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Nutritive value of ensiled Italian ryegrass and winter cereal mixture in dairy cow

MASTER'S THESIS (MS**c**)

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1. INTRODUCTION

The livestock industry, farmers' income and global food security have all been negatively affected by extreme weather conditions caused by climate change (Lipper et al., 2014). For example, heat stress costs the global cattle industry billions of dollars in revenue losses (Osei-Amponsah et al., 2019). In the United States alone, the livestock industry experiences economic losses of $1.69 to $2.36 billion annually because of heat stress, with the dairy industry accounting for $897 to $1500 million of these losses (Sejian et al., 2024; Sordillo, 2016).

The Carpathian Region's research indicates that the average annual temperature could rise by 1-4°C by the end of the 21st century (Dumitrescu et al., 2022; Probst & Mauer, 2023). From 2071 to 2100, the number of summer days per year could reach up to 60 in the northern part and over 100 in the southern part of the Carpathian Basin. Researchers project a rise in daytime temperatures of 3 to 5 degrees Celsius by 2071 (Ciupertea et al., 2017; Skarbit et al., 2022) and 5 to 7 degrees Celsius by the end of the 21st century. This could result in a higher temperature of 44 to 47°C between 2070 and 2099. Therefore, a rise in heat stress is predicted in both plants and animals. Irrigation can help lessen drought but managing heat stress is more challenging. Corn (Zea mays) is especially susceptible to heat stress and cannot tolerate high temperatures for prolonged periods of time due to its shallow root structure and high-water requirements during critical growth stages. Dairy cows are also susceptible to heat stress; even in sophisticated, well-equipped barns, it becomes an issue where the temperature is already above 24 ºC. According to Baumgard et al. (2011), climate alone does not lead to heat stress; dairy cows may experience heat stress due to rumen fermentation or decreased appetite, which can lead to various physiological changes such as hyperthermia and oxidative stress.

These alterations can cause a decrease of 25% to 40% in milk output and feed intake. Together with increased metabolic problems, delayed reproduction, reduced immune systems from high blood pressure and decreased milk production, these factors all lead to financial losses in the dairy industry.

Nevertheless, research indicates that during drought conditions, the yield stability of corn varies. Winter cereals and Italian ryegrass typically outperform corn in terms of yield stability when water is scarce (Scordia et al., 2019). Spring forages like Italian ryegrass (Lolium multiflorum Lam.) and winter cereals have high yield potential, producing more than 3 Mg ha-1 of dry matter. They consistently produce high-quality forage with total digestible nutrient (TDN) values above 700 g/kg when harvested at the boot stage and provided with 50 kg N/ha Harvesting at a later stage will increase forage yield but be of low quality, as higher fibre content in forage may lead to reduced protein and energy, decreased palatability and nutrient dilution (Landry et al., 2019). Furthermore, winter cereals have shown improved drought tolerance when treated with bacterial biostimulants, which produce higher carbon dioxide assimilation, reduced transpiration and better performance of the photosynthetic apparatus under drought stress (Hao-Kai et al., 2023). On the other hand, studies on Italian ryegrass found that specific proteins related to carbohydrate and amino acid metabolism, as well as signal transduction pathways, are differently accumulated in response to drought and this indicates the role of ryegrass in enhancing drought tolerance. Winter cereals and Italian ryegrass offer more stable yields compared to corn under drought conditions, making them the best alternatives for animal feed formulation.

However, there is limited knowledge about the nutritional value of different mixtures of Italian ryegrass and winter cereals (triticale, oats, wheat, and barley) silages.

Therefore, this study aims to assess the nutritional composition, fermentation characteristics and microbial counts of these two different mixtures.

1.1. Goal of the study

The main objective of this study is to evaluate the nutritional value of ensiled Italian ryegrass and winter cereal mixtures as feed sources for dairy cows. Specifically, the study aims to assess the nutritional composition, fermentation characteristics and microbiological counts of the ensiled forage mixtures, which include Italian ryegrass and various winter cereals such as triticale, barley, oat and wheat. The significance of this research lies in providing valuable insights into the potential of these forage mixtures to be used as high-quality feed options for dairy cattle.

2. LITERATURE REVIEW

Corn (Zea mays L.) belongs to the world's most important food, industrial and feed crop. According to the World Food and Agriculture Organization of the United Nations (FAO), more than 600 basic products and by-products are produced worldwide from corn (Fagodiya et al., 2020 & Christopher Michael et al., 2019). It is the principal ensiled crop in European countries, North America, New Zealand, and Australia (Allen et al. 2003; Keady et al. 2012) with average feeding rates of 3719.46 and 2449.40 Kg dry matter (DM) corn silage/cow/year in Europe and Northern America respectively, (Kleinmans et al. 2016). It has been known to be one of the best supplementation options, especially in dry seasons, because of its high production capacity per unit area, high green mass yield. Corn silage is typically composed of 25–35 % starch and 40–50 % neutral detergent fiber (NDF) which acts as a significant substrate for rumen fermentation, leading to the production of substantial amounts of propionic acid, which is a powerful source of energy (Hoffman et al., 2009 & NRC, 2001). In corn silage, the concentration and digestibility of starch in corn kernels are affected by hybrid type, harvesting stage, kernel processing and length of storage (Der Bedrosian et al., 2012; Ferraretto et al., 2015). Nutritional parameters such as starch content, crude protein (CP), ether extract (EE) and in vitro 30-h NDF digestibility of corn silage and fermentation parameters (lactic acid and ammonia) were the best predictors of mean daily milk yield of lactating dairy cows when fed corn silage-based diets (Tharangani et al., 2021). Higher digestibility of starch results in improved energy availability for dairy cows, thus increasing milk production (Ferraretto et al., 2015).

However, the variations that occur in the chemical composition of silage, as well as changes in fermentation characteristics, can influence intake and milk production (Khan et al., 2015). Heat stress can greatly affect the productivity and quality of silocorn, particularly in arid and high-temperate regions (Sabagh et al., 2020).

The occurrence of thermal waves, especially in the months of June, July, and August, can lead to a reduction in crop quality and even plant death (Ouda et al., 2022). Drought and heat stress can also affect the yield and composition of corn silage, with high temperatures inducing kernel abortion which potentially decreases fiber digestibility (Ferreira, 2016). It is evident that high temperatures hinder both vegetative and reproductive stages of a maize plant, i.e., from germination until maturity, but anthesis and silking stages are the most influenced by the increasing temperature after exceeding 35 °C during the time of pollen shed, which affects pollen reception: firstly, as pollen grains burst and silks do not emerge at all, resulting in very poor pollination and barren ears; and secondly, as initially emerging silks at the base of the ear affect as compared to the tip of the ear for late emerging silks (Waqas et al., 2021). Also, under heat stress conditions, plants accumulate more Reactive Oxygen Species (ROS) and lipid peroxidation, which alters the photosynthetic process and directly affects growth, flowering, and decreasing seed-filling duration, leading to smaller seed sizes, fewer numbered seeds, decreased leaf area expansion and poor-quality forage production (Hussain et al., 2019). Poor quality forages are characterized by low protein with high fiber and lignin content, which can negatively impact livestock productivity (Alemayehu et al., 2019).

Also, high temperature during grain filling can have a severe impact on grain quality, including changes in the accumulation and distribution of both macro and micronutrients, alterations in starch and protein composition.

It is of no doubt that heat stress has a significant effect on the dairy cow industry and this sector suffers from a significant financial burden when milk production decreases by 10 to 35% during the hot summer (Key et al., 2014). Heat stress, in the context of a dairy cow, can be thought of as the sum of all pressures that cause the cow to make modifications on scales from molecular to ecological to prevent physiological malfunction and better suit its environment (Sesay, 2023; Kadzere et al., 2002).

The ideal temperature range for lactating cows is 5 to 25°C where milk production is at its highest (Kadzere et al., 2002). There may be a 10%-40% drop in milk production if cows are exposed to atmospheric temperature above the maximum limit of their comfort zone (Michael et al., 2021). The cow is under heat stress when outside temperatures exceed 26°C and cannot cool off properly. Comfortable temperatures have a temperature humidity index (THI) of 70 or less and a stressful temperature of 75-78 and when THI increases to more than 70, growth hormone levels in milk from cows with low, medium, and high production decreases, indicating that growth hormone production was repressed to reduce metabolic heat output (Roushdy et al., 2018). High temperatures of 35°C reduce the plasma concentration of growth hormone and the rate at which it is secreted (Collier et al., 2008; Roushdy et al., 2018). During heat stress, the thyroid hormones triiodothyronine (T3) and thyroxine (T4) decrease, which they attributed to efforts to reduce their metabolic heat output (Kadzere et al., 2002; Settivari et al., 2007).

Heat stress increases digesta volume and water content in the rumen, making it more able to act as a pool of water to mitigate the impact of stress on rumen motility (Meneses et al., 2021). Milk production decreases due to HS because the rostral cooling region of the hypothalamus stimulates the medial centre of satiety, which impedes the lateral appetite center (Jose et al., 2020). The lactating cows' feed intake decreases at an atmospheric temperature (AT) of 26°C (Kadzere et al., 2002) and this decrease can reach 40% at an AT of 40°C (Tao & Dahl, 2013). In addition to consuming less feed, heat-stressed cows alter their feeding regimens to lower their endogenous heat output during the warmest hours. Depending on the degree of dietary fiber, ruminal fermentation contributes between 3 and 8% of the total endogenous heat produced by bovines (Min et al., 2019). Heat stress increases oxidative stress, which changes mammary secretory tissue cell metabolic and molecular activity, decreasing cellular efficiency for synthesizing milk components (Guo et al., 2019).

Exposed dairy cows to HS, decrease milk production by 35% in medium-lactation cows but only by 14% in early lactation cows this is due to the genetic potential, stage of lactation and degree of heat (Bernabucci et al., 2010). Clinical or subclinical health problems are caused by the impact of HS on the physiological processes of the high-producing cow as it is evident that 24% of cows who gave birth in the summer retained the placenta and developed postpartum metritis, while this occurred in just 8% of cows during the cooler months of the year (Temesgen et al., 2022). Additionally, cows with retained placenta and postpartum metritis were shown to have longer gestation periods than those without these symptoms (Dubuc et al., 2011). Compared to the colder months, scorching summer caused 11% greater ketosis in dairy cows (Wu, 2020).

Generally, the energy content of ruminant feeds has not been analyzed but charts prepared by the NRC or chemical analysis values are used instead (Abas et al., 2005)

The net energy content of forage feeds, particularly in the context of silage, is a critical factor in determining feed ratio. Pinho et al., (2011) found that the energy efficiency of forage production is influenced by factors such as defoliation systems and nitrogen application rates. This is further supported by Gordon (1999), who found that wilting and forage additives did not significantly impact energy utilization in grass silage. However, the digestibility of neutral detergent fiber (NDF) in maize silage was found to be a key determinant of dry matter intake and milk production in dairy cows (Krämer-Schmid, 2016).

In the context of forage-animal nutrition modelling, the animal's dietary energy is estimated mainly from the TDN of the forage used because its digestibility is essentially a function of its energy content (Woli et al., 2020). The TDN is directly related to digestible energy (DE) and is useful for characterizing animal feeds and forages. The TDN is the first limiting factor in accurately predicting the useful energy in a feedstuff because it is used to predict DE, which, in turn, is used to predict modelling efficiency, from which net energy may be predicted (Tedeschi and Fox, 2020). The evaluation of feeds for whole tract and ruminal digestion through in vivo experiments is time-consuming, laborious and expensive and requires large quantities of feed samples (Becker, 1998). For these reasons, other techniques have been developed in situ and in vitro, to predict the rumen degradation of feed.

The energy content of forage is influenced by fermentation characteristics during the ensiling process, which can affect digestibility and nutritional value (Grant & Ferraretto, 2018).

Some studies have replaced silage with hay to evaluate fermentation characteristics or pH, but these studies were often complicated by factors such as forage NDF content, digestibility, or other factors that affect dry matter consumption (Allen, 2000). Several studies have also shown that silage with higher acid concentrations and a lower pH, which are a result of the fermentation process, can limit the energy content in daily feed intake (Grant & Ferraretto, 2018). Proper fermentation can enhance energy content by increasing nutrient availability for animals, while improper fermentation can lead to nutrient losses and reduced energy content. Several fermentation characteristics, including pH, lactic acid production, and microbial activity, play a crucial role in ensuring high-quality forage for animal nutrition (Grant & Ferraretto, 2018). Studies have found that reducing the pH in silage mixtures prevents plant protein breakdown and inhibits spoilage microbe growth (Queiroz et al., 2018).

Additionally, nutrient preservation contributes to maintaining higher energy content in forage and different types of fermentation microbes in silage can impact the breakdown of fiber and other components, thereby influencing digestibility and energy availability to animals (Kim et al., 2021).

Italian ryegrass (*Lolium multiflorum*) is one of the fast-growing grass species with high forage yields, high fiber digestibility (NDFD), high crude protein and sugar content, palatability, resistance to winter hardiness, ease of establishment, high yield response to nitrogen and suitable for silage making (Baldinger et al., 2011; Alemayehu et al., 2021) However, Alemayehu (2019) reported that the yield of Italian ryegrass is not as high as winter cereals such as oats, but nutrient quality and palatability are greater which makes it more suitable for high-producing dairy cow feed.

In addition, there has been tremendous research work over the years by various researchers such as Van Duinkerken et al. (1999), Bender et al. (2016), Baldinger et al. (2011; 2014), Harper et al. (2017) in an attempt to replace corn silage with Italian ryegrass, annual ryegrass and winter cereals (wheat and triticale) silages. Van Duinkerken et al. (1999) reported that there is no significant difference in either feed intake or lactation performance between triticale whole crop silage and corn silage. However, the calculated intake of net energy for lactation was lower at the triticale-based ratio. The author concluded that the net energy value for lactation is underestimated for triticale whole crop silage. Harper et al. (2017) noted that at milk production of around 42 kg/d, wheat silage and triticale silage can partially replace corn silage without affecting DM intake, but milk yield may slightly decrease. For dairy farms in need of more forage, triticale or wheat double-cropped with corn silage may be an appropriate cropping strategy.

On the other hand, Bender et al. (2016) reported that substituting ryegrass silage for a portion or all of the corn silage in diets fed to lactating dairy cows can improve the yield of milk and components. Baldinger et al. (2011) reported that the inclusion of Italian ryegrass silage in the diet increased forage intake significantly (14.5 vs. 13.4 kg DM in the control group) and concentrate intake did not differ, but milk yield was slightly lower (20.3 vs 21.0 kg) owing to the low energy and protein concentration of Italian ryegrass silage.

They further reported that Italian ryegrass was indeed found to be highly palatable, confirming in principle its suitability as feed for organic dairy cows (Baldinger et al., 2011).Furthermore, higher energy and protein concentrations in Italian ryegrass forage would be necessary to translate the high intakes of Italian ryegrass silage into improved milk production as well.

However, harvesting at the appropriate time increases the amount of water-soluble carbohydrate (WSC) contents which is a determinant for the fermentation process during ensiling. Harvesting grass for silage at an early stage of maturity is expected to result in silage with a high concentration of energy and CP and may thereby be a prerequisite for high energy intake and production (Randby et al., 2012). This is further supported by the effectiveness of Italian ryegrass and barley mixtures in preventing Italian ryegrass lodging and increasing forage yield (Seo et al., 2010). In dairy farming systems, winter forage crops are the most frequently annual grasses grown in monoculture (Brown et al., 2018). Hence, the composition of Italian ryegrass at harvest has a great influence on silage fermentation products.

Andrighetto et al. (1997) reported that Italian ryegrass silage harvested at the flowering stage had higher butyrate and propionate contents and a lower concentration of lactic acid. Shao *et al*. (2007) found moderate lactic acid contents and higher ammonia nitrogen (NH_3-N) and butyric acid concentrations in vegetative Italian ryegrass silage. By contrast, Li and Nishino (2013) reported that 2,3-butanediol and ethanol were the predominant fermentation products in late-heading Italian ryegrass silage. These large differences in fermentation products could be mainly ascribed to the variations in the chemical compositions and phyllosphere microbiota of the harvested Italian ryegrass.

The phyllosphere is populated by a large and diverse microbiota of bacteria, fungi, yeast, archaea and other microorganisms that have commensal, pathogenic and mutualistic interactions with the host plant (Rastogi et al., 2013). Hoffman et al. (2011) reported that ensiling high-moisture corn for 240 days reduced zein protein subunits that cross-link starch granules and suggested that the starch-protein matrix was degraded by proteolytic activity over an extended ensiling period. Ensilability indices, such as DM and water-soluble carbohydrate concentrations and buffering capacity, indicated that satisfactory fermentations were likely if such crops were ensiled; buffering capacity, generally declined with advancing maturity. It is concluded that winter cereal grain (barley, wheat and triticale) DM yields and quality were relatively constant as ripening progressed from DM concentrations of around 550 to > 800 g/kg (Stacey et al., 2006).

Ensiling is an effective method to overcome the discrepancy between the seasonal imbalance of available forage and yearlong livestock production. It is based on solid-state lactic acid fermentation under anaerobic conditions whereby lactic acid bacteria (LAB) convert water-soluble sugars into organic acids, mainly lactic acid. As a result, the pH decreases, and the moist crop is preserved. However, this process is strongly affected by biomass characteristics such as buffering capacity, water-soluble carbohydrate (WSC), DM and epiphytic microorganisms (Guo et al., 2019).

Air is detrimental to silage because it enables plant respiration and the activity of aerobic spoilage microorganisms such as yeasts and moulds (Archundia et al., 2001). Therefore, many practices applied during ensiling, storage and feeding are intended to exclude air from the silage. Other practices that promote successful ensiling include the application of silage additives.

The ensiling process involves many steps that should be timed and controlled carefully to ensure successful ensiling with minimal losses (Weinberg et al., 2003)

The ensiling operation consists of the following steps: harvesting the crop at the optimal stage of maturity, wilting (if possible) to ensure adequate DM content depending on crop for a solid-state lactic acid fermentation, chopping, loading into a silo, compacting and sealing to exclude air, storing and finally unloading for feeding to the animals. Additives can be applied during the chopping or loading steps.

The biochemical and microbiological events that occur during ensiling can be divided into four distinct stages (Weinberg et al., 2008).

I. Aerobic processes during filling and immediately after sealing while air is still present between the plant particles and the pH is 6.0–6.5. In this stage, plant respiration continues as well as plant proteolysis and the activity of aerobic microorganisms such as enterobacteria, fungi and yeasts.
II. Fermentation, which is carried out by a dynamic succession of LAB which changes according to the conditions prevailing in the silage, starting with *Enterococcus* and *Leuco nostoc*, followed by *Lactobacillus* and *Pediococcus* species.

Lactic acid and organic acids accumulate and the pH decreases to below 5.0, depending on plant composition and buffering capacity.

III. Storage, during which the silage is sealed, little air penetrates, and only a few changes occur.

IV. Unloading for feeding, during which the silage is reexposed to air, reactivating aerobic microorganisms, mainly yeasts and moulds, that may spoil the silage.

Microbial counts are a crucial parameter in ensiling, as they can significantly impact the preservation of the crop and the growth of detrimental microorganisms.

The fermentation process during ensiling, particularly the production of lactic acid by lactic acid bacteria, plays a key role in controlling microbial activity (Muck, 2013). This process can inhibit the growth of spoilage microorganisms such as yeasts, moulds, and aerobic bacteria (Muck, 2013) However, the presence of certain microorganisms, such as Lactobacillus plantarum, can influence the quality and stability of the silage (Keshri et al., 2019). The gaseous environment and temperature during ensiling can also affect the microbial populations, with air being detrimental due to its potential to reactivate aerobic microorganisms (Weinberg et al., 2008). Therefore, understanding the microbial counts and dynamics during ensiling is crucial for ensuring the quality and stability of the silage.

3. MATERIALS AND METHODS

3.1. Experimental site and ensiling procedure

The trial was carried out on a medium-scale farm located at the Hungarian University of Agriculture and Life Science, Kaposvár Campus (46°22' N 17°48' E, 153 m altitude) Geodatos, (2020).

3.2. Forage mixtures

The following forage mixtures (commercial products, Agroteam S.p.a., Torrimpietre (RM), Via di Granaretto, 26, 00054 Italy) were studied: Texas: consisting of 50% of two cultivars of winter triticale + 40% of winter barley + 10% of winter wheat. Montana: consisting of 55% of three types of Italian ryegrass + 45% of winter oats.

3.3. Tillage and fertilizer application

The experimental field allotted 3 hectares to each mixture. Deep loosening and disc + cylinder cultivation were executed as stubble tillage. Before sowing, 351 kg/ha artificial fertilizer (NPK: 16:16:16) was applied. The seedbeds were prepared by the Kongskilde VibroFlex 7400 foldable tined thebble cultivator, mainly designed for primary tillage after harvest. The forage mixtures were sown on 29th September (75 kg seed/ha) with a depth of 3 cm with the John Deere 740 A type seed drill.

3.4. Plant protection and environmental conditions

No plant protection treatment was applied during the growing period and the annual precipitation was 425 mm (World Weather online/Kaposvár monthly climate average).

3.5. Harvesting

Cutting was executed on the 4 May. The BBCH (Biologische Bundesanstalt für Land-und Forstwirtschaft, 1997, a system for uniform coding of phenologically similar growth stages of all mono- and dicotyledonous plant species) code was 51-58, principal growth stage 5: Inflorescence emergence (main shoot)/heading. (Italian ryegrass: BBCH 51; oat: BBCH 51; triticale: BBCH 53; winter wheat: BBCH 52; winter barley: BBCH 58). The fresh mixtures with nutritional composition (Table 1) were wilted to 35% DM (24h) without any movement on the windrow. The wilted forage was chopped using a forage harvester (John Deere 7300) on a concrete surface with a theoretical chop length (TCL) of 9 mm (weight of wilted and chopped forage: 800 kg).

3.6. Fermentation process and storage

Wilted and chopped materials of (510 g) were packed into laboratory silo/glass jars with a capacity of 0.72 litter using a mechanical hand packer without any additives and the materials were ensiled for 90 days. Each treatment consisted of 5 laboratory silos. Then the silos were stored in a laboratory of the Hungarian University of Agriculture and Life Sciences at a temperature of 21 °C.

Table 1. Nutritional compositions of fresh forage mixtures right before ensiling (n = 20)

	Forage mixtures	
Components	Texas	Montana
Dry matter, g	184	168
Crude protein, g/kg DM	117	108
Neutral detergent fiber, g/kg DM	579	535
Total sugar, g/kg DM	166	168

3.7. Chemical analysis

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

While acetic acid, butyric acid, propionic acid and ethanol were measured by gas chromatography (Chrompack, Model CP 9002, The Netherlands) as described by Playne (1985).

Ammonia concentration was determined by a modified Berthelot method (Chaney and Marbach, 1962).

3.8. Microbiological counts

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

3.9. Statistical analysis

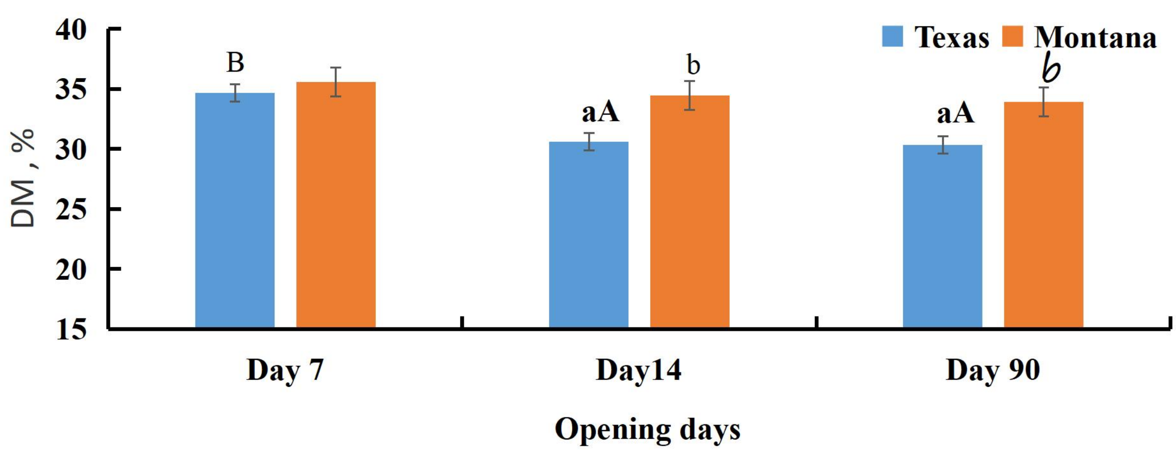
The experimental data were analyzed with one-way ANOVA (SAS Copyright © 2021 SAS Institute Inc. All Rights Reserved. | Release 3.1.0). In case of any significant treatment effects, the Tukey test checked the differences among the experimental groups (SAS OnDemeand for Academics, 2021) A significance level of $P<0.05$ was used. The variables for nutritional composition, fermentation characteristics and microbiological count among the three opening days, for the different crop mixtures and their interaction were computed using the following model: $Y_{ij} = \mu + \alpha_i + \beta_j + \gamma_{ij} + \varepsilon_{ij}$, where Y_i is the observation in the i^{th} different opening days, j^{th} crop mixture and their interaction, μ is the overall mean, α_i is the i^{th} opening days effect, β_j is j^{th} crop mixture effect, γ_{ij} is the interaction of opening days and crop mixture and ε_{ij} is the random error.

4. RESULTS AND DISCUSSION

4.1. Nutritional composition of ensiled mixtures

The DM content of ensiled mixtures was increased as compared to fresh green forage. This result is highly important from the silage-making point of view due to the challenge of producing high-quality forage as silage with avoiding DM losses. Crop mixture interaction shows a significantly higher ($p<0.05$) difference in the DM content between Texas and Montana on all opening days except day 7 (Figure 1). When compared to Texas, Montana had a significantly high content of DM on all opening days which implies that the Montana mixture of silage had the advantage of providing more nutrients for better performance compared to Texas. The statistical findings of the result also reveal that after day 7 of fermentation, there was no further significant decrease or increase of DM content in both Texas and Montana.

Figure 1. Dry matter content of mixture silages on opening days 7, 14 and 90



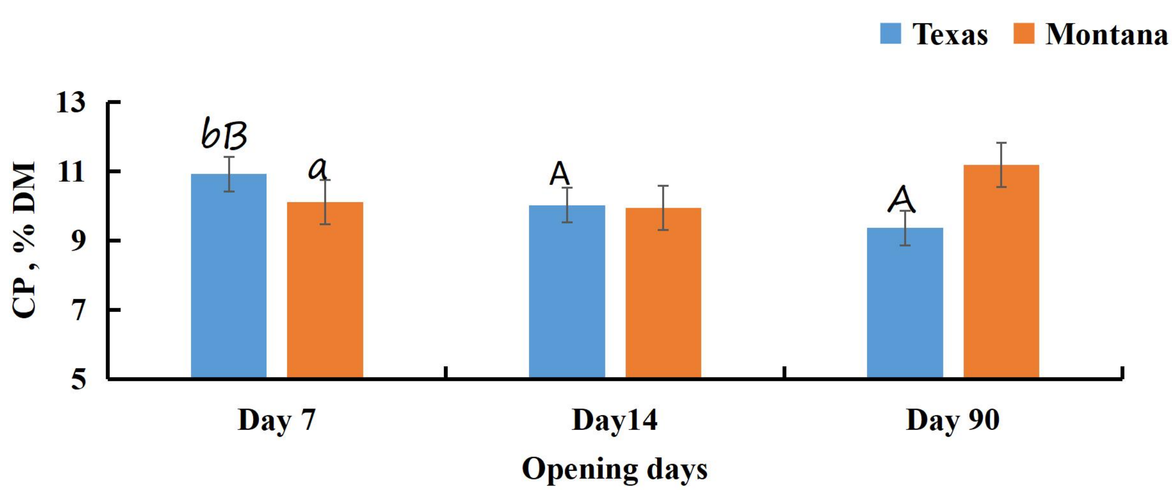
[a, b] Means with different superscripts different ($p<0.05$) (Crop mixture effect)

[A, B] Means with different superscripts different ($p<0.05$) (Opening days effect)

At the end of the fermentation period (day 90), the DM content obtained for both Texas (30.32%) and Montana (33.94%) silages fell within the optimal DM content of corn silage but was lower than the DM content of Italian ryegrass silage (36.5%) and winter cereals silage: barley (35.5%), oat (34.6%), triticale (32.0%) and wheat (33.3%) except for Montana which exceeded triticale and wheat silages (National Research Council (NRC) 2001). Our present result is in line with the findings of Bolsen. (1996) reported that once fermentation becomes stable at a very low pH, DM content in silage reduces.

Crop mixture interaction had no significant effects ($p>0.05$) on the crude protein content between Texas and Montana on all opening days except on day 7 (Figure 2). At day 90, Montana achieved the highest CP, content (11.18%) outperforming Texas (9.36%).

Figure 2. Crude protein content of mixtures silages on different opening days

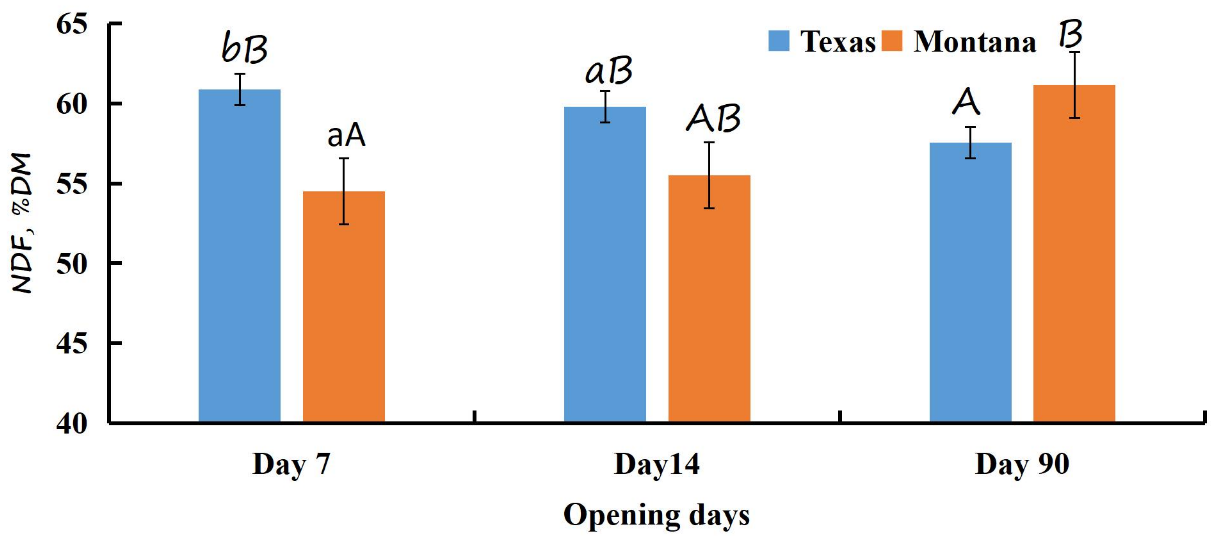


[a, b] Means with different superscripts different ($p<0.05$) (Crop mixture effect)

[A, B] Means with different superscripts different ($p<0.05$) (Opening days effect)

Results further indicate that CP content decreases with every increase in treatment duration in both Texas and Montana except on opening day 90 in Montana. The CP contents of both mixtures especially Texas were below the estimated values of Italian ryegrass silage (12.8%) and winter cereals silage: barley (12.0%), oat (12.9%), triticale (13.8%) and wheat (12.0%) (National Research Council (NRC) 2001). The high CP value in Montana than Texas may be due to the higher proportion of Italian ryegrass (55%) and Oat (45%) in the total mixed ensiled forage and Italian ryegrass has more protein than cereals (Baldinger et al. 2011, 2014; Byron seeds LLC. 2019; DLF seeds UK 2018). Crop mixture interaction had no significant (P>0.05) decreasing effects in the NDF content between Texas and Montana on all opening days except day 7 (Fig. 3). However, on all opening days except day 90, Montana had the lowest content of NDF suggesting that it may promote better feed intake and overall digestibility as compared to the Texas mixture.

Figure 3. Non digestible fibre (NDF) of mixture silages on different opening days



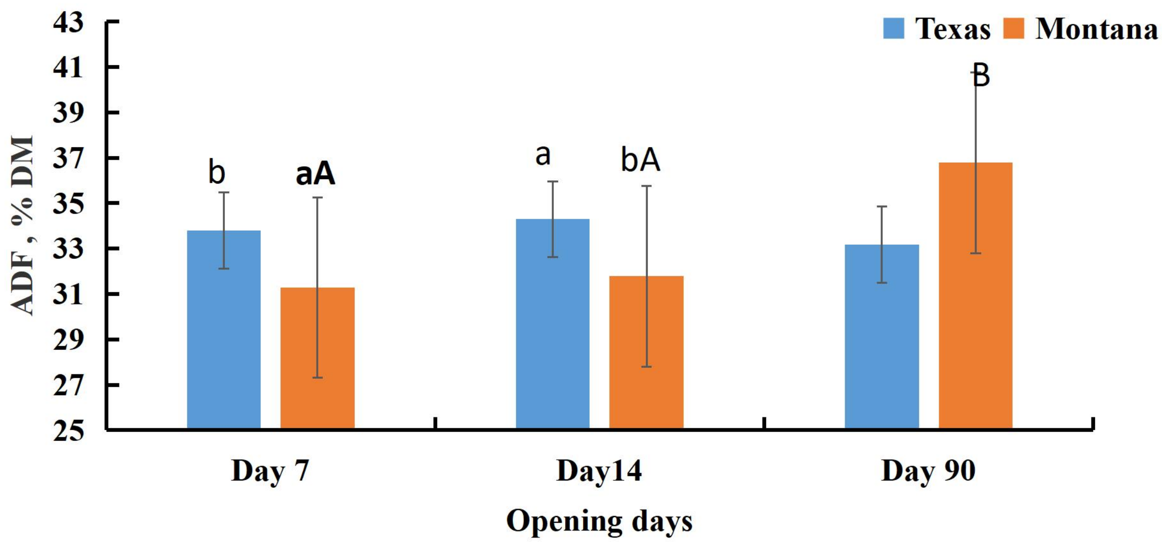
[a, b] Means with different superscripts different (p<0.05) (Crop mixture effect)

[A, B] Means with different superscripts different (p<0.05) (Opening days effect)

The NDF content in Texas kept on reducing with time while Montana gradually increased during each opening period this may be attributed to the fermentation conditions influencing nutrient breakdown rates. Crop mixture interaction had no significant (P>0.05) decreasing effects in the NDF content between Texas and Montana on all opening days except day 7. However, on all opening days except day 90, Montana had the lowest content of NDF suggesting that it may promote better feed intake and overall digestibility as compared to Texas mixture.

The statistical analysis of ADF content of crop mixture interactions shows a significant difference (p<0.05) between Texas and Montana throughout the fermentation period except day 90 (Fig. 4).

Figure 4. ADF content of mixture silages on different opening days



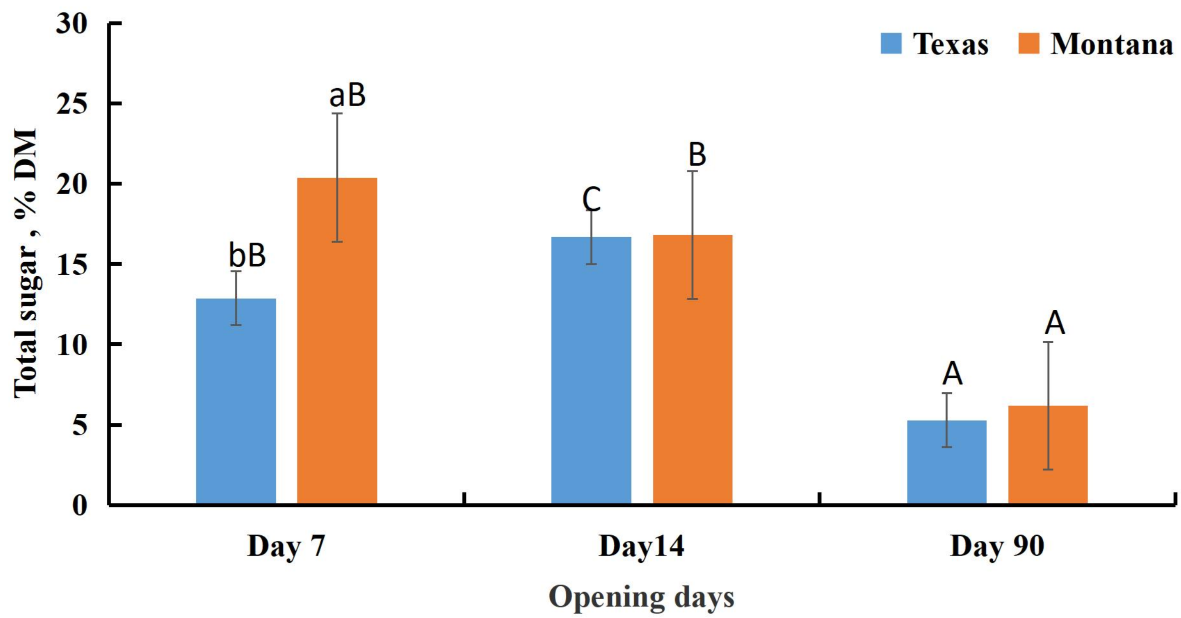
[a, b] Means with different superscripts different (p<0.05) (Crop mixture effect)

[A, B] Means with different superscripts different (p<0.05) (Opening days effect)

Results further reveal that Montana had low ADF content on opening day 7 and 14 as compared to Texas on all opening days. The high content of ADF in Texas as compared to Montana on day 7 and 14 implies low digestibility, low feed intake and low energy supply by Texas silage mixture which may eventually lead to low productivity. However, at the end of the 90 days of fermentation, Montana had the highest content of ADF as compared to any other day.

Crop mixture interaction on opening day 7 shows a significant difference ($p<0.05$) in the total sugar content between Texas and Montana when compared to days 14 and 90 (Figure 5).

Figure 5. The total sugar content of mixture silages on different opening days



[a, b] Means with different superscripts different ($p<0.05$) (Crop mixture effect)

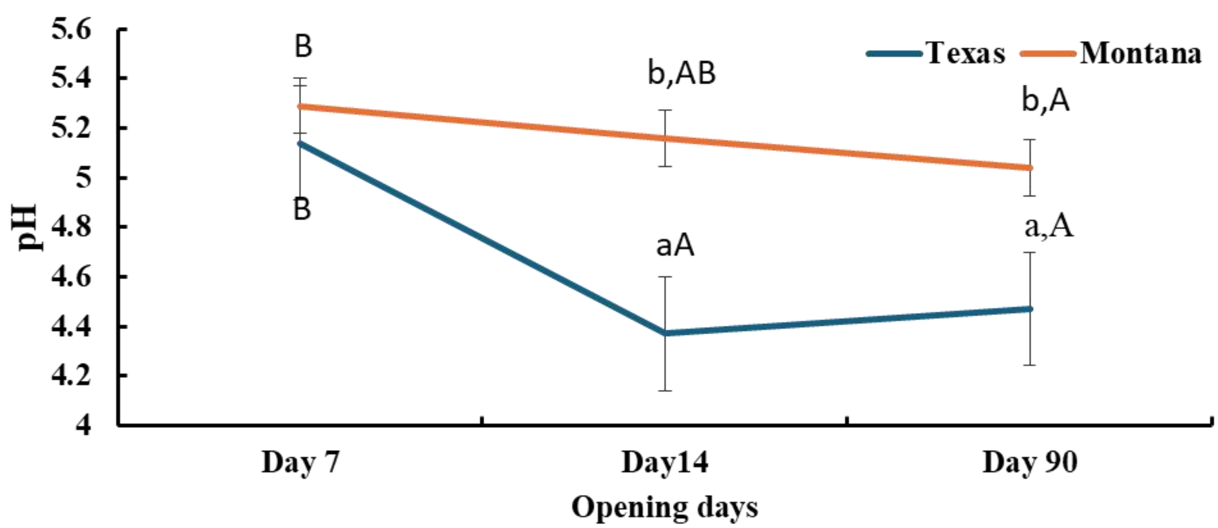
[A, B] Means with different superscripts different ($p<0.05$) (Opening days effect)

Results further reveal that Montana had the highest sugar content on all opening days as compared to Texas. However, the observed residual sugar which is assumed to be the source of energy for the rumen microbes was significantly affected by the fermentation process in both Texas (5.28%) and Montana (6.18) at the end of the 90 days of opening. The high total sugar content in Montana could be attributed to the high proportion of Italian ryegrass (55%) in the total forage mixture. As reported by Baldinger et al. (2014) the sugar content of Italian ryegrass is superior if it is harvested at the early stages. They further noted that Italian ryegrass harvested at the second cut had significantly higher (71.87%) sugar content than corn.

4.2. Fermentation characteristics of silage mixtures

From the analysis, it was evident that crop mixture interaction had a significant effect ($p<0.05$) in pH value between Texas and Montana on all opening days except day 7 of fermentation (Fig. 6).

Figure 6. The pH values of mixture silage on different opening days



[a, b] Means with different superscripts different ($p<0.05$) (Crop mixture effect)

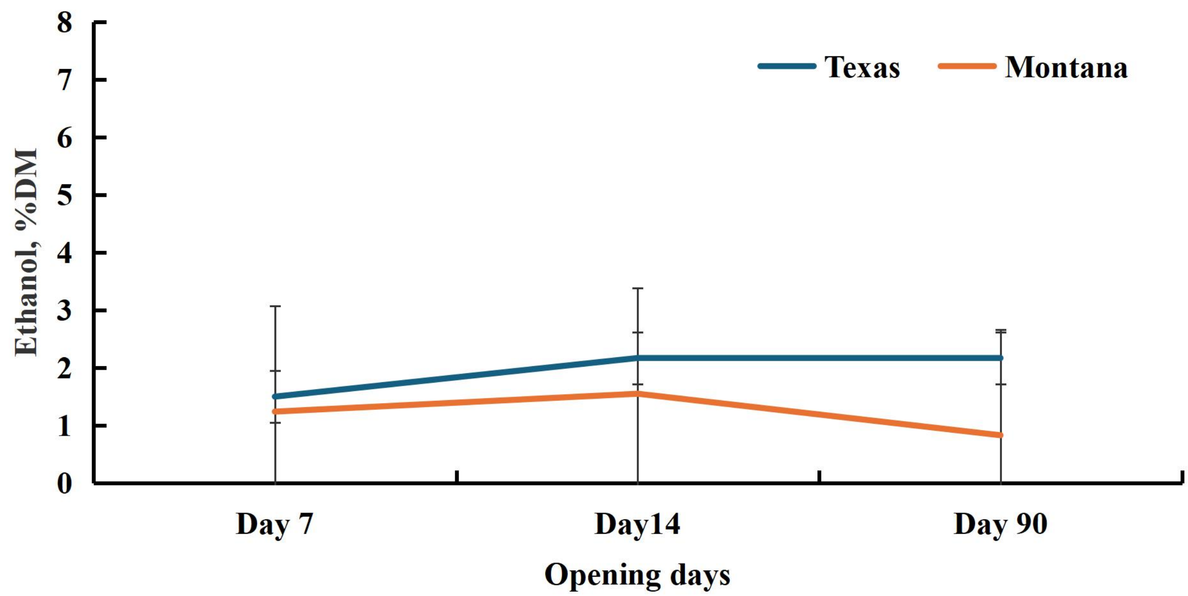
[A, B] Means with different superscripts different ($p<0.05$) (Opening days effect)

Throughout the fermentation period, Montana had a relatively high value of pH as compared to Texas however, the pH value of both mixture silages decreased at the end of the fermentation period (open day 90) as compared to day 7 this implies that the fermentation process has occurred properly. The reduction of pH was mainly caused by the rapid and intensive production of lactic acid. The rapid decrease in pH prevents the breakdown of plant proteins and helps to inhibit the growth of spoilage microbes. This finding is similar to other studies such as early-stage ensiling of Italian ryegrass (Shao et al. 2002), Italian ryegrass and Guinean grass silage (Shao et al. 2005), corn silage (Ulger and Kaplan 2017) and high moisture Italian ryegrass, Guinean grass and whole crop corn silages (Li and Nishino 2013).

Ethanol was detected during the storage period and the observed ethanol content for both ensiled mixture Texas and Montana was higher than the range (0.5-1.0% DM) reported by Kung and Shaver (2001). Results on the analysis of ethanol content show that the interaction of opening days and crop mixture type had no significant ($p>0.05$) effects between Texas and Montana throughout the experimental trial (Figure 7). Montana silage had lower ethanol content compared to Texas, indicating a lower yeast activity and a fermentation process dominated by lactic acid bacteria. However, Texas had the highest content of ethanol on all opening days as compared to Montana. Results further show that after day 7 of fermentation, Texas maintained a constant concentration level of 2.17% on both days 14 and 90 this may likely be due to a higher yeast population. The interaction of opening days and crop mixture type had no significant ($p>0.05$) effects on the fermentation characteristics of acetate content between Texas and Montana on all opening days except day 7 (Figure 8).

However, as compared to Texas, the Acetate content was consistently higher in Montana, further supporting the dominance of beneficial lactic acid bacteria during fermentation.

Figure 7. Ethanol content of mixture silages on different opening days



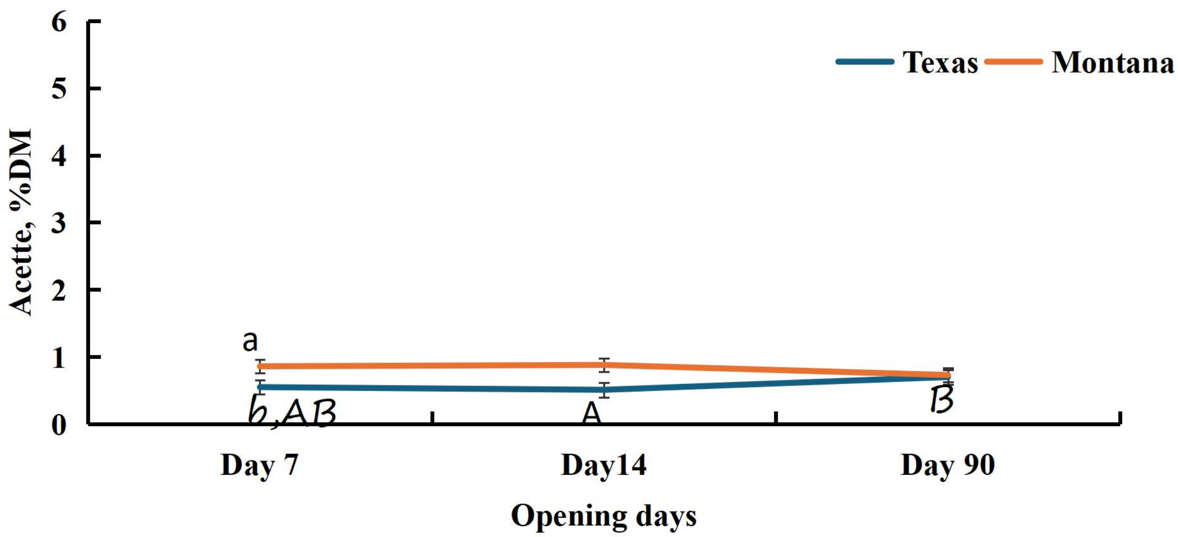
[a, b] Means with different superscripts different (p<0.05) (Crop mixture effect)

[A, B] Means with different superscripts different (p<0.05) (Opening days effect)

The low acetate concentration in both silages may be because of the dominance of homofermentative lactic acid bacteria during the fermentation process as observed also from the ethanol content. Results on the Lactate content of mixture silage show that crop mixture interaction had a significant ($p<0.05$) effect between Texas and Montana on all opening days except day 7 (Figure 9).

On all opening days except day 7 of fermentation, Texas had a relatively higher content of lactate as compared to Montana. As fermentation progresses, the lactate content in Montana initially increases from day 7 and peaks on day 14 when the fermentation is in its stable phase (Figure 9).

Figure 8. Acetate content of mixture silages on different opening days



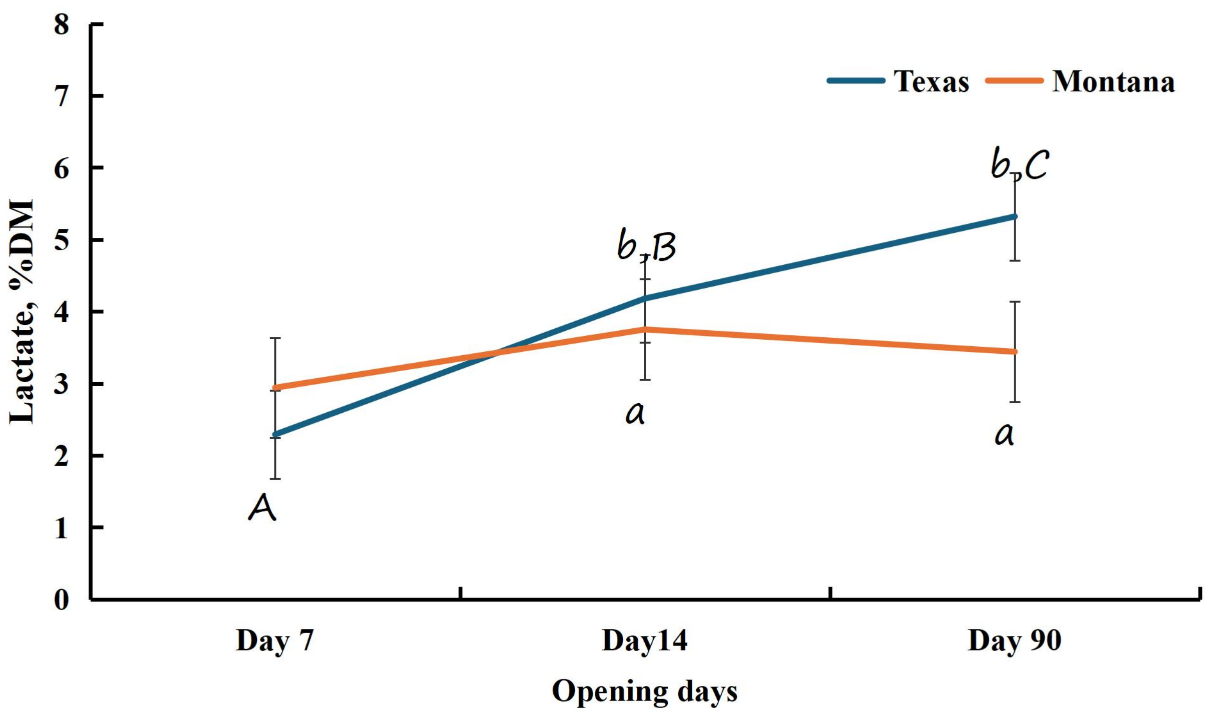
[a, b] Means with different superscripts different ($p<0.05$) (Crop mixture effect)

[A, B] Means with different superscripts different ($p<0.05$) (Opening days effect)

Statistical analysis showed that crop mixture interaction and opening days had no significant effects ($P>0.05$) in the ammonia-N content between Texas and Montana throughout the fermentation period (Figure 10). However, Texas had a relatively higher content of ammonia-N on all opening days except day 7 of ensilage.

Fermentation caused an increase in NH_3-N (g/100 g total N) at day 14 and 90 as compared to day 7 for both Texas and Montana. The NH_3-N/total N of both Texas and Montana was low (<6 g/100g total N) for all three opening days. As per the criteria after the end of the fermentation process when the NH3-N/total N is below 7 g/100 g total N, the silage could be categorized as excellent silage. Therefore, the silage ensiled throughout the fermentation period in the present study is categorized as an excellent-quality silage.

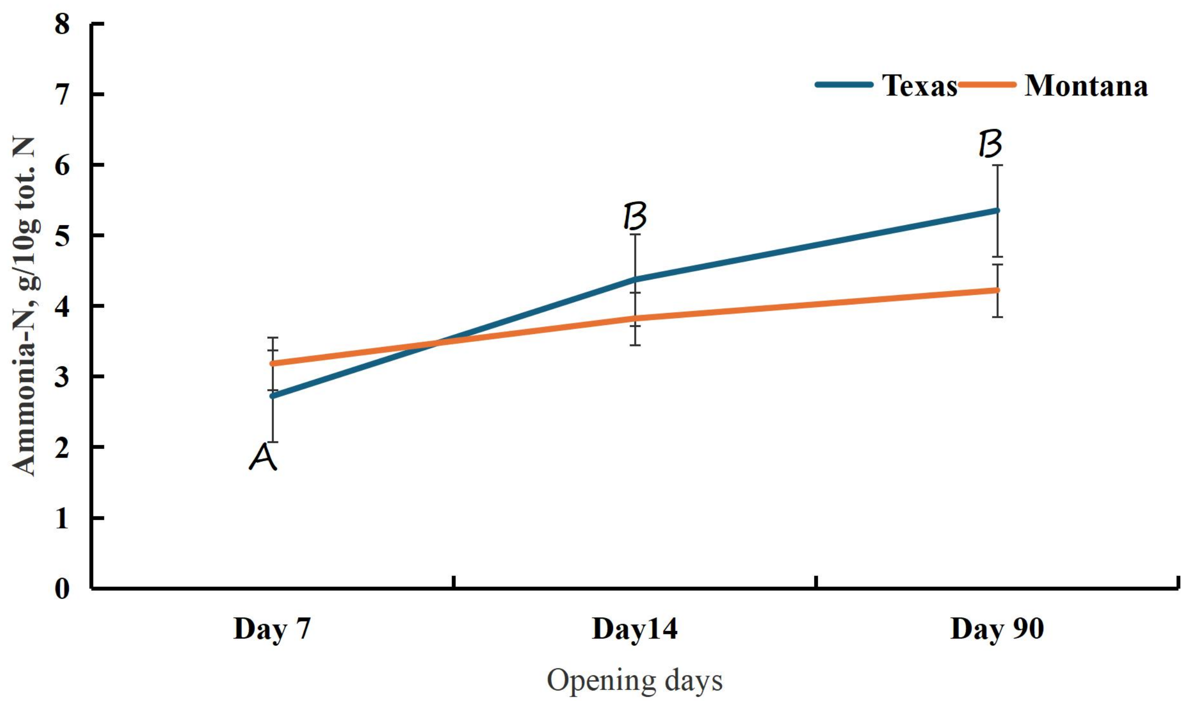
Figure 9. Lactate content of mixture silages on different opening days



[a, b] Means with different superscripts different ($p<0.05$) (Crop mixture effect)

[A, B] Means with different superscripts different ($p<0.05$) (Opening days effect)

Figure 10. Ammonia-N content of mixture silages on different opening days



[a, b] Means with different superscripts different ($p<0.05$) (Crop mixture effect)

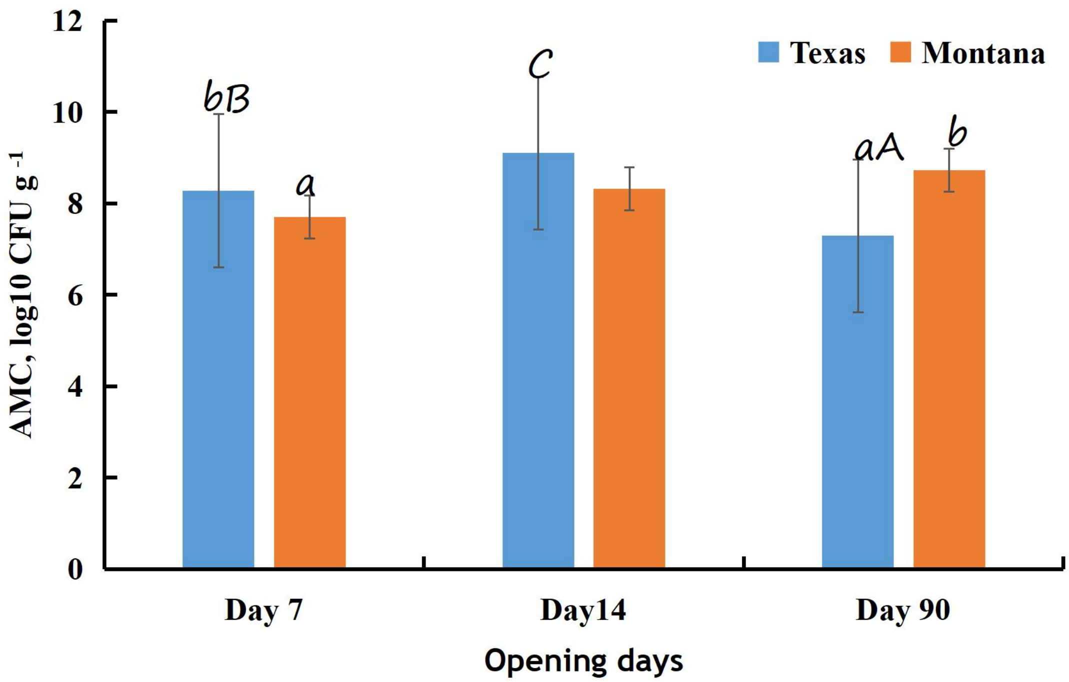
[A, B] Means with different superscripts different ($p<0.05$) (Opening days effect)

4.3. Aerobic mesophilic microorganism (AMC) and mould & yeast counts (Log10 CFU/g)

Crop mixture interaction had a significant effect ($P<0.05$) on the aerobic mesophilic microorganism count (Log_{10} CFU g-1) between Texas and Montana on all opening days except day 14 of fermentation (Figure 11). As compared to Montana, Texas had a relatively higher count of Aerobic mesophilic microorganism count (Log_{10} CFU g-1) on all opening days except day 90. However, the aerobic mesophilic microorganisms count (AMC) (Log_{10} CFU g^{1}) for both

Texas and Montana silages were above 7.00 (Log_{10} CFU g^1) or 1 x 10^6 (CFU g^1) at all opening days (7, 14 and 90) indicating that Texas and Montana silages exceeds the normal level (6.00 (Log_{10} CFU g^1) or 1 x 10^6 (CFU g^1) of the European decree (EN ISO 4833, Microbiological limits 65-2012 VM Decree Annex 12). This can lead to the growth of undesirable microorganisms, potentially pathogenic bacteria and reduced feed value (Zielińska, 2012).

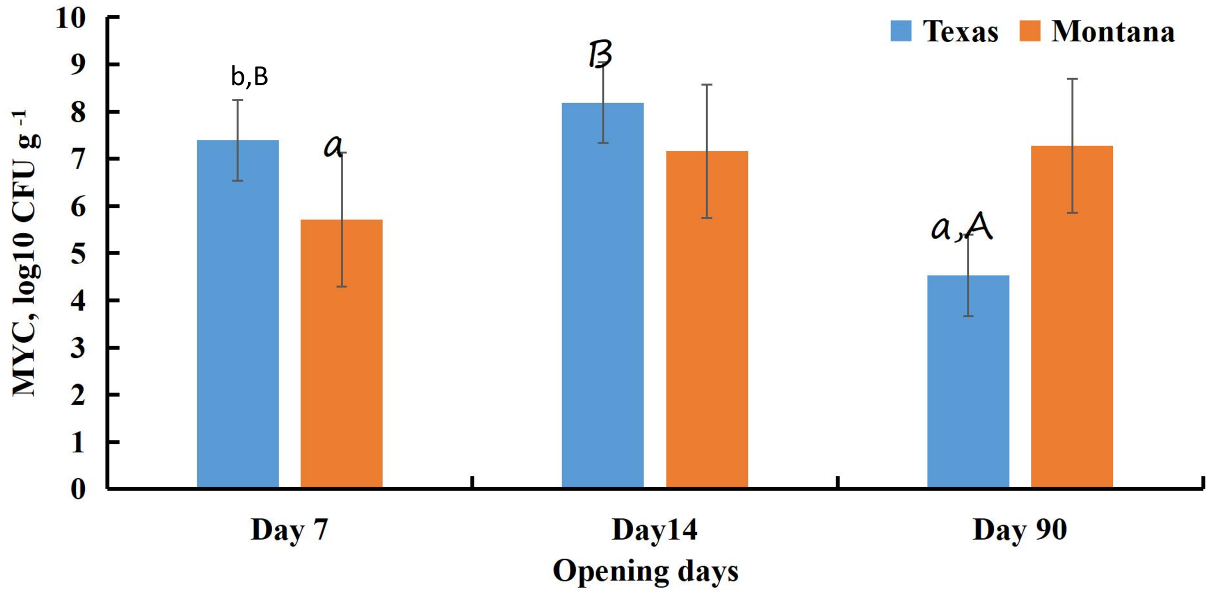
Figure 11. Aerobic mesophilic microorganism count of mixture silages on different opening days



[a, b] Means with different superscripts different ($p<0.05$) (Crop mixture effect)

[A, B] Means with different superscripts different ($p<0.05$) (Opening days effect)

Figure 12. Mould and yeast count of mixture silages on different opening days



[a, b] Means with different superscripts different ($p<0.05$) (Crop mixture effect)

[A, B] Means with different superscripts different ($p<0.05$) (Opening days effect)

The result of the analysis on silage mixture interaction showed that there were no significant effects on the mould and yeast count between Texas and Montana observed throughout the fermentation period except day 7 (Figure 12). Results further show that on days 7 and 14, Montana had relatively the lowest mould and yeast count as compared to day 90. The microbiological quality result indicates that the mould and yeast count (Log_{10} CFU g^1) of both Texas and Montana on all opening days was above 4.00 (Log_{10} CFU g^1) or 1 x 10^4 (CFU g^1). Chao et al., 2021 found that variation in CFU counts between two silages could be attributed to differences in bacterial communities and fermentation processes influenced by factors like moisture content, harvest time and ensiling conditions.

The mould and yeast count (Log_{10} CFU g^{1}) were above the limit recommended as a quality standard for animal feeds (3.00 (Log_{10} CFU g^{1}) or 1 x 10^4 (CFU g^{1}) (GMP 2008). However, the mould and yeast counts of Texas (4.53) on opening day 90 agree with Keller et al. (2013) who reported a mould count of 4.76 (Log_{10} CFU g^{1}) or 5.74 x 10^4 (CFU g^{1}) for corn silage at its post-fermentation phase.

5. CONCLUSION

In general, both types of ensiled forage mixtures yielded silage with exceptional nutritional qualities. When harvested at the appropriate stage (early heading), the mixtures of Italian ryegrass and winter cereals underwent effective fermentation. The elevated levels of crude protein, along with moderate concentrations of NDF and ADF, suggest that these mixtures are suitable for incorporation into the diets of dairy cattle.

The ensiled mixtures (Texas and Montana) yielded silage with favourable feed attributes. Both mixtures underwent effective fermentation processes. Limited fermentation, however, impeded the rapid and effective production of lactic acid, resulting in a more gradual decrease in pH than what is ideal for maintaining efficient fermentation.

Furthermore, it has been observed that the presence of ethanol after fermentation contributes to the survival of certain yeast strains. Further investigation is essential to improve the practical uses of the novel integrations of winter cereals and Italian ryegrass, as well as silages derived from winter cereals. It is also crucial to highlight that the two mixtures from Texas are designed for autumn sowing and early harvesting, making them suitable for integration into crop rotation systems.

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ABSTRACT

Climate change, particularly heat stress, is causing economic losses in the global livestock sector, particularly in regions like the Carpathian Basin and extreme heat occurrences in this region are anticipated to increase markedly by the end of the 21st century. The primary goals of this study were to determine the nutritional composition, fermentation characteristics and microbial counts of winter cereals and ensiled Italian ryegrass in dairy cow nutrition. Two distinct winter cereal-based diets were used in the study: Texas (which included 50% of two cultivars of winter triticale, 40% of winter barley and 10% of winter wheat) and Montana (55% of three varieties of Italian ryegrass and 45% of winter oats). The trial was carried out on a medium-sized farm at the Hungarian University of Agriculture & Life Science Kaposvar.

Results on the nutritional composition show that Montana exhibited higher DM (33.94%), CP (11.18%) and TS (6.18%) at the end of the fermentation period as compared to Texas with DM (30.32%), CP (9.36%) and TS (5.28%) indicating better nutrient availability and digestibility. The fermentation process proved effective in both Texas and Montana, with lactic acid production resulting in pH reductions to 4.47 and 5.04, respectively. Ethanol presence indicated elevated mould and yeast activities in Montana (7.27 Log_{10} CFU/g) relative to Texas (4.53 Log_{10} CFU/g). Furthermore, microbial counts (Log_{10} CFU/g) in both mixtures (7.29 for Texas and 8.73 for Montana) surpassed European standards, underscoring potential risks for feed contamination.

Inconclusion, both silage mixtures demonstrated potential as high-quality feed alternatives for dairy cattle, especially in regions susceptible to drought, despite certain limitations. Additional research is necessary to improve the incorporation of winter cereals and Italian ryegrass within crop rotation systems.