Piloting locally made microsilos to monitor the silage fermentation quality of conserved forage in Rwanda

Marie Louise Mukamuhirwa
School of Veterinary Medicine, University of Rwanda-Nyagatare Campus, P.O. Box 57, Nyagatare, Rwanda
Eric Hatungimana
School of Veterinary Medicine, University of Rwanda-Nyagatare Campus, P.O. Box 57, Nyagatare, Rwanda
Joos Latre
University for Applied Sciences and Arts-HoGent, Department Biosciences and Industrial Technology, Research Centre AgroFoodNature, Brusselsesteenweg 161 BE-9090 Melle, Belgium
Martin Ntawubizi
m.ntawubizi@ur.ac.rw
School of Veterinary Medicine, University of Rwanda-Nyagatare Campus, P.O. Box 57, Nyagatare, Rwanda https://orcid.org/0000-0002-1649-2447

Research Article

Keywords: Microsilos, Silage, Fermentation, Rwanda

Posted Date: April 30th, 2024

DOI: https://doi.org/10.21203/rs.3.rs-4339458/v1

Additional Declarations: The authors declare no competing interests.

Abstract

A good understanding of essential silage processes is key to success and large adoption by local farmers. This study aimed to test the use of a locally constructed micro silo mechanism with identified suitable forage species as an affordable quality check to evaluate the silage fermentation stages and effect on silage nutrient composition in the semiarid eastern part of Rwanda. Forage samples were filled in duplicate, considering 2 blocks (stations), resulting in 4 silage treatments * 3 repetitions, for a total of 24 microsilos. The treatments included the following steps: 1) two mono-grass (*Brachiaria Mulato*/ *Panicum coloratum*) treatments and two grass*legume mixtures (*Brachiaria Mulato** *Desmodium distortum*; *Panicum coloratum** *Desmodium distortum*). Silage fermentation was monitored for 6 weeks, and the following quality parameters were measured: dry matter (DM, %), pH, ammonia-nitrogen and crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF). Quantitative data were statistically analyzed with 95% CIs. The results indicated a consistent decrease in DM (%) due to fermentation losses. At silage maturity (D48), the average ammonia content [NH4+ -N (%)], CP and NDF were 0.08 ± 0.05%, 10.02 ± 2.61%, 62.92 ± 7.82 and 37.72 ± 5.78, respectively. As suggested in the results, silages made of 100% grasses had relatively higher NDF, ADF, pH, and DM levels, suggesting that the mixture of grasses and legumes had better fermentation characteristics than did the mono-grass silages. The use of microsilos for monitoring silage fermentation showed high consistency, yielding replicable quality-fermentation parameters.

Introduction

The growing livestock subsector in the East African Community (EAC) has made significant strides over the past decades, engaging nearly 50–70% of its working-age population, and is expected to expand in response to a growing demand for animal source food (ASF) products (AfDBank, 2020; MINAGRI, 2021). The sector, however, faces critical production and market-related constraints, with seasonal feeding deficit(s) representing a top conundrum. The high dependency of rainfall patterns on feed resource availability at smallholder farms (including green fodder crops, roadside weeds, tree leaves, cereal crop residues, and leguminous crop residues) is a significant limiting factor for livestock productivity and resilience (Lukuyu et al., 2009; Mutimura, et al., 2015).

Forage conservation (e.g., silage, hay) is believed to preserve feeds and address the seasonal variability (quantity/quality) of feed (Balehegn et al., 2022; Tufail et al., 2020). Ensiling, an established process of forage preservation, is based on spontaneous lactic acid fermentation under anaerobic conditions, where lactic acid bacteria ferment water-soluble carbohydrates (WSCs) to lactic acid and, to a lesser extent, to acetic acid (Elferink et al., 2000). The quality of silage not only is determined by the quality of the forage but also depends on silage management. A good understanding of essential silage processes is key to success and large adoption by local farmers (Weinberg & Muck, 1996; Schroeder, 2004).

The aim of this study was to pilot the use of a locally constructed micro silo mechanism with identified suitable forage species (grasses, legumes, and their mixtures) as an affordable quality check to evaluate

the silage fermentation stages and effect on silage nutrient composition in the semiarid eastern part of Rwanda.

Materials and methods

Study area

This study was conducted in the Nyagatare District, Eastern Province of Rwanda. This savannah region is approximately located at 1° 18' 0.00"S, 30° 19' 30.00"E and 1513.5 m from sea level, with an average annual rainfall of 827 mm and a relatively longer dry season, varying between 3 and 5 months (May-June to August-September). The prevailing semi-intensive livestock farming method is characterized by pasture grazing supplemented with cultivated fodder crops, roadside grass cuts, crop residues and a variety of farm byproducts.

Sampling

The grass species (*Brachiaria Mulato, Panicum coloratum*) and legume (*Desmodium distortum*) were randomly collected from 2 stations: 1) the Kinihira agrostology garden (University of Rwanda, Nyagatare Sector) and the Karama Forage Testing Station of the Rwanda Agriculture and Animal Resources Board (RAB, Karangazi Sector). The two collection sites are approximately separated by 30 km. The sampled species were identified based on their respective adaptability records and availability in the study area, indicating their preference for silage by local (including smallholder) farmers (Mutimura and Everson, 2012; Ndabikunze, 2004).

The forages were harvested at the maturity stage of their second cut, with a hand machete approximately 10 cm from the soil, and immediately transported to the laboratory for feed analysis at the University of Rwanda-Nyagatare Campus. Samples of forage (approximately 2 kg of mixed leaf, stem, and flower) were cut into palatable pieces (2 to 3 mm long) and dried in small amounts (100–200 g) using a microwave oven (WMS2014®, 230 V, 50 Hz, rated frequency, 700 W rated output) in a low-medium setting (420 W) for 20 minutes to reach an ideal DM ranging from 30–40% before filling them into microsilos.

Filling microsilos

The 24 microsilos used in this study were handcrafted, made of polyvinyl chloride (PVC, BENOL©) pipes, and sawed in a perpendicular way with dimensions of 35 cm in length and a 110 mm radius (**Fig. 1**). Each of them was sealed by two specific rubber caps for microsilos (IPCO®), one with a plain surface covering the bottom and the other equipped with a CO_2 valve covering the top. With equal dimensions, the microsilos filling density was calculated based on the dry matter content and the microsilo volume (density: DM/Vol, equivalent to 333.$^{24\ cm^3}$). An additional extension PVC pipe 25 cm long was prefabricated to serve as a buffer between the microsilos pipes and the compressor disk to protect the microsilos extremities and the handler during the silage compression process.

The microsilos were weighed with 2 caps before and after filling with chopped forages. This procedure was used to determine the weight of the ensiled materials via an electronic top-pan balance (Ohaus Scout® Pro Portable Electronic Balance 6000 g × 1 g). Forage samples were further filled in duplicate, considering 2 blocks (stations), resulting in 4 silage treatments * 3 repetitions, for a total of 24 microsilos. The treatments included the following steps: 1) two mono-grass (*Brachiaria Mulato*/ *Panicum coloratum*) treatments and two grass*legume mixtures (*Brachiaria Mulato***Desmodium distortum*; *Panicum coloratum** *Desmodium distortum*). The silage mixtures were made with a grass/legume ratio of 70/30. The details of sample filling and replication are highlighted in Table 1.

Table 1
Block arrangement of [1] microsilos in treatment and repetitions

Block 1 (Kinihira Site)		**Block 2 (Karama Site)**	
Block	Repetition	Block	Repetition
B	3	B	3
P	3	P	3
BD	3	BD	3
PD	3	PD	3
4	12	4	12

[1] *B: Brachiaria mulato; P: Panicum coloratum; BD: Brachiaria mulato*Desmodium distortum; PD: Panicum coloratum*Desmodium distortum*

Compacting microsilos

A silage air compressor (AIRCOMPACT© equipment) was used to compact the microsilos. The mechanism involved the use of a compressed air cylinder (XL.100/500 48 XL-100-0500-050 301, 75 301, 75) and its accessories (Axle for LB, rea hinge MP2, rear hinge LB2); 2 foot pedals (i/4” 2-stranden 48 FRH-07-520 83, 35 166, 70) with 2 air vents and 3 connectors; 1 coupling hose with adapters between pedals and compressor (65,00 65,00); 1 coupling hose with adapters between pedals and cylinder (82,00 82,00) both linked with a speed regulator; and a round disk (RVS 110 mm, with bore for 102,00). This air compressor system was coupled with a commercial air compressor (Airpress HL® 310/25; 8BAR pressure, 24 L, 220–240 V). The mechanism was mounted on a welded base frame (500 mm L/800 mm H) connected to the air compressor by Teflon tape to control the pumping of air into the compressed cylindrical piston disc and allow the latter to move down and up during the compaction of the silage material into the microsilos. The air compression mechanism is described in **Fig. 1**.

Approximately 2 kg of forage was added to the microsilos and compacted using a cylindrical pistol disc to avoid air space and spoilage of the silage. The top surface was sealed with a plastic cap with an inserted CO2 valve to allow extra gases to escape. Twenty-four microsilos were labeled and weighed for further monitoring (e.g., weight loss) during the fermentation process. After 6 weeks of silage

fermentation monitoring, the upper layers of the microsilos were opened, and approximately the top 3 cm of the silage samples were removed. Two hundred grams of each microsilos sample was collected, divided into 2 plastic bags of 100 grams each, labeled and immediately frozen at -80°C for further analyses.

Nutrient monitoring of silage contents

Dry matter (DM) for each sample (microsilo) was determined before (Day 1) and after (Day 48) ensiling using a commercial microwave oven (*MW*-WMS2014®, 230 V, 50 Hz, rated frequency, 700 W rated output-Herein referred to as **MW**) at a medium-high setting (600 W) for 20 min, as described by Oetzel et al. (1992). On Day 48 (silage maturity), the DM content of each sample was measured in duplicate using both the *MW* and a laboratory drying oven (Bov-30 V®, L 50 ~ 200°C; herein referred to as *OV*) at low temperature (55°C) for 48 hours for further comparisons. The DM content (%, *MW*) was calculated using the following formula: DM%= (weight of dry/weight of wet) * 100, while the DM content (%, *OV*) was estimated using the following formula: DM%= (W3-W2)/W1) *100 [**W1**: wet sample weight; **W2**: crucible weight; **W3**: dried sample weight with crucible weight].

pH determination was performed for all 24 microsilos contents using a HANNA edge pH meter. The pH meter was initially calibrated in buffer solution such that the pH was 7 or 4 before use. To prepare samples for pH measurement, 450 ml of distilled water was added to 50 g of each microsilo sample, and the mixture was left to withstand overnight. The extracted juice was used to measure the pH by emerging the electrode and waiting for 5 min before the measured values were recorded.

Ammonia-nitrogen and crude protein analysiss were performed using the Kjeldahl method (AOAC, 2007). Crude protein (CP) was considered based on the nitrogen content of the feed, where CP = N*6.25. The nitrogen content was further estimated by titration of the ammonium borate formed with standard sulfuric or hydrochloric acid using a suitable indicator to determine the endpoint of the reaction. The following calculations were considered: dilution factor (DF) **=** $14*100*10^{-3}$ (DF = 1.4); nitrogen content (%) = [titer HCl-blank] *N of acid*1.4/(sample weight) (% lab DM basis)] *100; and crude protein content (%) = nitrogen content × 6.25.

Insoluble fiber in feed, determined as neutral detergent fiber (NDF), which consists of cellulose, hemicelluloses, and lignin, was analyzed using the method described by Van Soest (Van Soest et al., 1991). The calculations were performed as follows: %NDF = $[(W_0 - W_t)/W_s]$ *100 (with Wo: fiber crucible weight; W_t: crucible weight, W_s: sample weight).

Acid detergent fiber analysis was performed using the method described by Van Soest (Van Soest et al., 1991), and calculations were performed using the following formula: % ADF= [(crucible weight + fiber) – (crucible weight of fiber)/(sample weight)]*100.

Data processing and analysis

The data processing and analysis were performed using Microsoft Excel 2019 and SPSS 16.0 under Windows 10 Pro. Quantitative data were analyzed using the general linear model (GLM): multivariate analysis. To evaluate the magnitude of differences within variables, the one sample t test was performed to detect potential significant differences from the mean, while paired sample t test analysis was performed to establish the statistical relationship between 2 separate variables. All tests were performed with a 95% confidence interval.

Results

The overall results are highlighted in Table 2 (Statistical description), with the means ± standard deviations for each parameter considered in this study. The maxima and minima values are also provided as additional indications of discrepancies in magnitudes among variables.

Table 2
Statistical description of the results

Parameter	Mean ± SD	Minimum	Maximum
DM Before Ensiling (%)	36.73 ± 0.92	35.50	37.50
DM After Ensiling-MW (%)[1]	28.63 ± 3.15	23.50	36.00
DM After Ensiling-OV (%)[2]	28.58 ± 3.71	23.00	36.00
Fermentation Loss on Day 8 (g)	6.79 ± 1.41	4.20	9.60
Fermentation Loss on Day 16 (g)	3.43 ± 1.31	1.00	6.70
Fermentation Loss on Day 24 (g)	1.97 ± 0.75	0.90	3.50
Fermentation Loss on Day 32 (g)	1.17 ± 0.33	0.80	2.10
Fermentation Loss on Day 40 (g)	1.22 ± 1.44	0.70	7.90
Fermentation Loss on Day 48 (g)	0.81 ± 0.14	0.60	1.10
Total Fermentation Loss (g)	15.39 ± 3.14	10.86	22.73
pH	4.74 ± 0.20	4.41	5.00
NH4+ -N (ppm)	820.71 ± 488.05	173.14	1761.64
NH4+ -N (%)	0.08 ± 0.05	0.02	0.18
Crude Protein (%)	10.02 ± 2.61	3.69	13.85
Neutral Detergent Fibers (%)	62.92 ± 7.82	51.00	79.50
Acid Detergent Fibers (%)	37.72 ± 5.78	25.09	47.73

[1] DM was measured after ensiling (day 48) using a microwave oven (MW).

[2] DM was measured after ensiling (day 48) using a laboratory drying oven (OV).

Dry matter variations

DM measurements were performed on day 1 (before ensiling, using a commercial microwave oven-*MW*) and on day 48 (silage opening in duplicate, using both a commercial microwave oven and a laboratory drying oven-*OV*). The results (Table 2) indicated relatively greater values on day 1 (mean ± SD: 36.73 ± 0.92%-*M*) than on day 48 (mean ± SD: 28.63 ± 3.15%-*MW*). Additional measurements on day 48 (in the *OV*-dried oven) resulted in slightly lower values (mean ± SD: 28.58 ± 3.71%).

Analysis via paired-samples t tests revealed a trend toward a negative correlation (r = -0.387; P = 0.062) between the day 1 DM values (before silage) and the day 48 DM values obtained by using a microwave oven. However, a significant negative correlation was observed between the day 1 DM values and day 48 DM values obtained after silage was cultivated in a laboratory drying oven, with r = -0.618 (P = 0.001).

The decreases in DM (%) for all 24 samples are also shown in Fig. 2 and Fig. 3, which are consistent with the 2 measured DM values (*MW* and *OV*) on day 48, which were highly significantly correlated (r = 0.552; P = 0.005). The 2 measurements of DM at silage opening did not significantly differ between the microwave treatment and regular drying oven treatment, as indicated by the t test analysis at the 95% confidence interval (P = 0.951).

Fermentation losses during the ensiling process

As indicated earlier in Table 2 and later in **Fig. 4**, the average fermentation losses registered between day 8 (first measurement) and day 48 (silage opening) seemed to be sharp at the beginning (mean ± SD: 6.79 ± 1.41 g) and gradually decreased in intensity beginning on day 16 (mean ± SD: 3.43 ± 1.3 g) as fermentation progressed to day 48. The results indicate a steady increase in fermentation losses from day 8 to day 16, estimated at 50.56%, which was followed by relatively slow decreases from day 24 to day 48. This may suggest a relatively more stabilized fermentation loss as silage maturation progresses.

Furthermore, Fig. 5 highlights the progression of fermentation loss, suggesting an important increase from day 8 to day 16 for most of the samples measured (n = 24). However, the one-sample t test indicated a very significant difference in total fermentation loss between 24 samples (P = 0.000).

Variations in pH values

The overall mean pH measured at silage maturity (day 48) was estimated to be 4.74 ± 0.20; however, this difference was considerable (Min = 4.41; Max = 5.00). Considerable variations were also observed in Fig. 6, where the pH decreased (y = − 0.0022 x + 4,76). The one-sample t test further indicated a very significant difference in pH between the 24 samples (P = 0.000).

Ammonia and crude protein contents

The values of ammonia content [NH4+ -N (%)], which indicate the level of protein degradation, are 0.08 ± 0.05% (mean ± SD), with the minimum and maximum values estimated at 0.02 and 0.18 g, respectively. The one-sample t test indicated a very significant difference in ammonia concentrations between the 24 samples (P = 0.000). Additionally, the measured crude protein content (%) suggested important variations among silage samples, with an overall mean ± SD of 10.02 ± 2.61%. Considerable magnitudes were also observed, from 3.69–13.85%. Furthermore, the one-sample t test confirmed a very significant difference between the 24 sample values (P = 0.000).

According to a separate paired-sample t test, the ammonia and crude protein contents (%) were not correlated (r = 0.373; P = 0.073) in the 24 samples of silage analyzed in this study. This is further highlighted in Fig. 7.

Silage digestibility and palatability

The silage digestibility parameters analyzed were neutral detergent fiber (%) and acid detergent fiber (%), which were found to be 62.92 ± 7.82 and 37.72 ± 5.78 (mean ± SD), respectively. Both parameters exhibited relatively important variations (Fig. 8), and the 2 variables were subsequently found to be significantly correlated (r = 0.668; P = 0.000).

In addition, as indicated in **Fig. 9**, a paired-samples t test indicated the absence of a linear relationship (correlation) between pH and NDF (r = 0.058; P = 0.808) or between CP and NH4 (r = 0.197; P = 0.410). Instead, the two variable pairs (parameters) were found to be very significantly different (P = 0.000).

Effects of the station and silage mixture

The effects of the station (**block**) and silage mixture (**treatment**) were evaluated on different quality parameters of the silage, and the results are suggested in Table 2. A significant effect of station was detected for pH (P = 0.000), ammonia content (P = 0.009) and crude protein content (P = 0.042), with higher values for the UR Nyagatare Farm, equivalent to 4.53 ± 0.02, 0.11 ± 0.01 and 11.41 ± 0.61, respectively (mean ± SE).

On the other hand, variations in key quality parameters were observed among the different types of forage mixtures, labeled combined-Treatment 1 (100% grass) and combined-Treatment 2 (70% grass + 30% legumes). A significant effect of treatment was found for DM (%) measured using a microwave oven after silage opening (P = 0.043) and for DM (%) measured in duplicate using a classical laboratory oven (P = 0.005), where mixtures of grass and legumes had higher DM contents (30.34 ± 1.02% and 31.30 ± 1.08%, respectively; mean ± SE). Another significant effect was detected for the neutral detergent fibers (P = 0.025), where samples composed of 100% grasses yielded greater amounts (65.36 ± 1.81%; mean ± SE) than mixtures of grasses + legumes.

Table 3
Effect of station and treatment (forage mixtures) on key quality parameters of silage

Parameter	Effect of station (block)			Effect of treatment (Mixtures)[1]		
	RAB Karama	UR Nyagatare		Treatment 1	Treatment 2	
	Mean ± Std. Error	Mean ± Std. Error	P value	Mean ± Std. Error	Mean ± Std. Error	P value
DM After Ensiling MW (%)	29.10 ± 0.91	28.78 ± 0.92	0.802	27.54 ± 0.80	**30.34 ± 1.02**	**0.043**
DM After Ensiling OV (%)	28.89 ± 0.96	29.38 ± 0.97	0.721	26.97 ± 0.85	**31.30 ± 1.08**	**0.005**
Total Fermentation Loss (g)	16.03 ± 0.85	13.95 ± 0.86	0.100	16.01 ± 0.75	13.96 ± 0.95	0.105
pH	4.90 ± 0.02	**4.53 ± 0.02**	**0.000**	4.75 ± 0.02	4.68 ± 0.03	0.066
NH4+ -N (%)	0.06 ± 0.01	**0.11 ± 0.01**	**0.009**	0.07 ± 0.01	0.10 ± 0.01	0.188
Crude Protein (%)	9.53 ± 0.61	**11.41 ± 0.61**	**0.042**	9.62 ± 0.53	11.31 ± 0.68	0.064
Neutral Detergent Fibers (%)	63.98 ± 2.07	59.63 ± 2.08	0.153	**65.36 ± 1.81**	58.26 ± 2.31	**0.025**
Acid Detergent Fibers (%)	37.28 ± 1.81	37.34 ± 1.82	0.983	38.58 ± 1.59	36.04 ± 2.02	0.334

[1]**T1: Brachiaria mulato (100%) and Panicum coloratum (100%), T2: Brachiaria mulato (70%) + Desmodium distrortum (30%) and Panicum coloratum (70%) + Desmodium distortum (30%)**

As suggested in Fig. 10, silages made of 100% grasses (Treatment 1: *Brachiaria mulato* (100%) and *Panicum coloratum* (100%)) had relatively greater proportions of neutral detergent fibers (%), acid detergent fibers (%), pH, total fermentation losses (g) and dry matter (DM%) than mixtures of Brachiaria mulato (70%), *Desmodium distrortum* (30%) and *Panicum coloratum* (70%) + *Desmodium distortum* (30%). The latter seemed to present higher levels of CP (%), ammonia (%), and DM (%) at silage opening.

In contrast, a detailed analysis (GLM: multivariate analysis) revealed no significant effects of the individual mixtures (N = 4; *Brachiaria mulato* (100%), *Panicum coloratum* (100%), *Brachiaria mulato* (70%) + *Desmodium distrortum* (30%) or *Panicum coloratum* (70%) + *Desmodium distortum* (30%) on any of the quality parameters of the silage in this study (Table 4). However, the effect of the station was maintained for the pH (P = 0.000), NH4+-N concentration (%; P = 0.009), crude protein concentration (%; P

= 0.038) and neutral detergent fibers (%; P = 0.005). Additionally, a trend (P = 0.057) was observed for total fermentation loss (TFL, g)

Table 4
Effect of station (block) and forage species on key quality parameters of silage

Parameter	Effect of station (block)			Effect of forage species + Mixtures[1]				
	RAB Karama	UR Nyagatare		BM	BMDD	PC	PCDD	
	Mean ± Std. Error	Mean ± Std. Error	P value	Mean ± Std. Error	Mean ± Std. Error	Mean ± Std. Error	Mean ± Std. Error	P value
DM After Ensiling MW (%)	29.08 ± 0.91	28.94 ± 0.93	0.917	28.13 ± 1.16	30.75 ± 1.51	26.90 ± 1.10	30.25 ± 1.38	0.272
DM After Ensiling OV (%)	28.74 ± 0.68	29.63 ± 0.70	0.376	26.38 ± 0.87	32.00 ± 1.14	27.27 ± 0.83	31.08 ± 1.04	0.599
Total Fermentation Loss (g)	16.12 ± 0.79	16.12 ± 0.79	0.057	17.37 ± 1.01	12.36 ± 1.32	14.84 ± 0.96	15.27 ± 1.20	0.371
pH	4.90 ± 0.02	4.54 ± 0.02	**0.000**	4.77 ± 0.03	4.73 ± 0.04	4.73 ± 0.03	4.64 ± 0.03	0.384
NH4+ -N (%)	0.06 ± 0.01	0.11 ± 0.01	**0.009**	0.05 ± 0.02	0.09 ± 0.02	0.09 ± 0.02	0.10 ± 0.02	0.261
Crude Protein (%)	9.57 ± 0.61	11.55 ± 0.62	**0.038**	10.06 ± 0.78	12.47 ± 1.02	9.27 ± 0.74	10.43 ± 0.93	0.291
Neutral Detergent Fibers (%)	63.54 ± 0.90	59.41 ± 0.91	**0.005**	59.34 ± 1.14	53.73 ± 1.49	70.50 ± 1.09	62.34 ± 1.36	0.857
Acid Detergent Fibers (%)	36.99 ± 1.12	37.26 ± 1.14	0.870	34.46 ± 1.42	31.88 ± 1.87	42.12 ± 1.36	40.04 ± 1.70	0.631

[1]BM: Brachiaria mulato (100%),

BMDD: Brachiaria mulato (70%) + Desmodium distrortum (30%),

PC: Panicum coloratum (100%),

PCDD: Panicum coloratum (70%) + Desmodium distortum (30%)

Discussion

The DM is an important factor for determining the nutritional content of forage before ensiling since it indicates the adequacy of wilting. A high forage DM (DM > 50%-55%) makes it difficult to achieve anaerobic conditions during the ensiling process, and silage is more susceptible to heating and mold growth (Piltz, 2016). In contrast, a high moisture content favors undesirable microorganisms, while a higher DM content impacts the compaction and reduction of the air present (Santos et al., 2006). In this study, the dry matter content of the fresh forages used for making silage ranged between 36% and 40%. However, at harvest, the dry matter content of the silage decreased to 26.5% and 32%, which is similar to the findings of recent studies showing that the ideal DM content is between 30 and 35% (McDonald et al., 1991a). The silage made of the [*Brachiaria mulato* (70%) + *Desmodium distrortum* (30%)] mixture (BD) had a greater DM content (32%) than did the silage made of *Brachiaria mulato* alone (B), measured at 26.5% DM.

Silage can be defined as an acidic fermentation product from ensiled materials (McDonald et al., 1991a), where pH is a good indicator of silage fermentation quality (Kung et al., 2018). Kung & Shaver, (2004a) recommended that good tropical silages have a pH of 4.3–4.7; however, this study indicated that the overall mean pH measured at silage maturity (day 48) was estimated to be 4.74 ± 0.20, which is considerable (Min = 4.41; Max = 5.00). Considerable variations were also detected, indicating a significant difference in pH between the 24 samples. Previous studies have suggested that a minimum pH of 4.4 is useful as a benchmark for good cassava peel silage (Asaolu, 1988), while silages of whole-plant corn and alfalfa were found to have a pH ranging between 3.14 and 5.43 (Kung et al., 2018). Furthermore, Ghedalia and Miron (2001) reported that the pH of silage from *alfalfa* spp. treated with $S0_2$ was 4.51, whereas that of silage from *Brachiaria mulato* and *Panicum coloratum* was 4.78 and 4.68, respectively. Therefore, the final pH of silage is likely affected by a series of factors, mostly including the DM of the ensiled forages, the ammonia produced during maturation and the nutritional value of the forage species (Kung et al., 2018).

Ammonia is produced during the clostridial fermentation of amino acids from ensiled forages (Charmley, 2001). In well-preserved silage, a small proportion of NH3 is produced, alongside other products such as nitrogen as part of amino acids and peptides (Harrison et al., 1994). Moreover, ammonia is a good indicator of fermentation. In this study, the ammonia concentrations in the silage from the four treatment groups ranged from 0.08–0.10%, while the ammonia content in the corn silage ranged from 5 to 7%, which falls within the normal range (Kung & Shaver, 2004a). Reports have suggested that an ammonia content less than 10% is desirable, while values greater than 15% can reduce silage intake, resulting in poor dairy cattle performance (Kung and Shaver, 2004a). In comparison, ammonia nitrogen concentrations ranging from 1.17–10.94% were reported for alfalfa silage by Sánchez-Duarte and García (2017); these values are higher than the ammonia content [NH4+ -N (%)] estimated to be between 0.02 and 0.18 g, which was recorded for the silos in this study. Additionally, the measured crude protein (CP, %) content suggested important variations among the silage samples, with an overall mean ± SD of 10.02 ± 2.61%. Considerable magnitudes were also observed, from 3.69–13.85%. Furthermore, a very significant difference was confirmed between the 24 sample values. According to our separate analysis, the

ammonia and crude protein contents (%) were not correlated in the 24 silage samples analyzed in this study.

Neutral detergent fibers (NDFs) consist of hemicellulose, cellulose, and lignin from whole plants, while acid detergent fibers (ADFs) consist of cellulose and lignin. After the silage was harvested, NDF and ADF were measured for all the silos. The results of this study showed that the percentage of neutral detergent fibers (%) and the percentage of acid detergent fibers (%) were 62.92 ± 7.82 and 37.72 ± 5.78 (mean ± SD), respectively. Both parameters exhibited relatively important variations, and later, the 2 variables were found to be strongly correlated. As suggested in the results, silages made of 100% grasses (Treatment 1: *Brachiaria mulato* and *Panicum coloratum*) presented relatively greater percentages of neutral detergent fibers (%), acid detergent fibers (%), pH, total fermentation losses (g) and dry matter (DM%) than did mixtures of *Brachiaria mulato* (70%) + *Desmodium distrortum* (30%) and *Panicum coloratum* (70%) + *Desmodium distortum* (30%). In previous studies, NDF was found to range from 53.7–70.6%, while significant variations among the treatments were found for NDF and ADF in mixed silages (grass * legumes); in contrast, the sole panicum maximum silage contained 59.67% NDF and 33.83% ADF (Okukenu et al., 2021). This finding indicates a certain similarity with findings from the current studies. Furthermore, Kung and Shaver (2004b) suggested that a higher content of NDF in silage is usually an indication of the presence of soluble nutrients that are degraded during silage fermentation, which affects the nutritive value of the silage. It is believed that microbial fermentation processes occurring in the silo produce an array of end products and can change many nutritive aspects of a forage as a result of fermentation losses. Therefore, delayed silo filling, prolonged wilting periods, and/or the method of compacting can cause a reduction in fermentable sugars, hence resulting in clostridial fermentation (Mills & Kung, 2002).

During this study, dry matter loss significantly varied among the treatments during the ensiling period, which was in accordance with the findings of previous studies (Kung et al., 2000), where untreated silage was subjected to prolonged fermentation due to its buffering capacity. Indeed, Hoffman et al. (2011) reported a decrease in starch-protein ratio in high-moisture corn due to degradation caused by proteolytic activity within the extended ensiling period. In summary, the mixture of BD and PD had higher levels of nutrients than did the single grasses.

Conclusion

Following regular monitoring of silage fermentation for 6 weeks, where quality parameters were measured, the results indicated a consistent decrease in DM (%) values due to fermentation losses. The results indicated a consistent decrease in DM (%) due to fermentation losses. At silage maturity (D48), the average ammonia content [NH4+ -N (%)], CP and NDF were 0.08 ± 0.05%, 10.02 ± 2.61%, 62.92 ± 7.82 and 37.72 ± 5.78, respectively. The discussion of the results suggested that silages made of 100% grasses had relatively greater NDF, ADF, pH, and DM contents than did the monocultured silages (*Brachiaria Mulato*Desmodium distortum*; *Panicum coloratum** *Desmodium distortum*), which exhibited better fermentation characteristics than did the monocultured silages (*Brachiaria Mulato/Panicum*

coloratum). The use of microsilos for monitoring silage fermentation showed high consistency, yielding replicable quality-fermentation parameters.

Declarations

Acknowledgments

We thank the HOGENT-UGENT Bottelare Research Station (Belgium), which donated the microsilos equipment and education support (manuals), through the Flemish Interuniversity Council (VLIR-UOS) project RW2019SIN260B125 between HOGENT and the University of Rwanda. A portion of the silage laboratory analyses was performed with the support (laboratory facility and consumables) of the Rwanda Agriculture and Animal Resources Board (RAB). The analysis of these data was performed by the first author of the MSc program at the University of Rwanda.

Author contributions

Marie Louise Mukamuhirwa (MSc): designed and implemented the study, performed the field and laboratory analyses, and drafted the MSc thesis and the first paper manuscript.

Eric Hatungimana: supervised the first author, provided support during the educational phase of the work (MSc thesis), and participated in editing the manuscript.

Joos Latre: Provided the required guidance and education manuals to design the experimental setup and provided substantial comments on the manuscript.

Martin Ntawubizi: cosupervised the first author, provided support during the educational phase of the work (MSc thesis), included statistical analysis of the results, and led the writing of the manuscript.

Funding

Microsilos equipment and research funds were provided by the (VLIR-UOS) project RW2019SIN260B125 through the HoGENT University of Applied Sciences, Gent-Belgium.

Available data will be available upon request.

Code availability Not applicable.

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest

The authors declare no competing interests.

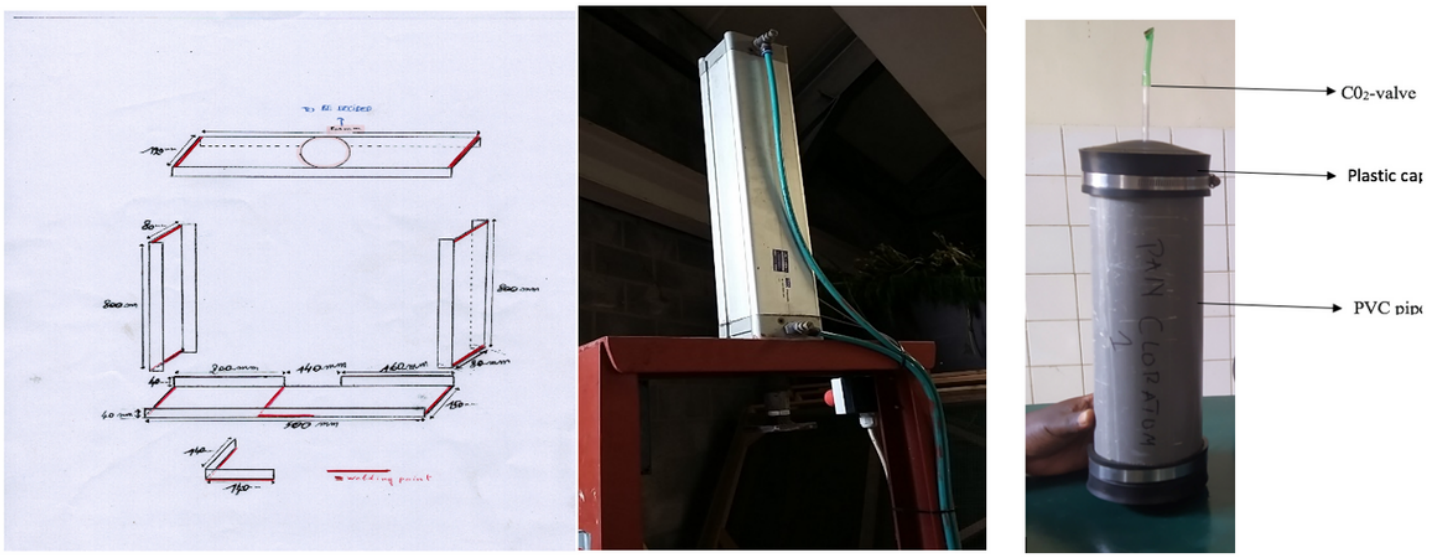
References

1. AfDBank (2020) African Development Bank, African Development Fund Project: Rwanda meat value chain trade competitiveness project, Republic of Rwanda: African development fund public disclosure authorized
2. AOAC (2007) Crude Protein Determination in Feed and Forages Macro-Kjeldahl Method. January,6–9.https://uwlab.soils.wisc.edu/wp-content/uploads/sites/17/2015/09/forage_CP.pdf
3. Asaolu VO (1988) Utilization of cassava peels and Gliricidia sepium in the diet of the West African Dwarf sheep. MPhil. thesis. Obafemi Awolowo University, Nigeria
4. Balehegn M, Ayantunde A, Amole T, Njarui D, Nkosi BD, Müller FL, Meeske R, Tjelele TJ, Malebana IM, Madibela OR, Boitumelo WS, Lukuyu B, Weseh A, Minani E, Adesogan AT (2022) Forage conservation in sub-Saharan Africa: Review of experiences, challenges, and opportunities. Agron J 114(1):75–99. https://doi.org/10.1002/agj2.20954
5. Charmley E (2001) Toward improved silage quality – A review. Can J Anim Sci 81(2):157–168. https://doi.org/10.4141/A00-066
6. Elferink SJWHO, Driehuis F, Gottschal JC, Spoelstra SF (2000) Silage fermentation processes and their manipulation. FAO Plant Production and Protection Papers, May 2014, 17–30
7. Ghedalia D, Miron J (2001) Digestion by sheep of monosaccharide constituents of direct cut alfalfa silage made with SO2-treated wheat straw. Anim Feed Sci Technol 92(3–4):175–183. https://doi.org/10.1016/S0377-8401(01)00260-7
8. Harrison JH, Blauwiekel R, Stokes MR (1994) Fermentation and utilization of grass silage. J Dairy Sci 77(10):3209–3235. https://doi.org/10.3168/jds.S0022-0302(94)77264-7
9. Hoffman PC, Esser NM, Shaver RD, Coblentz WK, Scott MP, Bodnar AL, Schmidt RJ, Charley RC (2011) Influence of ensiling time and inoculation on alteration of the starch-protein matrix in high-moisture corn. J Dairy Sci 94(5):2465–2474. https://doi.org/10.3168/jds.2010-3562
10. Kung L, Shaver R (2004a) Interpretation and use of silage fermentation analysis reports. 3(13):1–5

11. Kung L, Shaver R (2004b) Interpretation and use of silage fermentation analysis reports. January 2001
12. Kung L, Robinson JR, Ranjit NK, Chen JH, Golt CM, Pesek JD (2000) Microbial populations, fermentation end-products, and aerobic stability of corn silage treated with ammonia or a propionic acid-based preservative. J Dairy Sci 83(7):1479–1486. https://doi.org/10.3168/jds.S0022-0302(00)75020-X
13. Kung L, Shaver RD, Grant RJ, Schmidt RJ (2018) Silage review: Interpretation of chemical, microbial, and organoleptic components of silages. J Dairy Sci 101(5):4020–4033. https://doi.org/10.3168/jds.2017-13909
14. Lukuyu BA, Kitalyi A, Franzel S, Duncan A, Baltenweck I (2009) Constraints and options to enhancing production of high quality feeds in dairy production in Kenya, Uganda and Rwanda. ICRAF Working Paper no. 95. Nairobi, Kenya: World Agroforestry Centre. 34
15. McDonald P, Henderson AR, Heron S (1991a) The biochemistry of silage. (2nd ed.)
16. McDonald P, Henderson N, Heron S (1991b) The biochemistry of silage. Marlow (UK) Chalcombe Pub., 2 edition
17. Mills JA, Kung L (2002) The effect of delayed ensiling and application of a propionic acid-based additive on the fermentation of barley silage. J Dairy Sci 85(8):1969–1975. https://doi.org/10.3168/jds.S0022-0302(02)74273-2
18. MINAGRI 202. Republic of Rwanda, Ministry of Agriculture and Animal Resources, Annual Report (Issue September)
19. Mutimura M, Everson TM (2012) On-farm evaluation of improved Brachiaria grasses in low rainfall and aluminum toxicity prone areas of Rwanda. Int J Biodivers Conserv 4(3):137–154. https://doi.org/10.5897/ijbc10.121
20. Mutimura M, Ebong C, Rao IM, Nsahlai IV (2015) Nutritional values of available ruminant feed resources in smallholder dairy farms in Rwanda. Trop Anim Health Prod 47(6):1131–1137. https://doi.org/10.1007/s11250-015-0839-y
21. Ndabikunze SN (2004) Rwanda – Development of innovative forage production technologies for improved dairy productivity. Ministry of Agriculture and Animal Resources (MINAGRI) -
22. Oetzel GR, Villalba FP, Goodger WJ, Nordlund KV (1992) A Comparison of On-Farm Methods for Estimating the Dry Matter Content of Feed Ingredients. 1993 J Dairy Sci 76:293–299
23. Okukenu O, Okunogbe S, Afolabi R, Dele P, Bolanle A, Amisu A, Jolaosho A, Onifade O (2021) Influence of forage proportion and ensiling periods on physical characteristics and qualities of Panicum maximum (Ntchisi) and Stylosanthes. https://doi.org/10.21203/rs.3.rs-985205/v1. guianensis silage
24. Piltz AGK (2016) and J. W. Feed testing: assessing silage quality Feed testing : assessing silage quality (pp. 312–334)
25. Sánchez-Duarte JI, García Á (2017) Ammonia-N concentration in alfalfa silage and its effects on dairy cow performance: a meta-analysis. Revista Colombiana de Ciencias Pecuarias 30(3):175–184.

https://doi.org/10.17533/udea.rccp.v30n3a01
26. Santos EM, Zanine AM (2006) Tropical grasses silage. Colloquium Agrariae V 2, n. 1 (1st ed.)
27. Schroeder JW (2004) Silage Fermentation and Preservation. revised ebition. NDSU Extension Service, 1254(July), 1–8. https://www.ag.ndsu.edu/pubs/ansci/range/as1254.pdf
28. Tufail MS, Mbuku S, Mutimura M, Guo X, Piltz J (2020) Utilization of conserved forage to improve livestock production on smallholder farms in Asia and Africa. September 2015. https://uknowledge.uky.edu/igc/22/3-1/1/
29. Van Soest PJ, Robertson JB, Lewis BA (1991) Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. J Dairy Sci 74(10):3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2
30. Weinberg ZG, Muck RE (1996) New trends and opportunities in the development and use of inoculants for silage. FEMS Microbiol Rev 19(1):53–68. https://doi.org/10.1016/0168-6445(96)00025-3

Figures



a. plan of the frame **b. press on the frame** **c. sampled microsilo**

Figure 1

Description of the used silage compressing mechanism (a, b) with a sampled microsilo (c)

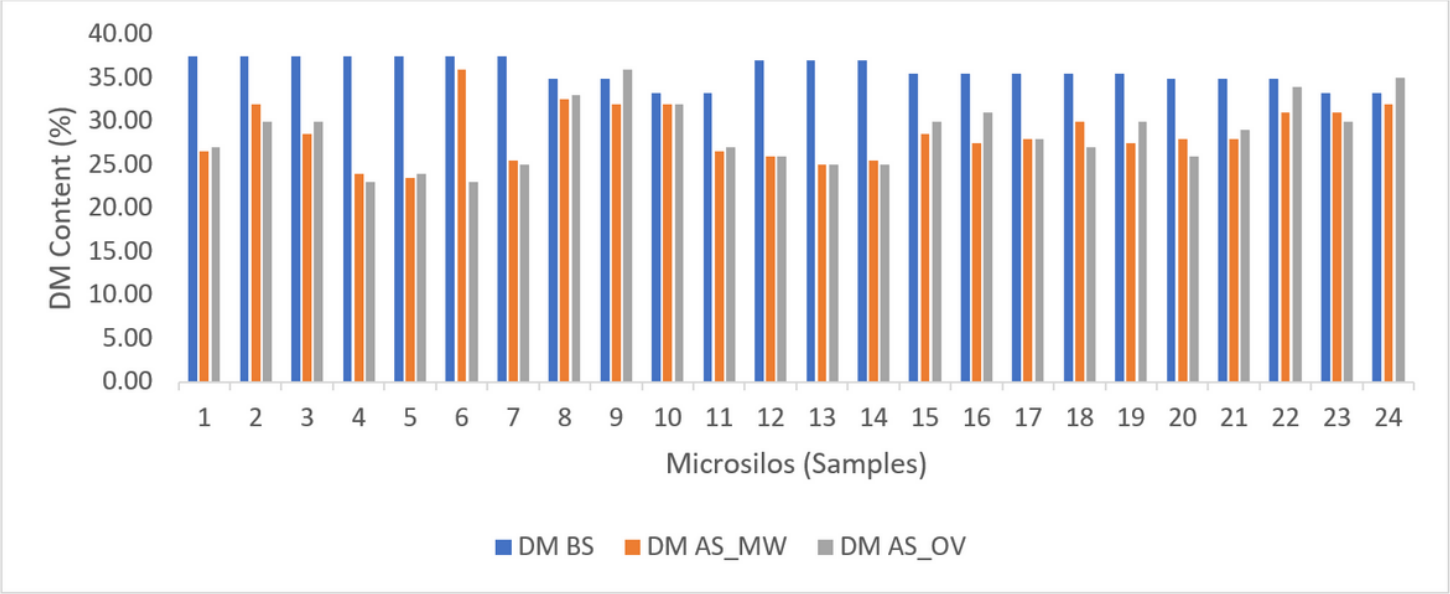


Figure 2

DM variations on day 1 (BS) and day 48 (AS; *MW*, *OV*) of the experiment

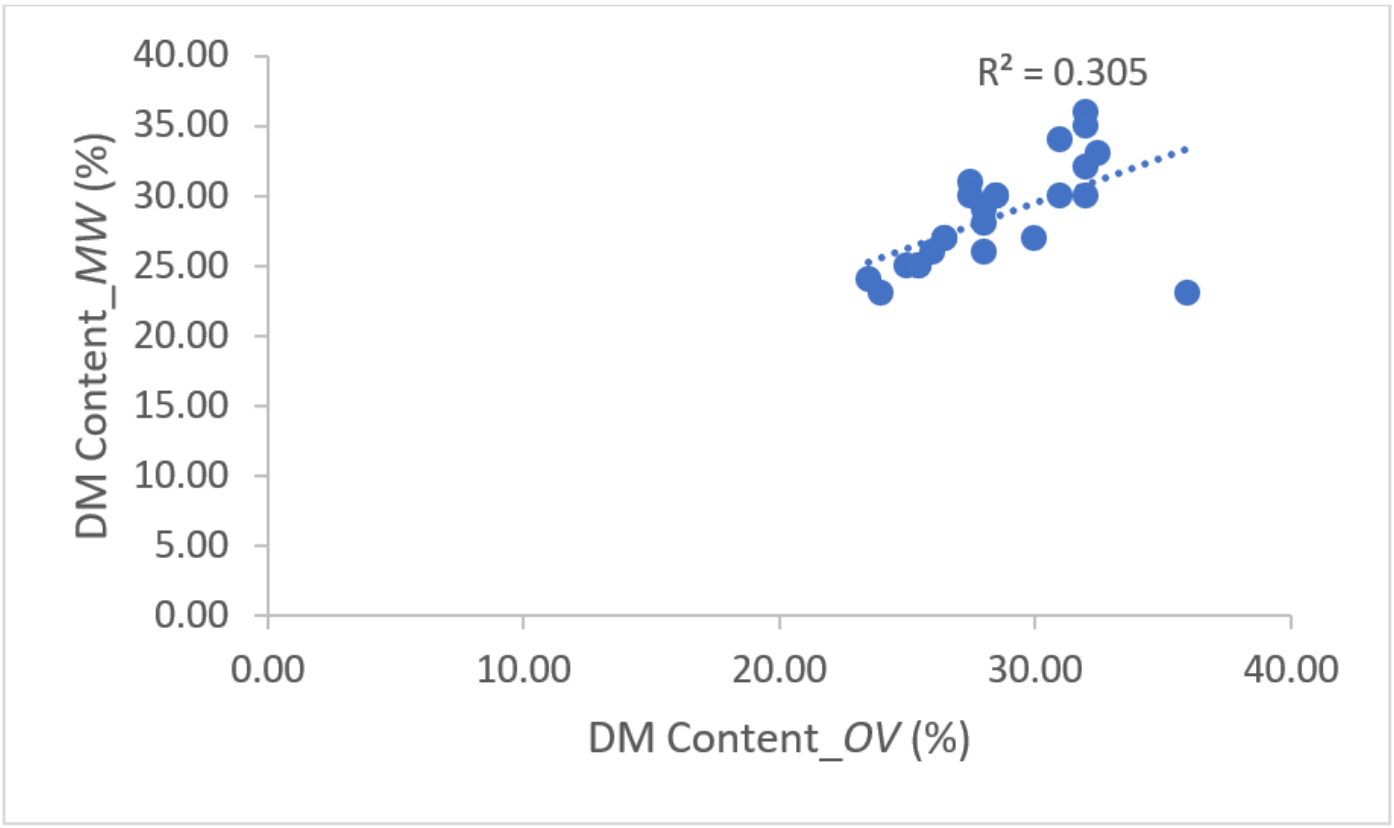
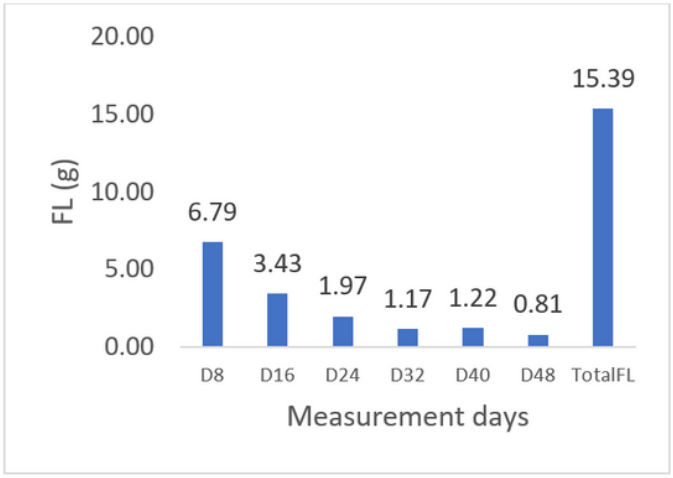
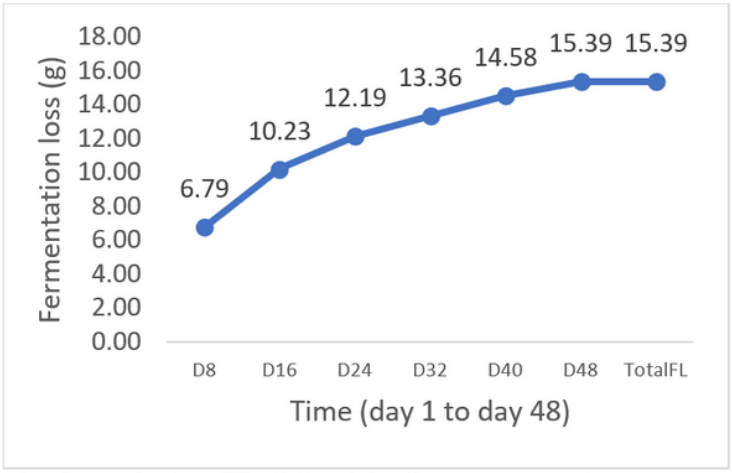


Figure 3

Linear relationship between DM-*MW* and DM-*OV* levels measured on day 48



a. FL (%) recorded at each measurement



b. Cumulating FL (%) with silage maturation process

Figure 4

Average fermentation loss records (a) and cumulating losses (b) from day 8 to day 48

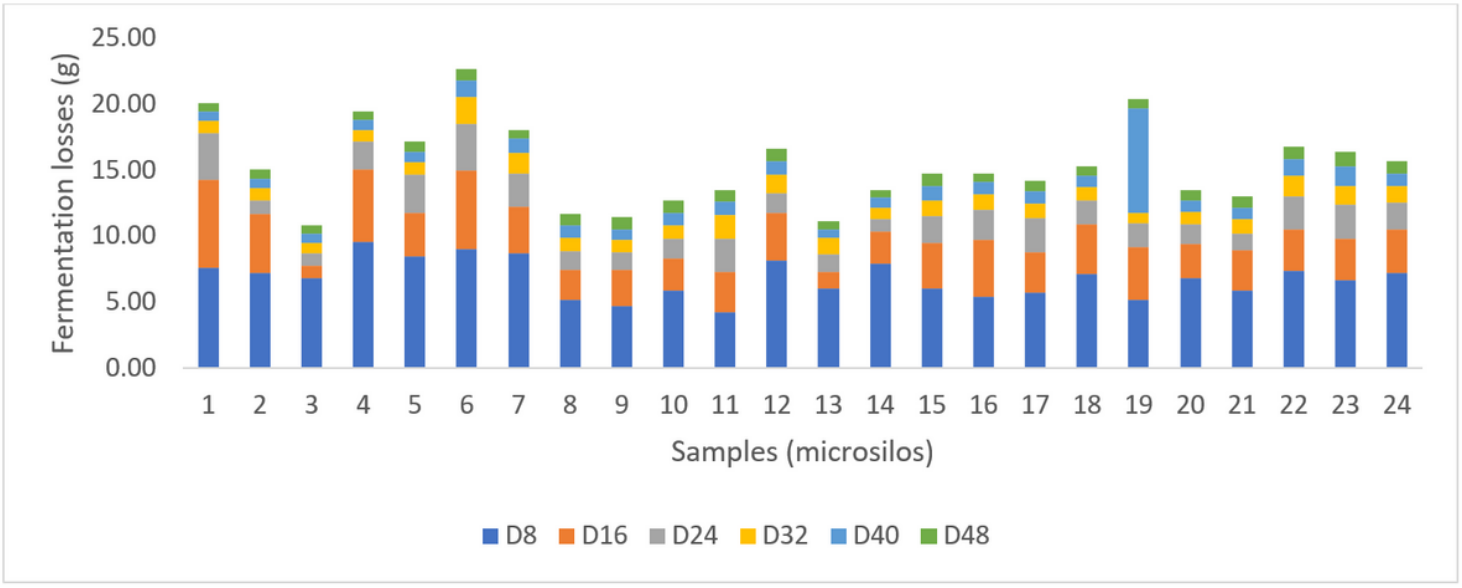


Figure 5

Proportions of fermentation losses by measurement day to the total FL

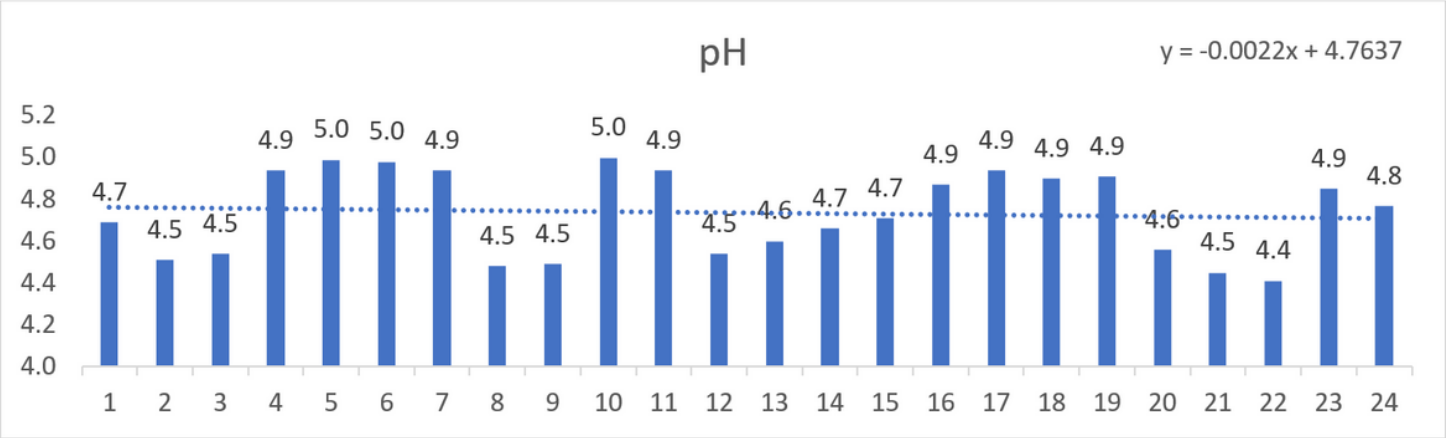


Figure 6

Variations in pH among 24 samples at silage maturity

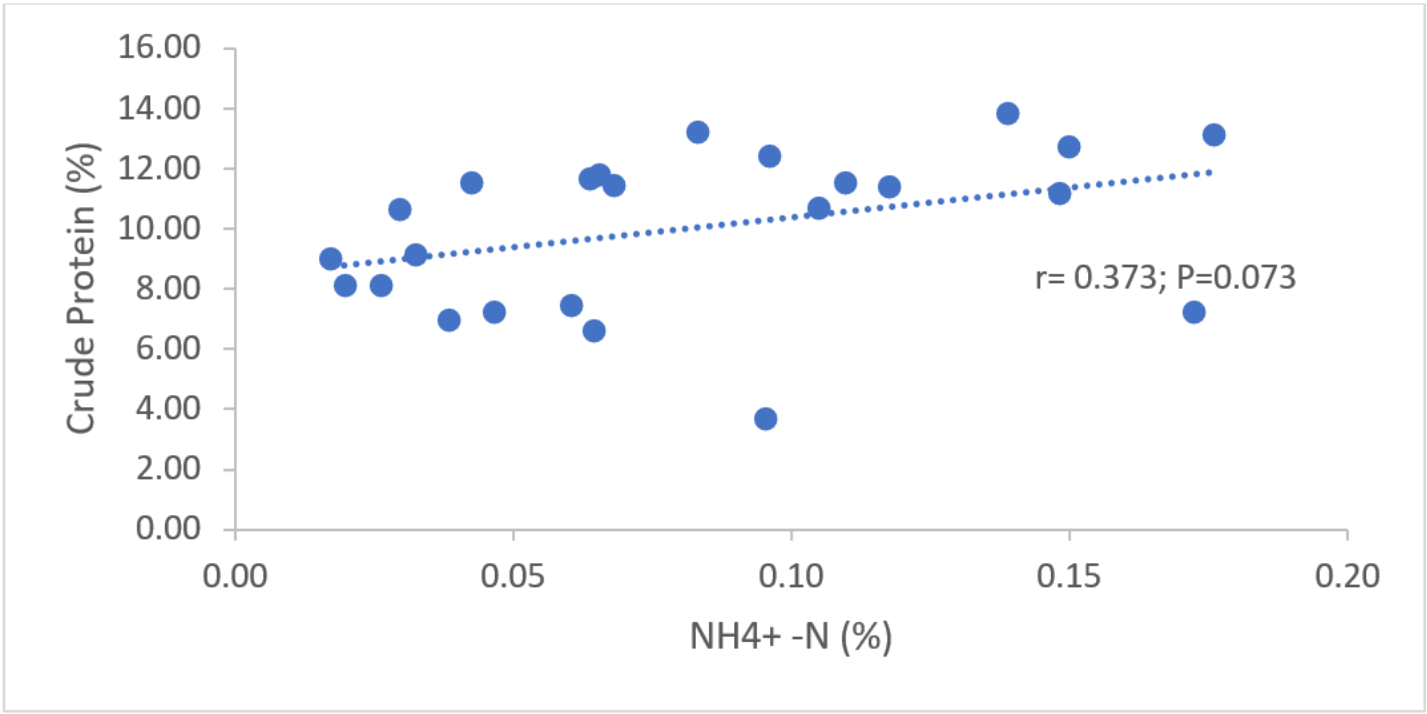


Figure 7

Relationships between ammonia and crude protein contents (%)

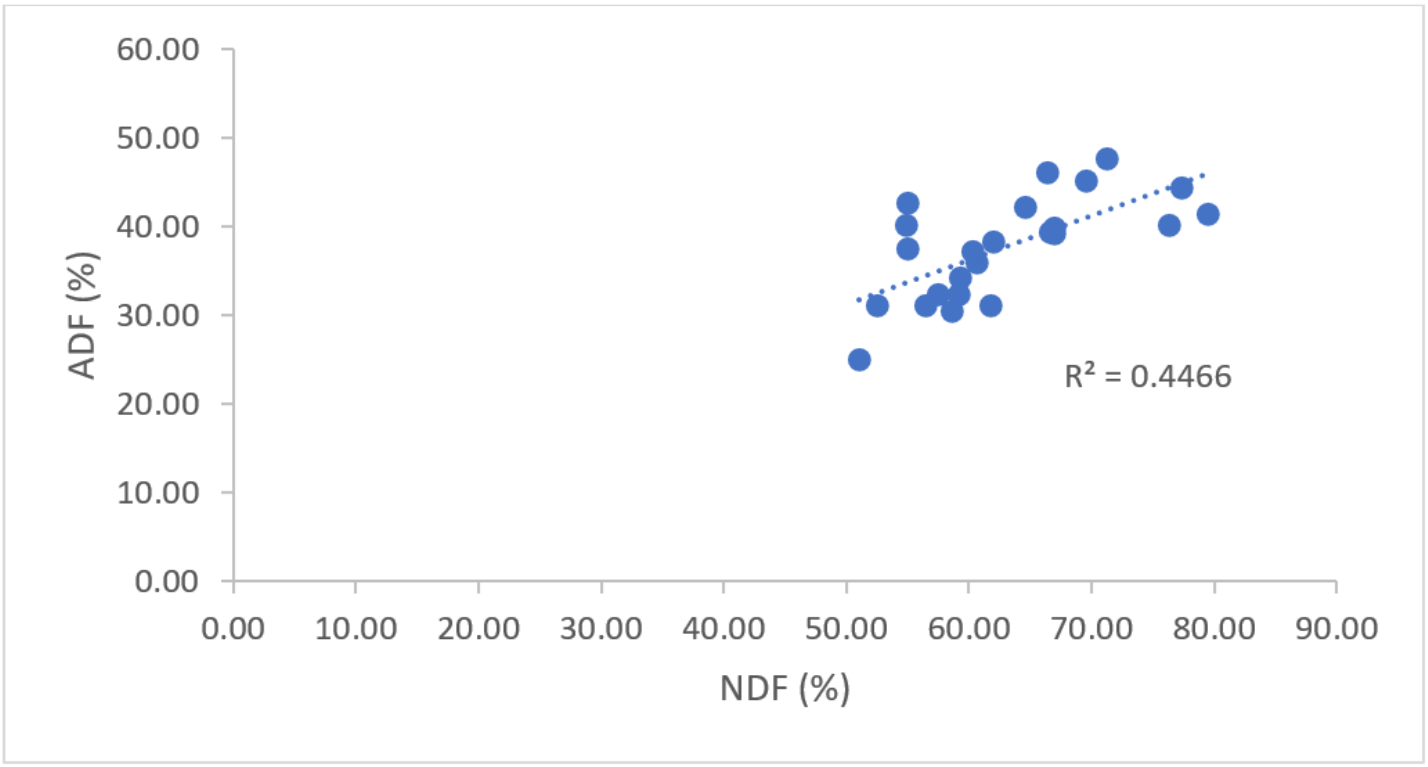
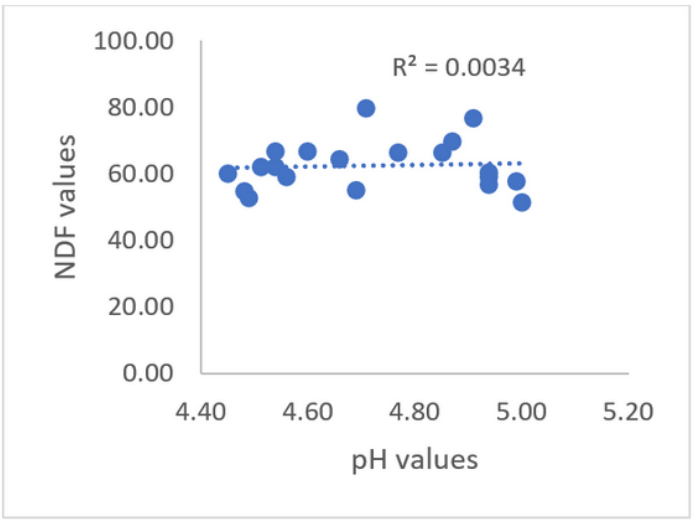
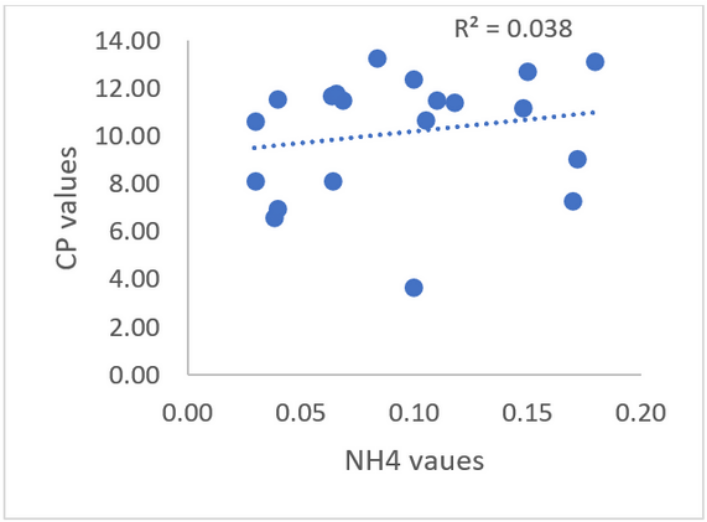


Figure 8

Relationships between NDF and ADF (%)



a. Linear relationship between pH and NDF



b. Linear relationship between CP and NH4

Figure 9

Correlation between pH and NDF (a) and between CP and NH4 (b)

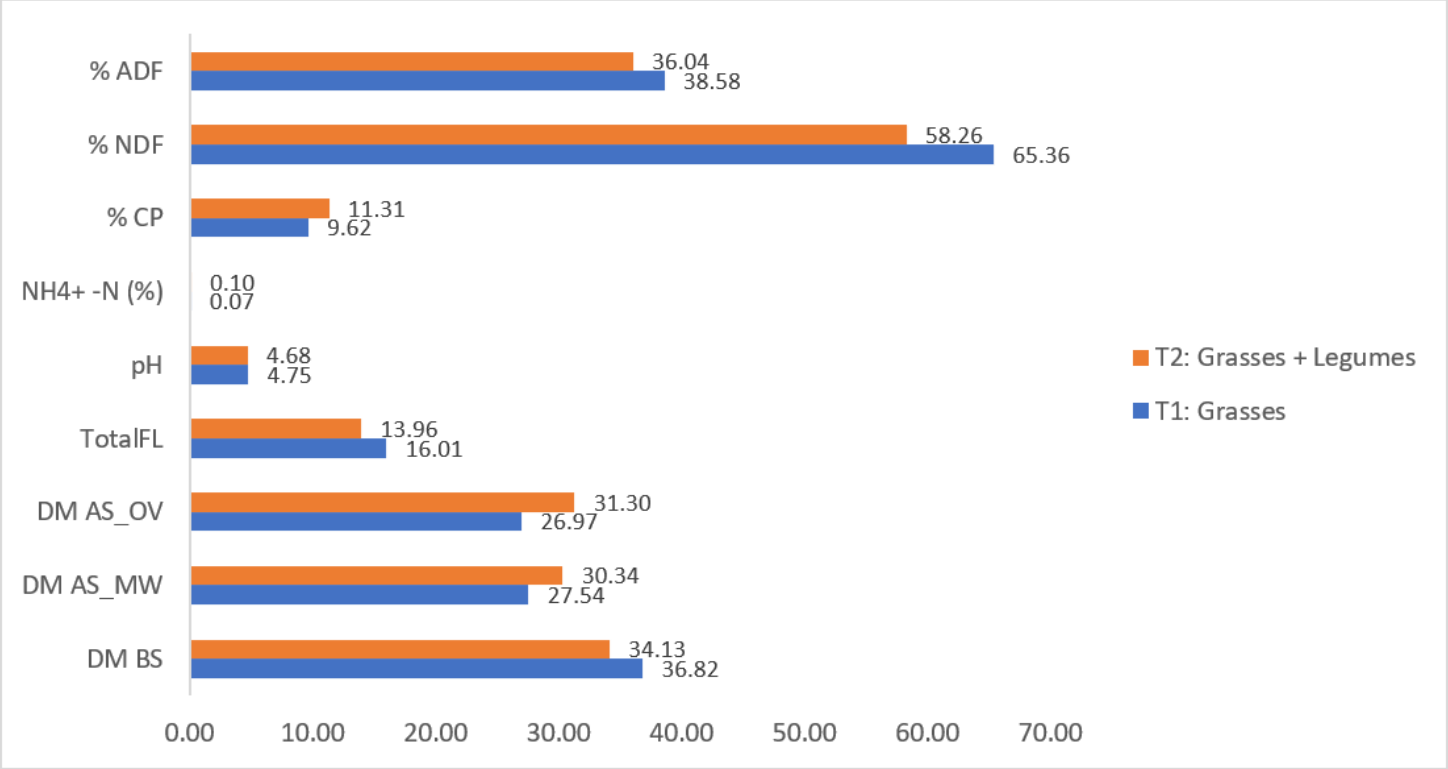


Figure 10

Quality parameters of silage as affected by treatment (forage mixture)