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Influence of Ensiling on Ruminal Degradability Of Whole Crop Cereal Mixtures with or without Italian Ryegrass

MASTER'S THESIS (MSC)

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1. INTRODUCTION

It is widely acknowledged that forage quality has a significant impact on dairy cattle production. Forage is an important component of the diet for lactating dairy cows, as it provides the necessary nutrients for milk production and overall health. High-quality forage, which is typically hay or silage made from legumes or grasses, can provide a good source of energy, protein, fibre, and other essential nutrients that are needed to support milk production (Brink et al., 2008).

Dairy cows require a balanced diet with a proper forage-to-concentrate ratio, and the quality of the forage can have a significant impact on milk production and overall cow health. Poor-quality forage may not provide enough nutrients to meet the needs of the cow, resulting in decreased milk production, poor body condition, and other health issues. Therefore, it is important for dairy farmers to ensure that their cows are receiving high-quality forage as part of their overall ration (Botha et al., 2008).

Due to its high yield, energy content, and digestibility, maize silage is the primary component of dairy cattle diets in many countries (Keim et al., 2013) including Hungary and other European countries and they are typically requiring significant amounts of irrigation or rainfall to grow successfully. These crops have high water requirements, and if they do not receive enough moisture, their growth and yield may be reduced. The climate in Hungary is generally defined by warm summers and cold winters, with moderate precipitation throughout the year. The amount and distribution of rainfall varies greatly by region, and some areas may experience drought conditions during the growing season, reducing forage production (Bakucs et al., 2020). Irrigation is frequently used to supplement rainfall and ensure that crops receive enough water for optimal growth. However, access to irrigation water may be limited in some areas, making high-quality forage production difficult. In these cases, farmers may need to rely on alternative forage sources or modify their management practises to make the best use of available water resources.

Furthermore, the weather uncertainty caused by the effects of climate change has caused a decrease in the production and production of certain agricultural products. Temperature, rainfall patterns, extreme weather events, and other factors have changed as a result of climate change, which can have an impact on crop yields and livestock production. Rising temperatures, for example, can cause heat stress in crops and livestock, reducing growth rates and increasing disease susceptibility (Campbell & Stafford Smith, 2000). Crop yields can also be affected by changes in precipitation patterns, as droughts and floods can damage crops and reduce yields. Further to that, rising temperatures can have an impact on the quality of forage crops, as hot and dry conditions can lead to decreased protein content and increased fibre levels, making forage less digestible for dairy cow (Braunwart et al., 2001). This has the potential to have an impact on the health and productivity of dairy cows that rely on forage as their primary food source.

In light of the problems, producers and policymakers want to find alternative sources of fodder to continue production and production of high-quality fodder. Introducing new forage mixtures can be a valuable way to supplement, rather than replace, a country's existing forage sources. Winter crops such as triticale, barley, oat, wheat, and rye may be viable alternatives to corn silage as a livestock feed source. These crops are typically planted in the fall and harvested in the spring, providing a source of forage during the winter months when pasture and hay are scarce. In addition to their ability to adapt to changing environmental conditions, these crops provide a variety of nutritional benefits to livestock. They are high in protein, fibre, and other essential nutrients, making them an excellent source of animal feed. Furthermore, some studies have shown that these crops can improve animal health and productivity by increasing milk yield and decreasing the incidence of digestive disorders. As mentioned by Ranck et al. (2020) these winter crops are widely used by mix with the main crop (corn silage) in order to increase the dry matter yield per hectare, milk production, net return on milk production and also nitrogen and phosphorus loss. Not only that, in dairy cow nutrition industries, the used of rye as silage are very popular due to the higher energy content that been provided compared to the annual cereals silages (Worku et al., 2021).

Italian ryegrass is a high-quality forage that is frequently included in cereal mixtures to increase protein content and nutritional value (Baldinger et al., 2010). Moreover, because of its high digestibility and protein content, the addition of Italian ryegrass to cereal mixtures can increase the ruminal degradability of the feed. The proportion of Italian ryegrass in the mixture, on the other hand, can affect its ruminal degradability. If the proportion of Italian ryegrass is too high, it may increase the rate of ruminal degradation, which can lead to a rapid release of energy and a decrease in feed efficiency (Baldinger et al., 2010). However, if the proportion of Italian ryegrass is too low, the overall protein content of the feed may be insufficient to meet the animal's nutritional requirements.

As for winter crops, by combining various crops among them can provide a good source of high-quality fibre and yield for heifers. Combining oats and barley, for example, can result in a high-fiber forage that is particularly appealing to cattle (Baron et al., 2015). This combination can also produce a good balance of energy and protein, making it an excellent source of nutrition for growing heifers. As mentioned by Kleimans et al. (2016) mixtures of forages between winter cereals have an outstanding yield, feed value and ruminal degradability. As example, results of degradability mixtures of barley and winter oat in the rumen is high which as results it improves the dry matter intake (DMI) of the animals.

Based on the information presented above, it was discovered that each Italian ryegrass and winter crop has distinct advantages in terms of ruminal degradability. Mixtures of both winter crop and Italian ryegrass can be an effective way in order to improve the ruminal degradability of the feed of ruminant as Italian ryegrass is a high-quality of forages that often included in winter mixtures crop. Therefore, in this experiment it is based on how the presence of Italian ryegrass will affect the ruminal degradability of dairy cows alongside winter crop mixtures.

1.1 OBJECTIVE OF THE STUDY

However, the ruminal degradability of nutrient content of sole winter cereal forages is well-known, there are not many available data about the ruminal degradability of forage cereal mixtures. Therefore, the objective of the experiment was to evaluate the *in sacco* ruminal degradability of two mixtures of winter-cereal silages with or without Italian ryegrass. It is known, that the *in sacco* ruminal degradability experimental method is a well-accepted method worldwide of studying the ruminal degradability of forages in ruminants. The *in sacco* ruminal degradability experimental method was used for this study as well.

2. LITERATURE REVIEW

2.1 Dairy Sector in Europe and Hungary

The dairy industry in Europe contributes significantly to the agricultural industry and the economy as a whole. The European Union is one of the world's largest dairy producers, exporting a diverse range of dairy products worldwide. The total annual milk production in the EU is estimated to be around 155 million tonnes. Germany, France, Poland, the Netherlands, Italy, and Ireland are the top producers. They account for nearly 70% of EU milk production (Augere-Granier, 2018).

There are over 650,000 dairy farms in the EU, with an estimated 23 million dairy cows. Germany, France, the Netherlands, Italy, and Ireland are the major dairy-producing countries in Europe (Bórawski et al., 2020). The primary products of the European dairy sector are milk and milk products such as cheese, butter, and yoghurt. In line with EU as largest dairy producers, Germany is the EU's largest milk producer, with an estimated output of 31.9 million tonnes of cow milk in 2021. France produced around 24.3 million tonnes of milk, while the Netherlands produced 13.7 million tonnes. The EU milk production is expected to reach 144.6 million tonnes in 2021. (no data for Luxembourg). Production fell by 0.2% compared to the compared to the previous year (Statistisches Bundesamt, 2022).

Further EU is a major exporter of cheese, with countries such as France and Italy bein g particularly well-known for their cheese production.

As world are well aware Europe is the world's largest producer of cow milk (32.69%), followed by Asia (30.18%) and the American continent (27.21%). In 2017, Hungary produced 1.967 million tonnes of milk, accounting for approximately 0.29% of global production and 0.89% of European production (Faostat, 2023). Since 2009, milk production in Hungary has steadily increased, reaching 1.924 million tonnes per year (HCSO, 2023; Kovács & Szűcs, 2020).

The Hungarian dairy sector has faced a number of challenges in recent years, including changes in the EU's Common Agricultural Policy, fluctuating milk prices, and increased competition from imports. As a result, the number of dairy farms in Hungary has decreased, as has overall milk production (Bakucs et al., 2013). However, the Hungarian government has taken steps to support the dairy industry, such as providing farmer subsidies and investing in modernising dairy processing facilities (Konig et al., 2007).

The most important overall characteristics of dairy farming in Hungary are as follows: production is based on large dairy herds having several hundred cows per farm unit; the overall management system is intensive, with indoor confinement; and the feeding technology is based on a corn silage-alfalfa haylage-dried or wet corn meal-based total mixed ration. Grazing is not currently a common practice on dairy farms in Hungary. There are several possible reasons for this situation: the period of substantial herbage growth is relatively short with a maximum of two months at the beginning of the grazing season; the nutritive value of grass decreases sharply in the spring; only animals requiring less-intensive feeding can be grazed (first of all dry cows, then heifers and low yield cows).

The carrying capacity of non-irrigated grassland will be reduced and the yield safety of silage corn will be compromised in the future if the expected climate change in Hungary is characterized by the increase of summer heat waves, a more extreme watercourse (with a decrease of 5%–10% in the summer rainfall average), and various fungal infections. Therefore, it is important to consider how crop production and feeding strategies can be adapted to this climate change in the long term, considering the different energy and nutrient needs of beef cows, and growing and fattening beef cattle and dairy cows.

2.2 Importance of Corn Silage in Dairy Nutrition

Corn silage is an important feed for high producing dairy cows. It is a high-energy feed that is rich in fiber that can help support milk production and overall cow health. Corn silage is a cost-effective source of energy that is also highly palatable and has a high productivity per hectare (Grieve et al., 1980; Phipps et al., 1992; Dhiman andSatter, 1997). These characteristics make corn silage a desirable forage source, particularly where land for growing feed is scarce. Corn silage is relatively good source of protein, although it may need to be supplemented with additional protein sources such as alfalfa or distillers' grains to meet the needs of high-producing animals (Owens et al., 1968). In previous study by Moreira et al., (2006) showed that by combine alfalfa and corn in silage with the ratio of 50:50 forage to concentrate ratio (dry matter basis) resulted in increased dry matter intake and milk yield.

Aside from providing energy for maintenance and lactation, coarser maize silage particles stimulate chewing and salivation, rumination, gut motility and health, regulate feed consumption, modulate feeding patterns and serve as the structural foundation for ruminal digestion. Since starch and fibre are the primary energy sources for dairy cows fed maize silage-based diets, improvements in digestibility of these nutrients may increase milk production or reduce feed costs through improved feed efficiency (Ferraretto, n.d.).

2.3 The Necessity of Alternative Forage Sources

Dairy farming requires a constant supply of high-quality forage to meet the nutritional needs of dairy cows. However, as the availability and cost of corn silage fluctuates. As the yield safety of corn silage will be compromised in the future if the expected climate changes in Hungary is characterized by the increase of summer heat waves and more extreme water courses; therefore, it is urgent to consider how crop production and feeding strategies can be adapted to this change in long term, considering the needs of high production lactating cows., many dairy farmers are turning to alternative forage sources to supplement or replace corn silage in their cow's diet. Based on this finding, acceptable other forages that can partly replace corn silage will be a critical point for the success of future dairy operations if corn production declines in dry continental areas of Central Europe as well. In this regard, attempts have been made; however, attaining forage species that are provide a balanced nutrient profile, reduce reliance on a single forage source, cheap, locally available, and acceptable by farmers, adapted to climatic stress, and equivalent with corn silage in terms of nutritional value, biomass yield, digestibility, and improved milk production is quite difficult but I'll try to list some options though.

2.3.1 Sorghum silage

Sorghum forage is a valuable alternative to corn silage and can be a suitable feed source for dairy cows. The whole-plant sorghum silage (WPSS) had received interest apart from corn because of its ability to be drought-tolerant and more efficient than corn (Staggenborg et al., 2008). Sorghum is a warm-season annual grass that is often used for forage production due to its high yield potential, drought tolerance, and ability to produce high-quality forage.

Depending on the intended use, sorghum forage can be harvested at the boot or soft-dough stage. Sorghum is typically harvested for dairy cows at the soft-dough stage, when the plant has a high moisture content and a good fiber-protein balance. In general, sorghum forage contains less protein but more fibre than maize silage. (Cattani et al., 2017). However, it can still provide adequate nutrition for dairy cows when used in combination with other forages, such as alfalfa or grasses. Sorghum forage can also be a cheaper alternative to maize silage because it can be grown in less favourable growing conditions (McCary et al., 2020)

In previous study, lactating dairy cows fed normal forage sorghum consumed less DM and produced less milk than cows fed traditional forages such as corn and alfalfa silages (Grant et al., 1995; Oliver et al.,2004). However according to other studies feeding a forage sorghum silage supplemented with corn meal as a total replacement for corn silage preserved milk composition and had no negative impact on milk coagulation properties. As a result, silages made from forage sorghums have the potential to replace maize silages in dairy cow diets. Feeding a forage sorghum silage supplemented with corn meal as a total replacement for corn silage maintained DMI, milk yield, and feed efficiency, which is defined as the cows' ability to convert feed to milk (Cattani et al., 2017). The development of new cultivars od sorghum that are more forage than grain types and have higher yields in digestible DM showed promise for the future of sorghum forages. However, the potential for sorghum silage in the daily diets of high producing dairy cows has not been adequately studied.

2.3.2 Grass Haylage and Silage

Grasses are a common alternative forage for dairy cows and, when managed properly, can provide a good source of nutrition. Perennial ryegrass, tall fescue, timothy, and orchardgrass are just a few of the grasses that can be used for forage production (Leverich et al., 2011). Grasses can be harvested as hay, haylage or silage, and the best time depends on the grass species and stage of growth. Grasses have a high nutrient content and can provide a good source of energy, protein, and fibre, all of which are important for cow health and milk production. It is also a type of forage that can be grown in a variety of climates and regions, making it a versatile forage option to replace the main one (Burke et al., 2007).

In previous study, it mentioned that the cow that given grasses silage diet may improve their health in certain instances as cow receives more effective fibre that may stimulate improved rumination (Leverich et al., 2011). Not only that, the inclusion of grass in a dairy cow diet can help reduce high non-fiber carbohydrate (NFC) concentrations commonly associated with corn silage-based rations. (Burke et al., 2007; Leverich et al., 2011). However, grasses mature quickly and lose quality faster than lucerne, so selecting a late maturing grass variety if mixed with a legume and harvesting on time are critical (Leverich et al., 2011). Grass silage, in particular, has a relatively low DMI potential, which limits its usefulness in the diets of high-producing dairy cattle (Burke et al., 2007). Therefore, grass silage varies greatly in terms of feeding value and preservation quality, and many studies show that combining grass silage with other forages improves dairy cow performance (O'Mara et al., 1998).

Annual ryegrass (Lolium multiflorum Lam. or Lolium perenne L. ssp. multiflorum (Lam.) Husnot) or also known as Italian ryegrass is a cool season annual bunchgrass native to southern Europe. It is related to perennial ryegrass (Lolium perenne L.). Both are found all over the world, including North and South America, Europe, New Zealand, and Australia. Italian ryegrasses have greater winter or early spring growth than perennial ryegrasses, allowing dairy farmers to overcome feed shortages during early lactation (middle of July to September), when cows may be underfed (Thom & Bryant 1996). This crop is cold weather resistant, capable of ensuring natural resowing, resistant to crop diseases, and has high animal acceptance when grown intercropped with other grasses and legumes (Cassol et al., 2011). Based on Gerdes et al. (2005), ryegrass is characterized by high productivity and excellent nutrition value to the livestock especially on ruminants.

Annual ryegrass has excellent nutritional qualities, including high palatability, digestible energy, protein, and minerals. In Italian ryegrass, the apparent digestibility of gross energy, dry matter, crude protein, ether extract, crude fibre, neutral detergent fibre, acid detergent fibre, crude ash, Ca, total P, and nitrogen free extract was 33.45%, 48.36%, 44.18%, 84.45%, 25.91%, 22.73%, 22.25%, 26.10%, 62.87%, 13.94%, and 61.42%, respectively (Song et.al., 20014). It showed that, the contents of crude fibre, neutral detergent fibre, and acid detergent fibre are higher in Italian ryegrass, while the contents of ether extract and total P are lower. Also Italian ryegrass is a fodder or cattle feed crop with high productivity and an excellent source of various nutrients such as Ca, Mg, K, P, crude protein, and crude ash with a favourable tetany ratio, K/(Ca+Mg) (Gerdes et al., 2005; Ball, Dennis, Carl and Dan, 2011). Italian ryegrass, like other millet species, is grown for livestock feed and bird seed. The Italian ryegrass, along with the English ryegrass, is the most widely planted grass (Lolium perenne L.). The feed value of grass is generally thought to be equivalent to that of grain sorghum or corn (Lyon et. al., 2008).

Ryegrass is so favourable among the farmers as the livestock feed because in the spring and fall, ryegrasses accumulate a lot of total usable carbohydrate. According to the study by Bailey (1964) ryegrass cultivars and discovered that Italian ryegrass forage particles were more rapidly broken down in the rumen than perennial ryegrass, resulting in a faster rate of passage and, as a result, greater feed consumption by sheep. Feed high in readily fermentable carbohydrates (soluble sugars and fructans) promotes the production of propionate and butyrate in the rumen, which animals can use more efficiently as energy than acetate accumulated in cellulose-rich feed. In addition, Ingalls et al. (1965) discovered that differences in intake determined 70% of the variation in animal production potential of forages, while digestibility determined only 30%. Raymond (1969) proposed that differences in intake were caused by the proportion of water-soluble or pepsin-soluble material and digestible fibre. Bailey (1964) demonstrated that the higher voluntary intake of Italian ryegrass compared to perennial ryegrass was due to the Italian type's higher content of water-soluble carbohydrates and lower cellulose content.

2.3.3 Alfalfa Haylage and Silage

Alfalfa is a highly nutritious legume forage that is rich in protein, energy, and minerals, making it an excellent source of feed for dairy cows. Alfalfa silage can be a good feed option for dairy cows, as it is high in protein, energy, and minerals, and has a digestible fiber content that is beneficial for milk production. Along with that alfalfa is a great forage for high-yielding cows. Cows efficiently use alfalfa's high levels of protein, calcium, and high-quality fibre to produce milk. Cows typically consume more alfalfa than grass because the fibre content of alfalfa is lower (Jennings, 2020). As mentioned, in term of digestibility alfalfa is highly digestible, cows can extract more nutrients from the feed, resulting in more efficient feed use and potentially higher milk production. Also in consistent quality, alfalfa silage is relatively easy to make and store, allowing for consistent feed quality throughout the year. This is especially important during times of limited forage availability (Dhiman and Satter, 1997).

While alfalfa silage can be a nutritious and productive feed option for dairy cows, it also has some potential disadvantages. These include bloat risk which alfalfa can cause bloat in ruminant animals, including dairy cows, if it is not properly manage (Martialis, 2015). Bloat occurs when gas builds up in the rumen, causing it to expand. This can be potentially fatal for the cow, and can also decrease milk production. Also high protein content can be fatal to dairy cows. Too much protein can lead to metabolic problems, such as ketosis, in dairy cows. Additionally, the excess protein can be excreted in urine, which can lead to environmental problems, such as nutrient pollution of waterways (Mertens, 1997).

2.3.4 Winter Annual Cereal Silages

Use of winter cereals as a forage in the rations of lactating dairy cows is increasing. These winter cereals include barley, wheat, triticale and oats, either alone or as mixtures. As compared to the corn silage, winter annual cereal silages at both the milky–dough and heading stage had a lower DM and relatively higher fibre fractions. On the other hand, the NRC (2001) nutritional composition table reported that cereal silages had a lower net energy for lactation

(NEl) content and higher fibre fraction (NDF and acid detergent fibre (ADF)) than corn silage. However, the DM content was comparable with corn silage, but the crude protein content was by far higher than for corn silage. Winter barley tends to have a lower sugar content and higher fiber content than other forage crops, which can make it more difficult to ferment.(Ahvenjärvi et al., 2006). During fermentation, winter barley can produce a range of organic acids, including lactic acid, acetic acid, and propionic acid. The production of lactic acid is particularly important, as it helps to lower the pH of the stored crop, inhibiting the growth of harmful bacteria and preserving the nutritional quality of the forage (Wolf, 2019). In previous study showed barley silage more stable than corn silage as it improving the dry mater intake (DMI), average daily gain (ADG) and feed conversion efficiency (FCE) of steers. Even though, corn forage had a lower pH than barley but the concentration of crude protein and water soluble carbohydrate (WSC) in barley was higher than in corn. The DMI of barley was 45% and 35% in corn at the time of ensiling.

Winter triticale has high sugar content, which makes it easily fermentable. During fermentation, lactic acid bacteria use the sugars in the triticale to produce lactic acid, which helps to lower the pH of the stored crop and inhibit the growth of harmful microorganisms (Au et al., 2010). The production of lactic acid is important in preserving the nutritional quality of the triticale and ensuring that it remains palatable for livestock. Triticale fermentation can also produce other organic acids, including acetic acid and propionic acid. Acetic acid can contribute to the preservation of the forage, while high levels of propionic acid can indicate that fermentation is imbalanced and may lead to reduced palatability and feed intake.(Li et al., 2019). A study showed that there were no changed in total of volatile fatty acid concentration when replaced barley with triticale and corn. As it resulted the lack of the effect between corn and triticale when replaced barley on total VFA showed that the fermentability of corn and triticale sama as barley. In this study, triticale did not significantly vary from the control diet in terms of total VFA production, indicating that the increase in pH may be related to the control diet's 8.5% numerically lower total VFA production (Au et al., 2010).

A study conducted showed the mixture of 30% barley and 70% triticale had a greatest DM recovery of 1011.1g kg^{-1} which also happen in the mixture of 10% barley/90% triticale and 100% triticale. This is because the ability of the forages to possess the ability to ensile when DM ranges from 300 to 400g (Wolf, 2019). Furthermore, the pH of the silage between these mixtures were in a good range from 3.77 to 4.57. In terms of ammonia, mixtures of 20% barley/80% triticale treatment had the highest ammonia level and similar to 100% triticale. A study conducted by Duniere et al. (2017) found that total volatile fatty acid (VFA) of barley and triticale silage were 29.09 g kg^{-1} and 17.08 g kg^{-1} respectively. Also triticale had the higher value of lactic acid content than barley silage (Wolf, 2019).

2.3.5 Winter Annual Cereal and Grass Silage Mixtures

These mixtures complete the forage sources of a dairy cows, rather than substitute the already existing varieties. Climate change is a risk due to the warmer and drier summers and the frequent occurrence of weather extremes, while the autumn-winter period becomes more rainier. Thus, the growing season avoids the warm and dry period. The cereals have a better drought tolerance than the corn. The wide harvest window (the harvest window for Italian ryegrass, triticale, barley, and wheat is wider than that for rye) results in a good quality of silage in the case of rainy weather or any technical failure. Fermentation is a natural process that occurs when forages, such as hay and silage, are stored under anaerobic conditions. During fermentation, microorganisms break down carbohydrates in the forage, producing organic acids and other byproducts. The fermentation characteristics of forages depend on several factors, including the type of forage, moisture content, pH, and the presence of microorganisms (Harrison et al., 1994). The fermentation process can affect the nutritional quality of forages, as well as their digestibility and palatability for livestock. One of the most important fermentation characteristics of forages is the pH of the material. In general, forages with a lower pH (more acidic) are considered to be of higher quality (Wolf, 2019), as this indicates that the fermentation process has produced a greater amount of lactic acid, which can improve digestibility and reduce the growth of harmful bacteria (Au et al., 2010). Another important fermentation characteristic of forages is the presence of specific microorganisms, such as lactic acid bacteria, which are responsible for producing lactic acid during the fermentation process (Wolf, 2019). These bacteria can help to preserve the forage and improve its nutritional value by breaking down complex carbohydrates into more easily digestible forms (Vwxeeoh et al., 1875). As known to have a satisfactory of long term storage of ensiled material the pH should be below 4.0 (Jaster, 1995). A study conducted using mixture of Italian ryegrass with winter cereal mixture which were triticale, oat, barley and wheat showed how the mixtures reacted in terms of pH, ammonia and lactic acid. Even though the pH of the mixture was not below 4.0 but it was reduced through 90 days and also in contrast the amount of lactic acid also increased. The mixture divided in two groups which both group Italian ryegrass and it showed that both of it had less than 75%. In which more than 75% considered as well fermented silage lactic acid as the proportion of lactic acid. As for the ammonia value both group showed the a total of below 7 g/100 g and it came to the silage that undergo 90 days fermentation was a good silage compared to the 7 and 14 days.

2.4 In sacco (*in situ*) method

In order to ensure the animals' requirement are met, dietary nutrient bioavailability of the feed or feed ingredient are two important information that the farmer have to know. there are several techniques had been developed for measuring nutrient digestibility which are *in situ, in vitro and in vivo*. Among those three methods, in situ or widely known as *in sacco* degradation method is often used in order to characterized the ruminal digestibility of a feed (Robinson et al., 1986; Nocek, 1988). We should say that the *in situ* technique is a widely adopted procedure to characterize the dynamics of degradation of feedstuffs as well as nutrients in the rumen and this technique is nowadays probably the most widely used method. There are several factors that affect the results in this method that include bag and pore size, sample size, bag material, bag insertion and removal procedures, rumen incubation time, number of replicate animals, animals, diet of the experimental animals, feeding level, feeding frequency, rinsing procedure, mathematical models and microbial contamination (Michalet and Ould, 1992; Vanzant, Cochran and Titgemeyer, 1998). *In sacco* method is carried out by placing a bag containing the study feeds are incubated for various incubation times in the rumen of the animals with controlled ruminal microbial flora and also variations in degradation rate among the feeds also only attributable to the specific characteristic of the feed (Noziere and Michalet., 1995). Dry forage samples are stored in bags with small pores before being inserted into the rumen of a fistulated animal. The forage is not consumed by the animal through conventional means, such as chewing, but is inserted directly through a punctured hole by the animal's side (directly into its rumen), where it is acted upon by the rumen's microbial population (Ali et. al., 2016). Ørskov et al. (1980) discovered that the nylon bag technique is not only an effective instrument for indexing the relative degradabilities of feedstuffs, but it can also be used to study rumen processes because the factors within the bag, or within the rumen, can be varied.

Moreover, Mehrez et al. (1977) investigated the rate of rumen fermentation in relation to ammonia concentration by varying the amount of urea added to a whole barley diet (from 0 to 10 g/kg diet) and then incubating these same diets in the animals' rumens in a nylon bag. They discovered that increasing the level of ruminal ammonia nitrogen from 10 to 24 mg% positively correlated with the disappearance of dry matter from the bag. This technique has been intensified since Mehrez & Ørskov (1977) critically assessed the factors causing the variability in DM and N degradability. They came to the conclusion that as long as the bags were large enough to allow free movement of substrate within, the technique could be extremely useful as a quick guide to determine nutrient disappearance, particularly the rate and extent of nutrient disappearance from the rumen.

The advantage from this technique is the measurement rumen digestion or feed components disappearance in relation to the time is straight forward and simple. The relationship between the extent of degradation or disappearance and the duration of time is defined by incubating the sample for a series of time periods. As a result, the rate of degradation (or disappearance) of feed in rumen can be calculated (Ørskov and McDonald, 1979). The importance of dry matter or nutrient effective degradability is recognised, particularly for true protein escape from the rumen (Van Soest et al., 1987). According to Haj-Ayed (2000), the effective degradability of dry matter and protein in vetch-oat hays was 65.8 and 79.3%, respectively. Furthermore, this technique is widely applied by the researcher across the world as it only requires fewer measurements, also relatively less labour intensive and cheaper compared to the vivo method (Ruba and Mohamed, 2008).

Nonetheless there are some downsides when using this technique. For example, this technique demonstrates potential sources of variation in the use of the in sacco method among different laboratories in terms of bag size, sample size, particle size, and time (h) of incubation used by various authors. The assumption that the N leaving the bag during washing in water is completely degraded at 0 h of incubation may not be correct. Excessive feed material loss at zero time will result in an overestimation of degradability (Ruba and Mohamed, 2008). Another downside of this technique has been reported by Huntington and Givens (1995), bag pore sizes less than 15 mm can reduce degradation by limiting microbial colonisation and diversity and trapping fermentation gases. However, bag pore sizes greater than 40 mm can result in soluble and undegradable particle losses. Furthermore, animal effects and bag incubation sequence contribute to differences in results between laboratories (Nocek, 1998). The pH inside the nylon bag has been shown to be lower than the pH outside the bag, particularly when small pore-sized bags are used (Marinucchi, 1998).

However, the *in sacco* method remains the standard method in most countries; this is likely due to the fact that degradability is measured in the rumen, making it more biologically reliable than in vitro methods (Hvelplund and Weisbjerg, 1998).

3. MATERIALS AND METHODS

3.1 Experimental site of the study

The trial was carried out on a medium-scale farm in Hungarian University of Agriculture and Life Sciences, Kaposvár Campus. Two different forage mixtures (commercial products, producer: *Agroteam S.p.a*., Torrimpietre (RM), Via di Granaretto, 26, 00054 Italy) were studied: “Montana” (45% of two cultivars of winter oats plus 55% of three types of Italian ryegrass; and “Texas”: 40% of winter barley plus 10% of winter wheat plus 50% of winter triticale). The experimental field was allotted for 30,000 m^2 for each experimental mixture. Deep loosening and disc plus cylinder cultivation were executed as stubble tillage. The soil preparation deep loosening machine subsoiler deep loose shovel adopts the special curved inverted trapezoidal design, which does not intermingle the soil layer during operation, and does not turn over the soil. After loosening the soil, the surface is smooth, maintaining the integrity of the vegetation. The 351 kg/ha artificial fertilizer (NPK: 16:16:16) was applied before sowing. This complex fertilizer enriched the soil with the main macro elements nitrogen, phosphorus and potassium. Seedbed was prepared by Kongskilde VibroFlex 7400 cultivator (lifted) for breaking up the ground and uprooting weeds. The two different forage mixtures were sown on 29th September (75kg seed/ha) with the depth of 3 cm with John Deere 740 A type seed drill (9 m grain drill, 3500 l seed hopper, seed monitoring system, 3 m transport width, hydraulically adjustable coulter pressure). Plant protection treatment was not applied during the growing period. The annual precipitation was 425 mm (World weather online/Kaposvár monthly climate average), just to mention the average annual precipitation across Hungary is 600 millimetres.

3.2 Harvesting and Preservation of Mixtures

Cutting was carried out at the heading stage of triticale based on the existing extended BBCH-scale (Meier, 2001) on 4th May (BBCH (Biologische Bundesanstalt für Land-und Forstwirtschaft) (1997) 51-58. (Italian ryegrass: BBCH51; oat: BBCH51; triticale: BBCH53; winter wheat: BBCH52; winter barley: BBCH58). It is known, that BBCH-scale is a scale used to identify the phenological development stages of a plant. A series of BBCH-scales have been developed for a range of crop species. Phenological development stages of plants are used in a number of scientific disciplines (crop physiology, phytopathology, entomology, plant breeding) and in the agriculture industry (timing of pesticide application, fertization, agricultural insurance). The BBCH-scale uses a decimal code system, which is divided into principal and secondary growth stages, and is based on the cereal code system (Zadoks scale). After cutting, the fresh forage mixtures were wilted to 35% DM (24 h) without any movement on the windrow to have a well fermented haylage. Wilting is one of the most important steps when making silage because wilting increases the dry matter percentage of the mixtures. This has an influence on silage quality and reduces the quantity of effluent produced. Normally the target should be to wilt the silage to a DM of between 28% and 32%, anyway we wilted a bit more. Wilting made the sugars more concentrated in the mixtures, allowing the resulting silage to stabilise at a higher pH; this means a lot less acid or inoculant is required to preserve the crop. 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.

3.3 Ruminal degradability study

After the 90 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 Hungarian University of Agriculture and Life Sciences, Kaposvár Campus, 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 NEl /kg DM; 14.40% CP, 39.06% NDF, 23.66% ADF, and 35.71% non-fibrous carbohydrate (NFC)] consisted of 5.50 kg per day of corn silage, 3.50 kg per day of alfalfa haylage, 3.50 kg per day of vetch-triticale haylage, 3 kg per day of cereal grain based concentrate, 1 kg per day of meadow hay and 0.75 kg per day of liquid molasses. The experimental cows consumed the daily allotted TMR with no daily feed refusal throughout the course of the experimental period. The cows had free access to water.

Rumen incubations were carried out according to Herrera-Saldana et al. (1990) nylon bags are inserted into the rumen at different time points and withdraw simultaneously. Nylon bags of 5×10 cm with pore size of 53 µm (Ankom, USA) were used. Approximately 5 g (on air dry matter basis) of samples were weighed into number-coded nylon bags. The nylon bags containing feed samples for each cow were either placed in the rumen simultaneously and removed from rumen at different time points (0, 2, 4, 8, 16, 24, 48 and 72 h). In each incubation, 60 bags per sample were used (5 bags × 4 replications per sample × 3 cows). Immediately after retrieval, all bags were placed in a bucket with cold tap water to stop microbial fermentation and then washed 5 times manually in cold tap water, followed by oven drying at 60°C for 48 h. The 0-h (five bags for each sample) incubation samples were not incubated in the rumen, but they were washed as described above. Residues from the bags were pooled within the incubation time and treatment.

3.4 Chemical analysis

Prior to chemical analysis, original feed samples and dried rumen-incubated residues were ground to pass through a 1-mm screen then ground by mortar and pestle to pass through a 1-mm screen. The original feed samples and rumen-incubated residues were analyzed for DM (AOAC 930.15) and CP (N×6.25; AOAC 2006) according to AOAC (1990). The NDF (determined with heat-stable α-amylase for original samples and rumen-incubated residues, and with sodium sulfite for rumen-incubated residues) and ADF concentrations were determined by the method of Van Soest et al. (1991).

This analysis predicts what nutrients the animals can use by distinguishing between cell walls and cell contents. Cell walls include cellulose and hemicellulose which are less digestible and lignin and silica, largely undigestible. Cell contents, include sugars, starches, vitamins, and minerals which are almost all digestible. Neutral detergent fiber (NDF) is determined when a sample is extracted with a neutral detergent solution. The cell contents are largely soluble and the cell wall components are insoluble. A detergent solution containing sulfuric acid is used in acid detergent fiber (ADF) analysis. Those cell components, like lignin, that are not soluble in this acid are even more useless to the animal.

3.5 Calculation and statistical analysis

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.

Soluble fraction (% of DM): This is the nutrient component of the cereal mixtures which is rapidly degradable in the rumen consisting mainly of starch and other portions of the feed sample that digest rapidly.

Potentially degradable fraction (% of DM): This is the nutrient component of the cereal mixtures which includes the slowly degradable and some degradable portions which might or might not be potentially degradable by the rumen microbes depending on certain factors like NDF and ADF.

Degradation rate (%/h^{-1}): The degradation rate of nutrient components in the rumen is the time it takes for the degradable DM of the feed mixture to be degraded by the rumen microbes per hour.

Rumen outflow rate (%): This represents the speed at which nutrient components of the cereal mixtures exits the rumen according to the rumen incubation time.

Effective degradability - 1% (ED_1): This is the rate at which the nutrient components of the cereal mixtures is efficiently degraded in the rumen at 1% rumen outflow rate per hour. This is used for testing dry dairy cows.

Effective degradability - 5% (ED_5): This is the rate at which the nutrient components of the cereal mixtures is efficiently degraded in the rumen at 5% rumen outflow rate per hour. This is used for testing beef cattle.

Effective degradability - 8% (ED_8): This is the rate at which the nutrient components of the cereal mixtures is efficiently degraded in the rumen at 8% rumen outflow rate per hour. This is used for testing high producing dairy cow.

NLIN program in SAS (version 9.4; SAS Institute, Inc., Cary, NC, USA) was used to calculate the values of *a*, *b*, and *c*.

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. Mean differences were considered significant at $P < 0.05$. Standard errors of means were calculated from the residual mean square in the analysis of variance.

4. RESULTS AND DISCUSSION

Table 1,2,3 and 4 showed the ruminal degradability of DM, CP, NDF and ADF (*a, b, k_p)* respectively and also effective degradability (ED) calculated for various rumen solid outflows rates (0.01, 0.05 and 0.08 h^{-1}).

4.1 Ruminal degradability of DM

DM degradation parameters showing (Table 1) that the potential degradable of DM for Montana and Texas mixtures were 62.75% and 64.90% respectively and the effective rumen DM degradability at 8% of rumen solid outflow rate (ED8) of Montana and Texas mixtures were 71.42% and 66.27% respectively.

The values of Montana mixtures as the presence of Italian ryegrass in terms of degradability is higher than reported by Andrighetto at al. (1993) which only 60.7%. As for the Texas mixtures, the lower in DM degradability compared to Montana could be contributed by the increasing the amount of fibre as barley and triticale are high in fibre. According to NRC (2001), oat, triticale and wheat are crops that contributed to lower DM degradability because of the fibre value as these crops may increase the fibre fraction in the mixtures. The effective DM degradability at 1% rumen outflow rate which defines the maintenance DM requirement for Montana was 75.45% and for Texas mixtures was 71.52%. These values it seems better than the one reported by Andrighetto at al., (1993).

Table 1 **Rumen degradation parameter and effective degradability of DM of Montana and Texas silage**

	Montana	**Texas**	**SEM**	***p* value**
Dry matter (DM)				
Soluble fraction, (% of DM)	13.32^{a}	$7.43^{b.}$	0.201	< 0.001
Potentially degradable fraction, (% of DM)	62.75	64.90	1.257	ns
Degradation rate, (% h^{-1})	1.00	0.78	0.120	ns
Effective degradability - 1, (%)	75.45^{a}	71.52^{b}	1.312	< 0.05
Effective degradability - 5, (%)	73.08^{a}	68.40^{b}	0.882	< 0.01
Effective degradability – 8, (%)	71.42^{a}	66.27^{b}	0.650	< 0.001

a,b Means with different superscripts differ

4.2 Ruminal degradability of CP

As for soluble CP fractions of Montana and Texas (Table 2) which were 18.38% and 7.44% were lower compared with the soluble CP fractions of Italian ryegrass silage (46.70%) (Andrighetto et al., 1993) and rye grass silage (49.00%). Montana mixture value of soluble fraction was comparable with the value of oat silage with the value of 83.7% (Saman, 2003). Not only that, Texas mixtures of soluble of CP fractions also lower when compared to the value of Italian ryegrass forage either in the first or second cut at the leaf stage of 20.6% and 19.2% respectively (Amrane-Michelet Doreau, 1993).

Based on previous study by de Oliveira et al. (2016), ensiling also can be one of the factor that may affect CP degradability as compared to the Italian ryegrass forage. This factor may be one of contributed even the crops had already been harvested at the right heading stage in order to maximum the protein utilisation by rumen microbes. On the other hand, the soluble CP fraction of Texas mixture 7.44% been compared with the value of soluble fractions of barley silage (83.7%) (Saman. 2003). As for the potential degradable CP fractions for both Montana and Texas mixtures (65.10% and 64.90%) were lower than Italian ryegrass when its pre-blooming stage (82.38%) but higher than early-blooming (64.85%) and late blooming (53.65%) stage (Fariani et al., 1994).

Aside from that, the degradability rate of Montana and Texas mixtures 1.27% and 0.78% respectively were higher than the Italian ryegrass forages in all three stages of pre-blooming, early-blooming and late blooming from previous study (Fariani et al., 1994). According to Worku et al, (2021), the increasing of degradation rate could be attributed to early heading stage of harvesting ahead of ensiling and also the incorporation of Italian ryegrass with winter cereals. As a consequence of the higher rate of CP degradability, it allows for the Montana and Texas mixtures to combine with other crops that high in fibre in order to improve forage utilization for dairy cow nutrition.

Table 2 **Rumen degradation parameters and effective degradability of CP of Montana and Texas silage**

	Montana	**Texas**	**SEM**	***p value***
Crude protein (CP)				
Soluble fraction, (% of DM)	18.38[a]	7.44[b]	0.358	< 0.001
Potentially degradable fraction, (% of DM)	65.10[a]	64.90	0.860	ns
Degradation rate, (% h^{-1})	1.27[a]	0.78[b]	0.071	< 0.001
Effective degradability - 1, (%)	82.98[a]	71.52[b]	0.798	< 0.001
Effective degradability - 5, (%)	81.03[a]	68.40[b]	0.668	< 0.001
Effective degradability – 8, (%)	79.70[a]	66.27[b]	0.631	< 0.001

[a,b] Means with different superscripts differ

4.3 Ruminal degradability of NDF and ADF

In dairy cow's nutrition, an ample amount of NDF is needed. This is because the ruminal degradability and amount of NDF of forages are diverge in its degradability in rumen and indirectly influences animal performance (Bender et al., 2016). Furthermore, in dairy cows diets it should be at least 25% of NDF according to study from Oba and Allen (1999). as mentioned by Pelletier et al., (2016) the wide range of winter or cool season of grasses are known to have a great fibre (NDF and ADF) degradability. In terms of potential ruminal degradable fractions of NDF for Montana and Texas (Table 3) 31.97% and 34.30% respectively were lower than the degradability fraction of Italian ryegrass (59.8%) (Andrighetto et al., 1993). Based on previous study, lower NDF values showed that the number measure of plant's cell wall contents is lower and make it more digestible and for ADF lower means the fibre concentration is low and digestibility and nutrient availability are increase (Sharpe, 2019). Moreover, according to Sharpe (2019), forages with NDF values of 65% or higher than that are not palatable while ADF level above 45% had less nutritive value. On top of that, study reported by Ali et at. (2014) showed the higher value of NDF degradability of grass silage which was 76.40% compared to Montana and Texas mixtures (31.97% and 34.30%).

The rate of ruminal of NDF degradability *(k_p)* of Montana and Texas mixtures were 0.03 and 0.04 respectively. As above, the value of these two mixtures were lower when compared to the value reported by Weisbjerg et al. (2017. In this report it showed that the grass/grass-clover silage and also whole crop cereal silage value were 0.016 and 0.015 respectively. In this study, the soluble ADF fractions of Monatana (8.01) and Texas (7.41) of the ensiled mixtures were low (Table 4). It is known, that ADF is a measure of the plant components in forages that are the least digestible by cattle, including cellulose and lignin. The potentially degradable as well as effective degradable ADF were low.

***Table 3* Rumen degradation parameters and effective degradability of NDF of Montana and Texas silage**

	Montana	**Texas**	**SEM**	***p* value**
Neutral detergent fibre (NDF)				
Soluble fraction, (% of DM)	9.58[a]	7.65[b]	0.536	< 0.01
Potentially degradable fraction, (% of DM)	31.97	34.30	9.188	ns
Degradation rate, (% h^{-1})	0.03	0.04	0.009	ns
Effective degradability - 1, (%)	34.20	32.52	4.467	ns
Effective degradability - 5, (%)	22.52	23.57	1.295	ns
Effective degradability – 8, (%)	19.14	19.73	0.788	ns

[a,b] Means with different superscripts differ

***Table 4* Rumen degradation parameters and effective degradability of ADF of Montana and Texas silage**

	Montana	**Texas**	**SEM**	***p value***
Acid detergent fibre (ADF)				
Soluble fraction, (% of DM)	8.01	7.41	0.887	ns
Potentially degradable fraction, (% of DM)	33.34	29.73	14.72	ns
Degradation rate, % h^{-1}	0.03	0.06	0.017	ns
Effective degradability - 1, %	32.05	32.96	4.992	ns
Effective degradability - 5, %	20.19[b]	23.97[a]	1.348	< 0.01
Effective degradability – 8, %	17.01[b]	20.58[a]	0.918	< 0.001

[a,b] Means with different superscripts differ

5. CONCLUSION

The ensiled mixtures in this experiment had high effective DM and CP degradability at the three rumen outflow rates and moderate DM and CP degradability potential.

In comparison to Texas combinations that only contained winter cereals, the Montana mixtures with Italian ryegrass demonstrated higher effective degradability (ED_8) and lower degradability of NDF and ADF (ED_8).

It is possible to feed Italian ryegrass and winter forages to dairy cows that are lactating because of the mixes' enhanced capacity for degradability.

In future studies, a small amount of exogenous enzyme may be added to improve the rumen degradability of NDF and ADF for lactating cow feed.

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ABSTRACT OF THESIS

The production of crops like maize, which is the primary source of protein for livestock, has been impacted by climate change because of the increasing weather. Italian ryegrass and winter forages have been suggested as replacements for maize crops as a solution to this issue.

This study was conducted with the objective of the study to evaluate *in sacco* ruminal degradability of two mixtures of winter cereals silages with or without Italian ryegrass.

The ruminal degradability trial was carried out with 3 multiparous non-lactating cannulated Hosltein-Freisian dairy cows. The two mixtures (Montana: 45% of two cultivators winter oats plus 55% of three types of Italian ryegrass; and Texas: 40% of winter barley plus 10% of winter wheat plus 50% winter triticale). After 90 days of fermentation, both mixtures were tested in term of rumen degradability of dry matter (DM), crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF).

Results showed *in situ* incubation Montana had highest value of potential degradable fraction (*b*) of DM, CP, and ADF which were 62.75%, 65.10% and 33.34% respectively while Texas has higher value of *b* in NDF of 34.30%.

The effective degradability at 0.08 (ED_8) rates of DM, CP, NDF and ADF of Montana 71.42%, 79.70%, 19.14% and 17.01% respectively while 66.27%, 66.27%, 19.73% and 20.58% for Texas respectively.

Based on the findings, it was concluded that feeding lactating cows a mix of Italian ryegrass with winter fodder could increase the capacity of lactating cow degradability.

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