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COMBINATION OF ITALIAN RYEGRASS AND WINTER CEREALS PROVIDES NEW ALTERNATIVE FORAGES IN DAIRY NUTRITION

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AA	Acetic Acid
ADF	Acid Detergent Fiber
ADIN	Acid Detergent Insoluble Nitrogen
ADL	Acid Detergent Lignin
AHBD	Agriculture and Horticulture Development Board (UK)
AMC	Aerobic Mesophilic Microorganism Mount
aNDF	Amylase Treated Neutral Detergent Fiber
ANOVA	Analysis of Variance
AOAC	Association of Official Agricultural Chemists
BA	Butyric Acid
BBCH	Biologische Bundesanstalt, Bundessortenamt and Chemical Industry
BMR	Brown Mid Rib
CA	Caproic Acid
CF	Crude Fiber
CFU	Colony Forming Unit
CP	Crude Protein
CRD	Completely Randomized Design
DC	Digestibility Coefficient
DE	Digestible Energy
DM	Dry Matter
DMD	Dry Matter Digestibility
DMI	Dry Matter Intake
dNDF	Digestible Neutral Detergent Fiber
ED	Effective Degradability
EE	Ether Extract
EN	Electronic Nose
EPD	Effective Protein Degradability
FA	Fatty Acid
FAO	Food and Agriculture Organization of the United Nation
FID	Flame Ionization Detectors
FOM	Fermentable Organic Matter
GMP	Good Manufacturing Practises
INRA	Institut National de la Recherche Agronomique
IPCC	Intergovernmental Panel Discussion on Climate Change
ISO	International Organization for Standardization

IVDMD	In-vitro Dry Matter Degradability
IVTD	In-vitro True Digestibility
LA	Lactic Acid
LAB	Lactobacillus Bacteria
LDA	Linear Discriminant Analysis
ME	Metabolizable Energy
MP	Metabolizable Protein
MPE	Energy Dependent Metabolizable Protein
MPN	Nitrogen Dependent Metabolizable Protein
NDF	Neutral Detergent Fiber
NDFd	Neutral Detergent Fiber Digestibility
NE	Net Energy
NE_g	Net Energy for Gain
NE_l	Net Energy for Lactation
NE_m	Net Energy for Maintenance
NFC	Non-Fiber Carbohydrates
NFE	Nitrogen Free Extract
NH_3-N	Ammonium Nitrogen
NIRS	Near Infrared Spectroscopy
NRC	National Research Council
OM	Organic Matter
OMd	Organic Matter Digestibility
PA	Propionic Acid
PCA	Principal Component Analysis
peNDF	Physically Effective Neutral Detergent Fiber
PFA	Process Adjustment Factor
RDP	Rumen Degradable Protein
RFQ	Relative Forage Quality
RI	Retention Indices
SCFAs	Short Chain Fatty Acids
SEM	Standard Error of the Mean
TCL	Theoretical Chop Length
tdCPf	Truly digestible Crude Protein for Forage
tdFA	Truly Digestible Fatty Acid
tdNDF	Truly Digestible Neutral Detergent Fiber
tdNFC	Truly Digestible Non Fiber Carbohydrates

TFA	Total Fermentation Acid
TMR	Total Mixed Ration
UDP	Rumen Undegradable Protein
USDA	United States Department of Agriculture
VA	Valeric Acid
VOC	Volatile Organic Compounds
WPCS	Whole Plant Corn Silage
WSC	Water Soluble Carbohydrate

1. INTRODUCTION

Preservation of forages for silage making has increased considerably since the 1960s and has become economically relevant for many farming systems in temperate areas of the world (Wilkins et al., 2005). This practise is common in European countries, North America, New Zealand and Australia with corn (*Zea mays L.*) is the principal ensiled crop (Allen et al., 2003; Keady et al., 2012; Campagnoli and Dell'Orto, 2013). Apart from corn, one of the commonest crops usually conserved for silage is grass. Both single species of grass (e.g., Italian ryegrass (*Lolium multiflorum L.*) or perennial ryegrass (*Lolium perenne L.*)) and mixed species of grasses and legumes are grown as silage crops (McDonald et al., 1991). Silage making is one of the most important sources of conserved forages and is a basic component of ruminant diet, and this approach is widely used for storing forage for feeding milk and meat producing animals (Cheli et al., 2013).

In recent years, difficulties occurring in corn cultivation (i.e., groundwater shortages, mycotoxin contamination) forced dairy farmers to consider alternative silages. The vegetation period of winter crops covers autumn, winter and early spring, when the soil conserves enough moisture for vegetation up to harvest because of generally higher winter precipitation, and preceding the dry summer period. However, the main vegetation period of corn in temperate zone covers the warmest and driest periods, which can decrease yield of whole plant corn dramatically. Finding acceptable alternative forage to replace whole crop corn silage will be a critical challenge for the success of future dairy operations if climate change induced factors continue to affect corn production, particularly in Europe. In this regard for the previous 20 years experimental studies had been conducted to replace whole crop corn silage with other alternative crop silage such as whole crop triticale silage (Van Duinkerken et al., 1999), annual ryegrass silage (Bernand et al., 2002), perennial ryegrass silage (Burke et al., 2007; Keady et al., 2008), whole crop rice silage (Ki et al., 2009), Italian ryegrass silage (Baldingar et al., 2012; 2014), different forage millet silage cultivars (Brunette et al., 2014), lucerne silage (Sinclair et al., 2015), whole crop sorghum silage (Colombini et al., 2015; Cattani et al., 2017; Khosrani et al., 2018), high sugar forage sorghum silage (Su-jiange et al., 2016) and wheat and triticale silage (Harper et al., 2017) considering the global climate change effect on yield and safety of corn production for silage making. However, all the studies reported limitations in their attempts to replace whole plant corn silage (WPCS). One of the reason difficult to replace whole corn silage is its outstanding energy content, fiber digestibility and fermentable characteristics both during silage making as well as ruminal fermentation. However, few of the experimental studies reported profound results with some limitation on certain critical characteristics to replace WPCS. For instance the use of whole crop cereal silages (Van Duinkerken et al., 1999; Ki et al., 2009; Harper et al. 2017), perennial ryegrass silage (Burke et al., 2007; Keady et al., 2008) and Italian ryegrass (Baldinger et al., 2012; 2014) was reported extraordinary result with minor limitation as compared to WPCS. Therefore, our hypothesis is based on those studies and we intended to study the mixtures of winter cereals and Italian ryegrass plus cereal grain mixture silages to complement the potential of the two mixture crops silage in the nutrition of dairy cows.

The components of the mixture complement each other's properties: the digestibility of barley, winter oats and Italian ryegrass is excellent, while wheat and triticale give high yields and triticale is an indicator plant for determining harvesting date. Triticale also contains perhaps the best quality fibers with highest proportion of NDF. This can be crucial in the nutrition of high yielding dairy cows fed high proportion of concentrate to provide enough precursors for milk fat synthesis. High fiber digestibility can improve dry matter intake (DMI) and milk production of dairy cows (Raffrenato and Van Amburgh, 2010; Grant, 2012) especially when fed at least 10 kg/day/cow under heat stress conditions (Orosz, 2019, unpublished data). It is also important for sustainable farming that the components of the mixture react differently to the amount of rainfall: with little autumn/winter rainfall, the mixture will become dominated by cereal components, while with abundant rainfall, the dominance of Italian ryegrass. In both cases, high yield biomass can be harvested with high digestibility, but the species composition of the mixture will be different from the original ratios. Another advantage is that it allows double cropping (Ketterings et al., 2015; Ranck et al., 2019), so two biomasses can be harvested per year. Winter wheat and winter rye will produce massive root systems and they lessen soil erosion during the winter, improve soil quality, and protect against nitrogen leaching and deflation. Italian ryegrass establishes rapidly and may reduce the risk of soil erosion (Baldinger et al., 2014). Legumes are not included in the present mixture due to its lower fiber digestibility and high buffering capacity than winter cereals or Italian ryegrass. However, the main trouble with legumes, that they contain high proportion of soluble N containing compounds, and increasing the NH_3 production in the rumen, which resulting in higher urea concentration in blood decrease the fertility (conception rate) of the cow.

Apart from measuring the nutritional constituents and fiber fractions, silages can be evaluated for volatile organic compounds (VOCs) that result from fermentation reactions to assess fermentation quality based on the content of undesired degradation products (Borreani et al., 2007) and VOCs resulting from the metabolism of undesirable microorganisms (bacteria, fungi, and yeast) (Campagnoli and Dell’Orto, 2013). Masoero et al. (2007) applied electric nose (EN) to analysis silage quality as a simple alternative method for evaluating volatile components. The EN exhibited advantages over some other analytical methods, including near-infrared spectroscopy (NIRS), for the evaluation of fermentation characteristics of silage and it can also useful at estimating total fatty acid and ammonia levels and buffering capacity (Campagnoli and Dell’Orto, 2013).

No studies have been conducted to determine the feed value, aroma profile, digestibility, degradability, ruminal fermentation and energy values of different combinations winter cereal based as well as Italian ryegrass (*Lolium multiflorum Lam.*) and winter cereal grain-based silages.

2. OBJECTIVES

- Evaluate nutritional composition of green forage mixtures of four winter cereals and Italian ryegrass combined with different cereal crop in different mixture.
- Investigate chemical composition, fermentation characteristics and microbiological quality of silages prepared from six mixtures of winter cereals; and Italian ryegrass combined with different winter cereals crop in different mixture silages.
- Evaluate aroma profile of four mixtures of winter cereals and Italian ryegrass plus winter cereals crop mixture silages using Electronic nose (E-nose) technique.
- Evaluate the *in sacco* ruminal degradability of six mixtures of Italian ryegrass plus winter-cereals and winter cereals-based silages.
- Evaluate the ruminal fermentation of four mixtures of Italian ryegrass plus winter-cereals and winter cereals-based silages.
- Evaluate the net energy (NE) and metabolizable protein (MP) content of a mixture of ensiled Italian ryegrass and winter-cereals.

3. LITERATURE REVIEW

Dairy farmers in many parts of the world rely on corn silage as a source of digestible fiber and readily fermentable energy for their cattle (Adesogan, 2006). However, climate change, which is currently characterized by increased atmospheric CO_2, rising temperature, and altered pattern of precipitation, is affecting corn production for silage making. Corn silage production is particularly affected by shortage of water, agronomic practices and environmental factors (including heat, moisture, and soil type). Farmers face several climatic challenges that can complicate corn silage production, including temperatures that reduce the rate of photosynthesis (Crafts-Brandner and Salvucci, 2002), and reduction in potential yields due to faster crop life-cycles. For instance, in Hungary according to a study of Kálmán and Rajki (2015), on an average of the year, the acreage devoted to silage corn production (80-90,000 ha/year) is usually enough to meet the needs of feeding the cattle population (818,000). However, at the same time, in extremely hot and dry years, the corn reacts very sensitively to the actual weather condition in Hungary. They further noted that the climate in Hungary has become more arid with extremities due to global climate change during the past decades. In Hungary, the arable crops are mostly not irrigated, therefore the yield reduction as a consequence of drought cannot be estimated in advance which currently affecting dairy farming. Rising temperature and shifting precipitation patterns will also alter the ability to meet crop water requirements, water availability, crop productivity, and costs of water access across the agricultural landscape (Getachew et al., 2016). Climatic factors cause not only loss of silage corn production but also other factors which aggravates crop failure as a whole. In this regard Kucharek and Raid (2005) and Samapundo et al. (2005) reported that climatic conditions are conducive for proliferation of many bacterial and fungal pathogens which cause stalk rot, smut, leaf blight and rust, and predispose to growth of mycotoxin producing fungi (Fusarium, Aspergillus, Penicillium). In addition to affecting crop growth and disease incidence, previous studies also showed that these climatic factors have adverse effects on silage fermentation and aerobic stability (Dewar et al., 1963; Muck, 1987; Garcia et al., 1989 and McDonald et al., 1991). For instance, rainfall at harvest can increase proteolysis in the silo (McDonald et al., 1991) and effluent production (Fransen and Strubi, 1998) thereby reducing dry matter recovery. According to Ashbell et al. (2002) and Weinberg et al. (2001) ensiling at high temperatures reduces lactic acid concentration, aerobic stability and increases pH and dry matter losses. In the last years, difficulties occurring in corn cultivation (i.e., groundwater shortages, mycotoxin contamination) have been forced dairy farmers to consider alternative silages. Mainly, because the yield safety of corn silage will be compromised in the future if the expected climate changes in Hungary will be characterized by the increase of summer heat waves and the more extreme water course. Therefore, it would be urgent to consider how crop production and feeding strategies can be adapted to this change in long term, taking into account the needs of the high producing dairy cows.

3.1. The importance of corn silage in dairy cow nutrition

Corn silage is a major dietary component for dairy cows in most parts of the world particularly in USA and Europe with average feeding rates of 2.70 and 4.10 tonne dry matter (DM) per cow per year, respectively (Kleinmans et al., 2016). The widespread use of corn silage implies that it has certain competitive advantages over other feedstuffs. This means over the long term, diets with corn silage must result in higher income over feed costs than do diets that include less commonly used feeds (McCuaghey et al., 2002). Corn silage produces more digestible energy per acre than other forages; therefore, corn silage is included in ruminant rations primarily as a source of energy. According to Swift (2004) the starch in corn grain accounts for approximately 45% of the energy value, and microbial digestion of cellulose and hemicellulose (NDF fraction) in the rumen contributes a further 25% to the energy value of corn silage. The remaining 30% of energy comes from sugars, pectin, organic acids, crude protein and ether extract. There is a substantial body of evidence from studies with lactating dairy cows that increasing digestibility, increased milk yield, milk protein concentration and higher yields of fat plus protein could be observed. According to Keady and Hanrahan (2013) the mean daily response for each 1 percentage unit increase in silage dry matter digestibility (DMD) result in 0.33 kg more milk production. The fiber digestibility of the stove and digestibility of starch in grain as well as the ratio of stove to grain explain the nutritional value of corn silage. Maturity at harvest has the greatest influence on NDF digestibility. The NDF digestibility in corn silage declines approximately 10.0 percentage units between the ½ milk-line to advanced black layer stages of maturity. Because corn silage has a high grain content, it is important that it also have adequate effective fiber to obtain successful utilization of the silage. Adequate physically effective NDF (peNDF, as the fraction of NDF that stimulates chewing and contributes to a ruminal digesta mat) in dairy cow diets is essential for good rumen function that results in proper digestion of the diet, and maintenance of animal health and milk fat production. Because corn silage is often chopped finely or processed through rollers, its peNDF is typically 85% of amylase-treated neutral detergent fiber (aNDF), but this can vary from 70 to 95%. The recommendation for peNDF in dairy rations is about 21% of dry matter, but this fiber requirement probably increases with increasing non-fiber carbohydrates (NFC) in the ration. The starch content of corn silage is mainly affected by stage of maturity of the plant at harvest (Johnson et al., 1999). The advancing maturity of the corn crop during the grain-filling period increases the content of starch (Phipps et al., 2000) but its digestibility can be decreased as kernels becomes harder, drier, and more vitreous (Keady, 2016). The feeding value of corn silage is mainly determined by intake and digestibility of silage (Huhtanen et al., 2002). There is no negative implication for corn silage digestibility and intake, except some reports (Charmley, 2001; Neto et al., 2009) which are suggested that ensiling process reduces the feeding value and digestibility of corn silages. Rations containing only corn silage as forage may limit the intake and production due to excess rapidly fermentable starch, low effective fiber, and/or slow rates of fiber digestion (Neto et al., 2009). Prolonged ensiling period increases digestibility of starch. Weakley (2016) reported that during storage, the digestibility of the starch will increase as the ensiling time increases. Typically, starch digestibility increases over the next 90 to 180 days, and by 180 days the digestibility will usually reach a plateau. On average, starch digestibility can increase 15 percentage units during this time. The upsurge in digestibility occurs because of the breakdown of prolamin proteins that protect the starch granules from microbial degradation. Proteolytic enzymes in the silage pile break down the prolamins holding the starch together during ensiling. This process allows for easier access to starch granules for microbial degradation in the rumen. On the other hand, protein degradability is also higher in the silage than the original green forage. According to González et al. (2007), it is generally accepted that proteins from silages have a higher efficient degradability than those of their original green forages as a consequence of the previous degradative actions of the ensiling microflora.

3.2. Effect of climate change on the production and quality of corn silage

Despite tremendous improvements in technology and crop yield potential, crop production remains highly dependent on climate, because solar radiation, temperature, and precipitation are the main drivers of crop growth. Plant diseases and pest infestations, as well as the supply of and demand for irrigation water are also influenced by climate (Tigchelaar et al., 2018). According to the report of United States Department of Agriculture, Foreign Agricultural Service (USDA, 2018) the area, yield and production of corn reduced or at least maintained constant for the last three years particularly in the EU and USA (Table 1). This could be attributed to the climate change which is expected to bring warmer weather, changes to rainfall patterns, and increased frequency of extreme weather. According to data of the Hungarian Meteorological Service there were 8.80 millimeters of precipitation on a national scale between 1 and 29 April 2020, 20% of the average April value (44 millimeters) calculated since the beginning of the measurements, so April 2020 can be considered as the third driest one ever in the rankings from 1901 (Hungarian Central Statistical Office, 2020). Despite the drought in the spring, repeated for many years, as well as the large quantity of precipitation falling by the time of the harvest, the average yield of corn and barley was close to record, but the average yield of wheat was also higher than the respective harvest results in the previous year (Hungarian Central Statistical Office, 2020).

Nearly 55% of cereal production was made up by corn in Hungary in 2020, and the harvested production of wheat, the main ear cereal in Hungary, accounted for 33% of total cereal production. The production of barley, grown on the third largest area, saw the highest yield in the last three years, while its share was 9.40% (Hungarian Central Statistical Office, 2020). The wheat production of 5.0 million tons was 6.80% less than in 2019. This mainly resulted from the harvested area in 2020 being 8.10% smaller, at 933 thousand hectares. At the same time, the average yield of 5.40 tons/hectare was an outstanding result, 1.50% higher than the previous year’s and 1.80% more than the average of 2015–2019. The harvested area of corn went below 1 million hectares again in 2020 (973 thousand hectares), by 5.30% over a year, but the 8.40 million tons of harvested production was 1.30% larger than in 2019 and 9.30% larger than the average of the previous five years. The average yield (of 8.60 tons) per hectare was 6.90% higher compared to 2019 and 15% higher than the average of the last five years preceding 2020. The 5.80% more corn was procured from producers in the first 11 months of 2020 than in the same period of 2019, at an average price of 49 forints per kilogram. The average yield of both wheat and corn has shown an increasing trend since 2010. The yield of corn per hectare has already exceeded 8.00 tons in the two years before 2020. As a result of rainfalls at the beginning of the summer, the average yield in 2020 approximated the high in 2016. The yield of wheat per hectare was higher as well than in earlier years, it has been more than 5.00 tons in every year since 2015.

Table 1. Area, yield and production of corn silage from 2015/16 - 2017/18

Item	Production year			
	2015/16	2016/17	2017/18	
			January	February
Area (million hectare)				
World	181.0	185.6	184.5	184.4
EU	9.25	8.65	8.47	8.47
USA	32.68	35.11	33.47	33.47
Yield (metric tons per hectare)				
World	5.38	5.79	5.66	5.65
EU	6.35	7.18	7.10	7.10
USA	10.57	10.96	11.08	11.08
Production (millions metric tons)				
World	973.4	1075.9	1044.5	1041.7
EU	58.75	61.45	60.09	60.09
USA	345.5	384.7	370.9	370.9

Source/ USDA / Foreign agricultural service, office of global analysis (February, 2018)

Results of a recent study revealed that climate change will increase the risk of corn crop failures across the world’s biggest corn-growing regions (Tigchelaar et al., 2018). According to this report much of the world’s corn goes into feeding livestock and making biofuels. In United States the mean total maize production is predicted to decline by 18% under 2 °C of global warming and by 46% with 4 °C of warming (Table 2).

Table 2. Predicted changes in total production in the top-four maize producing countries in response to a 2 °C and 4 °C warming (Tigchelaar et al., 2018)

Country	2 ^{o}C warming	4 ^{o}C warming
USA	- 17.80	- 46.50
China	- 10.40	- 27.40
Brazil	- 7.90	- 19.40
Argentina	- 11.60	- 28.50

Rainfall at harvest and high temperature during ensiling adversely affect the fermentation and quality of corn silage. Hot and humid conditions that occur during the corn growing season are responsible for production loss of corn for silage making (Adesogan, 2006). Corn silage producers in hot and humid regions need to adhere strictly to excellent silage making practices to overcome the adverse effects of moisture and temperature on corn silage production. Corn silages grown in hot and humid areas should be harvested at 34% DM to optimize DM yield, nutritive value, fermentation quality and reduce fungal infections. Higher stay-green rankings in corn hybrids resulted in greater moisture and crude protein (CP) concentrations and less in vitro dry matter digestibility (IVDMD) and starch concentrations (Arriola et al., 2005). Corn silage producers in hot and humid regions need to avoid harvesting corn in wet weather, and ensure that excellent silage management practices are followed to overcome these climatic challenges to quality silage production. In addition to climate change, factors like high demand of corn for different purposes; like other livestock feeds, particularly pig and poultry, raw material for most food, bioethanol/beverage and biogas industries and even for human consumption decrease the availability of corn for silage making for high producing dairy cows. The change in climatic condition particularly temperature and precipitation does not only affect the corn production but also quality of corn silage. According to the report by Phibro Animal Health Corporation (Sep 18, 2018), the effects of hurricanes and flooding can take their toll in corn crop harvesting, producing heavy rains that could delay harvest and force farmers to keep their silage corn in the field for a longer period of time. Delayed harvest may lead to altered DM content of the forage, which could lead to mould growth and stalk and ear rot; both of which may increase the opportunity for mycotoxin contamination. According to David and Gary (2018) report, FAO has estimated that 25% of the world's crops are affected by mycotoxins each year, with annual losses of around 1 billion metric tons of foods and food products. Climate change is conducive for the reproduction and proliferation of invasive pests and insects. In Africa the outbreak (2016) of the fall armyworm (*Spodoptera frugiperda*)/ the American armyworm, was an example of climate change effect (Niassy and Subramanian, 2018). The pest, an alien from the Americas (widely distributed in Eastern and Central North America, and in South America), was first reported in Africa in 2016 (Niassy and Subramanian, 2018). The outbreak started in São Tomé and Príncipe islands and Nigeria, and just two years spread to over 38 African countries. Cereal farmers across Sub-Saharan Africa are experiencing heavy losses due to the devastation by this invasive pest. In Africa it has caused huge losses to staple cereals, especially corn and sorghum, affecting food security and trade. According to recent a report, damage to corn alone is estimated to be between 2.50 to 6.20 billion USD per year (Niassy and Subramanian, 2018).

3.3. Replacement of corn silage with different crop silages

3.3.1. The use of new silo corn hybrids

The development of corn hybrids plays an important part in the worldwide success of corn silage, and the choice of suitable hybrid is the most important factor for profitable silage production. Plant breeders have made considerable advances in achieving earlier maturing maize varieties that are more reliable for a specific area (Dewhurst, 2013). The main criteria for selecting a hybrid variety are yield, precocity, and resistance to diseases, pests and lodging (Delmotte, 2010). Stalk characteristics are usually modified with the aim of increasing the digestibility of the fiber in corn silage. Grain characteristics can be altered through modifications in nutrient or starch composition (Ferraretto et al., 2015). Commonly there are two types of corn hybrids use in dairy cattle nutrition; these are the brown mid rib (bmr) and leafy (leafy) silo corn hybrids. According to Kung (2011), and Grant and Contanch (2012) nutrient composition of bmr corn hybrid silage is generally similar to the conventional hybrids with two important differences; the bmr is lower in lignin and has a significantly higher in vitro NDF digestibility. The in vitro fiber digestibility was greater in bmr corn silage than a conventional hybrid, DM intake of cows was greater with the bmr, but total tract digestion of the fiber did not differ between the hybrids. However, NDF digestibility did not increase because higher feed intake decreases the amount of time available for its microbial degradation (Martin et al., 2008). There are also hybrids with high fiber digestibility, such as waxy and stay-green types, which are rarely known. Waxy types have been used for silage but with inconsistent results (Roth and Heinrich, 2001). Some hybrids called “stay-green” maintain leafiness and have a slower DM accumulation in the grain (Arriola et al., 2012). Some hybrids intended for grain production have high yield and better degradability of DM and fiber, and thus also suitable for forage production. According to Dwyer et al. (1998) and Shaver (1983), corn silage produced from leafy hybrids is characterized by more leaves above the ear and, in some cases, higher grain moisture content or softer kernel texture. Leafy types have yields similar to those of grain types, but have softer kernels that dry more slowly. Such varieties may contain less starch and more fiber. Some leafy types were bred for silage production, while others have a faster drying rate, which requires for grain production (Roth and Heinrich, 2001). Corn hybrids traditionally have been selected for grain yield, but also for production of both grain and whole-plant corn silage (Bal et al., 2000). However, hybrids selected for high grain yield may not be the highest yielding for whole plant corn silage (WPCS) (Coors et al., 1994). Although differences in fiber concentrations and in vitro digestibility of WPCS produced from hybrids selected using conventional grain breeding strategies have been reported (Hunt et al., 1992). Feeding trials using corn hybrid silages to evaluate animal performance are limited. Hunt et al. (1993) and Barriere et al. (1995) reported improved weight gain and feed efficiency in beef steers, and DMI and milk yield in dairy cow, respectively, due to hybrid-related improvements in WPCS nutritive value. As reported by Bal et al. (2000) intake, digestion and milk production of dairy cows were not affected by corn hybrids. There are minimal benefits the feeding of leafy or low-fiber corn silage hybrids. Feeding bmr corn silage in a high-forage diet increased milk fat percentage and milk yield as compared to conventional corn silage diet (Bal et al., 2000).

3.3.2. Use of sorghum silage

Sorghum has been grown as a silage crop for many years. In general, under conditions of high temperature and moisture stress, the forage sorghums have given higher yields than corn (Rusche, 2015). Forage sorghum types range from sudan grass to traditional grain sorghum (Neto et al., 2017). In addition, forage sorghums can be bmr or photoperiod sensitive. The type and variety that best utilized will depend on its end use. For silage production, forage sorghums rather than sudan grass or sorghum-sudan grass hybrids are the best choice. Forage sorghums silage typically has lower energy values than corn silage, but their crude protein contents are similar (Table 3). Grant and Stock (1994) reported that, forage sorghum silage has less energy value because of a lower percentage of grain-to-forage, a higher undigested ratio of the grain, and lower digestibility of stalk. When compared to sorghum-sudangrass, forage sorghum silage is higher in energy and lower in protein. Other limitation to sorghum silage fed to cattle is the digestibility is generally less than that of corn, because corn has less lignin and more grain content. The higher lignin content and lower degradability of sorghum silage can result in less fiber digestion, lower DM intake and less milk produced in dairy cow (Cattani et al., 2017). However, bmr sorghum contains less lignin and offers higher digestibility. In this regard Oliver et al. (2004) reported that the total tract NDF digestibility of bmr6 and bmr18 variety is 54.40% and 47.90%, respectively. According to the same author the total tract DM digestibility of bmr6 and bmr18 is 62.90% and 69.10% respectively. Many of the bmr varieties, as well as some of the non-bmr varieties, have consistently had an in-vitro true digestibility (IVTD) value equal or greater 80.70% DM (McCollum et al., 2005) than that of corn. An important point is the variation among the varieties within each type. Utilization of sorghum forage as a total replacement for corn silage in dairy cow diets is possible. Brown mid rib hybrids likely offer the greatest advantage to lactating dairy cattle due to the increased fiber digestibility. Results of the study of Colombini et al. (2010) indicated that, although the rate of NDF degradability of bmr sorghum forage is faster, the effective rumen degradability of NDF in bmr sorghum forage is equal to corn silage. In a study that compared sorghum varieties and corn, total tract digestibility of starch in wild type, bmr6, bmr18, and corn silages were 85.70%, 82.30%, 79.70%, and 91.70%, respectively (Oliver et al., 2004). Development of new cultivars that are more forage than grain types have higher yielding in digestible DM shows promise for the future of sorghum forages. However, the potential for sorghum silage in the diets of high producing dairy cows has not been adequately studied, therefore additional research is needed in this area to fully address how these forages can be utilized in lactating dairy diets.

3.3.3. Use of corn-sorghum mixed silage

Although the yield potential of corn grown for silage is high, it is also sensitive to environmental stress. Dry conditions during any stage of corn growth can significantly reduce corn silage yields. In contrast to corn, forage sorghum possesses a much higher level of drought tolerance and water use efficiency (Grant and Stock, 1994; Getachew et al., 2016). Sowing mixtures of corn and forage sorghum may reduce the risk of low yields during years with below average rainfall and above average temperatures. There are two, often applied, farming techniques to plant mixed cropping: either two rows of maize or two rows of sorghum side by side or corn and sorghum planted in the same row upon each other (Kálmán and Rajki, 2015). Making mixed silage from corn and sorghum 1:1, corn increases the energy value of the silage blend and ensures the appropriate feed value for dairy cows. However, mixed silage has slightly lower dry matter and energy values than corn silage alone. Although, digestibility study report on corn and sorghum mixture silage is rare.

3.3.4. Use of winter cereal silages

Whole crop cereals for silage making are an exciting area of potential integration of the cropping and dairy industries. Research reports are not frequent on the potential of winter cereals for silage making, particularly in Europe. This could be attributed to the long tradition of using winter cereals as green forage, haylage as well as wrapped haylage. However, there are some reports (Table 4) in Hungary LPT Ltd. NIR Laboratory database (April 2013 – August 2017) compiled by Orosz et al. (2017), revealed the potential of early harvested winter cereal silages (boot-early heading, heading and milky dough stage). According to this report, at milky-dough stage, cereal silages have higher DM and lower fiber fraction content than its heading stage. However, NDF digestibility at this stage is lower than heading stage, but OM digestibility generally better for both stages. As compared to the corn silage (Table 3), cereal silage at both milky–dough and heading stages has lower DM and relatively higher fiber fractions (NDF, ADF and ADL). On the other hand, the NRC (2001) nutritional composition table (Table 3) reported that cereal silages have lower NE_l and higher fiber fraction (NDF and ADF) than corn silage. However, the dry matter content is comparable with corn silage, but the crude protein content is higher than corn silage. Digestibility reports are also not so frequent, particularly for whole total tract DM, NDF and ADF digestibility. Lyons et al. (2016) suggested that winter cereals, such as cereal rye and triticale, grown as double crops in corn silage rotations in the Northeast United States have the potential to increase on-farm forage production as well as provide many environmental, economic and nutritional benefits to dairy farms. The author further noted that winter cereals can provide a significant amount of additional, nutritious forage without greatly interfering with corn silage production. Winter wheat, winter triticale, and winter rye can be planted in autumn to produce good yields of high-quality forage in the following spring. Rye will grow and mature the quickest in the spring and must be managed to avoid over ripening. Wheat and winter triticale are easier to manage in spring because they mature later and more slowly than rye. Forage quality of winter cereals (winter wheat, winter triticale, and winter rye) will be excellent if harvested in the vegetative to boot stage of growth in the spring, with yields of 5.0 to 7.0 t DM/ha, depending on harvest stage. It fares well in years with extreme weather, such as in 2016, when a severe drought impacted corn silage throughout New York State (Lyons et al., 2016). Work is ongoing to determine specific sowing dates, harvest times, and fertilizer recommendations for winter cereal crops to ensure successful implementation of these rotations. However, very recently by using double cropping of winter rye for extra forage, farmers are looking for extra forage can plant winter rye following the harvest of many crops, particularly corn silage (Bagg, 2013).

Table 3. Energy and nutrient composition of corn and other crop silages (NRC, 2001)

Components	Energy and nutrient composition							
Silage type	NE_l (MJ/Kg)	DM (%)	CP (% DM)	EE (% DM)	NDF (% DM)	ADF (% DM)	ADL (% DM)	Ash (% DM)
Corn silage	6.57	35.10	8.80	3.20	45.00	28.10	2.60	4.30
Grass silage	4.56	36.20	16.80	2.40	58.20	35.20	6.60	8.70
Italian ryegrass silage	4.37	36.50	12.8	3.10	60.70	40.30	6.90	8.10
Sorghum silage	4.35	28.80	9.10	2.90	60.70	38.70	6.50	7.50
Barley silage	4.89	35.50	12.00	3.50	56.30	34.50	5.60	7.50
Oat silage	4.52	34.60	12.90	3.40	60.60	38.90	5.50	9.80
Triticale silage	4.60	32.00	13.80	3.80	59.70	39.60	5.80	9.70
Wheat silage	4.52	33.30	12.00	3.20	59.90	37.60	5.80	8.60

3.3.5. Use of Italian ryegrass silage

Italian ryegrass (*Lolium multiflorum Lam.*, *var. italicum*) evolved in the Mediterranean region, and in northern Italy, its cultivation as forage for livestock dates back as far as the 12th century (Baldinger et al., 2014). Both fresh and preserved Italian ryegrass is frequently used as forage for dairy cows and known for its high energy value and highly digestible fiber (Tamburini et al., 1995). Plant breeders have developed perennial ryegrass cultivars with an elevated concentration of water-soluble carbohydrates (WSC, also known as high sugar grasses) relative to conventional cultivars (Turner et al., 2006). This breeding has focused on increasing the accumulation of high molecular weight storage sugars (i.e. fructans), particularly in leaf blades rather than sheath bases

(Pavis et al., 2001). It is proposed that perennial ryegrass with high WSC may improve the balance and synchrony of the nitrogen and energy supply to the rumen (Miller et al., 2001). The CP and net energy content of new varieties of Italian ryegrass (e.g. the perennial Bahial hybrid, the one-year Suxyl variety) are high (175-179 g/kg DM and 6.25- 6.28 MJ/kg DM) (Lehel et al., 2011). High energy concentration due to good nutrient digestibility can be explained by relatively low lignin content of the grass hybrid silage (ADL: 20 and 27 g/kg DM) (Lehel et al., 2011). Reports about its positive effects on the forage intake of dairy cows are frequent (Bernard et al., 2002; Baldinger et al., 2011) and some researchers even report better feed efficiency than when feeding corn silage (Cooke et al., 2008). The sugar content of Italian ryegrass is good as compared to other grass silage provided that it is harvested in the early stages of harvesting. In this regard, Baldinger et al. (2014) reported that Italian ryegrass which is harvested at second cut had significantly higher (71.87 %) sugar content than corn silage. However, as the cutting day prolonged, such as the third cut the variation is not significant among them. According to Burke et al. (2007) and Keady et al. (2008) if perennial ryegrass silage replaced with corn silage, it has shown that increasing inclusion of corn silage positively affects the DM intake, milk yield, and milk protein content, while milk fat concentration is either not affected or decreased. Reports on digestibility and degradability on replacing corn silage with Italian ryegrass are not frequent. However, Bernand et al. (2002) reported that apparent DM digestibility declined linearly; whereas CP digestibility increased linearly as Italian ryegrass silage replaced with corn silage. They further noted that apparent digestibility of NDF and ADF was the highest for the diets in which ryegrass or corn silages provided all of the forage, resulting in a quadratic response. Their result on linear increments in apparent digestibility of CP is supported by other reports (González et al., 2007; 2009). The reason for this could be the fact that proteins from silages have a more efficient digestibility than those of their original green forages. This idea is also supported by González et al. (2007), who had an opinion that higher digestibility values are a consequence of the previous degradative actions of the ensiling microorganisms. On the other hand, Narasimaluhi et al. (1984), reported that apparent digestibility of DM, NDF and ADF of Italian ryegrass is 63.60%, 57.30% and 64.10%, respectively. According to the NRC (2001; Table 3) and Jacobs et al. (2009) CP content of Italian ryegrass silage is 12.80% and 12.50% respectively, which is higher as compared to other grass and cereals, even corn silage.

3.3.6. Winter cereal mixtures as alternative to corn silage

Information on seeding two or more winter cereal crops for silage making is not common. However, it has several benefits: increase both grain and forage biomass yield; reduce soil erosion and maintain the environment; increase the probability of crop survival at the time of weather extreme; increase overall productivity and product quality; and improve net return of productivity (Fouli et al., 2012; Ketterings et al., 2015; Larsen et al., 2018; Ranck et al., 2019). Nutrient content and digestibility of different winter-type whole crop cereal silages and mixed silages were studied in Hungary and promising results were achieved (Table 4). These results used as a base line for our study. Massive forage biomass yield with high quality silage is expected from polyculture winter cereal mixtures. For winter cereal mixtures: higher grain yields, limited weed pressure, soil erosion, and exposure to diseases and insect pets (Larsen et al., 2018) are expected, which are regularly serious threats to spring cereals. Additionally, the cereal mixtures are more drought tolerant and induce high forage yield. The individual cereal crops are complementing its own properties in cereal based sown mixtures: the nitrogen uptake of winter wheat is three times greater than winter barley (Hashem et al., 2000) and has faster growth rate than other mixture components as a result it gives high yield. However, the early growth rate of barley is higher than wheat (Cousens, 1996) and it suppress weed invasion during the early growth stage of the sown mixtures. The digestibility of barley and winter oats are excellent; as result it enrich the dry matter intake (DMI) and consequently improve milk production (Raffrenato and Van Amburgh, 2010; Grant, 2012). Winter triticale which is the dominant component of the current mixtures (40% in mixture A and 50% in mixture B, *see Chapter 4.1.*) has an excellent phosphorus removal capacity and induces environmental benefits by reducing phosphorus runoff (Brown, 2006). It also improves overall yield of the sown mixture. One of the limitations in sowing lone winter cereal mixtures is its limited growth rate and low cold tolerance ability at the time of unexpected winter shock (Larsen et al., 2018). There are few reports that modern varieties of winter cereals improve productivity at the time of weather extreme. Even through breeding significantly increases the grain and forage biomass yield of winter cereals, there is no progress in cold tolerance improvement has been realized over the same period (Larsen et al., 2018).

3.3.7. Silage of winter cereal-legume mixtures

Feeding mixed silages of cereals and legumes to ruminants is an established practice in many parts of the world. Compared to grass alone, grass-legume or cereal-legume intercrops are more productive on DM basis and can give higher DM intakes. The other advantages of such mixes also tend to have higher CP contents and therefore their utilization can reduce the requirement for protein supplements in livestock rations, including dairy cow. Intercropping the addition of peas to barley or other small grains including oat or triticale grown for forage does not necessarily improve yield, although it can increase yields from 0.00-0.50 tonnes DM per acre. The main reason for including peas is the positive effect on protein content and palatability of the resulting ensiled forage. Harvest timing of barley/pea forage also has a large impact on yield and quality. Timing of harvest is usually determined by the developmental stage of the oats or other small grain, which normally makes up most of the tonnage (Isleib, 2016). Harvesting at the boot stage of the barley results in higher protein content and improved digestibility this is most desirable if the forage is fed to dairy cattle. Expressed on a DM basis barley has 7.50-18.00% CP (Mustafa et al., 2000). These authors investigated degradability of nutrient of pea and barley silages in cannulated dairy cows. Pea silage had lower content of NDF, ADF, and starch but higher CP than barley silage (mid dough stage). Pea silage has higher effective ruminal degradability of DM than that of barley silage (mid dough stage). The rate of degradation and effective ruminal degradability of NDF was intermediate for pea silage and lowest for barley silage. According to Orosz, et al. (2017) cereal and legume mixed silage (e.g. wheat and pea, barley and pea, triticale and pea) at its milky-dough stage has lower DM and higher fiber fraction. However, the CP content, NDF and OM digestibility is higher than cereal silage alone at the same stage (Table 4). Due to their high protein content, the EU has promoted the production of field peas (*Pisum sativum*). Mustafa and Seguim (2004) studied on in vitro dry matter and NDF digestibility of silages made from whole crop-pea (*Pisum sativum L.*), pea-wheat (*Triticum aestivum L.*), pea-barley (*Hordeum vulgare L.*) and pea-oat (*Avena sativa L.*) mixtures harvested at 8 weeks and 10 weeks after seeding. Forty-five days after ensiling, all forages were well ensiled as indicated by low pH, water soluble carbohydrate and high lactic acid concentration. They further noted that regardless of forage type, CP and in vitro NDF digestibility were higher, while starch and ADL content were lower in 8 weeks than 10 weeks harvesting. The in vitro DMD of whole pea silage was higher than that of the three pea and cereal mixture silages in 8 weeks but was only higher than that of pea barley in week 10 harvest. For the pea and cereal mixtures, IVDMD was higher for pea-oat than pea-barley and pea-wheat in week 8 and was higher for pea-barley than pea-wheat in week 10. They concluded that silage from pea monoculture had similar forage yields and a generally higher nutritive value than silages from pea–cereal mixtures.

Table 4. Nutrient content and digestibility of different winter-type whole crop cereal silages and mixed silages in Hungary
(LPT Ltd. NIR Laboratory, NIR-database, April 2013 - August 2017; Orosz et al., 2017)

	Sample no.	DM	Crude protein	Ash	Crude fiber	NDF	ADF	ADL	NDFd[1] 48	dNDF[2] 48	Sugar	Starch	OMd[3] 48
		g/kg							g/kg DM				
Rye silage (in boot-early heading)	599	293	135	106	300	558	331	27	66	365	39	-	72
Triticale silage (in heading)	18	306	107	82	320	583	352	29	59	339	64	-	66
Triticale silage (milky-dough stage)	44	356	81	69	280	521	327	35	47	254	59	118	64
Oat silage (in heading)	14	323	110	154	291	535	324	31	60	315	31	-	68
Oat silage (milky-dough stage)	9	326	99	101	298	553	320	39	51	270	36	40	64
Barley silage (in heading)	15	317	133	127	304	551	328	30	60	327	35	-	67
Barley silage (milky-dough stage)	48	343	92	77	265	503	297	30	49	240	49	122	66
Wheat silage (in heading)	9	282	121	131	310	565	325	36	58	313	20	-	66
Wheat silage (milky-dough stage)	25	365	92	82	264	502	305	34	46	236	47	122	65
Oat and pea mixed silage (milky-	25	294	130	126	280	504	317	35	58	277	36	52	68
Wheat and pea mixed silage (milky-	35	232	159	101	281	532	317	34	53	275	41	32	69
Barley and pea mixed silage (milky-	29	218	148	87	249	498	250	30	53	227	37	102	70
Triticale and pea mixed silage	35	333	125	87	303	543	345	41	52	279	46	64	66

[1] NDF digestibility (*in vitro*, 48 hours incubation), [2] digestible NDF (*in vitro*, 48 hours incubation), [3] organic matter digestibility (*in vitro*, 48 hours incubation)

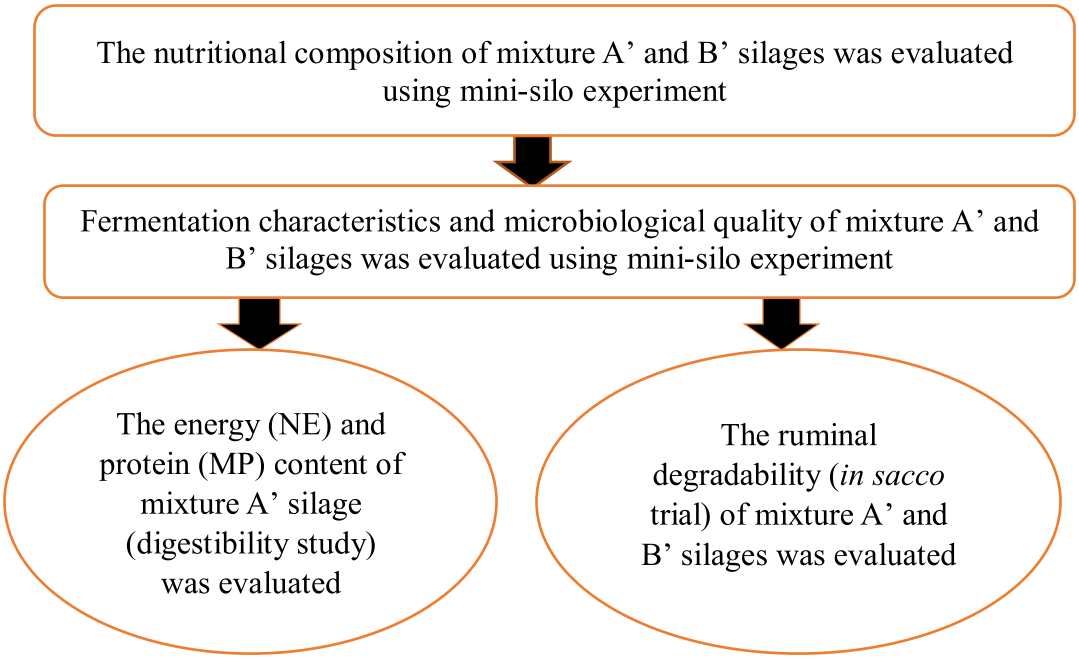
3.3.8. Winter cereal plus Italian ryegrass mixtures silage

Italian ryegrass (*Lolium multiflorum*) is one of the fastest growing grass species with extremely high yielding, fiber digestibility (NDFD), crude protein and sugar content, palatability, high relative forage quality (RFQ), resistance to winter hardiness, ease of establishment, high yield response to nitrogen and suitable for silage making (Baldingar et al., 2012, 2014; Bagg, 2013; DLF seeds, UK, 2018; Byron Seeds, LLC, 2019). However, Bagg (2013) reported that the yield of Italian ryegrass is not as high as winter cereals such as oats, but nutrient quality and palatability is greater which makes it more suitable for high producing dairy cow feed. For Italian ryegrass modern varieties with high fiber digestibility (particularly NDF) and vigorous cold tolerant had been realized (Bagg, 2013; Beaulieu, 2020). Sowing Italian ryegrass plus winter cereal complements each other properties and improves productivity. Italian ryegrass has excellent digestibility and nutritional profile, rapid germination, efficient nitrogen fertilizer utilization and excellent allelopathic effect to suppress weed invasion in the sown mixtures (DLF, Seeds and Science, 2018; Byron seeds, LLC. 2019; Beaulieu, 2020; Bohn et al., 2020). Additionally, it is very effective cover crop; boost cold hardiness, deep root system to prevent soil erosion and good drought tolerant. Italian ryegrass has also excellent and efficient N fertilizer utilization ability than winter cereals such as winter wheat. Hashem et al. (2000) reported that when Italian ryegrass seeded with barley and wheat with N fertilizer, it produces twice forage biomass yield per unit of N uptake at heading stage of mixture than winter wheat; even though the nitrogen uptake by winter wheat is three times greater than Italian ryegrass. Winter cereals and Italian ryegrass mixtures can be integrated in different farming system: monoculture with legumes (Donald, 1997; Francisco, 2005); intercropping of the mixtures with N fertilizer (Hashem, 2000) and/or with limited N fertilizer (Cousens, 1996; Donald, 1997); and double-cropping winter cereals with corn especially in a high density livestock area (Larsen et al., 2018). Winter cereals and Italian ryegrass can be successfully seed into monoculture legumes such as Kura and Persian clover (Donald, 1997; Francisco, 2005) without the use of herbicides and increased early spring or total season forage production the following year. Double-cropping winter cereals with corn (Ketterings et al., 2015, Ranck et al., 2019) are another option for producers. However, Goff et al. (2010) reported that to successfully implement double cropping the right varieties must be used for both crops in the system with an emphasis on earlier productivity and harvest of the winter cereal; and the potential of winter cereals to be inter seeded with a legume as a relay or cover crop to extend the growing season and provide benefits to subsequent crops. Larsen et al. (2018) reported that at high density livestock area double cropping involves a winter cereal (likely triticale or rye) and/or cool season crop (barley or wheat) with warm season crop (silage corn) possible to produce both forge biomass and grain yield at harvest. Fouli et al. (2012) reported that when winter cereal like rye and corn, both harvested as silage in a double crop led to an increase in yield of the entire system by 34% and 38% as compared to single crop corn and alfalfa harvested as silage, respectively. Furthermore, no negative effects were reported on the soil water balance associated with double cropping.

4. MATERIALS AND METHODS

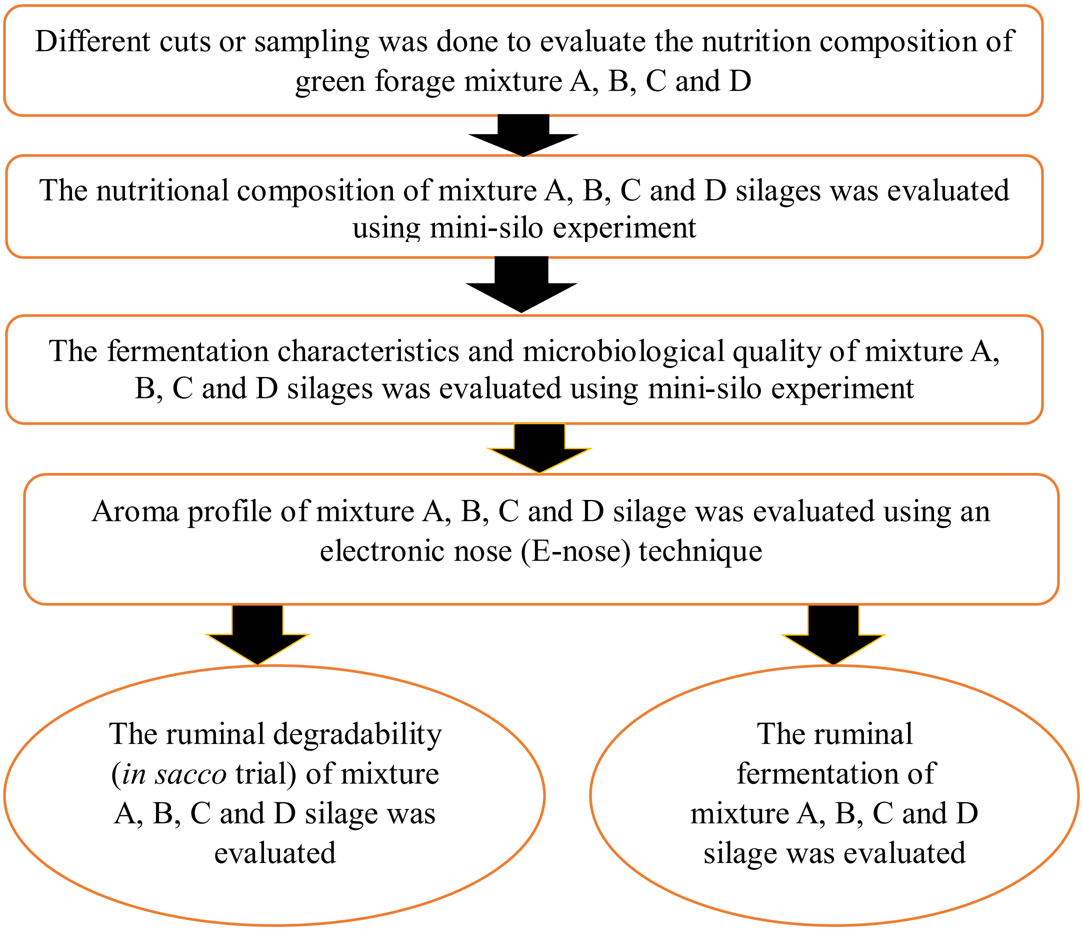
Experiment I

In Experiment I two mixtures of ensiled Italian ryegrass and winter cereals: mixture A : 40% of three cultivars of Italian ryegrass + 20% of two cultivars of winter triticale + 20% of two cultivars of winter oats + 15% of winter wheat + 5% of winter barley; and mixture B : 55% of three cultivars Italian ryegrass + 45% of two cultivars of winter oats were studied for chemical composition, fermentation characteristics, microbial counts, ruminal degradability. Additionally, the digestibility study (energy and protein evaluation) was conducted using mixture A silage.



Experiment II

In Experiment II, four mixtures: two winter cereal based (mixture A: 40% of two cultivars of winter triticale + 30% of two cultivars of winter oats + 20% of winter barley + 10% of winter wheat and mixture B: 50% of two cultivars of winter triticale + 40% of winter barley + 10% of winter wheat) and two Italian ryegrass plus cereal grain mixtures (mixture C: 55% of three types of Italian ryegrass + 45% of two cultivars of winter oats and mixture D: 40% of three types of Italian ryegrass + 30% of two cultivars of winter oat + 15% of two cultivars of winter triticale + 10% of winter barley + 5% of winter wheat) were studied for chemical composition, fermentation characteristics, microbial counts, ruminal degradability and ruminal fermentation study. Additionally, subsequent cuts were done to study the nutritional profile of the forage at different crop mixtures from leafy to early heading stage (harvesting stage). The quality of silages was evaluated for aroma profile using electronic – nose technique. The whole process of the study was shown in schematic diagram as follows:



Different cuts or sampling was done to evaluate the nutrition composition of green forage mixture A, B, C and D

The nutritional composition of mixture A, B, C and D silages was evaluated using mini-silo experiment

The fermentation characteristics and microbiological quality of mixture A, B, C and D silages was evaluated using mini-silo experiment

Aroma profile of mixture A, B, C and D silage was evaluated using an electronic nose (E-nose) technique

The ruminal degradability (*in sacco* trial) of mixture A, B, C and D silage was evaluated

The ruminal fermentation of mixture A, B, C and D silage was evaluated

Table 5. Composition of the investigated mixtures

Mixtures	A’	B’	A	B	C	D
Plant (% of kg seed)	**1st trial**		**2nd trial**			
Italian ryegrass (three cultivars)	40	55	--	--	55	40
Triticale (two cultivars)	20	--	40	50	--	15
Winter oats (two cultivars)	20	45	30	--	45	30
Winter wheat	15	--	10	10	--	5
Winter barley	5	--	20	40	--	10

4.1. Experimental site and ensiling procedure (Experiment I)

The trial was carried out on a large-scale farm (Galgamenti Agricultural Limited Company, Tura, Hungary - 47.593637 N, 19.576483 E, at 119 m altitude). Combined seed mixture of two different forage crops (commercial products, producer: *Agroteam S.p.a.*, Torrimpietre (RM), Via di Granaretto, 26, 00054 Italy) were studied: mixture A’ (40% of three cultivars of Italian ryegrass + 20% of two cultivars of winter triticale + 20% of two cultivars of winter oats + 15% of winter wheat + 5% of winter barley; and mixture B’ : 55% of three cultivars Italian ryegrass + 45% of two cultivars of winter oats). Experimental field was 30,600 m^2 (width: 36 m; length: 850 m) for each mixture. Deep loosening and disc plus cylinder cultivation were executed as stubble tillage after winter rapeseed (*Brassica napus L.*). Slurry (10 m^3/ha) and 300 kg/ha artificial fertilizer (NPK: 14:10:20) was applied before sowing on sandy soil. Seedbed was prepared by Kongskilde VibroFlex 7400 cultivator (lifted). The two different forage mixtures were sown on 11th September 2017 (mixture A’: 75 kg seed/ha; mixture B’: 75 kg seed/ha) with depth of 2-5 cm with John Deere 740 A type seed drill. Plant protection treatment was not applied during the growing season. The annual precipitation was 718 mm in 2017. The amount of precipitation during the vegetation period and during the time when the experiment lasted was shown in Table 6.

Table 6. Total precipitation (mm) by month during the growing season of 2017-2018

Months	Precipitation (mm)
September	167 mm
October	64 mm
November	51 mm
December	38 mm
January	17 mm
February	85 mm
March	55 mm
April	5 mm

Cutting was carried out in heading stage of triticale based on the existing extended BBCH-scale (Meier, 2001) [25th April, 2018, BBCH (Biologische Bundesanstalt, Bundessortenamt and Chemical Industry) 57-58], at 10 cm stubble height. After cutting fresh forage with nutritional composition (Table 7) were wilted (24h) without any movement on the windrow. Fresh forage was sampled by hand cut using a 1×1 quadrant. The wilted forage was chopped by a forage harvester (John Deere 7300) on concrete surface with theoretical chop length (TCL) of 9 mm (weight: 800 kg of harvested forage). Wilted and chopped material of 510 g were packed by hand into anaerobic glass jars capacity of 0.72 liter (total no. of laboratory silos = 30 (15/mixture), replicated five times) and stored in a controlled laboratory temperature at 21 °C. The applied density was 708 kg wilted material/m^3 (mixture A’: 200 kg DM/m^3; mixture B’: 219 kg DM/m^3).

Table 7. Nutritional compositions of fresh forage mixtures right before ensiling (n = 20)

	(g/kg DM)	
Components	mixture A’	mixture B’
Dry matter (g)	189	195
Crude protein	161	159
Neutral detergent fiber	485	519
Total sugar	137	138

mixture A’: 40% of three cultivars of Italian ryegrass + 20% of two cultivars of winter triticale + 20% of two cultivars of winter oats + 15% of winter wheat + 5% of winter barley mixture B’: 55% of three cultivars Italian ryegrass + 45% of two cultivars of winter oats

4.2. Experimental site and ensiling procedure (Experiment II)

The trial was carried out on a medium-scale farm (Kaposvár University, Hungary – 46°22' N 17°48' E, 153 m altitude (GeoDatos, 2020). Four different forage mixtures (commercial products, *Agroteam S.p.a.*, Torrimpietre (RM), Via di Granaretto, 26, 00054 Italy) were studied: mixture A (40% of two cultivars of winter triticale + 30% of two cultivars of winter oats + 20% of winter barley + 10% of winter wheat), mixture B (50% of two cultivars of winter triticale + 40% of winter barley + 10% of winter wheat), mixture C (55% of three types of Italian ryegrass + 45% of two cultivars of winter oat), mixture D (40% of three types of Italian ryegrass + 30% of two cultivars of winter oat + 15% of two cultivars of winter triticale + 10% of winter barley + 5% of winter wheat). The experimental field allotted 3 hectares to each mixture. Deep loosening and disc + cylinder cultivation were executed as stubble tillage. 351 kg/ha artificial fertilizer (NPK: 16:16:16) was applied before sowing. Seedbed was prepared by Kongskilde VibroFlex 7400 cultivator (lifted). The four different forage mixtures were sown on 29th September 2018 (mixture A: 75 kg seed/ha; mixture B: 75 kg seed/ha; mixture C: 75 kg seed/ha, mixture D: 75 kg seed/ha) with depth of 3 cm with John Deere 740 A type seed drill. Plant protection treatment was not applied during the growing period. The annual precipitation was 425 mm in 2018 (World weather online/Kaposvár monthly climate average). The amount of precipitation during the vegetation period and during the time when the experiment lasted was shown in Table 8.

Table 8. Total precipitation (mm) by month during the growing season of 2018-2019

Months	Precipitation (mm)
September	34 mm
October	25 mm
November	56 mm
December	43 mm
January	55 mm
February	30 mm
March	52 mm
April	116 mm

Cutting was carried out at the heading stage of triticale based on the existing extended BBCH-scale (Meier, 2001) on 4th May 2019 (BBCH (Biologische Bundesanstalt für Land-und Forstwirtschaft) (1997) 51-58. (Italian ryegrass: BBCH51; oat: BBCH51; triticale: BBCH53; winter wheat: BBCH52; winter barley: BBCH58). The fresh mixture A with nutritional composition (Table 9) were wilted to 35% DM (24h) without any movement on the windrow. The wilted forage was chopped by a forage harvester (John Deere 7300) on concrete surface with theoretical chop length (TCL) of 9 mm (weight of wilted and chopped forage: 800 kg). Wilted and chopped material of 510 g were packed into a laboratory silo/glass jars capacity of 0.72 liter using a mechanical hand packer without additives and ensiled for 90 days. Total number of laboratory silos were n=80 (20 (4 silages × 5 laboratory silo/ treatments) for fermentation quality + 60 (15/treatment) for aroma profile study). Then the silos were stored in a laboratory of Hungarian University of Agriculture and Life Sciences at a temperature of 21 °C.

Table 9. Nutritional compositions of fresh forage mixtures right before ensiling (n = 20)

	(g/kg DM)			
Components	mixture A	mixture B	mixture C	mixture D
Dry matter (g)	186	184	168	173
Crude protein	125	117	108	95
Neutral detergent fiber	566	579	535	532
Total sugar	168	166	168	140

mixture A: 40% of two cultivars of winter triticale + 30% of two cultivars of winter oats + 20% of winter barley + 10% of winter wheat; mixture B: 50% of two cultivars of winter triticale + 40% of winter barley + 10% of winter wheat; mixture C: 55% of three types of Italian ryegrass + 45% of two cultivars of winter oat; mixture D: 40% of three types of Italian ryegrass + 30% of two cultivars of winter oat + 15% of two cultivars of winter triticale + 10% of winter barley + 5% of winter wheat.

The remaining forage mixtures was stored as baleage for rumen fermentation study as follows: After cutting, the fresh forage mixtures were wilted to 35% DM (24h) without any movement on the windrow to have a well fermented haylage. During wilting the forage mixtures did not ted since tedding leaves the stems oriented at random while parallel stems will allow baling denser. Then the wilted forage with a capacity of 578 – 675 kg was wrapped (using forage harvester, John Deere 7300 fitted with cross wrap bale wrappers) without additives in plastic (within 2 hours to exclude air) using 6 mils of plastic and 50% overlap and 50% to 55% stretch. Wrapping was done in dry weather for plastic to stick. Then bales were stored in Hungarian University of Agriculture and Life Sciences dairy farms in a level concrete floor and the bales are arranged stacked to reduce sunlight exposure to save plastic and reduce sweating.

4.3. Sampling during growth (Experiment II)

In order to study nutritional composition, samples (n= 20, 5/treatment) from all sown forage mixture were taken on 1st week (1st ST), 2nd week (2nd ST), 3rd week (3rd ST), and 4th week (4th ST) of end of leafy stage on April 2019. At each sampling time, forage was cut at the sampling length of 1 cm above ground level from five replicates using quadrats (1m×1m) throughout the field. Subsamples (1 kg) of the forages were dried at 60 °C for 48 h and then ground through a 2 mm screen sieve.

4.4. Chemical analysis (Experiment I and II)

Samples from the different cuts were examined for DM, CP, CF, EE, ash, and total sugar content using AOAC (2006) protocol following specific procedure identification numbers 37 (Nitrogen), 39 (fat), 44 (fiber), 55 (sugars), 56 (mineral). Additionally, the nitrogen free extract (NFE) and organic matter (OM) were calculated as follows: 100% - (% EE + % CP + % Ash + % CF) and 100% - % ash, respectively.

Five laboratory silos per experimental mixtures were opened on 7, 14 and 90 days after ensiling (n=15/treatment). DM, CP, CF, neutral detergent fiber (NDF), acid detergent fiber (ADF), EE, ash, and total sugar content of all mixtures were determined. The chemical analyses of the fresh and mixtures silages were done following (AOAC, 2006) protocol and Van Soest et al. (1991) (ADF, NDF, ADL) following sodium sulphite assay. Approximately 25 g composite sample was taken from each laboratory silo immediately after opening. The sample silage was mixed with 100 ml of distilled water. After hydration for 10 min using blender, the diluted material was then filtered through cheese cloth and then pH was determined by using a digital pH meter (Metrohm 744, Switzerland). The lactate was analysed by high-performance liquid chromatography (HPLC) method developed by Megias et al. (1993).

Acetic acid, butyric acid, propionic acid and ethanol were measured by gas chromatography (Chrompack, Model CP 9002, The Netherlands) as described by Playne (1985). Ammonia concentration was determined by a modified Berthelot method (Chaney and Marbach, 1962).

4.5. Microbiological counts (Experiment I and II)

Aerobic mesophilic microorganism count (AMC) or mold and yeasts count of ensiled forage at the three opening days (7, 14 and 90 days) were determined at the Laboratory of Kaposvár University, Hungary following the standard laboratory protocols (EN ISO 4833-1:2013 and EN ISO 21527-1:2008) using a standard dispersion plate method (Pitt and Hocking, 2009). Total microbiological counts were expressed as colony forming units per gram (CFU g-1) and were transformed into log10 to obtain the lognormal distribution.

4.6. Aroma profiling with electronic nose (Experiment II)

Sample from fresh green forage and from each opening day (7, 14 and 90) were frozen (n=80, 20/treatment) before it sent to the laboratory of ADEXGO Ltd. in Herceghalom. Frozen samples were thawed and chopped with scissors. The smell fingerprints of the silage samples were acquired in 3 replicates by measuring 3-times 1 g of each into 20 mL headspace vials which were then sealed with a magnetic cup and a PTFE septum. The EN measurement was performed with a Heracles Neo 300 ultra-fast GC analyzer (Alpha MOS, Toulouse, France), specifically designed for the rapid analysis of volatile compounds. The EN was equipped with a PAL-RSI autosampler unit for standard handling the samples, generating headspace, and injecting the headspace into the Heracles analyzer unit, including an odor concentrator trap and two metal capillary columns (Restek MXT-5: length 10 m, ID 0.18 mm, thickness: 0.40 µm, low-polarity stationary phase composed of cross bond 5% diphenyl / 95% dimethyl polysiloxane; and Restek MXT-1701: length 10 m, ID

0.18 mm, thickness: 0.40 µm, mid-polarity stationary phase composed of cross bond 14% cyanopropylphenyl / 86% dimethyl polysiloxane (Restek, Co., Bellefonte, PA, USA). The volatile compounds were separated by both columns simultaneously and detected with two flame ionization detectors (FID). The autosampler and the analyzer were operated with the software AlphaSoft ver. 16 (Alpha MOS, Toulouse, France), and the same software was used for data acquisition and data transformations. The retention times of the volatiles recorded on both columns were converted to Kováts retention indices (RI) that relate the retention time of the investigated volatile molecules of a sample to the retention time of n-alkanes under the same conditions (Alpha MOS, 2018). The RI characterizes the volatile compounds on the specific columns and can be assigned to specific aroma recorded in the AroChemBase v7 of AlphaSoft software. In this study, "1-A" as an identifier after the RI refers to column MXT-5 and "2-A" refers to column MXT-1701. Before the analysis, a method was created with the following parameters of the PAL-RSI Autosampler and Heracles GC analyzer: Autosampler: incubation at 40 °C for 5 min with 500 rpm agitation to generate headspace, 1 mL of headspace injected into the Heracles analyser, flushing time between injections: 90 s; Analyzer: carrier gas: hydrogen, the flow of carrier gas: 30 mL/min, trapping temperature: 30 °C, initial oven temperature: 50 °C, the endpoint of oven temperature: 250 °C, heating rate: 2 °C/s, acquisition duration: 110 s, acquisition period: 0.01 s, injection speed: 125 µl/s, cleaning phase: 8 min.

4.7. Ruminal degradability (Experiment I and II)

After the ninety days of fermentation, the ensiled mixtures were subjected to ruminal degradability study. The ruminal degradability trial was carried out with three multiparous non-lactating Holstein-Friesian dairy cows (600±35 kg body weight) previously surgically fitted (ethical permission number - SOI/31/01044 – 3/2017) with a ruminal cannula (10 cm id., Bar-Diamond Inc., Parma, Idaho, USA) at the experimental dairy farm of Kaposvár University, Hungary. Cows were fed total mixed ration (TMR) formulated according to the dairy nutrient requirement and feeding standard (NRC, 2001) in equal portions at 8:00 and 14:00 on *ad libitum* basis. The baseline diet [9.12 kg dry matter intake (DMI)/day; 6.32 MJ NE_l /kg DM; 14.40% CP, 39.06% NDF, 23.66% ADF, and 35.71% non-fibrous carbohydrate (NFC)] consisted of 5.50 kg day^{-1} of corn silage, 3.50 kg day^{-1} of alfalfa haylage, 3.50 kg day^{-1} of vetch-triticale haylage, 3 kg day^{-1} of concentrate, 1 kg day^{-1} of grass hay and 0.75 kg day^{-1} of liquid molasses. The cows consumed the daily allotted TMR with no daily feed refusal throughout the course of the experimental period. Water was available *ad libitum*. Rumen incubations were carried out according to Herrera-Saldana et al. (1990). Nylon bags of 5×10 cm with pore size of 53 µm (Ankom, USA) filled with sample weight of 5.00 g (on air dry matter basis) was incubated for 0, 2, 4, 8, 16, 24, 48 and 72 h incubation times. In each incubation, 60 bags per sample were used (5 bags × 4 replications per sample × 3 cows). The 0 h samples were not placed in the rumen, but they were soaked and rinsed as described below. Removed bags were placed in cold tap water immediately after removal from the rumen, and they were washed by hand until the water was clear. After washing, the bags were dried in a forced air oven at 60 °C for 48 h, air equilibrated and weighed. Residues from the bags were pooled within time and animal, finely ground by mortar and pestle to pass through a 1-mm screen and retained in sealed containers to determine DM, CP, NDF and ADF. Feeds were analyzed for nitrogen according to Kjeldahl (AOAC, 2006), and thereafter, CP was determined by the total nitrogen (N) × 6.25. The NDF and ADF contents were residual portions after rinsing according to Van Soest et al. (1991).

4.7.1. Calculations and statistical analysis (Experiment I and II)

4.7.1.1. Calculations

Residues from the nylon bags at each incubation time were analyzed for DM, CP, NDF and ADF as described above. Ruminal nutrient disappearance data were used to determine nutrient degradation parameters using the equation (Ørskov and McDonald, 1979):

$$P = a + b\,(1 - e^{-ct}),$$

where P is the DM, CP, NDF or ADF disappearance (%) at time t, a is the soluble fraction (%), b is the potentially degradable fraction (%), and c is the rate of degradation of the b fraction (%/h). Effective degradability (ED) of DM, CP, NDF and ADF was then calculated according to the equation (Ørskov and McDonald, 1979):

$$ED = a + ((b \times c)/(k + c)),$$

where k is the rumen outflow rate assumed to be 1, 5 and 8%/h and a, b, and c are as described above. NLIN program in SAS (version 9.4; SAS Institute, Inc., Cary, NC, USA) was used to calculate the values of a, b, and c.

4.8. Ruminal fermentation (Experiment II)

The ruminal fermentation trial was carried out with three multiparous non-lactating rumen cannulated Holstein-Friesian dairy cows. The ruminal fermentation trial was conducted with mixtures silage following ninety days fermentation in the form of baleage capacity (578 – 675 kg) for all ensiled mixtures. Cows consumed TMR (control diet) as described in ruminal degradability (see *chapter 4.6*) plus ensiled mixtures by substitution 3.5 kg day^{-1} (experimental diet 1, 2, 3 and 4), instead of vetch-triticale haylage (Table 10 and Table 11). The daily ration of both the control and experimental diets were given in 2 instalments (8:00 am and 14:00 pm). The pre-feeding period lasted 14 days, which was followed by the 14-day experimental phase (Figure 1).

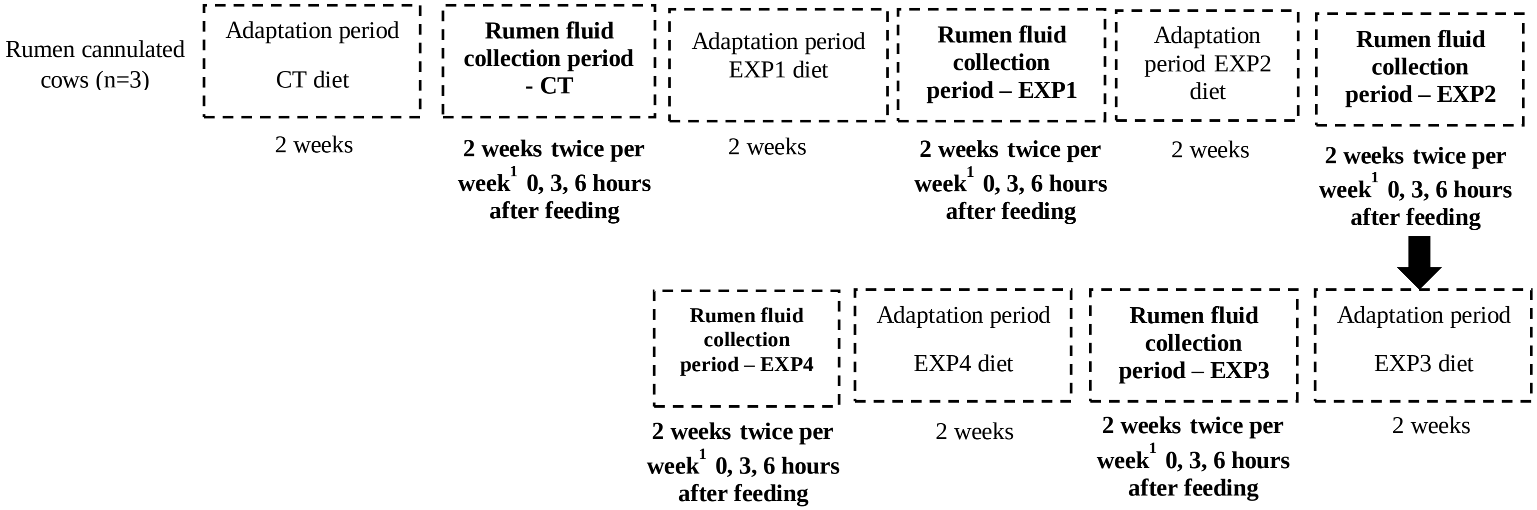


Figure 1. The experimental design for rumen fermentation study

Control diet (CT): 5.5 kg day^{-1} of corn silage, 3.5 kg day^{-1} of alfalfa haylage, 3.5 kg day^{-1} of vetch-triticale haylage (VTH), 3 kg day^{-1} of concentrate, 1 kg day^{-1} of grass hay and 0.75 kg day^{-1} of liquid molasses.

Experimental diet 1 (EXP1): CT + Mixture A silage (3.5 kg day^{-1}, replacing VTH)

Experimental diet 2 (EXP2): CT + Mixture B silage (3.5 kg day^{-1}, replacing VTH)

Experimental diet 3 (EXP3): CT + Mixture C silage (3.5 kg day^{-1}, replacing VTH)

Experimental diet 4 (EXP4): CT + Mixture D silage (3.5 kg day^{-1}, replacing VTH)

Mixture A-D (see Table 2)

[1] Rumen fluid collection twice (on Monday and on Wednesday) for each week and a total of four collection per two weeks

Rumen fluid sampling was performed twice a week (Monday and Wednesday). On sampling days, approx. 150 ml of rumen fluid samples were collected 3 times a day (immediately before morning feeding and then 3 and 6 hours thereafter, n=36/mixture) through the cannula using ruminal fluid collection device (Bar-Diamond Inc., Parma, Idaho, USA). The pH was measured immediately using a digital pH meter (Metrohm 744, Switzerland). Ammonia was determined by Berthelot method (Chaney and Marbach, 1962). Thereafter samples were centrifuged to analyse the volatile fatty acid (VFA) and lactic acid. The concentration of short chain fatty acids (acetic acid, propionic acid, iso-butyric acid, n-butyric acid, iso-valeric acid or n-valeric acid, iso-caproic acid, caproic acid) of rumen fluid and silages were measured by gas chromatography (Chrompack, Model CP 9002, The Netherlands) as described by (Playne, 1985). The lactate was analysed by high-performance liquid chromatography (HPLC) method developed by (Playne, 1985).

Table 10. Composition and calculated values of the baseline diet used in the rumen degradability and ruminal fermentation study as a control diet

Parameter	**Baseline/Control diet**
Ingredient, kg	***kg/ cow/ day***
Corn silage	5.50
Alfalfa haylage	3.50
Vetch-triticale haylage	3.50
Concentrate[1]	3.00
Grass hay	1.00
Molasses (liquid)	0.75
Calculated nutrients	**% DM**
Dry matter (%, as in feed)	47.42
Crude protein	14.40
Neutral detergent fiber	39.06
Acid detergent fiber	23.66
Acid detergent lignin	4.68
Ether extract	2.83
Non-fibrous carbohydrate	35.71
Starch	25.60
Sugar	6.06
Calcium	1.08
Phosphorus	0.40
Sodium	0.23
Vitamin A (IU kg^{-1})	8,725
Vitamin D (IU kg^{-1})	1,722
Vitamin E (mg kg^{-1})	43
Net energy for lactation (MJ kg^{-1} dry matter)	6.32

[1] Vitafort Co., Dabas, Hungary (“533-614”, dry matter: 88.00%, crude protein: 16.00%, NE_l MJ kg^{-1}: 6.74, crude fiber: 5.00%, ether extract: 2.90%, ash: 8.30%, starch: 42.71%, sugar: 2.34%, calcium: 1.71%, phosphorus: 0.57%, sodium: 0.66%, magnesium: 0.37%, vitamin A: 22,800 IU kg^{-1}, vitamin D: 4,500 NE kg^{-1}, vitamin E: 128 mg kg^{-1},

Table 11. Control and experimental diets and its compositions for ruminal fermentation study

Diets	Components
Control diet	5.50 kg day^{-1} of corn silage, 3.50 kg day^{-1} of alfalfa haylage, 3.50 kg day^{-1} of vetch-triticale haylage, 3 kg day^{-1} of concentrate, 1 kg day^{-1} of grass hay and 0.75 kg day^{-1} of liquid molasses.
Experimental diet 1	Control diet + Mixture A silage (3.50 kg day^{-1}, instead of vetch-triticale haylage)
Experimental diet 2	Control diet + Mixture B silage (3.50 kg day^{-1}, instead of vetch-triticale haylage)
Experimental diet 3	Control diet + Mixture C silage (3.50 kg day^{-1}, instead of vetch-triticale haylage)
Experimental diet 4	Control diet + Mixture D silage (3.50 kg day^{-1}, instead of vetch-triticale haylage)

Control diet: 6.32 MJ NE_l kg^{-1} DM; 14.40% CP; 39.06% NDF; 23.66% ADF and 35.71% non-fibrous carbohydrate (NFC)

Mixture A: 40% of two cultivars of winter triticale + 30% of two cultivars of winter oats + 20% of winter barley + 10% of winter wheat; Mixture B: 50% of two cultivars of winter triticale + 40% of winter barley + 10% of winter wheat; Mixture C: 55% of three types of Italian ryegrass + 45% of two cultivars of winter oat; Mixture D: 40% of three types of Italian ryegrass + 30% of two cultivars of winter oat + 15% of two cultivars of winter triticale + 10% of winter barley + 5% of winter wheat

4.9. Energy and protein evaluation

4.9.1. Digestibility study

Digestibility trial was conducted using mixture A’ silage (Experiment I) at the National Agricultural Research and Innovation Centre Research, Herceghalom, Hungary. Six wethers Hungarian Merino sheep (4 years of age) with an average body weight of 84.56±5.53 kg was housed in individual metabolic cages with slatted floors with *ad libitum* access to water. The trial consisted of a 10-days adjustment period followed by 5-days of complete faeces collection. The experimental ensiled mixture was offered as the sole feed to the sheep and fed two equal meals per day (07.00 and 15.00 h). A daily ration was determined on the basis of live weight (calculated as maintenance DM requirement, adjusted approximately by 2% higher than actual body weight). The sheep received 1.40 kg dry matter intake (DMI)/day plus daily 30 g mineral and vitamin premix (producer: Bábolna Takarmányipari Ltd., Nagyigmánd, Hungary) plus 10 g NaCl. Feed intake, feed refusal and faecal output were recorded daily during the collection period. A 25% sample of faeces was sub-sampled daily from each animal and pooled for each animal for chemical composition, dried in a forced air oven at 60 °C for 24 h and ground through a 1 mm screen to determine the DM percentage. Feed samples were taken at the beginning of adaptation period, and at the beginning and the end of collection period. Feed and faecal samples were analysed for DM, CP, CF, EE, ash, NDF, ADF according to the official methods of Hungarian Feed Codex (2004).

4.9.2. Calculation of digestibility, energy and protein values

4.9.2.1. Digestibility

The digestibility coefficient (DC, %) for nutrients was calculated for each animal on the basis of quantitative data for intake and output according to the classical formula: DC (%) = 100 × (NI-NE)/NI, where, NI represented the nutrient intake and NE expressed the nutrient excreted.

4.9.2.2. Energy evaluation

The net energy for lactation, maintenance and growth was calculated on the basis of digestible nutrients as suggested by the NRC (2001). The energy concentration was calculated as follows:

DE (Digestible Energy) of feeds using equation 2-8 (NRC, 2001)

DE_{1X} (Mcal/kg) = (tdNFC/100) * 4.2 + (tdNDF/100) * 4.2 + (tdCPf/100) * 5.6 + (FA/100) * 9.4 – 0.3

Where,

- Truly digestible NFC (tdNFC) = 0.98 (100 – [(NDF – NDICP) + CP + EE + Ash]) * PAF (NRC, 2001, equation 2-4a)
- Truly digestible CP for forage (tdcpf), tdcpf= CP*exp (-1.2*(ADICP/CP) (NRC 2001, equation 2-4b)
- Truly digestible FA (tdFA)= FA, If EE<1, then FA=0 (NRC 2001, equation 2-4d)

- Truly digestible NDF (tdNDF), tdNDF= 0.75*(NDF_n – L) *(1-(L/NDF_n)$^{0.667}$) (NRC 2001, equation 2-4e)
- Processing Adjustment Factors (PAF) is 1 for all other feeds (NRC, 2001, Table 2-1)
- Fatty acid (FA) = EE -1

Assume an 8% discount factor (i.e., multiply value from step 1 by 0.92)

$DE_P = DE_{1X} * 0.92$

ME (Metabolizable Energy) of feeds using equation 2-10 (NRC, 2001)

ME_P (Mcal/kg) = $[1.01 * (DE_P) – 0.45] + 0.0046 * (EE - 3)$

Where DE_P is Mcal/kg and EE in percent DM

NE_l (Net Energy for Lactation) of feeds using equation 2-12 (NRC, 2001)

NE_l (Mcal/kg) = $0.703 \times ME_P – 0.19 + ([(0.097 \times ME_P + 0.19)/97] \times [EE -3])$

where ME_P is expressed as Mcal/kg and EE in percent DM, EE – Ether extract, 1 mega calorie (Mcal) is equal to 4.184 mega joules (MJ).

Estimating Net Energy of Feeds for Maintenance and Gain

ME (Metabolizable Energy) of feeds (NRC, 1996)

$ME = DE_{1X} \times 0.82$

where DE_{1X} = Digestible energy

NE_m (Net Energy for maintenance) of feeds using equation 2-13 (NRC, 2001)

$NE_m = 1.37\ ME – 0.138\ ME^2 + 0.0105\ ME^3 – 1.12$ (Garrett, 1980)

where ME – Metabolizable energy

NE_g (Net Energy for growth) of feeds using equation 2-14 (NRC, 2001)

$NE_g = 1.42\ ME – 0.174\ ME^2 + 0.0122\ ME^3 – 1.65$ (Garrett, 1980)

where ME – Metabolizable energy

4.9.2.3. Protein evaluation

The protein evaluation was done following the Hungarian metabolizable protein system for ruminants (Schmidt et al., 1998). The formulas proposed for the calculation of protein values of feed was the follows:

MPE g/kg DM = 0.9 * (UDP - ADIN × 6.25) + 160*FOM × 0.8 × 0.8

MPN g/kg DM = 0.9 * (UDP - ADIN × 6.25) + RDP × 0.9 × 0.8 × 0.8

where MPE = energy dependent metabolizable protein; MPN = Nitrogen dependent metabolizable protein; UDP = Rumen undegradable protein; ADIN = acid detergent insoluble nitrogen;

RDP = Rumen degradable protein; FOM = Fermentable organic matter; FOM = DOM – (UDP + digestible fat + fermentation products + bypass starch), where DOM – Digestible organic matter.

4.10. Statistical analysis

4.10.1. Nutritional composition, fermentation characteristics and microbiological count

Data were analysed using the GLM procedure for ANOVA in SAS 9.1 software (SAS Inst. Inc., Cary, North Carolina, USA). Significant mean value differences were evaluated by Tukey's test following a post hoc comparison of means. A significance level of $P<0.05$ was used. Variables of nutritional composition of green forage mixtures were computed using the following model:

$$Y_i = \mu + \alpha_i + \varepsilon_i,$$

where Y_i is the observation in the i^{th} crop mixture effect, μ is the overall mean, α_i is the i^{th} crop mixture effect and ε_i is the random error.

Variables for nutritional composition, fermentation characteristics and microbiological count among the three opening days, different crop mixtures and their interaction were computed using the following model:

$$Y_{ij} = \mu + \alpha_i + \beta_j + \gamma_{ij} + \varepsilon_{ij}$$

where Y_i is the observation in the i^{th} different opening days, j^{th} crop mixture and their interaction, μ is the overall mean, α_i is the i^{th} opening days effect, β_j is j^{th}

crop mixture effect, γ_{ij} is the interaction of opening days and crop mixture and ε_{ij} is the random error.

4.10.2. Digestibility, *in situ* degradability and ruminal fermentation

Comparison of means of variables for digestibility of nutrients was carried out using the following model:

$$Y_i = \mu + \alpha_i + \varepsilon_i,$$

where Y_i is the digestibility of nutrients in the i^{th} sheep effect, μ is the overall mean, α_i is the i^{th} sheep effect and ε_i is the random error.

Comparison of means for degradability components were performed following model;

$$Y_i = \mu + \beta_i + \varepsilon_i,$$

where Y_i is the observation in the i^{th} silage type, μ is the overall mean, β_i is the i^{th} silage type effect and ε_i is the random error. Comparison of means for effective nutrient degradability was computed for 1%, 5% and 8% rumen outflow rates.

Comparison of the means of variables between treatments for rumen fermentation characteristics was computed by a two-way ANOVA following the model:

$$Y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij},$$

where Y_{ij} is the observation in the i^{th} treatment and j^{th} rumen fluid sampling period; μ is the overall mean; α_i and β_j is the i^{th} treatment and the j^{th} rumen fluid sampling period effects and; ε_{ij} were the random errors.

4.10.3. Multivariate data analysis

The multivariate data of the EN measurements describing the odour profiles of the feed samples were analysed with the AlphaSoft (ver. 16) software (Alpha

MOS, Toulouse, France). The chromatograms were transformed into a series of variables called sensors based on the identified chromatogram peaks (Kovacs et al., 2020). The name of a sensor originated from the location of the peak within the chromatogram and was identical to the respective retention indices (RI). The intensity of the sensor was calculated from the area under the respective chromatogram peak. Principal component analysis (PCA) was performed using the sensor data to detect outlier records and to describe the non-supervised clustering of the samples within the multidimensional space defined by the sensor variables (Naes et al., 2002). The PCA models were characterized by the discrimination index (%) between the classified groups, where positive values indicated group separations without overlapping on the examined plane of principal components. Supervised classification models were built using linear discriminant analysis (LDA) to find linear combinations of the sensor variables, optimally discriminating against the pre-defined groups (Naes et al., 2002). The accuracy of the LDA classification models was tested with leave-one-out cross-validation, when a single record was left out of the modelling process and was used for testing by predicting its group identity – the process was repeated iteratively until all samples were used for validation once (Naes et al., 2002). The cross-validations were evaluated based on the validation score, representing the ratio of correctly classified samples. The sensor selection function of AlphaSoft (provider, city, country) was used for tracing the most distinctive variables that show the largest capability to contribute to an LDA model identifying the actual pre-defined groups. The LDA calculations based on the selected sensors were also performed, and the impact of the sensors was evaluated by their orientations in PCA and LDA bi-plots. The volatile compounds described by the selected sensors were identified using the AroChemBase database (Alpha MOS, Toulouse, France).

5. RESULTS AND DISSCUSSIONS (EXPERIMENT I)

5.1. Nutritional composition of ensiled mixtures

The fermentation process affected ($p<0.05$) the DM and NDF content of both mixture silages at each opening day (Table 12). The mixture type did not affect ($p>0.05$) the DM, CP and total sugar contents of mixture silages at the three opening days (7, 14 and 90). However, the ADF (mixture A’) and NDF (mixture B’) contents was affected by the mixture type. The interaction of opening days and mixture type had significance effect ($p<0.05$) on nutritional composition (except total sugar and NFE contents) of mixture silages. At the end of 90 days fermentation all the nutrient contents of both silages were affected ($p<0.05$) except CP, total sugar and NFE; and mixture B’ silage had the highest DM, EE, CF, NDF and ADF contents than mixture A’ silage (Figure 2a, 2b, and 2c).

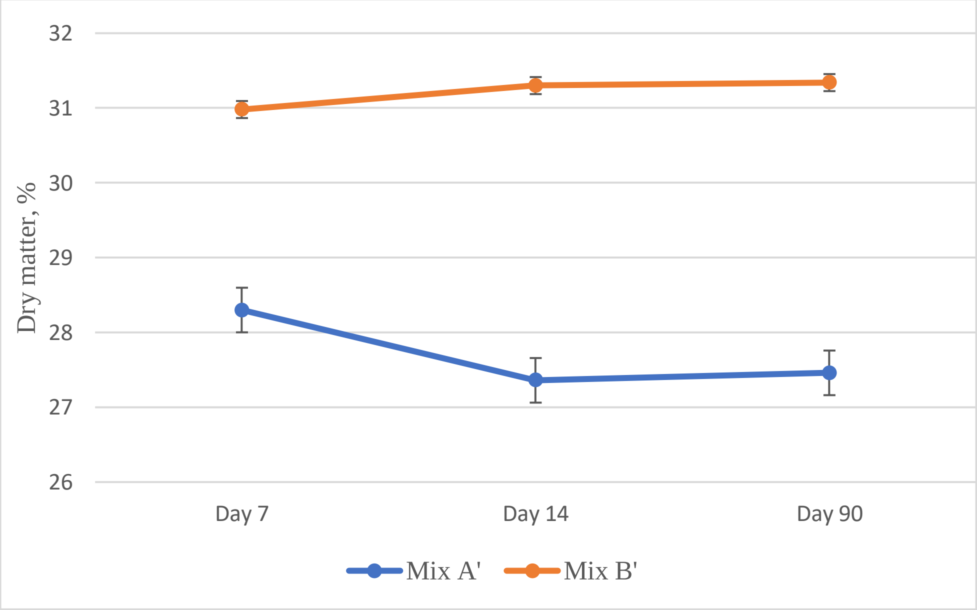
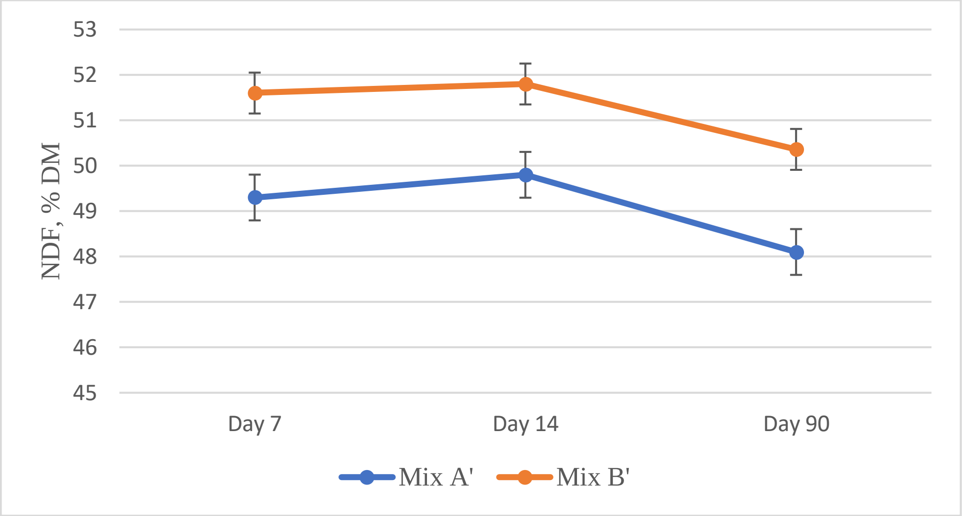


Figure 2. Line graph of nutrient composition of mixture A’ and B’ silage at day 7, 14 and 90

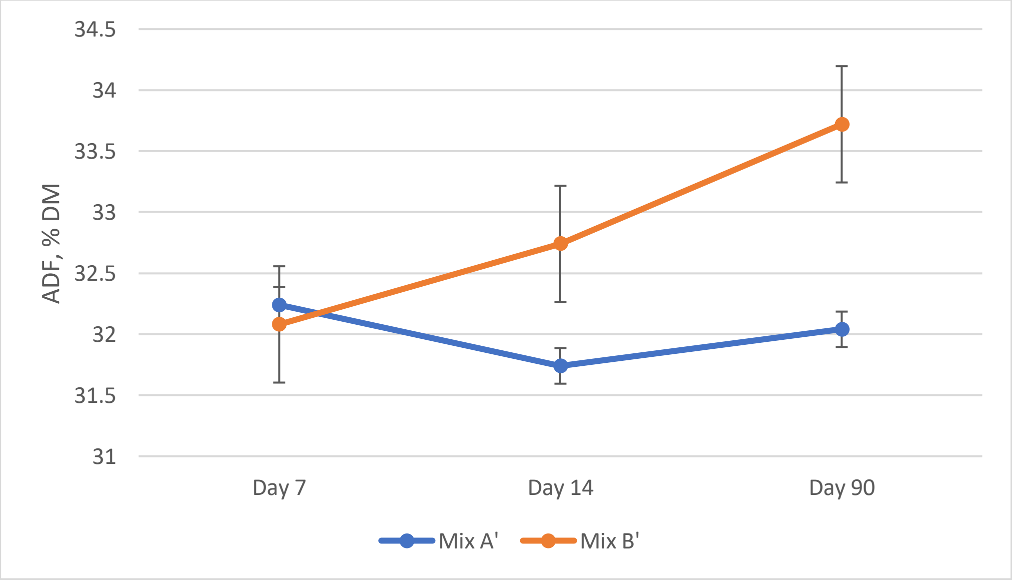
a) Dry matter content of mixture A’ and B’ silage at day 7, 14 and 90

Mix A’: 40% of three cultivars of Italian ryegrass + 20% of two cultivars of winter triticale + 20% of two cultivars of winter oats + 15% of winter wheat + 5% of winter barley.
Mix B’: 55% of three cultivars Italian ryegrass + 45% of two cultivars of winter oats.



b) NDF content of mixture A’ and B’ silage at day 7, 14 and 90

Mix A’: 40% of three cultivars of Italian ryegrass + 20% of two cultivars of winter triticale + 20% of two cultivars of winter oats + 15% of winter wheat + 5% of winter barley.
Mix B’: 55% of three cultivars Italian ryegrass + 45% of two cultivars of winter oats.



c) ADF content of mixture A’ and B’ silage at day 7, 14 and 90

Mix A’: 40% of three cultivars of Italian ryegrass + 20% of two cultivars of winter triticale + 20% of two cultivars of winter oats + 15% of winter wheat + 5% of winter barley.
Mix B’: 55% of three cultivars Italian ryegrass + 45% of two cultivars of winter oats.

Crude protein was well preserved attributed to lactic acid type fermentation (Tables 13). Well-preserved CP could improve the ruminal degradability of nutrients particularly fiber degradability (NDF and ADF) as well as improve protein dependent metabolizable energy (MPN) utilization. The DM content did not change greatly up to 90 days of ensilage, because the laboratory silos were in anaerobic conditions and had no seepage loss. At the end of fermentation, the DM content of mixture A' (27.46%) and mixture B' (31.34%) silages was lower than the DM content of Italian ryegrass silage (36.5%) and winter cereals silage: barley (35.50%), oat (34.60%), triticale (32.00%) and wheat (33.30%) silages (NRC, 2001). However, as compared to DM contents of winter cereals silage: triticale (30.60%) and wheat (28.20%) harvested at heading stage (Orosz et al., 2019), mixture B' had higher DM content. The observed CP contents of mixture A' (15.24%) and mixture B' (16.08%) silages was higher than the CP content of Italian ryegrass silage (12.80%) and winter cereals silage: barley (12.00%), oat (12.90%), triticale (13.80%) and wheat (12.00%) (NRC, 2001). The value was also higher than winter cereal silages: barley (13.30%), oat (11.00%), triticale (10.70%) and wheat (12.10%) harvested at heading stage (Orosz et al., 2019). The high CP value is a direct reflection of the quality of the present mixtures at the time of harvest (early heading stage) before ensiling as well as higher proportion of Italian ryegrass than cereal forage in the total mixed ensiled forage because Italian ryegrass has higher protein content than cereals (Baldinger et al., 2011, 2014; DLF seeds UK, 2018; Byron Seeds LLC, 2019). The total sugar content of fresh forage mixtures was higher than Italian ryegrass fresh green forage harvested at different growing period (Obayashi et al., 2008). This could also be attributed to high proportion of Italian ryegrass (40% in mixture A' and 55% in mixture B'). As reported by Baldinger et al. (2014) the sugar content of Italian ryegrass is superior provided that it is harvested at the early stages. They further noted that Italian ryegrass harvested at second cut had significantly higher (71.87%) sugar content than corn. The observed residual sugar which is assumed to be the source of energy for the rumen microbes was significantly affected by fermentation process. However, there was no difference (p>0.05) in sugar contents associated with opening days for both mixture silages after the 90 days ensiling process. After the end of fermentation, the ADF content was higher for both mixture silages. This result is consistent with the finding of Leão et al. (2017), who reported significant increase in ADF contents of winter cereals silages (triticale, barley, white and black oats) harvested at soft dough stage and subjected to different storage periods (60, 120 and 180 days).

Table 12. Nutritional composition of silage on 7, 14 and 90 opening days, at different crop mixtures and their interaction (n=5/silo opening day)

			Components (% DM)						
		DM (%)	CP	EE	CF	NDF	ADF	Sugar	NFE
Day 7	mixture A’	28.30^{b}	15.48^{b}	4.32^{A}	27.86^{A}	49.30$^{b, A}$	32.24	0.32	38.32
	mixture B’	30.98^{a}	15.84^{a}	4.06^{AB}	28.46	51.60^{a}	32.08^{B}	0.36	39.44
	SEM	0.805	0.217	0.245	0.577	0.502	0.761	0.278	0.801
Day 14	mixture A’	27.36^{b}	14.98^{b}	3.82^{B}	27.82^{A}	49.80$^{b, A}$	31.74	0.12	39.1
	mixture B’	31.30^{a}	15.50^{a}	3.82^{B}	28.62	51.80^{a}	32.74^{AB}	0.16	39.14
	SEM	0.833	0.34	0.13	0.593	1.024	0.781	0.223	0.865
Day 90	mixture A’	27.46^{b}	15.24	4.00$^{b, B}$	27.06$^{b, B}$	48.10$^{b, B}$	32.04^{b}	0.12	38.12
	mixture B’	31.34^{a}	16.08	4.20$^{a, A}$	28.60^{a}	50.36^{a}	33.72$^{a, A}$	0.04	37.98
	SEM	0.773	0.636	0.1	0.619	0.899	0.527	0.141	0.904
p value	Day 7	< 0.001	< 0.05	ns	ns	< 0.001	ns	ns	ns
	Day 14	< 0.001	< 0.05	ns	ns	< 0.05	ns	ns	ns
	Day 90	< 0.001	ns	< 0.05	< 0.01	< 0.01	< 0.01	ns	ns
SEM	mixture A’	0.593	0.330	0.127	0.478	0.570	0.679	0.196	0.736
	mixture B’	0.970	0.519	0.204	0.695	1.040	0.742	0.244	0.964
p value	mixture A’	ns	ns	< 0.001	< 0.05	<0.01	ns	ns	ns
	mixture B’	ns	ns	<0.05	ns	ns	<0.05	ns	ns
Interaction	SEM	0.804	0.435	0.17	0.596	0.838	0.711	0.221	0.858
(day×mixture)	p value	< 0.001	< 0.01	< 0.001	< 0.01	< 0.001	< 0.01	ns	ns

mixture A’: 40% of three cultivars of Italian ryegrass + 20% of two cultivars of winter triticale + 20% of two cultivars of winter oats + 15% of winter wheat + 5% of winter barley.
mixture B’: 55% of three cultivars Italian ryegrass + 45% of two cultivars of winter oats.
$^{a-b}$Means within a column with different superscripts different ($p < 0.05$) caused by opening days effect;
$^{A-B}$Means within a column with different superscripts different ($p < 0.05$) caused by mixture silage effect;
ns – not significant
DM – dry matter, CP – crude protein, EE – ether extract, CF – crude fiber, NDF – neutral detergent fiber, ADF – acid detergent fiber, NFE – nitrogen free extract, SEM – standard error of the mean.

5.2. Fermentation characteristics of ensiled mixtures

Both the type of mixtures and the interaction of opening days and mixture type had significance ($p<0.05$) effect on fermentation characteristics (except ethanol and acetic acid contents) of mixture silages (Table 13). At the end of 90 days fermentation, mixture A’ had lower ($p<0.05$) pH and higher ($p<0.05$) acetate and lactate contents than mixture B’ silage (Figure 3a, 3b and 3c). The pH values were also lower ($p<0.05$) for mixture A’ than mixture B’ silage during the early fermentation phase (opening day 7 and 14) (Table 13).

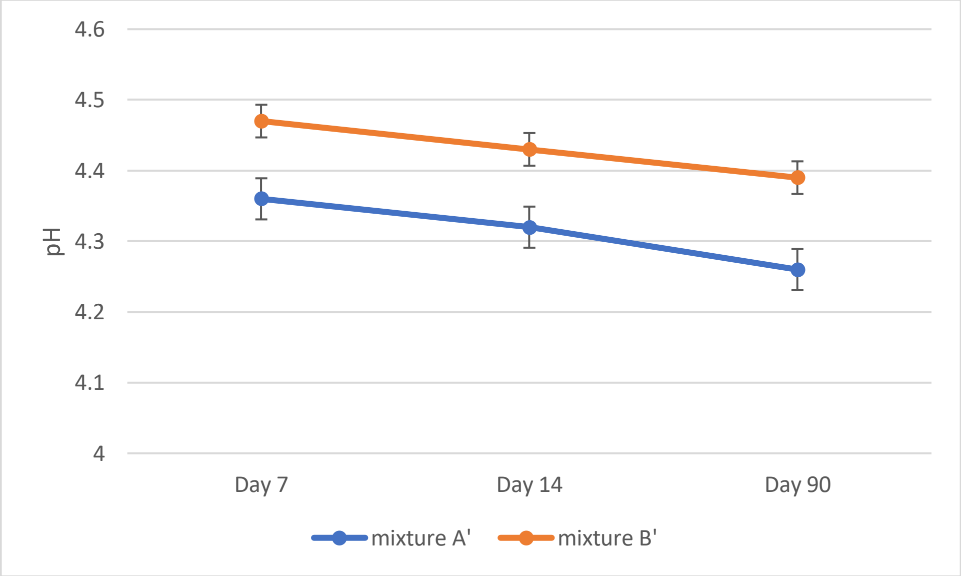
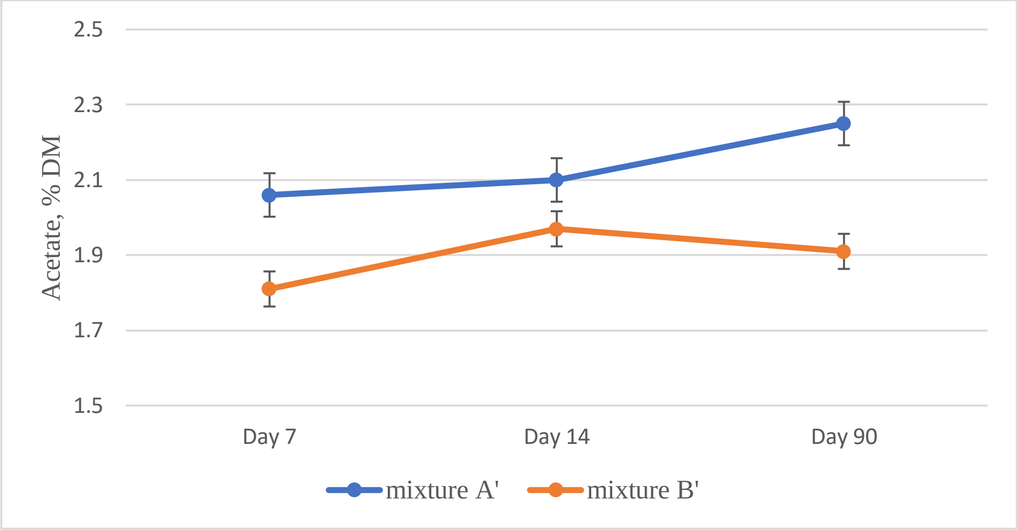


Figure 3. Line graph of fermentation end product contents of mixture A’ and B’ silages at day 7, 14 and 90

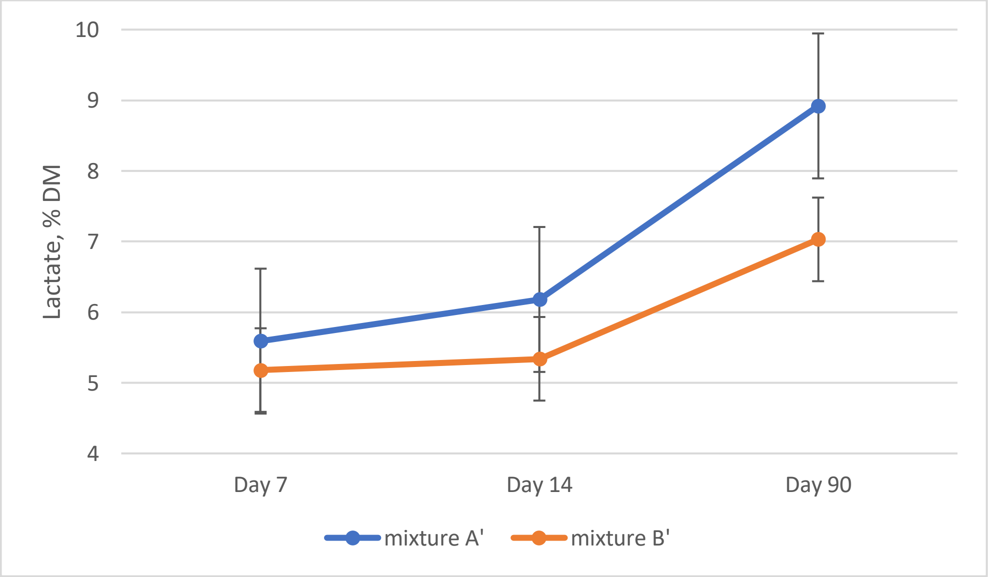
a) pH content of mixture A’ and B’ silages at day 7, 14 and 90

mixture A’: 40% of three cultivars of Italian ryegrass + 20% of two cultivars of winter triticale + 20% of two cultivars of winter oats + 15% of winter wheat + 5% of winter barley.
mixture B’: 55% of three cultivars Italian ryegrass + 45% of two cultivars of winter oats.



b) Acetate content of mixture A’ and B’ silages at day 7, 14 and 90

mixture A’: 40% of three cultivars of Italian ryegrass + 20% of two cultivars of winter triticale + 20% of two cultivars of winter oats + 15% of winter wheat + 5% of winter barley.
mixture B’: 55% of three cultivars Italian ryegrass + 45% of two cultivars of winter oats.



c) Lactate content of mixture A’ and B’ silages at day 7, 14 and 90

mixture A’: 40% of three cultivars of Italian ryegrass + 20% of two cultivars of winter triticale + 20% of two cultivars of winter oats + 15% of winter wheat + 5% of winter barley.
mixture B’: 55% of three cultivars Italian ryegrass + 45% of two cultivars of winter oats.

The rate and extent of reduction in pH was continuous within 90 days of ensiling for both silage mixtures (Figure 3a). The rapid decrease in pH prevents breakdown of plant proteins and helps to inhibit growth of spoilage microbes. This finding is similar with other studies such as early stage ensiling of Italian ryegrass (Shao et al., 2002), Italian ryegrass and Guinean grass silage (Shao et al., 2005), corn silage (Bal, 2006; Ülger and Kaplan, 2017) and high moisture Italian ryegrass, Guinean grass and whole crop corn silages (Li and Nishino, 2013). The reduction of pH was mainly caused by a rapid and intensive production of lactic acid. Fermentation products were limited to three principal products: lactic acid (LA), acetic acid (AA), and ethanol. Other short chain fatty acids (SCFAs) like propionic acid (PA), butyric acid (BA), valeric acid (VA) and caproic acid (CA) were below detectable concentrations (<0.1 g/kg of DM). Mixture A' had higher lactate, acetate and ethanol contents than mixture B'. However, there was no difference ($p>0.05$) in LA/AA, LA (% TFA) and NH_3 – N contents between mixture silages. Concentration of lactate gradually increased over time, reaching their highest values at day 90 for both ensiled mixtures. During the 90 days of fermentation, LA continued to be the major fermentation product (Figure 3c) resulting in a high LA/AA ratio over the storage periods. The observed percent of lactic acid per total fermentation acid (LA% of TFA) was above 72% for both mixture silages. During the 90 days of fermentation LA continued to be the major fermentation product with a small production of AA, resulting in the high value of LA/AA over the storage periods. These indicate that acidification was initiated by homofermentative lactic acid bacteria, and this was dominant during fermentation course.

Table 13. Fermentation characteristics of silage on 7, 14 and 90 opening days, at different crop mixtures and their interaction (n=5/silo opening day)

		Components						
		pH	Ethanol (% DM)	Acetate (% DM)	Lactate (% DM)	LA/AA	LA (% TFA)	NH_3-N (g/100 g total N)
Day 7	mixture A’	4.36$^{b, A}$	0.30	2.06	5.59^{B}	2.88^{B}	74.20^{B}	4.70^{B}
	mixture B’	4.47$^{a, A}$	0.11	1.81	5.18^{B}	2.68^{B}	72.75^{B}	4.20^{B}
	SEM	0.023	0.202	0.288	0.587	0.206	1.406	0.004
Day 14	mixture A’	4.32$^{b, B}$	0.20^{a}	2.1	6.18^{B}	2.95^{B}	74.60^{B}	3.80$^{b, B}$
	mixture B’	4.43$^{a, B}$	0.12^{b}	1.97	5.34^{B}	2.83^{B}	73.47^{B}	4.60$^{a, B}$
	SEM	0.022	0.018	0.427	0.596	0.401	2.872	0.005
Day 90	mixture A’	4.26$^{b, C}$	0.16^{a}	2.25^{a}	8.92$^{a, A}$	3.97^{A}	79.84^{A}	8.80^{A}
	mixture B’	4.39$^{a, C}$	0.11^{b}	1.91^{b}	7.03$^{b, A}$	3.67^{A}	78.59^{A}	8.10^{A}
	SEM	0.02	0.015	0.289	0.877	0.213	0.971	0.011
p value	Day 7	< 0.001	ns	ns	ns	ns	ns	ns
	Day 14	< 0.001	< 0.001	ns	ns	ns	ns	< 0.05
	Day 90	< 0.001	< 0.01	< 0.05	< 0.01	ns	ns	ns
SEM	mixture A’	0.026	0.165	0.202	0.688	0.203	1.243	0.008
	mixture B’	0.015	0.016	0.412	0.711	0.352	2.429	0.005
p value	mixture A’	< 0.001	ns	ns	< 0.001	< 0.001	< 0.001	< 0.001
	mixture B’	< 0.001	ns	ns	< 0.01	< 0.01	< 0.01	< 0.001
Interaction (day× mixture)	SEM	0.021	0.117	0.324	0.699	1.929	0.288	0.007
	p value	< 0.001	ns	ns	< 0.001	< 0.001	< 0.001	< 0.001

mixture A’: 40% of three cultivars of Italian ryegrass + 20% of two cultivars of winter triticale + 20% of two cultivars of winter oats + 15% of winter wheat + 5% of winter barley.

mixture B’: 55% of three cultivars Italian ryegrass + 45% of two cultivars of winter oats.

$^{a-b}$ Means within a column with different superscripts different ($p < 0.05$) caused by opening days effect;

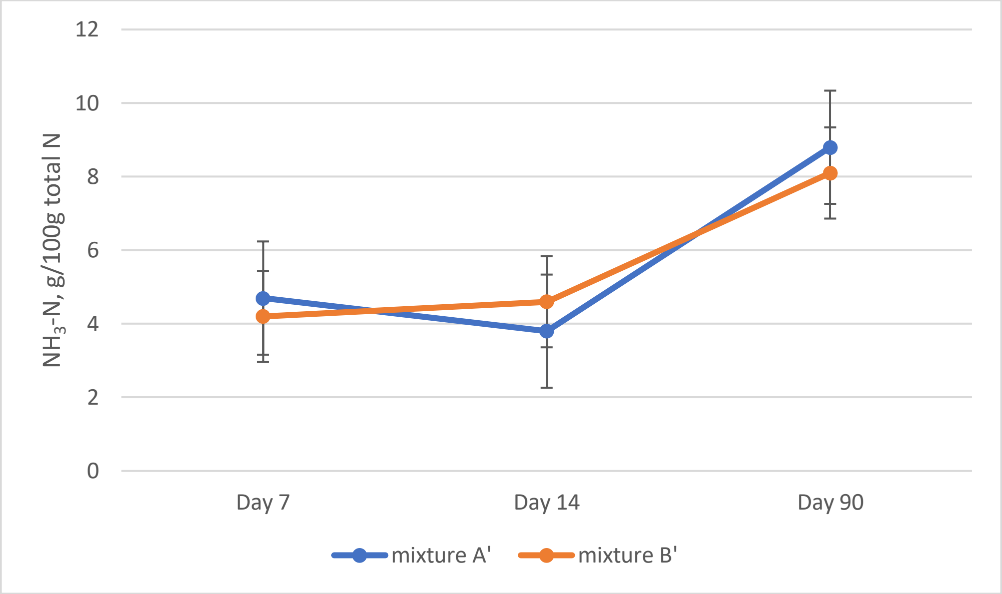
$^{A-B}$ Means within a column with different superscripts different ($p < 0.05$) caused by mixture silage effect;

ns – not significant

DM-dry matter, AA – acetic acid, LA – lactic acid, TFA – total fermentation acid, SEM – standard error of the mean

The LA/AA is a good efficiency indicator for silage fermentation (Jalc et al., 2009). This ratio ideally should not be less than 3:1, and the higher is better (Kung and Shaver 2001). In the present study, this ratio increased over time and the highest value of 3.97:1 and 3.67:1 was observed for both silages, respectively at the end of storage. Proportions of LA (% TFA) at opening day 90 were consistent with the report of AHDB (2012) for grass silage, which described that for well fermented silage lactic acid as the proportion of total acids should be >75%. However, proportions of LA at each opening day for both mixtures of silage were higher than the value reported by Kung and Shaver (2001), who stated that lactic acid should be the primary acid and should be at least 65 to 70% of the total fermentation acids in high quality silage. Ethanol was detected during the storage period probably due to the survival of some yeast throughout the ensilage period.

The PA, BA, VA and CA were undetectable over the ensiling period. This is attributed to the rapid reduction in pH because of the rapid production of LA, restricting the growth of clostridia and other bacteria (Henderson, 1993). The amount of NH_3-N (g/100 g total N) was very low (< 9 g/100 g total N) (Table 13). Mixture B' had higher NH_3-N (day 14) than mixture A' silage (Figure 3d). Fermentation caused an increase in NH_3-N (g/100 g total N) at day 90 as compared to day 14 for both mixture silages. However, there were no any negative results reported on fermentation products (Table 13) as well as microbial count (Table 14) associated with this change.



d) NH_3-N content of mixture A’ and B’ silages at day 7, 14 and 90

mixture A’: 40% of three cultivars of Italian ryegrass + 20% of two cultivars of winter triticale + 20% of two cultivars of winter oats + 15% of winter wheat + 5% of winter barley.
mixture B’: 55% of three cultivars Italian ryegrass + 45% of two cultivars of winter oats.

The NH_3-N/total N of mixture A’ and B’ was low (< 9 g/100 g total N) at the three opening days. As per the criteria after the end of fermentation process when the NH_3-N/total N is below 7 g/100 g total N, the silage could be categorized as excellent silage. Therefore, silage ensiled for 90 days in the present study may be categorized as excellent quality even though the NH_3-N/total N at day 90 was slightly higher than 7 g/100 g total N.

5.3. Microbiological quality

There was no difference ($p>0.05$) in mould and yeast count (Log_{10} CFU g^{-1}) in the silages, on all opening days except day 7 after opening the laboratory silos (Table 14). Mixture A’ had higher ($p<0.05$) mold and yeast count (Log_{10} CFU g^{-1}) than mixture B’ at opening day 7. However, there was no differences ($p>0.05$) in aerobic mesophilic microorganisms count (AMC) (Log_{10} CFU g^{-1}) between mixture A’ and B’ silages at each opening day. Both the mixture type and interaction of opening days and mixture type had significance effect ($p<0.05$) on aerobic mesophilic microorganisms count (AMC) (Log_{10} CFU g^{-1}) of mixture silages. The aerobic mesophilic microorganisms count (AMC) (Log_{10} CFU g^{-1}) for day 7 and 14 of both mixtures silage was in agreement with the normal level (6.00 (Log_{10} CFU g^{-1}) or 1×10^6 (CFU g^{-1}) of European decree (EN ISO 4833, Microbiological limits 65-2012 VM Decree Annex 12). As compared to day 7 and 14, significantly higher CFU counts were recorded at opening day 90 in both mixtures. This could be attributed to the presence of microbial population such as LAB and other anaerobes which would maintain the lactic acid type fermentation of ensiled material until day 90. The microbiological quality result indicates that the mould and yeast count (Log_{10} CFU g^{-1}) was low and there was no negative report on fermentation end products associated with mould and yeast count. The mould and yeast count (Log_{10} CFU g^{-1}) was consistent with the limit recommended as a quality standard for animal feeds (3.00 (Log_{10} CFU g^{-1}) or 1×10^4 (CFU g^{-1}) (GMP, 2008). Mould and yeast counts at different opening days were lower than the results obtained by González, et al. (2008) who found that 90% of their samples counts over 3.00 (Log_{10} CFU g^{-1}) or 1×10^4 (CFU g^{-1}) and Keller et al. (2013) who reported mould count of 4.76 (Log_{10} CFU g^{-1}) or 5.74×10^4 (CFU g^{-1}) for corn silage at its post fermentation phase.

Table 14. Microorganism count (Log$_{10}$ CFU g^{-1}) of silage on 7, 14 and 90 opening days, at different crop mixtures and their interaction (n=5/silo opening day)

		Components	
		Aerobic mesophilic microorganism count (AMC) (Log$_{10}$ CFU g^{-1})^{1}	Mould and yeast (Log$_{10}$ CFU g^{-1})^{1}
Day 7	mixture A’	6.05^{B}	2.41^{a}
	mixture B’	5.78^{AB}	2.03^{b}
	SEM	0.517	0.187
Day 14	mixture A’	5.33^{B}	2.56
	mixture B’	5.53^{B}	2.40
	SEM	0.405	0.749
Day 90	mixture A’	7.23^{A}	2.88
	mixture B’	6.52^{A}	1.40
	SEM	0.511	1.161
p value	Day 7	ns	< 0.05
	Day 14	ns	ns
	Day 90	ns	ns
SEM	mixture A’	0.424	0.962
	mixture B’	0.531	0.607
p value	mixture A’	< 0.001	ns
	mixture B’	< 0.05	ns
Interaction (day × mixture)	SEM	0.481	0.804
	p value	< 0.001	ns

mixture A’: 40% of three cultivars of Italian ryegrass + 20% of two cultivars of winter triticale + 20% of two cultivars of winter oats + 15% of winter wheat + 5% of winter barley.

mixture B’: 55% of three cultivars Italian ryegrass + 45% of two cultivars of winter oats.

$^{a-b}$ Means within a column with different superscripts different ($p < 0.05$) caused by opening days effect;

$^{A-B}$ Means within a column with different superscripts different ($p < 0.05$) caused by mixture silage effect;

ns – not significant

[1] Counting at silo opening; SEM – standard error of the mean, CFU – colony forming unit

5.4. Ruminal degradability

5.4.1. Ruminal degradability of DM

The soluble fraction, the slowly degradable DM fraction and its rate of degradation and effective DM degradability-8 (ED_8) were similar among the mixtures (Table 15). The most important factors affecting the DM degradability are the contents of the NDF and N-free extracts in the silage DM. The potential ruminal degradability for DM of mixture A' and B' silages was 39.41% and 39.16%, respectively and the effective rumen DM degradability at 8% rumen outflow rate (ED_8) of mixture A' and mixture B' were 48.47% and 48.23% respectively. These values were lower than the DM degradability of Italian ryegrass (60.70%) reported by Andrighetto et al. (1993). The low DM degradability in the present silage mixtures could be attributed to the inclusion of cereals which would increase the fiber fraction particularly oat, triticale and wheat (NRC 2001) as compared to sole Italian ryegrass forage used in the previous study. The NDF including cellulose and lignin seemed to protect the silage DM against ruminal digestion. However, the effective DM degradability at 1% rumen solid outflow rate (ED_1), which defines the maintenance DM requirement, were 64.61% (mixture A') and 65.58% (mixture B) better than the report of Andrighetto et al. (1993).

5.4.2. Ruminal degradability of CP

Mixture B' had a higher ($p< 0.05$) *in situ* soluble CP fraction, and lower ($p< 0.05$) potentially degradable CP fraction than mixture A'. The effective protein degradability (at 8% rumen outflow rate/h); EPD_8) was 67.26% (mixture A') and 67.19% (mixture B'). The potential ruminal degradability of CP for mixture A' and B' silages was 43.59% and 31.87% respectively and the effective rumen CP degradability at 8% rumen outflow rate (ED_8) of mixture A' and mixture B' were 67.27% and 67.04% respectively. Both the potential and effective degradability of CP in present mixture silages were lower than that of Italian ryegrass forage at 1st (81.40%) and 2nd (82.30%) cut of leaf stage, grazing (81.80%) and heading (82%) stages as well as CP degradability of alfalfa (81.40%) at first cut of its vegetative stage (Amrane and Michalet-Doreau, 1993). It was also lower than the CP degradability of Italian ryegrass forage at end of its heading stage (76.90%); alfalfa at 2nd cut of vegetative (77.90%) and end of budding (77.40%) stages reported by the same author. The low CP degradability in the present silage mixtures could be attributed to the inclusion of cereals similar to the case in DM degradability described above. On the other hand, ensiling could also affect the CP degradability (de Oliveira et al., 2016) as compared to pure stand Italian ryegrass forage even though both the present and previously used crops harvested at proper stage of maturity (heading) for maximum protein utilization by rumen microbes. The soluble fraction of mixture B' silage (50.82%) was higher than the soluble CP fraction (47%) of corn silage (Susmel et al., 1990) and ryegrass silage (49.05) at its vegetative stage (Valderrama and Anrique, 2011) as well as different cereal forages (Hadjipanayiotou et al., 1996; Turgut and Yanar, 2004). The soluble fraction of Italian ryegrass forage at 1st (20.60%) and 2nd (19.20%) cut of leaf stage as well as 2nd cut of vegetative (27.4%), early budding (24%), budding (18.40%) and end of budding (20%) stages of alfalfa (Amrane and Michalet-Doreau, 1993) were all lower than the value in mixture B' silage. Muazzez (2018) reported lower water-soluble CP fractions for mature alfalfa hay (50.82% *vs*. 37.26%) and normal corn silage (50.82% vs. 40.34%) than mixture B' silage, respectively. The soluble fraction of mixture A' was in the range of soluble fraction of grass silage 21-38% (Turgut and Yanar, 2004). However, the immediately soluble fraction of CP of both mixtures were lower than alfalfa (56.69%) and oat (68.47%) forages (Valderrama and Anrique, 2011) as well as different forage silages (53.70%) (Edmunds et al., 2012). The insoluble but potentially degradable CP fraction of both mixtures were lower than the slowly degradable CP fraction Italian ryegrass forage at 1st (74.80%) and 2nd cut (76.80%) of leaf stage as well as alfalfa silage at budding (74%) (Amrane and Michalet-Doreau, 1993), different cereal forages (Turgut and Yanar, 2004; Valderrama and Anrique, 2011); forage silages (Edmunds et al., 2012); alfalfa and ryegrass forage (Valderrama and Anrique, 2011); grass silage (Susmel et al., 1990; Turgut and Yanar, 2004) and corn silage (Susmel et al., 1990; Muazzez, 2018). Degradation rate of fraction 'b' at time t (c) for both mixtures were 0.620 and 0.083. These values were higher than the values in normal corn silage (Muazzez, 2018). The high degradation rate could be attributed to proper stage of harvesting (early heading) prior to ensiling as well as a higher proportion of Italian ryegrass than winter cereals in both mixture silages which improves protein recovery and increases degradability of ensiled material by rumen microbes. The degradability rate of mixture A' (0.620) is substantially higher than Amrane and Michalet-Doreau (1993) report for Italian ryegrass forage at 1st (0.142) and 2nd cut (0.140) of leaf stage, grazing stage (0.110), heading stage (0.103) and alfalfa forage at 1st (0.162) and 2nd cut (0.154) of vegetative stage, early budding (0.152), budding (0.166) and end of budding (0.137) stage. Valderrama and Anrique (2011) also reported lower 'c' parameter for alfalfa (0.197), oat forage (0.294) and rye grass forage (0.157) at vegetative stage as compared to value in mixture A' silage. Turgut and Yanar (2004) also reported lower 'c' values for alfalfa hay (0.113). This higher rate of CP degradability would make the current silage mixture attractive to combine in other higher fiber crops for better forage utilization in the nutrition of dairy cows. The effective protein degradability (EPD) values at 0.05 and 0.08 h^{-1} in both mixtures were higher than the EPD of corn silage (60.11% and 55.88%, respectively) at 0.05 and 0.08 h^{-1} rumen outflow rates reported by Muazzez (2018). The higher EPD in the present mixture silages could be attributed to either proper stage of harvesting (heading) prior to ensiling or higher proportion of Italian ryegrass in both mixture silages. Italian ryegrass has higher CP at the proper stage of harvesting i.e. 2nd cut (Baldinger et al., 2011) which is similar to

CP values at the end of 90 days fermentation period in present study. However, Valderrama and Anrique (2011) reported higher EPD values at 0.05 and 0.08 h^{-1} for alfalfa forage (88.25%, 85.16%), oat forage (90.80%) and rye grass forage (85.20%, 80.62%) at its vegetative stage. The EPD values at 0.05 h^{-1} rumen outflow rate of mixture B' silages (70.17%) was better than barley (69%, 61% and 56%); and oats (66%, 60% and 56%) at flowering, pod formation and early maturity respectively (Hadjipanayiotou et al., 1996). According to NRC (2001) recommendation, the maximum milk and milk protein yields occur when rumen degradable protein (RDP) is 12.20% of diet dry matter. Therefore, the observed EPD at the three rumen outflow rates were higher than the NRC (2001) requirement for maximum milk and milk protein yield.

5.4.3. Ruminal degradability NDF and ADF

The soluble NDF and ADF fractions of mixture A' and B' silages were low. Mixture A' had higher ($p<0.05$) *in situ* soluble NDF and ADF fraction than mixture B'. The effective ADF degradability at 0.08 h^{-1} rumen outflow rate (ED_8) was higher for mixture A' than mixture B'. However, there were no difference on effective NDF and ADF degradability at 0.01 h^{-1} rumen outflow rate (ED_1) between mixture A' and B' silages. The potentially degradable as well as effective degradable NDF and ADF at 0.01 and 0.08h^{-1} rumen outflow rate (ED_1 and ED_8) was high (Table 10). The potentially degradable NDF fraction, its degradation rate and effective ruminal degradability-8 (ED_8) were 80.23%. 0.017, and 18.68% (mixture A') and 94.35%, 0.014 and 16.37% (mixture B'). Potentially degradable ADF fraction, its rate of degradation and effective ruminal degradability (ED_8) were 85.18%. 0.017, and 19.45% (mixture A') and 87.26%, 0.014 and 16.44% (mixture B'). For effective rumen function sufficient NDF should be included in the diets of dairy cows. The NRC (1989) reported that large proportion of dietary NDF should come from forages and at least 25% of dairy ration should be composed of NDF (Oba and Allen, 1999).

Therefore, amount and ruminal degradability of NDF is a very important factor in the dairy cow's nutrition because forage NDF varies widely in its degradability in the rumen and NDF digestibility influences animal performance (Nousiainen et al., 2009; Zebeli et al., 2012; Bender et al., 2016). Grasses typically have higher NDF content as compared to corn and alfalfa silage (NRC, 2001; Bender et al., 2016). However, both alfalfa and corn silage have wider acceptance in dairy nutrition due to its higher DM digestibility as compared to grass silage (Bender et al., 2016). Identifying highly digestible and/or ruminal degradable (particularly NDF and ADF) grass has been a critical challenge in partial replacement of corn and alfalfa silage in the nutrition of dairy cows (Bender et al., 2016). Few research reports (Brink et al., 2010; Pelletier et al., 2010; Bender et al., 2016) revealed that modern varieties of cool season grasses have been selected to have greater fiber (NDF and ADF) digestibility. The high potential *in situ* NDF and ADF degradability of the present silage mixtures could be associated with those cultivars selected for high ADF and NDF degradability. The potential ruminal degradability, effective degradability at 0.01 h^{-1} (ED_1) and 0.08 h^{-1} (ED_8) of NDF and ADF were 77.39%, 53.82%, 18.68% and 76.95%, 53.93%, 19.41% (mixture A') or 89.07%, 53.92%, 16.34% and 84.97%, 53.16%, 16.42% (mixture B') respectively. The high potential and effective ruminal degradability could be associated with agronomic practices such as early harvesting (Hoffman et al., 1993; Rinne et al., 2002) as well as harvesting from spring growth (Rinne et al., 2002; Cherney et al., 2004; Pelletier et al., 2010).

Table 15. Degradable characteristics of silage (n=60)

	mixture A’	mixture B’	SEM	p value
Dry matter (DM)				
Soluble fraction (% of DM)	32.04	34.14	3.671	ns
Potentially degradable fraction (% of DM)	39.41	39.16	7.205	ns
Degradation rate (% h^{-1})	0.10	0.05	0.085	ns
Effective degradability-1 (%) (1)	64.41	65.58	5.911	ns
Effective degradability-5 (%) (1)	52.37	52.30	0.742	ns
Effective degradability-8 (%) (1)	48.47	48.23	1.255	ns
Crude protein (CP)				
Soluble fraction (% of DM)	28.65^{b}	50.33^{a}	1.779	< 0.001
Potentially degradable fraction (% of DM)	43.59^{a}	32.39^{b}	1.191	< 0.001
Degradation rate (% h^{-1})	0.62^{a}	0.09^{b}	0.041	< 0.001
Effective degradability-1 (%) (1)	71.55^{b}	79.20^{a}	1.697	< 0.01
Effective degradability-5 (%) (1)	68.99^{b}	70.75^{a}	0.523	< 0.05
Effective degradability-8 (%) (1)	67.26	67.19	0.703	ns
Neutral detergent fiber (NDF)				
Soluble fraction (% of DM)	5.17^{a}	4.09^{b}	0.408	< 0.05
Potentially degradable fraction (% of DM)	80.23	94.35	30.269	ns
Degradation rate (% h^{-1})	0.017	0.014	0.006	ns
Effective degradability-1 (%) (1)	54.11	54.45	10.620	ns
Effective degradability-5 (%) (1)	24.75	22.20	2.049	ns
Effective degradability-8 (%) (1)	18.68	16.37	1.202	ns
Acid detergent fiber (ADF)				
Soluble fraction (% of DM)	6.34^{a}	3.87^{b}	1.082	< 0.05
Potentially degradable fraction (% of DM)	85.18	87.26	32.53	ns
Degradation rate (% h^{-1})	0.017	0.014	0.007	ns
Effective degradability-1 (%) (1)	54.96	53.35	10.932	ns
Effective degradability-5 (%) (1)	25.28	22.31	2.007	ns
Effective degradability-8 (%) (1)	19.45^{a}	16.44^{b}	1.208	< 0.05

mixture A’: 40% of three cultivars of Italian ryegrass + 20% of two cultivars of winter triticale + 20% of two cultivars of winter oats + 15% of winter wheat + 5% of winter barley.

mixture B’: 55% of three cultivars Italian ryegrass + 45% of two cultivars of winter oats.

$^{a-b}$ Means within a row with different superscripts different (min. $p < 0.05$)

SEM – Standard error of mean; ns – not significant

(1) Effective degradability-1, -5 and -8 are calculated for various rumen solid outflow rates (0.01, 0.05 and 0.08h^{-1})

The higher effective ruminal NDF degradability (ED_8) of mixture A' (18.68%) as compared to mixture B' (16.34%) associated with lower NDF content (Table 12) attributed to acid hydrolysis of hemicellulose due to lactic acid type fermentation. Allen and Oba (1996) reported that enhancing NDF hydrolysis may stimulate rapid disappearance of NDF from the rumen, reduce physical fill, and allow greater voluntary feed intake. The potential ruminal NDF degradability of both mixtures were higher than NDF degradability of Italian ryegrass (59.80%) reported by Andrighetto et al. (1993). Ali et al. (2014) also reported lower NDF degradability of grass silage (76.40%) as compared to the present silage mixtures. The rate of ruminal NDF degradation (k_p) for mixture A' and mixture B' silages were 0.017 and 0.013 respectively. The value in mixture A' was higher than grass/grass – clover silage (0.016) as well as whole crop cereal silage (0.015) reported by Weisbjerg et al. (2007).

5.5. Energy and protein evaluation

5.5.1. Dry matter and nutrient digestibility

Some end-products of fermentation associated with poor fermentation, such as AA and BA and ammonia are associated with the decrease in the intake of silages and some changes resulting from the ensiling process influence the digestibility of silages (de Oliveira et al., 2016). However, the overall apparent digestibility of nutrients in the present study was better and above 67% attributed to absence of those undesirable fermentation end products like BA and ammonia (Table 13). On the other hand, the complement effect of ensiled materials could also be a reason for high digestibility as digestibility of barley, winter oats and Italian ryegrass is excellent. The apparent DM digestibility was 67.92% (Table 16) which is lower as compared to CP, NDF and ADF digestibility. The low DM digestibility could be associated with the inclusion of more winter cereals (60%) which has lower DM digestibility due to its high fiber content as compared to Italian ryegrass. On the other hand, grasses typically have higher NDF content and lower DM digestibility as compared to corn and alfalfa silage. The observed DM digestibility in the present study was higher than DM digestibility of alfalfa silage (Hassanet et al., 2014) and sorghum silage with different tannin content (Teixeira et al., 2014), but lower than DM digestibility of corn silage (Hassanat et al., 2014) and grass silage (Yan and Agnew, 2004). The apparent fiber digestibility and NDF or ADF were high (>69%). This could be attributed to lower lignification of cell wall contents (NDF and ADF) of ensiled mixtures. This high fiber digestibility particularly NDF digestibility is very important because NDF digestibility influences animal performance. Study reports reveal that for effective rumen function sufficient NDF should be included in the diets of dairy cows and large proportion of dietary NDF should come from forages and at least 25% of dairy ration should be composed of NDF (Allen and Oba, 1996). The apparent digestibility of OM, CP and NDF was better than grass silage reported by Yan and Agnew (2004). On the other hand OM, CP, NDF and ADF digestibility was higher than the OM (68.50%, 62.80% and 63.30%), CP (72.20%, 72.20% and 68.50%), NDF (69.80%, 59.70% and 50.70%) and ADF (69.10%, 58.30% and 48.30%) digestibility of oat silage at heading, early milk and early dough stages respectively (Wallsten et al., 2009). It was better than OM (69.40%, 66.70%), CP (69.30%, 66.60%), NDF (68.30%, 57.30%) and ADF (64.20%, 51.60%) digestibility of oat silage at early milk and early dough stages respectively (Wallsten et al., 2009). The observed OM and NDF digestibility were better than 48 hours incubation *in vitro* OM digestibility, NDF digestibility and digestible NDF of corn silage, alfalfa (silage, haylage and hay), ryegrass silage and grass silage reported by Orosz et al. (2019). However, the OM digestibility of the current study was lower than OM digestibility of corn silage (dough stage) and ryegrass silage reported by the same author. Additionally, the digestibility of OM and NDF value was comparable with ryegrass silage (before and in heading stage) as reported by Orosz et al. (2019).

Table 16. Digestibility coefficients (%) determined with sheep for the mixture of Italian ryegrass and winter cereal silage used as "mixture A' " in experiment I (n = 6)

Nutrient	Digestibility coefficient (%)	SD
Dry matter	67.92	1.48
Organic matter	72.12	1.12
Crude protein	73.44	1.42
Crude fat	70.47	3.03
Crude fiber	75.06	2.31
N-free extracts	69.76	1.21
Neutral detergent fiber	70.90	2.39
Acid detergent fiber	70.76	2.17

ensiled mixture – 40% of three cultivars of Italian ryegrass + 20% of two cultivars of winter triticale + 20% of two cultivars of winter oats + 15% of winter wheat + 5% of winter barley
SD – standard deviation

5.5.2. Energy concentration in ensiled mixture

Energy content of ensiled mixture and other silages are given in Table 17. All the values of net energy contents were better than other cereal silages. The high net energy values could be associated with higher digestible fiber and its fraction (Table 16) associated with proper stage of harvesting and high fiber digestible characteristics of Italian ryegrass which dominates the crop mixtures. The overall energy values of the current silage mixture were better than all conserved forages including alfalfa silage reported by the NRC (2001). The value for net energy for lactation (NE_l), net energy for maintenance (NEm) and net energy for growth (NEg) are all better than the values for alfalfa silage and also exceeds the values of good quality grass silage (Table 17). Thus, early heading harvest of the mixture was not accompanied by a decline in energy content. Ensiled mixture silage contains very low amount of starch, but the digestibility of the fiber was so favourable that its net energy content averaged

0.90 MJ/kg DM still exceeded the average energy content of cereal silages and appears to be similar to rye silage; however, the value was lower than the energy content of corn silage.

Table 17. Energy content (MJ kg^{-1}) of ensiled mixture and other silages (NRC, 2001)

	Energy value				
Forage types	DE	ME	NE_l	NE_m	NE_g
Ensiled mixture*	**11.42**	**9.36**	**5.37**	**5.74**	**3.32**
Alfalfa silage	10.96	8.28	5.02	5.39	3.01
Rye, annual, vegetative	11.40	8.70	5.36	5.73	3.31
Corn silage, normal (32-38%DM)	12.50	9.75	6.07	6.57	4.06
Grass silage, mid maturity(56-60%NDF)	10.70	8.03	4.85	5.23	2.85
Italian ryegrass silage	10.40	7.78	4.69	4.98	2.64
Sorghum silage	10.40	7.74	4.64	4.94	2.59
Barley silage, headed	11.20	8.49	5.19	5.56	3.18
Oat silage, headed	10.60	7.99	4.81	5.15	2.76
Triticale silage, headed	10.80	8.12	4.94	5.23	2.89
Wheat silage, early headed	10.70	7.99	4.85	5.19	2.80

* ensiled mixture – 40% of three cultivars of Italian ryegrass + 20% of two cultivars of winter triticale + 20% of two cultivars of winter oats + 15% of winter wheat + 5% of winter barley
DE – Digestible Energy, ME – Metabolisable Energy, NE_l – Net energy for lactation, NE_m – Net energy for maintenance, NE_g – Net energy for growth.

5.5.3. Protein evaluation

In ruminant nutrition accurate estimation of microbial protein is very essential as metabolizable protein synthesis is governed by microbial protein synthesis (Castillo-Lopez and Domínguez-Ordóñez, 2019). Availability of dietary carbohydrate, ruminally degradable protein and dietary fat are the main factors which influence ruminal microbial protein synthesis (Fernando et al., 2010). On the other hand, Abdulkarim and Kedir (2019) reported that the availability of energy generated by the fermentation of carbohydrates largely influence microbial protein synthesis. It further noted that on average, 20 grams of bacterial protein is synthesized per 100 grams of organic matter fermented in the rumen. Therefore, it seems to be appropriate that all feed should be given two metabolizable protein values namely the nitrogen-dependent metabolizable protein (MPN) and energy dependent metabolizable protein (MPE) which are the quantity of protein originated from the true digestible protein proportion of UDP and the digestible true microbial protein potentially synthesized from RDP and the fermentable organic matter (FOM) content of the feed respectively (Schmidt and Zsédely, 2011). Both metabolizable protein values should be calculated for ration because the production of animals is always limited by the lower value. Table 18 describe the protein evaluation values of ensiled mixtures. Both the nitrogen and energy dependent metabolizable protein values are higher than 88 g/kg DM. When a ration is formulated a protein balance (MPN-MPE, g) in a rumen should be considered and calculated (Schmidt and Zsédely, 2011). The nitrogen dependent metabolizable energy value is greater than the energy dependent metabolizable energy value implies that there are more nitrogen/protein as a source of energy than energy for the rumen microbes. The high nitrogen dependent metabolizable protein concentration as compared to energy dependent metabolizable protein attributed to digestibility of CP as well as proper stage of harvesting (early heading) of the ensiled mixtures.

Table 18. Protein evaluation values of ensiled mixtures

Silage	MPN (g/kg DM)	MPE (g/kg DM)
ensiled mixture*	97.03	88.87

*ensiled mixture – 40% of three cultivars of Italian ryegrass + 20% of two cultivars of winter triticale + 20% of two cultivars of winter oats + 15% of winter wheat + 5% of winter barley;

MPN – Nitrogen dependent metabolizable energy; MPE – Energy dependent metabolizable energy

6. RESULTS AND DISSCUSSION (EXPERIMENT II)

6.1. Nutritional composition

6.1.1. Nutritional compositions of green forage mixtures

The crop mixture (mixture A, B, C and D) did not affect ($p>0.05$) the CP contents of green forage at all sampling time (ST) (Table 19). Significance difference was also not found ($p>0.05$) between green forage in OM (3rd ST); CF and total sugar (2nd and 3rd ST) contents. However, significance change ($p<0.05$) was observed in DM (all ST); OM, CF and total sugar (1st ST); OM (2nd ST); and CF and total sugar (4th ST) contents. As compared to sole cereal mixtures (mixture A and B), IRG plus winter cereal mixtures (mixture C and D) had lower ($p< 0.05$) DM and OM content at 1st and 2nd cut as well as total sugar content at 1st cut. The CP content of green forage mixtures at all sampling time was higher than the CP content of Italian ryegrass green forage (Shao et al., 2007; Bande-Castro et al., 2010; Andrzejewska et al., 2018) and winter cereal (Shao et al., 2005; Jacobs et al., 2009) green forage right before ensiling. The increase in fiber content (Table 19) as the maturity advanced could be attributed to accumulation of stems and deposition of poorly digested lignin in both leaves and stems. Additionally, 100% inclusion of cereal (mixture A and B) as well as 55% (mixture C) and 40% (mixture D) will increase the fiber content. Cherney and Marten (1982) reported that acid detergent fiber, cell wall constituents and lignin concentrations of cereals increase with pre-heading maturation, but remain constant after heading.

Table 19. Nutritional composition of green forage mixtures before preservation (n = 20)

Components (% DM)	Green forage mixtures				SEM	P value
	mixture A	mixture B	mixture C	mixture D		
DM (%)						
Sampling time 1	17.95^{a}	17.72^{a}	16.06^{b}	16.77^{b}	0.814	< 0.01
Sampling time 2	18.00^{a}	17.72^{a}	16.11^{b}	16.57^{b}	0.820	< 0.01
Sampling time 3	17.60^{a}	17.44^{ab}	16.01^{b}	16.68^{ab}	0.861	< 0.05
Sampling time 4	17.90^{a}	17.56^{a}	16.04^{b}	16.79^{ab}	0.804	< 0.01
OM (%)						
Sampling time 1	88.68^{b}	87.97^{b}	85.49^{a}	85.46^{a}	1.027	< 0.001
Sampling time 2	88.77^{b}	87.80^{b}	85.70^{a}	85.31^{a}	0.862	< 0.001
Sampling time 3	86.64	85.75	84.23	85.79	1.396	ns
Sampling time 4	88.38^{ab}	87.49^{b}	85.55^{a}	87.26^{b}	1.110	< 0.01
CP (%)						
Sampling time 1	18.73	17.14	16.45	17.82	1.509	ns
Sampling time 2	15.36	16.86	14.67	14.18	1.664	ns
Sampling time 3	16.36	16.77	16.70	13.89	2.742	ns
Sampling time 4	12.68	11.38	12.57	12.03	0.996	ns
EE (%)						
Sampling time 1	4.37^{b}	3.98^{ab}	4.13^{ab}	3.62^{a}	0.337	< 0.05
Sampling time 2	3.72	3.86	3.53	3.14	0.546	ns
Sampling time 3	3.38	3.11	3.22	2.93	0.302	ns
Sampling time 4	3.01	2.69	2.77	2.73	0.253	ns
CF (%)						
Sampling time 1	15.89^{a}	17.33^{b}	17.14^{a}	17.31^{b}	0.773	< 0.05
Sampling time 2	20.97	22.07	22.15	21.29	1.152	ns
Sampling time 3	21.8	22.93	23.05	23.33	0.996	ns
Sampling time 4	24.79^{ab}	26.00^{a}	25.77^{a}	24.24^{b}	0.843	< 0.05
Ash (%)						
Sampling time 1	7.73^{a}	7.92^{a}	9.78^{b}	10.01^{b}	0.444	< 0.001
Sampling time 2	7.90^{a}	8.15^{a}	9.85^{b}	10.02^{b}	0.603	< 0.001
Sampling time 3	8.45^{a}	8.63^{a}	10.74^{b}	10.21^{b}	1.019	< 0.05
Sampling time 4	7.77^{a}	7.57^{a}	9.58^{b}	9.33^{b}	0.443	< 0.001

TS (%)						
Sampling time 1	25.57^{b}	26.71^{b}	23.45^{a}	21.34^{a}	2.941	< 0.05
Sampling time 2	21.73	17.87	19.63	19.27	3.962	ns
Sampling time 3	16.94	17.94	14.50	17.00	3.440	ns
Sampling time 4	16.01^{ab}	18.50^{b}	14.26^{a}	17.85^{ab}	2.088	< 0.01
NFE (%)						
Sampling time 1	53.66	53.61	52.47	51.22	2.624	ns
Sampling time 2	51.95	48.69	49.76	51.35	3.123	ns
Sampling time 3	49.99	48.51	46.27	49.62	4.394	ns
Sampling time 4	51.72	52.35	49.28	51.65	1.939	ns

mixture A: 40% of two cultivars of winter triticale + 30% of two cultivars of winter oats + 20% of winter barley + 10% of winter wheat; mixture B: 50% of two cultivars of winter triticale + 40% of winter barley + 10% of winter wheat; mixture C: 55% of three types of Italian ryegrass + 45% of two cultivars of winter oat; mixture D: 40% of three types of Italian ryegrass + 30% of two cultivars of winter oat + 15% of two cultivars of winter triticale + 10% of winter barley + 5% of winter wheat.

$^{a-c}$ Means within a row with different superscripts different ($p<0.05$) (Crop mixture effect)

ns=not significant ($p>0.05$)

Cutting time – 1st week end of leafy stage (1st cut), 2nd week end of leafy stage (2nd cut), 3rd week end of leafy stage (3rd cut), 4th week end of leafy stage (4th cut 4)

DM – dry matter, OM – organic matter, CP – crude protein, CF – crude fiber, EE – ether extract, TS – total sugars, NFE – nitrogen free extract, SEM – standard error of mean.

6.1.2. Nutritional composition of ensiled mixtures

The fermentation process caused significant change ($p<0.05$) on the nutritional composition of ensiled mixtures except ADF (mixture B and D); DM, CP (mixture C) (Table 20). The interaction of opening days and crop mixtures also caused significant change ($p<0.05$) on the nutritional composition of ensiled mixtures. At the end of 90 days of fermentation the DM content increased ($p<0.05$) (mixture A); decreased ($p<0.05$) (mixture B and D); and was not affected (Mixture C) (Table 20). However as compared to day 90 mixture A had higher ($p< 0.05$) at day 14 (Figure 4a).

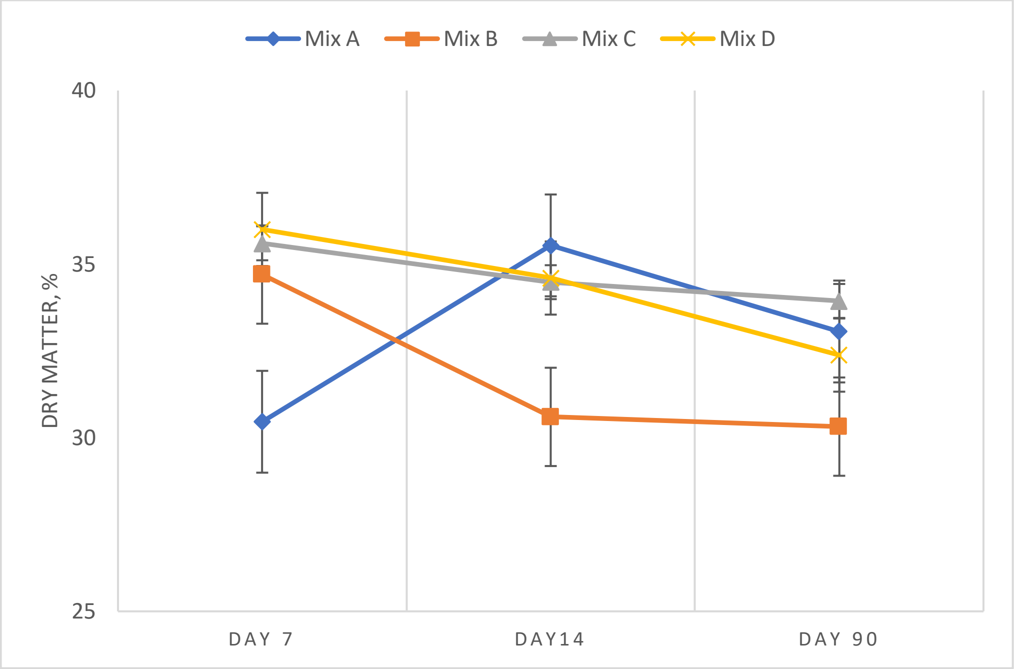
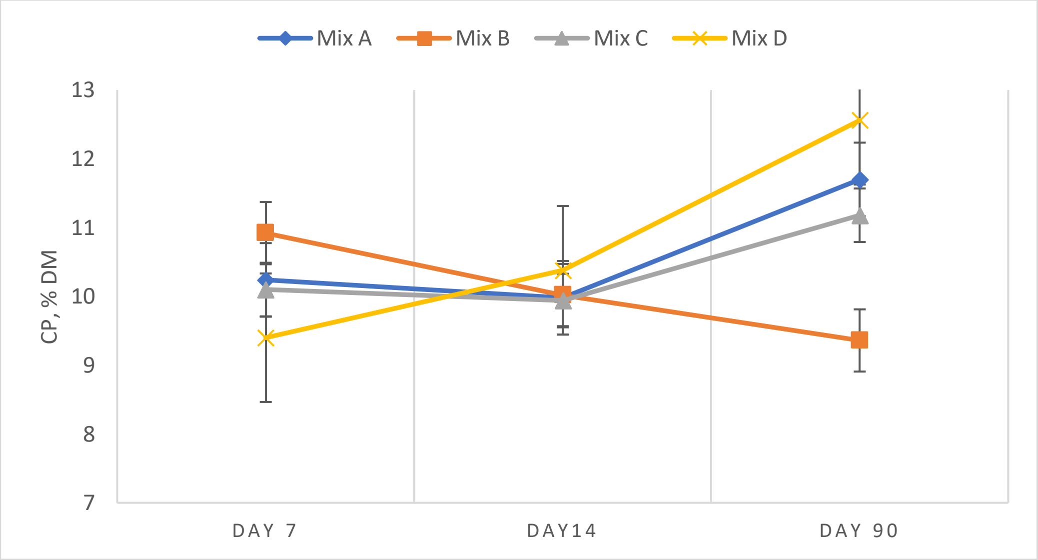


Figure 4. Line graph of nutrient composition of mixture A, B, C and D silages at opening day 7, 14 and 90 (Experiment II)

a) DM content of mixture A, B, C and D silages at opening day 7, 14 and 90 (Experiment II)

Mix A: 40% of two cultivars of winter triticale + 30% of two cultivars of winter oats + 20% of winter barley + 10% of winter wheat; Mix B: 50% of two cultivars of winter triticale + 40% of winter barley + 10% of winter wheat; Mix C: 55% of three types of Italian ryegrass + 45% of two cultivars of winter oat; Mix D: 40% of three types of Italian ryegrass + 30% of two cultivars of winter oat + 15% of two cultivars of winter triticale + 10% of winter barley + 5% of winter wheat.

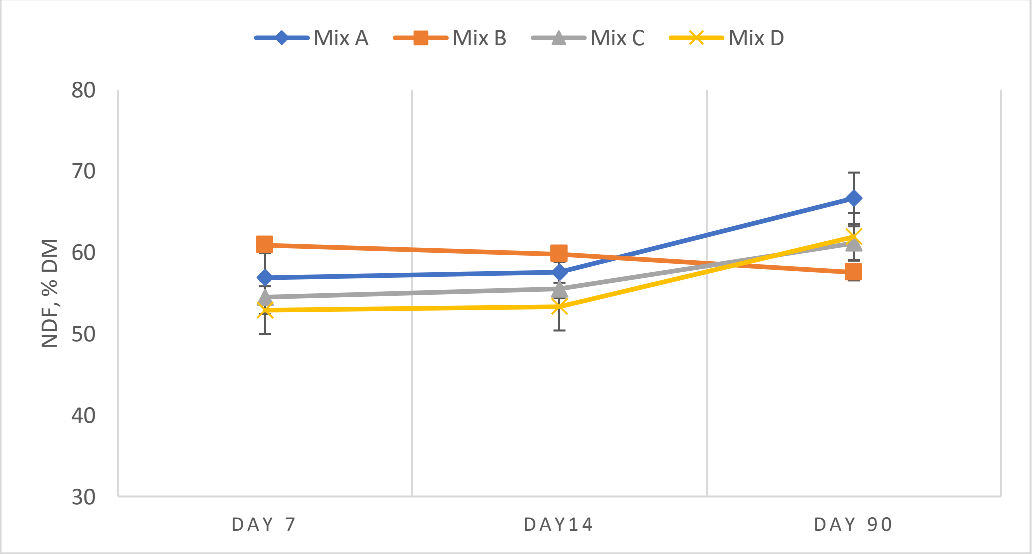
The DM content of ensiled mixtures was increased as compared to fresh green forage. This result is highly important from the silage making point of view due to the challenge in producing high quality forage as silage with avoiding DM losses (Kung and Shaver, 2001; Kung et al., 2018). The high DM recovery could be associated with lactic acid fermentation type in the presence of high sugar which encourages the homolactic bacteria to produce lactic acid and preserve the DM (Pahlow et al., 2003). The CP content increased ($p<0.05$) (mixture A and D); decreased ($p<0.05$) (mixture B); and was not affected (mixture C) (Figure 4b)



b) CP content of mixture A, B, C and D silages at opening day 7, 14 and 90 (Experiment II)

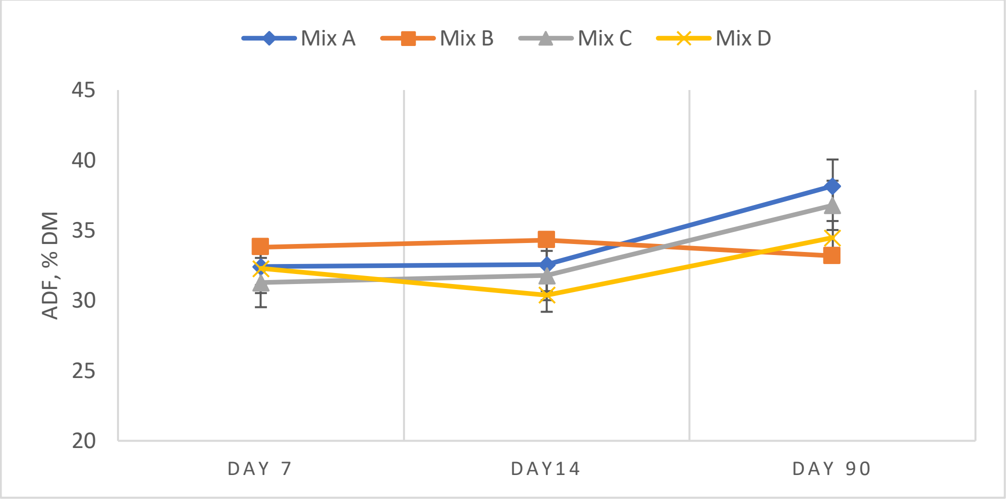
Mix A: 40% of two cultivars of winter triticale + 30% of two cultivars of winter oats + 20% of winter barley + 10% of winter wheat; Mix B: 50% of two cultivars of winter triticale + 40% of winter barley + 10% of winter wheat; Mix C: 55% of three types of Italian ryegrass + 45% of two cultivars of winter oat; Mix D: 40% of three types of Italian ryegrass + 30% of two cultivars of winter oat + 15% of two cultivars of winter triticale + 10% of winter barley + 5% of winter wheat.

At the end of 90 days fermentation, the CP contents of Italian ryegrass plus cereal grain-based silage (mixture C and D) was higher than Italian ryegrass silage (Yahaye et al., 2004; Yimiti et al., 2004). The high CP value is a direct reflection of the quality of the present mixtures at the time of harvest (early heading stage) before ensiling. The higher proportion of Italian ryegrass than cereal grain (mixture C and D) also resulted higher CP since Italian ryegrass has more protein than cereals (Baldinger et al., 2011, 2014; DLF seeds, UK, 2018; Byron Seeds, LLC, 2019). However, it was lower than the CP content of Italian ryegrass and winter cereal mixture silage and Italian ryegrass silage (Jacobs et al., 2009). The CP content of cereal-based mixture (mixture A and B) was higher than the CP content of triticale, oats, barley silage (Jacobs et al., 2009) and wheat, triticale, barley, white and black oats silage (Leão et al., 2017). Due to proper stage of harvesting of cereal mixtures (mixture A and B) and high proportion of Italian ryegrass (55% in mixture C and 40% in mixture D), the ensiled mixture had higher total sugar content. Baldinger et al. (2014), Italian ryegrass harvested at second cut had significantly higher (71.87%) sugar content than corn. The NDF content increased ($p<0.05$) (mixture A, C and D); and decreased ($p<0.05$) for mixture B silage (Figure 4c). The ADF contents increased ($p<0.05$) (mixture A and C); however, a significant increase in ADF were observed between opening day 14 and 90 (Figure 4d). The total sugar content was decreased ($p<0.05$) for all ensiled mixtures. However, a significant decrease was observed between day 14 and 90 (mixture A and C) and between day 7, 14 and 90 (mixture B and D) (Figure 4e). There was significant increase in ADF content of mixture A and C silage between day 14 and day 90. This result is consistent with the report of Leão et al. (2017), who reported significant increase in ADF contents of winter cereals silages.



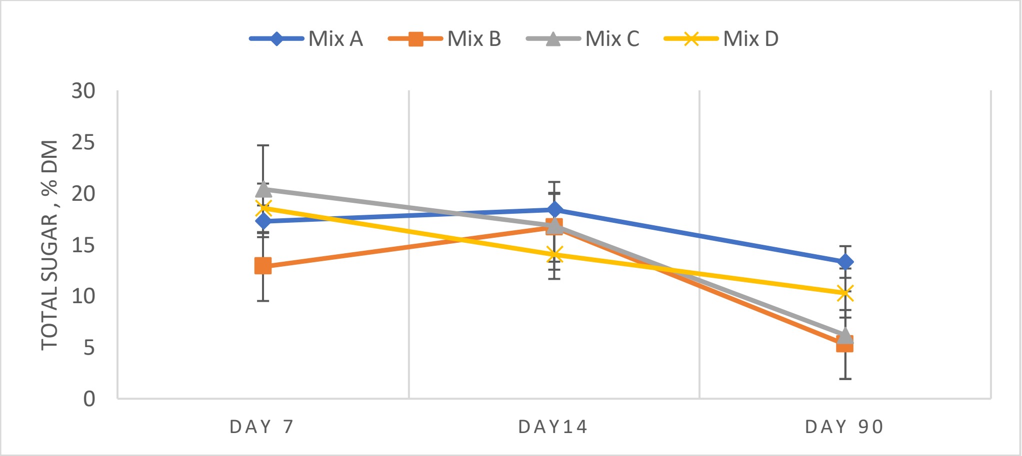
c) NDF content of mixture A, B, C and D silages at opening day 7, 14 and 90 (Experiment II)

Mix A: 40% of two cultivars of winter triticale + 30% of two cultivars of winter oats + 20% of winter barley + 10% of winter wheat; Mix B: 50% of two cultivars of winter triticale + 40% of winter barley + 10% of winter wheat; Mix C: 55% of three types of Italian ryegrass + 45% of two cultivars of winter oat; Mix D: 40% of three types of Italian ryegrass + 30% of two cultivars of winter oat + 15% of two cultivars of winter triticale + 10% of winter barley + 5% of winter wheat.



d) ADF content of mixture A, B, C and D silages at opening day 7, 14 and 90 (Experiment II)

Mix A: 40% of two cultivars of winter triticale + 30% of two cultivars of winter oats + 20% of winter barley + 10% of winter wheat; Mix B: 50% of two cultivars of winter triticale + 40% of winter barley + 10% of winter wheat; Mix C: 55% of three types of Italian ryegrass + 45% of two cultivars of winter oat; Mix D: 40% of three types of Italian ryegrass + 30% of two cultivars of winter oat + 15% of two cultivars of winter triticale + 10% of winter barley + 5% of winter wheat



e) Total sugar content of mixture A, B, C and D silages at opening day 7, 14 and 90 (Experiment II)

Mix A: 40% of two cultivars of winter triticale + 30% of two cultivars of winter oats + 20% of winter barley + 10% of winter wheat; Mix B: 50% of two cultivars of winter triticale + 40% of winter barley + 10% of winter wheat; Mix C: 55% of three types of Italian ryegrass + 45% of two cultivars of winter oat; Mix D: 40% of three types of Italian ryegrass + 30% of two cultivars of winter oat + 15% of two cultivars of winter triticale + 10% of winter barley + 5% of winter wheat

Table 20. Nutritional compositions of silage on 7, 14 and 90 opening days, different crop mixtures and their interaction (n = 15)

		Components (% DM)						
		DM (%)	CP	EE	CF	NDF	ADF	TS
Day 7	mixture A	30.46$^{a, A}$	10.24$^{ab, A}$	2.16^{A}	29.94$^{b, A}$	56.88$^{ab, A}$	32.42$^{ab, A}$	17.26$^{ab, B}$
	mixture B	34.70$^{b, B}$	10.92$^{b, B}$	2.30^{A}	29.82$^{b, A}$	60.88$^{b, B}$	33.80^{b}	12.86$^{b, B}$
	mixture C	35.60^{bc}	10.10^{a}	2.16^{A}	27.26$^{a, A}$	54.50$^{a, A}$	31.28$^{a, A}$	20.38$^{a, B}$
	mixture D	36.00$^{c, B}$	9.40$^{c, A}$	2.10^{A}	28.08$^{a, A}$	52.90$^{a, A}$	32.28^{ab}	18.54$^{a, C}$
	SEM	0.542	0.382	0.177	0.504	1.095	1.174	2.591
Day 14	mixture A	35.54$^{b, C}$	9.98^{A}	2.44^{B}	29.94$^{ab, A}$	57.56$^{bc, A}$	32.56$^{ab, A}$	18.38$^{c, B}$
	mixture B	30.60$^{a, A}$	10.02^{A}	2.58^{A}	31.38$^{b, B}$	59.78$^{c, B}$	34.30^{b}	16.68$^{b, C}$
	mixture C	34.48^{b}	9.94	2.52^{AB}	29.20$^{ab, A}$	55.50$^{b, AB}$	31.78$^{a, A}$	16.82$^{b, B}$
	mixture D	34.60$^{b, B}$	10.38^{A}	2.68^{B}	27.90$^{a, A}$	53.34$^{a, A}$	30.38^{a}	14.04$^{a, B}$
	SEM	1.052	0.749	0.157	1.287	1.685	1.232	0.658
Day 90	mixture A	33.06$^{b, B}$	11.70$^{b, B}$	2.96$^{a, C}$	35.10$^{b, B}$	66.66$^{b, B}$	38.16^{B}	13.30$^{c, A}$
	mixture B	30.32$^{a, A}$	9.36$^{a, A}$	3.56$^{b, B}$	30.32$^{a, A}$	57.54$^{a, A}$	33.18	5.28$^{a, A}$
	mixture C	33.94^{b}	11.18^{a}	2.78$^{a, B}$	34.20$^{b, B}$	61.14$^{ab, B}$	36.78^{B}	6.18$^{a, A}$
	mixture D	32.38$^{ab, A}$	12.56$^{b, B}$	3.74$^{b, C}$	34.10$^{b, B}$	61.92$^{ab, B}$	34.48	10.28$^{b, A}$
	SEM	1.199	0.935	0.201	1.434	3.313	3.817	1.479

p value	Day 7	< 0.001	< 0.001	ns	< 0.001	< 0.001	< 0.05	< 0.01
	Day 14	< 0.001	ns	ns	< 0.01	< 0.001	< 0.01	< 0.001
	Day 90	<0.01	<0.001	< 0.001	< 0.001	< 0.01	ns	< 0.001
SEM	mixture A	0.768	0.793	0.089	0.571	1.287	0.976	1.352
	mixture B	0.722	0.501	0.173	0.601	1.112	1.673	1.536
	mixture C	1.101	0.907	0.221	1.929	3.761	1.927	2.648
	mixture D	1.209	0.639	0.204	0.937	1.728	3.978	1.113
p value	mixture A	<0.001	<0.01	<0.001	<0.001	<0.001	<0.001	<0.001
	mixture B	<0.001	<0.01	<0.001	<0.01	<0.01	ns	<0.001
	mixture C	ns	ns	<0.01	<0.001	<0.05	<0.01	<0.001
	mixture D	<0.01	<0.001	<0.001	<0.001	<0.001	ns	<0.001
Interaction	SEM	0.973	0.726	0.179	1.150	2.237	2.413	1.764
	p value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

mixture A: 40% of two cultivars of winter triticale + 30% of two cultivars of winter oats + 20% of winter barley + 10% of winter wheat; mixture B: 50% of two cultivars of winter triticale + 40% of winter barley + 10% of winter wheat; mixture C: 55% of three types of Italian ryegrass + 45% of two cultivars of winter oat; mixture D: 40% of three types of Italian ryegrass + 30% of two cultivars of winter oat + 15% of two cultivars of winter triticale + 10% of winter barley + 5% of winter wheat.

$^{a-c}$ Means within a row with different superscripts different ($p<0.05$) (Crop mixture effect)

$^{A-C}$ Means within same column with different superscripts different ($p<0.05$) (Opening days effect)

ns=not significant ($P>0.05$)

The starch content of all four ensiled mixtures was below detectable concentration (< 0.10 g/kg DM). The crop mixtures affected ($p<0.05$) the DM, CP (except d14), NDF and total sugar (TS) of ensiled mixtures at the three opening days (Table 20). The low starch content could be associated with early harvest (early heading stage) before the cereal's onset starch accumulation such as early dough stage (mixture A and B) as well as very low starch content of Italian ryegrass (Baldinger et al., 2014) which dominates the IRG plus winter cereal mixtures (mixtures C and D).

6.2. Fermentation characteristics

Ensiling affected most of the fermentation characteristics of all ensiled mixtures except mixture C (only pH is affected) (Table 21). The pH values decreased ($p<0.05$) in all ensiled mixtures (Figure 5a).

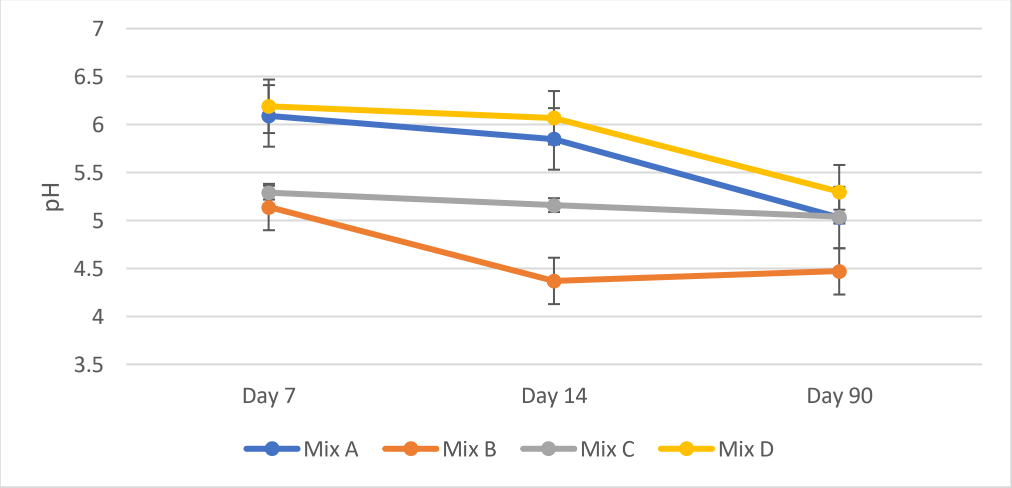
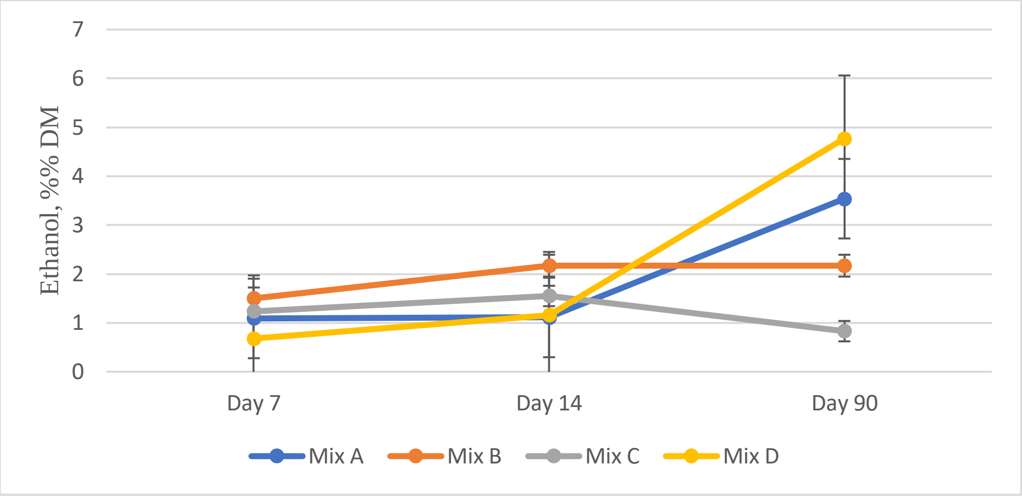


Figure 5. Line graph of fermentation end products content of mixture A, B, C and D silages at day 7, 14 and 90 (Experiment II)

a) pH content of mixture A, B, C and D silages at day 7, 14 and 90

Mix A: 40% of two cultivars of winter triticale + 30% of two cultivars of winter oats + 20% of winter barley + 10% of winter wheat; Mix B: 50% of two cultivars of winter triticale + 40% of winter barley + 10% of winter wheat; Mix C: 55% of three types of Italian ryegrass + 45% of two cultivars of winter oat; Mix D: 40% of three types of Italian ryegrass + 30% of two cultivars of winter oat + 15% of two cultivars of winter triticale + 10% of winter barley + 5% of winter wheat

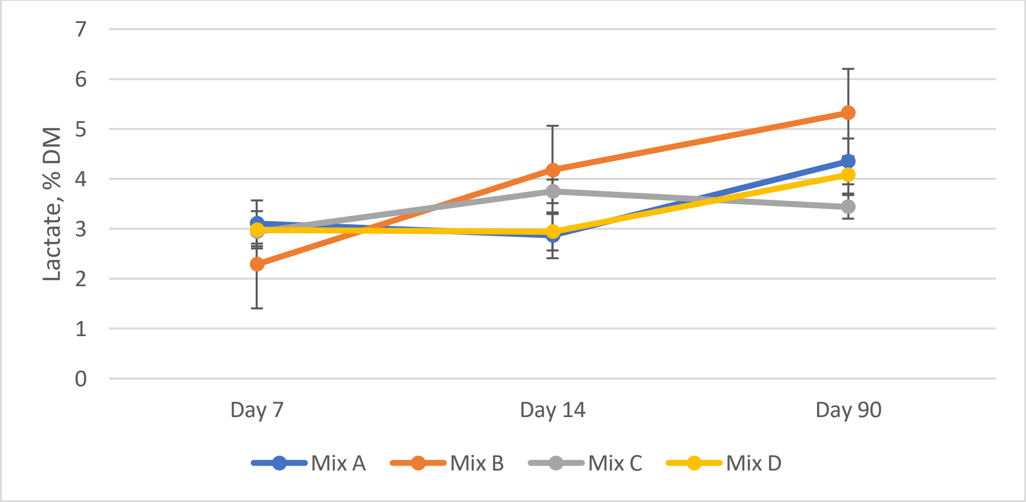
At the end of 90 days fermentation the pH content of ensiled mixtures (except mixture B) were not in a pH range of grass silage (25 – 35% DM) 4.3-4.7 reported by Kung and Shaver (2001). The high pH at day 90 could be associated with low lactic acid concentration 4.35 (mixture A), 5.32 (mixture B), 3.44 (mixture C) and 4.08% DM (mixture D) probably caused by restricted fermentation which result in low acidification and higher ethanol concentration (Kung et al., 2018). Kung and Stanley (1982) and Daniel et al. (2013) reports that restricted fermentation will occur when epiphytic yeasts converted sucrose into excessive ethanol. This could be the reason for excessive total sugar converted to high ethanol content at the end of 90 days fermentation (Figure 5b). As compared to the residual total sugar content (0.04 – 0.12% on DM basis) of Italian ryegrass and winter cereal mixture silage, excessive total sugar content (5.28 – 13.30% on DM basis) at end of the 90 days fermentation is an indicator of restricted fermentation in the present study. Kung et al. (2018) reported that during fermentation lactic acid contributes the most to the decline in pH because it is about 10 to 12 times stronger than any of the other major acids such as acetic and propionic acid found in silages. In all mixtures fermentation products were limited to three principal products: lactic acid (LA), acetic acid (AA), and ethanol (Table 16). Valeric acid (VA) and caproic acid (CA) concentrations were below detectable concentrations (<0.01%). The fermentation process did not affect (P>0.05) the ethanol contents of the ensiled mixtures except mixture D (Figure 5b).



b) Ethanol content of mixture A, B, C and D silages at day 7, 14 and 90

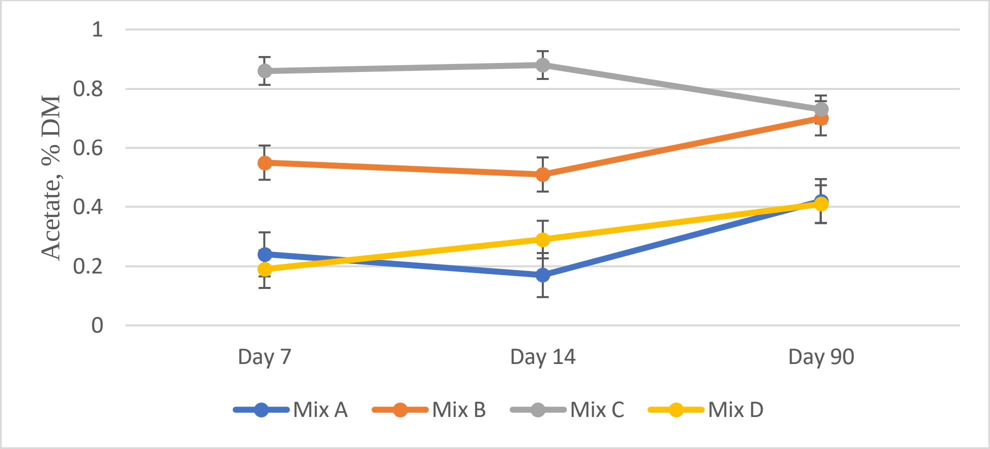
Mix A: 40% of two cultivars of winter triticale + 30% of two cultivars of winter oats + 20% of winter barley + 10% of winter wheat; Mix B: 50% of two cultivars of winter triticale + 40% of winter barley + 10% of winter wheat; Mix C: 55% of three types of Italian ryegrass + 45% of two cultivars of winter oat; Mix D: 40% of three types of Italian ryegrass + 30% of two cultivars of winter oat + 15% of two cultivars of winter triticale + 10% of winter barley + 5% of winter wheat

Concentrations of lactate gradually increased over time (except mixture C), reaching their highest values by day 90 (Figure 5c). The interaction of opening days and crop mixtures affected ($p<0.05$) all the measured fermentation products of mixture silage (Table 21). During the 90 days fermentation, lactic acid continued to be the major fermentation product with a small production of acetic acid (Figure 5d), resulting high LA/AA (mixture A and B) over the storage periods. However, LA/AA was not affected ($p>0.05$) in relation to the increase in fermentation period (mixture C and D). The observed percent of lactic acid per total fermentation acid (LA% of TFA) at each opening day for all silage mixtures was above 74%; the values were decreased ($p<0.05$) in relation to increase fermentation (mixture A and B) (Figure 5e). Even though lactic acid was dominant at the entire fermentation period for all ensiled mixtures, the observed lactic acid at the end of the 90 days fermentation was lower than the range (6 – 10% DM) of grass silage with DM ranges between 25 – 35% reported by Kung and Shaver (2001). However, Kung et al. (2018) suggests that lactic acid in commonly fed silages ranges from 2 to 4% of DM but can be considerably higher in silages with low concentration of DM (< 30%). Even if LA continued to be the major fermentation product with a small production of AA, the resulting LA/AA remain unaffected (p>0.05) over the storage periods except mixture B. This result indicated that even if acidification was initiated by homofermentative lactic acid bacteria, it was not dominant during the course of fermentation particularly around day 90. Additionally, continues increase in acetic acid production at each opening day would also affect this ratio. The lactic acid to acetic acid ratio is a good efficiency indicator for silage fermentation (Jalc et al., 2009). This ratio ideally should not be less than 3:1, and the higher it is the better (Kung and Shaver, 2001). In the present study, this ratio increased over time and at the end of storage the highest value of 10.39:1, 8.00:1, 5.80:1 and 10.27:1 was observed for mixture A, B, C and D silages, respectively.



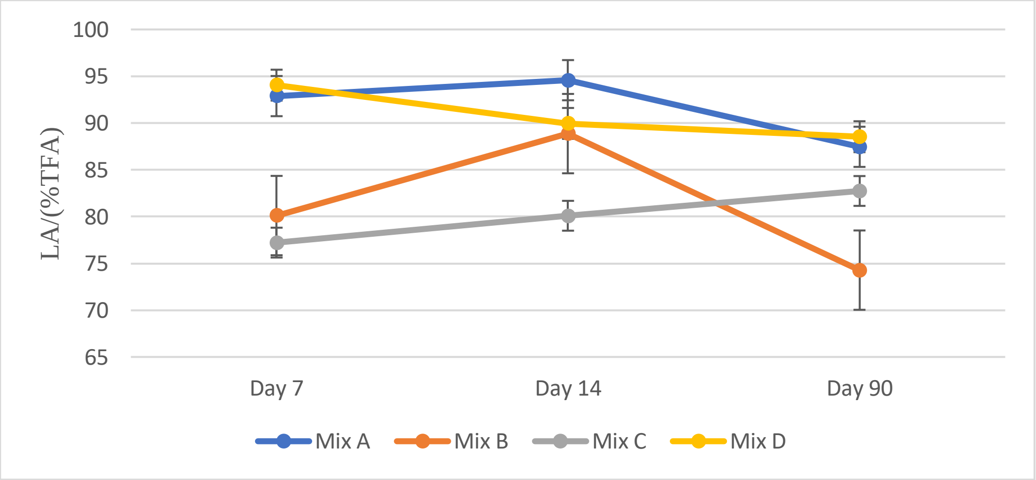
c) Lactate content of mixture A, B, C and D silages at day 7, 14 and 90

Mix A: 40% of two cultivars of winter triticale + 30% of two cultivars of winter oats + 20% of winter barley + 10% of winter wheat; Mix B: 50% of two cultivars of winter triticale + 40% of winter barley + 10% of winter wheat; Mix C: 55% of three types of Italian ryegrass + 45% of two cultivars of winter oat; Mix D: 40% of three types of Italian ryegrass + 30% of two cultivars of winter oat + 15% of two cultivars of winter triticale + 10% of winter barley + 5% of winter wheat



d) Acetate content of mixture A, B, C and D silages at day 7, 14 and 90

Mix A: 40% of two cultivars of winter triticale + 30% of two cultivars of winter oats + 20% of winter barley + 10% of winter wheat; Mix B: 50% of two cultivars of winter triticale + 40% of winter barley + 10% of winter wheat; Mix C: 55% of three types of Italian ryegrass + 45% of two cultivars of winter oat; Mix D: 40% of three types of Italian ryegrass + 30% of two cultivars of winter oat + 15% of two cultivars of winter triticale + 10% of winter barley + 5% of winter wheat



e) LA/(%TFA) content of mixture A, B, C and D silages at day 7, 14 and 90 (Experiment II)

Mix A: 40% of two cultivars of winter triticale + 30% of two cultivars of winter oats + 20% of winter barley + 10% of winter wheat; Mix B: 50% of two cultivars of winter triticale + 40% of winter barley + 10% of winter wheat; Mix C: 55% of three types of Italian ryegrass + 45% of two cultivars of winter oat; Mix D: 40% of three types of Italian ryegrass + 30% of two cultivars of winter oat + 15% of two cultivars of winter triticale + 10% of winter barley + 5% of winter wheat

Proportions of LA (% TFA) on 90th opening day were consistent (except mixture B) with the report of AHDB (2012) for grass silage, described that for well fermented silage lactic acid as the proportion of total acids should be >75%. Ethanol was detected during the storage period and the observed ethanol content for all ensiled mixture (except mixture C) was higher than the range (0.5 – 1.0% DM) reported by Kung and Shaver (2001). The detectable ethanol at day 90 for mixture A (3.54 % DM), mixture B (2.17% DM) and mixture D (4.77% DM) was unexpectedly higher than the normal range attributed to the survival of yeast (Table 22). Driehuis and van Wikselaar (2000) reported as high as 5 to 6% concentrations of ethanol in some Dutch grass silage. High concentrations of ethanol (>3–4% DM) affects aerobic stability of silages as some yeasts can assimilate lactic acid and cause off flavours in milk when fed in large quantities (Kung et al., 2018). The crop mixtures affected ($P<0.05$) the pH, acetate, lactate, NH3–N (except d90) contents at the three opening days. The amount of NH_3-N (g/100 g total N) was very low (<5.40 g/100 g total N) at the three opening days for all ensiled mixtures. Fermentation caused an increase ($p<0.05$) in NH_3-N (g/100 g total N) at day 90 as compared to day 7 and 14 (mixture A and C) and day 7 (mixture B). The NH3-N/total N of all ensiled mixture was low (< 5.35 g/100g total N) at the three opening days. As per the criteria after the end of fermentation process when the NH_3-N/total N is below 7 g/100 g total N, the silage could be categorized as excellent silage. The observed NH_3-N/total N at each opening days of all ensiled mixtures were below the ranges (8 – 12 NH_3-N, total N) reported by Kung and Shaver (2001).

Table 21. Fermentation characteristics of silage on 7, 14 and 90 opening days, at different forage mixtures and their interaction (n = 15)

		Components							
		pH	Ethanol (%DM)	Acetate (%DM)	Lactate (%DM)	TFA (%DM)	LA/AA	LA (% TFA)	NH_3-N (g/100g tot. N)
Day 7	mixture A	$6.09^{b, B}$	1.09	$0.24^{a, A}$	$3.11^{b, A}$	$3.36^{ab, A}$	16.55^{b}	$92.88^{b, B}$	$1.41^{a, A}$
	mixture B	$5.14^{a, B}$	1.50	$0.55^{b, AB}$	$2.29^{a, A}$	$2.85^{a, A}$	$4.14^{a, A}$	$80.11^{b, B}$	$2.72^{b, A}$
	mixture C	$5.29^{a, B}$	1.24	0.86^{c}	2.94^{ab}	3.80^{b}	3.46^{a}	77.22^{a}	3.18^{b}
	mixture D	$6.19^{b, B}$	0.68^{A}	$0.19^{a, A}$	$2.98^{ab, A}$	$3.17^{ab, A}$	17.04^{b}	94.05^{b}	$1.57^{a, A}$
	SEM	0.180	0.496	0.101	0.394	0.431	5.555	3.100	0.411
Day 14	mixture A	$5.85^{b, B}$	1.11^{a}	$0.17^{a, A}$	$2.87^{a, A}$	$3.04^{a, A}$	24.06^{c}	$94.58^{b, B}$	$1.65^{a, A}$
	mixture B	$4.37^{a, A}$	2.17^{b}	$0.51^{b, A}$	$4.18^{b, B}$	$4.76^{b, B}$	$8.25^{ab, B}$	$88.87^{b, C}$	$4.37^{b, B}$
	mixture C	$5.16^{b, AB}$	1.55^{ab}	0.88^{c}	3.75^{ab}	4.46^{b}	4.04^{a}	80.09^{a}	3.82^{b}
	mixture D	$6.07^{c, A}$	$1.16^{a, A}$	$0.29^{a, AB}$	$2.94^{a, A}$	$3.23^{a, A}$	14.80^{b}	89.96^{b}	$2.03^{a, A}$
	SEM	0.202	0.356	0.113	0.600	0.598	9.159	4.301	0.638
Day 90	mixture A	$5.03^{b, A}$	3.54	$0.42^{a, B}$	$4.35^{ab, B}$	$4.97^{a, B}$	10.39^{b}	$87.46^{b, A}$	3.99^{B}
	mixture B	$4.47^{a, A}$	2.17	$0.70^{b, B}$	$5.32^{b, C}$	$7.23^{b, C}$	$8.00^{ab, B}$	$74.28^{a, A}$	5.35^{B}
	mixture C	$5.04^{b, A}$	0.83	0.73^{b}	3.44^{a}	4.20^{a}	5.80^{a}	82.74^{b}	4.22
	mixture D	$5.30^{c, A}$	4.77^{B}	$0.41^{a, B}$	$4.08^{ab, B}$	$4.59^{a, B}$	10.27^{b}	88.54^{b}	4.42^{B}
	SEM	0.085	2.171	0.213	0.734	0.817	2.490	4.372	0.751
p value	Day 7	<0.001	ns	<0.001	<0.05	<0.05	<0.001	<0.001	<0001
	Day 14	<0.001	<0.05	<0.001	<0.05	<0.001	<0.05	<0.001	<0.001

	Day 90	<0.001	0.057	< 0.05	<0.05	<0.001	<0.05	<0.001	ns
SEM	mixture A	0.185	1.718	0.110	0.548	0.626	9.266	2.909	0.395
	mixture B	0.229	0.494	0.108	0.611	0.586	1.505	3.271	0.649
	mixture C	0.09	0.488	0.238	0.496	0.652	1.737	4.525	0.897
	mixture D	0.113	1.830	0.102	0.697	0.674	8.374	4.830	0.373
p value	mixture A	<0.001	ns	< 0.01	< 0.01	< 0.001	ns	< 0.01	< 0.001
	mixture B	< 0.01	ns	< 0.05	< 0.001	< 0.001	< 0.01	< 0.001	< 0.001
	mixture C	< 0.01	ns	ns	ns	ns	ns	ns	ns
	mixture D	< 0.001	< 0.01	< 0.05	< 0.05	< 0.01	ns	ns	< 0.001
Interaction	SEM	0.164	1.302	0.151	0.593	0.635	6.349	3.967	0.617
	p value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

mixture A: 40% of two cultivars of winter triticale + 30% of two cultivars of winter oats + 20% of winter barley + 10% of winter wheat; mixture B: 50% of two cultivars of winter triticale + 40% of winter barley + 10% of winter wheat; mixture C: 55% of three types of Italian ryegrass + 45% of two cultivars of winter oat; mixture D: 40% of three types of Italian ryegrass + 30% of two cultivars of winter oat + 15% of two cultivars of winter triticale + 10% of winter barley + 5% of winter wheat.

$^{a-c}$ Means within a row with different superscripts different (p<0.05) (Crop mixture effect)

$^{A-C}$ Means within same column with different superscripts different (p<0.05) (Opening days effect)

ns=not significant (p>0.05)

DM – dry matter, AA – acetic acid, LA – lactic acid, TFA – total fermentation acid, SME – standard error of mean.

6.3. Microbiological quality

There was no difference ($p>0.05$) in mould and yeast count (Log_{10} CFU/g) in the silages except mixture B (Table 22). The mould and yeast content decreased ($p<0.05$) by day 90 as compared to day 7 and 14 after opening the laboratory silos (mixture B). The fermentation process affected ($p<0.05$) the aerobic mesophilic microorganisms count (AMC) (Log_{10} CFU/g) of all ensiled mixtures except mixture C. As compared to day 7 and 14, lower ($p<0.05$) AMC were recorded at opening day 90. Both the crop mixtures and the interaction of opening days and crop mixtures affected ($p<0.05$) both mesophilic microorganisms count (AMC) and mould and yeast count (Log_{10} CFU/g) of all ensiled mixtures. For all ensiled mixtures, the AMC at each opening day was higher than the normal count 6.00 (Log_{10} CFU/g) or 1×10^6 (CFU/g) of European decree (EN ISO 4833, Microbiological limits 65-2012 VM Decree Annex 12). The mould and yeast count (Log_{10} CFU/g) at each opening day were higher than the limit recommended as a quality standard for animal feeds (3.00 (Log_{10} CFU/g) or 1×10^4 (CFU/g) (GMP, 2008) as a result higher level of ethanol was recorded at the end of the 90 days fermentation period. Mould and yeast count at different opening days were consistent with the results of González et al. (2008) who reported 90% of their samples counts over 3.00 (Log_{10} CFU/g) or 1×10^4 (CFU/g).

Table 22. **Microorganism count and/or mould and yeast count (Log_{10} CFU g^{-1}) of silage on 7, 14 and 90 days, at different forage mixtures and their interaction (n = 15)**

		Components	
		Aerobic mesophilic microorganism count (AMC) (Log_{10} CFU g-1) (1)	Mould and yeast count (Log_{10} CFU g-1) (1)
Day 7	mixture A	$7.93^{ab, B}$	7.29^{b}
	mixture B	$8.28^{b, B}$	$7.39^{b, B}$
	mixture C	7.70^{a}	5.71^{a}
	mixture D	$7.80^{a, AB}$	5.92^{a}
	SEM	0.271	0.601
Day 14	mixture A	$8.01^{a, B}$	6.91^{ab}
	mixture B	$9.10^{b, C}$	$8.19^{b, B}$
	mixture C	8.32^{ab}	7.16^{ab}
	mixture D	$8.29^{ab, B}$	5.61^{a}
	SEM	0.547	1.153
Day 90	mixture A	$7.22^{a, A}$	6.73^{ab}
	mixture B	$7.29^{a, A}$	$4.53^{a, A}$
	mixture C	8.73^{b}	7.27^{b}
	mixture D	$7.44^{a, A}$	5.03^{ab}
	SEM	0.601	1.414
p value	Day 7	<0.05	<0.001
	Day 14	<0.05	<0.05
	Day 90	<0.01	<0.05
SEM	mixture A	0.350	0.911
	mixture B	0.214	0.858
	mixture C	0.765	1.160
	mixture D	0.473	1.418
p value	mixture A	<0.01	ns
	mixture B	<0.001	<0.001
	mixture C	ns	ns
	mixture D	< 0.05	ns
Interaction	SEM	0.494	1.109
	p value	<0.001	<0.001

mixture A: 40% of two cultivars of winter triticale + 30% of two cultivars of winter oats + 20% of winter barley + 10% of winter wheat; mixture B: 50% of two cultivars of winter triticale + 40% of winter barley + 10% of winter wheat; mixture C: 55% of three types of Italian ryegrass + 45% of two cultivars of winter oat; mixture D: 40% of three types of Italian ryegrass + 30% of two cultivars of winter oat + 15% of two cultivars of winter triticale + 10% of winter barley + 5% of winter wheat.

$^{a-c}$Means within a row with different superscripts different ($p<0.05$) (Crop mixture effect)

$^{A-C}$ Means within same column with different superscripts different ($p<0.05$) (Opening days effect)

ns=not significant ($p<0.05$)

(1) Counting at silo opening; SME – standard error of mean, CFU – colony forming unit

6.4. Ruminal degradability

The ensiled mixtures (novel mixtures of winter cereals and Italian ryegrass plus winter cereals silages, cutting at the heading stage of wheat) had high effective degradable DM and CP at the three rumen outflow rates (ED_1, ED_5 and ED_8) and moderate potentially degradable DM and CP. The *in situ* degradability of the examined nutrient content (DM, CP, NDF, ADF) of the mixtures varied greatly depending on the proportion of cereals (mixtures A and B) and Italian ryegrass (mixtures C and D). The degradable fraction of DM and CP in the novel mixtures showed significantly different degradation values depending on whether 45% oats were associated with 40% Italian ryegrass (mixture C) or other cereals (15% triticale, 30% oats, 10% barley, 10% wheat) with 55% Italian ryegrass (mixture D). Significant difference was found in the effective degradability (ED_5, ED_8) of the NDF content of the two Italian ryegrass plus winter cereal silages (mixture C vs. mixture D).

6.4.1. Ruminal degradability of DM

There was variation ($p<0.05$) in soluble fraction, potentially degradable DM fraction, and effective DM degradability-1 (ED_1), degradability-5 (ED_5) and degradability-8 (ED_8) among the mixtures. However, rate of DM degradation was similar ($p>0.05$) among the mixtures. Mixture D had the highest soluble DM fraction (31.97 % of DM) and lowest potentially degradable DM fraction (42.52 % of DM) than other mixture silages. The effective degradable DM at the three rumen outflow rates (ED_1, ED_5, ED_8) for all ensiled mixture was higher and above 66%. The effective DM degradability at 1% rumen outflow rates (ED_1) was in the range of 71 -75%. Italian ryegrass plus winter cereal grain-based silage (mixture C, D) had higher ($p<0.05$) effective DM degradability-8 (ED_8) than cereal based silage (mixture A, B). The potential ruminal degradability for DM of both winter cereals and Italian ryegrass plus winter cereals ensiled mixtures was 63.76%, 64.90%, 62.75% and 42.52% for mixture A, B, C and D, respectively and the effective rumen DM degradability's at 8% rumen outflow rate (ED_8) were 67.96%, 66.27%, 71.42% and 69.97%, respectively. These values were higher than the DM degradability of Italian ryegrass (60.70%) reported by Andrighetto et al. (1993). The degradability of barley and winter oats are excellent; as result it enrich the dry matter intake (DMI) and consequently improve milk production (Raffrenato et al., 2010; Grant and Contach, 2012). The effective DM degradability at 1% rumen outflow rate (ED_1) which defines the maintenance DM requirement were 73.20% (mixture A), 71.52% (Mixture B), 75.45% (mixture C) and 73.87% (mixture D) by far better than the report of Andrighetto et al. (1993).

6.4.2. Ruminal degradability of CP

There was variation ($p<0.05$) in all degradable CP components among the mixture silage. Mixture A had higher ($p<0.05$) *in situ* soluble CP fraction (68.31% of DM), and lower ($p<0.05$) potentially degradable CP fraction (16.96% of DM) than other mixture silages. The effective protein degradability (at 8% rumen outflow rate/h; EPD_8) was 80.63% (mixture A), 66.27% (mixture B), 79.70% (mixture C) and 79.35% (mixture D). There was inconsistency on the potential ruminal degradable CP of both winter cereal and Italian ryegrass plus winter cereal-based silages where mixture A and D had lower value as compared to mixture B and C. The soluble fraction of mixture A (68.31%) silage was higher than the soluble CP fraction (47%) of corn silage (Susmel et al., 1990) and ryegrass silage (49.05) at its vegetative stage (Valderrama and Anrique, 2011), different cereal forages (Hadjipanayiotou et al., 1996; Turgut and Yanar, 2004) and mature alfalfa hay (37.26%) and normal corn silage (40.34%) (Muazzez, 2018). However, mixture D (45.22%) had lower value except mature alfalfa hay (37.26%). On the other hand, the soluble fraction of mixture A (68.31%) was comparable with the values in oat forages (68.47%)

(Hadjipanayiotou et al., 1996). Soluble CP fractions of mixture B (7.44%) and mixture C (18.38) silages were lower than Italian ryegrass forage at 1st cut (20.60%) and 2nd cut (19.20%) of leaf stage as well as 2nd cut of vegetative (27.40%), early budding (24%), budding (18.40%) and end of budding (20%) stages of alfalfa (Amrane and Michelet-Doreau, 1993). It was also lower than the range of soluble CP fraction of grass silage 21-38% (Muazzez, 2018). The potential ruminal degradability of CP was 16.96%, 64.90%, 65.10% and 36.58% respectively and the effective rumen CP degradability at 8% rumen outflow rate (ED_8) of was 80.63%, 66.27%, 79.70% and 79.35%, respectively. Both the potential and effective degradability of CP in present silage mixtures were lower than that of Italian ryegrass forage at 1st cut (81.4%) and 2nd cut (82.30%) of leaf stage, grazing (81.80%) and heading (82%) stages as well as CP degradability of alfalfa (81.40%) at first cut of its vegetative stage (Amrane and Michelet-Doreau, 1993). However, the effective CP degradability at 8% rumen outflow rate (ED_8) except mixture B was higher than the CP degradability of Italian ryegrass forage at end of its heading stage (76.90%); alfalfa at 2nd cut of vegetative (77.90%) and end of budding (77.40%) stages reported by the same author. The high effective CP degradability at 8% rumen outflow rate (ED_8) could be attributed to early harvest (heading) of all ensiled mixtures as well as the inclusion of more Italian ryegrass in Italian ryegrass plus winter cereal based fermented mixtures (55% in mixture C and 40% in mixture D). Italian ryegrass has higher CP at the proper stage of harvesting i.e. 2nd cut (Baldinger et al., 2011) which is similar to CP values at the end of 90 days fermentation period in present study. As compared to Italian ryegrass forage, the low potential degradable CP of ensiled mixtures particularly mixture A and D could be associated with ensiling, because ensiling affects the CP degradability (de Olivera et al., 2016). The potentially degradable CP fraction of all ensiled mixtures were lower than the slowly degradable CP fraction Italian ryegrass forage at 1st (74.80%) and 2nd cut (76.80%) of leaf stage as well as alfalfa silage at budding (74%) (Amrane and Michelet-Doreau, 1993), different cereal forages

(Turgut and Yanar, 2004; Valderrama and Anrique, 2011); forage silages (Edmunds et al., 2012); alfalfa and ryegrass forage (Valderrama, and Anrique, 2011); grass silage (Susmel et al., 1990; Valderrama and Anrique, 2011) and corn silage (Susmel et al., 1990; Muazzez, 2018). Degradation rate of CP fraction ‘b’ at time t (c) were 0.22 h^{-1}, 0.78 h^{-1}, 1.27 h^{-1} and 1.08 h^{-1}. These values were higher than the values in normal corn silage (Muazzez, 2018). The high degradation rate could be attributed to proper stage of harvesting (early heading) prior to ensiling for all mixtures as well as a higher proportion of Italian ryegrass than winter cereals in Italian ryegrass plus winter cereal-based mixture silages which improves protein recovery and increases degradability of ensiled material by rumen microbes. The degradability rate of all ensiled mixtures was substantially higher than Italian ryegrass forage at 1st (0.142 h^{-1}) and 2nd cut (0.140 h^{-1}) of leaf stage, grazing stage (0.110 h^{-1}), heading stage (0.103 h^{-1}) and alfalfa forage at 1st (0.162 h^{-1}) and 2nd cut (0.154 h^{-1}) of vegetative stage, early budding (0.152 h^{-1}), budding (0.166 h^{-1}) and end of budding (0.137 h^{-1}) stage (Amrane and Michelet-Doreau, 1993). Valderrama and Anrique (2011) also reported lower ‘c’ parameter for alfalfa (0.197 h^{-1}), oat forage (0.294 h^{-1}) and rye grass forage (0.157 h^{-1}) at vegetative stage as compared to value in mixture B, C and D silage. Turgut and Yanar (2004) also reported lower ‘c’ values for alfalfa hay (0.113 h^{-1}). This higher rate of CP degradability would make the current silage mixture attractive to combine in other higher fiber crops for better forage utilization in the nutrition of dairy cows. The effective protein degradability (EPD) values at 0.01, 0.05 and 0.08 h^{-1} of all ensiled mixtures were higher than the EPD of corn silage (60.11% and 55.88%, respectively) at 0.05 and 0.08 h^{-1} rumen outflow rates reported by Muazzez (2018). The higher EPD in the present mixture silages could be attributed to either proper stage of harvesting (heading) prior to ensiling in all mixtures or higher proportion of Italian ryegrass in Italian ryegrass plus winter cereals-based mixture silages. However, as compared to EPD values at 0.05 and 0.08 in the present ensiled mixtures, Valderrama and Anrique (2011) reported higher EPD values at 0.05 and 0.08 h^{-1} for alfalfa forage (88.25%, 85.16%) and oat forage (90.80%) at its vegetative stage. However, the EPD at 0.08 rumen outflow rate of rye grass forage (80.62%) was comparable with mixture A (80.63), and higher than mixture B, C and D (66.27%, 79.70% and 79.35%). The EPD values at 0.05 h^{-1} rumen outflow rate of all ensiled mixtures was better than barley (69%, 61% and 56%); and oats (66%, 60% and 56%) at flowering, pod formation and early maturity respectively (Hadjipanayiotou et al., 1996).

6.4.3. Ruminal degradability of NDF and ADF

In this study, the soluble NDF and ADF fractions of all ensiled mixtures were low. Mixture C had higher ($p<0.05$) *in situ* soluble NDF fraction than other mixture silages; however, there were no variation ($p> 0.05$) in *in situ* soluble ADF fraction as well as potentially degradable NDF and ADF fraction among mixture silages. The effective NDF degradability at 0.08 h^{-1} rumen outflow rate (ED_8) was low ($p< 0.05$) for mixture D but similar ($p> 0.05$) among mixture A, B and C silages. Mixture B had higher ($p< 0.05$) effective ADF degradability at 0.08 h^{-1} rumen outflow rate (ED_8) than other mixture silages. There was no difference ($p>0.05$) on effective NDF and ADF degradability at 0.01 h^{-1} rumen outflow rate (ED_1) between mixture A, B, C and D silages. The potentially degradable as well as effective degradable NDF and ADF at 0.01 and 0.08h^{-1} rumen outflow rate (ED_1 and ED_8) were low. The amount and ruminal degradability of NDF is very important factor in the dairy cow’s nutrition because forage NDF varies widely in its degradability in the rumen and NDF digestibility influences animal performance (Nousiainen et al., 2009; Zebeli et al., 2012; Bender et al., 2016). The low potential and effective ruminal degradability of NDF and ADF in our trial could be associated with high NDF and ADF contents of ensiled mixtures. The potential ruminal NDF degradability of all ensiled mixtures were lower than the NDF degradability of Italian ryegrass (59.8%) reported by Andrighetto et al. (1993). Ali et al. (2014) also reported higher NDF degradability of grass silage (76.4%) as compared to the present silage mixtures. The rate of ruminal NDF degradation (k_p) for all ensiled mixtures was higher than grass/grass – clover silage (0.016% h^{-1}) as well as whole crop cereal silage (0.015% h^{-1}) reported by (Weisbjerg et al., 2007).

Table 23. Ruminal degradability of nutrients at different crop mixture silage (n=96/mixture)

	mixture A	mixture B	mixture C	mixture D	SEM	p value
Dry matter (DM)						
Soluble fraction (% of DM)	10.28^{c}	7.43^{d}	13.32^{b}	31.97^{a}	0.201	< 0.001
Potentially degradable fraction (% of DM)	63.76^{a}	64.90^{a}	62.75^{a}	42.52^{b}	1.257	< 0.001
Degradation rate (%/h^{-1})	0.78	0.78	1.00	0.69	0.120	ns
Effective degradability-1 (%)	73.20^{ab}	71.52^{b}	75.45^{a}	73.87^{ab}	1.312	< 0.05
Effective degradability-5 (%)	70.09^{bc}	68.40^{c}	73.08^{a}	71.55^{b}	0.882	< 0.01
Effective degradability-8 (%)	67.96^{b}	66.27^{b}	71.42^{a}	69.97^{a}	0.650	< 0.001
Crude protein (CP)						
Soluble fraction (% of DM)	68.31^{a}	7.44^{d}	18.38^{c}	45.22^{b}	0.358	< 0.001
Potentially degradable fraction (% of DM)	16.96^{c}	64.90^{a}	65.10^{a}	36.58^{b}	0.860	< 0.001
Degradation rate (%/h^{-1})	0.22^{d}	0.78^{c}	1.27^{a}	1.08^{b}	0.071	< 0.001
Effective degradability-1 (%)	84.50^{a}	71.52^{c}	82.98^{ab}	81.46^{b}	0.798	< 0.001
Effective degradability-5 (%)	82.05^{a}	68.40^{c}	81.03^{ab}	80.19^{b}	0.668	< 0.001
Effective degradability-8 (%)	80.63^{a}	66.27^{b}	79.70^{a}	79.35^{a}	0.631	< 0.001
Neutral detergent fiber (NDF)						
Soluble fraction (% of DM)	6.96^{b}	7.65^{b}	9.58^{a}	7.51^{b}	0.536	< 0.01
Potentially degradable fraction (% of DM)	42.06	34.30	31.97	37.02	9.188	ns
Degradation rate (%/h^{-1})	0.02	0.04	0.03	0.02	0.009	ns
Effective degradability-1 (%)	38.07	32.52	34.20	31.73	4.467	ns
Effective degradability-5 (%)	22.26^{a}	23.57^{a}	22.52^{a}	18.41^{b}	1.295	< 0.01
Effective degradability-8 (%)	18.05^{a}	19.73^{a}	19.14^{a}	15.22^{b}	0.788	< 0.001
Acid detergent fiber (ADF)						
Soluble fraction (% of DM)	6.26	7.41	8.01	7.55	0.887	ns
Potentially degradable fraction (% of DM)	39.01	29.73	33.34	41.22	14.725	ns
Degradation rate (%/h^{-1})	0.03	0.06	0.03	0.02	0.017	ns
Effective degradability-1 (%)	36.25	32.96	32.05	30.63	4.992	ns
Effective degradability-5 (%)	21.91^{ab}	23.97^{a}	20.19^{bc}	17.48^{c}	1.348	< 0.01
Effective degradability-8 (%)	17.78^{b}	20.58^{a}	17.01^{bc}	14.65^{c}	0.918	< 0.001

mixture A: 40% of two cultivars of winter triticale + 30% of two cultivars of winter oats + 20% of winter barley + 10% of winter wheat; mixture B: 50% of two cultivars of winter triticale + 40% of winter barley + 10% of winter wheat; mixture C: 55% of three types of Italian ryegrass + 45% of two cultivars of winter oat; mixture D: 40% of three types of Italian ryegrass + 30% of two cultivars of winter oat + 15% of two cultivars of winter triticale + 10% of winter barley + 5% of winter wheat.

ns=not significant

6.5. Rumen fermentation

The replacement of ensiled mixtures with vetch-triticale haylage in TMR did not modify the rumen fermentation characteristics (Table 23); there was no variation ($p>0.05$) between control and experimental diets, even the inclusion of 40 – 55% Italian ryegrass (mixture C and D) did not cause variation. This implies that the level of inclusion of ensiled mixtures instead of vetch – triticale haylage did not cause any adverse effect on the rumen microbes for appropriate fermentation. This result also indicated that the inclusion level can be increased beyond this level in a total mixed ration formulation as far as an appropriate forage to concentrate ratio as well as energy requirement of dairy cows maintained depending its production status. However, the rumen ammonia nitrogen (NH_3-N) (Figure 6a) and butyric acid (BA) (Figure 6b) concentrations were affected ($p<0.05$) by sampling time (3hr) (Table 25).

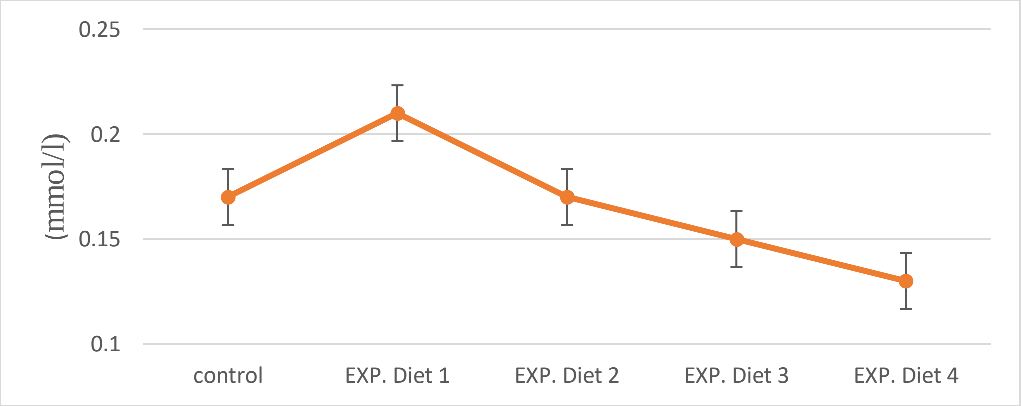
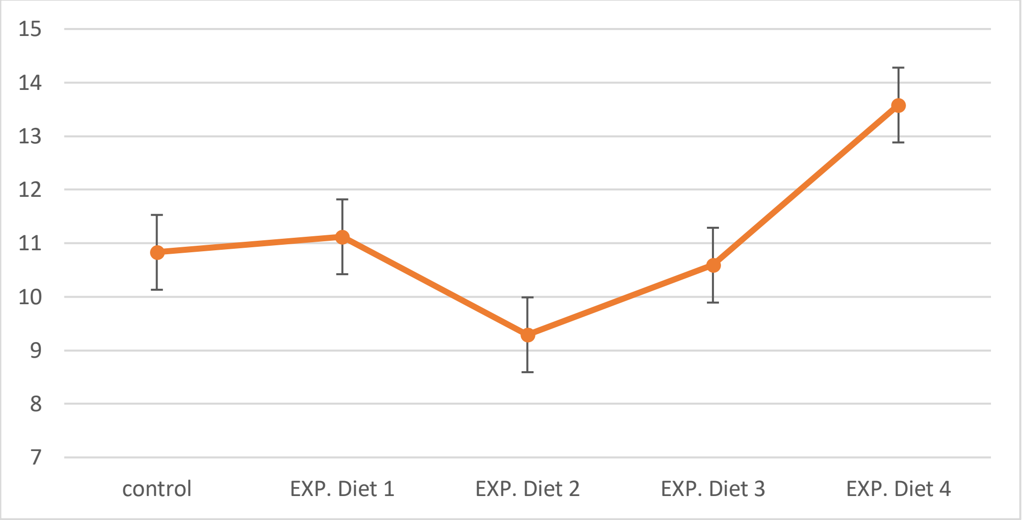


Figure 6. Line graph of rumen fermentation concentration of control and experimental diet at 3 hr sampling time

a) NH_3-N concentration of control and experimental diet at 3 hr sampling time

control: 5.5 kg day^{-1} of corn silage, 3.5 kg day^{-1} of alfalfa haylage, 3.5 kg day^{-1} of vetch-triticale haylage, 3 kg day^{-1} of concentrate, 1 kg day^{-1} of grass hay and 0.75 kg day^{-1} of liquid molasse; *EXP Diet 1*: Control diet + mixture A (40% of two cultivars of winter triticale + 30% of two cultivars of winter oats + 20% of winter barley + 10% of winter wheat) silage (3.5 kg day^{-1}, instead of vetch-triticale haylage); *EXP Diet 2*: Control diet + mixture B (50% of two cultivars of winter triticale + 40% of winter barley + 10% of winter wheat) silage (3.5 kg day^{-1}, instead of vetch-triticale haylage); *EXP diet 3*: Control diet + mixture C (55% of three types of Italian ryegrass + 45% of two cultivars of winter oat) silage (3.5 kg day^{-1}, instead of vetch-triticale haylage); *EXP diet 4*: Control diet + mixture D (40% of three types of Italian ryegrass + 30% of two cultivars of winter oat + 15% of two cultivars of winter triticale + 10% of winter barley + 5% of winter wheat) silage (3.5 kg day^{-1}, instead of vetch-triticale haylage)



b) Butyrate concentration of control and experimental diet at 3 hr sampling time

control: 5.5 kg day^{-1} of corn silage, 3.5 kg day^{-1} of alfalfa haylage, 3.5 kg day^{-1} of vetch-triticale haylage, 3 kg day^{-1} of concentrate, 1 kg day^{-1} of grass hay and 0.75 kg day^{-1} of liquid molasse; *EXP Diet 1*: Control diet + mixture A (40% of two cultivars of winter triticale + 30% of two cultivars of winter oats + 20% of winter barley + 10% of winter wheat) silage (3.5 kg day^{-1}, instead of vetch-triticale haylage); *EXP Diet 2*: Control diet + mixture B (50% of two cultivars of winter triticale + 40% of winter barley + 10% of winter wheat) silage (3.5 kg day^{-1}, instead of vetch-triticale haylage); *EXP diet 3*: Control diet + mixture C (55% of three types of Italian ryegrass + 45% of two cultivars of winter oat) silage (3.5 kg day^{-1}, instead of vetch-triticale haylage); *EXP diet 4*: Control diet + mixture D (40% of three types of Italian ryegrass + 30% of two cultivars of winter oat + 15% of two cultivars of winter triticale + 10% of winter barley + 5% of winter wheat) silage (3.5 kg day^{-1}, instead of vetch-triticale haylage)

The mean ruminal pH ranged between 6.93–7.06 and the mean ruminal NH_3-N concentration ranged from 0.068 to 0.107 mmol/L. The observed ruminal pH values of cows fed different dietary treatments were closer to neutral and ideal for all rumen microbes (Wales et al., 2004). Castillo-González et al. (2014) reported that within the ruminal ecosystem, the microorganisms coexist in a reduced environment and pH remains close to neutral. All the pH values (except experimental diet 2) were in the normal range of ruminal pH 6.0–7.0 (Wales et al., 2004), 5.5–7.0 (Krause and Oetze, 2006) and 6.2–7.0 (Queens land Government, 2013) depending on the diet and buffering capacity of saliva. Lack of variations in pH between control and experimental diets implies that the dietary treatment did not alter the rumen environment for conducive microbial function in this experiment, and efficient fermentation as a result the rumen microbes were able to adapt the given diet. This result was similar with Nur Atikah et al. (2018) report. A slight pH increment from the normal range in experimental diet 2 silage could not affect rumen cellulolytic processes of fiber and protein digestion as reported in Nur Atikah et al. (2018). Weimer (1996) found that a neutral pH is best to ensure the maintenance and growth of cellulolytic bacteria for optimal digestion of fibrous feed. Some diet changes improve manipulation of rumen fermentation for higher level of fermentation products (Castillo-González et al., 2014). Rumen NH_3-N concentration was higher ($p<0.05$) in experimental diet 1 as compared to experimental diet 4 and the ruminal BA concentration was higher ($p<0.05$) in experimental diet 4 than other dietary treatments (Table 25, sampling time 3 hr). The observed NH_3-N values for experimental diet 2, 3 and 4 were lower than the range of optimum NH_3-N level (0.09–0.21 mmol/L) that favours the ruminal microbial activity in animals fed with materials rich in lignocellulose (Nur Atikah et al., 2018). However, rumen NH_3-N values of experimental diet 1 was in the given range. The mean total VFA, AA, PA and BA were ranged from 99.39–108.3, 74.73–76.88, 16.96–21.60 and 7.72–10.49 mmol/L respectively (Table 24). The VFA concentration of the rumen was limited to acetic acid, propionic acid and butyric acid. Other investigated VFAs such as caproic and valeric acid concentration was lower and below detectable level (< 0.1 mmol/L). The total VFA and AA values were also higher than that of total VFA (92.80 mmol/L) and AA (48.60 mmol/L) of low roughage diets (Sutton et al., 2003). However, the PA values were lower than the PA (36.60 mmol/L) value of low roughage diets, additionally the BA values of experimental diets were lower than BA (11.00 mmol/L) and BA (8.80 mmol/L) of normal and low roughage diets, respectively.

Table 24. The rumen fermentation characteristics of control and experimental diets at different dietary treatments (n=36/treatment)

	Treatments						p value
Components	**Control**	**Exp. diet 1**	**Exp. diet 2**	**Exp. diet 3**	**Exp. diet 4**	**SEM**	**Treatment effect**
pH	6.98	6.94	7.06	6.98	6.93	0.27	ns
NH_3 -N (mmol/L)	0.09	0.10	0.08	0.07	0.06	12.50	ns
Total VFA (mmol/L)	103.7	103.1	99.3	103.1	108.3	17.10	ns
Acetate (mmol/L)	76.97	76.08	74.73	76.88	76.23	10.83	ns
Propionate (mmol/L)	17.36	17.45	16.96	17.14	21.60	4.50	ns
Butyrate (mmol/L)	9.36	9.61	7.72	9.08	10.49	2.29	ns

Control: 5.5 kg day^{-1} of corn silage, 3.5 kg day^{-1} of alfalfa haylage, 3.5 kg day^{-1} of vetch-triticale haylage, 3 kg day^{-1} of concentrate, 1 kg day^{-1} of grass hay and 0.75 kg day^{-1} of liquid molasse; *Experimental 1*: Control diet + mixture A (40% of two cultivars of winter triticale + 30% of two cultivars of winter oats + 20% of winter barley + 10% of winter wheat) silage (3.5 kg day^{-1}, instead of vetch-triticale haylage); *Experimental 2*: Control diet + mixture B (50% of two cultivars of winter triticale + 40% of winter barley + 10% of winter wheat) silage (3.5 kg day^{-1}, instead of vetch-triticale haylage); *Experimental 3*: Control diet + mixture C (55% of three types of Italian ryegrass + 45% of two cultivars of winter oat) silage (3.5 kg day^{-1}, instead of vetch-triticale haylage); *Experimental 4*: Control diet + mixture D (40% of three types of Italian ryegrass + 30% of two cultivars of winter oat + 15% of two cultivars of winter triticale + 10% of winter barley + 5% of winter wheat) silage (3.5 kg day^{-1}, instead of vetch-triticale haylage)

ns=not significant

Table 25. The rumen fermentation characteristics of control and experimental diets at different sampling times (n=36/treatment)

Sampling time	**Treatments**						
0 hour							
	Control	**Exp. diet 1**	**Exp. diet 2**	**Exp. diet 3**	**Exp. diet 4**	**SEM**	**p value**
pH	7.33	7.31	7.28	7.26	7.23	0.107	ns
NH_3 -N (mmol/L)	0.06	0.07	0.05	0.06	0.07	2.443	ns
Total VFA (mmol/L)	88.86	85.54	83.82	84.75	83.03	5.852	ns
Acetate (mmol/L)	66.92	64.32	64.18	64.25	62.85	5.126	ns
Propionate (mmol/L)	14.33	14.01	13.59	13.51	13.27	0.830	ns
Butyrate (mmol/L)	7.10	7.20	5.88	6.91	6.89	0.774	ns
3 hours (after feeding)							
pH	6.78	6.75	6.90	6.78	6.81	0.106	ns
NH_3 -N (mmol/L)	0.17 ^{ab}	0.21 ^{a}	0.17 ^{ab}	0.15 ^{ab}	0.13 ^{b}	5.925	< 0.05
Total VFA (mmol/L)	114.6	113.7	116.6	117.2	123.9	7.458	ns
Acetate (mmol/L)	83.85	81.07	86.34	86.27	87.16	6.401	ns
Propionate (mmol/L)	19.93	20.05	20.99	20.33	23.26	1.737	ns
Butyrate (mmol/L)	10.83 ^{b}	11.12 ^{b}	9.29 ^{b}	10.59 ^{b}	13.58 ^{a}	0.850	< 0.001
6 hours (after feeding)							
pH	6.83	6.77	6.94	6.91	7.41	0.707	ns
NH_3 -N (mmol/L)	0.05	0.05	0.03	0.03	0.02	3.964	ns
Total VFA (mmol/L)	108.1	110.2	97.72	107.4	117.9	12.682	ns
Acetate (mmol/L)	80.12	81.34	73.49	80.11	78.66	7.766	ns
Propionate (mmol/L)	17.84	18.30	16.22	17.55	28.29	8.880	ns
Butyrate (mmol/L)	10.15	10.53	8.00	9.76	10.99	1.409	ns

Control: 5.5 kg day^{-1} of corn silage, 3.5 kg day^{-1} of alfalfa haylage, 3.5 kg day^{-1} of vetch-triticale haylage, 3 kg day^{-1} of concentrate, 1 kg day^{-1} of grass hay and 0.75 kg day^{-1} of liquid molasse; *Experimental 1*: Control diet + mixture A (40% of two cultivars of winter triticale + 30% of two cultivars of winter oats + 20% of winter barley + 10% of winter wheat) silage (3.5 kg day^{-1}, instead of vetch-triticale haylage); *Experimental 2*: Control diet + mixture B (50% of two cultivars of winter triticale + 40% of winter barley + 10% of winter wheat) silage (3.5 kg day^{-1}, instead of vetch-triticale haylage); *Experimental 3*: Control diet + mixture C (55% of three types of Italian ryegrass + 45% of two cultivars of winter oat) silage (3.5 kg day^{-1}, instead of vetch-triticale haylage); *Experimental 4*: Control diet + mixture D (40% of three types of Italian ryegrass + 30% of two cultivars of winter oat + 15% of two cultivars of winter triticale + 10% of winter barley + 5% of winter wheat) silage (3.5 kg day^{-1}, instead of vetch-triticale haylage)

ns=not significant

6.6. Aroma profiling

In the knowledge driven phase of the EN data analysis, the retention indices of the major volatile compounds were identified using pure chemicals. The RIs of ethanol, AA and LA were 465, 569 and 439 on column MXT-5, and 565, 798 and 492 on column MXT-1701, respectively. The chromatograms of the silages fermented for 90 days were analyzed at these RIs. Figure 7 shows the measured intensities of the replicate samples of the four different mixture silages. The applied EN system is very sensitive on ethanol, thus, ethanol gave dominant peaks on both columns (Figure 7a). Figure 7b shows the zoomed image of the bar graph, where the intensities for AA and LA can be seen.

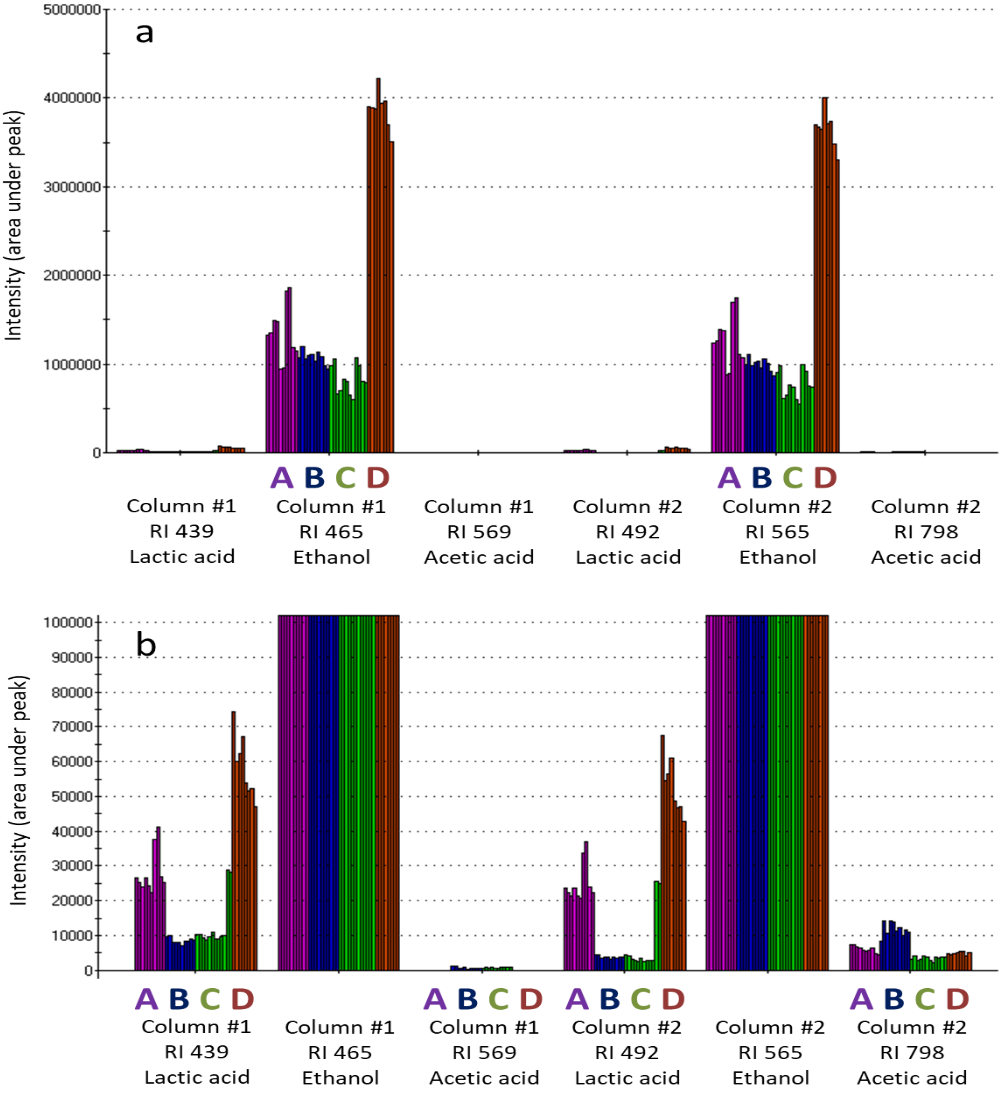


Figure 7. Bar graphs of the intensities measured with the electronic nose at the retention indices (RI) corresponding to lactic acid, acetic acid and ethanol for the ensiled mixtures at day 90, with the indication of mixture type (A, B, C, D), GC column type (#1: Restek MXT-5; #2: Restek MXT-1701), retention indices and respective chemicals. (a) showing intensity values corresponding to ethanol; (b) as a zoomed image of (a) to overcome magnitude differences showing intensity corresponding to acetic acid and lactic acid

mixture A: 40% of two cultivars of winter triticale + 30% of two cultivars of winter oats + 20% of winter barley + 10% of winter wheat; mixture B: 50% of two cultivars of winter triticale + 40% of winter barley + 10% of winter wheat; mixture C: 55% of three types of Italian ryegrass + 45% of two cultivars of winter oat; mixture D: 40% of three types of Italian ryegrass + 30% of two cultivars of winter oat + 15% of two cultivars of winter triticale + 10% of winter barley + 5% of winter wheat.

6.6.1. Comparing the aroma profiles of all mixtures at all stages

The EN chromatogram peaks used as sensor signals described the aroma profiles of the four mixtures at the fresh and three fermented stages. The PCA of the multivariate data showed repeatability of the aroma profiling of the 16 groups (Figure 8). The different types (mixtures A, B, C and D) of the freshly harvested samples formed one group with little variation along PC1 and PC2 describing 99.87% of the total variance of the sensor signals. The early stages of the fermentation (Day 7 and 14) resulted in an increase of the variation of the aroma profiles. However, all mixtures showed similar changes, since there was no clear separation of the mixture groups found at 7 or 14 days of the fermentation. The PCA performed with all the 16 groups was dominated by the aroma variation caused by the 90 days of fermentation. At this stage, there was a clear effect of the mixture type on the detected aroma profile. Mixture D had the most unique aroma. Its difference from the other mixtures at the 90-day fermentation stage or at any previous stages is described by the first principal component (PC1) which cover 99.56% of the total variance of the sensor signals. Comparingly, mixtures A, B and C on day 90 are more similar, showing a distribution along PC2, containing 0.31% of the total variance. Mixture C changed the least until the 90th day of the fermentation as its aroma profile remained similar to that of the previous stages. Mixture A and B changed more, but differently than mixture D, because the direction of the change in the PCA score plot if orthogonal, i.e. the samples of mixtures A and B on day 90 are different from samples on day 14 and the difference is caused by the aroma signals described by PC2. The influence of the composition of the different mixtures on the odour profile is nicely shown in fresh samples and in any stages of the fermentation by the very high ratio (≥95%) of correctly classified samples in the cross-validations of the classification models. In both types of classifications, (1) according to the sampling days of a single mixture, or (2) according to the mixture types on a single sampling day, each group was represented by five samples prepared in five different experimental silos. Since the samples of the different silos united in most of the classifications, it is nicely demonstrated that both the fermentation stages and the mixture types cause reproducible odour differences of the silages. As the e-nose measurements of all samples were performed randomly, it is highly unlikely that the clustering's were the result of some sample misrepresentations or systematic sample handling protocols.

The sensors identified as most influential in the classifications are collected in Table 26, with the indication of the possible volatile molecules causing the respective chromatogram peak, assigned in the AroChemBase database of retention indices of volatile chemicals. Effects of VOCs as markers of variation in silage fermentation quality in the voluntary feed intake of cattle was studied by Huhtanen et al. (2002). Muck (1998) confirmed that, the main components responsible for the characteristic smell of silages are Short chain fatty acids that evaporate quite easily when introduced to air. Apart from organic acids, other VOCs should be mentioned. Ethanol is obviously associated with an alcohol smell. The gradual change of mixture D during the fermentation (Figure 10g) is described by four sensors with retention indices of 600-1A, 492-2A, 639-2A, 670-2A. The difference of the mixtures A and B vs. mixtures C and D described in the initial stage (day 0, Figure 11a) is influenced by four sensors. Mixtures A and B are rich in volatiles at retention indices of 658-1A, 1046-1A, 1555-2A, while mixtures C is rich in volatiles at retention index 991-1A. The considerably different odour of mixture C on days 7 and 14 (Figure 12c, e) is caused by volatiles at retention index 506-2A. On day 90, Mixture B differed from the other samples based on its richness in volatiles at retention index of 1189-1A, while the unique odour of mixture D was dominated by volatiles at retention indexes of 960-1A, 541-2A, 951-2A.

Table 26. Identification of the possible chemical components responsible for the signals at the retention indices appeared in the various classification approaches

Retention index	Column	Appearance (in classification of)	1st identified volatile compound	2nd identified volatile compound	3rd identified volatile compound	4th identified volatile compound
489	1A	Day 7	diethyl ether	2-methyl-2-propanol	2-methyl-1-butene	3-chloropropene
600	1A	mixture D, Day 7	Hexane	di-isopropyl ether	2-butanol	2-methylfuran
658	1A	Day 0, Day 14	methyl butanone	1,1-dicholopropene	2-methylbutanal	tert-amylmethylether
711	1A	mixture C	ethylene glycol	propyl acetate	Acetoin	Benyotrifluoride
725	1A	mixture B, mixture C	diethoxy-1,1-ethane	2,2,3-trimethylpentane	3-methyl-3-buten-1-ol	3-penten-2-one
736	1A	mixture A	Thiazole	3-methyl-1-butanol	4-methyl-2-pentanone	Pyrazine
748	1A	mixture A	propionic acid	ethane dioic acid	dimethyl disulphide	isopropyl propanoate
801	1A	Day 7, Day 14	2-hexanol	Hexanal	3-hexanol	Octane
858	1A	mixture C	2,3-dimethylheptane	1,3-propanedithiol	3-methylbutanoic acid	methylthio-2-propanone
960	1A	Day 90	ethyl 3-	isopropyl 2-	2-heptanal	1-ethyl-3-methylbenzene
991	1A	Day 0	butyl butanoate	ethyl hexanoate	hexanoic acid	Trimethyl pyrazine
1046	1A	mixture C, Day 0	Limonene	Benzene acetaldehyde	Cineole	2-methyl-phenol
1189	1A	Day 90	methyl acetophenone	octanoic acid	ethyl octanoate	
492	2A	mixture B, mixture D	Acetaldehyde			
506	2A	Day 7, Day 14	Acetaldehyde			
541	2A	Day 90	Ethanol	Propenal		
600	2A	mixture A, mixture B	formic acid	2-propanol	Propanal	2-methylpropanal
639	2A	mixture D	2-methylpropanal	Butanal		
670	2A	mixture D	Butanal	ethyl acetate	butane2-one	butane-2,3-dione
698	2A	mixture A	butan-2-one	butane-2,3-dione		
744	2A	mixture B, Day 7	2-methyl-1-propanol	3-methylbutanal	ethyl propanoate	isopropyl acetate

869	2A	Day 14	propyl propanoate	ethyl butyrate	butyl acetate	propionic acid
951	2A	Day 90	Pirene	isoamyl acetate	propylene glycol	
1070	2A	mixture C	isovaleric acid	butyl butanoate	Cymene	Limonene
1101	2A	mixture C	Octanal	trimethyl pyrazine	alpha-terpinene	
1206	2A	Day 90	Acetophenone	Nonanal	ethyl-3-	
1366	2A	Day 0	ethylnonanoate	ethyl phenylacetate	Citronellol	phenylethyl acetate
1555	2A	Day 0	pentyl octanoate	methyl cinnamate	Indole	Eugenol

6.6.2. Comparing the aroma profiles of single mixtures at all stages

When the PCA was performed for each mixture, separately, then different variations of the dataset were highlighted (Figure 9). The patterns of aroma changing during the fermentation process is different in the different mixtures. Mixture A is very stable at the beginning, then, its samples on day 90 are very much different from the previous three stages, and more heterogeneous. Days 7 and 14 of mixture B are very heterogeneous but mostly similar, and these stages are different from the homogeneously unique aromas of the initial and end stages. In some aroma properties, day 90 is similar to day 0, since both are positioned in the same region along PC1 that is describing the major variance of the sensor signals. In mixture C, the initially homogeneous forage gets heterogenous by days 7 and 14 of fermentation. The aroma at these stages is highly similar. Samples of day 90 are different again, however, remain heterogenous. The initial stage of mixture D is very homogenous, and day 7 already differs significantly. The fermentation causes small change in the odour by day 14, but day 90 is appearing as a separate group, showing different odour pattern compared to the previous stages.

These changes can also be seen in the LDA graphs of Figure 10 prepared with the sensors most significant in the supervised classification of the given groups. Based on the cross-validation results of the LDA, the samples of days 7 and 14 have very similar odour in mixtures A, B. In mixtures C and D all the 4 days are separated as 93% and 95% of the samples were correctly identified in the cross-validation, respectively. Based on the LDA graphs, the aroma of mixtures A, B, and C changes differently in the first part of fermentation than in the final part, because the direction of day 0, day 7 and day 14 is different from that of day 14 and day 90. Mixture D is the only silage having a continuous change, as the samples of the different sampling days appear separately along one principal component (PC1). The results of LDA are similar, but somewhat different from

PCA, which difference arises from the fact that LDA is to find differences among the pre-defined groups, while PCA describes the variance of the sensor signals not regarding the group identity, thus, it does not work against the existing similarities of the groups. The LDA classifications were performed with the sensor selection approach of the AlphaSoft program. The right graphs (b, d, f, h) of Figures 10 show loadings of the LDA for mixtures A, B, C, and D. The loadings indicate the influence of the selected sensors on the linear discriminant factors defining the LDA planes in which we see the separation of the groups in the left graphs (a, c, e, g).

6.6.3. Comparing the aroma profiles of all mixtures at single stages

Figure 11 shows the results of PCA when data of the fermentation stages were used and the separation of the four mixtures are indicated. Mixtures A and B are very similar on day 0 and day 7, while all mixtures are similarly different on day 14. On day 90, mixtures A and B are similar again, and mixture C is also similar to these. Mixture D is different from all others at the final stage. The biggest different is seen between mixtures D and C, although in the previous stages the closest neighbour of mixture D was mixture C.

The results of the LDA to classify mixtures at each sampling time highlight some further differences (Figure 12). At the initial stage, each mixture can be identified, but there is a considerable difference between the winter cereals (mixtures A and B) and IRG plus winter cereals mixtures (mixture C and D). This difference is described by the most influential discriminant factor (DF1) which is dominated by the selected sensors representing high absolute values along the DF1 axis in Figure 4b. After one week of the fermentation, mixture C shows a very unique odour, and it is different from the rest of the samples along DF1. Mixtures A, B, and D align on the DF2. At day 14, the odour of mixture C remains unique, but the difference of the other mixtures is also increasing. At day 90, the difference already recognized in the initial stage can be seen along

DF2, as winter cereals (mixtures A and B) take high positive values, and IRG plus winter cereals mixtures (mixture C and D) take low negative values along this axis. The odour profiles of the two winter cereals mixtures are very similar (mixtures A and B are close in Figure 12g), but the odour profiles of the two IRG plus winter cereals mixtures are highly different from one another (mixtures C and D are far in Figure 12g).

Some identified volatile compounds (Table 26) have significant importance in silage odour characterization. Volatile compounds which are esters or have esterification potential may give pleasant odour outcomes in silages. Esters often also have characteristic smells because esters known to be odorants, they could affect the taste of silage and, consequently, feed intake (Campagnoli and Dell'Orto, 2013). Some authors consider esters more important than organic acids in defining the odour of ensiled mass. Mo et al. (2001) and Kristensen et al. (2010) expected esters to contribute to silage flavour due to their volatility. Furthermore, many esters have low odour thresholds and thus are perceived at concentrations of parts per million. The most abundant esters in silages include ethyl butyrate, acetate and propionate (Krizsan et al., 2007). Figueiredo et al. (2007) reported that ethyl esters being the predominant subclass of all esters and the most abundant class of VOCs in red clover silages. Mo et al. (2001) reported that ethyl esters are most abundant in grass silage. Ethyl propanoate at retention index 744-2A, characteristic in mixture B on day 14 (Figure 10c,d), and in mixture D on day 7 (Figure 12c,d), is an ethyl ester of propionic acid, ethyl butyrate at retention index 869-2A, characteristic in mixture B on day 14 (Figure 12e,f), is an ester formed from butyric acid and ethanol, and ethyl octanoate at retention index 1189-1A is an ester formed from caprylic acid and ethanol, and found to be characteristic for mixture B on day 90 (Figure 12g,h). These volatiles would likely produce pleasant fruity odours which could increase feed intake (Arena et al., 2006). Campagnoli and Dell'Orto (2013) also reported that ethyl lactate, which is characterized by a creamy odour with hints of fruit, has a weak negative influence on DMI. However, some off odour compounds were also identified is some silages. These were 3-methylbutanoic acid also called beta-methylbutyric acid (Morgan and Pereira, 1962), a branched chain alkyl carboxylic and 2-methyl-2-propanol, a simple alcohol with unpleasant camphor-like odour (Cometto-Muñiz and Cain, 1993) were found in fermented mixture C samples at retention index 858-1A (Figure 10e,f) and on day 7 in mixture A at retention index 489-1A (Figure 12c,d). The compounds with potential off odour formation may likely reduce the silage feed intake if they are found in significant amounts. Some authors have observed that ethyl acetate and ethyl lactate show a strong correlation with ethanol in fresh and well-fermented silages (Campagnoli and Dell'Orto, 2013) were found in fermented mixture D (Figure 8) at retention index of 670-2A and fermented samples of mixture A and D at retention index of 1189-1A, 541-2A, 1366-2A (Figure 11a,d).

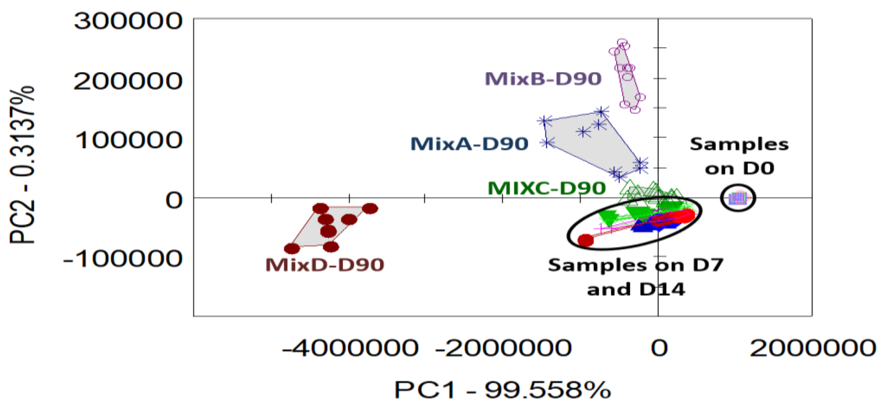


Figure 8: PCA score plot of the aroma profile of all (4) mixtures (mixture A, B, C and D) measured on all (4) days (Day 0, 7, 14, 90)

mixture A: 40% of two cultivars of winter triticale + 30% of two cultivars of winter oats + 20% of winter barley + 10% of winter wheat; mixture B: 50% of two cultivars of winter triticale + 40% of winter barley + 10% of winter wheat; mixture C: 55% of three types of Italian ryegrass + 45% of two cultivars of winter oat; mixture D: 40% of three types of Italian ryegrass + 30% of two cultivars of winter oat + 15% of two cultivars of winter triticale + 10% of winter barley + 5% of winter wheat.

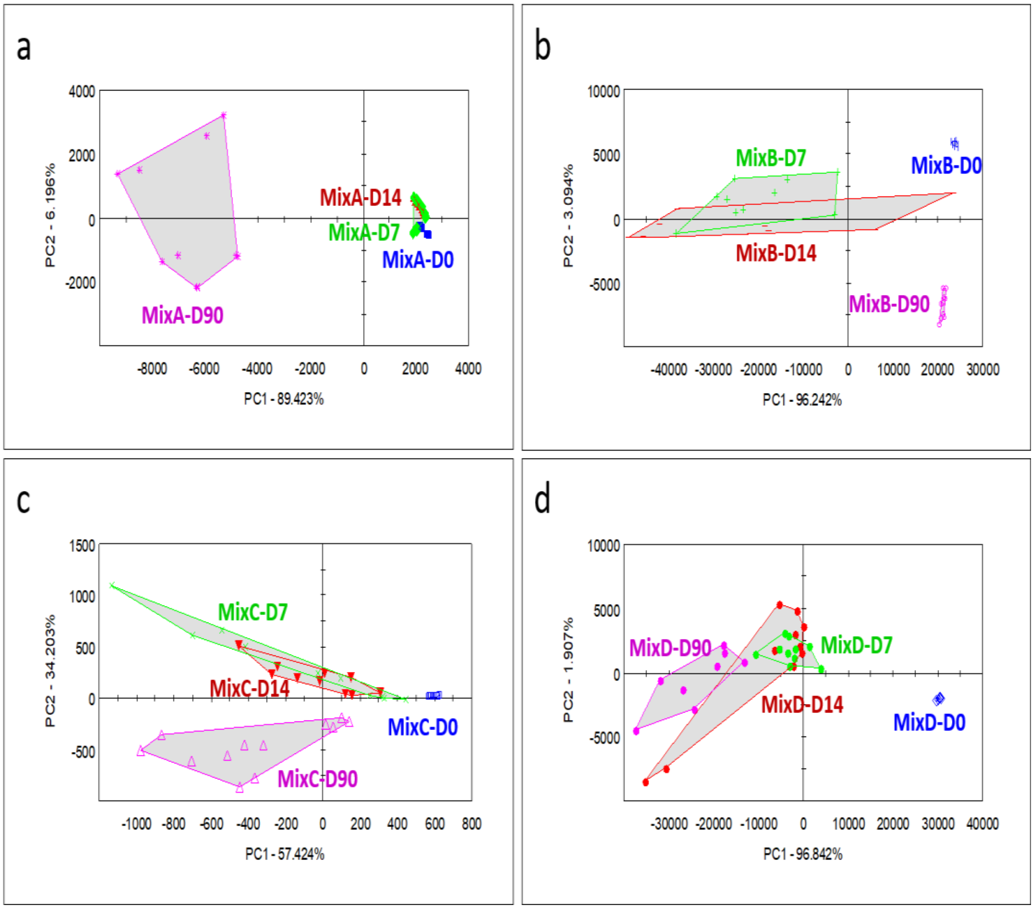


Figure 9: PCA score plots calculated from the aroma profiles of mixture A (a), mixture B (b), mixture C (c) and mixture D (d), indicating the fermentation days (Day 0, 7, 14, 90) within each plot

mixture A: 40% of two cultivars of winter triticale + 30% of two cultivars of winter oats + 20% of winter barley + 10% of winter wheat; mixture B: 50% of two cultivars of winter triticale + 40% of winter barley + 10% of winter wheat; mixture C: 55% of three types of Italian ryegrass + 45% of two cultivars of winter oat; mixture D: 40% of three types of Italian ryegrass + 30% of two cultivars of winter oat + 15% of two cultivars of winter triticale + 10% of winter barley + 5% of winter wheat.

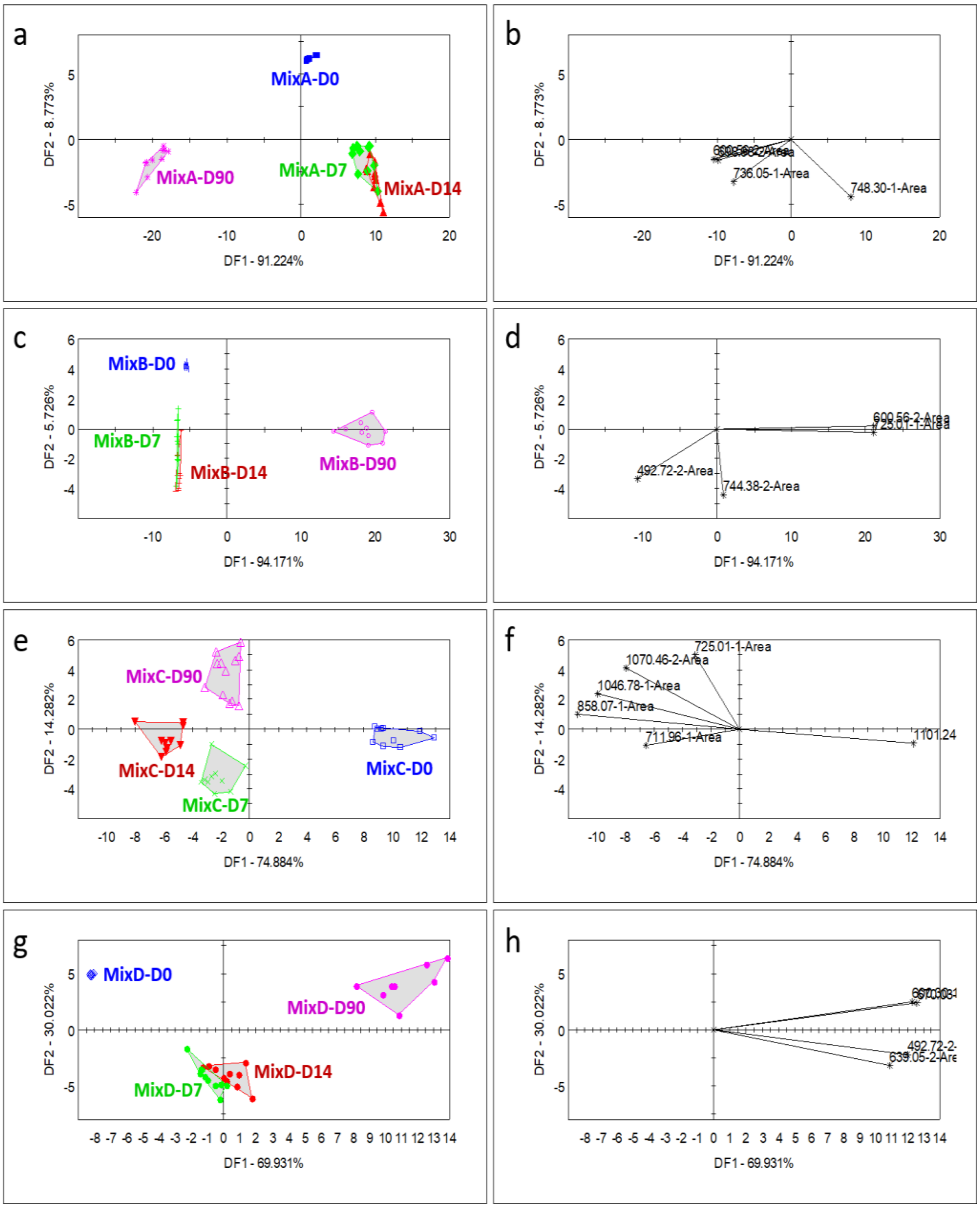


Figure 10: LDA classifications of the fermentation stages (Day 0, 7, 14, 90) within the different silage mixtures (a: mixture A; c: mixture B; e: mixture C; g: mixture D), and the loading plots (b, d, f, h) showing the impact of the selected dominant sensors on the relevant discriminant factors

mixture A: 40% of two cultivars of winter triticale + 30% of two cultivars of winter oats + 20% of winter barley + 10% of winter wheat; mixture B: 50% of two cultivars of winter triticale + 40% of winter barley + 10% of winter wheat; mixture C: 55% of three types of Italian ryegrass + 45% of two cultivars of winter oat; mixture D: 40% of three types of Italian ryegrass + 30% of two cultivars of winter oat + 15% of two cultivars of winter triticale + 10% of winter barley + 5% of winter wheat.

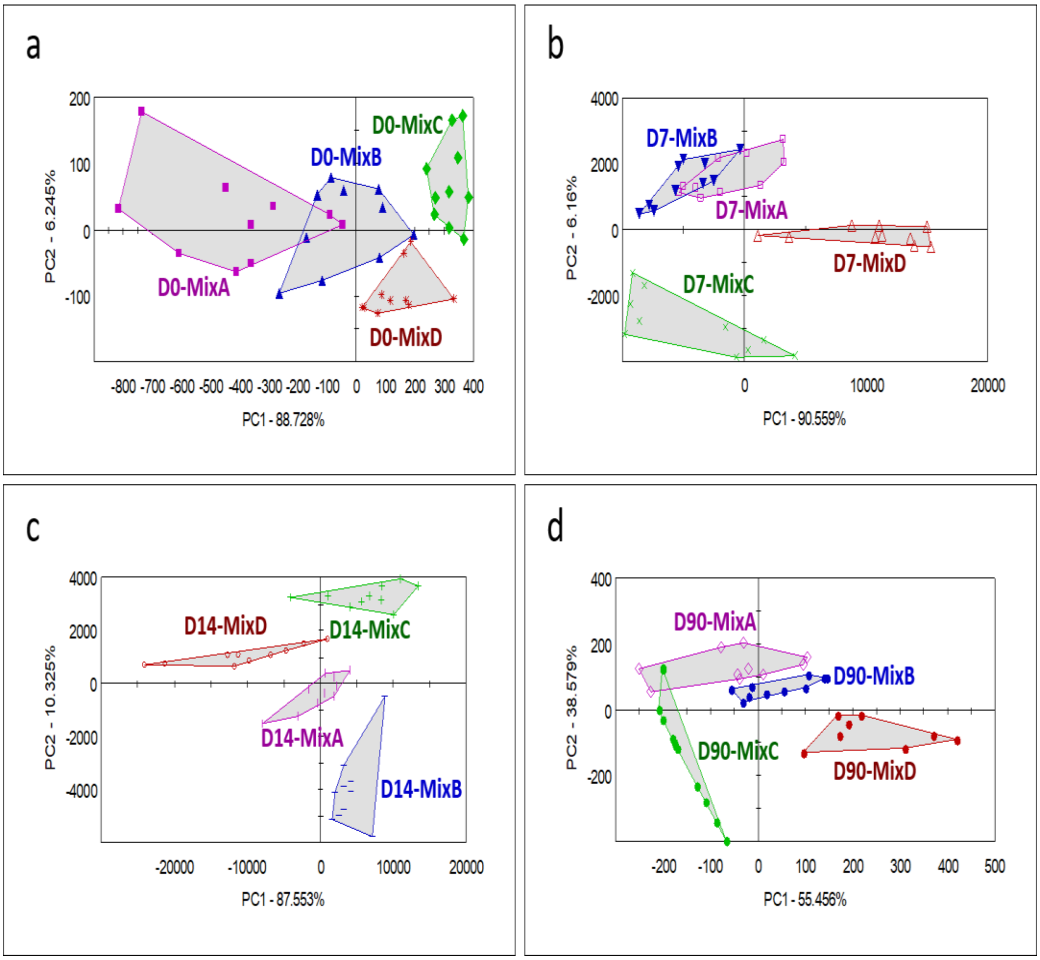


Figure 11: PCA score plots calculated from the aroma profiles of the silages at different fermentation stages (a: Day 0; b: Day 7; c: Day 14; d: Day 90), indicating the type of mixture (mixture A, B, C and D) within each plot

mixture A: 40% of two cultivars of winter triticale + 30% of two cultivars of winter oats + 20% of winter barley + 10% of winter wheat; mixture B: 50% of two cultivars of winter triticale + 40% of winter barley + 10% of winter wheat; mixture C: 55% of three types of Italian ryegrass + 45% of two cultivars of winter oat; mixture D: 40% of three types of Italian ryegrass + 30% of two cultivars of winter oat + 15% of two cultivars of winter triticale + 10% of winter barley + 5% of winter wheat.

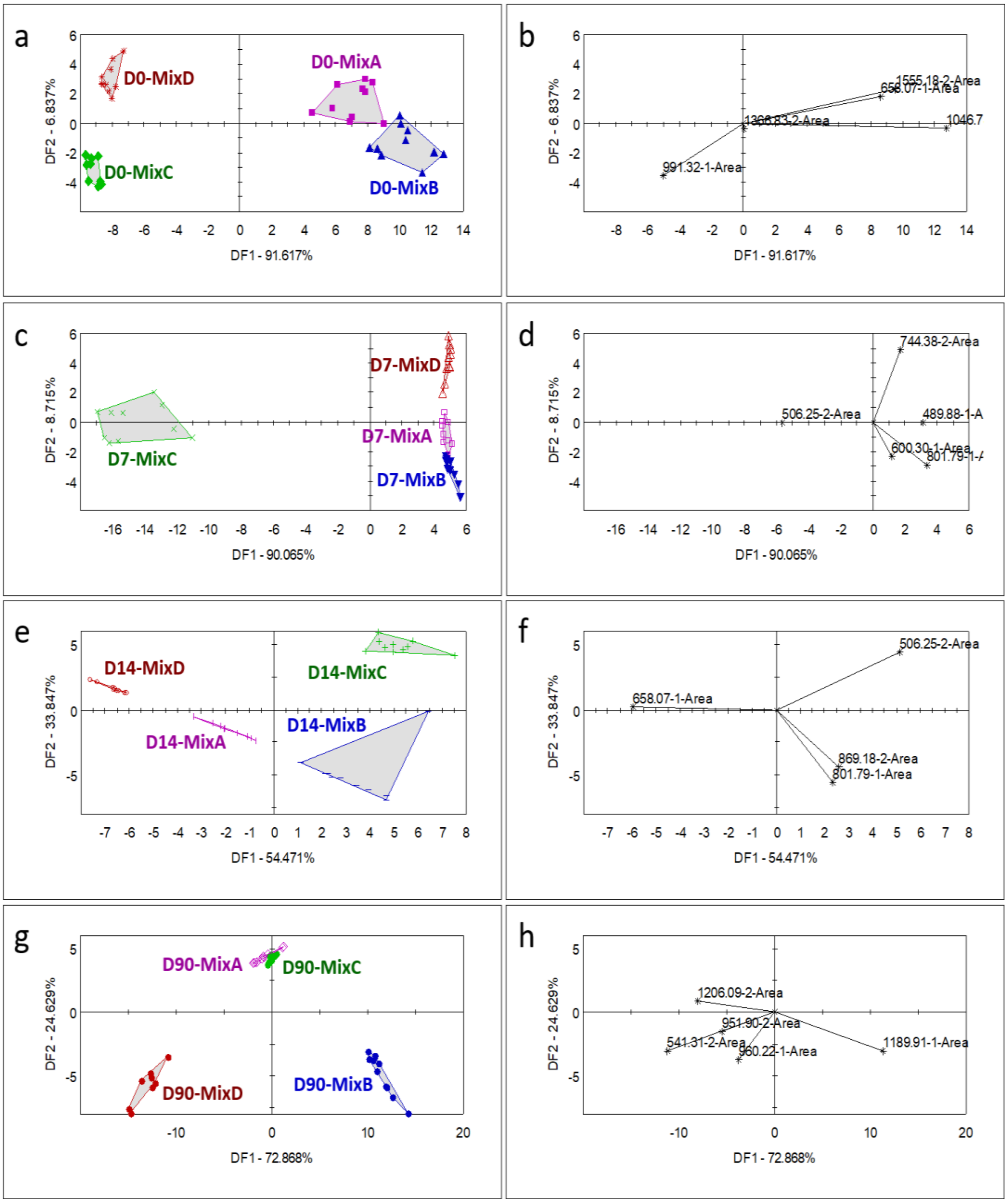


Figure 12: LDA classifications of the types of silages (mixture A, B, C and D) at the different fermentation stages (a: Day 0; c: Day 7; e: Day 14; g: Day 90), and the loading plots (b, d, f, h) showing the impact of the selected dominant sensors on the relevant discriminant factors

mixture A: 40% of two cultivars of winter triticale + 30% of two cultivars of winter oats + 20% of winter barley + 10% of winter wheat; mixture B: 50% of two cultivars of winter triticale + 40% of winter barley + 10% of winter wheat; mixture C: 55% of three types of Italian ryegrass + 45% of two cultivars of winter oat; mixture D: 40% of three types of Italian ryegrass + 30% of two cultivars of winter oat + 15% of two cultivars of winter triticale + 10% of winter barley + 5% of winter wheat.

7. CONCLUSIONS AND RECOMMONDATIONS

In experiment I the ensiled Italian ryegrass and winter cereals mixtures produces silage with an excellent feed characteristic, digestibility, degradability and energy contents. With proper stage of harvesting (early heading), Italian ryegrass and winter cereal mixtures were fermented well. Lactic acid was the dominant fermentation acid produced (>72% of the total acids) resulting the ensiled mixtures to underwent lactic acid fermentation type. This high concentration of lactic acid was most effective in lowering pH, and helps reduce protein breakdown as well as strongly restricts the production of undesirable VFAs. The efficient fermentation improves the recovery of most nutrients and reduces the NH_3-N and ethanol production. As a result, the produced ensiled mixtures had well preserved crude protein, moderate NDF and ADF content, and high effective protein degradability potentially degradable NDF fraction. The high potentially degradable fiber fractions can be applied in the diet may increase diet NDF rumen degradability having positive effect on dry matter intake and performance of the dairy cow. The ensiled mixtures had also good apparent nutrient digestibility attributed to proper stage of harvesting as well as inclusion of more Italian ryegrass and improved the energy concentration (NE_l, NEm and NEg) of ensiled mixture. Generally, the inclusion of 40% IRG in cereal mixtures produced well fermented silage with excellent feed value; therefore 40% inclusion of IRG can be taken as a maximum threshold level otherwise more inclusion of IRG (up to 55%) could not cause further significant quality advantages. The results also imply that the ensiled mixtures can be ensiled without silage additives under practical condition and included in dairy cattle diets at a suitable level to replace partially other components (e.g. corn silage, alfalfa haylage). It further noted that due to good apparent nutrient digestibility particularly NDF, NDF and ADF degradability as well as high energy concentration; the ensiled mixture can be included in the nutrition of high producing dairy cows and complete the forage sources of a country. It also important to notice that the mixtures are autumn sowing and early harvest forages, therefore it can be well integrated into crop rotation, double cropping can also be done with short-growing corn, combined with sorghum or Sudan grass. However, further studies are needed to confirm the effect of the mixture of Italian ryegrass and winter cereals on performance of dairy cows and on the composition of milk. In general, in areas where little autumn/spring precipitation, mixture A' (40% IRG + 60% winter cereals) can be recommended; and with abundant rainfall mixture B' (55% IRG + 45% winter cereals) recommended. Otherwise both mixtures can be recommended in different agronomic condition particularly in area with feed shortage and high livestock density.

In experiment II, the fermentation process underwent lactic acid fermentation type for all ensiled mixtures. However, restricted fermentation affected the output of some fermentation end products with lower lactate production as a result pH was not dropped as rapid as possible. Both the opening days and the mixture types cause reproducible odour differences in the ensiled mixtures. The ensiled mixtures both at day 0 and different opening days, expressed its richness in VOCs at different retention indexes. Most of those VOCs was dominated by esters and have esterification potential compounds which give pleasant odour outcomes consequently contribute to silage flavour due to their volatility. Ethyl esters which is a product of an ester formed from Short chain fatty acids and ethanol are the most abundant esters in many silages was reported in the present ensiled mixtures due to the presence of desirable SCFAs and ethanol. These volatiles would likely produce pleasant fruity odours which has no negative effect on silage intake and could increase the intake of ensiled mixtures. However, some off-odour compounds like 3-methylbutanoic acid also called beta-methylbutyric acid, a branched chain alkyl carboxylic and 2-methyl-2-propanol, a simple alcohol with unpleasant camphor-like odour found in fermented mixture C (Italian ryegrass plus winter cereals mixtures) may likely reduce its intake.

The ensiled mixtures had high effective degradable DM and CP at the three rumen outflow rates (ED_1, ED_5 and ED_8) and moderate potentially degradable DM and CP. The 40 – 55% inclusion of Italian ryegrass on cereal grain (mixture C and D) caused higher effective degradability (ED8) of DM and CP (except mixture A) and lower NDF and ADF (except mixture C) degradability (ED8) over winter cereal mixture silages (mixture A and B). The replacement of ensiled mixtures with vetch – triticale haylage in TMR did not cause any deleterious effect on rumen environment as pH remain similar with control diets. In general, the ensiled mixtures had comparable feeding value as well as in situ degradable and ruminal fermentation quality. The inclusion of 40-55% IRG in mixture C and D did not cause any significant quality and performance advantages over pure winter cereal mixtures (mixture A and B). This implies that as far as the mixture crops harvested at proper stage (early heading) it has good fermentable characteristics; and feeding value. However restricted fermentation affects the output of fermentation products such as medium LA and higher ethanol content. Therefore, the use of silage additives such as Lactobacillus bacteria (LAB) recommended in future use practical use. Inclusion of winter cereal mixtures and winter cereals and Italian ryegrass mixtures in dairy cow ration had no effect on rumen fermentation parameters. Therefore, when formulating cattle feed, special attention should be paid to the tested nutrients and ruminal degradability data available for the mixture used. Further experiments should be performed to improve the practical use of the novel mixtures of winter cereals and Italian ryegrass plus winter cereal-based silages. The optimal proportions of winter cereals (60-45 %) plus Italian ryegrass (40-55% IRG) mixtures as well as pure winter cereal mixtures used in present mixtures can be established in future use. Electronic nose as applied in this study could be a practically useful rapid analytical technology to characterize fermentation, identify and quantify the most abundant volatile compounds of silages, giving a good description of the sensible smell. The technology also gives the possibility to identify the characteristics of false fermentation processes rapidly. Feeding dose field trials should be performed to determine the effects of the mixtures on the production parameters.

8. NEW SCIENTIFIC RESULTS

1. Nutritive values (NE_m, NE_l, NE_g MPN and MPE) in the combination of Italian ryegrass and winter cereals (triticale oat, wheat and barley) silages tested in our experiments were higher than the values reported for these silages alone in NRC (2001).
2. The detailed analysis of chemical composition, nutritive value and fermentation characteristics of silages of Italian ryegrass and winter cereals in comparison with winter cereal mixtures support that this feedstuff can be successfully included in the ruminant feeding. According to the results of *in vivo* digestibility trial Italian ryegrass and winter cereal mixture (associated with harvesting at early heading stage) has high digestibility and nutritive value and would be a good option for ruminant.
3. An inclusion of about 40-45% of Italian ryegrass causes higher effective degradability of dry matter and crude protein than cereal crop mixture silages alone tested at 8 % of rumen content flow rate.
4. Inclusion of winter cereal mixtures and winter cereals and Italian ryegrass mixtures in dairy cow ration had no effect on rumen fermentation parameters.
5. The opening days and the mixture types of silages cause reproducible odour differences in the ensiled mixtures. Silages made from different combination mixtures of Italian ryegrass and winter cereals expresses richness in volatile organic compounds (VOCs) at different retention indexes. Most of those VOCs were dominated by esters and have esterification potential which give pleasant odour outcomes consequently contribute to silage flavour due to their volatility. As demonstrated by these results, the applied electronic nose technology is a useful tool to describe the quality of ensiled forages. The technology may be used in practical applications to identify defects or preferred smell for certain reasons.

9. SUMMARY

Poor climatic conditions such as drought, high summer heat waves, ground water shortage and mycotoxin contamination increase the risk of corn crop failures across the world's leading corn-growing regions. This situation is currently altering the crop cultivation practices in many areas of the world. Study report on sensitivity of silage corn to climate change reveals that, management practices in relation to silage corn production governed by the current climate and local conditions must change considerably by the warming climate. It is urgent now to consider how crop production and feeding strategies can be adapted to this change in long term, taking into account the nutrient requirements of high producing lactating cows. Accordingly, interest in new alternative forage supplying technologies has increased in recent years. However, finding and robust application of acceptable alternative silage to replace corn silage is still critical issue to the success of future dairy industry. Options like using new forage corn hybrids, new irrigation systems (such as sprinkler and drip water) in areas where shortage of water, partial replacement/changes of corn silage preparation in the diet (using whole dwarf and brown mid rib sorghum, corn plus sorghum silage mixes, winter-type early harvested cereals like rye and triticale), intensive annual and perennial grasses, winter-type cereal plus legume mixtures (barley plus pea, wheat plus pea and triticale plus pea) and winter-type cereals plus grass mixtures (wheat, oats, triticale and winter barley plus e.g. Italian ryegrass) are among the potential ones.

In the present study six ensiled mixtures (4 Italian ryegrass and cereal grain as well as 2 winter cereal mixtures) were tested at two different phases of experiment. In the first phase of the experimental a study of two mixtures of Italian ryegrass and winter cereals were examined to evaluate the nutritional composition, fermentation characteristics, microbial count, digestibility, energy and protein quality and ruminal degradability.

As a general summary, both ensiled mixtures of forages produced silage with excellent feed characteristics. With proper stage of harvesting (early heading), Italian ryegrass and winter cereal mixtures were fermented well. High crude protein, moderate NDF and ADF content, and high effective protein degradability, potentially degradable of NDF fraction implies that these mixtures could be included in dairy cattle diets. The high potentially degradable fiber fractions in mixture A (40% Italian ryegrass and 60% winter cereals) and mixture B (55% Italian ryegrass and 45% winter cereals) applied in the diet may increase diet NDF rumen degradability having positive effect on dry matter intake and performance of the dairy cow. The apparent nutrient digestibility of all nutrients was better and above 67%. The higher fiber digestibility particularly NDF improved the energy concentration (NE_l, NE_m and NE_g) of ensiled mixture (Italian ryegrass 40% + two types of triticale 20% + two types of oats 20% + wheat 15% + barley 5%).

In the second phase of experiment four mixtures (two winter cereal based and two Italian ryegrasses plus cereal based silages) were studied to evaluate nutritional composition, fermentation characteristics, microbial count, ruminal fermentation and *in sacco* degradability and aroma profile to evaluate silage quality. As a summary, all the ensiled mixtures (40-55% Italian ryegrass plus 45-60% winter cereal) produced silage with good feed characteristics. Like in first phase, both winter cereals tested and Italian ryegrass plus winter cereal crop mixtures were fermented well. However, restricted fermentation affected the rapid and efficient production of lactic acid; as a result, pH was not reduced as rapid as possible to maintain efficient fermentation. Additionally, at the end of fermentation, ethanol was reported attributed to the survival of some yeast. This would affect the aerobic stability of the silage. Therefore, use of additives which facilitate hydrolysis of sugars such as *Lactobacillus* bacteria (LAB) is recommended in future use. The aroma profile study reveals that the aroma profile of the different mixtures changed differently during the fermentation process. At the end of the 90-day fermentation, winter cereal mixture silages had similar aroma pattern, and mixture C (55% Italian ryegrass plus 45% winter cereal) was also similar to winter cereal silages. However, mixture of 40% Italian ryegrass plus 60% winter cereals had different aromatic pattern than other ensiled mixtures. The ensiled mixtures had high effective degradable DM and CP at the three rumen outflow rates (ED_1, ED_5 and ED_8) and moderate potentially degradable DM and CP. The *in situ* degradability of the examined nutrient content (DM, CP, NDF, ADF) of the mixtures varied greatly depending on the proportion of cereals and Italian ryegrass. The degradable fraction of DM and CP in the novel mixtures showed significantly different degradation values depending on whether 45% of oats were associated with 55% of Italian ryegrass or other cereals (30% oats, 15% triticale, 10% barley, 5% wheat) with 40% of Italian ryegrass. Similarly, a significant difference was found in the effective degradability (ED_5, ED_8) of the NDF content of the two mixtures containing Italian ryegrass plus winter cereal silages. Therefore, when formulating the cattle feed ration, special attention should be paid to the tested nutritive and ruminal degradability data available on the mixture used. Due to high effective degradable DM and CP of the well-preserved silages might be successfully included in high producing lactating cows. However, the low potential and effective ruminal degradable NDF and ADF should be considered with proper ration formulation particularly for high producing lactating cows. Further experiments should be performed to improve the practical use of the novel mixtures of winter cereals and Italian ryegrass plus winter cereal-based silages. The optimal proportions of winter cereals plus Italian ryegrass mixtures should be established in these experiments, besides determining the effect of phenological phase at cutting on the nutrient content of the mixtures and their ruminal degradability. Feeding dose field trials should be performed to declare the effects of the mixtures on production and reproduction parameters.

It also important to notice that all the six mixtures are autumn sowing and early harvest forages, therefore it can be well integrated into crop rotation, double cropping can also be done with short-growing corn, combined with sorghum or Sudan grass. Further studies are needed to confirm the effect of the mixture on performance of dairy cows and on the composition of milk. On the other hand, due to long tradition of farmers using corn silage particularly in Europe, replacing corn with other silage crops could not be an easy task even best forage species is found in the future. Therefore, different extension approaches should be implemented for the adoption of new feed and feeding system by the farmers before disseminating the new technology.

10. ÖSSZEFOGLALÁS

A szélsőségesen változó időjárási és éghajlati viszonyok (pl. aszály, nyári hőhullámok, talajvízhiány), valamint a mikotoxin szennyezettség növekedése hatással lehet a Világ bármely pontján a kukoricatermesztésre. Az említett nehézségek a megszokott növénytermesztési gyakorlatot akár alapjaiban is megváltoztathatják. A silókukorica klímaérzékenységéről szóló kutatási munkák kiemelik azt, hogy a korábban alkalmazott munkaszervezési feladatokat az éghajlati és a helyi viszonyokhoz igazodva jelentősen meg kell változtatni. Fontos mérlegelni, hogy a széleskörben elterjedt, a bőtejelő tehenek növekvő energia- és táplálóanyag igényét is szem előtt tartó növénytermesztési és takarmányozási stratégiák hogyan igazíthatóak hosszú távon az említett változásokhoz. Éppen ezért, az utóbbi években megnőtt az érdeklődés az új, alternatív takarmány-előállítási technológiák iránt. A kukoricaszilázs gazdaságos helyettesíthetőségének (részleges vagy akár teljes) meghatározása és beillesztése a tej és tejtermékek előállítását szolgáló élelmiszeripari-láncba sürgető kérdés. Megoldás lehet a vízhiányos területeken az új silókukorica hibridek, a korszerű öntözőrendszerek (pl. esőztető és csepegtető öntözés) használata. További lehetőség az egyéb erjesztett tömegtakarmányok (pl. szárazságtűrő cirokfajták, kukorica-cirok keverékszilázsok, őszi gabonakeverékek – pl. rozs, tritikálé), intenzív egynyári és évelő fűfélék, gabona-pillangós keverékek (pl. borsó és árpa, búza és borsó, tritikálé és borsó stb.), továbbá őszi gabona és fűkeverékek (pl. búza, zab, tritikálé, őszi árpa stb. olasz perjével társítva) beillesztése a napi takarmányadagba.

A PhD munkám során összesen hat, különböző komponensekből álló, erjesztett tömegtakarmány (négy olaszperje és őszi gabonakeverék, illetve két önálló őszi gabonakeverék) vizsgálatára került sor. A munka első szakaszában az olaszperje és őszi gabonák keverékéből készült szilázsok táplálóanyag-összetételének, erjedésdinamikai jellemzőinek, valamint a mikrobaszám, az emészthetőség, az energia- és fehérjeérték, illetve a táplálóanyagok bendőbeli lebonthatóságának a megállapítása volt a cél. Kísérleti eredményeink alapján általánosságban kijelenthető, hogy mindkét tartósított keverékből kiváló minőségű és takarmányértékű szilázst lehet előállítani. A megfelelő fenológiai fázisban (kalászhányás előtt) történő betakarítást követően az olaszperjéből és őszi gabonakeverékekből álló keverékből jó minőségű, stabil állapotú szilázst sikerült készítenünk. A tartósított keverékszilázs kimagasló nyersfehérje-, mérsékelt NDF- és ADF-tartalommal, valamint jelentős bendőbeli fehérjelebonthatósággal, illetve potenciálisan lebontható NDF-frakcióval rendelkezett.

A vizsgálat első fázisában tesztelt, eltérő olaszperje-gabonahányadú (40% olaszperjét és 60% őszi gabonaszilázst, valamint az 50% olaszperjét és 50% őszi gabonaszilázst tartalmazó) keverékszilázsok potenciálisan lebomló rostfrakciójának magas hányada növelheti az etetett takarmányadag NDF-tartalmának bendőbeli lebonthatóságát, ami egyben pozitív hatású lehet a tejelő tehenek szárazanyagfelvételére és teljesítményére. Az olaszperje-gabonaszilázs keverék fontosabb táplálóanyag-tartalmának látszólagos emészthetősége a 67%-ot meghaladta. A kedvező rostemészthetőség, különösen az NDF-frakcióé javította a vizsgált keverék (40% olaszperje + 20% tritikálé + 20% őszi zab + 15% őszi búza + 5% őszi árpa) számított energiatartalmát (NE_l, NE_m és NE_g).
A PhD munkám második fázisában négy keverékszilázs (két őszi gabona alapú és két olaszperje-gabonaszilázs alapú) táplálóanyagtartalmának, mikrobiológiai és erjedésdinamikai jellemzőinek, továbbá *in sacco* lebonthatóságának értékelésére került sor. Továbbá a keverékek minőségének meghatározása illatanyagaik szenzoros (elektronikus orr) úton történő vizsgálatával is megvalósult. Általánosságban megállapítást nyert, hogy a keverékszilázsok jól erjedtek és minőségük is megfelelő volt. Ugyanakkor a silózási segédanyag használatát nélkülöző tartósítás ebben e kísérletben nem eredményezett kívánatos mértékű tejsavképződést, így a tartósított keverékszilázsok pH-értéke

a kívánatosnál magasabb volt. Az erjedés végén a modellsilókban elszaporodó élesztőgombák tevékenységének következtében a silóbontást követően a mért etanoltartalom nagyobb volt, mint a jó szilázsoktól elvárt érték, ami a szilázsok aerob stabilitását negatívan befolyásolhatja. Az eredmények alapján a gabona- és az olaszperje-gabona alapú keverékszilázsok esetében a tejsavas erjedést és a stabil szilázs előállítását biztosító silózási segédanyagok (pl. tejsavtermelő baktériumok) használata javasolt. Az elektronikus orral végzett aromavizsgálatokból kiderült, hogy a különböző keverékek aromaprofilja a fermentációs folyamat során eltérő módon változott. A 90 napos fermentáció végén, a modellsiló megbontásakor, az őszi gabona keverékszilázsok aroma mintázata eltért a többi keveréktől. A vizsgált keverékszilázsok szárazanyag- és nyersfehérje-tartalmának tényleges bendőbeli lebonthatósága jelentős mértékű volt valamennyi vizsgált bendőtartalom óránkénti kiáramlási sebessége mellett (ED_1, ED_5 és ED_8), ugyanakkor a potenciálisan lebontható szárazanyag- és fehérjemennyiség mérsékeltebb volt. A keverékek vizsgált táplálóanyag-tartalmának (szárazanyag, nyersfehérje, NDF, ADF) *in situ* lebonthatósága nagymértékben változott a gabona komponens részaránytól függően. Ennek megfelelően a szárazanyag és a nyersfehérje bendőben lebomló hányada a vizsgált keverékekben szignifikánsan ($p<0,05$) eltérő volt attól függően, hogy 45% zab és 55% olaszperje vagy ettől eltérő gabonafélék (15% tritikálé, 30% zab, 10% árpa, 5% búza) alkottak egy keveréket 40% részarányú olaszperjével kiegészítve. Hasonlóképpen szignifikáns különbség ($p<0,05$) volt igazolható a két 55% illetve 40% olaszperjét tartalmazó keverékszilázs NDF-tartalmának tényleges lebonthatóságában (ED_5, ED_8). Éppen ezért a szarvasmarha takarmányadagjának formulázásakor különös figyelmet kell fordítani az alkalmazott keverékről rendelkezésre álló, vizsgált táplálóanyag- és bendőbeli lebonthatósági adatokra. A megfelelően tartósított, stabil olaszperje-gabona, illetve őszi gabonaszilázsok alkalmazása a nagy részarányú bendőben lebomló szárazanyag- és nyersfehérje-tartalom miatt eredményes lehet a nagy tejtermelésű tehenek takarmányadagjában. Ugyanakkor az alacsony potenciális és ténylegesen lebontható NDF- és ADF-tartalmuk miatt a keverékeket csak megfelelő körültekintéssel lehet használni a tejelő teheneknél. További kísérletek szükségesek az őszi gabonafélékből összeállított keverékekkel, valamint az újszerű olaszperje-gabona keverékszilázsokkal a gyakorlati használat javításának érdekében. Ezekben a kísérletekben meg kell határozni az őszi gabonafélék és az olaszperje keverékek optimális részarányát, azt a vágáskori legkedvezőbb fenológiai fázist, amely a bendőbeli lebomlási értékek tekintetében a legjobb eredményt adja. Üzemi etetési vizsgálatokat kell végezni, hogy a napi adagban a növekvő részarányú keverékszilázsoknak a tejelő tehenek termelési és szaporodásbiológiai eredményeire gyakorolt hatását pontosabban lehessen elemezni.

Végezetül fontos kiemelni azt is, hogy a PhD munka keretében vizsgált mind a hat keverék őszi vetésű és korai betakarítású takarmánynövényekből áll, ezért jól integrálhatók a vetésforgóba, így pl. beilleszthetők kettős termesztésben rövid tenyészidejű kukorica után, cirokkal vagy akár szudáni fűvel kombinálva is. További vizsgálatokra van azonban szükség, hogy az erjesztett keverékszilázsok etetésének a tejelő tehenek teljesítményére és a tej összetételére gyakorolt hatását pontosan lehessen értékelni. Ugyanakkor a kukoricaszilázst hosszú évtizede sikeresen használó termelőknek, különösen az európai régióban, a silókukorica más, jó hatékonyságot mutató tömegtakarmány növényekkel történő helyettesítése nem könnyű feladat. Éppen ezért, az ilyen típusú alternatíva széleskörű elterjesztése előtt a kidolgozásra kerülő új takarmányozási technológiának a tejelő tehenek tartásával foglalkozó gazdák általi elfogadtatására, a gazdálkodók meggyőzésére, a helyi adottságokat is figyelembe vevő megközelítésből kell kiindulni.

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13. SCIENTIFIC PAPERS AND LECTURES ON THE SUBJECT OF THE DISSERTATION

13.1. Peer-reviewed papers published in foreign scientific journals

Alemayehu, W., Tóthi, R., Orosz, Sz., Fébel, Kacsala, L., Drew V., Tóth, T. (2021). Novel mixtures of Italian ryegrass and winter cereals: influence of ensiling on nutritional composition, fermentation characteristics, microbial counts and ruminal degradability. Italian Journal of Animal Science. Vol. 20 (1). https://doi.org/10.1080/1828051X.2021.1924883

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13.2. Peer-reviewed papers published in Hungarian scientific journals in English

Alemayehu, W., Tóth, T., Orosz, Sz., Tóthi, R. (2019). Potential forage resources as alternatives to partial or total substitution of corn silage in dairy cattle nutrition: A review. Állattenyésztés és Takarmányozás. 68(2):109-127.

13.3. Proceedings in English

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14. OTHER PUBLICATIONS

14.1. Peer-reviewed papers published in foreign scientific journals

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14.2. Peer-reviewed papers published in Hungarian scientific journals and magazines

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15. CURRICULUM VITAE

Alemayehu Worku was born on the 4th of September in 1984 in Jimma, Ethiopia. He grew up in Jimma city Oromiya region of Ethiopia. In 2000 he graduated from secondary education. He joined the Department of Animal science, College of Agricultural Sciences, the then Alemaya University in Dire Dawa, Ethiopia. In 2004 he obtained his degree of agricultural science in the field of animal science. From 2005 to 2014 he worked in Federal agricultural technical vocational and educational training college at different academic and administrative positions. From 2011 to 2013 he completed a Master of Science degree in animal production at the Haramaya University, College of Agricultural and Environmental Science, Dire Dawa, Ethiopia. From 2015 – 2017 he worked as department head of Animal and Range science, college of agricultural science, Arba Minch University. He started his PhD study in 2017 at the then Kaposvar University, Hungary following his PhD scholarship award by the Stipendium Hungaricum.

Since 2015, he is employed by Arba Minch University, college of Agricultural Science, Department of Animal and Range Science as lecturer.