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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Determination of Nutritional Quality and Aerobic Stability of Sorghum, Maize, and Sorghum-Maize Mixture Silages

Sorgum, Mısır ve Sorgum-Mısır Karışımı Silajlarının Besin Kalitesi ve Aerobik Stabilitesinin Belirlenmesi

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Abstract

The current study aimed to determine the nutritional quality and aerobic stability of sorghum, maize, and their mixture silages without any additives. Sorghum and maize were harvested at dough stage from a local farm in Tekirdağ. Fresh plant materials were chopped to a length of 2-3 cm and packed into polythene bags, and vacuumed. A total of 12 vacuum-packed silos (4 replications in each treatment) were prepared with sorghum (S), maize (M), and a mixture of sorghum-maize (SM) forage (w:w, 50:50 according to dry matter) and stored at room temperature for 60 days. The chemical and microbiological composition of silages with the rate of aerobic deterioration upon aerobic exposure were evaluated. Based on the pH and ammonia nitrogen concentration, all silages could be classified as good quality. The water-soluble carbohydrate level of the SM group increased due to mixing S and M forages, leading to improved lactic acid content. The NDF and ADF values of silages varied between 520.52-588.32 and 234.98-309.01 g kg^{-1}, and the differences between silages were significant ($P<0.01$). The Hemicellulose/Cellulose ratio of S, M, and SM silages were found 0.94, 1.49, and 1.18, respectively. The lactobacilli and yeast content of silages were significant and varied between 5.18-7.41 and 5.18-7.29 log cfu g^{-1}, and the highest and lowest values were observed in SM and S silages, respectively ($P<0.01$). No visible mold was detected in all silages after 5 days of aerobic exposure ($P>0.05$). The pH, CO_2, and yeast numbers were varied in groups between 4.88-6.74, 55.71-119.33 g kg^{-1}, and 8.40-9.01 log cfu g^{-1}. It was concluded that it is possible to improve the nutritional and fermentation characteristics of sorghum and maize silage by ensiling their mixture. However, it is highly recommended that silage additives should be used to guarantee and strengthen the fermentation and aerobic stability of silage mostly made by a mixture of these two energetic forage crops.

Keywords: Sorghum, Maize, Silage, Aerobic stability, Silage quality

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Öz

Bu çalışmada, katkı maddesi içermeyen sorgum, mısır ve bunların karışımından oluşan silajların silaj kaliteleri ve aerobik stabilite özelliklerinin belirlenmesi amaçlanmıştır. Sorgum ve mısır hamur olum döneminde Tekirdağ'daki lokal bir işletmeden temin edilmiştir. Taze bitki materyalleri 2-3 cm uzunlunda doğranarak ve polietilen torbalara konulmuş ve vakumlanmıştır. Sorgum (S), mısır (M) ve sorgum-mısır karışımından (kuru madde düzeyine göre 50:50) her bir muamele grubu için 4 tekerrür olmak üzere toplamda 12 adet vakumlanmış paket silo hazırlanmış ve oda sıcaklığında 60 gün silolanmıştır. Silajların kimyasal ve mikrobiyolojik kompozisyonları ile oksijene maruz kalması sonrası aerobik bozulma oranları birlikte değerlendirilmiştir. Elde edilen pH ve amonyak nitrojen konsantrasyonuna göre tüm silajlar iyi kaliteli sınıfında yer almıştır. S ve M'nin karışımından oluşan SM grubunda, suda çözünebilir karbonhidrat oranının artması laktik asit içeriğinin artmasını sağlamıştır. Silajların NDF ve ADF içeriğindeki farklar önemli olmakla birlikte sırasıyla 520.52-588.32 ve 234.98-309.01 g kg^{-1} arasında değişim göstemiştir ($P<0.01$). Hemiselüloz/selüloz oranı S, M ve SM gruplarında sırasıyla 0.94, 1.49 ve 1.18 olarak bulunmuştur. Silajların laktobasil ve maya içerikleri arasındaki farklar önemli olup 5.17-7.41 ve 5.18-7.29 log kob g^{-1} arasında değişiklik göstermiş ve en yüksek ile en düşük değerler sırasıyla SM ve S silajlarında gözlemlenmiştir ($P<0.01$). Beş günlük aerobik maruziyet sonrasında tüm silaj gruplarında küf tespit edilmemiştir ($P>0.05$). Silaj gruplarında pH, CO_2 ve maya sayısı sırasıyla 4.88-6.74, 55.71-119.33 g kg^{-1} ve 8.40-.01 log kob g^{-1} arasında değişmiştir. Sorgum ve mısırın birlikte silolanması ile besin ve fermantasyon kalitesini iyileştirmenin mümkün olduğu sonucuna varılmıştır. Bununla birlikte enerji bakımından zengin bu iki bitkinin birlikte silolanmasını garanti altına almak ve fermentasyon ve aerobic stabilitesini güçlendirmek amacıyla katkı maddelerinin kullanılması önerilmektedir.

Anahtar Kelimeler: Sorgum, Mısır, Silaj, Aerobik stabilite, Silaj kalitesi

1. Introduction

Supplying high-quality roughage to livestock year-round results in high milk and meat production. Meeting these animals' nutrient demands with high-quality roughage in dry seasons and preserving them from deterioration in the rainy seasons is among the most crucial constraint for livestock production. To overcome this constraint, the crop-livestock integration system intercorporate two types of annual grasses; one is forage to provide feed for livestock from late summer to early spring, and the other is a grain-producing crop to provide straw (Nakao et al., 2018). Although Turkey's ecological conditions allow the cultivation of many forage crops being suitable for silage production, maize and sorghum species and their hybrids are the most cultivated ones (Demirel et al., 2003).

Maize's popularity is derived from its high yielding potential, high energy concentration, palatability, and easy application in the total mixed ration and it's all forms (e.g., grain, hay, silage) represent a principal and essential feed source for farm animals on a global scale (Szymańska et al., 2014). Feed produced from maize is also characterized by relatively low nitrogenous content and biological value (Daniel et al., 2008). Therefore, it should be used together with other roughage and concentrated feed.

Sorghum (*Sorghum bicolor* L. Moench) is characterized by drought resistance and salinity tolerance, high water-soluble carbohydrates, and greater biomass yield than maize (Rocateli et al.,2012; Pinho et al., 2015). Moreover, dual-purpose sorghum hybrids can reach 68% more dry matter (DM) yield than wild ones due to their number of panicles (Da Silva et al., 2012). The nutritional quality of sorghum is influenced by the genotype, specific size, life cycle, and breeding purpose is classified into four main groups according to their stem, number of panicles, and stem-leaf ratio:grain, forage, grazing, and broom (Neto et al., 2017; Perazzo et al., 2014). In addition to these factors, plant height, maturity stage at harvest, chopt length, and additives also influence sorghum's nutritional quality of ensiling (Zurak et al., 2018; Da Silva et al., 2012). It was stated in the literature that some sorghum varieties did not meet the nitrogen requirements of ruminants when fed without a concentrate-free diet and resulted in insufficient total digestible nutrients (Kaewpila et al., 2021). The cell wall components of sorghum silages (ADF and ADL content) decrease ruminants' acceptance rate and reduce forage digestibility (Thomas, 2013).

On the other hand, sorghum's production cost is lower than maize in a single seeding with more than one cut, and the nutritional quality is 10-15% lower than maize (Carvalho et al., 2016). Also, sorghum's high moisture content and thick stem structure make it more suitable for ensiling than hay production (Liang et al., 2018). One main challenge to make a good quality sorghum silage is that its low DM content. It is possible to increase the DM content of sorghum ensiling with maize without any preservatives. Therefore, this study aimed to investigate the fermentation and nutritional quality, and aerobic stability of sorghum (S), maize (M), and sorghum-maize mixture (SM) silages without any preservatives.

2. Materials and Methods

Sorghum cultivar of ES Hyperion (Euralis) and maize hybrid of BC 678 (BC Institut) were harvested at dough stage from a local farm in Tekirdağ in 2019, and then study material was immediately transferred into the Animal Feed and Nutrition Laboratory of Tekirdag Namık Kemal University for silage preparation and further analysis. The total precipitation and average temperature of the experimental year were 299.0 mm and 15.7°C, respectively. Since the total precipitation was not sufficient, forage water demands were supplied by irrigation fortnightly *(Figure 1).*

At the laboratory, fresh plant materials were chopped to a length of 2-3 cm and packed into polythene bags, and sealed by a vacuum sealer (CAS CVP-260PD) (Tan, 2021; Büyüktosun and Tan, 2015). A total of 12 vacuum-packed silos (4 replications in each treatment) were prepared with sorghum, maize, and a mixture of sorghum-maize forage (w:w, 50:50 according to DM) stored. The chemical and microbiological composition of S, M, and SM ensiling was given in *Table 1*.

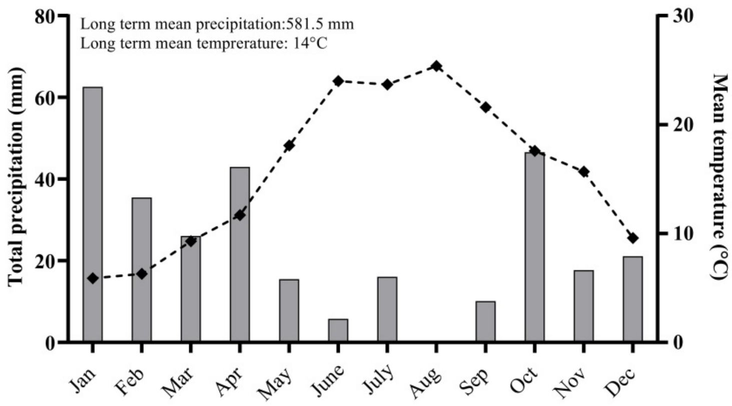

Figure 1. Monthly total precipitation and mean temperature in 2019 at Tekirdag, Turkey.

The vacuum-packed silos were opened at the end of 60 days of ensiling, and pH, DM, WSC and lactic acid content of silages were determined immediately (Anonymous, 1986; Chen et al., 1994; Koç and Çoşkuntuna, 2003). The ammonia nitrogen (NH_3-N) content of silages was carried using the micro distillation method (Anonymous, 1986). Aerobic stability test in bottle systems for 5 days described by Asbell et al. (1991) was subjected simultaneously. At the end of the aerobic stability test, change in pH, production of carbon dioxide (CO_2), number of yeast and mold were used as an indicator. Enumeration of LAB, yeast, and mold of silages was determined using MRS (de Man, Rogosa and Sharpe) and potato dextrose agar, according to Seale et al. (1990) and presented on fresh and wet silages basis.

Table 1. Chemical and microbiological composition of starting materials

Item	S	M	SM
DM, g kg^{-1}	250.2	330.0	283.2
pH	5.8	6.0	6.3
CP, g kg^{-1} DM	80.02	61.10	72.85
WSC, g kg^{-1} DM	122.0	135.7	130.0
Lactobacilli, log10 cfu g^{-1}	2.18	3.00	2.78
Yeast, *log10 cfu g^{-1}*	5.18	6.90	6.10
Mould, *log10 cfu g^{-1}*	<10	<10	<10

DM: Dry Matter, CP: Crude Protein, WSC: Water Soluble Carbohydrate, cfu: colony-forming unit

The proximate analysis of fresh material and silages was performed according to AOAC (1990). The Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) of silages was determined according to Van Soest et al. (1991).

The digestible dry matter (DDM) and dry matter intake (DMI) of silages were calculated using NDF and ADF values by the given equations below (Moore and Undersander, 2002). Then DDM and DMI values were used to calculate the relative feed value (RFV) of silages by the following equations (1, 2, 3) developed by Van Dyke and Anderson (2002).

$$DDM\ (\%) = 88.9 - 0.779 \times ADF \quad \text{(Eq.1).}$$
$$DMI\ (\%) = 120 \div NDF \quad \text{(Eq.2).}$$
$$RFV = DDM \times DMI \times 0.775 \quad \text{(Eq.3).}$$

The effect of treatments on fermentation quality and nutritive value of silages were analyzed using the GLM procedure of Minitab (2014) statistical package programs, and least-squares means were compared using Tukey's multiple comparison tests. The following statistical model (Eq.4) was used:

$$y_{ij} = \mu + a_j + e_{ij} \quad \text{(Eq.4).}$$

Where y_{ij}= observed value; μ = overall mean; a_i= effect of treatment; e_{ij}= effect of the experimental error.

3. Results and Discussion

An adequate level of DM, WSC, and buffering capacity (BC) of ensiled plant material is required to achieve good quality silage, and it was stated in the previous studies that the DM content of ensiled plant material is desired to be around 300-350 g kg^{-1} to ensure sufficient fermentation in the silo (Neto et al., 2017). A desirable WSC level is also required for a rapid decrease in pH at the initial phase of the fermentation and produces more lactic acid bacteria (LAB) to protect silage microflora against spoilage (Başkavak et al., 2008). The silo's fermentation is often checked with the measuring pH and NH_3-N level due to its practical application. Kaya and Polat (2010) have classified the silages within the pH range between 3.7-4.2 as good quality. Also, Veriato et al. (2018) considered the silages as very good (<100 g kg^{-1}), good (100-150 g $kg^{-1)}$, medium (150-200 g kg^{-1}), and bad (>200 g kg^{-1}) quality according to their NH_3-N content. The DM and WSC content of fresh S, M, and SM silage was 250.2, 330.0, and 283.2 g kg^{-1}; 122.0, 135.7, and 130.0 g kg^{-1}, respectively *(Table 1)*. Considering the WSC, the levels observed in this investigation are lower than those reported by Neto et al. (2017) but higher than Alhaag et al. (2019).

The pH of silages was significant (P<0.05) and below 4.2, which means all silages could be classified as good quality dealing with obtained values. The highest (4.13) and the lowest (3.90) pH values were observed in the SM and S groups. The DM of silages was found significant (P<0.01) in the present study. Also, the differences among the silage groups in NH_3-N content were significant (P<0.01); all silages could be classified as good quality (NH_3-N<100 g kg^{-1} TN). The NH_3-N parameter is also used to determine the level of proteolysis in silages. The NH_3-N is mainly derived from amino acid catabolism and degradation products of some biochemical processes such as deamination, decarboxylation, and oxidation and reductions, resulting in heating in the silo mass (Veriato et al., 2018). In contrast, well-preserved silages with low proteolytic activity may improve microbial protein synthesis in the rumen (Sucu et al., 2016).

In the current study, the S group's LA content was recorded significantly higher than the M and MS groups (P<0.01). It was also suggested that the LA content of silages must be greater than 20 g kg^{-1} to yield good quality silages (Kaya and Polat, 2010). The WSC content of pre-ensiled material also affects the lactic acid (LA) content of silages. In general, high WSC may induce more LA production during ensiling. The WSC of the SM group was increased due to mixing S and M forages, leading to improved LA content *(Table 2)*. The results derived from this study were in agreement with the findings of Kaplan (2013) and Pinho et al. (2015), who stated increased level of WSC enhanced the LA content of silage and accounted for a lower pH value.

Table 2. Fermentation quality and chemical composition of silages

Item	Treatments			P
	S	M	SM	
DM	247.05±7.06^{c}	328.72±9.73^{a}	275.31±1.93^{b}	**
pH	3.90±0.02^{b}	4.05±0.01^{ab}	4.13±0.14^{a}	*
CP	75.44±2.91^{a}	60.72±1.46^{c}	69.46±1.17^{b}	**
Ash	67.34±0.60^{a}	35.84±2.85^{c}	52.45±0.84^{b}	**
NDF	554.43±3.92^{b}	520.52±4.02^{c}	588.32±10.19^{a}	**
ADF	309.01±12.03^{a}	234.98±4.33^{b}	294.57±2.56^{a}	**
ADL	47.32±3.20	42.80±2.44	46.61±3.67	NS
H_{cell}	245.41±15.63^{b}	285.53±8.26^{a}	293.75±10.64^{a}	*
Cell	261.69±9.04^{a}	192.18±2.99^{c}	247.96±6.13^{b}	**
WSC	15.35±0.82^{a}	2.95±0.16^{c}	4.41±0.32^{b}	**
NH_3-N	86.24±2.18^{a}	74.97±2.94^{b}	85.82±2.10^{a}	**
LA	39.54±8.84^{a}	12.02±2.54^{b}	19.29±1.00^{b}	**
RFV	108.74±0.95^{b}	126.13±0.43^{a}	104.28±1.83^{c}	**
DDM	64.83±0.94^{b}	70.60±0.34^{a}	65.95±0.20^{b}	**
DMI	2.16±0.02^{b}	2.31±0.02^{a}	2.04±0.04^{c}	**

^{a,b,c} Values within a row with different superscripts differ significantly at P<0.05

DM: Dry Matter, g kg^{-1}; CP: Crude Protein, g kg^{-1} DM; Ash, g kg^{-} DM; NDF: Neutral Detergent Fiber, g kg^{-1}DM; ADF: Acid Detergent Fiber, g kg^{-1}DM; ADL: Acid Detergent Lignin, g kg^{-1}DM; Hcell: Hemicellulose, g kg^{-1}DM; Cell: Cellulose, g kg^{-1}DM; WSC: Water Soluble Carbohydrate, g kg^{-1}; NH_3-N: Ammonia Nitrogen, g kg^{-1}TN; LA: Lactic Acid, g kg^{-1}DM; RFV: Relative feed value; DDM: Digestible Dry Matter, %; DMI: Dry Matter Intake, %; NS: Not significant (P>0.05); *: P<0.05; **: P<0.01; ***: P<0.001

Differences with respect to the CP and ash content among the silage groups were found significant (P<0.01). This result was expected because it was stated by some researchers in the earlier studies that the forage quality may strongly be affected by species, genotype, maturity at harvesting period, steam/leaf ratio, number of panicles (Cândido et al., 2002; Da Silva et al., 2012). The results were consistent with an adequate CP level for the functioning of ruminal microbiota (Pinho et al., 2015). Ash referring to mineral content in forages, influenced by maturity, is found higher in seed than those of other parts. The ash content of silages ranged between 35.84 to 67.84 g kg^{-1}, and the differences among the treatments were significant (P<0.05). The obtained results were lower than the findings reported by Sucu et al. (2016) for M silage but higher than S silage. These results were also determined higher than the findings stated by Dundar et al. (2020) for S and SM silages.

The NDF and ADF values of silages, indicative of the amount of forage fiber, were varied between 520.52-588.32 and 234.98-309.01 g kg^{-1}, and the differences among the silages in terms of NDF values were found significant (P<0.01). Some researchers reported that the level of NDF is above 600 g kg^{-1}, NDF negatively affects the DM consumption of ruminants, if the ADF level is above 300 g kg^{-1}, ADF hinders feed fragmentation and digestion by ruminal bacteria (Gonçalves et al., 2010; Veriato et al., 2018). Based on the obtained results, fiber levels of silage groups were close to those reported for sorghum and maize silages. On the other hand, the ADL content of silages did not differ in the groups (P<0.05). This situation may be derived from the harvesting of these crops at the same maturity stages. Several authors emphasized that the lignin content of forages increased with the advancement of the stage of maturity (Ferreira and Mertens, 2007; Gonçalves et al., 2010; Nurk et al., 2016).

Ruminants can benefit more from feeds with a high hemicellulose (H_{cell})/cellulose (Cell) ratio due to the easy degradation of H_{cell} than Cell. The H_{cell} and Cell values of silages were determined significant (P<0.05 and P<0.01). In the present study, H_{cell}/Cell ratios of S, M, and SM silages were found as 0.94, 1.49, and 1.18, respectively. The results from this study for S silage were lower than the findings of Veriato et al. (2018), who evaluated the seventeen S genotypes but higher than those noted by Dundar et al. (2020), who applied different irrigation levels for ensiled material.

The differences in terms of RVF, DDM, and DMI between among the silage groups were significant (P<0.01). While the M silages had the highest values, the SM silages showed the lowest value (except for DDM in S silages). Based on the results from the present study, S (108.74) and SM (104.28) silages were categorized in the second quality class, while the M (126.13) silages were evaluated in the first class according to legume, grass, or mixed hay quality standards developed by American Forage and Grassland Council (Kaplan et al., 2014). Similar RFV values of forage S and M were also stated by Neves et al. (2015) and Kızılşimşek et al. (2020).

Kaewpila et al. (2021) indicated that to promote lactic acid fermentation, the fresh form of ensiling material must include at least 5 log cfu g^{-1} epiphytic bacteria. Otherwise, harmful bacteria should not be controlled during the ensiling period without any silage additives. In this study, the number of epiphytic bacteria was smaller than the recommended values. The microbiological composition of silages was presented in *Table 3*. After 60 days of the ensiling period, the number of *lactobacilli* and yeast content was significant among the silage groups (P<0.01).

Table 3. Microbiological composition of silages

Item	Treatments			P
	S	M	SM	
Lactobacilli, log cfu g^{-1}	5.18±0.16^{c}	6.04±0.74^{b}	7.41±0.15^{a}	**
Yeast, *log cfu g^{-1}*	5.18±0.17^{b}	5.73±0.81^{b}	7.29±0.12^{a}	**
Mold, *log cfu g^{-1}*	<10	<10	<10	NS

^{a,b,c} Means in the same row with different superscripts differ significantly at P<0.05
NS: Not significant; **: P<0.01

Mixing S and M forages increased the number of *lactobacilli* and mold content of silages. The *lactobacilli* and yeast content of silages varied between 5.18-7.41 and 5.18-7.29 log cfu g^{-1} and the highest and lowest values were observed in SM and S silages, respectively. No mold growth was detected in all silage groups. It was observed that silages with a higher *lactobacilli* content were of a lower pH value compared to others. Inconsistent with this expectation, SM silages had the most elevated pH and *lactobacilli* content. It was attributed by Khota et al. (2017) to natural bacteria present in pre-ensiled material, which may produce a different amount of short-chain fatty acids during the fermentation process, and the interaction effect of S and M forages. Filya et al. (2004) reported that the *lactobacilli* number as 6.7 and 7.0, and yeast number as 5.8 and 5.5 log cfu g^{-1} in sorghum and maize silages ensiled for 60 days, respectively. Sucu et al. (2016) found similar results in a study that aimed to determine the effect of ensiling density on the nutritive value of S and M silages. The obtained results from this study were in consistent with the above reports mentioned by some researchers. The aerobic stability parameters of silages are given in *Table 4*. No visible mold was detected in all silages after 5 days of aerobic exposure (P>0.05).

Table 4. Aerobic stability parameters of silages

Item	Treatments			P
	S	M	SM	
DM, *g kg^{-1}*	252.61±0.98^{c}	342.60±7.24^{a}	283.91±3.24^{b}	**
pH	4.88±0.25^{c}	6.74±0.03^{a}	6.35±0.01^{b}	**
CO_2, *g kg^{-1} DM*	55.71±9.93^{b}	118.78±5.07^{a}	119.33±0.98^{a}	**
Yeast, *log cfu g^{-1}*	8.40±0.15^{c}	9.08±0.03^{a}	8.71±0.02^{b}	**
Mold, *log cfu g^{-1}*	<10	<10	<10	NS

^{a,b,c} Values within a row with different superscripts differ significantly at P<0.05

DM: Dry Matter; NS: Not significant; **: P<0.01

The pH, CO_2, and yeast numbers varied in silage groups between 4.88-6.74, 55.71-119.33 g kg^{-1}, and 8.40-9.01 log cfu g^{-1}. Vissers et al. (2007) noted that to start the deterioration process, the pH and yeast numbers in silages must be above 5 and 7 after exposure to air, respectively. However, the differences among the silages with respect to pH, CO_2, and yeast numbers were significant (P<0.01) (Toruk et al., 2010). As seen in *Table 4*, the deterioration process appeared in all silage groups. Ruppel et al. (1995) reported that a decrease of exposed time of silages to air without spoiling before removal from the silo caused these results. On the other hand, one unexpected finding was the highest CO_2 level (119.33 g kg^{-1}) observed in SM silages. This unexpected result could be attributed to the acceleration of microbial activity at high pH and the increased risk of O_2 due to negative gas pressure in the silo (Li et al., 2017).

4. Conclusions

In this study, fermentation and nutritional characteristics of two forage crops, maize and sorghum, frequently used by livestock enterprises, and their mixtures were evaluated without any additives. Results showed that to improve the nutritional and fermentation characteristics of silages, sorghum and maize may be ensilled as a mixture. On the other hand, it was highly recommended that silage additives should be used to guarantee and strengthen the fermentation and aerobic stability of silages mostly made by a mixture of these two energetic forage crops.

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