UNIVERSITÀ DEGLI STUDI DELLA TUSCIA | DIPARTIMENTO DI SCIENZE AGRARIE E FORESTALI

75th EAAP Annual Meeting
1/5 September 2024 - Florence, Italy

# Effect of conservation length on fermentation characteristics of teff silage as alternative forage for Mediterranean livestock farming systems

**R. Primi,** R. Spina, F. Luziatelli, M. Ruzzi, U. Bernabucci, F. Manganello, C. Evangelista, F. Lazzari, R. Ruggeri, F. Rossini, A. Benelli, R. Moscetti, A. Brunetti, I. Bottoni, S. Ceci, G. Fabbrizi, P. P. Danieli, B. Ronchi

agritech
National Center for Technology in Agriculture

1st September 2024

*Session 5. Innovative and emerging feed and forage resources*

# Introduction

- **Climate change** impacts ruminant livestock farming by affecting **feed** availability and quality, requiring **adaptation strategies** to sustain production (Benitez-Alfonso *et al*., 2023).

- **Drought-tolerant forage crops** are critical for meeting animal nutritional needs in arid and semi-arid (i.e., Mediterranean) regions, potentially replacing vulnerable conventional crops (Kakabouki *et al*., 2021).

- **Teff** [*Eragrostis tef*, (Zucc.) Trotter, Poaceae], a minor ("orphan") warm-season C4 plant traditionally cultivated in Ethiopia and Eritrea, have the potential to offers high yield (~5.5 t DM/ha, Ruggeri *et al*., 2024) and quality as a **summer forage**, is adaptable to **various climates**, and can serves as an **emergency or double crop** option immediately following wheat or barley (Woldeyohanne *et al*., 2020)

- Teff is a **fast-growing crop** (it can provide a first harvest 40–45 days after sowing and is able to persist for over 5 months with multiple mowings when an amount of water is available), relatively **pest and disease resistant** (Kakabouki *et al*., 2021).

# Introduction

- Tef is gaining popularity as an alternative summer forage grass in various regions worldwide, including Australia, Canada, Kenya, the USA, South Africa, India, Pakistan, Yemen, and South America.

- In Europe, it is marginally cultivated in the Netherlands and in Spain

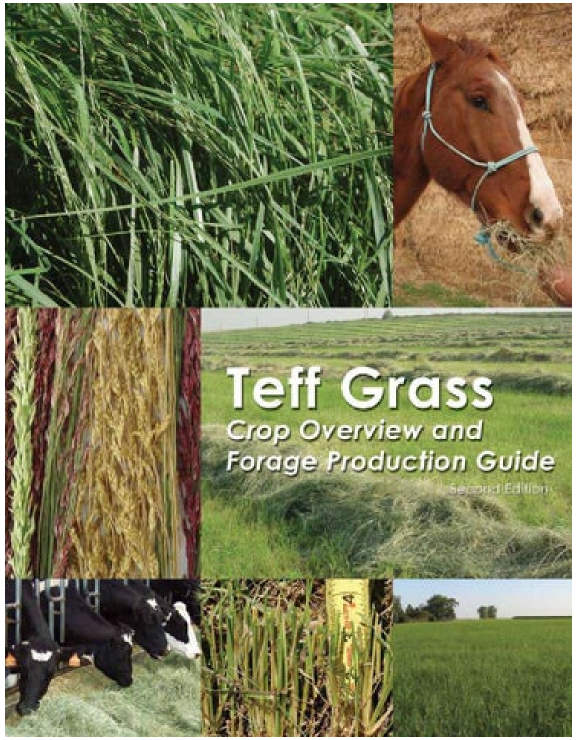
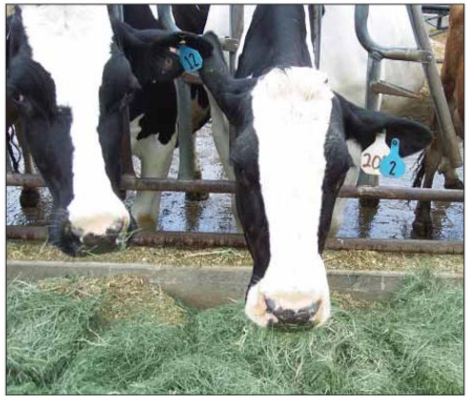



**Table 4.** Chemical composition of a composite sample of Moxie teff harvested at either the early-heading (EH) or late-heading (LH) stages of maturity

| Item | EH | LH |
|---|---|---|
| DM | 84.1 | 81.9 |
| Nutrient content, % DM | | |
| OM | 86.9 | 87.15 |
| CP | 17.6 | 15.4 |
| Starch | 0.40 | 0.65 |
| ADF | 35.2 | 35.6 |
| NDF | 58.6 | 60.0 |
| TDN | 58.6 | 57.7 |
| Calcium, % | 0.63 | 0.67 |
| Phosphorus, % | 0.29 | 0.27 |
| Magnesium, % | 0.28 | 0.28 |
| Potassium, % | 2.55 | 2.31 |
| Sulfur, % | 0.28 | 0.29 |
| Sodium, % | 0.06 | 0.05 |
| Chloride, % | 0.51 | 0.43 |
| Iron, mg/kg | 723 | 439 |
| Manganese, mg/kg | 59.5 | 58.5 |
| Zinc, mg/kg | 38.0 | 35.0 |
| Copper, mg/kg | 11.0 | 11.5 |
| Nitrate ion, % | 1.66 | 1.34 |

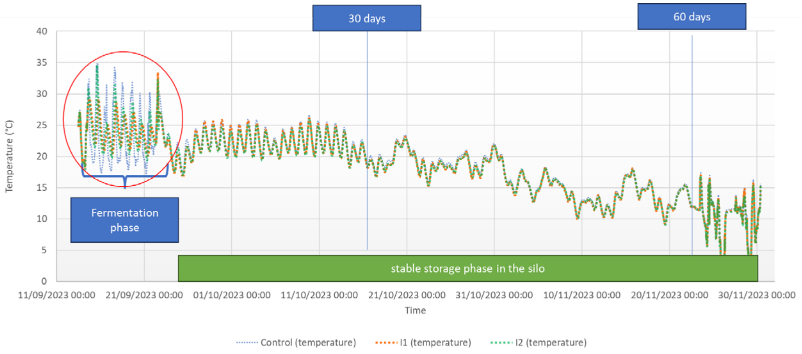
Ream *et al*. 2020. Applied Animal Science, Volume 36, Issue 5



- Very few studies available for nutritional characteristics

# Aim of the research

- Our study was aimed to assess the fermentative characteristics of whole crop teff silage and to examine the impact of different (short) conservation periods and the effects of various inoculants on the aerobic stability, fermentation profile, and microbial count of silages.
- Here we present only **results of different conservation periods** (30 and 60 days of ensiling, without inoculants)



# Material and methods


- Teff *var*. Moxie® (Barenbrug, The Netherlands) seeds were planted <u>as second crop after wheat</u> harvesting at the experimental farm of the University of Tuscia, Viterbo, central Italy, altitude 310 m a.s.l.):
  - Sowing: July 1, 2022
  - Fertilization: 100 kg N/ha
  - Water supply: 200 mm.

- Whole teff crop was harvested at approximately 30% dry matter content and chopped to a theoretical cutting length of 4 cm.
- No pre-wilting

# Material and methods

- 5 samples of fresh forage were analyzed, for water activity, chemical profile and microbial count (Borreani *et al*., 2014).
- The chopped material was ensiled in quadruplicate in 20 L plastic silos with gas-release lids (density: 400 kg/m³) and a temperature logger inside.

- Silos were opened 30 and 60 days after ensiling.
- 8 samples of silages were analyzed, for water activity, chemical profile, microbial count, and aerobic stability* (Borreani *et al*., 2014).
- Silos weights were measured pre and post ensiling to calculate weight and DM losses.


*Aerobic stability was defined as the number of hours the silage remained stable before its temperature increased by 2 °C above room temperature (Ranjit and Kung, 2000).

# Material and methods

## Statistical data analysis

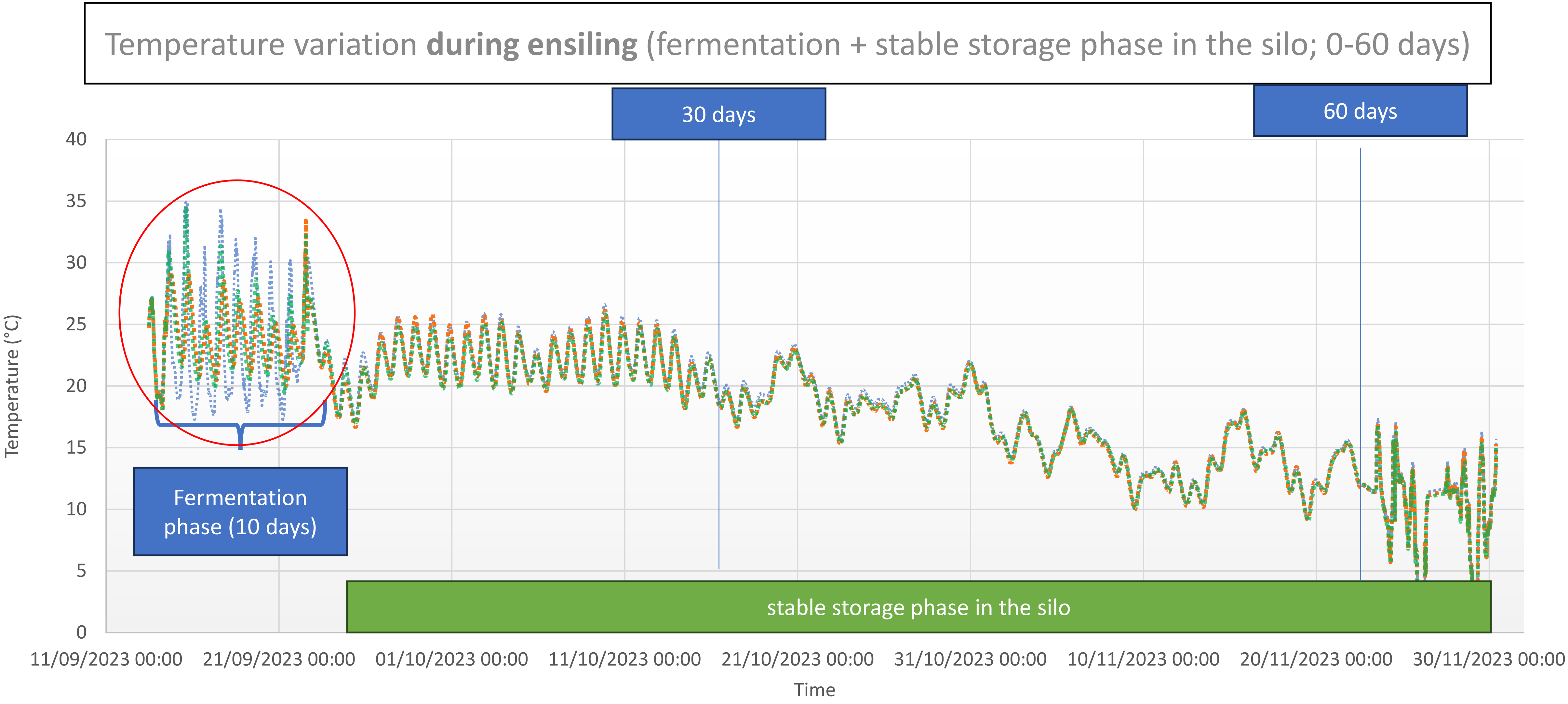
- Two-sample Student's unpaired t-test was performed to compare the means between the two groups (30 *vs* 60 days of conservation periods)
- Correlations between the fermentative, chemical, and microbial profile were determined using Pearson's test
- *P*-values below 0.05 were considered statistically significant.
- All statistics were performed with XLSTAT® (Lumivero, 2024)

Chemical and microbial characteristics of teff silages at ensiling

| Parameters | Mean | SD | Min | Max |
| --- | --- | --- | --- | --- |
| Dry matter (%) | 30.6 | 0.6 | 30.2 | 31.3 |
| pH | 6.1 | 0.0 | 6.1 | 6.1 |
| Water activity ($a_w$) | 1.0 | 0.0 | 1.0 | 1.0 |
| Buffering capacity (mEq/kg DM) | 5.5 | 0.0 | 5.4 | 5.5 |
| Crude protein (% of DM) | 13.1 | 0.2 | 12.8 | 13.3 |
| Ash (% of DM) | 11.8 | 0.2 | 11.5 | 12.1 |
| Starch (% of DM) | 6.0 | 0.6 | 5.3 | 6.7 |
| Ether extract (% of DM) | 1.4 | 0.1 | 1.4 | 1.6 |
| Crude fibre (% of DM) | 31.8 | 1.6 | 29.7 | 33.8 |
| aNDFom (% of DM) | 65.3 | 0.3 | 64.8 | 65.6 |
| ADF (% of DM) | 52.0 | 2.8 | 48.8 | 56.1 |
| Hemicelluloses (% DM) | 14.2 | 2.2 | 11.1 | 16.7 |
| ADL (% of DM) | 7.7 | 0.2 | 7.5 | 8.1 |
| Nitrogen-free extract (% of DM) | 41.9 | 1.7 | 40.0 | 44.5 |
| Non-fiber carbohydrates (% of DM) | 8.4 | 0.4 | 8.0 | 8.9 |
| Lactic acid bacteria ($log_{10}$ cfu/g) | 5.5 | 0.2 | 5.3 | 5.8 |
| Yeast ($log_{10}$ cfu/g) | 2.5 | 1.2 | 0.0 | 3.3 |
| Moulds ($log_{10}$ cfu/g) | 2.9 | 2.3 | 0.0 | 4.8 |

Native microorganisms

# Results




All silages were well fermented.

# DM and nutritional parameters of teff silage after the two conservation periods

| Parameters | 30 days | 60 days | P-value |
|---|---|---|---|
| Dry Matter (DM) (%) | 28.08 (27.56-28.46) | 27.19 (26.57-27.63) | ↓ 0.001 |
| Ash (% of DM) | 10.91 (10.48-11.13) | 11.10 (10.89-11.44) | 0.097 |
| Crude protein (% of DM) | 14.53 (13.16-15.57) | 8.04 (7.23-8.78) | ↓ <0.0001 |
| Ether extract (% of DM) | 2.14 (1.97-2.26) | 1.85 (1.54-2.20) | ↓ 0.006 |
| Crude fibre (% of DM) | 32.09 (31.17-33.31) | 32.51 (32.10-33.31) | 0.135 |
| aNDFom (% of DM) | 61.39 (59.96-62.56) | 62.12 (60.17-63.28) | 0.182 |
| ADF (% of DM) | 49.37 (46.84-54.15) | 50.10 (48.69-51.75) | 0.533 |
| Hemicelluloses (% of DM) | 12.02 (5.96-15.58) | 12.02 (9.32-13.41) | 0.999 |
| ADL (% of DM) | 6.90 (6.50-7.83) | 7.63 (7.17-8.06) | ↑ 0.002 |
| Nitrogen-free extract (% of DM) | 40.34 (37.86-41.62) | 46.52 (45.91-47.29) | ↑ <0.0001 |
| Non-fiber carbohydrates (% of DM) | 11.03 (9.57-12.32) | 16.90 (15.78-18.29) | ↑ <0.0001 |
| Starch (% of DM) | 8.85 (7.39-11.39) | 7.02 (6.18-8.21) | ↓ 0.013 |

# Aerobic stability, microbiology and weight/DM loss of teff silage at different conservation periods

| Parameters | 30 days | 60 days | P-value |
| --- | --- | --- | --- |
| pH | 4.36 (4.33-4.41) | 4.40 (4.36-4.41) | 0.027 |
| Water activity ($a_w$) | 0.98 (0.98-0.99) | 0.98 (0.98-0.98) | 0.027 |
| Buffering capacity (mEq/Kg DM) | 28.13 (27.25-29.90) | 43.40 (34.90-50.00) | 0.000 |
| Aerobic stability (h) | 511.50 (363.00-740.00) | 617.00 (530.00-740.00) | 0.143 |
| LAB ($Log_{10}$ CFU/g) | 7.44 (6.95-7.80) | 7.33 (7.11-7.52) | 0.257 |
| Yeasts ($Log_{10}$ CFU/g) | 2.19 (1.86-2.56) | 0.49 (0.00-1.43) | <0.0001 |
| Moulds ($Log_{10}$ CFU/g) | 0.28 (0.00-1.26) | 0.24 (0.00-0.95) | 0.878 |
| Lactic acid (relative %) | 38.07 (36.99-39.47) | 19.46 (5.08-38.16) | 0.006 |
| Nitrate (Mg/L) | 24.13 (10.00-38.00) | 34.13 (24.00-45.00) | 0.049 |
| Ammonium (Mg/L) | 70.38 (67.00-73.00) | 68.13 (60.00-73.00) | 0.186 |
| Weight loss (%) | 0.82 (0.72-0.95) | 0.83 (0.73-0.91) | 0.802 |
| DM loss (%) | 10.72 (7.68; 9.00) | 12.13 (10.44; 13.93) | 0.000 |

RESULTS

# Pearson’s correlation coefficients of chemical and microbiological characteristics of teff silages

| Variables | Days | DM | Ash | CP | Fat | CF | aNDFom | ADF | Hemicel. | ADL | NFE | NFC | Starch | pH | Aw | Buffering capacity | Aerobic stability | LAB | Yeast | Molds | Nitrate | Ammonium | Weight loss | DM loss (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DM | **-0,755** | | | | | | | | | | | | | | | | | | | | | | | |
| Ash | 0,429 | -0,334 | | | | | | | | | | | | | | | | | | | | | | |
| CP | **-0,980** | **0,669** | -0,404 | | | | | | | | | | | | | | | | | | | | | |
| Fat | **-0,656** | **0,737** | -0,220 | **0,607** | | | | | | | | | | | | | | | | | | | | |
| CF | 0,391 | **-0,506** | 0,270 | -0,404 | -0,242 | | | | | | | | | | | | | | | | | | | |
| aNDFom | 0,351 | -0,300 | -0,137 | -0,365 | **-0,617** | 0,082 | | | | | | | | | | | | | | | | | | |
| ADF | 0,171 | 0,177 | 0,352 | -0,175 | -0,098 | -0,258 | -0,046 | | | | | | | | | | | | | | | | | |
| Hemicelluloses | 0,000 | -0,286 | -0,370 | -0,002 | -0,178 | 0,263 | 0,470 | **-0,903** | | | | | | | | | | | | | | | | |
| ADL | **0,712** | **-0,643** | 0,086 | **-0,770** | **-0,543** | 0,438 | 0,457 | -0,121 | 0,303 | | | | | | | | | | | | | | | |
| NFE | **0,967** | **-0,637** | 0,321 | **-0,983** | **-0,643** | 0,248 | 0,417 | 0,208 | -0,005 | **0,756** | | | | | | | | | | | | | | |
| NFC | **0,949** | **-0,645** | 0,424 | **-0,963** | **-0,499** | 0,404 | 0,109 | 0,185 | -0,117 | **0,704** | **0,936** | | | | | | | | | | | | | |
| Starch | **-0,604** | 0,187 | -0,188 | **0,676** | **0,555** | -0,081 | **-0,583** | -0,376 | 0,081 | **-0,604** | **-0,714** | **-0,555** | | | | | | | | | | | | |
| pH | **0,550** | -0,487 | **0,576** | **-0,501** | -0,291 | 0,236 | -0,243 | 0,420 | -0,476 | 0,204 | 0,461 | **0,599** | -0,144 | | | | | | | | | | | |
| Aw | **-0,577** | 0,165 | **-0,612** | **0,567** | 0,230 | -0,165 | 0,061 | **-0,819** | **0,750** | -0,132 | **-0,534** | **-0,601** | 0,458 | **-0,635** | | | | | | | | | | |
| Buffering capacity | **0,890** | **-0,844** | 0,389 | **-0,863** | **-0,890** | 0,452 | **0,515** | 0,154 | 0,086 | **0,739** | **0,854** | **0,788** | **-0,636** | **0,505** | -0,452 | | | | | | | | | |
| Aerobic stability | 0,383 | -0,460 | -0,040 | -0,470 | -0,265 | **0,718** | 0,197 | -0,368 | 0,410 | **0,755** | 0,387 | 0,459 | -0,253 | 0,128 | 0,086 | 0,467 | | | | | | | | |
| LAB | -0,301 | -0,009 | 0,063 | 0,391 | -0,259 | -0,377 | 0,124 | -0,156 | 0,191 | -0,488 | -0,327 | -0,445 | 0,285 | -0,118 | 0,345 | -0,090 | -0,477 | | | | | | | |
| Yeast | **-0,868** | **0,670** | -0,391 | **0,833** | **0,597** | -0,286 | -0,389 | -0,075 | -0,101 | **-0,598** | **-0,831** | **-0,777** | 0,454 | **-0,498** | 0,473 | **-0,774** | -0,287 | 0,086 | | | | | | |
| Moulds | -0,042 | 0,159 | -0,038 | -0,019 | 0,371 | 0,397 | -0,070 | -0,202 | 0,148 | -0,085 | -0,071 | 0,020 | 0,113 | -0,106 | 0,048 | -0,168 | 0,287 | -0,166 | 0,076 | | | | | |
| Nitrate | **0,499** | -0,225 | 0,373 | -0,456 | **-0,563** | -0,092 | 0,418 | 0,421 | -0,193 | 0,056 | **0,503** | 0,363 | **-0,610** | 0,207 | -0,482 | **0,509** | -0,365 | 0,183 | -0,338 | -0,268 | | | | |
| Ammonium | -0,348 | 0,156 | -0,018 | 0,396 | 0,237 | -0,012 | -0,346 | -0,021 | -0,130 | -0,170 | -0,424 | -0,323 | 0,161 | -0,253 | 0,089 | -0,302 | -0,102 | -0,070 | 0,404 | -0,128 | -0,287 | | | |
| Weight loss | **-0,068** | 0,095 | 0,205 | 0,183 | -0,040 | **-0,611** | -0,015 | 0,375 | -0,337 | -0,415 | -0,097 | -0,202 | -0,005 | 0,000 | -0,176 | -0,120 | **-0,870** | 0,408 | 0,077 | -0,439 | 0,558 | 0,227 | | |
| DM loss | **-0,794** | **0,983** | -0,332 | **0,728** | **0,763** | **-0,542** | -0,366 | 0,175 | -0,312 | **-0,680** | **-0,693** | **-0,688** | 0,242 | -0,481 | 0,189 | **-0,884** | **-0,517** | 0,006 | **0,736** | 0,105 | -0,242 | 0,291 | 0,192 | |
| Lactic acid | **-0,725** | **0,668** | -0,245 | **0,735** | **0,865** | -0,400 | **-0,649** | -0,059 | -0,226 | **-0,655** | **-0,738** | **-0,615** | **0,615** | -0,314 | 0,283 | **-0,902** | -0,473 | -0,053 | **0,710** | 0,096 | -0,470 | **0,531** | 0,269 | **0,771** |

Aside from the effect of conservation period:

- the pH was negatively correlated with the CP and positively correlated to ash and NFC
- negative correlations were detected between the DM content and CF, ADL, nitrogen-free extract and non-neutral detergent fiber carbohydrate and buffering capacity;
- the CP content was positively correlated with the DM, fat, starch, yeast count, DM loss and lactic acid and negatively with ADL, NFE and NFC, pH.
- lactic acid was negatively correlated with the fiber fractions and buffering capacity and positively with DM, CP, fat and starch
- DM losses were found to be strongly correlated positively to the DM content

# Discussions

- Our findings indicate that teff can be successfully ensiled without pre-wilting or inoculants, achieving stable fermentation even at 30% dry matter.
- The longer conservation periods led to increased aerobic stability and better preservation of carbohydrates, though there was a reduction in crude protein content.
- These results align with previous studies, such as those by Ferrero et al. (2019), which observed similar trends in dry matter losses and nutrient preservation over extended conservation periods.

# Future directions

- We are currently conducting further studies to
  - assess the effects of various inoculants (i.e., *Pediococcus pentosaceus, Lactobacillus plantarum, Lactobacillus hilgardii, Lactobacillus buchneri, Xylanase, Beta-glucanase*)
  - perform a comprehensive metabolomics analysis to better understand the metabolic changes during ensiling

- Further studies are expected to evaluate:
  - the fermentation characteristics in vitro and under farm conditions
  - the effect on livestock production through *in vivo* studies

# Conclusions

- Teff shows great promise as a sustainable forage option for Mediterranean livestock systems, particularly in regions affected by climate change.
- The ability to produce high-quality silage without the need for extensive inputs makes teff a valuable crop for the future.

# Thanks for your kind attention

Any questions?

This study was carried out within the Agritech National Research Center and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022). This manuscript reflects only the authors' views and opinions, neither the European Union nor the European Commission can be considered responsible for them.

Finanziato dall'Unione europea NextGenerationEU | Ministero dell'Università e della Ricerca | Italiadomani

agritech
National Center for Technology in Agriculture
Plan. Plant. Planet.