterion of the achieved coefficient of determination of the calibration ensemble, the prediction of the content of most forage quality parameters was excellent (R^2 = *0.90* or more), or applicable to common agricultural practice /R^2 = *0.75–0.89*/ (Dvořáček et al. 2014). Our results are in agreement with the results of the study by Bruno-Soares et al. (1998), where the R^2 values of the calibration model for dry sorghum forage for the parameters CP, NDF, ADF, OMD, fat range from *0.87 to 0.98*.

CONCLUSIONS

The results of the study demonstrate, that NIR spectroscopy is a modern tool suitable for measuring the quality of dry sorghum forage in the parameters (CP, fiber, fat, NDF, ADF, OMD). It is a measurement technique, that is very fast (sample determination takes about 2 minutes), sufficiently accurate and that can determine the different components (parameters) of the material (forage) under analysis from only one sample measurement (dried, ground forage sample). Precise regression modelling using modern statistical multivariate methods such as PLSR, MPLSR, etc. can achieve very good results for the prediction of forage quality parameters (see Results). Sufficiently robust calibration models for the prediction of forage quality parameters represent a high potential in the future management and control of forage harvesting and sorghum ensiling.

ACKNOWLEDGEMENTS

This paper was made possible with financial support from the Ministry of Agriculture, institutional support MZE–RO0423, and the NAZV project QK22010251 "Innovation of sorghum cultivation technology for use in ruminant nutrition as an adaptation measure leading to stabilization of roughage production under the changing climate of the Czech Republic".

REFERENCES

Bruno-Soares, A. M., I. Murray, R. M. Paterson, and J. M. F. Abreu. 1998. Use of near infrared reflectance spectroscopy (NIRS) for the prediction of the chemical composition and nutritional attributes of green crop cereals. Animal Feed Science and Technology 75(1):15–25.

Cattani, M., N. Guzzo, R. Mantovani, and L. Bailoni. 2017. Effects of total replacement of corn silage with sorghum silage on milk yield, composition, and quality. Journal of Animal Science and Biotechnology 8(1).

Dvořáček, V., A. Prohasková, and L. Štočková. 2014. Efektivní využití blízké infračervené spektroskopie s Fourierovou transformací pro hodnocení technologických vlastností pšenice. Metodika pro praxi. Praha.

Fahey, G. C., ed. 1994. Forage Quality, Evaluation, and Utilization. Wiley.

FAOSTAT. 2023, February 19. FAOSTAT - Crops and livestock products - Sorghum. https://www.fao.org/faostat/en/#data/QCL.

Fardin, F., B. Sani, P. Moaveni, G. Afsharmanesh, and H. Mozafari. 2023. Nutritional value and agronomic traits of forage sorghum under drought stress. Biocatalysis and Agricultural Biotechnology 48 (March 2023):102624.

González-Martín, M. I., O. Escuredo, I. Revilla, A. M. Vivar-Quintana, M. Carmen Coello, C. P. Riocerezo, and G. W. Moncada. 2015. Determination of the mineral composition and toxic element contents of propolis by near infrared spectroscopy. Sensors (Switzerland) 15(11):27854–27868.

ISO 16472. 2012. Krmiva - Stanovení obsahu neutrálně detergentní vlákniny po úpravě vzorku amylázou (aNDF). 19.

Manley, M. 2014. Near-infrared spectroscopy and hyperspectral imaging: Non-destructive analysis of biological materials. Chemical Society Reviews 43(24):8200–8214.

Marsalis, M. A., S. v. Angadi, and F. E. Contreras-Govea. 2010. Dry matter yield and nutritive value of corn, forage sorghum, and BMR forage sorghum at different plant populations and nitrogen rates. Field Crops Research 116(1–2):52–57.

Mertens, D. R. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: Collaborative study. Journal of AOAC International 85(6):1217–1240.

Míka, V., Kohoutek, J., Pozdíšek J., Němcová P. 2009. Stanovení ELOS (enzymaticky rozpustné organické hmoty) v píci s využitím tuzemských celuláz z *Trichoderma reesei*. Praha: Výzkumný ústav rostlinné výroby, v.v.i., 28 s. ISBN 978-80-8701-54-6.

Mirahki, I., M. R. Ardakani, F. Golzardi, F. Paknejad, A. Mahrokh, and S. Faraji. 2023. Yield, Water Use Efficiency and Silage Feeding Value of Sorghum Cultivars as Affected by Planting Date and Planting Method. Gesunde Pflanzen (2023).

Muselík, J. 2012. Aplikace blízké infračervené spektroskopie ve farmaceutické analýze. Chemicke Listy 106 (2012):10–15.

Nerušil, P., L. Menšík, and V. Jambor. 2018. Vývoj kalibračních rovnic k predikci parametrů výživné hodnoty konzervované kukuřičné siláže pomocí techniky NIRS. Praha: Výzkumný ústav rostlinné výroby, v.v.i., Praha 6 - Ruzyně.

OECD. 2017. Sorghum (Sorghum bicolor). In Safety Assessment of Transgenic Organisms in the Environment (OECD Consensus Documents), 29–68. Paris: OECD Publishing.

Rosati, V. C., C. K. Blomstedt, B. L. Møller, T. Garnett, and R. Gleadow. 2019. The Interplay Between Water Limitation, Dhurrin, and Nitrate in the Low-Cyanogenic Sorghum Mutant adult cyanide deficient class 1. Frontiers in Plant Science 10 (November 15, 2019).

Smith, C. W., and R. A. Frederiksen. 2000. Sorghum: Origin, History, Technology, and Production. Wiley.

Wang, H. W., Z. B. Wu, and J. Meng. 2006. Partial Least Squares Regression: Linear and Nonlinear Methods. Beijing: National Defence Industry Press.

Yang, Z., G. Nie, L. Pan, Y. Zhang, L. Huang, X. Ma, and X. Zhang. 2017. Development and validation of near-infrared spectroscopy for the prediction of forage quality parameters in Lolium multiflorum. PeerJ 5 (2017):e3867.

MYCOTOXIN LEVELS IN MAIZE SILAGE AND THEIR EFFECT ON HEALTH, PRODUCTION AND REPRODUCTION OF COWS

ILLEK, J.[1], JAMBOR, V.[2], SYNKOVÁ, H.[2], RULFOVÁ, L.[3], KUMPRECHTOVÁ, D.[4], KADEK, R.[1], STAFFA, A.[1], ANDĚLOVÁ, J.[5]
[1] University of Veterinary Sciences Brno, Faculty of Veterinary Medicine, Brno, Czech Republic
[2] Nutrivet, s.r.o., Czech Republic
[3] Volac Agrobest, s.r.o., Czech Republic
[4] Institute of Animal Science Prague, Czech Republic
[5] Veterinary Centre Susice, Czech Republic

INTRODUCTION

Nutrition is essential for production and reproductive health of dairy cows. Despite significant advances in dairy cow nutrition, there are still many deficiencies that cause health problems, reduce performance, affect milk composition and impair fertility. In addition to various imbalances in essential nutrients, minerals and vitamins, dietary factors are also negatively impacting. The deterioration of the quality of preserved forage due to contamination by fungi and mycotoxins, as well as a number of other factors, is a serious problem.

Excessive levels of mycotoxins, mainly deoxynivalenol, zearalenone and T2 toxin, which are mainly produced by *Fusarium* fungi and cause serious animal health problems, are found in preserved forage, especially in maize silage.

The aim of this study was to determine the concentration of mycotoxins in maize silages from different farms and to evaluate the health status of dairy cows, milk production and reproduction. A total of 60 samples of maize silage were examined in 2021 and 2022. The health status of dairy cows, in particular the incidence of subclinical mastitis, laminitis, metabolic disorders and reproductive disorders was monitored on two farms with above-limit mycotoxin levels in maize silage and on one farm where mycotoxin levels were low and below the limit value. We considered the limit value for deoxynivalenol to be 5000 μg/kg, for zearalenone 500 μg/kg and for T2 toxin 100 μg/kg.

METHODS

In three Holstein herds with an average milk yield of 10,200 kg to 11,500 kg, performance, milk composition, lameness incidence, fertility, embryonic mortality and metabolic profile were monitored. The diet of lactating dairy cows was the same in the 3 herds studied and consisted of maize silage at 26 kg to 28 kg, alfalfa silage at 8 kg to 10 kg, concentrate at 9 to 12 kg, mineral and vitamin supplements. The prevalence of lameness in the herds was determined in all lactating cows by clinical observation and expressed as %. Performance and reproduction results were obtained from the farm records. Rumen fluid (by probe) and tail vein blood were collected from 10 cows in each herd. Samples of rumen fluid, blood and serum were analysed at the Large Animal Clinical Laboratory of VETUNI Brno. pH, total volatile fatty acid concentration and individual acids (acetic, propionic, butyric and valeric) were measured in the rumen fluid. Hemoglobin, hematocrit, erythrocyte and leukocyte counts were determined in blood samples. In serum, total protein, albumin, urea, glucose, NEFA, BHB, AST, GMT, Bilirubin, GSH-Px, betacarotene, Vit E., T3, T4 were analysed. Milk yield, milk fat and protein percentages and somatic cell count were measured. Milk analyses were carried out in the dairy herd improvement laboratory.

RESULTS

As the results show, the proportion of mycotoxin contaminated maize silage samples is significant. Feeding maize silage with elevated mycotoxin content in farms A and B had a negative impact on health, production and reproduction. There was a lower average daily milk yield, significantly higher lameness in cows and significantly higher somatic cell counts in milk in herds A and B. Significant differences were also found for conception rate. While in herd C the conception rate was 44.8 %, in herd A it was 36.5 % and in herd B only 32.3 %.

Significant changes were also found in the metabolic profile of the selected cows. Significant differences were evident in the rumen fluid. The cows with increased concentration of mycotoxins in the fed maize silage (A, B) had decreased concentration of total volatile fatty acids and especially propionic acid compared to herd C cows. Some changes were also found in haemoglobin concentration, with the value at the lower end of the reference range, especially in herd B. Changes were also found in the serum metabolic profile, namely in the concentration of total protein, albumin, bilirubin and AST. These findings are indicative of impaired liver function caused by mycotoxins, especially T2 toxin. Significantly lower serum concentrations of beta carotene and vitamin E were found in the herds A and B. These micronutrients are important antioxidants and play a significant role in metabolism, immunity and mammary health. Also in our observation, an association can be found between beta carotene, vit, E concentrations and somatic cell counts. A remarkable finding can also be observed in the concentration of thyroid hormones. In dairy cows with increased intake of mycotoxins, the concentration of T4 hormone was significantly lower.

Farm	A	B	C
Lameness during lactation (%)	28	32	6
Daily milk yield (kg/d)	32.6	30.5	34.4
Somatic cell count (thous./ml)	335	372	160
First-service conception rate (%)	36.5	32.3	44.8
Metabolic profile:			
Rumen fluid			
pH	6.34	6.31	6.42
VFA (mmol/l)	107.1	99.8	115.7
Acetic acid (%)	68.3	63.2	74.6
Propionic acid (%)	17.8	15.6	24.3
Butyric acid (%)	18.5	18.2	15.4
Valeric acid (%)	2.3	2.8	1.4
Ammonia (mmol/l)	11.6	16.3	12.8
Infusoria (thous./ml)	218	186	386
Blood/serum			
Hemoglobin (g/l)	94.2	88.6	128
Hematocrit (1/1)	0.31	0.34	0.36
Erythrocytes (T/l)	5.42	5.14	6.12
Leukocytes (G/l)	6.23	6.88	7.82
Total protein (g/l)	70.3	68.4	78.8
Albumin /g/l)	33.4	31.8	35.2
Urea (mmol/l)	5.32	5.24	5.48
Glucose (mmol/l)	3.28	3.12	3.42
Bilirubin (μmol/l)	6.12	6.28	4.36
BHB (mmol/l)	0.94	1.16	0.82
AST (ukat/l)	1.52	1.76	1.12
GMT (μmol/l)	0.48	0.56	0.38
GSH-Px (μkat/l)	863	748	886
Beta carotene (μg/l)	3.28	3.86	6.42
Vitamin E (μmol/l)	4.32	3.84	5.22
T3 (nmol/l)	1.78	1.73	1.78
T4 (nmol/l)	52.84	48.35	68.5

CONCLUSION

Monitoring revealed that maize silage was contaminated with mycotoxins Above limit values were found in a significant number of samples analysed. Deoxynivalenol concentrations above the limit were found in 26 samples. 20 samples exceeded limits for zearalenone and 17 for T2 toxin.

In farm A the concentrations of deoxynivalenol, zearalenone and T2 toxin were 7230 μg/kg, 612 μg/kg and 165 μg/kg, in farm B 6320 μg/kg, 782 μg/kg and 315 μg/kg and in farm C 324 μg/kg, 120 μg/kg and 68 μg/kg, respectively.

Increased concentrations of mycotoxins in maize silage negatively affected the health of the dairy cows, which was manifested by a high proportion of lame cows and an increased incidence of subclinical mastitis and impaired fertility. Mycotoxins had a negative effect on rumen fermentation processes - reduced propionic acid synthesis and reduced number of infusoria in rumen fluid. Feeding of maize silage with excessive concentrations of mycotoxins negatively affected the metabolic profile of dairy cows. Liver function was impaired, as evidenced by increased bilirubin BHB and elevated AST values. Significantly lower serum levels of beta carotene, vit. E and even T4 were found.

REFERENCES

Available on request

CORN SILAGE PROCESSING SCORE IN HUNGARY (2013-2022)

OROSZ[1], S., BALOGH[2], K.
[1]Livestock Performance Testing Ltd., Gödöllő, Hungary
[2]Hungarian University of Agriculture and Life Sciences, Institute of Physiology and Nutrition, Department of Feed Safety, Gödöllő, Hungary

INTRODUCTION

Kernel pocessing has many advantages. Processed corn silage (harvested at 2/3 Milk Line) fermented faster and have a lower pH after 10 days compared to the un-processed silage (Harrison et al, 1997). Kozakai et al. (2007) have found more rapid and greater colonization of the rumen bacteria fed processed silage compared to unprocessed control. The net energy concentration of the processed corn silage were greater than the unprocessed corn silage (Johnson e al, 2003). Processing of corn silage improved dry matter intake, starch digestion and lactation performance (Bal et al, 2000b; Ferraretto et al, 2018a). Processing during harvest may increase starch digestibility of corn silage (Bal et al., 2000a). Ferraretto and Shaver (2012) found 5.9% and 2.8% greater total-tract starch digestibility when corn silage was processed using 1-3-mm roll gap settings compared with 4-8-mm processed or unprocessed. Processing may reduce the amount of starch excreted in the faeces (Johnson et al., 1996), but not all silage processed is effective in improving the digestibility of starch (Johnson et al., 2003), therefore quantitative methods were required to describe physical properties of starch in corn silage. *In vitro* disappearance from corn silage indicates that starch >4.75 mm is negatively affect dry matter fermentation, suggesting that this starch is poorly fermented *in vivo*. Therefore the proportion of total starch that is in particles smaller than one quarter of the kernel (<4.75 mm) provides a quantitative measure of kernel fragmentation in corn silage. The Corn Silage Processing Score (CSPS) was developed to characterize the quality of corn grain processing (in corn silage) by forage harvesters (USDA). The CSPS is the percentage of starch content in cracked seed passing through the coarse 4.75 mm screen (Ferreira and Mertens, 2005). The rating categories are: CSPS < 50% inadequate; 50-70% adequate-average; CSPS >70% optimally processed corn silage. CSPS can change during fermentation process, as Ferraretto et al (2015) observed improvement of CSPS (30% and +7%) after 30 or 120 d of fermentation, compared with unfermented samples. The potential value of the CSPS depends on the type of the processing rollers, roller surface geometry and physical condition, turning speed difference of the 2 rollers, the gap between the rollers, the flow intensity of fresh material passing through the roller-gap in a certain time (the capacity and speed of the self-propelled chopper harvester, the size of the corn adapter). In this context, CSPS is influenced by yield, dry matter content (seed hardness) and phenological phase of corn plant (Ferrareto and Shaver, 2012). Recognising the importance of kernel processing, the authors investigated Corn Silage Processing Score (CSPS) of 2904 corn silage samples derived from Hungarian large scale farms between 2013 and 2022.

MATERIAL AND METHODS

Routine laboratory samples derived from large scale farms in Hungary (n= 2904) between 2013-2022. Results based on routine laboratory NIR analyses (Livestock Performance Testing Ltd, Hungary). 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). Spectra were determined according to the guidelines of NEN-EN- ISO 12099:2010 guidelines (Q-Interline Quant FT-NIR analyser). Starch content was determined by Near-InfraRed Spectroscopy. Reference starch method: NEN-EN-ISO 15914. Corn silage processing score (CSPS) was determined on dried samples with Ro-Tap Sieve shaker according to Ferreira and Mertens (2005).

RESULTS AND DISCUSSION

Samples from different areas of Hungary collected by farmers during 2013-2022 were assessed (Table 1). The weakest results were measured in 2013 and 2014. There was no significant difference between the average annual values within the period of 2016-2019. In 2020, there was a significant improvement compared to the previous years in the CSPS value. In 2022, the full season has not yet been completed, so the final result could change. An 17.6% improvement in CSPS has been measured during the 10 years since the analyses were introduced (based on 2904 data).

Table 1 Corn Silage Processing Score (CSPS) of corn silage in Hungary, %

	Harvest year									
	2013	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**	**2020**	**2021**	**2022**
Mean	55a	57a	60b	64c	66c	65c	66c	69d	71d	72d
SD	11.5	12.1	10.1	12.5	11.5	12.7	11.0	10.1	9.8	10.0
n	147	263	278	299	279	384	353	367	392	142

Different letters within the row significantly differ p≤ 0,05

Ratio of inadequately processed corn silages decreased by 24% between 2013 and 2022. While there was no CSPS above 80% in 2013 and 2014, the rate of excellent quality reached 22% in 2022. The rate of unacceptable CSPS value was 28% in 2013, compared to only 4% in 2022.

Table 2 Distribution of corn silage according to the Corn Silage Processing Score (n=2904).

SCPS	Harvest year									
	2013	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**	**2020**	**2021**	**2022**
	% of total samples									
<50%	28	26	16	12	10	13	10	4	2	4
50-59%	37	29	26	28	21	19	19	15	11	6
60-69%	28	32	45	27	30	32	34	34	28	28
70-79%	7	14	12	20	30	26	28	33	41	40
>80%	0	0	1	13	9	10	8	14	17	22

CONCLUSIONS

Ferraretto et al. reported (2018b) that producers have increased their use of seed processing over the years, but poorly processed silage can still occur in the United States. Hungary's progress has been intense over the last decade: only 4% unacceptable processing in 2022. The introduction of the laboratory method has had an impact on the improvement of CSPS in Hungary. The quantitative measurement of the efficiency of cracking (kernel processing) had positive effect on the chopper harvester service and operation, moreover it has become a further monitoring point during harvesting. The measured value of processing score allows to give the modified starch digestibility and the corrected net energy content, which helps to optimise the feed ration of dairy cows. Ferraretto et al. reported (2018a) 4.6 % higher values for shredlage processor than for conventional silages, so shredlage technology improved kernel processing and fermentation patterns compared with traditional silages. Therefore our future research should be toward further investigations of new processors and adequate processor settings.

REFERENCE

Bal, M. A., R. D. Shaver, K. J. Shinners, J. G. Coors, J. G. Lauer, R. J. Straub, and R. G. Koegel. (2000a). Stage of maturity, processing and hybrid effects on ruminal in situ dissapearence of whole plant corn silage. Anim. Feed Sci. and Technol. 86:83-94.

Bal, M.A., Shaver, R.D., Jirovec, A.G., Shinners, K.J., Coors, J.G. (2000b). Crop processing and chop length of corn silage: effects on intake, digestion, and milk production by dairy cows. J. Dairy Sci. 83, 1264–1373.

Ferraretto, L. F., and R. D. Shaver. (2012). Meta-analysis: Impact of corn silage harvest practices on intake, digestion and milk production by dairy cows. Prof. Anim. Sci. 28:141–149

Ferraretto, L. F., B. A. Saylor, J. P. Goeser, and K. A. Bryan (2018b). Case Study: Effect of type of processor on corn silage processing scorein samples of whole-plant corn silage. Prof. Anim. Sci. 34:293–298

Ferraretto, L. F., G. S. Dias Junior, L. C. de Resende, and R. D. Shaver. (2015). Effect of ensiling on kernel processing score in whole plant corn silage harvested with varied processors and settings. J. Dairy Sci. 98(Suppl. 2):689

Ferraretto, L. F., R. D. Shaver, and B. D. Luck. (2018a). Silage review: Recent advances and future technologies for whole-plant and fraction-ated corn silage harvesting: A contemporary review. J. Dairy Sci.101:3937–395

Ferreira G., and D.R. Mertens (2005). Chemical and Physical Characteristics of Corn Silages and Their Effects on In Vitro Disappearance. J. Dairy Sci. 88:4414–4425.

Harrison, J.H., L. Johnson, C. Hunt, J. Siciliano-Jones, and K.J. Shinners. (1997). Use of kernel-processed silage in dairy rations. Proceedings of the Silage:Field to Feedbunk North American Conference, Hershey, Pennsylvania.

Johnson, L. M., J. H. Harrison, D. Davidson, W. C. Mahanna, and K. Shinners (2003). Corn silage management: Effects of hybrid, chop length, and mechanical processing on digestion and energy content. J. Dairy Sci. 86:208–231.

Johnson, L., J. H. Harrison, K. A. Loney, D. Bengen, R. Bengen, W. C. Mahanna, D. Sapienza, W. Kezar, C. W. Hunt, T. Sawyer, and M. Bieber (1996). Effect of processing of corn silage prior to ensiling on milk production, component yield, and passage of corn grain into manure. J. Dairy Sci. 79 (Suppl. 1):149. (Abstr.)

Kozakai K., T. Nakamura, Y. Kobayashi, T. Tanigawa, I. Osaka, S. Kawamoto, and S. Hara (2007). Effect of mechanical processing of corn silage on in vitro ruminal fermentation, and in situ bacterial colonization and dry matter degradation. Can. J. Anim. Sci. 87: 259–267.

Schwab E. C., R. D. Shaver, J. G. Lauer, and J. G. Coors (2003). Estimating silage energy value and milk yield to rank corn hybrids. Animal Feed Science and Technology 109: 1–18.

EFFECT OF CORN SHREDLAGE ON FEED QUALITY, FATTENING PERFORMANCE, HEALTH AND CARCASS VALUE OF FLECKVIEH BULLS

BUNK[1], L., FENSKE[1], K., KORTE[1], H., WESTEDARP[1], H.
[1]Faculty of Agricultural Sciences and Landscape Architecture, University of Applied Sciences Osnabrück, Germany

INTRODUCTION

Since the beginning of maize cultivation for silage, there has been a discussion in research about the optimal chop length and processing of maize silage with regard to the best possible ensilability and compactability, as well as efficient nutrient utilization by ruminants (PRIES et al. 2018). Short chop lengths of 5 to 10 mm have so far become established by German feedlots (DÖRING 2016). The crop can be compacted and ensiled well under these conditions (PRIES 2016). However, shorter chop lengths are associated with a decrease in physical effectiveness of fibre. Thus, longer chop lengths of corn silage are discussed from a nutritional point of view, especially for high-concentrate diets (SPIEKERS et al. 2009). The silage corn harvesting process, known under the brand name shredlage®, harvests maize with lengths of 26 to 30 mm and crushes it with special corncracker rollers. In the result, the technology is said to improve the fiber effectiveness and digestibility through intensive processing of the residual plant and maize kernels (DÖRING 2016). The effects of feeding shredlage to finishing bulls have not yet been investigated. For this reason, a feeding trial was conducted for the first time on a practical farm in Germany/Münster-Amelsbüren from 20th November 2019 to 20th July 2020.

MATERIAL AND METHODS

Early September 2019 65.3 ha of silo maize were harvested with two chopping chains running in parallel. For the conventional corn silage, a Claas Jaguar 970 forage harvester with the Multi Crop Cracker (MCC) Classic L, 30 % speed difference and 8 mm theoretical length of cut (TLC) was used. The shredlage was harvested by a Claas Jaguar 950 with the MCC Shredlage, 50 % speed difference and 26 mm TLC. The crop was stored in a silage clamp with 50 m length, 20 m width and 4 m high walls. One half of the silo was filled with short-cut corn silage and the other half with shredlage. The short-cut corn silage was compacted by a Claas Xerion 3300 with a total rolling weight of 18 tons (t). In contrast, the shredlage was rolled by a Claas Xerion 4000 with 22 t.

The feeding trial started on 20th November 2019 with a total of 72 Fleckvieh bulls. The animals were divided into two feeding groups based on their live weight of Ø 382 ± 33 kg. The experimental group received a total mixed ration (TMR) with shredlage without straw and the control group received a TMR with short-cut corn silage and straw. The TMR contained short-cut corn silage or shredlage, triticale/rye, grain maize, concentrates, minerals and straw (only in control group). The experimental and control rations had comparable energy and nutrient contents.

The animals were housed in a barn in single area pens on full slats with rubber overlay. Six pens were available for each of the experimental and control groups. Six animals were housed in each pen.

The feed intake was measured and documented daily as a group mean value. All the animals were weighed individually at the beginning, on the 64th day and again on the 176th day of the trial. The rumen pH of six animals per group was measured using rumen boluses (smaxtec animal care GmbH) and recorded over 150 days of the trial. In ten different deadlines, the washing of manure from selected animals of both groups was performed with a three-part sieve (Nasco Digestion Analyzer). The proportions of the three fractions were visually estimated according to the method of COTANCH and DARRAH (2012). At the end of the trial animals were slaughtered in the slaughterhouse of Westfleisch SCE mbH in Hamm.

Density measurements were taken at the cut surface of the corn silage on five dates. Sampling of the 9.8 cm diameter drill cores was done with a drilling cylinder at nine defined points, each distributed over the cut surface of the short-cut corn silage and shredlage.

RESULTS

Dry matter (DM) intake of animals in control group was 10.5 kg and in shredlage group 10.2 kg DM per day. Live weights were similar on day 64 at about 480 kg in both groups ($p = 0.9624$). On day 176, the bulls fed shredlage weighed 648.6 kg and the bulls fed short-cut corn silage 656,0 kg ($p = 0.4005$). The average daily gain in the first 176 days was 1.55 kg for the control group and 1.51 kg for the shredlage group ($p = 0.4005$).

Table 1 shows the data for the carcass value of the animals of both groups. A nominal difference of about 6 kg in carcass weight was found in favor of animals in shredlage group, but this difference could not be statistically confirmed. As conformation grade, the bulls fed shredlage received on average an R+ and the bulls fed short-cut silage an R to R+. This was a trend difference ($p = 0.0647$). For fat grade, animals in shredlage group received a 2+, while that of the control group tended more toward a 3-. The lower fat grade of the shredlage group compared to the control group was significant ($p = 0.0421$).

Over an acquisition period of 150 days, the daily mean pH of the animals in shredlage group was numerically higher at 6.51 ± 0.11 compared to 6.37 ± 0.21 of the animals in control group ($p = 0.1907$). None of the animals fed shredlage had a ph value of under 6.0 during the diurnal pattern. 2 animals of control group fell below this value in the evening.

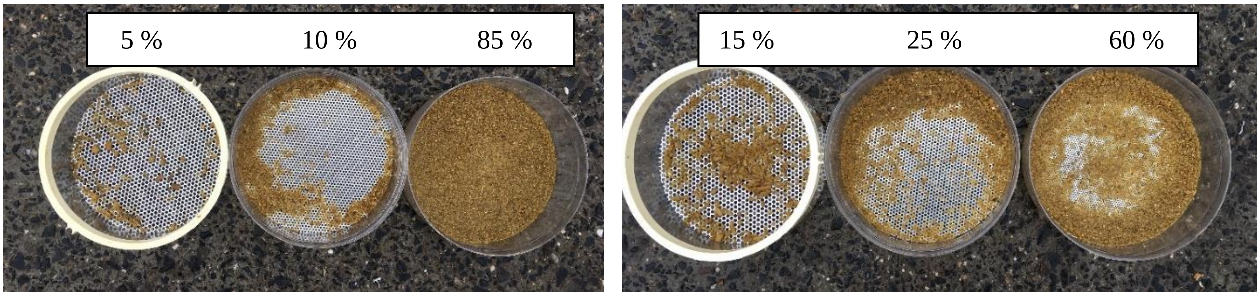
Tab. 1: Carcass value of control group and shredlage group

	Control n=34	Shredlage® n=34	p
Carcass weight (kg)	397.1 $_{3,16}$	402.9 $_{3,18}$	0.2006
Conformation grade[1]	8.6 $_{0.17}$	9.0 $_{0.17}$	0.0647
Fat grade[2]	6.7^{a} $_{0,18}$	6.2^{b} $_{0,18}$	0.0421

Least Squares Means $_{\text{Standard error}}$
[ab]: Different superscripts indicate significant differences (p≤0.05)
[1]: R = 8, R+ = 9
[2]: 2+ = 6, 3- = 7

Less feces residues were found in the top and middle sieves of the bulls fed shredlage compared to bulls fed short-cut silage (Fig. 1). Visually estimated, the proportion of feces fraction >4.76 mm in the shredlage group was 5 % on each sampling date, while the top sieve fractions of the control group varied between 5 %, 10 % and 15 %. The proportion of feces fraction >2.38 mm was mostly less than 20 % for the shredlage group, while for the control group this proportion was estimated to be ≥ 20 %. The feces fraction >1.59 mm took proportions of 75 to 85 % in the shredlage group. In contrast, the control group had noticeably lower proportions, often below 70 %.

Fig. 1: Feces residues of shredlage group (left) and control group (right) on 232th day of trial



No significant differences were found in density between the silages. In the entire silo, density of the short-cut corn silage was about 224 kg DM/m^3 and of the shredlage 216 kg DM/m^3 (p = 0.3201). In the critical top layer, the density of the short-cut corn silage was approximately the same at 183 kg DM/m^3 and just under 182 kg DM/m^3 of the shredlage.

CONCLUSIONS

With an increased rolling weight shredlage can be compacted just as well as short-cut corn silage. The feed quality was not affected.

Feeding corn shredlage had a positive effect on carcass value of the bulls. The animals had a significantly lower fat grade.

Lower fecal residue levels in the top and middle sieves of the shredlage group were an indication that the animals digested the ration better.

The omission of fodder straw and the saving of concentrates (here: 300 g DM/animal/day) can be allowed with shredlage in corn silage-based rations.

The feed of shredlage offers the potential to contribute to a ruminant-friendly feeding and thus to reduce the incidence of acidosis in beef cattle.

REFERENCES

COTANCH, K., DARRAH, J. (2012): Fecal fractions of the Nasco Digestion Analyzer/Cargill Manure Screener. The William H. Miner Agricultural Research Institute Farm Report, 7 – 8.

DÖRING, G. (2016): Kurzschnitt, Langschnitt, SHREDLAGE – neue Trends in der Fütterung? In: ETTLE, T., STRAET, D. (Hrsg.) "DMK-Tagung Futterkonservierung und Fütterung - Tagung des Ausschusses Futtterkonservierung und Fütterung im Deutschen Maiskomitee e.V. (DMK) am 05./06. April 2016 an der LfL in Grub". Schriftenreihe der Bayerischen Landesanstalt für Landwirtschaft, 27 – 37.

PRIES, M. (2016): Erfahrungen mit der Shredlage-Silagebereitung und Stand der Versuchsvorhaben. In: ETTLE, T., STRAET, D. (Hrsg.) "DMK-Tagung Futterkonservierung und Fütterung - Tagung des Ausschusses Futtterkonservierung und Fütterung im Deutschen Maiskomitee e.V. (DMK) am 05./06. April 2016 an der LfL in Grub". Schriftenreihe der Bayerischen Landesanstalt für Landwirtschaft, 38 – 42.

PRIES, M., DENIßEN, J., SPEIT, J. - H. (2018): Silier- und Fütterungsversuche mit Shredlage-Maissilage im Vergleich zur Maissilage herkömmlicher Häcksellänge. 45. Viehwirtschaftliche Fachtagung, 65 – 73.

SPIEKERS, H., ETTLE, T., PREIßINGER, W., PRIES, M. (2009): Häcksellänge und Strukturwert von Maissilage. Übers. Tierernährg. 37, 91 – 102.

OPPORTUNITIES AND LIMITS FOR PORTABLE NIRS EQUIPMENT: COMPARISON BETWEEN TWO PORTABLE NIRS SYSTEMS AND WET CHEMISTRY ANALYSIS OF GRASS SILAGE ENSILED AT LOW AND HIGH DRY MATTER LEVELS

EISNER1, I., SOMMER1, C., OHL2, S.
[1]Chr. Hansen A/S, Boege Alle 10/12, 2970 Hoersholm, Denmark. [2]Department of Forest [2]Landwirtschaftskammer Schleswig-Holstein, Lehr- und Versuchszentrum Futterkamp, Gutshof, 24327 Blekendorf, Germany.
Correspondence: deivei@chr-hansen.com

INTRODUCTION

Many different quality parameters change with time during the ensiling of forage. Typically, however, a forage analysis on the silage is made only once and acts as the basis for planning the composition of the Total Mixed Ratio (TMR) throughout the entire feedout of the silage. Portable NIR equipment have an appeal as they allow on-farm silage analysis, enabling changes or adaptions to the TMR composition to be made (Berzaghi and Marchesini, 2014). The purpose of this study was to evaluate the quality/precision in key parameters of relevance at silage opening as well as during feedout when determined by two different portable NIR equipment.

MATERIALS AND METHODS

The herbage originated from two parts of one field located in the northwest of Germany. The grass was cut in the afternoon of May 30, 2021. One part of grass was picked up with a round baler after approximately 18 hours of wilting at a dry matter (DM) content of 201 g/kg (WET). Another part of the grass was picked up after a wilting period of approximately 36 hours at 460 g/kg DM (DRY). Six bales per treatment were prepared. The control bales (CON) were treated with water. HF treated bales were inoculated with a bacterial suspension, containing only homofermentative lactic acid bacteria. DUAL treated bales were inoculated with a bacterial suspension, containing a mixture of homo- and heterofermentative lactic acid bacteria. DRY bales were sampled for proximate analysis, forage hygiene, fermentation and aerobic stability test after 65 days of storage. WET bales were sampled after 92 days of storage. Samples were analysed after exposure to air on Day 0, and Day 7 for dry matter (DM, drying and correction for volatile compounds)), crude protein (CP, Dumas) and ash (ashing at 550°C) content by wet chemistry (CHEM) and in parallel using portable NIRS equipment from two different manufacturers (NIRS A and NIRS B), following the instructions for use provided by the producers.

RESULTS AND DISCUSSION

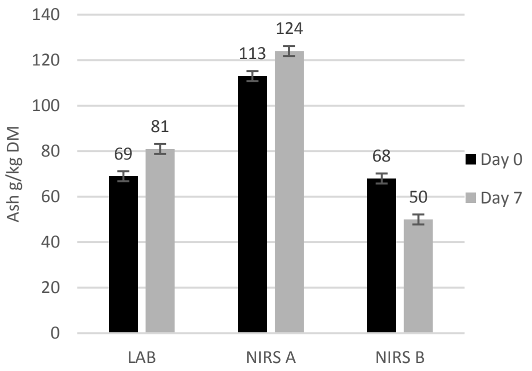
Significant differences were found between the method of analysis in freshly opened WET silages for all three nutritional values (Table 1). NIRS A and NIRS B overestimated DM content on average by 12 and 38 g/kg DM, and CP by 56 and 23 g/kg DM, respectively, compared to CHEM. A significant interaction was found between the treatments and the method of analysis for CP and ash contents, showing that analysis results from various devices were affected by treatment. The ash content was overestimated by NIRS A on average by 46 g/kg DM. NIRS B underestimated ash content by 15 g/kg DM in the CON silage ($P < 0.0001$), but delivered similar results in HF and DUAL silage compared to CHEM. Our results are in accordance with the study of Cherney et al. (2021), who found poor accuracy of nutritive value calibrations of a portable NIR in haylage.

Table 1. Effect of analysis method on quality parameters of grass silage ensiled without or with bacterial inoculants

	Low DM level									Effects (P-Values)		
	CON			HF			DUAL			I	E	I x E
	CHEM	A	B	CHEM	A	B	CHEM	A	B			
DM g/kg	210	237	245	209	228	239	200	219	248	*	***	ns
CP g/kg DM	137	210	169	153	184	171	146	210	164	ns	***	***
Ash g/kg DM	82	126	67	69	113	68	70	120	68	**	***	**

I = effect of bacterial inoculants; E = effect of the methods of analysis; CON = untreated grass silage, HF = grass silage treated with the mixture of homo-fermentative lactic acid bacteria; DUAL = grass silage treated with the mixture of homo- and heterofermentative lactic acid bacteria; CHEM = wet chemistry; A = NIRS A; B = NIRS B; DM = dry matter; CP = crude protein; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.0001$

HF WET silage showed a temperature increase in the samples of 2.7 °C above ambient at aerobic exposure on day 7, indicating the onset of organic matter degradation due to aerobic deterioration. An increase in ash content should occur as a result of organic matter disappearing (Ashbell and Weinberg, 1992). CHEM and NIRS A showed an increase in ash content by 12 g/kg DM and 11 g/kg DM from Day 0 to Day 7 ($P < 0.001$), but NIRS B showed a decrease in ash content by 12 g/kg DM ($P < 0.0001$, Figure 1). Depending on the speed of the water evaporation in relation to water accumulation as a final product of organic matter degradation, changes in DM content may occur at aerobic exposure. All methods of the analysis showed a decrease in DM content between Day 0 and Day 7 of aerobic exposure by 1.7 % DM absolutely for CHEM, 2.0 % DM for NIRS A, and 2.9 % DM for NIRS B ($P < 0.0001$, Figure 2).



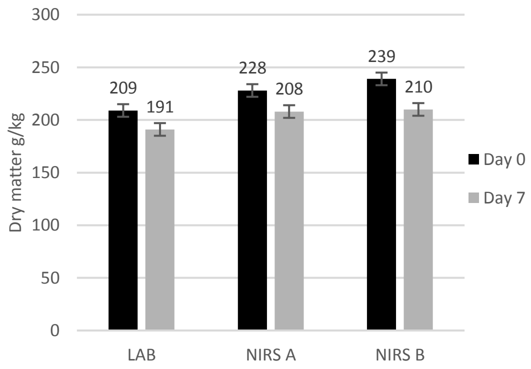


Figure 1. Results of the analysis for ash content at day 0 and day 7 of aerobic exposure of aerobically unstable silage by wet chemistry (CHEM), NIRS device A, and NIRS device B

Figure 2. Results of the analysis for dry matter content at day 0 and day 7 of aerobic exposure of aerobically unstable silage by wet chemistry (CHEM), NIRS device A, and NIRS device B

In DRY silages, NIRS B showed similar values to CHEM for DM, CP and ash content (Table 2). NIRS A significantly overestimated CP by 9 g/kg DM ($P < 0.001$) and ash by 14 g/kg DM points ($P < 0.0001$). All DRY samples remained stable till Day 7 of aerobic exposure. CHEM showed an increase in DM from 45.1 to 48.3 % ($P < 0.01$), whereas NIRS A and NIRS B did not show any changes in DM during aerobic exposure of the samples.

Table 2. Effect of analysis method on quality parameters of grass silage ensiled without or with bacterial inoculants

	High DM level									Effects (P-Values)		
	CON			HF			DUAL			I	E	I x E
	CHEM	A	B	CHEM	A	B	CHEM	A	B			
DM g/kg	447	461	446	463	467	448	443	447	443	ns	ns	ns
CP g/kg DM	135	147	134	137	144	137	138	151	136	ns	**	ns
Ash g/kg DM	75	90	72	73	86	78	74	90	78	ns	***	**

I = effect of bacterial inoculants; E = effect of the methods of analysis; CON = untreated grass silage, HF = grass silage treated with the mixture of homo-fermentative lactic acid bacteria; DUAL = grass silage treated with the mixture of homo- and heterofermentative lactic acid bacteria; CHEM = wet chemistry; A = NIRS A; B = NIRS B; DM = dry matter; CP = crude protein; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.0001$

CONCLUSION

The results of the comparison of WET silage demonstrate that portable NIRS equipment may not deliver reliable results if the DM of forage moves too far away from "standard conditions" to the extremes, or the silage is aerobically unstable. More accurate calibration, based on datasets including a sufficient number of silages in the low and high DM levels, and at different stages of aerobic deterioration, is required to make this technology useful on a daily basis.

REFERENCES

Ashbell, G., and Z. G. Weinberg (1992). Top silage losses in horizontal silos. *Canadian Journal Agricultural Engineering.* 34:171–175.

Berzaghi, P., and G. Marchesini (2014). Rapid methods of analysis of silages to improve feeding management in Dairy Farms. *Proceedings of 16th International Symposium Forage Conservation, Brno:* 76-81.

Cherney, J.H., Dibman, M.F., and D.J. Cherney (2021). Handheld NIRS for forage evaluation. *Computers and Electronics in Agriculture.* 190: 106469.

1,2-PROPANEDIOL IN MAIZE SILAGES

MITRÍK[1], A., MITRÍK[1], T., KOŘÍNEK[2], D.
[1]FEED LAB s.r.o., Magurská 2631/3, 052 01 Spišská Nová Ves
[2]SCHAUMANN ČR s.r.o. Volyně
Correspondence: andrej.mitrik@feedlab.sk, phone: +421 948 842 229

ABSTRACT

Propane-1,2-diol (propanediol) represents an important glucoplastic substance for the nutritional requirements of highly productive cows, and its natural synthesis during silage fermentation is of great importance. The aim of this paper was to monitor the fermentation activity of two combinations of homo- and hetero-fermentative lactic acid bacteria (LAB) in two preparations (preparation 1: L. buchneri, L. rhamnosus, L. plantarum; preparation 2: L. buchneri, L. rhamnosus, L. diolivorans). Propanediol production was monitored in maize silages prepared at the following combinations levels: cutting technology, hybrid, vegetation development, type of silage fermentation and length of fermentation time. Preparation 1 had a demonstrably positive influence on the production of propanediol in maize silages from whole plants (average content of 4.93 g.kg-1). Propanediol production in the silage alternative with preparation 2 (average content of 1.33 g.kg-1) was higher when compared to the negative control (average content of 0.11 g.kg-1). The production of propanediol decreased with increasing dry matter content, which is directly related to the advancing vegetative development of plants. The type of silage fermentation and vegetation development had a statistically demonstrable effect on the level of propanediol production in maize silages. The fermentation activity of the Lactobacillus buchneri strain used depended on its combination with other homofermentative and/or heterofermentative LAB strains.

Keywords: Zea mays, hybrids, harvesting technology, vegetation stages, dry matter content, silage, 1,2-propanediol, Lactobacillus buchneri

INTRODUCTION

The development and course of silage fermentation is fundamentally influenced by the fermentation's microflora. It is primarily formed by epiphytic bacteria. Targeted inoculation and guidance of the course of silage fermentation is achieved by adding various additives, in which different types and strains of lactic acid bacteria (hereinafter LAB) are most often used. These are isolated from nature and their selection depends on their unique fermentation characteristics, and their potential to interact with one another.

The aim of this work was to monitor and evaluate the dynamics of the production of propanediol and other fermentation metabolites in silages of whole plants of different maize hybrids (Zea mays) using two combinations of homofermentative and heterofermentative LAB, which contained one homofermentative and one heterofermentative strain (Tab. 1). We monitored changes in the composition of the fermentation profile at the following levels: 7 different silage maize hybrids, 2 material cutting technologies, 4 points of vegetation development in over a time interval of 34 days and 3 lengths of the fermentation process.

Table 1 Characteristics of preparations

PREPARATION	0	1	2
L.buchneri 1k2075		+	+
L.diolivorans 1k20752			+
L.plantarum 1k2079		+	
L.rhamnosus 1k20711		+	+
KTJ/1g		min. $3.0x10^{11}$	min. $2.5x10^{11}$
dosing		1g/1t	1g/1t

* *$g.kg^{-1}$; grey background: matched strains of lactic acid bacteria*

MATERIALS AND METHODOLOGY

Seven (7) different silage maize hybrids (FAO 200 – 530) from KWS SEMENA s.r.o. were sown on 28/04/2021 in four repetitions on the plot in Bátka: altitude 182 m above sea level - 48°21'45.9"N 20°11'55.7"E (Fig. 1). Sampling was carried out at an interval of 34 days on four dates (12/08/2021; 19/08/2021; 2/9/2021; 13/09/2021). From each cut sample, a roughly 30 kg coarse sample was taken from at least 10 places. The coarse samples were transported to the laboratory immediately after collection, where each of them was again thoroughly mixed and laboratory samples were taken from this material for nutrient analysis, and at the same time three silage alternatives (Tab. 2) were ensiled in the laboratory, each in two replications for three different periods of silage fermentation (90, 150 and 240 days). After the prescribed fermentation time, the samples were opened.

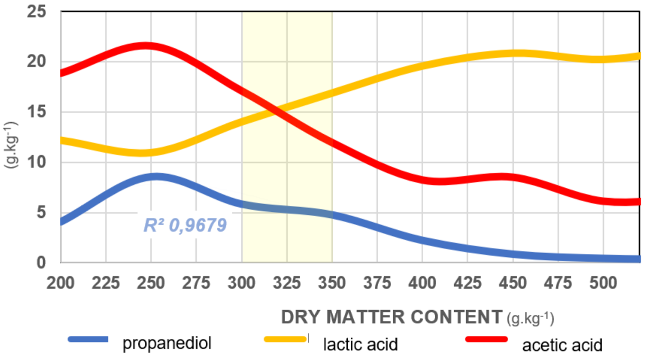
RESULTS AND DISCUSSION

The dry matter content in all three silage alternatives (Tab. 4) did not show statistically significant differences. Each of the groups consisted of an extensive set of 168 silage samples (Tab. 4), which testifies to a broad and standard starting base for each group. The addition of silage additives affects the presence of L. buchneri in maize silages (Mitrík et al., 2019; Kaluzová et al., 2022). We found very significant statistical differences (P<0.01) in the content of propanediol between the silage alternatives. Preparation 1 reached significantly the highest content of 4.9 g.kg-1, which is also in accordance with the results of other authors (Weiss et al., 2005; Kleinschmit and Kung, 2006; Arriola et al., 2021; Huang et al., 2021), indicating very good performance of the L. buchneri strain in combination with two homofermentative strains. Despite using the same strain of L. buchneri in preparation 2, the production of propanediol was almost 3.8 times lower. The control contained only trace amounts of propanediol, which did not exceed 1.00 g.kg-1 even in the maxima, which is also in accordance with the results of Kleinschmit and Kung (2006). The results show that the use of a particular strain/species and its combination with other LAB affects the success of propanediol production. We found a statistically significant correlation (P<0.01) between propanediol and lactic acid (r -0.356), acetic acid (r 0.415) and ethanol (r -0.381). The fermentation parameters and their ratios when using preparation 1 point to the successful course of the secondary production of acetic acid from lactic acid, which is also confirmed by:

- the lowest lactic acid content,
- the highest acetic acid content,
- higher ethanol content than preparation 2,

which is in agreement with the description of fermentation pathways characteristic of L. buchneri (Oude Elferink et al., 2001; Krooneman et al., 2002; Rooke and Hatfield, 2003).

Chart 1 **Propanediol and dry matter content**



Homofermentation supported by the inclusion of L. plantarum in preparation 1 most likely supported higher lactic acid formation in the first stages of fermentation, thus creating the basis for its secondary fermentation by the L. buchneri strain. At the level when using the silage alternative with preparation 1, we also found statistically significant (P<0.01) correlations between dry matter content and the following fermentation products: propanediol (r -0.590), lactic acid (r 0.629), acetic acid (r -0.627).

Propanediol peaked (8.58 g.kg-1) at a dry matter content of 250 g.kg-1 (Chart 1) and dropped at a dry matter content of 300 to 350 g.kg-1 to a level of approximately 5.0 g.kg-1. At a dry matter content of 400 g.kg-1, propanediol production dropped to 2.21 g.kg-1 and continued to drop below 1.00 g.kg-1 thereafter. These results again indicate that the intensity of the secondary fermentation of lactic acid to acetic acid and propanediol decreases with increasing dry matter content.

CONCLUSION

The same L. buchneri strain was able to increase propanediol production almost 3.8-fold under the same conditions if it was inoculated in combination with two homofermentative LAB strains. The combination of two heterofermentative LAB strains (L. buchneri and L. diolivorans) with one homofermentative strain (L. rhamnosus) did not produce increased amounts of acetic acid. The results achieved and the differences between the preparations indicate that the performance of the same L. buchneri strain in the production of propanediol depends, with great probability, on its action in combination with other Lactobacillus species.

Preparation 1 had a demonstrably positive influence on the production of propanediol in maize silages (average content 4.93 g.kg-1), fulfilled the declared properties and is strongly assumed to positively influence the health of highly productive cows. The production of propanediol in the silage alternative with preparation 2 (average content 1.33 g.kg-1) was higher than in the negative control (average content 0.11 g.kg-1), but only approximately at a third of the level compared to preparation 1. The results of this work significantly indicate that the nutrient characteristics of silage hybrids in individual vegetation stages can create different fermentation starting points for the course of the silage process, and this issue will require further monitoring.

REFERENCES

Arriola, K.G., Oliveira A.S., Jiang Y., Kim D., Silva H.M., Kim S.C., Amaro F.X., Ogunade I.M., Sultana H., Pech Cervantes A.A., Ferraretto L.F., Vyas D., Adesogan A.T. (2021). Meta-analysis of effects of inoculation with Lactobacillus buchneri, with or without other bacteria, on silage fermentation, aerobic stability, and performance of dairy cows. J. Dairy Sci. 104:7653–7670. https://doi.org/10.3168/jds.2020-19647

Huang, Z.;Wang, M., Ke, W., Guo, X (2021). Screening of High 1,2-Propanediol Production by Lactobacillus buchneri Strains and Their Effects on Fermentation Characteristics and Aerobic Stability of Whole-Plant Corn Silage. Agriculture 2021, 11, 590. https://doi.org/10.3390/agriculture11070590

Kalúzová, M., Kačániová, M., Bíro, D., Šimko, M., Gálik, B., Rolinec, M., Hanušovský, O., Felšöciová, S., Juráček, M. (2022) The Change in Microbial Diversity and Mycotoxins Concentration in Corn Silage after Addition of Silage Additives. Diversity 2022, 14, 592. https://doi.org/10.3390/d14080592

Kleinschmit D. H., Kung L. Jr. (2006): The Effects of Lactobacillus buchneri 40788 and Pediococcus pentosaceus R1094 on the fermentation of Corn Silage; J. Dairy Sci. 89:3999–4004

Krooneman, J., Faber, F., Alderkamp, A.C., Qude Elferink, S.J.H.W., Driehuis, F. and Cleenwerck, I. (2002). Lactobacillus diolivorans sp. nov. a 1.2–propanediol–degrading bacterium isolated from aerobically stable maize silage. International Journal of Systematic and Evolutionary Microbiology, 2002; 52, 639–46.

Mitrík T., Kořínek D., Mitrík A. (2019). Heterofermentative LAB and 1,2-propanediol production in maize silages with lower dry matter content; 18th INTERNATIONAL SYMPOSIUM FORAGE CONSERVATION, Brno, 2019

Oude-Elfrink S. J. W. H., Krooneman J., Gottschal J.C., Spoelestra S. F., Faber F., Driehuis F. (2001). Anaerobic Conversion of Lactic Acid to Acetic Acid and 1,2-Propanediol by Lactobacillus buchneri; APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Jan. 2001, p. 125-132

Rooke, J.A. and Hatfield, R.D., 2003. Biochemistry of ensiling. In: Buxton, D.R., Muck, R.E. and Harrison, J.H. (eds.) Silage science and technology. Vol. 42. Wiley, Hoboken, NJ, USA.

Weiss K., Kroschlewski B., Auerbach H. (2005). Effects of air exposure, temperature and additives on fermentation characteristics, yeast count, aerobic stability and volatile organic compounds in corn silage; J. Dairy Sci. 99:8053–8069

CORN SILAGE 2012-2022 (DRY CONTINENTAL REGION OF EUROPE)

OROSZ[1], S., BALOGH[2], K.
[1]*Livestock Performance Testing Ltd., Gödöllő, Hungary*
[2]*Hungarian University of Agriculture and Life Sciences, Institute of Physiology and Nutrition, Department of Feed Safety, Gödöllő, Hungary*

INTRODUCTION

Difficulties in corn production are expected in many European countries, especially in dry continental regions. There is a high probability that weather extremes will become more frequent during the summer compared to the long term average (1960-1990): lower precipitation, more heat stress days (average temperature > 25ºC), longer dry periods, the soil water shortage may have a higher chance in the near future. This is exacerbated by the increasing frequency of Aspergillus spp. infections and aflatoxin contamination in the field of corn plant due to extreme weather conditions (Dobolyi et al, 2013). The authors show variability of corn silage yield and nutritive value to highlight the attention to the threat that is already affecting the dry continental areas. The yield, nutrient content and digestibility of corn silages between 2012-2022 (n= 4898) reported by the authors.

MATERIALS AND METHODS

Routine laboratory samples derived from large scale farms in Hungary (n= 4898) between 2012-2022. 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* ($aNDFomd_{48}$), DOM (digestible orgnic matter), FOM (fermentable organic matter) are given based on the spectra 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 (AKI, 2012-2022).

Data (Table 2) are expressed as mean ± standard deviation (SD). GraphPad InStat 3.05 software (GraphPad Software, San Diego, CA, USA) was used for the statistical evaluation. Data were analyzed by one-way ANOVA and Tukey-Kramer multiple comparison post hoc test ($p < 0.05$).

RESULTS AND DISCUSSION

The forage yield range was found 17.0-33.5 ton AF/ha for Hungary during the last 11 years between 2012 and 2022 (Table 1). Silage yields were more than 30 t/ha at only 5 years out of 11 years. Extremely low yields had been found in 2012 and 2022 (19.3 ton AF /ha and 17.0 ton AF/ha, respectively).

Table 1 The silage (as fed -AF) and dry matter (DM) yield of corn, as whole crop plant in Hungary (harvested during August-September of 2012-2022).

		2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022
Silage yield	t AF/ha	19.3	22.5	31.1	24.3	30.6	26.7	30.7	32.0	33.5	27.4	17.0
DM yield	t DM/ha	7.4	7.4	11.1	8.6	11.0	9.8	11.7	11.8	11.9	9.6	5.7
Cropland	ha	87,952	76,867	68,440	71,822	70,707	62,776	56,900	57,563	53,198	54,989	87,952

Dry matter content varied between 328-381 g/kg (Table 2). The higher the dry matter content, the higher the starch content (r= 0.91), the higher the digestibility of organic matter (r= 0.62), while there is negative correlation between DM-content and the NDF-content (r= -0.90), the fibre digestibility ($NDFd_{48}$ r= -0.50), the digestible fibre content (r= -0.70).

Starch content ranged between 207-360 g/kg DM bertween 2012-2022. Tthe corn plant was harvested with the lowest starch content in 2022 (207±111g/kg DM) after the extremely hot and dry summer, associated with higher fibre content (2022:475±75g/kg DM aNDFom) and better fibre digestibility (2022:58±4%NDF $dNDF_{48}$) compared to the normal years. Low starch content was associated with extreme high deviation, indicating high variability of corn silage under heat stress and drought. Corn silage typically consists of 250-350 g/kg DM starch (NASEM, 2021). Starch content was over 300 g/kg DM at only 6 years out of 11 years. The poor results show that extreme weather can significantly reduce the nutritional value of corn silage, compromising the adequate starch- and energy supply of high producing dairy cows.

The aNDFom content ranged between 356-475 g/kg DM with aNDFom digestibility of 49-58% during 2012-2022 (Table 2). The lower the silage yield, the higher the NDF content (r= -0.90), the better the fibre digestibility (r= -0.50), and the more digestible fibre in the silage (r= -0.70). Degradable $aNDFom_{48}$ content of corn silage was found 180-277 g/kg DM. It can be concluded that the average of degradable $aNDFom_{48}$ content of corn silage (217 g/kg DM, n= 4898) was about 40% lower than that of whole-crop rye cut at the boot stage (2012-2022: 353 g/kg DM, n= 1326; un-published). Therefore corn silage is not considered to be an optimal degradable aNDFom source compared to early cut whole-crop cereals for dairy cattle.

Table 2 Nutrient content, fiber degradability ($NDFd_{48}$), degradable fiber ($dNDF_{48}$) and organic matter digestibility (OMd_{48}) of corn silage harvested in the period of 2012-2022 (n= 4898).

Year of the harvest		Dry matter	Crude protein	Crude fiber	Total starch	aNDFom[1]	ADF	ADL	$NDFd_{48}$[2]	$dNDF_{48}$[3]	OMd_{48}[4]
		g/kg	g/kg DM						%NDF	g/kg DM	%
2012	Mean	361bc	84d	217de	229a	450f	250e	19d	54cd	216bc	73a
n=103	SD	69	16	27	87	51	28	3	4	90	2
2013	Mean	328a	75c	216d	257b	444f	250e	18c	54d	242d	73a
n=724	SD	58	11	28	72	55	31	3	4	43	2
2014	Mean	357b	73b	168a	360f	356a	198a	17b	50b	180a	75c
n=526	SD	52	8	22	55	42	25	2	4	31	2
2015	Mean	352b	75c	195c	299c	411e	229d	18c	53c	220c	74b
n=617	SD	56	10	28	72	56	32	3	4	44	2
2016	Mean	359bc	70a	172a	357f	367b	206b	18c	49a	180a	75c
n=441	SD	49	8	23	51	42	26	4	4	31	2
2017	Mean	367bc	74b	184b	319d	393cd	217c	18c	53c	210b	75c
n=453	SD	52	9	24	60	45	27	3	4	34	2
2018	Mean	381d	69a	185b	337e	388c	215c	19d	54d	209b	76d
n=370	SD	53	8	23	57	43	26	3	4	35	2
2019	Mean	369cd	72b	188b	318d	394cd	219c	18c	54d	214b	76d
n=463	SD	63	9	30	74	55	32	3	4	42	2
2020	Mean	354b	73b	186b	317d	393cd	216c	17b	55e	218bc	76d
n=411	SD	47	9	23	52	42	27	3	3	28	2
2021	Mean	349b	76c	185b	299c	402d	216c	15a	56f	226c	77e
n=462	SD	58	10	28	73	53	30	3	2	33	2
2022	Mean	336a	87d	224e	207a	475g	256e	18c	58g	277e	75c
n=290	SD	59	19	40	111	75	41	4	4	56	3

[1]aNDFom - amylase treated ash corrected NDF; [2]$NDFd_{48}$ – 48 hours *in vitro* degradability of amylase treated ash corrected NDF;[3]$dNDFd_{48}$ - 48 hours *in vitro* degradable aNDFom, , [4]organic matter digestibility: *in vitro* 48 hours incubation; a,b Means with different letters in the same column differ significantly ($p < 0.05$).

CONCLUSIONS

The prevalence of extremely low yield and poor nutritive values with high variability show that corn silage is at high risk in the dry continental region and strategic plant management decisions are needed to increase the forage bank safety and silage quality for dairy cattle. The removal of corn silage from the feed ration in high risk areas may be justified, but there are other tools of stabilising the feed bank maintaining of corn silage. Adjusting stubble height can be a tool to increase the starch content of corn silage while considering dry matter loss. In addition, double cropping has become a viable option, helping autumn-sown and early spring-harvested whole crop cereals to reduce the risk of corn silage yield losses due to summer drought and to maintain biomass yield per ha per year. In the dry continental regions, the introduction of irrigation in corn fields for silage should be considered in the near future. Additionally, the use of summer drought-tolerant crops (*Aspergillus* spp. resistant BMR sorghum, BMR sudan grass and blends) at least for dairy replacements (eg. heifers), should be under consideration in dry continental regions.

REFERENCES

AKI (2012-2022). Annual report on agricultural field operations in Hungary. Research Institute of Agricultural Economics. Budapest. Volume XVII-XVII. ISSN 1418 2130

Dobolyi, C. S., Sebok, F., Varga, J., Kocsube, S., Szigeti, G., Baranyi, N., et al. (2013). Occurrence of aflatoxin producing Aspergillus flavus isolates in maize kernels in Hungary. Acta Alimentria 42, 451–459. doi: 10.1556/AAlim.42.2013.3.18

NASEM (2021). Nutrient Requirements of Dairy Cattle: Eighth Revised Edition. National Academies of Sciences, Engineering, and Medicine. Washington, DC: The National Academies Press. https://doi.org/10.17226/25806.

Posters

LABOUR STUDIES BY THE FORAGE PRODUCTION ON PEAT GRASSLANDS BEFORE, DURING AND AFTER REWETTING

MAČUHOVÁ, J., WOORTMAN, A., THURNER S.
Institute for Agricultural Engineering and Animal Husbandry, Vöttinger Str. 36, 85354 Freising

INTRODUCTION

Peatlands worldwide occupy about 3% of land area and contain approximately between 500 and 700 billion tons of Carbon (Yu et al., 2010). Besides Carbon storage and sequestration, peatlands deliver a range of other ecosystem services including water regulation, biodiversity protection (peatlands host many unique and rare habitats and species), natural risk mitigation, food and fuel, and recreation opportunities (Page and Baird, 2016). The peatlands are frequently intensively used for feed or food production. They are usually drained to be used for this purpose. However, the draining of peatlands results in the loss of the peatland Carbon storage function and, critically, the release of greenhouse gases to the atmosphere, which contributes to global warming (Page and Baird, 2016)).

To reduce or prevent greenhouse gas emissions from drained agriculturally used peatlands and to preserve the peat organic matter, there is an effort to rewet as many of drained peatlands as possible in Germany. At present the key factor by realisation of this goal is that the peatlands can still be used for agriculture after rewetting and, if privately owned, it should remain so. However, even when used as grassland, rewetted peatlands cannot be used at the same intensity and lower quality products can be expected compared to their use in drained state.

This study is part of a larger ten years lasting joint project of 3 institutions (Landkreis Ostallgäu in Bavaria, University of Applied Sciences Weihenstephan Triesdorf, and Bavarian State Research Centre for Agriculture). The joint project evaluates feasibilities of rewetting (e.g. rewetting and water management, legal effects, and sustainable farming practices) and consequences of rewetting of peat grasslands on biodiversity, greenhouse gas emissions, and farm economy in the area of the district Ostallgäu. The aim is to involve private farms with drained or uncontrolled wetted peat grasslands in the joint project willing to manage these grasslands (or part thereof) long-term in rewetted form (with groundwater level ideally approximately 10 cm below the soil level).

When estimating economic aspects of rewetting for the landowners and farmers, an important point is an evaluation of its consequences on work organisation and demands. It can be expected that the rewetting of peat grasslands will require changes in technical equipment and production processes, even if the harvested product will be used or should be used for the same purpose, e.g. as a feed (silage or hay) for cattle. There are studies available regarding the labour input or requirement for silage and hay production on mineral soils or drained peatlands (Eichhorn 1999, Schick and Stark 2002, Ammann and Wyss 2007, BWagrar 2015, Mačuhová et al. 2019). However, there is hardly any information regarding labour input or requirement for harvest of materials for feeding or bedding from rewetted or wet peat grasslands.

The aim of this part of the joint project is to examine work situation (labour input, used technical equipment, conditions during harvest) by forage production from peatlands before rewetting (if possible), in course of rewetting, and in the desired rewetted state. Furthermore, changes in yield and the quality of the harvested material are to be evaluated.

MATERIAL AND METHODS

Therefore, labour studies should be performed during the harvest of forage or bedding material from peat grasslands before, during, and after rewetting on participating farms. As already mentioned, the whole joint project lasts ten years, i.e. until the end of the year 2031. As the farms will be consecutively included in the joint project, the labour studies will start on these farms and continue (depending on necessity and possibilities) until the year 2030.

Participating farms

For the recruitment of farms farming on peat grasslands and with interest to participate in the study, the rewetting management, and later management of wetting, the cooperation partner Landkreis Ostallgäu is responsible. The project started at the beginning of the year 2022; and based on a preliminary project, one farm could be included in the study already in the year 2022. A second private farm should be included in the project in the year 2023. In total, up to 12 farms and 60 ha of total grassland peatland area should be involved in the study. In case that not enough private farms can be recruited for the joint project, also not private peat grassland areas will be included. Not private farms participating in the joint project will be included in labour studies only when managed by private farmers and producing feed (or using the land as pasture) or bedding material.

Data recording

The recording of labour data and other essential information about the harvest is performed using work diaries and by exact time measurement. The data should be recorded not just for the tasks performed directly during harvest but also for set-up works before and after harvest (i.e. sharpening of mower blades, cleaning of machines, and repair works) as well as other tasks needed for the management of grassland such as weed control.

Recording of labour input using work diaries

Data recording using work diaries is performed by farmers for the whole harvesting process and for all cuts. Data as for example duration of individual tasks, used machinery inclusive work width of devices, duration and reasons for disturbance when occurred, and weather conditions should be recorded. Data recording using work diaries will be performed every harvest season during the entire participation time of one farm in the join project.

Exact time measurements, sampling of harvested material, and additional measurements

Besides data recording using work diaries, exact time measurements are carried out randomly during some cuts or during all cuts (depending on personal availability) throughout the harvest on measure plots (and control plots when necessary). Besides recording of working time for individual harvesting task, the recording of relevant influence variables (e.g. humidity and shear strength of the soil, distances, fresh mass yield, and dry matter of harvested material) is performed. Moreover, harvesting material is sampled for quality analysis.

RESULTS AND DISCUSSION

In the year 2022, first labour studies were performed on the participating farm during 3 harvesting periods (2 cuts) on an already partially uncontrolled waterlogged plot (measure plot) and 3 harvesting periods (3 cuts) on drained peat grassland (control plot/s) located next to the measure plot (to evaluate control conditions and work situation). The harvested material was used to produce field dried hay, silage, or grass cobs.

Data have not been evaluated yet, however, first considerations regarding work management can already be done. Due to the farmer participation in different state nature conservation programs (e.g. for preserving animal species (insects and ground nesting birds)) and repeatedly occurred uncontrolled rewetting of one part of the measure plot in last few years, this plot has been already since 2017 semi-intensive used (up to three cuts per year) and the technical equipment was already adapted to be used also for the harvest of material from rewetted areas. Therefore, for example, a special track with deeper gravity centre and wider tyres and a double blade mower has been used for the mowing. As well, the loader wagon was adjusted to be used also on waterlogged grassland peatlands. An additional tyre per axle and side can be installed on both axles of the loader wagon and even another two per side on the first axle when harvesting material from the wetted areas.

Due to the already mentioned uncontrolled wetting of one area of the measure plot, the water level occasionally ranged to even a few centimetres above the soil level in previous years. When this occurred, the mowed material was harvested from the rewetted areas shortly after mowing and transported to another field to be dried up to needed dry matter, or this area was mowed later when conditions allowed it. Due to the dry weather conditions during the summer 2022, the whole area could be dried up to needed dry matter at measure plot. The rewetting management system should enable to better regulate the water level over the entire measure plot (to prevent long-lasting water levels above or too deep below the soil level on some areas), and even enable to lower the water level for harvest. The works on the rewetting management system should be performed in course of this winter.

CONLUSIONS

In conclusion, the labour studies should give an overview about changes in labour input, work processes, and needed machinery and technical equipment for management (especially for the harvest) due to rewetting of peatland grassland on investigated farms. Moreover, the information about changes in quality of harvested material should be given.

This information should finally contribute to the evaluation of the technology and processes used, claims on work organisation, and work demands to manage rewetted peatland grasslands. Based on these findings, work management recommendations for practice should be drawn up.

ACKNOWLEDGEMENT

This study is supported by the Federal Ministry for the Environment, Nature Conservation, Nuclear Safety and Consumer Protection (Funding code 67MP004B). The authors would like to thank the farmer for participation in the study.

REFERENCES

Ammann, H.; Wyss, U. (2007): Feuchtheu als mögliche Konservierungsart für Raufutter. ATB-Berichte, Nr. 685

BWagrar (2015): Verfahrenskosten für die Grassilagebereitung. http://www.bwagrar.de/Aktuelles/Verfahrenskosten-fuer-die-Grassilagebereitung,L0lNR19aT09NP0FJRD0yNDEzMjI4JkZJRD0yNDEzMjMwJk1JRD01MTY0NA.html, online available 21.04.2015

Eichhorn, H. (Hg.) (1999): Landtechnik. Stuttgart, Ulmer, 7. Ed., 688 p.

Mačuhová, J., Haidn, B., Thurner, S. (2019): Labour input for production of barn dried hay and grass silage and for feeding of cows on Bavarian dairy farms. In: KTBL (Hrsg.), 14th Conference Construction, Engineering and Environment in Livestock Farming, 24. 26.09.2019, Bonn, pp. 315-320.

Page, S. E. and Baird, A. J., 2016. Peatlands and global change: Response and resilience. Annual Review of Environment and Resources 41, 35-57.

Schick, M.; Stark, R. (2002): Arbeitswirtschaftliche Kennzahlen zur Raufutterernte. FAT Berichte Nr. 588

Yu, Z., Loisel, J., Brosseau, D. P., Beilman, D. W., and Hunt, S. J., 2010. Global peatland dynamics since the Last Glacial Maximum, Geophysical Research Letters 37, 5 p., L13402

THE NUTRITIONAL VALUE OF YELLOW LUPINE FODDER COMPARED TO DOMESTIC ALTERNATIVES

JANČÍK, F., HOMOLKA, P., KUBELKOVÁ, P., LOUČKA, R., KOUKOLOVÁ, V., TYROLOVÁ, Y., VÝBORNÁ, A.
Institute of Animal Science, Prague, Přátelství 815, 10400

ABSTRACT

Legumes are one of the most important sources of nitrogenous substances for the nutrition of ruminants, not only in the form of concentrate, but also as a source of fodder for the production of silage. In the conditions of the Czech Republic, peas and beans are the most commonly grown annual legumes for silage purposes. There are also less common alternatives to these crops such as lupins. In our work, we focused on comparing the quality of yellow lupine forage with peas and broad beans. In addition to the basic parameters of the chemical composition, we also compared the digestibility, determined by the *in vitro* enzymatic method. The first results obtained show that yellow lupine had a lower yield of dry matter. Furthermore, it achieved a comparable content of nitrogenous substances with peas, but lower compared to broad beans.

INTRODUCTION

Field pea (*Pisum sativum* L.) is an annual plant that is grown in many parts of the world. It is highly valued for its high crude protein content. Pea cultivation is also beneficial for improving soil fertility by nodule bacteria (Rhizobia) which are able to introduce atmospheric nitrogen into the soil (Kwabiah, 2004). Broad bean (*Vicia faba*) is a very important crop, not only for its rich protein content, but it is also efficient in biological nitrogen fixation with economic and ecological benefits and is an excellent raw material for silage production (Li et al., 2022). Lupine (*Lupinus* spp.) can be used as feed for livestock in the form of seeds, fodder and silage (Panasiewicz, 2022). A good ingredient for mixtures with spring cereals is yellow lupine, which is characterized by a high content of protein (over 40%), rich in lysine, and fat (over 50%), which significantly increases the nutritional value of feeds from such mixtures. In addition, it has low water and soil requirements thanks to a well-developed root system (Księżak et al., 2018).

The aim of the study was to evaluate the yield and quality parameters (chemical composition and dry matter digestibility) of the fodder of these legumes, depending on the harvest date.

MATERIALS AND METHODS

Broad bean (variety Merkur), field pea (variety Gambit) and yellow lupine (variety Salut) were compared in this study. The forage was collected from each crop at growth stages: 1) Full flower, 2) End of flower, 3) Seed filling, and 4) Full seed - waxy maturity. Forage samples were collected in three replicates from each crop and collection date. The fodder yields were measured for each sampling. The forage samples were dried at 55 °C for 48 hours. This was followed by milling to a particle size of 1 mm for laboratory analysis and determination of digestibility by the *in vitro* method. The dry matter of all samples was determined by drying at 105 °C for 6 hours. The ash was determined by burning the sample at 550°C for 6 hours. The organic matter (OH) was calculated according to the formula: OH = 100 – ash. The fat was determined by the method according to AOAC 2003.05. Nitrogen was determined by the Kjeldahl method (AOAC 976.05) and crude protein (CP) was calculated as N × 6.25. Crude fiber was determined by two-step hydrolysis according to AOAC 962.09 and is presented without ash content. The neutral detergent fiber (NDF) was determined according to AOAC 2002.04 and was determined using sodium sulfide and with α-amylase and is reported ash free. The acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined according to AOAC 973.18 and are reported ash free. All analyzes of fiber content and fiber fractions were determined using the ANKOM220 Fiber Analyzer (ANKOM Technology Corporation, Macedon, NY, USA). Dry matter digestibility was determined by the *in vitro* method using a two-stage incubation. The GLM procedure of SAS program (SAS Institute Inc., Cary, North Carolina, USA, 2002) was used for statistical evaluation. The effects of forage type and harvest date were entered into the model as fixed and repetition as a random effect. Significant differences were assessed using the Tukey–Kramer test.

RESULTS AND DISCUSSION

The average yield of dry matter was comparable for beans and peas (7.7 and 7.5 t/ha, respectively) and about 2 t/ha lower for lupine. Looking at the harvest, there was an increase in yield from the first to the third harvest, and there was no significant difference between the third and fourth harvest. For CP yield, the highest yield was found in broad bean, followed by pea and the lowest average yield was found in lupine (1.39; 1.04 and 0.78 t/ha, respectively). In terms of harvest date, average CP yield values were comparable from late flowering to waxy maturity. The steepest increase in forage dry matter yield can be observed in beans, followed by peas, and the lowest was in lupine. For all crops, there was a significant increase in post-harvest dry matter yield at the seed filling stage. A further increase has already occurred only for peas. Beans and lupins experienced a slight reduction in yield, which was apparently caused by increased leaf fall that could not be replaced by grain ripening. Similar to the dry matter yield, there was an increase in the values of the CP yield for all crops, where again the most significant increase was for beans, followed by a slight decrease in the last harvest. The highest yield of dry matter and CP in bean was achieved at the seed-filling stage (10.7 and 1.9 t/ha), as well as in lupine (5.8 and 0.9 t/ha). For peas, the highest yield of dry matter and CP was found in the stand at wax maturity (9.6 and 1.2 t/ha). Compared to our results, Soufan and Al-Suhaibani (2021) reported a significantly lower forage dry matter yield in pea monoculture (4.8 t/ha).

The dry matter content was the highest in the pea fodder, lower in the bean and lowest in the lupine. This indicator is also an important factor for possible harvesting for silage, where forage with lower dry matter will need a longer time to wilting, also taking into account the morphology of the plants. The dry matter content also increases during ripening, in our case from 150 to 220 g/kg on average. The content of organic matter was higher in peas (214 g/kg DM). There was no difference in OM content between beans and lupins. The average fiber content was comparable for beans and peas (229 and 222 g/kg DM). Lupine showed a fiber content of approx. 60 g higher. There were no significant differences between harvest dates. The highest CP content was found in beans (186 g/kg DM) and 30 to 40 g lower for lupins and peas. There was a gradual decrease in the CP content within the different harvest dates, but the difference was not evident from the second to the fourth harvest. Between the first and second harvest, there was the most significant increase in the fiber content of lupine, with subsequent stabilization of values around 310 g/kg DM. For beans, the crude fiber content was balanced during all harvests, around 220 to 240 g/kg DM, similarly for peas, where from the second to the fourth harvest, the crude fiber content was from 190 to 220 g/kg DM. The CP content decreased during ripening (by 53, 51 and 25 g/kg DM) in both beans, peas and lupins.

In addition to the standard parameters of the chemical composition, we also performed an analysis of the fiber fractions, which describe the forage quality in more detail. The highest content of NDF and ADF was contained in lupine forage (438 and 362 g/kg DM, respectively). For beans and peas, the NDF content was comparable. The ADF content was the lowest in peas (271 g/kg DM). The NDF and ADF values of pea forage, comparable to our results, were also found in the experiments of Mustafa et al. (2002). On the other hand, the ADL content was highest in beans, lower in lupins and the lowest in peas. Compared to our results, significantly higher ADL content for pea forage is reported by Mustafa et al. (2002) in the range of 85 to 98 g/kg DM. In terms of harvest, differences were found only between the NDF content in the first versus the last harvest. In terms of ADF and ADL, the values in terms of harvest did not differ significantly. In lupine, there was a gradual increase in NDF content with minimal difference between the third and fourth harvest. Bean and pea NDF content decreased slightly (apparently related to pod and grain growth), with both crops increasing between the third and fourth harvest. In bean, for example, in comparison with the results of Li et al. (2022) significantly lower NDF content (370 to 420 g/kg DM vs. 540 g/kg DM) and comparable ADF content (300 to 330 vs. 320 g/kg DM in Li et al., 2022) in each of our harvests. The dry matter digestibility of peas was higher than that of lupine (74.2 vs. 71.5 %). For broad beans, the dry matter digestibility was between that of peas and lupins (74.2 %). In vitro digestibility of dry matter in pea forage was also determined in their work by Mustafa et al. (2002) and found slightly lower values (67 to 74%) compared to our results (70 to 79%), which may be due to the already mentioned lower NDF and ADL contents.

CONCLUSIONS

In conclusion, we can state that in our growing conditions, yellow lupine failed to reach the yield or quality parameters of our traditional legumes, broad bean and pea. Yield and CP content was the highest for bean. Dry matter digestibility and content were the best for pea. From the point of view of the harvest date, in general, for all tested leguminous species, the most advantageous harvest is at the time of seed filling. A later harvest already provides lower yields and nutrient digestibility for bean and lupine. Conversely, with peas, the harvest window can be from the end of flowering to wax maturity, when digestibility has even increased.

DEDICATION

This paper was supported by the Ministry of Agriculture of the Czech Republic (Project No. QK21010344 and MZE-RO0723)

REFERENCES

Ksiezak J, Staniak M, Bojarszczuk J (2018). Evaluation of mixtures of yellow lupine (Lupinus luteus L.) with spring cereals grown for seeds. Applied Ecology and Environmental Research 16(2):1683-1696.

Kwabiah AB (2004). Biological efficiency and economic benefits of pea-barley and pea-oat intercrops. Journal of Sustainable Agriculture 25(1): 117-128.

Li H, Zeng T, Du Z, Dong X, Xin Y, Wu Y, Huang L, Liu L, Kang B, Jiang D, Wu B, Yang W, Yan Y (2022). Assessment on the Fermentation Quality and Bacterial Community of Mixed Silage of Faba Bean With Forage Wheat or Oat. Frontier Microbiology 13:875819.

Mustafa AF, Seguin P, Ouellet DR, Adelye I (2002). Effects of Cultivars on Ensiling Characteristics, Chemical Composition, and Ruminal Degradability of Pea Silage. Journal of Dairy Science 85:3411–3419.

Panasiewicz K (2022). Chemical Composition of Lupin (Lupinus spp.) as Influenced by Variety and Tillage System. Agriculture 12: 263.

Soufan W, Al-Suhaibani NA (2021) Optimizing Yield and Quality of Silage and Hay for Pea–Barley Mixtures Ratio under Irrigated Arid Environments. Sustainability 13: 13621.

THE IMPACT OF GRASS SILAGE DENSITY ON OXYGEN PENETRATION IN WALLED BUNKERS IN THE UK

MARLEY, C. A., MARLEY, G.
Alltech UK, Ryhall Rd, Stamford, PE9 1TZ. UK

Keywords: Oxygen, Air, Concentration, Density, Stability, Homolactic

INTRODUCTION

Opening of silage bunkers and drive over piles immediately leads to exposure of forage to atmospheric oxygen with the subsequent growth of aerobic and facultative anaerobic organisms. Aerobic microbial growth results in the loss of dry matter and nutritive value from the silage, primarily through fungal growth which led to the use of heterolactic treatments targeting fungal inhibition since the 1990s. Gerlach recently highlighted the negative impact on intake of acetic acid from heterolactic treatments, in addition to the nutritional losses associated with their fermentation (Borreani). Management practises (compaction, sheeting and feed out practises) have significantly improved within the past 15 years raising the question as to whether modern silage management practises can be used to overcome aerobic growth during silage feed out allowing the farm to focus on a more efficient homolactic fermentation with associated better maintained feed values, intake and both animal and farm efficiency, as well as improved farm carbon sustainability.

MATERIAL AND METHODS

Multiple grass bunkers were assessed over a 2 month period from July – August 2022 representing variable cuts, ages, harvesting and compaction methods. Bunkers were assessed for density, temperature and oxygen concentration at 4 points – Centre Base (CB) of silo (1m from the base), Top Left (TL, 1m from the top, 1m from the left wall), Top Centre (TC, 1m from the top) and Top Right (TR, 1m from the top, 1m from the right wall). Measurements were at depths of 10, 25, 50 and 75cm behind the face. Temperature was measured using a K-Type 1m thermometer with oxygen concentration measured using an Eijkelkamp Soil Oxygen Content System. A single density was measured through coring using the Dairy One Forage Master Probe.

RESULTS AND DISCUSSION

Measured oxygen concentration results are directly compared to the oxygen concentration at the centre base of the silage at the stated depth with the difference between the readings quoted – the recorded oxygen concentration at CB is quoted. It proved impossible to read oxygen concentrations below approximately 28% DM due to silage liquour being drawn into the analyser. Oxygen concentration results are presented in Table 1.

Table 1 – Oxygen concentrations (%) at different depths across 15 grass silages

Farm	Oxygen Concentration (%) at Stated depth (cm) and Location															
	CB				TL				TC				TR			
	10	25	50	D	10	25	50	D	10	25	50	D	10	25	50	D
1	0	0	0	194	15.7	5.7	0	184	12.7	5.4	0.2	212	18.6	13.8	1.5	194
2	9.6	1.5	0	150	2.4	1.5	3	172	-0.1	3.8	0.2	178	WP	WP	WP	150
3	16.9	8	0	128	0.1	1.1	3	135	-2.5	1.1	4.8	151	-3.2	-3.6	1.2	128
4	15.4	8	0	128	3.3	7.8	10.8	148	1.2	-0.8	0.4	163	2.8	10.2	14.1	128
5	20.7	20.5	19.7	74	19.6	17	7.7	37	20.1	19.7	19.7	44	19.4	18.7	17.8	74
6	8.4	3.2	0.4	167	2.1	1.4	0.7	180	2.6	1.3	0.4	167	2.8	1.9	1.4	167
7	11.1	5.2	0	220	4.7	1.2	0	192	4.3	0.5	0	183	4.5	1.1	0	220
8	8.7	3.3	0	174	14.2	5.9	1.6	109	3.2	0.3	0	151	2.9	1.4	0	174
9	17.1	6.6	0.6	277	-2.7	-0.7	4	245	-5.7	-2.7	1.4	269	-3	-4.8	1.2	277
10	12.3	5.3	0	200	2.8	1.6	1.5	165	4.6	-2.1	0	191	2.8	8.8	0.6	200
11	9.3	7	0	194	10	1.6	1.7	184	6	-1	1.8	212	3.1	-1	0.6	194
12	11.2	4.6	0	255	0.1	1.1	0.4	246	2	-1.2	0.4	240	0.9	0.8	0	255
13	9.1	3.5	0.1	188	0.6	-1.0	-0.1	169	1.2	1.2	0.5	177	1.4	0.9	0.6	162
14	7.6	2.3	0	229	2.2	1.0	0.3	175	2.4	-1.0	0	188	2.6	-1.4	0.3	194
15	6.5	1.1	0	233	1.3	2.2	0.5	174	1.2	1.6	0	190	1.4	1.5	0	195
Mean*	10.2	4.3	0.1	195.5	4.1	2.2	2.0	177.0	2.4	0.5	0.7	190.9	2.9	2.3	1.7	188.4
Min*	0	0	0	128	-2.7	-1	-0.1	109	-5.7	-2.7	0	151	-3.2	-4.8	0	128
Max*	17.1	8	0.6	277	15.7	7.8	10.8	246	12.7	5.4	4.8	269	18.6	13.8	14.1	277

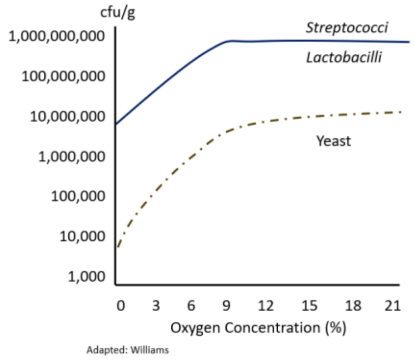
Depth measured in cm. D = Density $KgDMm^{-3}$. WP = Wet Probe. *data is screened for Farm 5

No oxygen (0% O_2) was recorded at a 75cm depth bar one farm with very low density silage being fed with a grab. All other farms were fed with either block-cutter or defacer.

Silage densities vary considerably between farms but clear trends are observed with the density of the silage being greatest at the base of the bunkers and the shoulder areas of the bunkers typically showing a lower density than the central area of the bunker. Weight of silage within the bunker mathematically will result in a higher density at the base of the bunker and drivers frequently roll less at the shoulder so as to not damage side plastic / walls or because the wall area of the bunker will only ever get one 'tyre width' compaction when other areas will get two 'tyre width' compactions as the compacting machinery moves across the surface.

Average oxygen concentration 10 cm behind the face at the centre base of the bunker was 10.2%, dropping to 5.6% once at 25cm behind the face. Williams showed yeast to be starting to be metabolically active at 3% oxygen which is the average oxygen concentration 50 cm behind the face at the base of the bunker. Oxygen concentrations are modestly higher to the at the top sides of the bunker compared to the central area, reaching between 0.6% and 2.1% total oxygen on average 50 cm behind the face and dropping to 0% (zero) at a depth of 75 cm.

Aerobic instability in silage is caused by the outgrowth of aerobic organisms and resulting in dry matter, energy and nutritive loss. Silages have inherent stability between 1 – 3 days depending on forage type, dry matter, sugar content, hygiene through ensiling, compaction, oxygen management through storage and oxygen management through feeding.



CONCLUSION

Silage block cutters typically range from a cutting depth of 0.8 m to 1m meaning that correct usage of a block cutter will stay ahead of oxygen penetration into the bunker if crossing the face every day, and, if it is necessary to take 2 days crossing the face then removal of half a block of grass silage (0.4 – 0.5 m) will stay ahead of aerobic instability without taking into consideration the farm specific inherent stability achieved from ensiling. Many farms currently using heterolactic treatments that potentially reduce intake, dry matter and feed value recovery can achieve more efficient fermentations and nutrient preservation through the use of homolactic inoculants without adversely impacting silage stability through maintenance of good ensiling, storage and feedout management.

This work has been undertaken with grass silage at a maximum of 44% dry matter content.

REFERENCES

Borreani *et al*, 2018. Silage review:Factors affecting dry matter and quality losses in sialge. *Journal of Dairy Science*, **101**

Gerlach *et al*, 2021. A data analysis on the effect of acetic acid on the DM intake in dairy cattle. *Animal Feed Science and Technology*, **272**

William, A. G. et al, 1994. The effect of oxygen concentration on changes in the microbial population, temperature and dry matter content in grass silage. Grass and Forage Science, **49**, 2

THE IMPACT OF EGALIS FERMENT ON BALED SILAGE IN NORWAY

TONJE EILERAAS, T.,[1], EIKEFJORD, L-A[1], MARLEY, G.[2]
[1] *Alltech Norway, Indre Horrnesvika 9, 6809 Førde, Norway*
[2]*Alltech UK, Ryhall Rd, Stamford, PE9 1TZ*

Keywords: Bale, Grass, Digestibility, Yield, Intake, Palatability, Egalis Ferment,

INTRODUCTION

Dry matter intake (DMI) and milk yield are positively correlated to neutral detergent fibre digestibility (NDF-D), with each 1% improvement in NDF-D being associated with a 0.17Kg increase in DMI and a 0.25Kg increase in 4% fat corrected milk (Oba; Kendall), with intake of silage also being negatively correlated to the level of acetic aicd in the diet (Gerlach) as well as dry matter and nutritive loss (Borreani).

MATERIAL AND METHODS

Second cut Timothy (*Phleum pratense*) was baled using a C441r Model 2020 John Deere baler to a 1000Kg approximate fresh bale weight post a 24 hour wilt under 10 layers of white plastic, either untreated or treated with Egalis Ferment at 1,000,000 cfu/g (4l/T liquid application) and stored for 10 weeks unstacked. Individual bales were cored in triplicated horizontally through the bale with a composite sample of each bale being analysed through dry NIR for nutritional profile. Statistical analysis was run using Minitab 21 after data assessment for normality of distribution with significance declared at $p \geq 0.05$.

RESULTS AND DISCUSSION

All silages appeared to have fermented well with no visible signs of damage to the plastic and no visible surface growth of yeast or mould on either the treated or untreated bales.

Table 1. Mean Fermentation profile

	DM	pH	NH_3N	NDF-D 30hr	Ethanol	Lactic Acid %	Acetic Acid %	LA : TVFA
Un	31.5	4.12	81.4	32.2	7.6	70.6	7.6	8.8
EF	32.9	3.96*	77.0	34.4*	6.2	78.4	4.6*	15.9*

* Denotes significance at $p \geq 0.05$

Treated and untreated timothy silage was well fermented with no visible difference in spoilage. No aerobic assessments were run.

Treatment with Egalis Ferment resulted in a statistically improved maintenance of fibre digestibility compared to untreated bales of 2.2%, equating to an extra 0.55Kg of 4% fat corrected milk per day. Egalis Ferment resulted in a statistically significant shift in the fermentation profile toward a more homolactic fermentation, with protection of more protein as indicated by lower levels of ammonia, and protection of more dry matter from field to feed passage as indicated by the lower ethanol, acetic and ammonia levels. Egalis Ferment resulted in a significantly lower, more stable pH in the final silage.

No butyric acid was observed in any of the silages.

Combing the fermentation data and digestibility data it can be strongly suggested that the speed of fermentation was faster with Egalis Ferment, driven through a homolactic fermentation (shown by the higher LA, lower AA and elevated LA:AA), resulting in a faster final pH and greater NDF-D (as analysed).

CONCLUSION

With good field, wilting and baling management, high quality baled timothy silage can be produced without the use of inoculant. Under the optimal baling practices in use Egalis Ferment significantly enhanced the fermentation of the timothy resulting in more feed value being protected, a more palatable silage being produced and an improvement in 4% fat corrected milk of 0.55Kg / cow / day

REFERENCES

Borreani *et al*, 2018. Silage review:Factors affecting dry matter and quality losses in sialge. *Journal of Dairy Science*, **101**

Kendall. C., Leonardi,C., Hoffman, P. C. & Combs, D. K. 2009. Intake and milk production of cows fed diets that differed in dietary neutral detergent fiber and neutral detergent fiber digestibility. *Journal of Dairy Science* 92(1), 313-323

Gerlach *et al*, 2021. A data analysis on the effect of acetic acid on the DM intake in dairy cattle. *Animal Feed Science and Technology*, **272**

Oba M. & Allen, M. S.. 1999. Evalutation of the importance of the digestibiity of neutral detergent fibre from forage: Effects on dry matter intake and milk yield of dairy cows. *Journal of Dairy Science* **82**, 589-596

THE IMPACT OF EGALIS FERMENT ON LOW DRY MATTER GRASS ENSILED IN MINI-SILOS

MARLEY, G.
Alltech UK, Ryhall Rd, Stamford, PE9 1TZ. UK

Keywords: Egalis Ferment, Grass, Digestibility, Yield, Intake, Palatability, Egalis Ferment,

INTRODUCTION

Ensiling of low dry matter (DM) grass offers specific fermentation challenges that can result in a butyric fermentation, high nutritive and dry matter losses and lead to the production of unpalatable silage due to the gas space in the silage becoming rapidly filled with effluent and thus no longer capable of sustaining glycolysis. A limited fermentation can occur that then allows the outgrowth of Clostridial species often present under lower DM conditions. Borreani highlighted the increased efficiency of a homolactic fermentation compared to heterolactic, and the ability to protect more DM and nutrient, with Gerlach further highlighting the additional negative impacts of heterofermentative fermentations with the produced acetic acid reducing DM recovery, feed value and final animal dry matter intake.

MATERIAL AND METHODS

First cut rye grass (*Lolium perenne L*) was cut with a Krone mower conditioner and collected, untreated, after a 6 hour wilt using a Krone 320GL forage wagon. A 40Kg sample was taken from the third untreated forage wagon. Collected rye was homgenised and sampled pre ensiling (Table 1). Forage was split into 20Kg aliquots and then sprayed with either water at 10mls/Kg or Egalis Ferment at 1,000,000 cfu/g forage and 10mls/Kg. 2Kg aliquots were ensiled in 5l buckets double lined with plastic liners and compacted by hand. Liners were sealed air tight, a 2Kg dry sandbag placed on top and snap lids closed. Five replicates per treatment (Un = Untreated, EF = Egalis Ferment) were ensiled. Mini silos were weighed and stored for a 76 day period unstacked. On opening each replicate was weighed, homogenised and sampled for dry NIR analysis.

A further 30 x 100ml sterile jars were ensiled with 75g of grass per treatment for daily pH assessment – jars were opened daily in triplicate with individual replicates being homogenised, sub sampled for 30.0g and maccerated in 270mls sterile water prior to pH assessment. Statistical analysis was run for normality with subsequent ANOVA using Minitab 21 with significance declared at $p \geq 0.05$.

RESULTS AND DISCUSSION

The fresh grass sample (Table 1) confirmed the ensiling DM to be low (21%) and the rye to have been harvested hygienically due to the low ash content. Ethanol soluble carbohydrate was recoded at 13.6%.

<u>Table 1. Profile as Ensiled (%DM)</u>

	DM	Ash	CP	ADF	aNDF	ESC	ME (mj/Kg DM)
Un	21.0	7.32	12.6	30.7	56.0	13.6	10.69

Table 2 presents the mean daily pH fall which showed a statistical significant difference at every sampling point with Egalis Ferment producing a statistically faster rate of pH fall compared to the untreated silage, and producing a statistically significant lower final pH compared to the untreated silage. The enhanced rate of fermentation achieved through treatment with Egalis Ferment leading to the likely inhibition of germination of clostridial spores.

<u>Table 2. Mean pH Fall</u>

	Day 0.5	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day7	Day 8	Day 9
Un	6.83	6.50	6.18	5.90	5.45	4.94	4.81	4.63	4.48	4.37
EF	6.65*	6.17*	5.27*	4.73*	4.34*	4.13*	3.99*	3.95*	3.95*	3.93*

All of the untreated replicates showed more visible surface fungal growth than the Egalis Ferment treated replicates, and the odour of the untreated silage was butyric.

Table 3 presents the statistically significant results of the losses and fermentation characteristics of the silages on opening. The enhanced rate of fermentation observed with Egalis Ferment led to a reduced level of proteolytic activity within the treated silage. It is impossible to say whether this was from reduced enterobacterial activity at the start of the fermentation or from reduced Clostridial activity longer term through storage. The Egalis Ferment led to a significant reduction in the production of alcohol either through aerobic yeast activity at the start of the fermentation or through reduction in the heterofermentative lactic acid bacterial activity (or both). A statistically lower, more stable final pH was achieved through the treatment by the statistically significant shifting of the fermentation to a more efficient, homofermentative fermentation as indicated by the statistically higher levels of lactic acid and statistically lower level of acetic acid. The ratio of desirable to undesirable acids was statistically improved through treatment and the production of butyric acid was reduced by over 70%.Total losses of silage were reduced by 47% through treatment with Egalis Ferment.

Table 3. Mean Fermentation Data (%DM)

	DM	NH_3N	Eth	pH	TVFA	LA	AA	BA	LA:VFA	FW Loss g/Kg
Un	19.6	1.77	2.4	4.41	11.0	6.5	2.8	1.7	1.4	21.5
EF	19.3	1.37*	1.1*	3.82*	12.8*	10.4*	1.9*	0.5*	4.3*	11.5*

CONCLUSION

Ensiling of low dry matter grass without treatment frequently leads to a challenged fermentation that produces a silage with high DM losses, high protein breakdown and a low palatability in the final silage due to the undesirable fermentation products. In this trial Egalis Ferment showed an ability to statistically significantly overcome the negative challenges of ensiling low dry matter grass and led to the recovery of more DM, more protein and a significantly more palatable silage. Egalis Ferment is an effective product for ensiled grass inclusive of low dry matter.

REFERENCES

Borreani *et al*, 2018. Silage review:Factors affecting dry matter and quality losses in sialge. *Journal of Dairy Science*, **101**

Gerlach *et al*, 2021. A data analysis on the effect of acetic acid on the DM intake in dairy cattle. *Animal Feed Science and Technology*, **272**

THE IMPACT OF OXYGEN PENETRATION ON TEMPERATURE OF GRASS SILAGE

MARLEY, C. A, MARLEY, G.
Alltech UK, Ryhall Rd, Stamford, PE9 1TZ. UK

Keywords: Oxygen, Air, Concentration, Density, Stability, Temperature

INTRODUCTION

Opening of silage bunkers immediately exposes the silage to atmospheric oxygen and allows aerobic and facultative anaerobic organisms that had previously been dormant to become metabolically active and increase in their numbers. Within this outgrowth lactate assimilating yeast and acetic acid bacteria are especially undesirable due to their production of heat and associated loss of energy from the silage. Marley (2023) and Losand (2003) showed that with increasing silage density the oxygen penetration into the silage is reduced.

MATERIALS AND METHOD

Multiple grass bunkers were assessed over a 2 month period from July – August 2022 representing variable cuts, ages, harvesting and compaction methods. Bunkers were assessed for density, temperature and oxygen concentration at 4 points – Centre Base (CB) of silo (1m from the base), Top Left (TL, 1m from the top, 1m from the left wall), Top Centre (TC, 1m from the top) and Top Right (TR, 1m from the top, 1m from the right wall). Measurements were at depths of 10, 25, 50 and 75 cm behind the face. Temperature was measured using a K-Type 1 m thermometer. A single density was measured through coring using the Dairy One Forage Master Probe. Temperatures are compared to the average base temperature point for each farm. Bunkers were either untreated or homolactic treated with farms taking between 1 and 3 days to cross the face.

RESULTS AND DISCUSSION

Silage temperatures at different locations were compared to the average base temperature at each farm and presented in Table 1.

Table 1 – Temperature of Silage (°C) compared to basal comparative

Farm	Temperature Difference (°C) at Stated depth (cm) and Location															
	CB				TL				TC				TR			
	10	25	50	D	10	25	50	D	10	25	50	D	10	25	50	D
1	0.1	-0.3	0	194	7.1	6.1	5.1	184	5	4.6	5.2	212	8	8.1	9.7	194
2	-0.1	0	0.1	150	0.6	0.7	0.7	172	3.2	4	4.3	178	0.3	2.8	3.6	150
3	-0.1	-0.3	0.4	128	-3.1	-2.3	-2.1	135	-7.5	-7.3	-7.5	151	-7.6	-8	-8	128
4	0	-0.9	0.4	128	2.1	-1.3	-1.6	148	3.8	3.5	3.1	163	4.7	2.5	1.6	128
5	-2.5	1.6	0.7	74	16.5	22.5	27.5	37	1.6	6.3	1.8	44	3.7	3.9	4.3	74
6	-1.9	0	0.7	167	-2.9	-3.1	-2.8	180	-1.5	-1	0	167	-2	-0.3	-0.9	167
7	0.4	-0.3	0	220	15.7	13	11.3	192	7	10.6	6.2	183	2.8	2.8	4.6	220
8	-1.2	-0.3	0.3	174	8.9	9.1	8.8	109	6.8	6.9	8.4	151	4	5.6	6.4	174
9	0	-0.2	1.1	277	1.4	2.4	1.6	245	4.6	-0.2	3.7	269	4.6	0.5	3.3	277
10	3	-1.3	-0.9	200	5.5	5	3.3	165	3	3	3.4	191	3	3.7	3.3	200
11	0.2	0	0.1	194	4.2	3.1	1.3	184	3.1	2.2	2	212	3.5	2.9	2.9	194
12	-0.2	0	0.2	255	1.6	-0.5	-0.7	246	0.3	-0.2	-0.4	240	-0.4	-0.9	-1.1	255
13	-0.1	0.1	0	188	3.2	1.1	-0.3	169	1.2	0.3	1.1	177	1.1	1.5	1.2	162
14	-0.3	0.1	0.2	229	4.8	2.2	1.1	175	2.8	2.1	0.6	188	-0.2	-0.3	-0.6	194
15	-0.2	-0.1	0.3	233	2.9	1.4	0.4	174	0.9	1.4	1.4	190	1	-0.1	1.1	195
Mean	-0.2	-0.1	0.2	195	4.6	4.0	3.6	177	4.4	2.3	2.4	191	1.8	1.6	2.1	188
Min	-2.5	-1.3	-0.9	128	-3.1	-3.1	-2.8	109	-4.5	-7.5	-7.3	151	-7.6	-8	-8	128
Max	3	1.6	1.1	277	16.5	22.5	27.5	246	26.3	7	10.6	269	8	8.1	9.7	277
Mean*	0.0	-0.3	0.2		3.7	2.6	1.9		2.3	2.1	2.3		1.6	1.5	1.9	
Min*	-1.9	-1.3	-0.9		-3.1	-3.1	-2.8		-7.5	-7.3	-7.5		-7.6	-8	-8	
Max*	3	0.1	1.1		15.7	13	11.3		7	10.6	8.4		8	8.1	9.7	

* Screened for Farm 5. Depth measured in cm

Negligible temperature difference (<0.5 °C) was seen between temperatures recorded at 50 and 75 cm depths.

Silage densities vary significantly but expected trends are observed with increasing density at the base of the bunker due to the additional compaction from the grass above and lower densities at the edges of the bunker as compaction drivers are wary of damaging the side walls / side plastic by driving too close to the wall.

One farm showed low silage density (Farm 5) and this is reflected by exceedingly high temperatures behind the face compared to the base of the bunker. Summary results are shown with and without Farm 5 but the interpretation is purely for results 'without Farm 5'.

The average temperature 25 cm behind the face of every bunker was consistently less than 3°C below the average temperature at the base of the corresponding bunker (with 3°C being the EFSA defined temperature difference of aerobic instability) indicating that even when silage was heating directly at the face it was not heating behind the face irrespective of taking between 0.5 and 3 days to cross the face.

Aerobic microbial growth starts to occur at approximately 4% oxygen concentration within silage (William et al). The penetration of oxygen behind the face of untreated or homolactic treated UK grass silage bunkers has shown negligible temperature increases 25 cm behind the face of the bunker irrespective of taking 0.5 – 3 days to cross the face.

CONCLUSION

To overcome the negative impact of silage heating from aerobic growth of lactate assimilating yeast heterofermentative organisms have historically been used in the knowledge that they increase fermentation nutritional and dry matter losses. Gerlachs summary assessment of published feed intake trials correlated to acetic acid levels showed a reduction in dry matter intake from using Heterolactic bacteria due to the production of acetic acid – this is in addition to the nutritive and dry matter losses that occurred through use of Heterolactic strains such as *L buchneri, L brevis* and *L hilgardii*. This work shows that silage can be treated with homolactic inoculant and consistently maintain aerobic stability with "2022" UK levels of compaction and management and achieve 3+ days of aerobic stability under farm conditions.

This work has been undertaken with grass silage ranging from 32% - 44% dry matter content.

REFERENCES

Borreani *et al*, 2018. Silage review:Factors affecting dry matter and quality losses in sialge. *Journal of Dairy Science*, **101**

EFSA guidelines The Feed Additives Regulation (**Regulation (EC) No 1831/2003 of September 2003**

Gerlach *et al*, 2021. A data analysis on the effect of acetic acid on the DM intake in dairy cattle. *Animal Feed Science and Technology*, **272**

Marley *et al*, 2023. The impact of Grass Silage Density on Oxygen Penetration in Walled Bunkers in the UK. Proceedings ISFC 2023

William, A. G. et al, 1994. The effect of oxygen concentration on changes in the microbial population, temperature and dry matter content in grass silage. Grass and Forage Science, **49**, 2

THE IMPACT OF EGALIS FERMENT ON LOW DRY MATTER GRASS ENSILED IN MINI SILOS IN SPAIN

PRADO[1], H, MARLEY[2], G.
[1]*Alltech Spain, Pol. Industrial el Pozambrón, 19115 Almoguera, Guadalajara, Spain*
[2]*Alltech UK, Ryhall Rd, Stamford, PE9 1TZ. UK*

Keywords: Egalis Ferment, Grass, Digestibility, Yield, Intake, Palatability.

INTRODUCTION

Low dry matter (DM) grass offers specific fermentation challenges that can undesirably impact grass fermentation both in speed of fermentation and end products, potentially resulting in the formation of butyric acid, high nutritive and dry matter losses and lead to the production of unpalatable silages. A limited fermentation can occur that may then allow the further outgrowth of Clostridial species with increasing storage time and an ongoing decline in silage quality and palatability through storage. Borreani highlighted the increased efficiency of a homolactic fermentation compared to heterolactic, and the ability to protect more DM and nutrient, with Gerlach further highlighting the additional negative impacts of heterofermentative fermentations with the produced acetic acid reducing DM recovery, feed value and final animal dry matter intake.

MATERIAL AND METHODS

Second cut rye grass (*Lolium perenne L*) was cut with a Krone mower conditioner and collected, untreated, after a Claas Jaguar 840. A 40Kg sample was taken from the third untreated trailer. Collected rye was homgenised and sampled pre ensiling (Table 1). Forage was split into 20Kg aliquots and then sprayed with either water at 10mls/Kg or Egalis Ferment at 1,000,000 cfu/g forage and 10mls/Kg. 2Kg aliquots were ensiled in 5l buckets double lined with plastic liners and compacted by hand. Liners were sealed air tight, a 2Kg dry sandbag placed on top and snap lids closed. Five replicates per treatment (Un = Untreated, EF = Egalis Ferment) were ensiled. Mini silos were weighed and stored for a 75 day period unstacked. On opening each replicate was weighed, homogenised and sampled for dry NIR analysis.

Statistical analysis was run for normality with subsequent ANOVA using Minitab 21 with significance declared at $p \geq 0.05$.

RESULTS AND DISCUSSION

The fresh grass sample (Table 1) confirmed the ensiling DM to be low (23.8 %) and the rye to have been harvested hygienically due to the low ash content. Ethanol soluble carbohydrate was recoded at 0.7% with water soluble carbohydrate recorded at 5.1% making the grass easy to ensile.

Table 1. Profile as Ensiled (%DM)

	DM	Ash	CP	ADF	aNDF	ESC	WSC	ME (mj/Kg DM)
Un	23.8	8.79	13.3	35.3	53.8	0.7	5.1	9.34

All of the untreated replicates showed visible surface fungal growth and a darkening of the final silage on opening than the Egalis Ferment treated replicates which remained free of surface growth.

Table 2 presents the statistically significant results of the losses and fermentation characteristics of the silages on opening. The enhanced rate of fermentation observed with Egalis Ferment led to a reduced level of proteolytic activity within the treated silage. It is impossible to say whether this was from reduced enterobacterial activity at the start of the fermentation or from reduced Clostridial activity longer term through storage. The Egalis Ferment led to a significant reduction in the production of alcohol either through aerobic yeast activity at the start of the fermentation or through reduction in the heterofermentative lactic acid bacterial activity (or both). A statistically lower, more stable final pH was achieved through the treatment by the statistically significant shifting of the fermentation to a more efficient, homofermentative fermentation as indicated by the statistically higher levels of lactic acid and statistically lower level of acetic acid. The ratio of desirable to undesirable acids was statistically improved through treatment and the production of butyric acid was reduced by over 70%.Total losses of silage were reduced by 47% through treatment with Egalis Ferment.

Table 3. Mean Fermentation Data (%DM)

	ADICP	uNDF120	uNDF240	LA	AA	BA	LA:TVFA
Un	1.43	20.1	18.6	8.6	2.7	0.2*	2.7
EF	1.32*	18.8$^{p\ 0.055}$	17.2*	10.0*	2.1*	0	4.3*

CONCLUSION

Ensiling of low dry matter grass without treatment frequently leads to a challenged fermentation that produces a silage with high DM losses, high protein breakdown and a low palatability in the final silage due to the undesirable fermentation products. In this trial Egalis Ferment showed an ability to statistically significantly overcome the negative challenges of ensiling low dry matter grass and led to the recovery of more DM, more protein and a significantly more palatable silage. Egalis Ferment is an effective product for ensiled grass inclusive of low dry matter.

REFERENCES

Borreani *et al*, 2018. Silage review:Factors affecting dry matter and quality losses in sialge. *Journal of Dairy Science*, **101**

Gerlach *et al*, 2021. A data analysis on the effect of acetic acid on the DM intake in dairy cattle. *Animal Feed Science and Technology*, **272**

EFFECTS OF A MIXTURE OF LACTIC ACID BACTERIA CONTAINING LACTOBACILLUS DIOLIVORANS ON AEROBIC STABILITY OF MAIZE SILAGE AFTER SHORT TIME OF STORAGE

KRAMER[1], E., SPRAFKE[2], J., NELLES[2], M.
[1]ISF GmbH, Pinneberg
[2]University Rostock, Faculty of Agricultural and Environmental Sciences, Department Waste and Resource Management, Rostock

Keywords: aerobic stability, *Lactobacillus diolivorans*, storage time

INTRODUCTION

Heterofermentative lactic acid bacteria (LAB), e.g., *Lactobacillus buchneri* are widely used to improve aerobic stability in silages. Nevertheless, a minimum time of storage of at least 6 to 8 weeks is needed for a significant effect on aerobic stability. Since several years new heterofermentative strains such as *Lactobacillus diolivorans* are used to improve aerobic stability even after a short time of storage of 14 days. The aim of the present study was to assess the short-term effect of a LAB mixture containing *L. diolivorans* on aerobic stability of whole plant maize silage after 14, 49 and 90 days of storage in the years 2020 and 2021.

MATERIAL AND METHODS

Fresh maize material from the years 2020 (breed: *KWS Bernardino*) and 2021 (breed: *AgroMais Amaveritas)* was ensiled in laboratory mini silos (crude nutrients and number of lactic acid bacteria, yeasts and molds: Table 1).

Table 1: Crude nutrients and number of lactic acid bacteria, yeasts and molds of the fresh maize of 2020 and 2021

Parameter	**2020**	**2021**
Dry Matter (%)	29.5	32.8
Water soluble carboydrates (% DM)	12.2	10.0
Crude ash (% DM)	3.5	2.4
Crude fibre (% DM)	23.6	17.5
Starch (% DM)	28.9	35.7
Lactic acid bacteria (cfu/g FM)	4.0E+03	4.8E+07
Yeasts (cfu/g FM)	4.0E+06	2.2E+05
Molds (cfu/g FM)	1.4E+04	8.0E+04

Treatments consisted of an untreated control and a treated variant with a mixture of homo – and heterofermentative LAB (*L.rhamnosus*, *L.buchneri* and *L.diolivorans*: *Bonsilage Speed M*) at one application rate of 250.000 CFU/ g FM in a total of three replications. After 14, 49 and 90 days of fermentation at a constant storage temperature of 20°C, the silages were exposed to fresh air after opening the mini silos. Maize samples were analyzed to determine forage quality (pH-value, fermentation pattern and number of yeasts and molds) as well as aerobic stability according to Honig (1996). For statistical evaluation, means and standard deviations were calculated for each parameter. The data were examined by SPSS evaluation including Mann Whitney UTest for significant differences ($P<0.05$) between the control and the treatment group.

RESULTS

The LAB mixture containing *L. diolivorans* led to significantly higher ($p< 0.05$) amounts of lactic acid and acetic acid and a lower pH after 14 days of storage in both years. Acetic acid and n-propanol was higher ($p <0.05$) at day 49 and 90 in both years, while propionic acid was increased only after 90 days of storage in both years ($p<0.05$). The LAB mixture containing *L.diolivorans* enhanced aerobic stability significantly ($p < 0.05$) for more than 4 (2020) and 3 days (2021, respectively) after 14 days of storage, while aerobic stability was improved for more than 3 days after 49 days of storage in both years. After 90 days of storage, both variants were stable during the test of aerobic stability in the years 2020 and 2021, respectively. Furthermore, number of yeasts were significantly decreased after 14 and 49 days of storage in both years with LAB mixture containing *L.diolivorans.*

Table 2 Silage parameters of the maize silages 2020 and 2021 after different days of storage for control (a) and treated LAB mixture (b)

Year 2020	14		49		90	
	a	b	a	b	a	b
DM$_c$ (%)	28.9	28.8	28.2	27.3	27.9	27.5
LA (% FM)	1.2^{a}	1.7^{b}	1.5	1.6	1.7^{a}	1.4^{b}
AA (% FM)	0.4^{a}	0.6^{b}	0.4^{a}	0.8^{b}	0.5^{a}	0.9^{b}
PD (% FM)	0.0^{a}	0.1^{b}	0.0^{a}	0.1^{b}	0.0^{a}	0.2^{b}
Pol (% FM)	0.0	0.1	0.0	0.1^{b}	0.0	0.1^{b}
PA (% FM)	0.0	0.0	0.0	0.0	0.0	0.1^{b}
pH	4.14^{a}	3.93^{b}	3.71	3.66	3.75^{a}	3.81^{b}
Yeasts (cfu/ g FM)	8.0 E+04^{a}	3.2 E+03^{b}	4.2 E+03^{a}	1.0 E+02^{b}	1.0 E+02	1.0 E+02
Molds (cfu/ g FM)	7.0 E+02^{a}	2.0 E+02^{b}	1.0 E+02	1.0 E+02	1.0 E+02	1.0 E+02
AS (days)	4.1^{a}	8.2^{b}	6.6^{a}	>10^{b}	>10	>10

Year 2021	14		49		90	
	a	b	a	b	a	b
DM$_c$ (%)	32.9	32.3	31.7	30.8	31.6^{a}	30.2^{b}
LA (% FM)	0.9^{a}	1.1^{b}	1.8	1.9	1.9^{a}	1.7^{b}
AA (% FM)	0.4^{a}	0.6^{b}	0.6^{a}	0.9^{b}	0.6^{a}	1.1^{b}
PD (% FM)	0.0^{a}	0.1^{b}	0.0^{a}	0.4^{b}	0.0^{a}	0.3^{b}
Pol (% FM)	0.0	0.0	0.0^{a}	0.2^{b}	0.0^{a}	0.3^{b}
PA (% FM)	0.0	0.0	0.0	0.0	0.0	0.0
pH	4.01^{a}	3.91^{b}	3.81^{a}	3.72^{b}	3.83^{a}	3.75^{b}
Yeasts (cfu/ g FM)	1.1 E+03^{a}	2.0 E+02^{b}	2.2 E+03^{a}	1.0 E+02^{b}	1.0 E+02	1.0 E+02
Molds (cfu/ g FM)	1.0 E+02	1.0 E+02	1.0 E+02	1.0 E+02	1.0 E+02	1.0 E+02
AS (days)	2.9^{a}	6.3^{b}	5.8^{a}	9.4^{b}	>10	>10

DM$_c$ = DM corrected, LA=Lactic acid; AA= Acetic acid; PD= 1,2-Propandiol; Pol= n-Propanol; PA= Propionic acid; AS= Aerobic stability (max. length: 10 days);^{a},^{b} symbolize significant differences ($p < 0.05$)

DISCUSSION

The results indicate a special fermentation pattern of the LAB mixture containing *L. diolivorans*. In contrast to Krooneman et al. (2002) a rapid production of lactic acid, acetic acid and n-propanol was observed - even after 14 days of storage. Furthermore, a significant improvement of aerobic stability was observed. The use of a LAB mixture containing *L.diolivorans* is stated as a good alternative for farmers which have to open the silage immediately after harvest.

CONCLUSIONS

The results of this study clarify the potential of improving aerobic stability of maize silage even after a short time of storage with using a LAB mixture containing *L. diolivorans*.

REFERENCES

Honig, H. 1990. Evaluation of aerobic stability. In: Lindgren, S. & Pettersson, K. (eds.), Proceedings of the EUROBAC Conference, Uppsala, Sweden, 1990, special issue, pp. 76-82.

Krooneman, J., Faber, F., Alderkamp, A. C., Elferink, S.J., Driehuis, F., Cleenwerck, I., Swings, J., Gottschal, J. C., Vancanneyt, M. & Nousiainen, J. 2002. *Lactobacillus diolivorans* sp. nov., a 1,2-propanediol degrading bacterium isolated from aerobically stable maize silage. International Journal System Evolution Microbiology, 52, 639-646.

THE QUALITY OF MAIZE SILAGES TREATED WITH BIOSTABIL MAYS

LOUČKA[1], R., JAMBOR[2], V., JENKINS[3], V., MUELLER[3]. I.
[1]Institute Animal Science Prague
[2]NutriVet Ltd. Pohořelice
[3]Biomin Holding GmbH, Erber Campus 1, 3131 Getzersdorf, Austria

INTRODUCTION

The use of effective silage additives is increasingly important. The aim of the work was to assess the quality of maize silages treated with BioStabil Mays (BSM) - Silage Inoculant.

MATERIAL AND METHODS

Maize forage (hybrid Agro Vitallo FAO 280) was harvested from a commercially operated farm in Knínice u Pohořelic, Czech Republic. The forage was harvested at a DM level of 41 to 42 %, with a forage cutter (KRONE) and a kernel processor set at 8 mm of theoretical cutting length and 25 cm cutting height. At ensiling, forage was separated into two piles and the following two treatment were applied:

1) CTL- Control: No inoculant applied,
2) BSM- Silage Inoculant BioStabil Mays HC applied at 1x 10^5 cfu/g FM.

A dilution of the test product in 10 mL of distilled water per kg of fresh forage (5 ml BioStabil Mays HC + 45 ml water per 5 kg FM - amount for 4 L glass bottles) was applied to one of the two piles (B). On the other pile, the same amount of distilled water 10mL was applied to fresh forage, but without the test product (C). Treatments were applied using a manual sprayer. For both trials, glass jars (mini silos) with a volume of 4 L were prepared within 4 h after harvesting. For each treatment and opening time point (42 days and 92 days), 4 replicates were prepared.

Fresh forage at ensiling was analysed for pH, microbial contamination (yeast and molds), starch digestibility and NDF digestibility (*in situ* in rumen fistulated cows, fermented for 24 hours).

Silage at day 42 and day 92 for microbial contamination (yeast and molds), starch digestibility and NDF digestibility (*in situ* in rumen fistulated cows, fermented for 24 hours), fermentation profile (lactic acid, acetic acid, propionic acid, butyric acid, alcohols - 1,2 propanediol, 1- propanol, NH_3 and ethanol), DM loss (not corrected for volatiles). Analysis were carried out according to AOAC 2005.

Aerobic stability was tested for 7 days after opening the silos after 42 days and 92 days of fermentation, with three replicates at 25°C (room temperature). Temperature was measured every 15 minutes with data loggers. Aerobic stability was defined as the time needed until silage temperature reached 2°C above room temperature. pH was measured up front and after 7 days of aerobic exposure.

To analyze for pH and DM losses of silages, fresh forage was filled in plastic bags, from which air was removed (VaxSys). Plastic bags were opened after 2, 3, 7, 14, 21,42 and 92 days

Statistical Analysis was carried out using STATISTICA 10.1 (StatSoft, Inc. 2011, Tulsa, OK, USA), program. Data was analyzed by ANOVA multifactorial (or monofactorial) procedure and by the subsequent POST-HOC Tukey HSD test. The significance level was set to $\alpha = 0.05$.

RESULTS AND DISCUSSION

Fresh Forage

There were only minor differences in the nutrient values of the fresh forage when ensiling Control and BioStabil Mays treatment groups. The sugar content was high enough in both variants, to ensure a desired fermentation to lactic acid and respective drop in pH. The dry matter content was slightly higher than recommended for silage in both treatment groups.

Nutrient value of silages

Only minor differences of nutrient value between treatments could be observed after 42 and 92 days of fermentation. Content of ADF tended to be higher for BioStabil Mays group after 42 days ($p=0.055$) and after 92 days ($p=0.090$) of fermentation. Energy content of silage and methane production remained unaffected by treatment. With an increasing maturation stage of the silage, the potential milk production per ton of dry matter increased, independent of dietary treatments.

Fermentation profile

Already two days after ensiling the fresh forage, the pH started to drop and decreased continuously. After only 7 days, the ensiled forage reached the pH level of finished silage. In BioStabil Mays group, the pH was significantly lower compared to Control group after 3 days of fermentation ($p<0.01$) and after 7 days of fermentation ($p<0.05$).

The pH of BioStabil Mays treated silage tended to be lower after 42 days ($p=0.069$) and after 92 days of fermentation ($p=0.090$) compared to Control group. Between d 42 and d 92 the pH decreased significantly ($p<0.001$) in both treatments. No differences in volatile fatty acid – composition (VFA) between treatments could be observed after 92 days of fermentation. However, after 42 days of fermentation, the fermentation profile showed higher contents of acetic acid ($p<0.001$) and propionic acid ($p<0.001$) and a higher content of total VFA ($p<0.001$) in the

Control group compared to the BioStabil Mays group. The ratio of lactic acid to total VFAs (LA/VFA) was higher in the BioStabil Mays group compared to the Control group ($p<0.001$). On day 42 Amino- Nitrogen ($N\text{-}NH_2$ %) tended to be higher ($p=0.069$) in the BioStabil Mays group. No effect on Ammonia- Nitrogen could be observed on d 92, but formol titration was higher in the BioStabil Mays group compared to Control ($p=0.005$), with no effect on Amines ($N\text{-}NH_2$ %).

Dry matter losses were significantly reduced in the BioStabil Mays group at all sampling time points (d 42; $p=0.013$ and after 92 days $p=0.033$). After 92 days of fermentation, dry matter losses were reduced by 33 % (10.64 vs. 7.11%) in BioStabil Mays group.

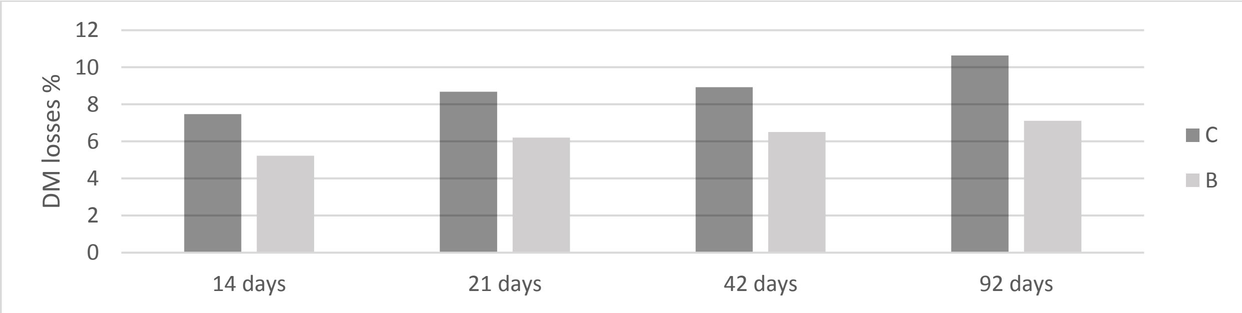


Figure 1: Dry matter losses (%) in plastic bags for Control (C) and Biostabil Mays treatment (B).

Digestibility

No significant differences between treatments group were found on day 42 and day 92 for digestibility of organic matter, as well as digestibility of NDF.

Microbial contamination

The total number of microorganisms (CPM) and the contents of micromycetes (both yeasts and fungi) decreased with the time of storage in the silo, while the content of LAB increased.

Significant higher LAB was found in fresh forage of BioStabil Mays treatment ($p< 0.05$) and after 42 days ($p< 0.01$) of fermentation. No differences between treatment groups in LAB could be observed after 92 days of fermentation. Higher CPM were found in BioStabil Mays group only after 42 days of fermentation ($p<0.01$). No differences between treatments in yeasts, molds and micromycetes could be observed at any sampling time point.

Aerobic Stability

Aerobic stability (HONIG, 1991) was defined as the time needed until silage temperature reached 2°C above the room temperature. After 42 days of fermentation, aerobic stability (≥2°C) was improved by 35.6 hours ($p<0.01$) as well as aerobic stability (≥3°C) by 33.5 h. The time until the maximal temperature was reached was reduced by 23.5 hours ($p<0.01$) and was 4°C lower compared to Control group ($p=0.04$). After 92 days of fermentation aerobic stability (≥3°C) was improved by 11.7 h ($p<0.001$). The time until the maximum temperature was reached tended ($p=0.07$) to be lower for Biostabil Mays treatment and had a lower maximum temperature by 2°C ($p=0.03$).

CONCLUSIONS

Only minimal differences in nutritional values of fresh forage between treatment and Control without silage inoculant was observed, even after fermentation of 42 and 91 days. BioStabil Mays treated silage had significantly lower pH compared to Control after 7 days of fermentation, tended to reduced Ammonia (%) levels after 42 days of fermentation, resulted in higher content of LAB and yeast and significantly reduced dry matter losses by 33 % after 92 days of fermentation. The trial confirmed that BioStabil Mays improves aerobic stability by 35.6 h after 42 days of fermentation.

ACKNOWLEDGMENT

Thanks to Biomin Holding GmbH and the project QK18180137 for supporting the trial.

REFERENCES

AOAC (2005) Association of Official Analytic Chemists. Official methods of analysis. 18th ed. Gaithersburg, USA

HONIG H. (1991) Reducing losses during storage and unloading of silage. Landbauforschung Voelkenrode. Sonderheft (Germany, FR).

EFFECT OF HARVEST DATE OF 9 PEA VARIETIES ON NUTRITIONAL VALUE AND BIOGASABILITY

LOUČKA, R.[1], JAMBOR, V.[2], JANČÍK, F.[1], HOMOLKA, P.[1], DOSTÁLOVÁ, R.[3]
[1]Institute of Animal Science. Prague Uhříněves
[2]NutriVet s.r.o. Pohořelice
[3]AGRITEC, výzkum, šlechtění a služby, s.r.o. Šumperk

INTRODUCTION

The benefits of legumes and their mixtures with cereals (LOS) as a source of high-quality forage can only be achieved if they are made into high-quality and healthy silage with a high nutritional value, and if the nutritional value of the produced silage is determined as accurately as possible, and further that these silages will be well included in feed rations. The problem is that those who calculate feed ration proposals for cattle have only outdated or piecemeal information about the nutritional value of legumes and LOS. This was also the reason why the NAZV project QK21010344 "Domestic protein crops in cattle nutrition" was created.

From the ÚKZÚZ Methodology for utility value tests (ZUH), which is valid from 1 August 2019 and has been updated for the year 2022, information on the nutritional values of peas for ensiling purposes cannot be read. The methodology is intended for the study of the pea grain. Thus, the basis for zootechnicians and feed manufacturers remains the "VÚVZ Pohořelice feed catalog" from 1995 (Zeman et al. 1995). In this catalog, peas are listed as silage with a dry matter (DM) content of 18%, or crude fiber content 22% and digestible nitrogenous substances 13% in DM. The starch content is not listed at all. These data are not applicable for the compilation of feed rations for high-performance dairy cows.

Individual creeping peas are sown as a monoculture at a dose of 250 to 300 kg/ha. The advantage is the simple method of establishment, on the other hand, in the first phase of growth, it does not create perfect shading and is therefore not suitable for arid areas. When harvesting in wetter areas, there is a risk of pulling out the plants with their roots instead of clean mowing. The lower harvested DM in this crop mostly necessitates a two-phase harvest. However, care must be taken that the DM of the cut is not too high. At about 30% DM, the peas are not heated and the beta-carotenes remain in them. At a higher DM content above 35%, it can be assumed with high probability that the silage will have very low aerobic stability and will mold after opening the silo (especially when stored in a bag), because a lot of sugars remain in the silage even after fermentation. The principle of the excellent feeding effect of pea silage is that the storage protein of the pea seed is not fed, but the whole plant, while the seed proteins in the waxy-milky maturity are functional, not storage, and the silage is also characterized by high palatability.

According to Lu et al. (2019) field pea has a relatively new type of plant proteins including globulin, albumin, prolamin and glutelin. Globulin and albumin are the major storage proteins in pea seeds.

Field pea *Pisum sativum* L. was grown in a field trial with the aim of obtaining information on nutritional values and the suitability of the harvest date for conservation by ensiling.

MATERIALS AND METHODS

The field experiment with pea varieties was sown on AGRITEC plots in Šumperk with a HEGE small-plot seeder in three repetitions. 7 varieties of semi-leafness pea (HÚ), suitable for ensiling (Gambit, Impuls, Trendy, Avatar, Saxon, Protin, Atoll) were selected for the experiment. The leaf type Protecta and the semi-leafness type Turnia (HLP) were assigned to them. During the growing season, the experiment was treated with registered herbicides against monocotyledonous and dicotyledonous weeds. Before flowering, an insecticide was also applied against aphids and preventively against grain blight. The cultivation and silage technology is described in detail in the publication Loučka and Tyrolová (2013). Sampling was carried out on two dates: 11/07 and 26/07/2022. All chemical analyzes were carried out in the agricultural laboratory of NutriVet Ltd. in Pohořelice according to AOAC (2005).

Statistica 10 (StatSoft, Tulsa, OK, USA) was used to evaluate the results by analysis of variance (ANOVA) with a multivariate design.

RESULTS AND DISCUSSION

A significant difference between the first and second sampling was in the DM content. At the first sampling on 7/11, the DM content of 286 ± 39 g/kg for all varieties was still low for silage, but manageable with the use of a suitable silage preparation, preferably a chemical one based on formic acid. However, in the second sampling on 7/26, just 14 days after the first, the DM content was already 697 ± 67 g/kg, i.e. so high that ensiling such a mass would not be possible. It follows from this that it is necessary to watch the harvest date very carefully and to harvest the stands on time. This is emphasized, for example, by Bal et al. (2000). A significant difference between semi-leafness peas and other types of peas was in the starch content (in the first sampling 245 vs. 214, respectively in the second sampling 270 vs. 215 g/kg DM) and ash content (in the first sampling 58 vs. 71, respectively in the second sampling 69 vs. 78 g/kg DM). The difference between the two samplings was also in the digestibility of organic matter (65.5 vs. 70.8%). From the point of view of nutritional value and potential milk production, the newly bred Atoll variety had the highest NEL, while the Protin variety had the lowest NEL. For use in biogas stations, the leaf type Protecta variety from the first harvest is best suited. For processing into silage, the Gambit semi-leafness pea variety is best suited not only because it has the highest starch content at the time of the first harvest and has a favorable ratio between NDF and ADF, which is hemicellulose, which is easily decomposed by lactic acid bacteria during silage fermentation. The starch contents are in accordance with the study by Dostálová and Horáček (2009).

Tab. 1: Chemical analyzes of peas (DM in g/kg; nutrients in g/kg DM)

Index	HÚ		HLP		SEM			P-value		
	2. odběr	1. odběr	2. odběr	1. odběr	HÚ	HLP	O	D	O	D x O
DM	707,8	296,5	658,1	247,3	20,6	38,5	21,8	0,131	**0,001**	0,992
Protein	162,1	133,8	150,9	147,0	5,78	10,81	6,13	0,911	0,084	0,182
NDF	392,5	403,1	385,9	382,4	21,8	40,7	23,1	0,682	0,914	0,833
ADF	279,9	285,9	258,8	277,0	16,1	30,1	17,0	0,543	0,623	0,804
Starch	269,7	244,6	214,7	213,7	9,9	18,6	10,6	**0,012**	0,397	0,435
Ash	68,9	58,4	77,7	71,5	1,62	3,03	1,72	**0,001**	**0,004**	0,401
DNDF24	45,8	38,6	41,7	45,1	3,03	5,67	3,21	0,79	0,687	0,268
DOM24	70,7	64,9	71,2	67,5	1,10	2,05	1,16	0,37	**0,012**	0,522
NEL	5,71	5,50	5,57	5,63	0,07	0,14	0,08	0,90	0,504	0,237
Milk	1803	1737	1755	1775	23,1	43,3	24,5	0,89	0,522	0,231
Methane	398	412	393	413	11,1	20,8	11,8	0,88	0,338	0,860

HÚ = semi-leaf pea; HLP = leaf pea and white pea; D = type of forage; O = collection order; DNDF24 = % fiber digestibility in 24 hours of incubation; DOM24 = % digestibility of organic matter in 24 hours of incubation; NEL = net energy of lactation in MJ/kg DM; Milk = potential milk production in kg/t DM; Methane = measured methane production in kg/t DM

Shortly after the second sampling (June 29, 2022), the grain yields at harvest were between 3.86 and 5.25 kg per 10 m^2, with the highest yield for the Saxon variety and the lowest for the Impuls variety. The pea yields of the Protecta leaf variety were comparable to those of creeping pea.

Tab. 2: Yields of pea varieties (kg/10 m2) after the second sampling at grain harvest

Index	Gambit	Impuls	Trendy	Avatar	Saxon	Protin	Atoll	Protecta	Turnia
Výnos	4,51	3,86	4,93	4,74	5,25	4,83	4,84	4,25	4,91

CONCLUSIONS

In the Šumperk region, the beginning of July is a good time for harvesting. For ensiling, semi-leafness peas of the Gambit variety were the most suitable. The nutritional values and yields of the pea variety Protecta were comparable to those of the semi-leafness pea. In the next year, it is necessary to focus not only on comparing varieties and determining a suitable harvest date, but also on solving the harvesting technology. If the peas are harvested for ensiling at a suitable stage, they do not reach the required DM content for a good fermentation, but a two-phase harvest is not suitable (difficult loading and usually a higher ash content).

The contribution was created thanks to the support of the NAZV project QK21010344.

REFERENCES

AOAC – Association of Official Agricultural Chemists. Official methods of analysis. 18th ed. Washington, DC: AOAC International; 2005.

Bal MA, Shaver RD, Shinners KJ, Coors JG, Lauer JG, Straub RJ, Koegel RG. Stage of maturity, processing, and hybrid effects on ruminal in situ disappearance of whole-plant corn silage. Anim. Feed Sci. Tech., 2000 July 31;86(1-2):83-94.

Dostálová R. a Horáček J. Sledování změn obsahu škrobu a rezistentního škrobu u hrachu Chem. listy. 2009; 103:753-782.

Loučka R. a Tyrolová Y. Správná praxe při silážování hrachu. Certifikovaná metodika MZe, 2013, 29 s.

Lu ZX, He JF, Zhang YC, Bing DJ. Composition, physicochemical properties of pea protein and its application in functional foods, Critical Reviews in Food Science and Nutrition. 2019:1-12.

Zeman L. a kol. Katalog krmiv. VÚVZ Pohořelice, 1995, 465 s.

EFFECTS OF A MIXTURE OF LACTIC ACID BACTERIA CONTAINING LACTOBACILLUS DIOLIVORANS ON AEROBIC STABILITY OF GRASS SILAGE AFTER SHORT TIME OF STORAGE

KRAMER[1], E., SPRAFKE[2], J., NELLES[2], M.
[1]ISF GmbH, Pinneberg
[2]University Rostock, Faculty of Agricultural and Environmental Sciences, Department Waste and Resource Management, Rostock

Keywords: aerobic stability, *Lactobacillus diolivorans*, storage time

INTRODUCTION

Heterofermentative lactic acid bacteria (LAB), e.g., *Lactobacillus buchneri* are widely used to improve aerobic stability in silages. Nevertheless, a minimum time of storage of at least 6 to 8 weeks is needed for a significant effect on aerobic stability. Since several years new heterofermentative strains such as *Lactobacillus diolivorans* are used to improve aerobic stability even after a short time of storage of 14 days. The aim of the present study was to assess the short-term effect of a LAB mixture containing *L. diolivorans* on aerobic stability of grass silage after 14, 49 and 90 days of storage in first cut grass silage in the years 2020 and 2021.

MATERIAL AND METHODS

First cut grass material (*Festuca arundinaceae*) from the years 2020 and 2021 was ensiled in laboratory mini silos (crude nutrients and number of lactic acid bacteria, yeasts and molds: Table 1).

Table 1: Crude nutrients and number of lactic acid bacteria, yeasts and molds of the fresh grass of 2020 and 2021

Parameter	**2020**	**2021**
Dry Matter (%)	35.3	43.2
Water soluble carboydrates (% DM)	12.8	9.4
Crude protein (% DM)	19.5	13.9
Crude ash (% DM)	9.5	8.1
Crude fibre (% DM)	25.9	28.4
NDForg (% DM)	51.6	56.7
ADForg (% DM)	30.0	33.7
Lactic acid bacteria (log cfu/g FM)	6.4	7.5
Yeasts (log cfu/g FM)	5.4	5.9
Molds (log cfu/g FM)	4.2	3.9

Treatments consisted of an untreated control and a treated variant with a mixture of homo – and heterofermentative LAB (*L.plantarum*, *L.buchneri* and *L.diolivorans*: *Bonsilage Speed G*) at one application rate of 250.000 CFU/ g FM in a total of three replications. After 14, 49 and 90 days of fermentation at a constant storage temperature of 20°C, the silages were exposed to fresh air after opening the mini silos. Forage samples were analyzed to determine forage quality (pH-value, fermentation pattern and ammonia-N) as well as aerobic stability according to Honig (1996). For statistical evaluation, means and standard deviations were calculated for each parameter. The data were examined by SPSS evaluation including Mann Whitney U-Test for significant differences ($P<0.05$) between the control and the treatment group.

RESULTS

The LAB mixture containing *L. diolivorans* led to significantly higher ($p< 0.05$) amounts of lactic acid, acetic acid, n-propanol and a lower pH after 14 and 49 days of storage. NH_3-N was significantly reduced with LAB mixture at all opening days in 2020, while it was significant lower only at opening day 14 in 2021. In addition, the LAB mixture containing *Lactobacillus diolivorans* enhanced aerobic stability significantly ($p < 0.05$) for more than 3 days after 14 days of storage in both years, while aerobic stability was improved for more than 5 (2020) and 6 (2021) days after 49 days of storage. After 90 days of storage, both variants were stable during the test of aerobic stability in 2020 (>10 days each) and 2021 (9.6 days in control and >10 days with the LAB mixture, respectively, Table 2). Butyric acid was not detected at all. NH_3-N was significantly reduced with LAB mixture at all opening days in 2020, while it was only significant lower at opening day 14 in 2021.

Table 2 Silage parameters of the grass silages 2020 and 2021 after different days of storage for control (a) and treated LAB mixture (b)

Year 2020	14		49		90	
	a	b	a	b	a	b
DM_c (%)	33.0	34.0	32.8	32.8	31.4	31.9
LA (% FM)	1.2[a]	1.8[b]	1.6[a]	2.1[b]	1.7[a]	2.0[b]
AA (% FM)	0.2[a]	0.7[b]	0.5[a]	0.9[b]	0.7[a]	1.0[b]
PD (% FM)	0.0[a]	0.1[b]	0.0[a]	0.2[b]	0.0[a]	0.1[b]
Pol (% FM)	0.0	0.0	0.0	0.2[b]	0.0[a]	0.3[b]
PA (% FM)	0.0	0.0	0.0	0.0	0.0[a]	0.1[b]
pH	4.58[a]	4.44[b]	4.39[a]	4.24[b]	4.42[a]	4.32[b]
NH3-N/ total-N (%)	7.1[a]	6.3[b]	8.0[a]	7.1[b]	9.9[a]	8.8[b]
AS (days)	2.6[a]	5.9[b]	3.9[a]	>10[b]	9.6	>10

Year 2021	14		49		90	
	a	b	a	b	a	b
DM_c (%)	42.3	42.6	42.0	42.1	41.8	41.3
LA (% FM)	1.9[a]	2.5[b]	2.1[a]	2.2[b]	2.1	1.9
AA (% FM)	0.4[a]	0.6[b]	0.7[a]	0.9[b]	0.7[a]	1.2[b]
PD (% FM)	0.0[a]	0.1[b]	0.0[a]	0.3[b]	0.0[a]	0.3[b]
Pol (% FM)	0.0[a]	0.1[b]	0.0[a]	0.2[b]	0.0[a]	0.4[b]
PA (% FM)	0.0	0.0	0.0	0.0	0.0	0.1[b]
pH	4.4[a]	4.1[b]	4.3[a]	4.2[b]	4.3[a]	4.4[b]
NH3-N/ total-N (%)	7.2[a]	6.2[b]	9.2	9.1	10.3	10.6
AS (days)	3.5[a]	6.6[b]	4.4[a]	>10[b]	>10	>10

DM_c = DM corrected, LA=Lactic acid; AA= Acetic acid; PD= 1,2-Propandiol; Pol= n-Propanol; PA= Propionic acid; AS= Aerobic stability (max. length: 10 days);[a],[b] symbolize significant differences ($p < 0.05$)

DISCUSSION

The results indicate a special fermentation pattern of the LAB mixture containing *L. diolivorans*. In contrast to Krooneman et al. (2002) a rapid production of lactic acid, acetic acid and n-propanol was observed - even after 14 days of storage. Furthermore, a significant improvement of aerobic stability was observed. The use of a LAB mixture containing *L.diolivorans* is stated as a good alternative for farmers which have to open the silage immediately after harvest.

CONCLUSIONS

The results of this study clarify the potential of improving aerobic stability of grass silage even after a short time of storage with using a LAB mixture containing *L. diolivorans*.

REFERENCES

Honig, H. 1990. Evaluation of aerobic stability. In: Lindgren, S. & Pettersson, K. (eds.), Proceedings of the EUROBAC Conference, Uppsala, Sweden, 1990, special issue, pp. 76-82.

Krooneman, J., Faber, F., Alderkamp, A. C., Elferink, S.J., Driehuis, F., Cleenwerck, I., Swings, J., Gottschal, J. C., Vancanneyt, M. & Nousiainen, J. 2002. *Lactobacillus diolivorans* sp. nov., a 1,2-propanediol degrading bacterium isolated from aerobically stable maize silage. International Journal System Evolution Microbiology, 52, 639-646.

FORAGE QUALITY AND YIELDS OF *FESTUCA ARUNDINACEA* SCHREB. IN 4-CUT MANAGEMENT (INTENSIVE) IN A LONG-TERM FIELD EXPERIMENT

MENŠÍK, L.[1], NERUŠIL, P.[1], PLISKOVÁ, J.[1], KOLOMAZNÍKOVÁ, J.[2], KYSELÝ, D.[2]
[1] Crop Research Institute /CRI/ Prague 6 - Ruzyně, Grassland Research Station /GRS/ Jevíčko, K. H. Borovského 461, 569 43 Jevíčko, Czech Republic
[2] AGROKOP HB Ltd., Kyjovská 3607, 580 01 Havlíčkův Brod

INTRODUCTION

Grasslands are considered as most valuable economic elements in landscapes and agroecosystems. Grasslands ensure a high quality forage production and at the same time other non-productive functions (Lei et al 2020). The production and quality of forage and arable crops in currently strongly affected by global climate change (GCC) (Mirzabaev et al 2023), so farmers must adapt selection of new grass species and varieties that are able to cope with dry conditions during the growing season. According to long-term meteorological measurements, provided by the Czech Meteorological Office (1961-2021), a significantly increasing trend in temperature was recorded over the last 50 years in the Czech Republic, resulting in 2°C higher mean temperature (Menšík et al. 2022a). On the other hand, the average precipitation stayed unchanged, and significant effect of uneven distribution over the growing season was recorded, increasing soil evaporation and the occurrence of drought episodes (Rožnovský 2022). To compensate deficit of roughage, caused by warmer and dryer weather conditions, farmers must search for solutions, such as utilization of thermophilic C4 plants (e.g., sorghum /Menšík et al. 2022b/, millet /Hermuth et al. 2023/) and drought-tolerant grasses in the *Poaceae* family, which currently include newly bred varieties of tall fescue and some intergeneric grass hybrids (festulolium). Therefore, seeds of grass mixtures containing such species become interesting for farmers restoring and establishing grassland areas. Such properties could offer tall fescue (*Festuca arundinacea* Schreber.), which has been less used in the past because of its lower feeding value and voluntary intake of forage by animals. The high persistence and production capacity of *Festuca arundinaceae*, including its intergeneric hybrids, is therefore an important biological prerequisite for inclusion in perennial intensive forage mixtures on arable land. This paper evaluates the yield and selected forage quality parameters of new French varieties of tall fescue in the sixth crop year (2021, 2022) and analyze its suitability for intensive use under the conditions of region in the Czech Republic (Mala Haná, Boskovice Furrow).

MATERIALS AND METHODS

The small-plot experiment with tall fescue /TF/ was established in 2016 with a quick-renewal permanent grassland (oat type /*Arrhenatheretum*/, harvested three times per year using the large-scale technology) on the experimental plot of the CRI, GRS Jevíčko (elevation 342 m above sea level, average annual temperature 8.4 °C and average annual rainfall 559 mm (Czech Hydrometeorological Institute Ostrava /CHI/, meteorological station Jevíčko, period 1991–2020), soil type: brown soil modal. Variants of the experiment: (1) control (inland variety, DLF Seeds Hladké Životice); (2) variety FR1; (3) variety 'Callina'; and (4) variety FR3 (all three RAGT Semences, France).

Fertilizers, applied between 2017-2022, consisted of mineral nitrogen, phosphorus and potassium. Mineral nitrogen was applied as limestone ammonium nitrate (180 kg ha^{-1} applied in three doses in spring, after 1st and 2nd cutting), phosphorus as superphosphate (30 kg ha^{-1} P), and potassium as potassium salt (60 kg ha^{-1} K), both applied in spring. Harvesting was done using the small-plot forage harvester MPZ–115 and Wintersteger Cibus F/S. Dry matter (DM) production was determined on the basis of green matter yield and laboratory determined dry matter of harvested forage in the 1–4th cut. Using the NIRS instrumentation technique (Mika 1997), the main forage quality parameters were determined: crude protein content (CP), fiber content, water soluble carbohydrates (WSC), net energy of lactation (NEL) and organic matter digestibility (OMD). Potential milk production (PMP) was determined according to dry matter content and net energy of lactation /NEL/ production in each individual cutting between 2021–2022. Data were statistically evaluated (descriptive statistics, ANOVA) using the STATISTICA 14.0 (StatSoft, Inc. 2021).

RESULTS AND DISCUSSION

With the temperature of 8.3°C, the year 2021 was the second coldest year in the region of Mala Hana (Jevíčko) in the last ten years Precipitation in 2021 (544 mm) was evaluated as average, corresponding with 97% of the long-term normal (1991-2020).In contrast, September was exceptionally dry, with only one-fifth of the normal rainfall, making it the fourth driest September since 1961 (meteorological station Jevíčko, CHI Ostrava). The period of 2022 was average in terms of precipitation and temperature when compared to the long-term average. The total precipitation was 564 mm and the average air temperature was 0.9 °C higher than long-term mean (111 % of the long-term average). The DM production of all varieties was balanced in 2021, which was close to the 30-year normal in terms of meteorological characteristics. The highest DM production for all cuts (sum of cuts) was provided by 'Callina', 12.4 t ha^{-1}, while the lowest in Control, 12.0 t ha^{-1}. Differences in DM production among the varieties were insignificant ($\alpha < 0.05$). The CP content was balanced (130–178 g kg^{-1}) across all cuts and variants in 2021, with no statistically significant differences between cuts. WSC content was balanced between the variants within each cut, with the highest WSC content determined in the 3rd cut (no statistically significant differences between variants and within cuts). The NEL varied from 5.5 to 6.6 MJ kg^{-1}. OMD was higher in the 1st, 3rd and 4th cut when compared to the 2nd cut. OMD ranged from 64 to 71% (with no statistically significant differences within cuts). Potential milk production (PMP) was comparable between the analyzed variants varied from 23.3 to 23.7 thousand kg milk per ha in 2021. In the warmer year of 2022 (average annual air temperature almost 1°C higher than normal, above-average temperatures in the summer and occurrence of significantly dry period), the differences in yields between the evaluated variants were higher. The highest DM production in 2022 was found in the 'Callina' variant (9.3 t ha^{-1}), while the lowest DM production was found in the Control (8.0 t ha^{-1}). The CP content was balanced in 2022 in the 1st and 2nd cut and among variants (130–134 and 182–188 g kg^{-1} dry matter, respectively), without statistically significant difference ($\alpha < 0.05$) between the variants and within the 1st and 2nd cut. In the 3rd and 4th cut, the CP content was different (higher content /172–174 g kg^{-1}/ recorded in the Control and FR1 variants, lower in the 'Callina' and FR3 variants /158–162 g kg^{-1}). The WSC content was balanced between the variants in the 1st and 4th cut, while in the 2nd and 3rd cut, the WSC content was higher in the 'Callina' and FR3 variants. The NEL varied from 5.5 to 6.4 MJ kg^{-1} of dry weight. The OMD ranged from 65 to 72%. Higher OMD in the 2nd, 3rd and 4th cut was recorded in the 'Callina' and FR3 variants (no statistically significant differences between variants and cuts). Potential milk production (PMP) was balanced between the Control, FR1 and FR3 variants (15.5–15.7 thousand kg ha^{-1} milk), with the highest PMP of 17.5 thousand kg ha^{-1} milk per ha in 2022 for the 'Callina' variant.

CONCLUSIONS

On the basis of the research carried out in the period 2017–2022 and the multicriteria evaluation of the obtained results (see Menšík et al. 2019, 2021, 2022, 2023), we can conclude that the French varieties 'Callina' and FR3 are efficient varieties, suitable for intensive use in the Czech Republic. The French late-season varieties have a winter character, significantly differ from the Control variety, and are characterized by a great foliage with broad leaf blades, finer surface and tendency of dropping rather than erection (unlike some early-season varieties). A positive finding (demonstrated by long-term monitoring in the conditions of the Mala Hana region /Boskovice Furrow/) is the fact, that despite the unfavorable weather conditions in climatically less favorable years 2018, 2019 and 2020, characterized by intensively warm and dry summer, these French varieties have retained their persistence (resistance), resulting in high yields in the following years (4–6 crop years). By slightly delaying the onset of vigorous growth in the growing season and extending the metering interval, they also maintain favorably high forage quality and high levels of production for longer time, which is better matched to the optimum harvest maturity of most of the widely grown, forage-valuable grass species. In view of the results, late-season varieties of tall fescue can be recommended as one of the carrier components of seed of perennial grass mixtures for drought-affected areas.

ACKNOWLEDGEMENTS

This paper was supported by contract research of AGROKOP HB, Ltd., Havlíčkův Brod and the Ministry of Agriculture, institutional support MZE-RO0423.

REFERENCES

Hermuth J., Menšík L., Svoboda P., Nesvadba Z., Frydrych J., Bradáčová L., Škrobák R., Liebich D., Lošák M., Mrkvicová E., Šťastník O., Pavlata L., Rysová J., Enochová H., Děd M., Podrábský M., Zavel O. 2023. Pěstební technologie a praktické využití českých vyšlechtěných odrůd béru italského Ruberit a Rucerus. Ověřená technologie. Praha: Výzkumný ústav rostlinné výroby, v.v.i., Praha – Ruzyně. 66 pp. ISBN 978-80-7427-387-2

Hervieu B. 2002. Multi-functionality: a conceptual framework for a new organisation of research and development on grasslands and livestock systems. In. Durand, J.L. et al. (Eds.). Multi-function grasslands: Quality Forages, Animal Products and Landscapes. British Grassland Society, U.K., vol. 7, p. 1–2.

Lei T., Feng J., Zheng C., Li S., Wang Y., Wu Z., Lu J., Kan G., Shao C., Jia J., Cheng H. 2020. Review of drought impacts on carbon cycling in grassland ecosystems. Frontiers of Earth Science, 17.

Menšík L., Nerušil P., Hermuth J., Menšík L., Podrábský M. 2022b. Čirok a bér pro výrobu objemných krmiv. Krmivářství 26(2): 24−26. ISSN 1212–9992.

Menšík L., Nerušil P., Kyselý D. 2019. Možnosti uplatnění nově vyšlechtěných francouzských odrůd kostřavy rákosovité pro intenzivní pícninářství v podmínkách ČR. Pícninářské listy. Agriprint, s.r.o., Olomouc, pp. 58–61.

Menšík L., Nerušil P., Kyselý D. 2021. Produkce a kvalita píce intenzivně využívaných víceletých porostů pozdních odrůd kostřavy rákosovité v letech 2019–2020 v oblasti Malé Hané. Pícninářské listy 2021, s. 90–95.

Menšík L., Nerušil P., Kyselý D. 2022a. Produkce píce porostů pozdních odrůd kostřavy rákosovité. Pícninářské listy 2022: 32–34.

Menšík L., Nerušil P., Kyselý D. 2023. Možnosti intenzivního využití festulolií a kostřav v oblasti Boskovické brázdy – výsledky za rok 2022. Pícninářské listy 2023. (v tisku).

Mirzabaev A., Olsson L., Kerr R.B., Pradhan P., Ferre M.G.R., Lotze-Campen H. 2023. Climate Change and Food Systems. In: von Braun, J., Afsana, K., Fresco, L.O., Hassan, M.H.A. (eds) Science and Innovations for Food Systems Transformation. Springer, Cham. https://doi.org/10.1007/978-3-031-15703-5

Rožnovský J. 2022. Změny teploty vzduchu a srážek za poslední desetiletí na území ČR. In Menšík L., Kunzová E., Madaras M. Současné hospodaření na zemědělské půdě v měnících se podmínkách prostředí – SOM (půdní organická hmota), 2. ročník. Praha: Výzkumný ústav rostlinné výroby, v.v.i., Praha – Ruzyně, p. 10−11. ISBN 978-80-7427-401-5.

FERMENTATION QUALITY OF MAIZE SILAGE WITH ADDITION OF *L. CASEI* AND *L. BUCHNERI*

JURÁČEK, M., BÍRO, D., KALÚZOVÁ, M., ŠIMKO, M., GÁLIK, B., ROLINEC, M., HANUŠOVSKÝ, O., MIČEK, A.

Slovak University of Agriculture in Nitra, Faculty of Agrobiology and Food Resources, Institute of Nutrition and Genomics, Department of Animal Nutrition, Trieda A. Hlinku 2, 94976 Nitra, Slovakia; xkaluzova@uniag.sk

INTRODUCTION

Achieving high performance, especially in dairy cows, due to the requirements of ruminal digestion, requires stable and long-term balanced feeding, especially because of the need to maximize the use of the components of the fibre complex for energy needs and to ensure the conditions for a high degree of proteosynthesis. Such parameters are fulfilled by high-quality preserved feeds which show minimal changes in nutrient content and quality during storage. Successful silage fermentation is intended to create an acidic environment under anaerobic conditions, suppressing the decomposing activity of plant enzymes and unwanted microorganisms and thus promoting the full development of lactic acid bacteria until the final stabilization of the feed. During the ensiling process, fermentation products are formed from fermentable carbohydrates, of which lactic acid is crucial in acidifying the mass and lowering the pH to a preservative value. In the conditions of Slovakia, maize silage is the main source of energy in the rations of ruminants in the most productive areas (Bíro et al., 2020). For these reasons, continuous attention is paid to maize in terms of selection of suitable hybrids and silage technology (Bíro et al., 2020; Jančík et al., 2022; Jambor et al., 2019; Orosz et al., 2016). Most farms have switched to year-round feeding of conserved forages and therefore their quality is the most decisive factor for the profitability of livestock production. The course of the fermentation process is influenced not only by the dry matte content and quality composition, but also by the addition of effective silage additives (Bíro et al., 2020; Doležal et al. 2012.).

The aim of this work was to investigate the effect of silage additive (*L. casei* and *L. buchneri*) addition on dry matter content and fermentation parameters of maize silage.

MATERIALS AND METHODS

The silage experiment with maize was carried out in the Laboratory of Feed Preservation in cooperation with VPP SPU s.r.o. Kolíňany, farm Oponice. The experiment included silage stay-green hybrid maize with FAO number 480, with dent type of kernel. The maize crop was harvested with a Class Jaguar self-propelled chopper at the milky-wax stage of grain maturity, with the chopper set to a theoretical chop length of 20±2 mm. First, the untreated mass (control: variant C) and then the treated mass (experimental variant A) was taken after starting the pressure preservative dispenser. The preservative in liquid form was sprayed into the mass at a dose of 1 g of additive in 10 ml per 1 ton of mass (1.1x1011 CFU.g-1). After sampling, the mass was filled into plastic mini-bags (n=3) from which the air was immediately sucked out with a Foodsaver powerful vacuum packer (FSGSSL0300) and sealed hermetically. The silage mini-bags were stored in standard conditions at a constant temperature of 22 ± 2 °C. After 9.5 months of filling, the silage mini-bags of both variants were opened and average samples were taken for chemical analysis. Dry matter was determined by drying the sample at 103 ± 2 ˚C. Lactic acid (LA), acetic acid (AA) and butyric acid (BA): were determined by isotachophoretic method - EA 100 analyzer (Villa Labeco), pH: electrometrically. The alcohols content was determined by the microdiffusion method, iodometric titration. Acidity of water extract (AWE): determination by alkalimetric titration of the water extract to pH 8.5 (the resulting AWE is expressed in mg KOH per 100 g of feed). Fermentation products (FP) were determined by calculating FP = lactic acid + volatile fatty acids + alcohols.

The results were statistically evaluated and processed by IBM SPSS 26.0. the descriptive statistics by Oneway Anova and differences between the control and experimental variant by independent samples T-Test were expressed ($P < 0.05$).

RESULTS AND DISCUSSION

Dry matter content was significantly ($P < 0.05$) lower in the experimental variant compared to the control. The difference of 9.17 g as dry matter loss during fermentation and storage was 2.26%. This confirmed the known fact that heterofermentative LAB added to silage in the form of additives increase dry matter and energy losses, which is their adverse effect. This fact is also pointed out by Loučka et al. (2020), according to whom increased acetic acid production, when heterofermentative LAB are used, increases the aerobic stability of silages, but the price for this positive effect is a relatively large loss of dry matter and a reduction in the energy value of silage as a feed for ruminants. The lactic acid content in the control group was 59.23 g in 1 kg dry matter and was significantly ($P < 0.05$) 10% lower in experimental group A. This fact indicates that *L. buchneri* transformed a part of the lactic acid into acetic acid as expected. This was manifested by a statistically significant increase ($P < 0.05$) in acetic acid content in the experimental variant, where the acetic acid content practically doubled from 13.30 g to 26.33 g, which is in agreement with the findings of Rajčáková et al. (2018). This also resulted in a narrowing of the lactic acid to acetic acid ratio from 4.45:1 to 2.02:1. Similar conclusions based on the results of additive experiments containing both homo- and heterofermentative LAB were presented by Gallo et al. (2018) and Szutz et al. (2018). It can be considered as a positive result that butyric acid was not observed in any of the variants despite higher harvested dry matter. At a higher dry matter, the mass is harder to press and it is necessary to shorten the cut so that enterobacteria and butyric acid bacteria do not become established at the beginning of fermentation. They are also buffered by the rapid drop in pH when the fermentation is properly directed. In this context, Bíro et al. (2011) point out that ensiling maize at a dry matter content higher than 40% is not a guarantee of quality silage, due to the possibility of the development of undesirable microflora and fermentation at higher temperatures, causing more oxygen locked in the silo. The digestibility of organic matter is reduced due to the higher lignin content and possible Maillard reaction. The pH value found in the control variant was 3.88 and in the experimental variant 3.97. The differences were statistically significant ($P < 0.05$), with the lower pH in the experimental group being related to the higher lactic acid content. It can be concluded that the pH value in the silages of both variants was at the required level and the silages were very well preserved. Based on the fermentation quality assessment by Mitrik (2021), both C and A variant silages met the parameters for the 1st quality class. The acidity of water extract was significantly ($P < 0.05$) higher in the experimental variant compared to the control. The alcohols content was significantly ($P < 0.05$) higher in the control group 6.82 g as compared to 1.74 g in the experimental group. The total amount of fermentation products was fairly balanced in both the treatments in spite of the difference in pH value. The observed parameters of acidity of water extract, alcohols content and fermentation products in maize silages treated with biological additives (containing *L. buchneri*) agree with the results of experiments published by Juráček et al. (2018).

Table 1 Effect of *L. casei* and *L. buchneri* addition on dry matter and fermentation parameters of maize silage

n=3		DM	LA	AA	LA:AA	BA	pH value	AWE	Alcohols	FP
C	$\bar{x}$	405.50[a]	59.23	13.30[a]	4.45:1	/	3.88[a]	1584.00[a]	6.82[a]	79.35
	S.D.	0.75	4.02	0.81	/	/	0.01	46.95	0.23	3.22
A	$\bar{x}$	396.33a	53.23	26.33[a]	2.02:1	/	3.97[a]	1804.00[a]	1.74[a]	81.3
	S.D.	0.77	6.53	0.92	/	/	0.02	100.70	0.34	5.70

C: control, A: additive (*L. casei*, *L. buchneri*), DM: dry matter (in $g.kg^{-1}$), LA: lactic acid, AA: acetic acid, BA: butyric acid, AWE: acidity of water extract, FP: fermentation products, /LA, AA, BA, Alcohols and FP in $g.kg^{-1}$ of DM/, [a] the values with identical superscripts in a column are significantly different at $P < 0.05$

CONCLUSIONS

The results confirmed that the addition of *L. casei* and *L. buchneri* significantly reduced the dry matter content of maize silages, confirming higher dry matter losses during fermentation. The inoculated silages were characterized by a lower lactic acid content and almost twice the acetic acid content, confirming the activity of *L. buchneri*, which efficiently transformed part of the lactic acid into acetic acid and increased the aerobic stability of the silage. The lower lactic acid concentration in the silages with the addition of the additive was reflected by significantly higher pH value. The addition of *L. casei* and *L. buchneri* was effective in reducing the alcohols content of maize silages.

REFERENCES

Bíro, D. et al. 2011. *Kukuričná siláž – významný energetický komponent kŕmnej dávky pre prežúvavce*. [Maize silage – an important energy component of the feed ration for ruminants], Slovenský chov, 7, p. 28.

Bíro, D. et al. 2020. *Konzervovanie a úprava krmív*. [Conservation and adjustment of feeds], 2nd ed. Nitra: SUA in Nitra, pp. 223.

Doležal, P. et al. 2012. *Konzervace krmiv a jejich využití ve výživě zvířat*. [Forage conservation and their use in animal nutrition], 1. ed. Brno: MU in Brno, pp. 307.

Gallo, A. et al. 2018. Effect of inoculation with *Lactobacillus buchneri* LB1819 and *Lactococcus lactis* O224 on fermentation and mycotoxin production in maize silage compacted at different densities. In *Animal Feed Science and Technology*, 246, pp. 36–45.

Jambor, V. et al. 2019. Selection of maize hybrids with the use of harvestlab on john deere forage harvester. In *Forage Conservation*, Brno: MU in Brno, pp. 214-215.

Jančík, F., et al. (2022). Shredlage processing affects the digestibility of maize silage. In *Agronomy*, 12(5), 1164. https://doi.org/10.3390/agronomy12051164.

Juráček, M. et al. 2018. *Konzervačné aditíva a ich vplyv na nutričnú a fermentačnú kvalitu siláží*. [Conservative additives and their influence on the nutritional and fermentation quality of silages], 1. ed. Nitra: SUA in Nitra, 2018. pp. 67.

Loučka, R. et al., 2020. *Jak zajistit vhodnou fermentaci v silážích a v bachoru dojnic*. [How to ensure appropriate fermentation in silages and in the rumen of dairy cows], Praha: Agrární komora České republiky, pp. 78.

Mitrík, T. 2021. *Ensilage: Illustrated manual for expert and beginner alike*. Wageningen Academic Publisher. pp. 280.

Orosz, S. et al., 2016. Corn silage processing score in Hungary between 2013-2015. In *Forage Conservation*. Nitra: NAFC, pp. 155-156.

Rajčáková, Ľ. et al. 2018. The effect of silage additives on quality of the mixture silages of maize and dendromass. In Slovak *Journal of Animal Science*, 51(3), pp. 111-118.

Szucz, P. et al. 2018. Application of some lactic acid bacteria strains to improve fermentation and aerobic stability of maize silage. In *Review on Agriculture and Rural Development*, vol. 7, (1-2), pp. 127-132.

This publication was supported by the Scientific Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic and the Slovak Academy of Sciences, project no.1/0321/23.

EFFECT OF RAKE TYPE AND SETTINGS ON CLOVER QUALITY AND LOSSES

LOUČKA[1], R., JAMBOR[2], V.
[1]Institute of Animal Science Prague
[2]NutriVet Ltd. Pohořelice

INTRODUCTION

With the use of more powerful and precise mechanization, the introduction of fodder is significantly accelerated, the cuttings are better cut, the silage products are more evenly applied and the matter in the silage area is better stratified, smothered and anaerobically isolated from the external environment. Mowing machines equipped with various finger or rubber conditioners and powerful rotor spreaders and rakes are already used when harvesting fodder. One of the latest innovations is the forage belt rake. Its finger picker system works with higher picking efficiency than conventional rotary rakes. More important than efficiency, however, is lower dustiness during their work (and thus the assumption of a lower buffering capacity of forage) and reduced contamination with undesirable microflora (which competes with lactic bacteria). Mowed forage dries faster when the weather is dry. Under such conditions, however, dust may accumulate during the treatment of mowed vegetation. As soon as the dust gets into the forage, the proportion of ash in it increases, which results in an increase in the buffering capacity of the silage. The fermentation process then takes place more slowly and with much greater losses.

The main influence on the buffering capacity is the content of mineral substances in the dry matter. In alfalfa, an increase in the crude ash content by 1% increases the buffering capacity by 0.55 - 1.36 mol / liter, an increase in the crude protein content by 1% increases the buffering capacity by 0.15 - 0.39 mol / liter (Stepanova and Volovik, 2021). The high buffering capacity of alfalfa requires the accumulation of a much larger amount of lactic acid than is needed to acidify the clover. However, in the case of meadow clover, data on increasing the buffering capacity by increasing the content of ash or crude protein cannot be found in the literature, so it is necessary to be satisfied with at least these numbers for a basic orientation.

In this regard, mechanization that follows the terrain well, does not tear up turf and therefore does not stir up dust (or only very little) is so-called priceless, especially if the fodder is harvested in poor conditions. Unfavorable weather is associated with the postponement of the harvest and the aging of the stand, or the ensiling of insufficiently rotten forage. The main factor influencing the natural fermentation quality of silages is mainly water-soluble carbohydrates (WSC), which, more than other nutrients in the plant, are subject to daily fluctuations. Harvesting clover in the afternoon will increase WSC by up to 10% compared to harvesting in the morning. This is because sugars accumulate during the day and are consumed at night when they are transpire (Bayatkouhsar et al. 2022). In addition to higher WSC content, forage harvested in the afternoon also tends to have higher dry matter (DM), organic matter (OM), crude protein (CP), starch and lower pH, NDF and ADF than forage harvested in the morning ($P<0.05$). Forage cut in the afternoon also has a higher potential for gas production than forage cut in the morning. When ensiling rotten clover, it is not enough that it has a high WSC content, there is often a problem with poorer fermentation quality, which is caused by the buffering properties of ashes and unwanted bacteria that enter the silage with dust or clay when harvesting from the field or meadow.

For finished protein silages with a higher content of buffering substances and the so-called critical dry matter content, or water activity, secondary fermentation can occur (in about 3 weeks after placing the cuttings in the silage trough), or to the so-called collapse, when, even under anaerobic conditions, clostridia can start to multiply and consume lactic acid, while the pH starts to increase again and thus the quality starts to decrease. This undesirable process can only be prevented by timely feeding of such silage.

The aim of the experiment was to confirm that when harvesting clover with a poorly adjusted rotor rake, a lot of dirt and dust gets into the cuttings, which can significantly deteriorate the quality of the silage. Another goal was to confirm the hypothesis that silage quality can be better when using a belt rake than a rotary rake.

MATERIALS AND METHODS

Two successive experiments were carried out. In the first experiment, clover was freshly cut (with a dry matter content of 12%), during wilting (with a dry matter content of 28%) and after wilting (with a dry matter content of 34%) and was harvested with a well- and poorly adjusted rotary rake and then with a belt rake. Nutritional values (AOAC, 2005) and clostridia abundance were evaluated. In the second experiment, it was harvested after rotting (with a dry matter content of 30–35%) with a well-adjusted (RG) and badly adjusted (RB) rotary rake, as well as a belt rake (PS) and a belt rake, where 170 g of clay was added to 5 kg of cuttings, taken from a field under clover (PSH). The cut from these four variants was ensiled in special containers. After 60 days, the silages were opened and chemically analyzed for nutritional values (AOAC, 2005), indicators of fermentation quality and clostridia abundance. Dry matter losses were also determined by weighing the chops before ensiling and the silage after opening the silos.

RESULTS AND DISCUSSION

In the first experiment, it was shown that the differences in nutritional values were not significant between the individual variants, but the differences in the content of clostridia were significant. While for silage after harvesting with a poorly adjusted rotary rake there was a log of 6.67, after harvesting with a well-adjusted one it was only log 5 and after harvesting with a belt rake it was log 4.33. The SEM (error of the mean) value was 0.27.

Important results from the second attempt are in tab. 1. The differences between the quality of silage after harvesting with a poorly adjusted and well-adjusted rotary rake, but also between them and a belt rake, were not significant. Significant differences were found between the variant after the addition of clay (PSH) and the other variants. The PSH variant had a higher dry matter and ash content, which was subsequently reflected in higher dry matter losses (12.52 vs. 5.34%), i.e. more than twice as high. The lower content of nitrogenous substances and ADF indicates a higher scraping of leaves when using a rotary rake, compared to a belt rake. An important finding is that after the addition of clay, the potential production of milk and biogas in the form of methane decreased significantly. trend (P-value = 0.1).

Tab. 1: Differences between clover harvesting technologies

Index	RB	RG	PS	PSH	SEM	P-value
Dry matter g/kg	308.9[a]	300.4[a]	314.2[a]	349.1[b]	7.4	0.01
Crude protein g/kg DM	181.7	186.3	194.5[b]	162.5[a]	5.4	0.02
NDF g/kg DM	398.0	379.0	377.9	365.5	9.4	0.19
ADF g/kg DM	309.6[b]	306.3.[b]	298.7	275.7[a]	6.4	0.02
Ash g/kg DM	117.5[a]	115.1[a]	112.1[a]	187.8[b]	7.8	0.01
Potential milk kg/t DM	1479	1519[b]	1524[b]	1390[a]	26.5	0.02
Potential methane l/kg DM	458.4[b]	442.0	461.0[b]	380.4[a]	15.4	0.02
pH	4.49	4.49	4.52	4.43	0.06	0.78
Lactic acid %	2.75	3.23	3.02	2.90	0.24	0.57
Acetic acid %	0.89	0.98	0.88	1.05	0.05	0.15
Lactic acid / Volatile Fatty Acids	2.81	3.18	3.18	3.17	0.15	0.28
$N\text{-}NH_3$ %	6.15	6.96	6.36	7.95	0.92	0.54
Clostridia log	4.33	3.33	3.33	6.33	0.83	0.10
DM losses %	7.88[a]	7.73[a]	5.34[a]	12.52[b]	0.98	0.01

RB = badly adjusted rotary rake; RG = well-adjusted rotary rake; PS = belt rake; PSH = belt rake with clay

CONCLUSIONS

There were more clostridia in silages when a rotary, poorly adjusted machine was used than when a well-adjusted machine or belt rake was used. In another experiment, the harvesting variant using a belt rake increased the dry matter content and ash content in the dry matter, and conversely decreased the protein and ADF content in the dry matter. The highest losses were when using a belt rake after adding 170 g of clay per 5 kg of cuttings. On the contrary, the lowest losses, although not significant, were when using a belt rake without adding clay.

The contribution was created thanks to the support of the MZE RO0723 project.

REFERENCES

AOAC (2005) Association of Official Analytical Chemists. Official methods of analysis. 18th ed. Gaithersburg, USA

Bayatkouhsar, J., Rezaii, F., Ghanbari, F., & Rahchamani, R. (2022). Morning vs. the afternoon time of alfalfa, clover and barley harvest affects the chemical composition and nutritional value of the silage. Iranian Journal of Applied Animal Science, 12(1), 11-21.

Stepanova, G.V. and Volovik, M.V. (2021): Dependence of the buffer capacity on the chemical composition of dry matter of alfalfa. In. IOP Conf. Ser.: Earth Environ. Sci. 901, 012044.

INFLUENCE OF UREA ADDITION ON THE FERMENTATION PROCESS AND NUTRITIVE VALUE OF ENSILED SHREDDED HIGH MOISTURE CORN GRAIN

DOLEZAL, P., KOLECKAR, J., DVORACEK, J.**; JAMBOR, V.****
Mendel University in Brno
** Mikrop Cebín, Stock company*
***Association of professional Services, Ltd., Skalice n. Svitavou*
**** NutriVet, Ltd., Pohorelice*

ABSTRACT

The aim of this work was to determine the effect of the urea addition on the parameters of the fermentation process and the nutritive value of shredded high moisture corn grain. In a model experiment, was shredded corn grain prepared with urea at a dose of 3 kg.t^{-1}. The results of the experimental variant were compared with the untreated control. The addition of urea had a significant effect on lower dry matter losses compared to the control variant. Compared to the control silage, silage with urea had a lower average amount of lactic acid (10.63 g.kg-1), a lower total value of fermentable acids in 1 kg of dry matter (17.52 g.kg^{-1}) and a smaller LA/VFA ratio (3.55). The average percentage of lactic acid from all acids in the silage was 77.88 %. A higher value of ammonia was also recorded in the experimental silage.

Keywords: Moisture grain corn, silage, urea, fermentation process, silage aditive, nutritive value

INTRODUCTION

Moist maize grain corn has a favorable nutritive value and high OH digestibility (Woodacre, 2004). The quality of moist corn grain silage is influenced by a number of factors, especially dry matter content, particle structure, grain endosperm type, as well as the preservation method, i.e. the use of silage additives. The effect of the addition of biological additives on the final quality of wet corn grain silage has been studied by a number of authors (Dolezal, Zeman, 2005; Bíro et al., 2009b; Schaefer et al., 1989; Kung et al. (2004) Dordevic et al., 2020; Souza et al., 2020; et al.). The influence of chemical preservatives and their effect on the quality of moist corn grain silage were studied, for example, by Bíro and Juracek (2003), Bíro et al. (2006), Volkov et al., 1999; Pyrochta et al., (2005), Gálik et al. (2010) and others. The preservative effect of urea in the preservation of silage corn was studied by Shirley et al. 1972. Later experiments also confirmed that the addition of urea in a dose of 5 kg/ton increases not only the content of N-substances in the silage, NH_3-N, but does not have a negative effect on the fermentation process or on the subsequent fermentation, as there is also an increase in the production of acetic acid (Baintner et al. 1985; Pahlow, 1979; Huber, 1983, Knabe et al. 1984; Dolezal, 2001) others.

MATERIALS AND METHODS

The model experiment on maize grain corn containing 668.5 g of dry matter per kg on average was carried out in the laboratories of the Mendel University of Agriculture and Forestry in Brno. The maize grain corn was ensiled into 9 L experimental laboratory silos. Three separate batches of fresh maize corn were collected. Both control maize corn (untreated) and the maize corn supplemented with urea 3.0 kg.t^{-1}were pressed into experimental silos. Untreated maize grain corn was used as a negative control. The average weight of the compacted mass was 800 kg/m^3. The silos were closed and stored at 20±25 ºC prior to analysis. Three replicated silos of each treatment were incubated for a period of 180 days. The silos were then opened and representative sub-samples (6) analysed for DM, fermentation parameters and nutritive value. Dry matter was determined at 105 °C (drying till constant weight) according to Czech National Standard 467092-42). All analytical procedures including the preparation of aqueous extracts were described previously (Doležal, 2002). The samples were analyzed for the content of the volatile fatty acids, lactic acid, ammonia, pH values, titration acidity according to Decree No. 124/2001 Coll., which establishes the requirements for sampling and the principles of methods of laboratory testing of animal feed. The nutritive value of the silages were determined according to the methods by AOAC (2000). Results were evaluated by means of variance analysis using the software Statgraphic (ver. 5.0).

RESULTS AND DISCUSSION

The results of the model test of ammoniated moist corn grain are shown in Tables 1 and 2. From the results (Table 1), it is clear that the addition of urea at a dose of 3 kg/t of grain caused certain changes in the formation of fermentation products, specifically a statistically inconclusively higher pH value was recorded, lower production of LA and total acids in dry matter and higher production of ethanol. The results achieved did not confirm earlier tendencies from experiments with the preservation of the whole corn plant (Baintner et al. 1985, Doležal, 2001 and other).

Table 1: The effect of urea addition on the indicators of moist corn silage (g/kg)

		DM	pH	TA	LA	AA	LA/AA	∑ Acids/DM	Ethanol
Control Silage	Mean	662.40±6.03	3.66±0.048	1372.33±76.95	19.30±1.43	3.30±0.38	5.89±0.29	33.00±5.12	0.70±0.08
		a	**a**	**b**	**b**	**ab**	**b**	**b**	**b**
Experiment. Silage	Mean	664.31±10.66	3.85±0.027	954.83±98.64	10.60±1.14	3.00±0.19	3.55±0.542	17.50±1.34	0.10±0.08
		a	**a**	**a**	**a**	**ab**	**a**	**a**	**a**

Fermentation was affected in experimental silages with the addition of urea, which was manifested by lower titration acidity (TA), lower production of lactic acid (LA) and acetic acid (AA), statistically significantly lower ($P<0.05$) content of fermentable acids in dry matter. The addition of urea as a silage additive significantly reduced ethanol production compared to the control silage.

Ammonia treatment of moist silage corn grain increased the crude protein content and a higher crude fiber (CF) content as well as ADF and NDF was found in the experimental silages compared to the control silage. The starch content was also reduced in the experimental silages compared to the control variants. It turns out that the higher grain dry matter content compared to whole-plant silage corn conservation could have influenced the course of fermentation processes.

Table 2: The effect of urea addition on the nutritive value of moist corn silage (g/kg DM)

		CP	F	CF	ASH	ADF	NDF	Starch
Control Silage	Mean	99.04±1.321	55.06±2.174	26.84±3.185	19.42±2.311	27.93±3.875	81.5±5.538	733.21±4.629
		a	**a**	**a**	**ab**	**a**	**a**	**b**
Experimental Silage	Mean	111.25±3,581	53.05±0.488	33.77±4.095	18.43±0.529	35.58±2.732	90.66±6.134	724.73±14.18
		a	**a**	**a**	**ab**	**a**	**ab**	**a**

CONCLUSIONS

The chemical treatment of mechanically treated moist maize grain with urea (experimental group) was compared with the fermentation values of the control silage. A higher pH value and a lower titration acidity were found in the experimental silage, which were supported by a lower formation of organic acids in the dry matter and a lower alcohol production. No statistically significant difference was found in the nutritional value between the two groups of silages, with the exception of a lower starch content in the experimental silage.

REFFERENCES

AOAC (2000): Official methods of Analysis. 17th ed. Oficial Methods of Analysis of AOAC.

Bíro, D., Juráček, M. (2003): Conservation of maize corn with high moisture by organic acids. Forage conservation: 11th international Scientific Symposium, Nitra, p.122-123.

Bíro, D., Juráček, M., Gálik, B., Šimko, M., Kačániová, M. (2006): Influence of chemical inhibitors on fermentation process and hygienic quality of high moisture corn. Slovak J. Anim. Sci., 39:108-112.

Doležal, P. (2002): Vliv přídavku Lactobacillus plantarum DSM 12771 na kvalitu siláží silně zavadlé vojtěšky a trávy. Acta univ. agric. et silvic Mend. Bruno, 2002, 5, s. 37-44.

Doležal, P., Zeman, L. (2005): Effect of differentforms of bacterial inoculants on the fermentation process of ensiled crushed maize moisture grains. Czech J. Anim. Sci., 50:201-207.

Pyrochta, V., Doležal, P., Doležal, J. (2005): Vliv směsi organických kyselin a mikrobiálního inokulantu na fermentační proces laboratorních siláží z mačkaného vlhkého zrna kukuřice. Acta Univ Agric Silvic Mendel Brunnens 53:107-115.

MONITORING OF SELECTED QUALITY PARAMETERS IN LG MAIZE HYBRID

JANČÍK, F., TYROLOVÁ, Y., KUBELKOVÁ, P.
Institute of Animal Science, Prague, Přátelství 815, 10400

INTRODUCTION

During the growing season of 2020, an experiment comparing the quantitative and qualitative parameters of silage maize hybrids of the Limagrain company was carried out on the fields of the Institute of Animal Science. One anonymous hybrid (FAO 310, used like a Control) and the LG 31.277, LG 31.272, LG 31.235, LG 31.268 hybrids were used in the experiment.

The aim of the experiment was to assess both the yield and especially the quality parameters of the tested hybrids, especially with regard to the digestibility of the neutral detergent fiber.

MATERIALS AND METHODS

The experimental plots were sown on 17/04/2020 in three repetitions for each hybrid. Yield parameters were determined by sampling ten plants from each plot. Whole plants and their individual parts were weighed, the number of grains per stick was counted and the dry matter was determined. After drying the grains, the weight of thousand seed (WTS) was also determined. For trial silages, a total of 20 bags (3 x 6 bags; plus two extra) of each hybrid were created using the Vacsy system method. Silages were opened after two months and processed for chemical and *in situ* analyses. In addition to fermentation analysis, the content of dry matter, organic matter (OM), crude protein (CP), fiber, neutral detergent fiber (NDF), acid detergent fiber (ADF), fat and starch were determined. The *in situ* method, focused on the rumen digestibility of dry matter, OM, NDF and starch, was carried out on two milking cows of the Holstein breed. Incubation times of 6 and 24 hours were used to determine starch digestibility, and times of 24 and 48 hours were used to determine NDF digestibility. The digestibility of dry matter and OM were calculated for all mentioned incubation times. Each sample was incubated in the rumen of cows in a total of six replicates (3 bags per cow). After incubation, the bags were washed for about 20 minutes (until the water was clear). Subsequently, the residues after incubation were transferred to dishes and dried at 50 °C and further analysed for the content of dry matter, OM, NDF and starch. All results were statistically evaluated using the GLM procedure of the SAS program. To assess the yield elements and the chemical composition of the cut, the hybrid was entered into the model as a fixed effect and the repetition as a random effect. For the rumen digestibility results, hybrid and cow were entered into the model as fixed effects and as random effects hybrid and replicate in incubation. When significant differences were found, these differences were assessed using the Scheffe test.

RESULTS AND DISCUSSION

The yield of the whole plant with the cob was without significant differences, but the numerically lowest values were achieved by LG 31,277 (hereinafter referred to as 277) and LG 31.268 (hereinafter referred to as 268). The numerically highest yield of ears was achieved by the hybrid LG 31.272 (hereinafter only 272), followed by the hybrid LG 31.235 (hereinafter only 235), then 268, and the lowest was 277. The average number of grains in the ear was also not significantly different, however, numerically higher values were achieved by hybrids 272 and 277, and lower 268 and 235. The hybrid 235 demonstrably had the highest HTS value compared to all other hybrids. This also corresponded with the weight of grains in the ear, where hybrid 235 had a higher weight compared to hybrids 277 and 268.

A higher content of CP in the silage was found in hybrid 235 compared to hybrid 272. No significant difference was found in starch content between the hybrids. In the fiber content, NDF and ADF in the silage, no differences between the hybrids were found, just like in the fresh chopped plants.

Dry matter digestibility was higher in hybrid 235 (especially at incubation times of 6 and 48 hours). At the incubation time of 24 hours, the digestibility of dry matter was lower in Control and hybrid 272. The digestibility of OH was also the highest in hybrid 235, only at the incubation time of 24 hours, hybrids 268 and 277 showed similar values. For starch digestibility after 6 hours of incubation, hybrid 268 reached a lower value. Other hybrids had comparable values. After 24 hours of incubation, starch was completely digested in all hybrids. Significant differences were found in NDF digestibility. After 24 hours of incubation, higher values were found for hybrids 268, 277 and 235. A lower value was found for hybrid 272 and the lowest value was found for the control. After 48 hours of incubation, NDF was most digestible in hybrids 235 and 268. Lower, mutually comparable levels were achieved by hybrids 277 and 272. The lowest NDF digestibility value after 48 hours of incubation was found for the control hybrid.

CONCLUSIONS

From the point of view of yield parameters, the tested hybrids were comparable in all parameters, with the exception of HTS, where hybrid 235 stood out. Also, from the point of view of chemical composition, the hybrids were comparable both in the evaluation of fresh chopped plants and silage. For silages, hybrid 235 was comparable to the others in terms of starch digestibility. Overall, from the point of view of NDF digestibility of corn silages, all LG hybrids showed significantly higher values compared to the control hybrid.

Název:	19TH INTERNATIONAL SYMPOSIUM FORAGE CONSERVATION
Autoři:	Ing. Václav JAMBOR, CSc., Prof. MVDr. Leoš PAVLATA, Ph.D., Prof. MVDr. Ing. Petr DOLEŽAL, CSc., Prof. Ing. Jiří SKLÁDANKA, Ph.D., MVDr. Rudolf ŽITŇAN, DrSc., Ing. Lubica RAJČAKOVÁ, PhD., Doc. Ing. Petr HOMOLKA, Ph.D., Ing. Radko LOUČKA, CSc., RNDr. Jan NEDĚLNÍK, PhD., Ing. Ladislav MENŠÍK, Ph.D.
Editoři:	Ing. Václav Jambor, CSc., Ing. Soňa Malá
Vydala:	Mendelova univerzita v Brně, Zemědělská 1, 613 00 Brno
Tisk:	Mendelova univerzita v Brně, Zemědělská 1, 613 00 Brno
Vydání:	První, 2023
Náklad:	170 ks
Počet stran:	136
ISBN	978-80-7509-919-8

Texty neprošly jazykovou úpravou.