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Fermentation Characteristics and Nutritional Value of *Avena sativa* Genotypes Ensiled with or without Napier Grass (*Pennisetum purpureum*)

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Abstract: The objective of this study was to investigate the fermentation characteristics and nutritional value of *A. sativa* genotypes ensiled either solely or in combination with *P. purpureum* 16791. Three different *A. sativa* genotypes (*SRCPX80AB2806*, *ILRI_5527A*, and *ILRI_5526A*) were harvested at the dough stage and ensiled solely or in combination with equal parts of *P. purpureum* 16791 with the addition of 3% molasses for all treatments in a completely randomized design with three replications over a period of 45 days. *P. purpureum* harvested at 60 days was ensiled and used as a control treatment. All treatments were evaluated for fermentation characteristics (pH, temperature, physical properties, and flieg point) and subjected to chemical analysis. The results showed that ensiling *A. sativa ILRI_5527A*, in combination with equal parts of *P. purpureum* 16791, produced the best silage with a significantly lower pH of 3.52. Optimal temperature (25 °C), nutrient losses based on the total dry matter (2.17%), gas (3.74%), and effluent (4.28%) were significantly ($p < 0.0001$) lower for T6 compared to the others. The dry matter recovery rate of T6 was significantly ($p < 0.0001$) higher than that of the others. T6 ranked first in the quality of physical properties (smell, color, mold, and texture). The highest dry matter (24%), organic matter (96.80%), crude fat (3.32%), and metabolizable energy (10.05 MJ/kg DM) were recorded for T6. The flieg score for T6 silage (96.6%) was also better than the others. In conclusion, ensiling *A. sativa ILRI_5527* with equal parts of *P. purpureum* 16791 and the addition of 3% molasses improved fermentation characteristics and silage quality.

Keywords: silage; *Avena sativa* genotypes; *Pennisetum purpureum* 16791; molasses; forage

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1. Introduction

Inadequate feed supply, in terms of quality, quantity, and seasonality, is a major constraint to ruminant production in sub-Saharan Africa [1,2]. In sub-Saharan Africa, the dominant feeding systems for sheep and goats are based on pastures and, in the aftermath, roadside grasses [2,3] and crop byproducts. They often contain less than the minimum content of metabolizable energy required for maintenance [4]. As a result, animals suffer weight loss, lower birth weight, lower disease resistance, and invariably reduced reproductive and productive performance [5]. Thus, there is an urgent need to use new research technologies to boost animal production. Improved feeding techniques include supplementation and the provision of improved forage and feed preservation, including ensiling. Feed preservation contributes to better feed quality, ensures longer shelf life, and maintains nutritional quality and palatability.

The two basic ways of forage preservation are hay and silage [6]. Ensiling is a method of preserving wet season forage for use as animal feed during dry periods. Silage preparation is based on the anaerobic fermentation of water-soluble carbohydrates into organic acids, primarily lactic acid. The fermentation process stops at pH values below 4.2 and

protects the moist forages from spoiling by germs [7]. Silage processing is a potential option to ensure the supply of high-quality fodder in the event of feed scarcity [8].

Cereal forages have different developmental stages, and the dough stage is the best harvest time for maximum yield and nutritive quality traits [9,10]. Harvesting *A. sativa* for silage at the dough stage increases the yield two-fold due to grain formation at the dough stage [11]. Napier grass (*P. purpureum*) is a popular tropical grass for silage production due to its high biomass yield per unit area, high levels of water-soluble carbohydrates, its ability to withstand frequent cutting and rapid regeneration, tolerance to intermittent drought, and good palatability [12–14]. It provides adequate digestible dry matter per hectare and supports a significant increase in weight per animal [14].

Several researchers have reported that adding molasses to forage silage improves fermentation, dry matter, and lactic acid content while lowering pH and ammoniacal nitrogen concentration [15,16]. The addition of 4% molasses to *P. purpureum* grass silage has been found to affect fermentation parameters, crude protein, and structural carbohydrate breakdown [15]. Harvesting *A. sativa* at the dough stage and *P. purpureum* at 60 days produces significant amounts of soluble carbohydrates. Despite the critical feed shortage and wide adoption of both *A. sativa* and *P. purpureum* forages in Ethiopia, little work has been conducted to evaluate the various preservation methods without compromising their quality. Furthermore, there is insufficient scientific information on the chemical composition, silage quality, and feeding benefits of combining *A. sativa* and *P. purpureum* with the inclusion of molasses to obtain more fermentable carbohydrates. Therefore, this study aimed to evaluate the fermentation characteristics and nutritive value of *A. sativa* genotypes ensiled either solely or in combination with equal parts of *P. purpureum* grass with the addition of 3% molasses as an additive for all treatments.

2. Materials and Methods

2.1. Study Site

This experiment was conducted at the Bonga Agricultural Research Center, Modeyo Bonga Sheep Multiplication Substation, located in Decha District, Kaffa Zone, and Southwest Ethiopia. The study site lies 455 km southwest of Addis Ababa at an altitude of 1809 m above sea level. The annual average rainfall is 1839 mm. The maximum and minimum temperature of the area is 23.1 °C and 14.1 °C, respectively. The soil type is loam soil [17,18].

2.2. Planting and Harvesting of Experimental Forages

A. sativa was sown on a 1 ha plot maintaining a 20 cm spacing between rows using the drill method at a seed rate of 100 kg/ha. Blended fertilizer (NPS 100 kg/ha) of nitrogen, phosphorus, and sulfur at an amount of N 19%, P 38%, and S 7% were applied with 50% urea (50 kg/ha) at planting, while the remaining 50% urea was applied at the mid tilling of the crop. *P. purpureum* 16791 was planted 75 cm within rows and 1 m between plants, with a fertilizer rate of 100 kg/ha NPS and 100 kg/ha Urea (split application). Planting was performed by cutting the stem at 2–3 nodes at an angle of 45°. The basal 2 nodes were buried for planting on a plot size of 1 ha to use at regrowth after 60 days of age [19,20] for silage [21,22].

2.3. Forage Preparation and Ensiling

After harvest, the forages were manually chopped with a knife to a theoretical cut length of 2–3 cm to adequately exclude oxygen from the hollow stems in the silage [20], control effluent production, and facilitate forage compaction in the silo [22]. The forage was harvested with a large knife and mechanically chopped to ensure the materials were fine and uniform [23]. The subsamples were wilted, chopped to particle sizes of 2–3 cm, and mixed thoroughly according to the treatment regimen given in Table 1. Triplicate samples of 250 g pre-silage were oven-dried at 60 °C for 72 h [24] and stored for laboratory chemical analysis. The chopped materials were weighed and thoroughly mixed with 3% molasses in a ratio of 150 mL of molasses to 300 mL of water [25].

Table 1. Treatment silages made by ensiling chopped *A. sativa* genotype alone and in combination with equal parts of *P. purpureum* 16791 and with the addition of 3% molasses.

Treatments
Sole *Avena sativa SRCPX80AB2806* (T1)
Sole *Avena sativa ILRI_5527A* (T2)
Sole *Avena sativa ILRI_5526A* (T3)
Sole *Pennisetum purpureum* 16791 (T4)
50% *Avena sativa SRCPX80AB2806* + 50% *Pennisetum purpureum* 16791 (T5)
50% *Avena sativa ILRI_5527A* + 50% *Pennisetum purpureum* 16791 (T6)
50% *Avena sativa ILRI_5526A* + 50% *Pennisetum Purpureum* 16791 (T7)

Representative samples of the chopped forage were loaded into individual treatment laboratory bucket silos made of PVC tubes (30 cm high, 30 cm in diameter, 21,195 cm^3 volume) lined internally with a polyethylene film. The chopped silage materials were thoroughly mixed until homogeneous. The 7-treatment silages shown in Table 1 were made by manual filling, packing, and layering. Each layer was compacted by continuous pounding to remove air. Pressed with a wooden stick, all treatment silos were filled to the same average packing density of 7 kg per 16 L plastic PVC bucket silo [6]. All treatments were ensiled over a period of 45 days. After 45 days of fermentation, the silos were opened, and the upper (top 9 cm) and lower (bottom 9 cm) silages were discarded. Finally, samples were taken to examine their physical quality, chemical composition, and fermentation characteristics [24].

2.4. Parameters of Treatment Silages Evaluation

To assess the quality of the treatment silage, flieg points were calculated using the pH values and the DM content of the silage according to the equation developed by [26]. Flieg points = 220 + (2% DM − 15) − 40 × pH. The flieg score with values 81–100 = very good, 61–80 = good, 41–60 = medium, 21–40 = low, and 0–20 = poor. An early system for assessing silage quality was the Flieg point scheme, which awarded points according to the relative amounts of lactic, acetic, and butyric acids; the higher the ratio of lactic and acetic acids to butyric acid, the higher the score and the better the quality [26].

A thermometer was installed at the center of each silo to measure the temperature (°C). The silage temperature is a critical parameter for the management of the silage process. An anaerobic condition with low and stable temperatures is imperative for quality silage production. Nevertheless, when the silo is opened or if there are any defects (e.g., on the lid or on the walls), the anaerobic environment is converted into an aerobic state. This leads to intensive decomposition processes leading to an increase in temperature in the problematic silage layers [27]. High temperatures in a silo are most often the result of aerobic deterioration, especially after several months of fermentation [28].

The pH of the silage extract was measured with a digital pH meter and calibrated with buffer solutions (pH 4 and 7). The aerobic stability and fermentation profile of silage are both evaluated by pH [29,30]. Since pH measurement is a simple and inexpensive method, it can be used to determine whether silage is fermenting or spoiled [29]. pH probe electrodes can be used to efficiently monitor the pH in experimental silos or agricultural silos [30]. An increase in silage temperature, the growth of lactate-assimilating yeasts, and an increase in silage pH follow. Ultimately, the latter leads to the development of opportunistic bacteria and molds that thrive in oxygen and increase heating and spoiling [28].

The visual assessment of the silage quality was assessed with reference to Table A1 in Appendix A. A panel discussion with six trained experts on silage quality indices and silage quality scales was included in the assessment of the physical properties of the silage. Mold growth was monitored while the silo was opened. The color, smell, and texture were assessed after the silo contents had been emptied. Organoleptic properties can be used to

assess silage quality as the volatility of many fermentation end products produces a variety of distinct odors [28]. Lactic acid, the primary organic acid produced during fermentation, is almost odorless: well-fermented silages should not smell particularly overpowering. However, since acetic acid is formed in the second-highest proportion after lactic acid and is quite volatile, most silage has a moderate smell of vinegar. Smelling silage with extremely high concentrations of acetic acid often causes the eyes and nose to sting. Wet silage with high levels of acetic acid also smells of vinegar and has a yellow tint, especially in the silo bottom, where compaction further increases the moisture content [28,31].

2.5. Chemical Analysis

Subsamples of the fresh forage and treatment silages were oven dried and ground in a Wiley mill to pass through a 1 mm sieve [32]. The dry matter and nitrogen (N) content of the samples was determined according to the method of [31]; then, the N concentration was multiplied by a factor of 6.25 to obtain CP. The neutral detergent fiber (NDF) was determined according to [33]. Acid detergent fiber (ADF) and acid detergent lignin (ADL) levels were determined according to [31]. ME = 5.34 − 0.1365CF + 0.6926 NFE − 0.0152 NFE^2 + 0.0001 NFE^3, where NFE is a nitrogen-free extract [34].

The total dry matter loss (TDML) was calculated by DM weight loss in the silage (DM of forage − DM of silage)/DM forage × 100 [35]. Effluent losses = E = [[(empty bucket weight + sand weight at opening (kg) − empty bucket weight (kg)) − (empty bucket weight + sand weight at sealing (kg) − weight empty bucket (kg))]/forage mass at sealing (kg)] × 100 [36].

$$E = [(PVf - Tb) - (PVi - Tb)]/MFi \times 100 \quad (1)$$

Gas losses (% DM) = G = (weight of full silo at sealing − the weight of full bucket at opening)/(forage mass at sealing (kg) − forage dry matter concentration at closing (%)) × 100 [36].

$$G = (Wfss - Wfbo)/(Fms \times Fdmcc) \times 100 \quad (2)$$

RMR = dry matter recovery = dry matter recovery rate (%) = [forage mass at opening (kg) × forage dry matter concentration at opening (%)]/[forage mass at sealing (kg) × forage dry matter concentration at sealing (%)] × 100 [36].

$$DMRR\ \% = [(FMO\ (kg) \times FDMCO\ (\%))/(FMS\ (kg) \times FDMCS\ (\%))] \times 100 \quad (3)$$

2.6. Statistical Analysis

An analysis of variance was used to test the statistical significance of treatments using the general linear model procedure of the SAS program version 9.4 [37] with a 5% probability with the model:

$$Y_{ijk} = \mu + A_i + E_{ijk}$$

where: Y_{ijk} = response variable; μ = overall mean; A_i = treatment effect; E_{ijk} = random error.

3. Results

3.1. Chemical Composition of Forage and Treatment Silage

The chemical composition of the forage used for silage making and the treatments prior to sealing are shown in Table 2. The treatments contained less dry matter, while the individual forages had over 90% dry matter. The crude protein content of all materials except molasses was 10% above the thresholds required for animal maintenance. The overarching characteristics of silage affecting fermentation can be distinguished. One of the factors is the nature of the raw material, which is determined by the chemical composition of the crop [38].

Table 2. Average chemical composition of ingredients and pre-ensiling treatments (%).

Treatments (n = 3)	DM	Ash	EE	OM	CP	NDF	ADF	ADL	ME
Avena sativa SRCPX80AB2806	91.33	6.14	1.97	93.86	11.46	42.70	19.28	3.43	8.44
Avena sativa ILRI_5527A	91.66	4.70	2.57	95.30	12.35	39.33	14.57	2.70	8.41
Avena sativa ILRI_5526A	91.25	7.22	1.28	92.78	9.97	51.11	21.88	4.02	8.30
Pennisetum purpureum 16791	90.96	6.35	1.94	93.65	14.37	57.31	28.71	4.80	8.30
Molasses	72.43	13.38	ND	86.62	3.27	0.43	0.12	0.09	14
T1	23.91	5.64	1.81	95.10	11.45	40.70	18.28	3.23	9.75
T2	24.76	6.72	2.47	93.28	12.33	37.11	13.88	2.32	9.92
T3	22.96	4.75	2.5	95.25	10.07	50.16	20.84	4.12	9.70
T4	23.19	6.16	2.62	93.84	14.25	55.15	26.44	5.17	9.88
T5	25.37	5.85	2.13	94.15	11.37	47.31	24.71	4.8	9.88
T6	26.70	4.2	3.07	95.80	13.29	42.33	22.57	3.70	10.11
T7	22.96	4.29	3.03	95.71	10.21	52.21	25.75	4.06	9.68

DM—dry matter, EE—ether extract, CP—crude protein, NDF—neutral detergent fiber, ADF—acid detergent fiber, ADL—acid detergent lignin, ME—metabolizable energy, ND—not determined, T1: 100% of chopped *A. sativa SRCPX80AB2806*, T2: 100% of chopped *A. sativa ILRI_5527A*, T3: 100% of chopped *A. sativa ILRI_5526A*, T4: 100% of chopped *P. purpureum* 16791, T5: 50% of chopped *A. sativa SRCPX80AB2806* + 50% chopped *P. purpureum* 16791, T6: 50% of chopped *A. sativa ILRI_5527A* + 50% chopped *P. purpureum* 16791, T7: 50% of chopped *A. sativa ILRI_5526A* + 50% chopped *P. purpureum* 16791.

3.2. Silage pH, Temperature, and Dry Matter Loss

The pH, temperature, and dry matter loss are shown in Table 3. The pH of the pre-ensiled materials from the treatments with the lowest pH was T6, and the highest was T2 ($p < 0.0001$). However, the final pH did not follow a trend similar to that seen during pre-ensiling. The result from the beginning shows that T6 initially had a low buffering capacity, which meant that T6 needed less acid than the rest of the treatments to lower its pH. T3, T5, and T7 had relatively higher final pH, temperature, and losses of DM, GL, and EL compared to the rest of the treatments. However, the DMRR followed an opposite trend, with T6 showing the highest value, followed by T2. There is a difference between the pH of the treatments on day 1 and day 45 ($p < 0.0001$). The pH T3 > T7 > T4 > T1 > T2 > T5 > T6 shows the pH with different values to preserve the silage. The temperature of the treatments on day 45 shows a trend of T3 > T7 > T4 > T1 > T2 > T5 > T6 that varies between treatments ($p < 0.0001$). The total DM loss of the treatments ranged from 2.17% (T6) to 2.67% (T7). Mixing *P. purpureum* 16791 increased gas loss from T1, T2, and T3 by 5.74%, 7.43%, and 4.39%, respectively. The lowest effluent treatment was T6 (4.28%), and the highest was T3 (10.04%). The highest total DM recovery rate for which the highest score was obtained was with treatments *A. sativa 5527A* mixed with *P. purpureum* 16791, followed by T5 and T2.

Table 3. pH, temperature, gas loss, dry matter recovery, and effluent loss of *A. sativa* silage with or without *P. purpureum* 16791 with 3% molasses inclusion.

Parameter	T1	T2	T3	T4	T5	T6	T7	SE	p
pHi	5.72 ^{d}	5.72 ^{d}	5.80 ^{a}	5.74 ^{b}	5.71 ^{e}	5.70 ^{f}	5.73 ^{c}	0.01	<0.0001
pH 45th day	4.71 ^{d}	4.52 ^{e}	5.01 ^{a}	4.81 ^{c}	4.22 ^{f}	3.52 ^{g}	4.91 ^{b}	0.01	<0.0001
Temperature (°C)	27.53 ^{d}	26.70 ^{e}	30.22 ^{a}	28.60 ^{c}	25.80 ^{f}	25.00 ^{g}	29.73 ^{b}	0.20	<0.0001
TDML%	2.47 ^{d}	2.33 ^{e}	2.55 ^{b}	2.53 ^{c}	2.23 ^{f}	2.17 ^{g}	2.67 ^{a}	0.01	<0.0001
GL (%DM)	4.18 ^{d}	4.04 ^{e}	4.55 ^{a}	4.31 ^{c}	3.94 ^{f}	3.74 ^{g}	4.35 ^{b}	0.01	<0.0001
EL (kg/t FM)	7.09 ^{d}	6.38 ^{e}	10.04 ^{a}	8.52 ^{c}	5.57 ^{f}	4.28 ^{g}	9.28 ^{b}	0.20	<0.0001
DMRR (%)	59.16 ^{d}	70.60 ^{c}	34.18 ^{g}	49.16 ^{e}	79.91 ^{b}	96.30 ^{a}	46.79 ^{f}	0.42	<0.0001

pHi—initial pH, TDML—total dry matter loss, GL—gas loss, EL—effluent loss, DMRR—dry matter recovery rate. Means in the same row with different superscript letters differ significantly at $p < 0.05$. T1: 100% of chopped *A. sativa SRCPX80AB2806*, T2: 100% of chopped *A. sativa ILRI_5527A*, T3: 100% of chopped *A. sativa ILRI_5526A*, T4: 100% of chopped *P. purpureum* 16791, T5: 50% of chopped *A. sativa SRCPX80AB2806* + 50% chopped *P. purpureum* 16791, T6: 50% of chopped *A. sativa ILRI_5527A* + 50% chopped *P. purpureum* 16791, T7: 50% of chopped *A. sativa ILRI_5526A* + 50% chopped *P. purpureum* 16791.

3.3. Physical Properties

The physical properties of silage assessed by the six trained experts' analysis of the response rating are shown in Table 4. There was a significant difference ($p < 0.0001$) between the treatments in their physical properties. The highest scores in odor, color, and texture were recorded for T6, followed by T5, T2, and T1, compared to the rest of the treatments. T3 had the lowest score for all parameters. The trend of the silage quality rating for odor (T6 > T5 > T4 > T2 > T4 > T1 > T7 > T3), color (T6 > T5 > T2 > T1 > T4 > T7 > T3), moldiness (T6 > T5 > T2 > T1 > T4 > T7 > T3), and texture (T6 > T5 > T2 > T1 > T4) for the treatments differed significantly ($p < 0.0001$). The overall physical properties parameter of the silage result shows that T6 is the top treatment identified as meeting the requirement of acceptable quality silage.

Table 4. Mean score values (scale 1–4) for physical properties of *Avena sativa* silage ensiled with or without *P. purpureum* 16791.

Parameter	T1	T2	T3	T4	T5	T6	T7	SE	*p*-Value
Smell	3.00 ^{c}	3.16 ^{c}	2.00 ^{e}	3.00 ^{c}	3.50 ^{b}	4.00 ^{a}	2.50 ^{d}	0.33	<0.0001
Color	3.30 ^{d}	3.50 ^{c}	2.00 ^{g}	3.10 ^{e}	3.70 ^{b}	4.00 ^{a}	2.90 ^{f}	0.05	<0.0001
Moldiness	3.30 ^{d}	3.50 ^{c}	2.70 ^{g}	3.10 ^{e}	3.70 ^{b}	3.93 ^{a}	2.90 ^{f}	0.06	<0.0001
Texture	3.30 ^{d}	3.50 ^{c}	2.00 ^{g}	3.10 ^{e}	3.70 ^{b}	4.00 ^{a}	2.90 ^{f}	0.05	<0.0001

Means in the same row with different superscript letters differ significantly at $p < 0.05$. T1: 100% of chopped *A. sativa SRCPX80AB2806*, T2: 100% of chopped *A. sativa ILRI_5527A*, T3: 100% of chopped *A. sativa ILRI_5526A*, T4: 100% of chopped *P. purpureum* 16791, T5: 50% of chopped *A. sativa SRCPX80AB2806* + 50% chopped *P. purpureum* 16791, T6: 50% of chopped *A. sativa ILRI_5527A* + 50% chopped *P. purpureum* 16791, T7: 50% of chopped *A. sativa ILRI_5526A* + 50% chopped *P. purpureum* 16791.

3.4. Change in Dry Matter and Nutrient Composition

The chemical composition of the fermented silage for the seven treatments after day 45 of ensiling is shown in Table 5. There were significant differences ($p \leq 0.0001$) between the treatments. During ensiling, an acceptable DM (moisture) and OM composition existed to facilitate the fermentation process. T3 and T7 had the lowest values of OM compared to the other treatments. T6 had the best DM and ME and the second-best CP content. T4 (sole *P. purpureum*) had the best CP content of 14.02%. The ME of T1 was enhanced by 1.34% and T2 by 1.82%, while in T3, no change occurred due to mixing *A. sativa* with *P. purpureum* 16791. Mixing A. *sativa* with *P. purpureum* 16791, the CP of T1 decreased by 1.74%, the CP of T2 improved by 7.55%, and the CP of T3 improved by 1.39%. The fiber composition followed the order of T4 > T7 > T3 > T5 > T6 > T1 > T2, with some variations between the fiber fractions. ADF followed the trend of T4 > T5 > T3 > T7 > T1 > T2 > T6.

Table 5. Chemical composition (%) and ME (MJ/kg DM) of ensiled *A. sativa* genotypes with or without *P. purpureum* 16791 with 3% added molasses in all treatments.

Parameter	T1	T2	T3	T4	T5	T6	T7	SE	*p*
DMi (FW)	23.91	24.76	22.96	23.19	25.37	26.7	22.96		
DM (FW)	21.00 ^{d}	22.00 ^{c}	19.00 ^{f}	20.00 ^{e}	23.00 ^{b}	25.00 ^{a}	21.00 ^{e}	0.01	<0.0001
Ash	4.64 ^{d}	3.75 ^{e}	5.72 ^{a}	4.85 ^{c}	3.29 ^{f}	3.2 ^{g}	5.16 ^{b}	0.04	<0.0001
OM	95.36 ^{d}	96.25 ^{c}	94.28 ^{g}	95.15 ^{e}	96.71 ^{b}	96.80 ^{a}	94.84 ^{f}	0.04	<0.0001
EE	2.72 ^{d}	2.84 ^{c}	2.03 ^{g}	2.69 ^{d}	3.28 ^{b}	3.32 ^{a}	2.35 ^{f}	0.01	<0.0001
CP	11.45 ^{d}	12.32 ^{c}	10.03 ^{fg}	14.02 ^{a}	11.25 ^{ef}	13.25 ^{b}	10.17 ^{fg}	0.07	<0.0001
NDF	39.70 ^{f}	36.12 ^{g}	49.15 ^{C}	54.31 ^{a}	46.21 ^{d}	40.03 ^{e}	51.15 ^{b}	0.01	<0.0001
ADF	17.28 ^{e}	12.84 ^{e}	20.88 ^{c}	25.71 ^{a}	24.74 ^{b}	15.57 ^{f}	19.44 ^{d}	0.01	<0.0001
ADL	3.06 ^{e}	2.15 ^{f}	4.06 ^{c}	5.06 ^{a}	4.6 ^{b}	3.50 ^{d}	4.01 ^{c}	0.05	<0.0001
ME	9.72 ^{d}	9.89 ^{b}	9.67 ^{e}	9.85 ^{c}	9.85 ^{c}	10.07 ^{a}	9.67 ^{e}	0.005	<0.0001

DM—dry matter, FW—fresh weight, EE—ether extract, CP—crude protein, NDF—neutral detergent fiber, ADF—acid detergent fiber, ADL—acid detergent lignin, ME—metabolizable energy. Means in the same row with different superscript letters differ significantly at $p < 0.05$. T1: 100% of chopped *A. sativa SRCPX80AB2806*, T2: 100% of chopped *A. sativa ILRI_5527A*, T3: 100% of chopped *A. sativa ILRI_5526A*, T4: 100% of chopped *P. purpureum* 16791, T5: 50% of chopped *A. sativa SRCPX80AB2806* + 50% chopped *P. purpureum* 16791, T6: 50% of chopped *A. sativa ILRI_5527A* + 50% chopped *P. purpureum* 16791, T7: 50% of chopped *A. sativa ILRI_5526A* + 50% chopped *P. purpureum* 16791.

3.5. Flieg Score of Silage from A. sativa Genotypes with or without P. purpureum 16791 with 3% Added Molasses

The quality of the silage treatments was determined using the flieg point index, which was computed using the pH and DM of the silages measured at the end of ensiling (see Figure 1). Although the highest flieg point was recorded from T6, the results are inconsistent between the treatment groups. There was no consistent increase or decrease when prepared either solely or in combination between *A. sativa* genotypes and *P. purpureum*.

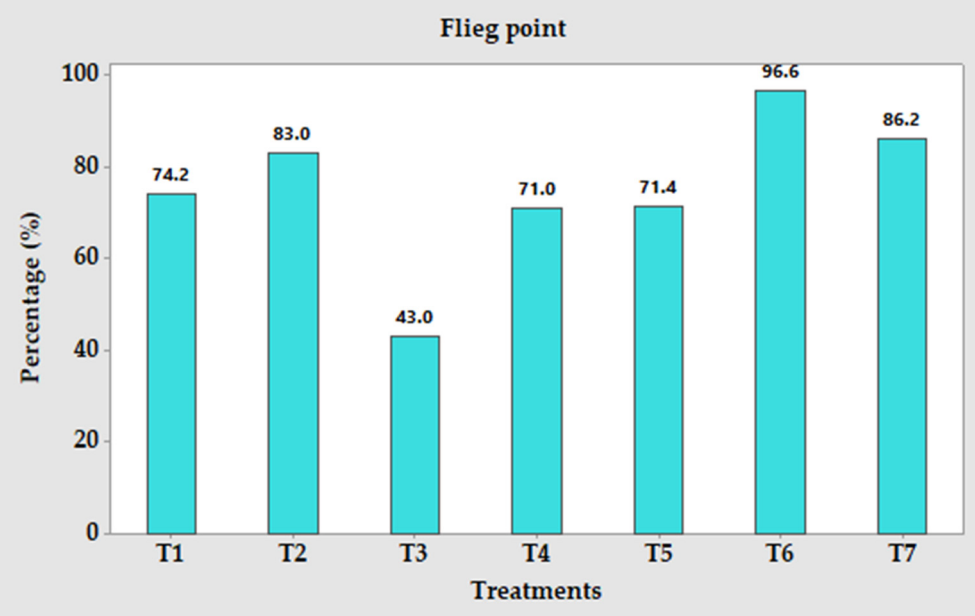

Figure 1. Quality of silage according to flieg point.

4. Discussion

One of the factors that affect the ensiling process is the DM content of the plant. It predicts the insolubility of forage crops [38]. The DM content of the plants before ensiling range from 22.96% to 26.70% (Table 2). After ensiling, the DM content ranges from 19% to 24% (Table 5). The DM content is critical to producing good quality silage. There was up to 3.74% variability in the treatment values at the baseline and 3% variability at day 45. These variations are likely due to the differences in each *A. sativa* genotype used and the properties they exhibited when mixed with *P. purpureum* 16791.

The results of this study indicate that T6 probably had sufficient soluble sugar, which created an optimal environment for the successful fermentation by microorganisms. T6 had the lowest pH on the 45th day of fermentation, showing that this treatment had soluble carbohydrates that paved the way for fermentation by lactic acid bacteria. In addition, all treatments had a pH in the range of 3.52–5.01, indicating good preservation and in the range of good to average quality silage [39]. A low pH (below 4) indicated high-quality silage [15,35]. The relatively highest pH value of 5.80 was measured for T3 (100% *A. sativa ILRI_5526A* ensiled with 3% molasses), indicating that this specific genotype had less soluble carbohydrates and a high buffering capacity as the ammonia delayed a drop in pH and increased DM loss [40].

The silage treated with *A. sativa ILRI_5526A* (T3) had higher temperature values ($p < 0.0001$) compared to the others, indicating that it was more susceptible to deterioration. This is probably due to the soluble carbohydrate content of the genotype and the creation of a less favorable environment for microbial fermentation compared to the others. A temperature of 25 °C was recorded for T6. This is an indicator of good silage where adequate fermentation has occurred [41]. According to [42], the temperature of the silage in small silos should be similar to the ambient temperature or a few degrees warmer. From this study, the amount of heat generated was low, indicating that little aerobic degradation

occurred. The browning (Millard) reaction occurs when aerobic oxidation generates too much heat and creates a protein and carbohydrate combination that prevents protein and fiber digestion [43].

These high temperatures are the result of aerobic bacteria oxidizing the extra air trapped in the forage mass. Importantly, these temperatures should drop quickly as more packaging forces air out of the mass and fermentation takes place. Heat-damaged protein can result from sustained high temperatures in excess of 45–50 °C [28]. Temperatures in the core of the silo typically drop gradually 25–30 °C once the active phase of fermentation is over. Big bale and bag silos are examples of small silos that are designed to cool faster than larger silos. Rarely, especially after several months of storage, is the temperature above 35 °C. Huge masses of fodder act as insulation in large silos, resulting in very slow heat dissipation and in core silage temperatures often remaining high for long periods of time [28].

The total dry matter loss was lower ($p < 0.0001$) for silage made by ensiling 50% *A. sativa ILRI_5527A* + 50% *P. purpureum* 16791 with the addition of 3% Molasses (T6) compared to the others. A comparatively higher total dry matter loss of 2.67% was recorded for the treatment made by ensiling 50% chopped *A. sativa ILRI_5526A* + 50% chopped *P. purpureum* 16791 with the addition of 3% molasses (T7), suggesting that desirable microbes for quality silage are present in T6 compared to the others.

The ensiling of 50% *A. sativa 5527A* and 50% *P. purpureum* 16791 with the addition of 3% molasses (T6) resulted in a significant reduction in gas losses ($p < 0.0001$). Secondary fermentation by entero bacteria, clostridium bacteria, and aerobic microorganisms causes gas losses in silage. These microbes typically thrive in poorly fermented silage, while well-fermented silage with high levels of lactic acid fermentation produces minimal nutrient content [28]. This happens when the sugar content is particularly high. Yeast development leads to alcohol fermentation, which leads to gas losses in the form of ethanol. According to [41], moisture loss through the stomata peaks soon after cutting and stops within 2 h as a result of complete stomata closure. However, moisture loss through the cuticle can still occur after that.

Effluent production was lower for T6 than for the others. Abundant effluent production in silage results in high losses of organic substances such as sugars, organic acids, and proteins [36]. The absence of organic substrates reduces the nutritional value of silage [44]. Increasing the DM content in the silage by adding certain genotypes of the two forages (T6) led to a reduction in effluent production. Reduced effluent production refers to the reduction in nutrient losses due to percolation. Silage effluent is thought to transport nitrogen molecules, sugars, organic acids, and mineral salts [41]. Effluent (or leachate) may be produced typically when the ensiled crop has a high moisture content [45]. The moisture of pre-ensiled crop moisture can be influenced by plant factors [41]. There is high variability in reported effluent production rates for different crops with different moisture contents [45]. Fermenting a crop is said to lower the pH to inhibit putrefactive bacteria, thus preserving the protein content of the fodder [41].

The mean odor score for T6 was higher ($p < 0.0001$) than for the others. T6 treatment had a pleasant, sweet, and sour odor. A relatively lower odor score ($p < 0.0001$) was recorded for T3. It was characterized as irritating, offensive, and acidic in odor, attributed to high ammonia production. A high concentration of ammonia indicates excessive protein breakdown during fermentation [46]. According to [43], good silage smells similar to milk due to the lactic acid content. The result of this study showed that T6 had a good forage quality at the beginning of ensiling, which is supported by [6], which stated that the odor of the silage was influenced by the fresh forage.

According to [47], good silage has a light green to yellow or brownish-green color, depending on the silage raw material. The mold coverage score of T6 was better ($p < 0.001$) than the others. The lowest mold was absorbed by T6 compared to the rest of the treatments: a good quality score result. T6 had an excellent texture score ($p < 0.0001$) characterized

by a fluffy and soft texture. T6 had the best physical properties based on the parameters measured in this study.

Silage from T6 had the highest ($p < 0.0001$) level of organic matter percentage and ether extract, whereas T3 had the lowest level of organic matter and ether extract. According to [39], the nutrient content of the raw material and the microorganisms involved in fermentation influence the decrease in DM and OM contents during ensiling.

Silage made from sole *P. purpureum* (T4) had the highest ($p < 0.0001$) crude protein content compared to the other treatments. Two of the treatments (T3 and T7) had the lowest crude protein levels. The crude protein content of all the treatment groups was above the minimum crude protein requirement of ruminants [48]. The high nutrient content of T6 was likely due to enhanced microbial growth during the silage process, resulting in an increased microbial population. According to [49], the microbes are single-cell protein sources that can increase the crude protein content in the silage.

The fiber fractions (NDF, ADF, and ADL content) of T1, T2, and T6 were comparable and lower ($p < 0.001$) than the others. The lower proportion of NDF (<40%) makes these treatments a suitable feed resource for ruminants. A low fiber content improves nutrient utilization by animals [50]. ADF and ADL levels critically affect feed quality. Research has shown a negative correlation between their high levels and the potential digestibility of a feed. As ADF increases, the feed becomes less digestible. This study showed that T6 had the lowest content of ADF and ADL. This has the potential to increase intake and digestibility by animals [51]. According to [50], heterofermentative bacteria convert simple glucose into organic acids (acetic, lactic, propionic, and butyric), which leads to a drop in fiber concentration. According to Figure 1, T6 had the highest flieg point, followed by T4. The high flieg point record from T6 was attributed to the higher DM and lower pH of the silage. Measured by the flieg point, a DM, and pH-dependent value, all treated silages were in the range of medium to very good silage.

The selection of the cereal, A. *sativa*, and the grass, *P. purpureum*, in this study is a good combination as it produces high-quality silage with little nutrient loss in 45 days. Legumes were excluded from this study because of their high buffering capacity, which reduces the quality of silage due to high CP and mineral contents which delay the drop in pH and increase nutrient loss [52].

Forages with a high CP content, such as legumes, can be blended with low CP forages before or after ensiling in order to satisfy the ruminant's need for CP. The problem is their low water-soluble carbohydrate (WSC) content, high buffering capacity, and substantial proteolysis during ensiling [38,53]. Due to legumes' high buffering capacity, which negatively affects silage quality due to the plant's crude protein and high mineral concentration, it takes a long time to drop the pH level and resulting in high nutrient losses [52]. So far, different studies have been conducted using legumes for their potential as a crude protein (CP) source only. In this study, we used *P. purpureum* 16791, which had a relatively good source of protein with acceptable amounts of water-soluble carbohydrates). It yielded a high biomass per unit area. *A. sativa* was used in combination since it has technical characteristics that favor its use for silage making. *P. purpureum* 16791 has sufficient soluble carbohydrates with molasses to support or replace the deficiency. The study agrees with [52] that any fodder, which has sufficient amounts of fermentable carbohydrates, can be ensiled.

5. Conclusions

There were notable effects of using *A. sativa* in combination with *P. purpureum* 16791 on the nutritive value and fermentation properties of their silage. The results show that the blends of 50% *A. sativa ILRI 5527A* and 50% *P. purpureum* 16791 + 3% molasses for 45 days resulted in higher silage compared to sole silages of the species. The actual effect of feeding the silage combinations to ruminants on their voluntary feed intake and production performance requires further investigation.

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Appendix A

Table A1. A description of physical qualities and a scale (1–4) for evaluating silage quality.

Rating Scores	Smell	Color	Texture	Moldiness	pH
1. (Bad)	Rancid and musty smell/pungent	Dark/deep brown	Putrefactive and agglutinative	Highly moldy	>5.0
2. (Moderate)	Irritative/offensive; alcohol, acidic	Brown (Medium)	Slightly viscous/slimy	Medium	4.4–5.0
3. (Good)	Light acidic (pleasant)	Brown, yellow	Medium (loose, soft, and firm)	Slightly moldy	4.1–4.3
4. (Excellent)	Pleasant and sweet-acidic (very pleasant)	Light/greenish yellow/Olive green	Loose and soft, Firm	Without mold	≤4.0

Source: [5,49,50].

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