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Effects of Different Additives on Some Chemical Parameters and In Vitro Digestibility of *Leucaena leucocephala* Silage

Mert ÖZMEN[1], Kadir Emre BUĞDAYCI[2] ✍
[1]Burdur Mehmet Akif Ersoy University Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Diseases, Burdur/Türkiye, [2]Burdur Mehmet Akif Ersoy University Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Diseases, Burdur/Türkiye
[1]https://orcid.org/0009-0001-0685-5005, [2]https://orcid.org/0000-0002-1715-6904
✉: kebugdayci@mehmetakif.edu.tr

ABSTRACT

This study investigated the effects of various additives on the physical and chemical properties, feed value, and in vitro digestibility of *Leucaena leucocephala* silage. The experiment consisted of one control group and three treatment groups, which were supplemented with 5% molasses, 5% barley, and 0.5% formic acid, respectively. Each group included eight replicates. The plant material was chopped (2.5–5 cm), treated with the respective additives, and vacuum-packed (200 g per bag). Following a 60-day silage fermentation period, analyses were conducted to evaluate physical properties, ammonia nitrogen, pH levels, nutrient composition, feed value, in vitro crude protein degradability (IVCPD), and in vitro digestibility. According to DLG scores (≥17.62±0.29), all groups produced high-quality silages. While treatments did not significantly affect non-structural nutrients, a notable increase in crude ash content was observed in the 5% barley group. The addition of 5% molasses to *Leucaena* silage significantly reduced crude fiber ($p<.005$) and acid detergent fiber ($p\leq.001$) levels, while also increasing the Flieg score. All feed value parameters except dry matter intake (DMI) increased significantly with the addition of 5% molasses ($p\leq001$). No significant changes were observed in the metabolisable energy of *Leucaena* silage. The addition of 5% molasses significantly increased in vitro true dry matter digestibility (IVTDMD) and in vitro true organic matter digestibility (IVTOMD) ($p\leq.001$), while it did not affect IVCPD. In conclusion, the silage additives evaluated in this study did not negatively affect silage quality, and the inclusion of 5% molasses yielded the most favorable silage quality parameters.

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Farklı Katkı Maddelerinin Leucaena leucocephala Silajının Bazı Kimyasal Parametreleri ve In Vitro Sindirilebilirliği Üzerine Etkileri

ÖZET

Bu çalışma, çeşitli katkı maddelerinin *Leucaena leucocephala* silajının fiziksel ve kimyasal özellikleri, yem değeri ve in vitro sindirilebilirliği üzerindeki etkilerini araştırmıştır. Araştırma, bir kontrol grubu ve sırasıyla %5 melas, %5 arpa ve %0.5 formik asit ile ilave edilen üç deneme grubundan oluşmuştur. Her grup sekiz tekerrürden oluşturulmuştur. Bitki materyali 2,5-5 cm boyutunda kesilmiş, ilgili katkı maddeleri ile işlenmiş ve vakumlanarak paketlenmiştir (200 g torba). 60 günlük silaj fermentasyon süresinin ardından, fiziksel özellikler, amonyak azotu, pH seviyeleri, besin kompozisyonu, yem değeri, in vitro ham protein parçalanabilirliği (IVHPP) ve in vitro sindirilebilirliği değerlendirmek için analizler yapılmıştır. DLG puanlarına göre (≥17.62±0.29), tüm gruplar yüksek kaliteli silaj sınıfında yer almaktadır. Uygulamalar yapısal olmayan besin maddelerini önemli düzeyde etkilemese de, %5 arpa grubunun ham kül içeriğinde önemli düzeyde bir artış gözlemlenmiştir. *Leucaena* silajına %5 melas ilavesi, ham selüloz

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(p<.005) ve asit deterjan fiber (p≤.001) düzeylerini önemli düzeyde azaltmış ve Flieg skorunu artırmıştır. Kuru madde tüketimi (KMT) dışında incelenen tüm yem değeri parametreleri %5 melas ilavesi ile önemli düzeyde artmıştır (p≤.001). *Leucaena* silajının metabolize olabilir enerjisinde önemli bir değişiklik gözlemlenmemiştir. %5 melas ilavesi, in vitro gerçek kuru madde sindirilebilirliği (IVGKMS) ve in vitro gerçek organik madde sindirilebilirliğini (IVGOMS) önemli düzeyde arttırmıştır (p≤.001), IVHPP'yi etkilememiştir. Sonuç olarak, bu çalışmada değerlendirilen silaj katkı maddeleri silaj kalitesini olumsuz etkilememiş ve %5 melas ilavesi en olumlu silaj kalite parametrelerini vermiştir.

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INTRODUCTION

Feed resources are expected to become increasingly limited due to global warming. Habermann et al. (2022) highlighted that rising temperatures lead to a decrease in crude protein content and an increase in structural carbohydrates and lignification in forage crops, ultimately reducing their digestibility. It has been suggested that natural resources should be utilized alongside cultivated forage crops, with forest areas and tree species being integrated into local vegetation systems (Seidavi et al., 2020). According to Avcıoğlu et al. (2009), leguminous forage crops encompass herbaceous, shrub, and woody species, playing a vital role in reforesting unproductive lands. Leguminous forage trees are particularly valuable for animal nutrition, offering high-quality roughage. Leucaena leucocephala, mesquite (Prosopis laevigata), and huisache (Acacia farnesiana) leaves and pods are reported to provide significant feed potential for ruminants due to their low crude fiber (CF) content, high crude protein (CP) levels, and moderate in vitro fermentation properties (Zapata-Campos et al., 2020).

Leucaena leucocephala is a fast-growing, multipurpose leguminous species cultivated in tropical and semi-tropical regions. It thrives under average temperatures of 22–30°C and annual rainfall exceeding 650 mm. With its high growth potential, the plant spreads effectively in humid and semi-humid environments (Çakmakçı et al., 2001). Its high protein content (22–29%) and balanced amino acid composition make it an excellent feed source, particularly for ruminants. Research conducted in Çukurova reported annual yields of 443–714 kg da^{-1} green leaves and 47–78 kg da^{-1} crude protein. Moreover, its condensed tannin content reduces ruminal protein degradation while increasing bypass protein rates (Barry and Duncan, 1984; Ülger et al., 1990).

Numerous studies have demonstrated the potential of *Leucaena leucocephala* as silage for animal nutrition (Phesatcha and Wanapat, 2016; Andrade et al., 2019). The addition of 2% molasses and 1% urea to Leucaena silage increased total gas production, propionic acid, and volatile fatty acid levels (Phesatcha and Wanapat, 2016). Similarly, Andrade et al. (2019) found that incorporating Leucaena into sorghum silage enhanced CP content while reducing hemicellulose and neutral detergent fiber (NDF) levels. Sutaryono et al. (2023) reported that adding 4% molasses to maize-Leucaena silage improved fermentation quality, increased CP, and reduced pH and ammonia nitrogen (NH_3-N) levels compared to maize silage. Furthermore, increasing the proportion of Leucaena (0%, 20%, 40%, 60%) in maize-Leucaena silage mixtures raised CP levels and supported the growth of lactic acid bacteria (Arslan et al., 2017). Araújo et al. (2022) similarly observed that increasing Leucaena content (0%, 20%, 40%, 60%, 80%) in elephant grass-Leucaena mixtures significantly increased dry matter (DM), ether extract (EE), and CP levels while reducing fermentation losses. Additionally, Leucaena has been reported to positively influence digestibility in mixed silages (Putra et al., 2017; Mohamed et al., 2000). For instance, Putra et al. (2017) observed that adding 10% Leucaena enhanced crude protein degradability, while 20% Leucaena improved dry matter and organic matter digestibility in native grass-Leucaena silage with inoculant (4% *Lactobacillus plantarum*). Similar effects were reported for barley grain-Leucaena silage (Mohamed et al., 2000).

There are few studies investigating the use of *Leucaena leucocephala* as forage or silage in animal nutrition. Barros-Rodríguez et al. (2013) reported that in sheep fed 80% elephant grass (Pennisetum purpureum) and 20% Leucana leucocephala forage for 60 days, the addition of Leucaena decreased rumen pH, increased the level of NH_3 released in the rumen, dry matter digestibility, and feed intake. Similarly, Castro González et al. (2022) reported that rumen microbial protein production and nitrogen utilization were balanced when different ratios of Leucana leucocephala leaves were added to calf diets at an optimum level of 20%, while the benefit of higher ratios decreased. The researchers emphasized the importance of providing carbohydrate balance in rations to which leucaena was added. Giang et al. (2016) evaluated the effects of dietary inclusion of *Leucaena leucocephala* silage at 0%, 30%, 60%, and 100% levels in dairy cattle diets, and reported that the highest dry matter intake and nutrient digestibility were achieved at the 60% inclusion level. Additionally, ruminal ammonia nitrogen and blood urea nitrogen concentrations increased with higher levels of silage inclusion, whereas rumen pH remained unaffected.

Leucaena leucocephala is a drought-tolerant, self-regenerating leguminous shrub with high protein content, making it a valuable forage option for arid and semi-arid regions. However, its relatively low content of fermentable carbohydrates can limit silage fermentation quality and digestibility. To address this limitation, various additives such as carbohydrate sources (e.g., molasses and barley) and fermentation-modifying agents (e.g., formic acid) can be used to improve the ensiling process. While such additives have been widely studied in other forage species, their effects on Leucaena silage remain underexplored. In particular, the impact of carbohydrate sources and organic acids on Leucaena silage has not been sufficiently investigated, highlighting the need for further research to address this gap. The aim of this study was to evaluate the in vitro digestibility, in vitro crude protein degradability, and silage quality parameters of Leucaena leucocephala silage using different additives. Based on the chemical composition of Leucaena, it was hypothesized that the inclusion of different additives, including carbohydrate sources and organic acids, would lead to observable changes in fermentation characteristics, nutritional value, and in vitro digestibility of the silage.

MATERIALS and METHODS

The *Leucaena leucocephala* plants used in this study were collected from Muratpaşa, Antalya, in June 2023, during the pre-flowering stage. Leaves and twigs were hand-harvested from five different trees to represent the general population. The study was conducted in four groups: one control group (no additives) and three experimental groups. The experimental groups included: 1) 5% molasses, 2) 5% barley mash, and 3) 0.5% formic acid, with additives applied based on the wet weight of the samples. The inclusion levels of the additives were determined based on commonly used rates in silage production to improve fermentation quality and preserve the material. (Bugdayci et al., 2024). Molasses and barley mash were used as sources of fermentable carbohydrates to support the activity of lactic acid bacteria. Formic acid was applied as a preservative to enhance silage stability and inhibit the growth of undesirable microorganisms. Each group consisted of eight replicates. For each replicate, 200 g of silage material was vacuum-packed in a 25 × 35 cm bag with a thickness of 110 microns. The air was removed using a vacuum sealer (Sonkaya, SMVK150) to create an anaerobic environment, and the silage was stored at room temperature for 60 days to allow fermentation.

Determination of Nutrient Content of Groups

The dry matter (DM), crude ash (CA), crude protein (CP), and ether extract (EE) contents of fresh *Leucaena* leaves and twigs, as well as silage samples, were analyzed using methods specified by AOAC (2019). Crude fiber (CF) was determined following the method by Crampton and Maynard (1938), while neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were analyzed using Goering and Van Soest's (1970) methodology. The metabolizable energy (ME) of the silages was calculated using the equation reported by Güngör et al. (2008).

- $ME\ (kcal\ kg^{-1}\ DM) = 3464.70 - 58.10 \times ADF + 27.99 \times CF$

The levels of nitrogen-free extract (NFE), non-structural carbohydrate (NSC), and hemicellulose were calculated using the formulas reported by Ahsan (2023).

- $Nitrogen\text{-}free\ extract\ (NFE) = 100 - (CA + CP + EE + CF)$
- $Non\text{-}structural\ carbohydrate\ (NSC) = 100 - (NDF + CP + CA + EE)$
- $Hemicellulose = NDF - ADF$

If the values obtained are CA = 8%, CP = 14%, EE = 4%, CF = 25%, ADF = 30%, and NDF = 40%, the formulas yield the following results.

- $ME = 3464.70 - 58.10 \times 30 + 27.99 \times 25$
- $ME = 3464.70 - 1743 + 699.75 = 2421.45\ kcal\ kg^{-1}\ DM$
- $NFE = 100 - (8 + 14 + 4 + 25) = 49\%$
- $NSC = 100 - (40 + 14 + 8 + 4) = 34\%$
- $Hemicellulose = 40 - 30 = 10\%$

Determination of Feed Value and DLG Score

The feed value parameters, including Flieg score (Moselhy et al., 2015), dry matter intake (DMI) as a percentage of live weight, digestible dry matter (DDM), relative feed value (RFV), net energy for lactation (NEL), and total digestible nutrients (TDN), were calculated using the following formulas. Additionally, the DLG silage scoring system was applied based on the criteria presented in Table 1 (DLG, 1987). This evaluation method was also previously used by Karadeniz and Saruhan (2021).

- *Flieg score* = 220 + [2 × *DM* (%) - 15] - (40 × *pH value*)
- *Digestible Dry Matter* (%) *(DDM)* = 98.9 - (0.779 × *Acid detergent fiber*)
- *Dry Matter Intake* (% *Live weight*) *(DMI)* = 120 ÷ *Neutral detergent fiber*
- *Relative Feed Value (RFV)* = *DDM* (%) × *DMI* (%) × 0.775
- *Net Energy Lactation (Mcal* kg^{-1}*) (NEL)* = [1.044 - (0.0119 × *ADF*)] × 2.205
- *Total Digestible Nutrients (TDN)* = (-1.291 × *ADF*) + 101.35

If the values obtained are DM = 35%, pH = 4.2, ADF = 30% and NDF = 40% the formulas yield the following results.

- *Flieg score* = 220 + [2 × 35 - 15] - (40 × 4.2) = 220 + 55 - 168 = 107
- *DDM* = 98.9 - (0.779 × 30) = 98.9 - 23.37 = 75.53%
- *DMI* = 120 ÷ 40 = 3.00% live weight
- *RFV* = 75.53 × 3.00 × 0.775 ≈ 175.41
- *NEL* = [1.044 - (0.0119 × 30)] × 2.205 = [1.044 - 0.357] × 2.205 ≈ 1.515 Mcal kg^{-1}
- *TDN* = (-1.291 × 30) + 101.35 = -38.73 + 101.35 ≈ 62.62%

Table 1. DLG Silage Evaluation Criteria
Çizelge 1. DLG Silaj Değerlendirme Kriterleri

Observation	Score
Odor	
No buttery or acidic smell, only a slightly sour, fruity, or bread-like odor	14
A mild buttery odor, strong sourness, or musty smell	8
Moderate buttery or strong musty odor	4
Strong buttery odor with a noticeable ammonia scent	2
Rotten or unpleasant odor with strong mustiness	0
Structure	
Normal leaf and stem structure	4
Slight distortion in leaf structure	2
Significant deformation of leaves and stems, slightly moldy	1
Rotten leaves and stems, heavily moldy and contaminated	0
Color	
Green forage color, with a slight brownish tint in wilted silage	2
Yellow or dull brown color	1
Significant discoloration, ranging from light yellow to very dark	0

Ammonia Nitrogen (NH_3-N) Analysis and pH Analysis

Ammonia nitrogen levels were determined using the Kjeldahl method (Kjeldahl, 1883). For this, 25 g of each silage sample was mixed with 125 ml of 0.1 N sulfuric acid in a blender. The homogenized mixture was filtered through a double layer of cheesecloth, and NH_3-N was calculated using the following formula.

$$NH_3\text{-}N\ (mg\ 100ml^{-1}) = \frac{17031\ mg \times titrisol\ normality \times\ titrisol\ spent\ (ml)}{10 \times Sample\ weight\ (ml)} \times 100$$

For pH analysis, 25 g of each silage sample was blended with 100 ml of distilled water for 5–10 minutes. The mixture was filtered through a double layer of cheesecloth, and the pH of the filtrate was measured using a pH meter (Hanna HI 9124) as described by Buğdaycı et al. (2023).

In Vitro Digestibility and *In Vitro* Crude Protein Degradability of Samples

The in vitro true dry matter digestibility (IVTDMD), organic matter digestibility (IVTOMD), ADF digestibility (IVTADFD), NDF digestibility (IVTNDFD), and crude protein degradability (IVCPD) of the control and experimental groups were determined using the ANKOM DaisyII incubator. For this analysis, two parallel samples from each replicate were placed in device-compatible bags, which were then incubated in a bottle containing rumen fluid. The rumen fluid was obtained from healthy cattle fed a diet consisting of barley and corn silage. The incubator was filled with carbon dioxide and maintained at 39°C for 48 hours. At the end of the incubation period, IVTDMD, IVTOMD, IVTADFD, IVTNDFD, and IVCPD values were calculated.

Statistical Analysis

Statistical analyses were conducted using SPSS version 18.0 (IBM Corp., Armonk, NY, USA) (SPSS, 2013). The Kolmogorov-Smirnov test was applied to evaluate the normality of data distribution. Differences among groups were analyzed using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test to identify significant differences between group means. Results are expressed as marginal means accompanied by their standard errors. A p-value less than 0.05 was considered statistically significant (Dawson and Trapp, 2001)

RESULTS

The structural and non-structural nutrient content of *Leucaena leucocephala* leaves and branches is shown in Table 2.

Table 2. Nutrient content of *Leucaena leucocephala* leaves and twigs (on a dry matter basis).
Çizelge 2. Leucaena leucocephala yaprak ve dallarının besin içeriği (Kuru madde bazında).

Non-structural nutrient content, %		Structural nutrient content, %	
DM	36.88	CF	15.10
CA	9.81	NDF	47.49
CP	23.67	ADF	29.72
EE	2.26	ADL	9.66
NFE	44.90	Hemicellulose	17.17
NSC	16.77		

DM: Dry matter, CA: Crude ash, CP: Crude protein, EE: Ether extract, NFE: Nitrogen-free extract, NSC: Non-structural carbohydrate, CF: Crude fiber, NDF: Neutral detergent fiber, ADF: Acid detergent fiber, ADL: Acid detergent lignin, Hemicellulose = NDF - ADF.

The DLG score (sum of odor, color, and structure values) was similar in the control and barley groups, while the molasses and formic acid groups had significantly lower DLG scores ($p \leq .001$) compared to the control and barley groups. There was no significant difference ($p > .05$) among the groups in terms of NH_3-N levels. With regard to silage pH, the barley and formic acid groups had similar pH values; both were significantly higher than the control group and significantly lower than the molasses group ($p \leq .001$). The Flieg score of the molasses group was significantly ($p \leq .001$) higher than those of the control and other experimental groups. The effects of different additives on silage physical quality parameters, NH_3-N, pH, and Flieg score are presented in Table 3.

Table 3. Effect of different additives on the physical properties of Leucaena leucocephala silage.
Çizelge 3. Farklı katkı maddelerinin Leucaena leucocephala silajının fiziksel özellikleri üzerine etkisi.

	Experimental groups				
	Control group	Molasses group	Barley group	Formic acid group	*p*
NH_3-N	15.09±0.22	15.56±0.67	17.13±0.38	15.88±0.78	.081
pH	5.52±0.02^{a}	4.37±0.03^{c}	5.05±0.01^{b}	4.94±0.12^{b}	.001
Color	1.95±0.04^{a}	1.62±0.10^{b}	1.83±0.07^{ab}	1.66±0.09^{b}	.020
Odor	13.25±0.18^{a}	12.58±0.26^{b}	13.54±0.12^{a}	12.29±0.19^{b}	.001
Structure	3.91±0.05	3.62±0.11	3.87±0.06	3.66±0.09	.050
DLG	19.12±0.17^{a}	17.83±0.36^{b}	19.25±0.19^{a}	17.62±0.29^{b}	.001
Flieg	52.59±1.1^{c}	100.95±2.3^{a}	75.40±2.0^{b}	78.77±6.1^{b}	.001

Molasses group: 5% molasses addition, Barley group: 5% barley addition, Formic acid group: 0,5% formic acid addition; NH_3-N: Ammonia nitrogen, mg $100ml^{-1}$; DLG silage clasification: 18-20: Very good, 14-17: Good, 10-13: Fair, 5-9: Poor value, 0-4: Poor; *DLG score = Color + Odor + Structure; Flieg = (205 + (2 × DM%) - (40 × pH)).

^{a,b,c} Values within a row with different superscripts differ significantly at $p < .05$

The highest dry matter (DM) content (36.32%) was recorded in the barley group, while the control group had the lowest (34.24±0.34%) ($p < .05$). The lowest crude ash (CA) value (9.85±0.12%) occurred in the barley group, which differed significantly from the molasses, formic acid, and control groups ($p < .001$).

Although no significant difference ($p > .05$) in crude fiber (CF) was observed between the control and barley groups, CF levels in the molasses and formic acid groups were significantly lower than the control ($p < .005$). In terms of

ADF, the control group exhibited the highest value (22.26±0.34%), whereas the molasses group had the lowest (19.67±0.55%) (p<.001). There were no significant differences among the control and experimental groups for CP, EE, NFE, NSC, NDF, ADL, hemicellulose, or ME. Table 4 shows the effects of different additives on the nutrient composition of *Leucaena* silage.

The highest values for digestible dry matter (DDM), relative feed value (RFV), net energy for lactation (NEL), total digestible nutrients (TDN), and Flieg score were observed in the molasses group. In these parameters, the molasses group had either significantly higher or comparable values relative to the barley and formic acid groups. In addition, the molasses group displayed significantly higher IVTDMD, IVTOMD, and IVTADFD compared to the control group (Table 5).

Table 4. Effect of different additives on the nutrient composition of Leucaena leucocephala silage (on a dry matter basis).

Çizelge 4. Farklı katkı maddelerinin Leucaena leucocephala silajının besin maddesi bileşimi üzerine etkisi (Kuru madde bazında).

	Experimental groups				
	Control group	Molasses group	Barley group	Formic acid group	p
DM	34.24±0.34^{b}	35.37±0.34^{ab}	36.32±0.82^{a}	35.83±0.43^{ab}	.044
CF	16.91±0.49^{a}	14.54±0.29^{b}	15.29±0.69^{ab}	14.68±0.37^{b}	.004
EE	3.01±0.17	3.63±0.42	3.74±0.20	3.45±0.15	.218
CP	22.77±0.49	22.93±0.14	22.35±0.24	22.27±0.17	.349
CA	10.81±0.23^{a}	10.88±0.30^{a}	9.85±0.12 b	10.24±0.76^{ab}	.002
NFE	42.12±0.57	43.75±0.65	43.73±0.79	44.21±0.45	.110
NSC	17.87±0.82	19.07±1.30	18.88±0.89	17.95±0.63	.735
ADF	22.26±0.34^{a}	19.67±0.55^{b}	21.20±0.36^{ab}	21.02±0.36^{ab}	.001
NDF	45.52±0.71	43.47±1.05	45.16±0.77	46.07±0.66	.142
ADL	4.96±0.25	4.90±0.37	5.78±0.13	5.38±0.33	.129
Hemicellulose	23.25±0.45	23.80±1.17	23.96±0.85	25.04±0.74	.512
ME (kcal kg^{-1})	2644.27±21.94	2728.64±28.06	2660.99±30.86	2654.02±14.71	.075

Molasses group: 5% molasses supplemented, Barley group: 5% barley supplemented, Formic acid group: 0.5% formic acid supplemented; DM: Dry matter, CF: Crude fibre, EE: Ether extract, CP: Crude protein, CA: Crude ash, NFE: Nitrogen-free extract, NSC: Non-structural carbohydrate; ADF: Acid detergent fiber, NDF: Neutral detergent fiber, ADL: Acid detergent lignin, Hemicellulose = NDF - ADF, ME (kcal kg^{-1} DM) = 3464.70 - 58.10 × ADF + 27.99 × HS.

[a,b,c] Values within a row with different superscripts differ significantly at p<.05

DISCUSSION

The current study found that the DM content of *Leucaena* leaves and twigs ensiled with different additives was 36.88%. This value is consistent with findings from other research indicating that the DM content of *Leucaena leucocephala* leaves ranges from 31.66% to 38.0% (Santana et al., 2015; Phasatcha and Wanapat, 2016; Voltolini et al., 2019; Mohamed et al., 2000). The CA level of the plant's leaves (9.81%) was in line with values reported by Santana et al. (2015) and Voltolini et al. (2019), although other studies have noted CA contents of 6.21% and 7.6% (Phasatcha and Wanapat, 2016; Mohammed et al., 2000). This discrepancy likely results from variations in leaf-to-stem ratio, plant species, or environmental and harvesting conditions. Literature shows CP values for *Leucaena* ranging from a low of 18.6% (Voltolini et al., 2019) to a high of 25.94% (Mohamed et al., 2000), likely influenced by harvest timing. In parallel with the current results, Santana et al. (2015) recorded an EE value of about 2.5%. The CF content measured here approximates the 13.09% reported by Mohamed et al. (2000), although Santana et al. (2015) noted a higher CF of 26.53%. Zapata-Campos et al. (2020) determined a hemicellulose level of 19.1%, exceeding the findings of this study, which may be due to differences in harvest timing, plant maturity, or analytical methods. Studies have also reported that ADF and NDF in *Leucaena* can range from 19.9% to 46.5% and 33.15% to 63.1%, respectively (Voltolini et al., 2019; Mohamed et al., 2020), differences which may arise from plant species and harvest times.

Reports on the physical quality parameters of *Leucaena* silage remain limited. In this work, the DLG score of all groups was 17 points or higher according to silage classification criteria. Similarly, Aykan and Saruhan (2018) obtained a DLG score of 17.75 for forage pea silage without additives. However, Gül et al. (2015) found that alfalfa silages prepared with 5% molasses, 10% barley crush, or both had low or medium physical quality, whereas the control silage was classified as poor.

Table 5. Effect of different additives on feed value, in vitro degradability, and in vitro digestibility values of Leucaena leucocephala silage (on dry matter basis).

Çizelge 5. Farklı katkı maddelerinin Leucaena leucocephala silajının yem değeri, in vitro parçalanabilirlik ve in vitro sindirilebilirlik değerleri üzerine etkisi (Kuru madde bazında).

	Experimental groups				
	Control group	Molasses group	Barley group	Formic acid roup	*p*
Feed value results					
DMI	2.64±0.16	2.78±0.70	2.66±0.04	2.61±0.03	.102
DDM	81.55±0.27^{b}	83.57±0.43^{a}	82.38±0.28^{ab}	82.51±0.28^{ab}	.001
RFV	167.33±3.18^{b}	180.41±4.60^{a}	170.43±3.13^{ab}	167.10±2.51^{b}	.027
NEL	1.71±0.01^{b}	1.78±0.01^{a}	1.74±0.01^{ab}	1.75±0.01^{ab}	.001
TDN	72.60±0.44^{b}	75.95±0.71^{a}	73.98±0.47^{ab}	74.20±0.47^{ab}	.001
In vitro results					
IVCPD	45.81±1.70^{a}	42.04±1.97^{ab}	35.58±2.10^{b}	36.76±2.04^{b}	.001
IVTDMD	79.20±0.64^{b}	82.76±0.76^{a}	79.28±0.58^{b}	79.17±0.62^{b}	.001
IVTOMD	79.80±0.60^{b}	83.64±0.74^{a}	80.35±0.49^{b}	80.39±0.62^{b}	.001
IVTNDFD	57.69±1.17	60.36±1.35	58.72±1.01	62.52±1.53	.054
IVTADFD	33.75±0.56^{b}	40.51±1.45^{a}	37.57±1.13^{ab}	37.45±1.56^{ab}	.004

Molasses group: 5% molasses supplemented, Barley group: 5% barley supplemented, Formic acid group: 0.5% formic acid supplemented, DMI: Dry matter intake (% body weight), DDM: Digestible dry matter (%), RFV: Relative feed value, NEL: Net energy lactation (Mcal kg^{-1}), TDN: Total digestible nutrients (%), IVCPD: In vitro crude protein degradability, IVTDMD: In vitro true dry matter digestibility, IVTOMD: In vitro true organic matter digestibility, IVTNDFD: In vitro true neutral detergent fiber digestibility, IVTADFD: In vitro true acid detergent fiber digestibility.

^{a,b,c} Values within a row with different superscripts differ significantly at $p<.05$

Several studies (Ferreira et al., 2016; Mohamed et al., 2000) have reported lower pH in *Leucaena* silages, with and without molasses, compared to the present study. Here, the control group showed the highest pH, while the molasses group had the lowest. This difference may result from the high soluble carbohydrate content in molasses, which promotes rapid acidification via lactic acid bacteria. Consequently, the Flieg score was highest in the molasses group. Although no significant difference ($p>.05$) was observed among the additives regarding NH_3-N levels, the NH_3-N value in the molasses group was roughly 3.5 times higher than that reported by Mohamed et al. (2000). This discrepancy may be related to several factors, such as differences in analytical methods, the high content of non-protein nitrogen (NPN) compounds in molasses, the rapid fermentation of soluble carbohydrates, or variations in molasses composition depending on processing conditions.

The CA value in the control group (10.81%) exceeded previously reported values for untreated *Leucaena* silage (Mohamed et al., 2000), potentially reflecting soil mineral content or fertilizer application. The lower CA ratio in the barley group compared to the other groups could be attributed to the barley's inclusion level and its lower CA content. Although the CP value observed here is consistent with Phasatcha and Wanapat (2016), Ferreira et al. (2016) reported a CP of 16.77% for untreated *Leucaena* silage. Khaing et al. (2016) noted 27.5% CP in the same silage, and Mohamed et al. (2000) reported 25.94% CP in *Leucaena* silage containing about 3% molasses. Giang et al. (2016) recorded 25.1% CP in *Leucaena* silage with 1% urea and 2% molasses. These variations could stem from differences in plant species, the portion of the plant ensiled, soil nitrogen content, or silage-making technique. In this study, the additives did not alter the crude oil value of *Leucaena* silage. However, Mohamed et al. (2000) found that adding about 3% molasses raised the EE content to 5.13%, whereas Khaing et al. (2016) reported a lower level of 0.97%. This discrepancy might relate to plant species, harvest timing, and the ratio of leaves, stems, and seeds. The CF content in all groups resembled that reported by Mohamed et al. (2000).

Results indicated that the pH of the molasses and formic acid groups was significantly lower than that of the control. Although the rate of pH reduction was not quantified, the faster pH decline in the molasses group may be attributable to several factors, including its higher sugar content, which likely supported more rapid lactic acid bacterial growth compared to barley. In contrast, formic acid likely contributed to the pH reduction through its direct acidifying effect. A rapid pH drop can accelerate the degradation of cellulose and hemicellulose fractions, consistent with the significantly lower ADF level in the molasses group. The minimal structural carbohydrate in molasses, along with changes in silage mass ratio, may also play a role. Correspondingly, Phasatcha and Wanapat (2016) observed that 1–2% molasses supplementation reduced the ADF content in *Leucaena* silage by about 4%. However, Khaing et al. (2016) and Ferreira et al. (2016) documented ADF values of 34.95% and 46.6%, respectively, which may reflect different *Leucaena* species or harvest periods.

Studies on *Leucaena* silage report varying NDF and ADL values. Phasatcha and Wanapat (2016) noted that 1–2%

molasses supplementation lowered the NDF content by about 4%, whereas Giang et al. (2016) and Mohamed et al. (2000) reported NDF levels of 36.2% and 33.15% for molasses-supplemented *Leucaena* silages. These values align relatively well with the current findings, yet Ferreira et al. (2016) recorded 63.03% in untreated silage, and Khaing et al. (2016) found 50.1%. Such discrepancies likely stem from diverse plant materials and harvest schedules. In the present study, ADL values in the control and experimental groups were roughly two-thirds of the 8.16% reported by Mohamed et al. (2000). Because ADF represents difficult-to-digest structural components, an increase in ADF generally reduces metabolic energy (ME). Conversely, lower ADF usually correlates with higher digestibility, as shown by the significantly higher IVTADFD in the molasses group than in the control. Enhanced digestibility translates into greater energy availability for livestock. Nevertheless, no significant differences (p>.05) were observed among the groups with respect to ME, possibly because the ME formula includes both CF and ADF.

The molasses group exhibited higher feed value parameters (DDM, RFV, NEL, TDN) compared to the barley and formic acid groups. This difference may be attributable to several factors, including the presence of soluble carbohydrates in molasses reducing the proportion of structural carbohydrates, the increased energy availability, and the potential stimulation of fermentative microbial activity. Indeed, DDM, NEL, and TDN were notably elevated in the molasses group relative to the control, corresponding to the decrease in ADF. IVTDMD and IVTOMD also rose significantly in the molasses group, while IVTNDFD remained statistically unchanged (p>.05). Giang et al. (2016) reported an IVCPD of 60.70% for Leucaena silage enriched with 1% urea and 3% molasses. In the present study, the IVCPD for the 5% molasses group was 42.04±1.97%. This situation is likely attributable to the use of urea in the study by Giang et al. (2016), as urea promotes the activity of proteolytic bacteria, thereby increasing the pH. Additionally, the high starch content in the group supplemented with 5% barley may have influenced the microbial fermentation dynamics, potentially slowing down the rate of protein degradation. The lower protein degradability observed with barley addition to Leucaena silage compared to the control group may be attributable to several factors, including the starch content of barley, which could interfere with microbial activity and protein breakdown.

For this reason, it may have caused slower fermentation compared to molasses. Molasses is rich in simple sugars (sucrose, glucose, fructose). However, formic acid may have inhibited the activity of some bacteria. On the other hand, the addition of formic acid to Leucaena silage may have caused denaturation of soluble protein fractions found in plants, particularly those that play an important role in legumes, such as albumins and globulins. This denaturation could have contributed to the observed decrease in in vitro protein degradability compared to the control group.

Research on IVTDMD, IVTADFD, and IVTNDFD in *Leucaena* leaves is somewhat scarce. However, results parallel the findings of Zhang et al. (2018), who reported an IVDMD of roughly 72.20% in Guiana stylo (*Stylosanthes guianensis*), another legume adapted to tropical and subtropical regions. In the present research, IVTDMD in the molasses group rose significantly (a 4.49% increase) compared to both the control and other experimental groups, likely reflecting the high sugar content of molasses and improved fermentation quality. Indeed, Phasatcha and Wanapat (2016) noted a 31.55% jump in IVDMD when 2% molasses was added to *Leucaena* silage. Studying mulberry leaf silage supplemented with 3% molasses, Dong et al. (2020) reported an IVDMD of 53.6% (a 10.3% increase) and an IVTNDFD of 35.4% (a 1.3% increase) compared to the control—although their overall values remained lower than those recorded for the molasses group here. This difference might be attributed to the higher CP in *Leucaena* leaves compared to mulberry leaves, as well as to mulberry's broader array of antinutritional factors (oxalic acid, tannins, saponins). The lower IVTDMD and IVTOMD values of barley and formic acid additives compared to molasses may result from the slower fermentation of barley carbohydrates and the low formic acid inclusion level (0.5%), which may have had a limited effect on fiber digestibility. Notably, the molasses group had the lowest ADF content and the highest IVTADFD. By contrast, the other experimental groups and the control showed similar IVTADFD, reflecting the relationship between decreased structural carbohydrates and enhanced digestibility.

CONCLUSION

This study demonstrated that all *Leucaena leucocephala* silage groups achieved high physical quality based on DLG scores. Among the tested additives, 5% molasses supplementation yielded the most favorable outcomes in terms of digestibility and feed-value parameters. The reduction in ADF content in this group was associated with improvements in IVTDMD, IVTOMD, and IVTADFD, highlighting the potential of molasses to enhance the nutritional value of *Leucaena* silage.

While the barley and formic acid treatments influenced some chemical parameters, their effects were less pronounced compared to molasses. The observed differences among treatments suggest that the type and inclusion level of additive play a crucial role in silage quality. Based on these findings, it is practically recommended to supplement Leucaena leucocephala silage with 5% molasses on a fresh matter basis to improve fermentation, digestibility, and feed value. For practical applications, diluting molasses with water at a 1:1 ratio is advised to ensure ease of handling and uniform distribution across the forage material. In this case, 10 kg of diluted molasses solution should be applied per 100 kg of fresh forage to maintain the same effective molasses concentration, thereby preserving silage dry matter within the optimal range for fermentation and avoiding excessive moisture or effluent losses. This method can be readily implemented by farmers under field conditions. Future studies should focus on optimizing inclusion rates and exploring the effects of harvest timing and additive combinations to refine silage quality further.

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Contribution Rate Statement Summary of Researchers

The authors declare that they have contributed equally to the article.

Conflict of Interest

The authors declare that there is no conflict of interest between them.

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