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Ruminal degradability characteristics of different forage mixtures in dairy cows

MASTER'S THESIS (MSC)

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INTRODUCTION

Forage is the most critical component of the diet of dairy cows because of its impact on nutrient consumption and dry matter which ultimately impacts the nutrients available for the animal for growth and development as well as production of products like meat and milk. Forage quality is based on its ruminal degradability, nutrient content and digestibility. The higher these qualities are in the forage provided to the animal the higher the productivity of the animals. As much as forage quality is a fundamental aspect of dairy cattle production; most farmers are not able to offer high-quality forage for their dairy cattle mainly because of the high cost of production as well as global warming that is affecting the planet now. Global warming is now one of the most serious issues affecting 21st-century farmers as the high temperatures and unreliable rainfall lead to low production of forage as well as low-quality forage production because of higher levels of lignin content. Climate change can affect the reliability and quantity of production of forage, water demand for production of forage crops and rangeland vegetation patterns. Developing countries and those that are dependent on agriculture are more vulnerable to the changes that come with climate change because of the predominance of agriculture and livestock production in their economies

To counter this problem dairy cattle farmers, must come up with strategies that will increase the amount of forage produced as well as the quality of the forage being provided to the cattle regarding their ruminal degradability, digestibility, nutrient content, and effect on productivity of milk. Some of the farmers are employing strategies such as replacing some of the traditional forages with choices that are easy to produce and still have a positive impact on productivity, improving feed processing, and many others. One of the growing means of challenging this problem of forage quality is integrating different forages such as winter cereals in double cropping systems has been seen as a viable solution if it is done carefully as it’s a way of optimizing forage production in the rations of dairy cows (Ketterings et al., 2015). If forage management is conducted appropriately risks associated with this method such as reduced milk production can be avoided.

Winter cereals are a viable solution to forage production because they are more stable in drought and cold conditions when compared to traditional forages like corn. Besides, double cropping winter forages like Italian ryegrass increases diversification of forages which in turn reduces the risk of loss of production in case one of the forages is affected. Apart from that winter cereals when mixed with Italian ryegrass can be harvested in early spring after plants and they make it possible to plant silage corn, sorghum, corn and Sudan grass making it possible to harvest forages twice a year. It is critical to understand the effects of mixing winter cereals such as ryegrass regarding their ruminal degradability, nutrient value and effect on production in dairy cattle (Chaudhry, 2007).

LITERATURE REVIEW

Feeding covers about 60% of the production cost in dairy production. For dairy cows to produce enough milk and have a return on the investment feeding should be taken seriously at all stages of production of the high-producing animals to ensure that there are adequate nutrients for growth and development, maintenance and production. Different feeding materials can be used to feed the animals and they can be in the form of grains, silage, hay and many more. About 80% of a cow's diet over its lifetime comes from forages. One of the most common feeding materials in high-producing dairy production is corn silage. Corn silage is popular in many parts of the world because of its high nutritive value and easy production and processing. It has a high energy and protein content. Besides, corn silage is easy to digest and hence good for maintaining the animal's body condition. Some of the benefits of maize silage in dairy cows include improving milk production through its high energy and protein content which maintains the body condition of the cow and provides the nutrients required for milk production. Corn silage contains about 25% dry matter. It is relatively poor in protein (5-10% of DM) and rich in fibre (15-27% of DM), with a highly variable starch content (18-37% of DM). It is low in lignin and lipids (about 2-3% of DM each) Thomas et al. (2001).

Also, corn silage improves milk quality through its high protein content, fibre and essential nutrients which are critical in high-producing cows especially during lactation when the nutrient demand is high. The other benefit of corn silage is its high digestibility and palatability. When corn silage is produced from corn that has been harvested at the right stage it will produce silage that is easily available, palatable and digestible in the gut of the cow. With these factors in place the feed intake will be high and subsequently the productivity of that animal regarding milk production. On top of that, corn silage has few complications associated with other feeding materials such as acidosis and bloat. Unlike grain-based feeds corn silage has a high fibre content that helps buffer the cow's rumen and reduce rapid fermentation which causes acidosis.

As much as corn silage forms an integral part of most high-producing dairy operations there are other forage sources in the diet of dairy cows. One of the main sources of forage for dairy cows is grasses. Different grasses are grown and processed to provide the animals with the necessary nutrients to sustain their growth and production. Grasses belong to the Gramineae family which can be annuals or perennials depending on the region they are grown as well as the climatic conditions. Some of the common grasses used in dairy production include smooth bromegrass, tall fescue, perennial ryegrass, timothy grass, reed canary grass, and orchard grass.

Others native to other regions like Africa include Rhodes grass, Sudan grass, Kikuyu grass, Nappier grass, and Bracharia. These grasses have different nutrient values and when harvested at the right time and mode of processing and preservation can provide the animals with the required amount of nutrients that can sustain milk production. For instance, the Italian ryegrass has a DM of 16%, CP content of 12%, ADF of 35% and NDF of 50%. These qualities make it a good source of forage for dairy production (Miller et al., 2001).

The other source of forages are the legumes which forage in pods and creeping or large tap roots. Legumes can be perennial or annual and serve as an important source of proteins for dairy cows. They also improve the soil as they stimulate biological activities in the soil, improve soil aeration, soil structure, and water-holding capacity and reduce soil erosion. Annual forage legumes include varieties such as the 40-10 forage pea as well as vetches. Forage legumes include biennials such as white blossom sweet clover and yellow blossom sweet clover, and perennials such as alfalfa, alsike clover, birdsfoot trefoil, cicer milkvetch, red clover, purple prairie clover, sesbania, stylo, lablab, leucaena, sainfoin and white clover. However, lucerne is the most common leguminous forage for dairy cows with a DM of about 15.8%, CP of about 24.6%, and CF of about 20.1% (Raeside et al., 2012). Alfalfa is generally considered the queen of forages in many parts of the world because of its outstanding protein level and balanced amino acid profile which is good for dairy production.

According to (Mauries, 2003) the amino acid profile of lucerne is favourable when compared to that of soybean meal and it has a higher protein per unit area yield than soybean making alfalfa a good source of forage for dairy production. It also has a good amount of energy but is slightly lower than that of many types of grass but none less should not be underestimated (Bruce et al., 2008). Lucerne can be processed upon maturity to alfalfa hay, silage or dehydrated alfalfa. Dehydrated alfalfa hay was found to be the best way to assist in stabilizing and drying the alfalfa while preserving its high protein content and vitamins. Besides dehydration is a good source of beta carotenes and xanthophylls for dairy and poultry farmers. (Renaud, 2002). Despite alfalfa being the most common legume in dairy cattle production; it requires careful management when feeding the livestock as it presents the risk of bloating. Bloating occurs when fine legumes digest quickly and create a frothy bloat in the rumen of the animal. This condition can be managed by mixing alfalfa with other forages like grasses.

The other essential leguminous forage is bird'sfoot trefoil effectively uses excess water and reduces drainage problems and salinity. There is also white clover, which is susceptible to drought because of its lack of tap roots. Legumes can be established for grazing or as stored forage such as hay, silage, green feed, or turned under as green manure to improve fertility and organic matter in soils. Unsurprisingly, legumes have higher protein levels than grasses, but their digestibility tends to reduce as they go past the age of maturity. They also typically require more careful management compared to grass stands because of factors like fertility and the soil. However, they are more persistent and high-yielding than grasses.

The ability of farmers to produce enough forage for their animals throughout the year is limited by the climatic conditions of the location the farm is located. Since feeding accounts for about 60% of the total production cost many farmers try to use available resources such as growing their forage crops to reduce the amount of money spent on feeding the animals and maximize profits. With the ongoing climate change, there has been an overall increase in environmental temperature which affects every phase of production and growing season. Climate change which has the potential to impact quantity and quality of forages produced by farmers for sustainable of livestock production. Based on the current situation and data collected by climate change experts it's expected that the crops and plants that are applied as forage for animals will continue to face warmer temperatures due to elevated greenhouse gasses in the atmosphere such as carbon dioxide. There is also the threat of changing precipitation patterns and when interplayed with the rising temperatures culminate to a negative impact on forage growth and yield. Increasing CO2 levels increase dry matter production in C3 plants that C4 plants. The quantum response to this phenomenon depends on the interactions among the soil moisture, crop, and nutrient availability in the soil. As the agricultural sector where forage production falls is the largest user of freshwater the decreasing supplies with negatively affect forage crop production which will subsequently affect livestock production negatively. This aspect is because of the variability in the amount and frequency of precipitation received in an area. Whenever the precipitation received is low, the amount of feed available for the animals will be low which ultimately lowers production (Ketterings et al., 2015). To counter this problem strategies such as double cropping can be put in place to help farmers produce more fodder with the varying temperatures and precipitation. Double cropping is the sowing of several crops on the same farm in the same year so that more yields can be generated from the same field in the same year. It's the harvesting of two or more crops in a calendar year for instance barley in winter and soybeans in fall. For dairy farms with a small area but many cows this is a critical aspect. Double cropping anyway has a significant role in preventing wind and water erosion, also in nitrogen retention through fall mulching. For a long time, we (in Hungary) were almost alone in Europe with this technology. In Western Europe, this production system is best known in the former East German territories, where the first rye seeds came from. The rest are northern and Western countries that are still harvesting the winter cereals at the early dough stage

(BBHC 83-85) which would make double cropping impossible. However, it has a large quantity and good quality grass that can be grazed or ensiled is the reason, because of the rain, but it is not digestible in mountainous, Scandinavian areas fiber is the limiting factor in terms of milk production, but the lack of starch (with the cultivation of corn in many places they have difficulties due to the cool climate). The most important factor determining the ability to double crop a farm is the crops' harvesting stage. The harvesting should be done at an appropriate stage but at the same time be able to allow the next crop to be sown in time (Liebert et al., 2023). Winter cereals like triticale and ryegrass can be seeded and harvested for silage in early spring and allow double cropping because of the many advantages associated with them. Fall rye is an idea double cropping forage and can easily be included in forage rotations. When seeded between two forage crops the winter cereal creates additional yield with average yield equivalent to an excellent first cut. Besides, since it has an allelopathic effect and can grow vigorously it can easily suppress weeded when planted in fall so much that it eliminates the need for herbicides. On top of that winter cereals like fall ryegrass when planted are beneficial to the health of the soil and since it covers the soil during winter it reduces soil erosion and nutrient losses as well as boosting rainfall infiltration, microbial activity and porosity of the soil.

The most significant change in attitude is in the fields the phenological stage was done in harvesting technology with the early dough stage. Farmers for decades lived under the spell of starch, believing that cereals harvested in dough stage cereals have the highest percentage of net energy. This thinking was wrong. Laboratory technology with the development (of fibre digestibility and dynamics with its novel measurements and the differentiated assessment of fibre it became clear that the easily digestible fibre is more accessible (due to reduced cell wall effect). Net energy from nutrients significantly increases harvested around the heading stage energy content of cereals. So much so that the rye its energy content is higher when in the heading stage (6.0-6.5 MJ/kg), than at the early dough stage (5.0-5.5 MJ/kg). The nutrient content of winter rye gets altered very quickly. The palatability and quality reduce fast as the maturity advances. It is thus important to harvest the crop at the right time of growth. When harvesting fall rye for silage, the early-boot stage is the best stage for maximum potential. Besides, harvesting at the early boot stage or heading stage will ensure there is rapid regrowth after harvest from the crown buds and provide a compromise between highest yield and highest quality. According to the NRC data annual ryegrass harvest at fresh and mature stage has 58% TDN, 2.56 (Mcal/kg) DE, 2.10 (Mcal/kg) ME, 1.24 NEm and 5.8% CP. At the full bloom stage 55% TDN, 2.43 (Mcal/kg) DE, 1.99 (Mcal/kg) ME, 1.14 NEm and 5.8% CP showing a reduction in the nutrient content of the rye grass from the fresh mature stage (Hannaway et al. 1999). Therefore, the period of harvesting is essential in determining the quality of forage regarding their nutrient content and their digestibility in the animal which in return determines the growth and productivity of the animal. The most essential element of the quality of plants and forage is the growing stage which can help to identify the optimum time of harvest for optimum productivity (Mohajer et al., 2012). Different species and varieties of plants have different harvest times and qualities, meaning there are different digestibility levels when mixed with different crops. Therefore, the harvesting time also determines the mixtures that can be made and their qualities regarding nutrient content and degradability in the cow's rumen. Understanding the degradability of different crops at different harvesting stages also helps avoid losses associated with making mixtures of forages harvested in the wrong harvesting stage. Ultimately it helps avoid production losses in dairy cows.

To understand the nutrient value of the crop at different stages of growth different agronomical technologies are applied. The most common one is the BBCH scale for cereals which describes the phenological development of cereals using the BBCH scale (Lancashire et al., 1991). There are different BBCH scales for a range of crop species where the growth stages of each plant have different codes. This scale creates a framework to understand the phenology of cereals in germination, leaf development, tillering, stem elongation, booting, inflorescence emergence, flowing anthesis, ripening and senescence. It helps determine the net energy content of forage feeds which is of great importance in the feed ration, it determines the amount of silage/sage that can be fed. From these scales, the nutritive value of the foraging stage at that stage can be determined regarding nutrient content, degradability, mixtures, and the amount needed to fulfil the animal’s needs.

Much research on nutritive value and degradation has been done on different crops except for winter cereal silages. This aspect is because they are not usually grown or given good care to warrant research on their characteristics and effects on productivity. Besides, their production is low, unpredictable and expensive. Despite that, there has been some research work done in these growing fields, albeit fewer, which aims at evaluating the digestibility, chemical composition, quality, fermentation and feeding value of different winter cereal silages (Mohammed, 2008). Livestock producers can use winter cereals to establish pasture that can supplement perennial pasture or raise the carrying capacity of their forage farms for livestock operations. Besides, they can fall seeded, grazed, or overwintered and harvested or grazed in spring. Livestock farmers who want to use early spring pasture need to consider fall rye as their first choice. Winter wheat and triticale can also be applied in winter and growing areas and they can stay green and continue producing forage until it snows as longs as the early frosts is not severe. Some of the notable winter cereal silages include crops like wheat, barley, rye, winter oats, and winter triticale. Research has focused on the harvesting stage of these crops and their nutritive value at different harvesting stages. However, practical data shows that most winter cereal silages are harvested at the heading stage instead of the usual dough stage. The early heading stage has high water-soluble carbohydrates or sugars which enhances fermentation. When winter cereals mixtures are grown and fermented well as silage, they have high yields and can sustain production in dairy cattle because of their high nutritive value and rumen degradability. For instance, barley and winter oats have high ruminal degradability that increases dry matter intake and subsequently productivity (Kleinmans et al., 2016). Winter triticale and Italian grass when produced and ensiled well have a high yield potential, high energy content and digestibility which translates to positive productivity. This aspect is because of the high-water soluble carbohydrates that balance energy and nitrogen supply in the cattle's rumen (Miller et al., 2001). Italian grass varieties such as the one-year Suxyl variety have high dry matter of as much as 6.28MJ/kg. The high energy content of this grass is because of high nutrient digestibility and low lignin content which improves rumen degradability and silage quality when mixed with other cereals (Lehel et al. 2011). According to Worku et al. 2021 the CP content of Italian rye grass was 12.8%, DM was 36.5% which makes it highly valuable winter cereal for dairy cattle production. Baldinger et al 2014 reported that the sugar content of rye grass harvested at the early stages is high and nutritive. They further indicated that the Italian ryegrass in chopped and processed in the second cut had significantly higher sugar content that corn hence the application of this silage can help reduce the over reliance on corn silage in dairy production. it is the difference in nutritive value of the forages that makes it possible or demands for forage mixtures in ensiled forage mixtures. When different forages such as winter cereal forages are harvested and made into mixtures for livestock production there are many benefits that can be accrued from this strategy which include nutritional balance. Difference crops have varying nutrient compositions and hence mixing them creates a feed or silage that is more balanced and nutritively more beneficial to the livestock that single forage feed as the animals will be able to access a wide variety of proteins, vitamins, minerals and carbohydrates. Besides, it increases the palatability of the forage. Some forages have appealing tastes while other have smell and taste that if given alone can reduce intake and hence mixing the forages into one feed eliminates or subdues these negative characteristics within the mixture create a superior feed that the animals can enjoy. Apart from that, the mixing of forages increases the fiber content of the feed when the some of the forages used have a lower fiber content. Mixing the forages allows farmers to adjust the fiber content of the o meet the specific dietary requirements of the livestock and thereby ensuring proper gut health and digestion. Moreover, by mixing forages, farmers can often achieve a more cost-effective feed option compared to purchasing single forages. This can be particularly beneficial when certain forages are expensive or in short supply. also, mixtures reduce the chance for nutritional deficiencies happening in the case that the single forages have lower nutrient content to sustain the requirements of the livestock for optimum productivity. Mixing mitigates this problem by providing a broader spectrum of nutrients. The differences in nutrient quality and characteristics of the different winter cereals make the mixtures have differences in aspects such as ruminal degradability when mixed. For instance, the inclusion of 40-55% Italian grass influences the fermentation and ruminal degradability of the ensiled mixture (Worku et al. 2021). According to Lyons et al. (2016) mixing different cereals in silage mixtures has the merit of compensating for the deficient nutrients in some of the cereals in the mixture, reducing the need for costly concentrates, improving rumen environment, increasing in situ fermentation and degradation and overall nutrient supply to the animal.

The ruminal characteristics are different at various stages of growth of any species of forage. According to Fariani et al. (1994) and Sullivan (1973), the stage of growth is one of the most crucial factors that contribute to the nutritive value of forage because of the physiological and morphological changes that occur in each stage of development. As forages mature their cells' cytoplasmic portion degrades in value and its qualities like the number of lipids, proteins, carbohydrates, and soluble minerals reduce. Besides, the digestibility also reduces. These facts translate to the ruminal degradation of the forage. However, despite the factor that digestibility and the quality of forage are high at the initial stages; the dry matter per unit area is at its lowest. Hence to optimize on these factors the stage of harvest must be considered. According to Fariani et al. (1994), the heading stage of the Italian ryegrass showed high ruminal degradation that reduced with maturity of the grass. Degradation of DM was high at early and mid-blooming stages and low at late blooming stages. The increasing quantities and changes in the composition of cell wall contents are the reasons for the reduction in ruminal degradation. In the studies conducted by Aufrere et al. (2002) when compared to fresh fodder taken at the end of heading (0.705), the DegN of perennial ryegrass silage (0.760) and wrapped huge bales harvested at 42% DM (0.739) were greater ($P < 0.05$). $P < 0.05$) DegN values were found to be lower for wrapped big bales of perennial ryegrass harvested at 58% DM (0.667) and hay (0.536) than for the other forages. Therefore, the stage of harvesting is fundamental and in determining the degradability of the forage in the case of Italian ryegrass the best stage is at

42% DM or at the heading stage. According to the study conducted by Hadjipanayiotou et al. (1996), the in-situ degradability of oats, barley, and vetch harvested at heading, milk stage, and early maturity and determined using three mature rumen fistulated Damascus goats; the CP contents and DM degradability decreased with advancement in maturity. Hence due to the shift in the CP degradability with maturity its essential to consider the stage of growth or maturity when mixing the forages to make silage.

To understand the rumen degradation of different forages as affected by varied factors it's good to use the right method of evaluation. Ingested feeds are subjected to microbial degradation in the rumen with the end products of that process being amino acids, ammonia, volatile fatty acids, and peptides which are sued to supply protein and energy for the ruminant's tissues. The nutritive value of the feeds depends on its nutrient content and degradation in the rumen hence the need to understand the degradability of the feedstuffs being applied. For this purpose, a routine method to determine and foresee the nutrient degradation of the feeds taken by the animals is required to formulate rations to give the animal ruminally required amounts of undegraded and degraded nutrients. Three major methods are used in degradability and digestibility studies, and they include in vivo studies, in vitro studies and in sacco studies. In in vivo method degradability of the feedstuffs can be determined either from measuring the amount of nutrients finding their way into the duodenum or abomasum of fistulated animals (*in vivo*) or from the measurements of N and DM disappearance from synthetic porous bags suspended in the rumen of fistulated animals (*in sacco* or *in situ*). There are also the *in vitro* methods that use rumen fluid obtained from fistulated animals, to estimate either digestibility or gas production. Also, there are other *in vitro* methods that involve proteolytic enzymes which are either commercially extracted from non-rumen sources (for example, ficin from fig latex or sap or protease from *Streptomyces griseus*) or extracted from mixed rumen micro-organisms. However, the most widely applied method in analysing rumen degradability is the *in sacco* method (Mohammed et al., 2008). The method was pioneered by Quin et al and has now become basic in the evaluation of the use of concentrates, forages, and high-protein feeds. Despite the intensive criticism by Mehrez and Orskov (1977), the method has since been applied widely because of its simplicity and reliability in assessing the degradability of protein and DM in the rumen. This method is based on rumen incubation of substrate (feed) in nylon or dacron bags followed by rinsing and analysis of the residue. Small pores in the bag allow microbes to enter the bag whilst a variable portion of the feed is retained in the bag. The results are used to estimate the ruminal effective degradation (ED) that is used in several protein evaluation systems. Unlike methods like the in vivo method, the *in sacco* or *in situ* method is not laborious, expensive and can be used to evaluate more than one feedstuff at a time (Mehrez & Orskov, 1977). Besides, when compared to the in vivo method the *in sacco* method is not very much associated with errors related to using digesta flow rate markers and inherent animal variations. The demerit of the *in sacco* method includes its inability to be reproduced in laboratories due to variations in proteolytic activity between animals because of differences in physiology and diet (Chaudhary, 2007). Hence, the results of these studies cannot be accurately and equally applied in all situations unless the factors are standardized which is hard to do. It is also facing a lot of backlash from animal welfare programs due to its application of fistulated animals. This leads to the use of only a few sample sizes which limits the accuracy and reproducibility of the results (Mohamed and Chaudhry, 2008). Lindberg 1981 conducted studies on cannulated cows to understand the effectiveness of the in-situ method in the study of degradation of barley and other feedstuffs and found it to be effective in rumen degradation studies.

GOAL OF THE STUDY

Different forage crops have different qualities including digestibility and degradation in the rumen due to differences in individual variability. Understanding the ruminal degradation of the forages and their mixtures goes hand in understanding the nutritive value of the feeds to the animals and their effect on the productivity of the animals. This study was carried out to evaluate the *in sacco* ruminal degradability of two different mixtures of winter-cereals-based silages.

MATERIALS AND METHODS

Experimental site

The trial was conducted on a medium-scale farm (Hungarian University of Agriculture and Life Sciences, Kaposvar Campus, Hungary – 46°22' N 17°48' E, 153 m altitude (GeoDatos, 2020). The different forage mixtures that were studied are Missouri (30% of two cultivars of winter oat + 40% of two cultivars of winter triticale + 10% of winter barley + 20% of winter wheat), Montana (45% of two cultivars of oats + 55% of Italian ryegrass, as well as the forage mixtures (commercial products, Agroteam S.p.a., Torrimpietre (RM), Via di Granaretto, 26, 00054 Italy), were studied. The experimental field was allocated 3 hectares to each mixture of cultivars involved in the study. The deep loosening and disc cylinder cultivation were executed as stubble tillage. To boost the fertility of the soil, 351 kg/ha artificial fertilizer (NPK: 16:16:16) was applied before sowing. The seedbed was prepared by the Kongskilde VibroFlex 7400 cultivator. The forage mixtures were sown on 29th September (75 kg seed/ha) with a depth of 3 cm with a John Deere 740 A type seed drill. Plant protection treatment was not applied during the growing period. The annual precipitation was 425 mm (World Weather Online/Kaposvár monthly climate average).

Harvesting and conservation

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. (oat: BBCH51; triticale: BBCH53; winter wheat: BBCH52; winter barley: BBCH58). 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 a 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. The wrapping was done in dry weather for plastic to stick. Then bales were stored in Hungarian University of Agriculture and Life Sciences dairy farms on a level concrete floor and the bales were arranged stacked to reduce sunlight exposure to save plastic and reduce sweating.

Ruminal degradability study

The ensiled mixtures were subjected to a 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, Hungary. Cows were fed a 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 an 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 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 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) were 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).

Calculations and statistical analysis

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.

A comparison of means for degradability components was performed following the model;

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

where Y_i is the observation in the i^{th} silage type, m is the overall mean, β_i is the i^{th} silage type effect and e_i is the random error.

A comparison of means for effective nutrient degradability was computed for 1%, 5% and 8% rumen outflow rates. Outflow rates are highly correlated with the level of feeding. Higher feed intakes result in shorter rumen retention times and faster outflow rates. Different outflow rates have been described relative to three typical feeding situations: 0.02/h for animals fed at maintenance, a low level of production, 0.05/h for animals fed at higher levels, but less than twice maintenance, e.g. calves, beef cattle, sheep and low yielding dairy cows. 0.08/h for high-yielding cows fed at more than twice maintenance.

RESULTS AND DISCUSSION

Ruminal degradability of dry matter

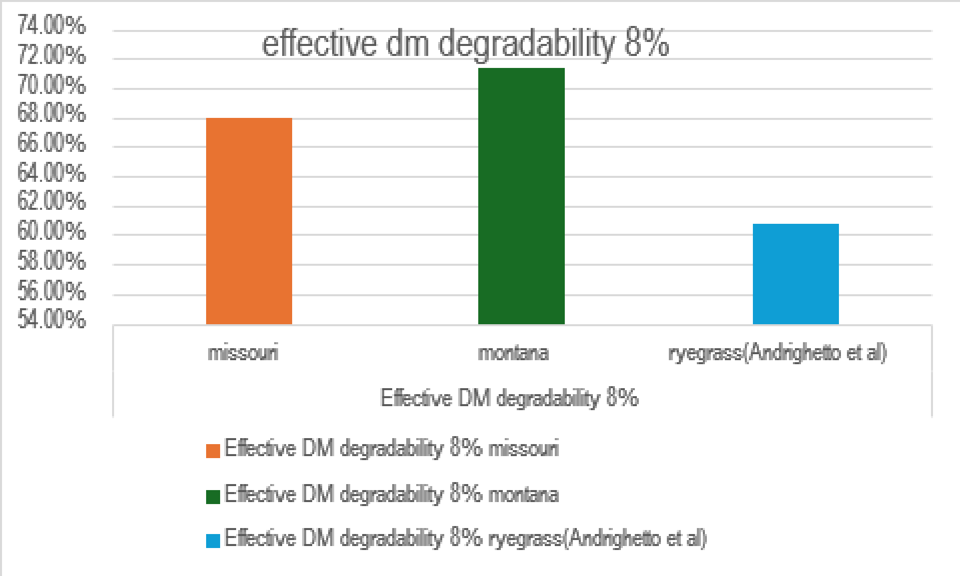
DM degradation parameters show (Table 1) that the potential degradable fraction of DM for Montana and Missouri mixtures were 62.7% and 63.7% respectively. The effective DM degradability at 1% rumen solid outflow rate (ED1), which defines the maintenance DM requirement was 73.2% and 75.4%, respectively which were higher than those reported by Andrighetto et al. (1993). However, the effective DM degradability at 8% rumen solid outflow rate (ED_1), which defines the high-yielding cows fed at more than twice maintenance was 67.9% and 71.4%, respectively. These values were higher than the DM degradability of Italian ryegrass (60.7%) reported by Andrighetto et al. (1993).

Table 1 Rumen degradation parameter and effective degradability of DM of Montana and Missouri silage mixtures

	Missouri	Montana	SEM	p-value
Soluble fraction (% of DM)	10.2[a]	13.3[b]	0.201	≤0.05
Potentially degradable fraction (% of DM)	63.7	62.7	1.257	ns
Degradation rate (%/h^{-1})	0.78	1.00	0.120	ns
Effective degradability-1 (%)	73.2	75.4	1.312	ns
Effective degradability-5 (%)	70.0 [a]	73.0 [b]	0.882	≤0.05
Effective degradability-8 (%)	67.9[a]	71.4[b]	0.650	≤0.05

Missouri: 40% of two cultivars of winter triticale + 30% of two cultivars of winter oats + 20% of winter barley + 10% of winter wheat; Montana: 45% of two cultivars of winter oats + 55% of Italian ryegrass; ns=not significant, [a,b] Means with different superscripts differ (P ≤0.05).

Graph 1 Effective DM degradability at 8%



As compared to Missouri, Montana had a significantly higher soluble DM fraction which means that the Montana mixture had a better chance of providing the animal with more nutrients for productivity compared to Missouri. There was no effect of mixture type on the fractional degradation rate of DM per hour., but the effective dry matter degradability (at 8% rumen solid outflow rate/h) of Montana was significantly higher than in Missouri.

Besides, the mixtures had a high potentially degradable DM fraction even though the difference was not significant. The results correspond with the study done by Worku et al 2021 which showed similar values in some of the mixtures used in the study for soluble fraction, potentially degradable fraction, effective degradability and degradation rate. In Work et al 2021 studies mixture A (40% of two cultivars of winter triticale + 30% of two cultivars of winter oat + 20% of winter barley + 10% of winter wheat) and mixture C (55% of three types of Italian ryegrass + 45% of two cultivars of winter oat) which were similar to the forages used in Montana and Missouri mixtures showed corresponding values (Worku et al. (2021). The similarity of the results and the study by Worku et al. (2021) is because of the similarity in feedstuffs used in the mixture and application of the same methodology.

The lower values of the study could result from the high amount of lignin and cellulose from the inclusion of cereals in the mixtures. However, these results showed higher figures when compared to the study conducted by Ma et al 2021 which showed that the ED of DM for barley, ryegrass and oats were 40.89%, 46.53% and 44.90% respectively.

Ruminal degradability of CP

The crude protein's soluble fraction (% of DM) had a significant difference between the two mixtures since Missouri recorded 68.3% while Montana recorded 18.3% at ($p < .05$).

There was also a hugely significant difference between the CP potentially degradable DM fraction where Missouri showed 16.9% while Montana showed 65.1% at ($p < .05$).

Besides, there was a significant difference in the mixtures crude protein degradation rate since Missouri has a rate of 0.22 while Montana showed 1.27 at significance levels of ($p < .05$). About effective degradation significant differences were seen at ED 8% with Missouri showing 80.6% while Montana showed 79.7% at a significance level of ($p < .05$).

Table 2 Rumen degradation parameter and effective degradability of CP of Montana and Missouri silage mixtures

	Missouri	Montana	SEM	p-value
Soluble fraction (% of DM)	68.3[a]	18.3[b]	0.358	< 0.05
Potentially degradable fraction (% of DM)	16.9[a]	65.1[b]	0.860	< 0.05
Degradation rate (%/h^{-1})	0.22[a]	1.27[b]	0.071	< 0.05
Effective degradability-1 (%)	84.5	82.9	0.798	ns
Effective degradability-5 (%)	82.0	81.0	0.668	ns
Effective degradability-8 (%)	80.6[a]	79.7[b]	0.631	< 0.05

Missouri: 40% of two cultivars of winter triticale + 30% of two cultivars of winter oats + 20% of winter barley + 10% of winter wheat; Montana: 45% of two cultivars of winter oats + 55% of Italian ryegrass; ns=not significant, [a,b] Means with different superscripts differ (P ≤0.05).

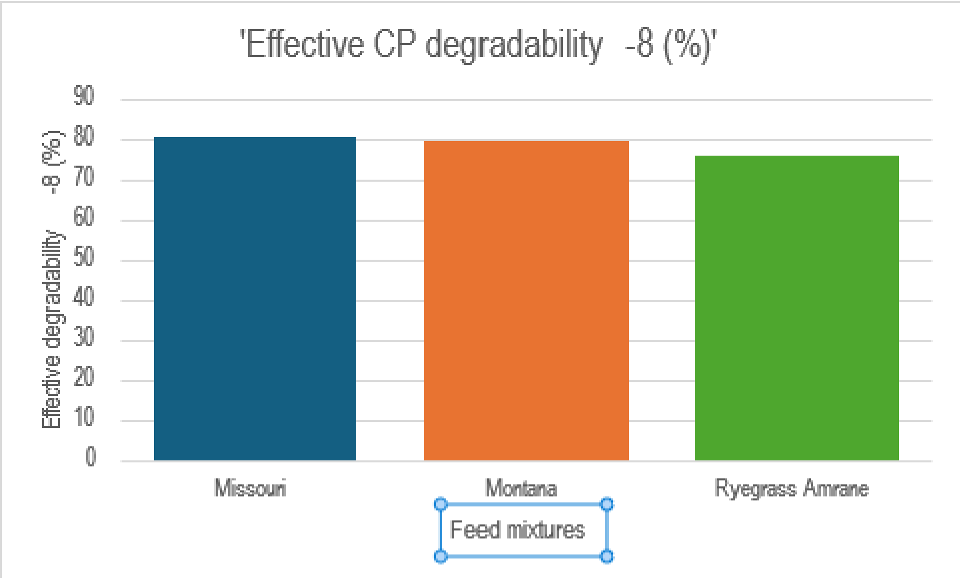
The crude protein soluble fraction represents the portion of crude protein that is quickly degraded in the rumen. The Crude protein's soluble fraction (% of DM) had a significant difference between the two mixtures since Missouri recorded 68.3% while Montana recorded 18.3% at ($p < .05$). The Missouri mixture therefore presented with the best prospect in solubility and provision of proteins to the animal because of its high soluble fraction. This soluble fraction of CP for Montana is higher than the results found in studies done by Valderrama et al (2011) which carried out in situ experiments to check rumen degradation kinetics of high protein crops. Valderramma et al. 2011 showed ryegrass degradability values of 49% which is lower than the 68.3% recorded in the Missouri mixture. The Montana mixture had a significantly lower degradability than Missouri mixture. However, the Valderrama studies were based on the vegetative stage of the ryegrass while the present study is based on the mature heading stage of the ryegrass supporting the fact that the heading stage is the best stage for high nutritive value of feeds (Kohn et al., 1995). On top of that the soluble fraction % of Missouri (68.3%) was similar to the studies done by Hadjipanayiotou et al. 1996 on oat forages which had a soluble fraction of 68.47%.

There was also a huge significant difference between the CP potentially degradable DM fraction where Missouri showed 16.9% while Montana showed 65.1% at ($p < .05$).

Besides, there was a significant difference in the mixture crude protein degradation rate since Missouri has a rate of 0.22 while Montana showed 1.27 at significance levels of ($p < .05$).

About effective CP degradation significant differences were seen at ED 8% rumen outflow rate with Missouri showing 80.6% while Montana showed 79.7% at significance level of ($p < .05$). The effective CP degradability at 8% rumen outflow rate was higher than that of Italian ryegrass fodder at the end of the heading stage (76.9%) recorded by studies done by (Amrane and Michelet-Doreau 1993). The high CP degradability at 8% rumen outflow rate can be attributed to early harvesting of all ensiled combinations and the inclusion of greater Italian ryegrass. According to Baldinger et al. (2011), Italian ryegrass had a greater CP at the second cut during harvesting, which corresponds to the CP values at the end of the 90-day fermentation period in our study.

Graph 2 Effective degradability of CP at 8% rumen outflow rate



The effective protein degradability for maximum milk protein and yield according to the National Research Council (2001) is 12.2% of the diet. In this case study the EPD is higher than 12.2% in both mixtures hence they are both effective in ensuring high production of milk and proteins. However, the Montana mixture has a higher potential degradable fraction than the Missouri mixture but on the other side, the Missouri mixture has higher figures when it comes to effective degradation at 1%, 5%, and 8%. The effective CP degradation figures in the experiment were higher than those recorded by individual ingredients in a study conducted by Ma et al. (2021) which showed that the ED for ryegrass, barley, and oats was 54.46%, 48.54%, and 50.34% respectively. Therefore, the mixing of the cereals increased the rate of degradation making the mixtures more nutritive and highly likely to support high milk-producing cattle.

Ruminal degradability of NDF and ADF

The soluble NDF fraction for the Montana mixture was higher than that of Missouri at ($p < .05$). However, there were no significant differences in the potentially degradable fractions, and effective degradability at 1%, 5%, and 8%. Regarding acid detergent fibre, the mixtures showed no significant differences in all ruminal degradability aspects studied. Potentially degradable fraction, rate of degradation and effective ruminal degradability at ED1, ED5, and ED8 for the Missouri mixture were 39.0%, 0.03%, 36.2%, 21.9%, and 17.7% while that for the Montana mixture was 33.3%, 0.03, 32%, 20.1%, and 17%. In this study, the soluble NDF and ADF fractions of all ensiled mixtures were low. Information on NDF and ADF ruminal degradability is essential because it determines animal performance (Bender et al. 2016). After all, they stipulate the plant cell wall portions including lignin, hemicellulose and cellulose and the general energy potential of the feed. ADF and NDF content also influence digestibility, feed intake and rate of passage of the feeds in the gut which is critical in determining the performance of the animals regarding productivity and growth. Unlike corn and alfalfa silages, grass silages have a higher NDF content which usually poses a challenge for nutritionists and farmers in coming up with the right formulations to satisfy the needs of the animals for maximum productivity. The soluble NDF fraction for the Montana mixture was higher than that of Missouri at ($p < .05$). However, there were no significant differences in the potentially degradable fractions, and effective degradability at 1%, 5%, and 8%. The low soluble DM fractions can be attributed to the stage of harvest which in this case was the heading stage. Early milk stages of harvesting have high soluble ADF and NDF fractions compared to late-stage harvesting. The Montana mixture had 55% Italian grass and 40% cultivars of oats which contributed to its higher soluble ADF and NDF fractions compared to the Missouri mixture that had 30% winter oats, 40% winter triticale, 10% winter barley, and 20% winter wheat.

Table 3 Rumen degradation parameter and effective degradability of NDF and ADF of Montana and Missouri silage mixtures

Neutral detergent fibre (NDF)	Missouri	Montana	SEM	p-value
Soluble fraction (% of DM)	6.9 [a]	9.5 [b]	0.536	< 0.05
Potentially degradable fraction (% of DM)	42.0	31.9	9.188	ns
Degradation rate (%/h^{-1})	0.02	0.03	0.009	ns
Effective degradability-1 (%)	38.0	34.2	4.467	ns
Effective degradability-5 (%)	22.2	22.5	1.295	ns
Effective degradability-8 (%)	18.0	19.1	0.788	ns
Acid detergent fibre (ADF)				
Soluble fraction (% of DM)	6.2	8.0	0.887	ns
Potentially degradable fraction (% of DM)	39.0	33.3	4.725	ns

Degradation rate (%/h^{-1})	0.03	0.03	0.017	ns
Effective degradability-1 (%)	36.2	32.0	0.992	ns
Effective degradability-5 (%)	21.9	20.1	0.348	ns
Effective degradability-8 (%)	17.7	17.0	0.918	ns

The study also showed that the potentially degradable as well as effective degradable NDF and ADF at 0.01 and 0.08h^{-1} rumen outflow rates (ED_1 and ED_8) were low. The theoretical ruminal NDF degradability of all ensiled combinations was lower than that of Italian ryegrass (59.8%), as reported by Andrighetto et al. 1993. The same higher NDF degradability values (76.4%) were reported by Ali et al. (2014) than the current grass silage. However, the rate of ruminal NDF degradation for both Montana and Missouri mixtures were higher than the whole crop cereal silage reported by Weisbjerg et al. (2007).

The amount and ruminal degradability of NDF is a very important factor in the dairy cow's nutrition because first and foremost NDF is the precursor for volatile fatty acids which provide cows with energy for maintenance and production. It also influences the extent and rate of digestion of fibre in the rumen with preference being given to highly degradable NDF sources. However, very highly degradable NDF sources cause metabolic problems like acidosis. Research has shown that the amount and degradability of NDF in the diet can impact milk production and composition (Shi et al. 2023). While too little NDF can compromise rumen function and reduce milk yield, excessively high levels or poorly degradable NDF can limit dry matter intake and hinder milk production efficiency. Besides, adequate NDF intake is necessary for promoting proper chewing behaviour and saliva production, which helps maintain rumen pH and prevent digestive disorders. Additionally, sufficient fibre in the diet supports optimal body condition and overall cow health (Shi et al. 2023).

Regarding acid detergent fibre, the mixtures showed no significant differences in all ruminal degradability aspects studied. Potentially degradable fraction, rate of degradation and effective ruminal degradability at ED1, ED5, and ED8 for Missouri were 39.0%, 0.03%, 36.2%, 21.9%, and 17.7% while that for Montana were 33.3%, 0.03, 32%, 20.1%, and 17%. The results from the study showed similar results to ruminal effective degradation studies conducted by Ma et al (2021) which showed that the ED for ryegrass, barley, and oats was 36.36%, 30.68%, and 29.36% respectively.

CONCLUSIONS

The ensiled mixtures in this experiment had high effective dry matter (DM) and crude protein (CP) degradability at the three rumen outflow rates and moderate DM and CP degradability potential. In comparison to the Missouri mixture that only contained four varied species of winter cereals, the Montana mixtures with Italian ryegrass demonstrated higher effective degradability (ED8) and lower degradability of NDF and ADF (ED8). It is possible to feed Italian ryegrass and winter forages to lactating dairy cows because of the mixtures' enhanced capacity for degradability. Fibre degradability in the rumen can be assisted by the use of ensiling additives, or possibly using exogenous fibre-degrading enzymes, even in the event of harvesting in a later phenological phase. To confirm this, it is recommended to carry out additional feeding experiments.

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ABSTRACT OF THESIS

This study was conducted using multiparous non-lactating rumen-cannulated Holstein-Friesian dairy cows with the objective of evaluating the in-sacco ruminal degradability of two different winter cereal-based diets namely Missouri (30% of two cultivars of winter oat + 40% of two cultivars of winter triticale + 10% of winter barley + 20% of winter wheat) and Montana mixture (45% of two cultivars of oats + 55% of Italian ryegrass. In sacco ruminal degradation studies were conducted to understand the degradability of CP, DM, NDF and ADF of the mixtures. The effective DM degradability at 8% rumen solid outflow rate (ED_1), which defines the high-yielding cows fed at more than twice maintenance was 67.9% and 71.4%, respectively, the ED of CP at 8% was 80.6% for Missouri while Montana showed 79.7% at a significance

level of ($p < .05$) and the effective ruminal degradability at ED1, ED5, and ED8 for the Missouri mixture were 39.0%, 0.03%, 36.2%, 21.9%, and 17.7% while that for the Montana mixture was 33.3%, 0.03, 32%, 20.1%, and 17%. These results suggest that the mixtures had improved degradation in the rumen and could be applied as a source of feed to replace partially or supplement traditional forages in high producing dairy cows.