BUKTI KORESPONDENSI JURNAL

MIXED LEUCAENA AND MOLASSES CAN INCREASE THE NUTRITIONAL QUALITY AND RUMEN DEGRADATION OF CORN STOVER SILAGE

JOURNAL OF ADVANCED VETERINARY AND ANIMAL RESEARCH
ISSN 2311-7710 (Electronic)
http://doi.org/10.5455/javar.2023.j660

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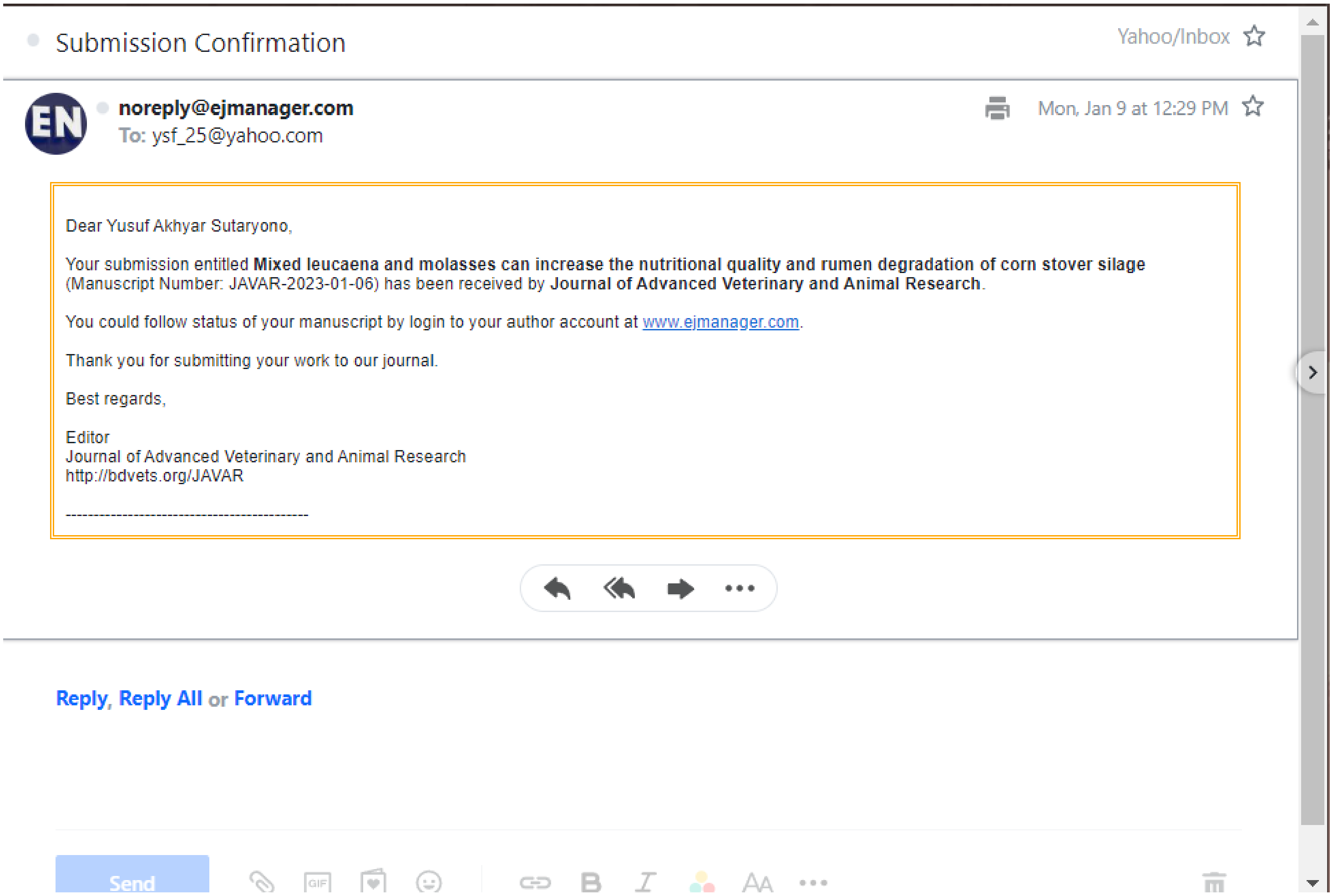

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Mixed leucaena and molasses can increase the nutritional quality and rumen degradation of corn stover silage

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Abstract : Objective: The study was conducted to determine the effect of leucaena at different proportion and doses of molasses on the Nutrient Quality, silage fermentation characteristic, and In vitro Digestibility of corn stover silage. Materials and Methods: The study was designed in a completely randomized factorial design 3 3 pattern. The first factor was the proportion addition of leucaena i.e. L0 (0%), L15 (15%), L30 (30%), and L45 (45%) of inclusion of leucaena on the DM basis of corn stover. The second factor was the dose of inclusion of molasses, i.e. M2 (2%), M4 (4%), and M6 (6%) on the fed basis of silage. Each treatment had five replications. Variables: The variables observed included chemical composition (DM, OM, CP, CF, hemicellulose, ADF, and NDF), silage fermentation characteristics (pH and NH3-N), dry matter digestibility (DMD), and organic matter digestibility (OMD) under in vitro conditions. Results: The result shows that the inclusion of leucaena in the proportion of 30% to 45% is very effective in increasing and improving the chemical composition of corn stover silage, significantly suppresses the content of crude fiber, and increases the CP content of the silage. Likewise, the inclusion of molasses at a dose of 4% also positively contributed to the quality of the resulting silage, especially its effect in suppressing the buffer capacity of proteins resulting in low pH values and NH3-N concentrations in silage. Conclusions: It was concluded that the inclusion of leucaena in 30% to 45% and the inclusion of molasses at a dose of 4% is very effective in increasing and improving the chemical composition, silage fermentability characteristics, and digestibility of corn stover silage.

Keywords : corn stover, silage, leucaena, water-soluble carbohydrate.

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ORIGINAL ARTICLE,

Mixed leucaena and molasses can increase the nutritional quality and rumen degradation of corn stover silage

Statement of novelty: Revealed a strong interaction between various additions of leucaena and various doses of molasses. The addition of leucaena up to 45% and molasses at a dose of 4% has significantly improve the quality of corn stover silage compared to the addition of 15% Leucaena at all doses of molasses inclusion.

Mixed leucaena and molasses can increase the nutritional quality and rumen degradation of corn stover silage

ABSTRACT

Objective: The study was conducted to determine the effect of leucaena at different proportion and doses of molasses on the Nutrient Quality, silage fermentation characteristic, and In vitro Digestibility of corn stover silage. **Materials and Methods**: The study was designed in a completely randomized factorial design 3*3 pattern. The first factor was the proportion addition of leucaena i.e. L0 (0%), L15 (15%), L30 (30%), and L45 (45%) of inclusion of leucaena on the DM basis of corn stover. The second factor was the dose of inclusion of molasses, i.e. M2 (2%), M4 (4%), and M6 (6%) on the fed basis of silage. Each treatment had five replications. **Variables:** The variables observed included chemical composition (DM, OM, CP, CF, hemicellulose, ADF, and NDF), silage fermentation characteristics (pH and NH3-N), dry matter digestibility (DMD), and organic matter digestibility (OMD) under in vitro conditions. **Results:** The result shows that the inclusion of leucaena in the proportion of 30% to 45% is very effective in increasing and improving the chemical composition of corn stover silage, significantly suppresses the content of crude fiber, and increases the CP content of the silage. Likewise, the inclusion of molasses at a dose of 4% also positively contributed to the quality of the resulting silage, especially its effect in suppressing the buffer capacity of proteins resulting in low pH values and NH_3-N concentrations in silage. **Conclusions:** It was concluded that the inclusion of leucaena in 30% to 45% and the inclusion of molasses at a dose of 4% is very effective in increasing and improving the chemical composition, silage fermentability characteristics, and digestibility of corn stover silage.

Keywords: corn stover, silage, leucaena, water-soluble carbohydrate.

INTRODUCTION

Feed availability for cattle in West Nusa Tenggara Province fluctuates between the rainy and dry seasons. This fluctuation than the growth and body weight of Bali cattle raised by farmers in this province. With high feed availability in the rainy season, the body weight growth is very high, but during the dry season, the body weight of cattle will decrease rapidly due to less quantity and quality of feed availability **[1]**.

The high fluctuation of feed availability in this region needs to be addressed with the use of feed that is available in large numbers, easy to be accessed, and cheap. The most widely known in this region is corn. The availability of corn stover in this region is extensive due to the large amount of land planted with corn. Until recently, most of this corn stover was left wasted, returned to the soil, and burned **[2]** . Burning this biomass wastes organic matter potential for cattle feed **[3]**, and causes massive environmental pollution due to the high carbon released into the atmosphere **[4]**.

The use of corn stover as feed has drawbacks, Approximately 50–70% of corn stover is composed of cellulose, hemicellulose, and lignin which affects the utilization efficiency **[5]**. Especially, its low protein content, so it needs to be mixed with other high protein feeds, one of which is quite widely available in this area is leucaena. Adding protein is expected to respond positively **[6]**. However, adding high-protein materials in the silage has its problems. The problem is the buffering capacity by the material protein component, which can inhibit achieving a low pH that supports conservation **[7]**. Hence, it is necessary to consider adding water-soluble carbohydrates to overcome them. The addition of biological additives is intended to increase the efficiency of the fermentation process **[8]**, produce high lactic acid, lowers pH, reduce proteolysis, and finally can improve livestock performance **[9,10]**. Based on this, research on the use of corn stover mixed with leucaena and molasses to be used as feed ration for cattle fattening was then conducted before harvested. This study aimed to test the effect of several inclusions of leucaena and the doses of molasses in increasing and improving the chemical composition, silage fermentability characteristics, and digestibility of corn stover silage.

MATERIALS AND METHODS

Silage Preparation Process

The material used in this experiment is corn stover, Leucaena, and molasses. Corn stover was collected randomly from corn stover fields in the Central Lombok district, while molasses was obtained from a molasses trader in Mataram city. Corn stover and leucaena leaves were then chopped to 3-6 cm in size. Before chopping the corn stover, leucaena leaves were let dry under the shade for 6 hours to achieve a water content of approximately 65%. The experiment was conducted on a laboratory scale. Silage was made from mixed corn stover and leucaena in a 5kg mixture, with a leucaena proportion of 0, ,15, 30 and 40% of the total mix. Molasses were applied in doses o,f 2, 4 and 6% into the corn stover and leucaena. All materials were mixed well, placed into a plastic container, pressed and vacuumed to reduce oxygen in the silo, and then sealed. Finally, all silos (plastic containers) were placed in a sterile room and left for fermentation for 21 days before being harvested.

Sample analysis procedure

Before the ensilage process, silage materials were sampled for analysis of the nutrient content sample was analyzed for dry Matter (DM), organic matter (OM), crude Protein (CP), and crude fiber (CF) content according to the procedure of **[11]**. Hemicellulose (HSL), neutral detergent fiber (NDF), and acid detergent fiber (ADF) content were analyzed according g to the procedure described by Van Soest et al. (1991) in Table 1. Fermentation characteristics were analyzed based on pH and NH3-N. Analysis of pH silage followed the procedure **[12]** using a pH meter (Metrohm 691 pH electrode). Analysis of NH3-N concentration follows the procedure of **[13]** using a spectrophotometer with a reading wavelength of 640 nm. In vitro digestibility was analyzed according to the methods developed by **[14]**. In vitro tubes were filled with samples consisting of rumen fluid and artificial saliva solution (McDougal solution) with 1: 4 ratios. When the solution was filled into the tubes, the CO2 was provided simultaneously to enable the anaerob condition in tubes that will be incubated. Incubation process were conducted in the waterbath at 39-40^{o}C for 48 hours.

Experimental Design

The study was designed in a completely randomized factorial design 3*3. The first factor was the proportion addition of leucaena, as follows: L0 (0%), L15 (15%), L30 (30%), and L45 (45%) of inclusion of leucaena on the DM basis of corn stover. The second factor was the dose of inclusion of molasses, as follows: M2 (2%), M4 (4%), and M6 (6%) on the fed basis of silage. Each treatment had five replications. Hence there were 60 experimental units.

Data Analysis

The variables observed included chemical composition (DM, OM, CP, CF, hemicellulose, ADF and NDF), silage fermentation characteristics (pH and NH_3-N), dry matter digestibility (DMD) and organic matter digestibility (OMD) under In vitro conditions. All data obtained were then processed with Statistical Product and Service Solutions (SPSS) software version 20 based on the design used. If there are differences between treatments, Duncan New Multiple Range Test (DNMRT) was applied.

RESULTS

Effect on the chemical composition of silage

The results showed that the increase of leucaena proportion substantially affects the value of DM, CP, CF, hemicellulose, acid detergent, and neutral detergent fiber of corn stover silage ($p < 0.05$; Table 2). Specifically, the increase of leucaena increased the CP content and decreased CF and its fractions. The CP content in L0, L15, L30 and L45 were 6.11%, 7.98%, 8.85% and 11.09 respectively ($p < 0.05$).

A similar result is shown by adding a dose of molasses, where increasing the dose significant affected corn stover silage nutrient content except for hemicellulose content. The most potent effect of molasses was indicated by the neutral detergent fibre value increase of 64.54%, 62.92%, and 60.76% for M2, M4, and M6, respectively ($p < 0.05$; Table 2). A significant effect of interaction between leucaena and molasses was also shown by DM, OM, CP, CF, hemicellulose and neutral detergent fiber ($p < 0.05$).

Effect on silage fermentability quality

The experiment showed no effect of the interaction of leucaena and molasses on the silage fermentation quality. Although the addition of leucaena and molasses significantly affected the pH and NH_3-N of the silage ($p < 0.05$; Table 2), there was no interaction effect on the value of pH and NH_3-N of the silage. The pH value increased significantly in line with the increase of leucaena proportion in the silage (3.58 to 4.07 on average; $p < 0.05$), but there was a significant decrease when molasses was added (3.87 to 3.70 on average; $p < 0.05$) with the lowest value is in L0 treatment.

The increase of leucaena proportion increased the NH_3-N concentration of silage ($p < 0.05$), the value of NH_3-N concentration caused by the increase of leucaena proportion were 5.2 mg/100 ml (L0), 6.24 mg/100 ml (L15), 6.90 mg/100 ml (L30), and 8.27 mg/100 ml (L45) (Table 2). On the other hand, the NH_3-N concentration decreased with the increase of molasses dose ($p < 0.05$) with pH values of 7.87 mg/100 ml, 6.61 mg/100 ml, and 5.76 mg/100 ml for M2, M4 and M6 respectively.

Effect on dry matter and organic matter digestibility

Dry matter and organic matter digestibility of the silage increased linearly with the increase of the proportion of leucaena and molasses dose ($p < 0.05$; Table 2). Moreover, there was a significant interaction between leucaena and molasses ($p < 0.05$) in affecting the DMD and OMD of silage. Dry matter and organic matter digestibility of silage increased significantly with the leucaena proportion of 45% of total silage (49.10% and 50.87%, respectively). The dry matter and organic matter digestibility values with the addition of molasses at 2% were 42.63% and 44.37%, while 4% were 44.06% and 45.40% ($p < 0.05$).

DISCUSSION

Chemical Composition of silage

The result showed the significant effect of leucaena and molasses addition on the silage quality ($p < 0.05$). The increase of dry matter content was the direct effect of Leucaena addition on the silage. Another researcher also reported a similar result: increased DM content in a silage mixture of corn husk and leucaena in a ratio of 75:25, with an average increase of 1.56 – 2.04% of dry matter content **[15]**. In this regard, mixing several materials in silage or feed-making could increase the dry matter content compared to the dry matter content used as single feed material. Organic matter decreased slightly in line with the increased dose of molasses ($p < 0.05$), contrary to the effect of molasses dose that increases dry matter content. The decrease of silage organic matter affected by the increase of molasses dose was presumed caused by the use of organic matter by lactic acid bacteria (LAB) during the ensilage process. The rate of LAB population increase during the ensilage process might be very high; hence there was a need for high energy that caused the high use of organic matter. Those were in line with **[16]** that the loss of organic matter during the making of silage primarily originated from carbohydrate fractions which are nitrogen free extracts with the main component of amylum and sugars used by bacteria to produce organic acids, mainly lactic acid. There was a significant interaction between leucaena and molasses on dry matter and the organic matter content of the silage. The best interaction was shown at the treatment of 45% leucaena and 6% molasses with dry matter value of 95,97% ($p <$ 0,05).

The crude protein content of silage increased with the increase of leucaena proportion in silage ($p < 0.05$); in this case, mixed feed materials had an associative effect. This result was confirmed by **[17]**, which reported that adding leucaena to 37% in making cactus silage produced the highest Nitrogen. Furthermore, **[18]** described that nitrogen content was higher in mixed silage compared to common silage comprised of Cerealia content. An increase in silage's protein content also occurs caused by the increased number of additive molasses applied. The higher the molasses concomitant with, the higher crude protein content of silage. This result was associated with the LAB growth as a direct amount of molasses as a source for water-soluble carbohydrates (WSC) in the silage. Increased dose of molasses means more energy is available for LAB to grow and proliferate. With high proliferation rate of LAB would contribute to the increase the crude protein content of silage.

Nevertheless, Hu et al. **[19]** reported no effect of LAB inoculation on the crude protein content of corn silage. The highest crude protein content at 11.89% found in this experiment is enough to fulfil cattle need for the basic need of cattle. Furthermore, Putra et al. **[20]** stated that to fulfil the basic need of cattle, it needs a minimum of 12% crude protein content in its ration.

Low crude fibre content of silage on leucaena proportion of 45% was caused by the increase of leucaena proportion in the corn stover silage. Leucaena has low CF content compared to corn stover; hence, the higher the leucaena, the lower the crude fiber (Table 1). The crude fibre content of corn stover only silage was 30.50%, while the silage with 15%, 30% and 45% was 28.52%, 26,43% and 25.39% respectively ($p < 0.05$). A similar result was reported by **[21]** that a decreased crude fiber in Elephant grass silage in line with the increase of Leucaena of 20-30%. Furthermore, the inclusion leucaena of 30% to the base material of native grasses silage decreased the crude fiber content of the silage **[20]**.

Moreover, the increase of molasses as silage additive showed a significant effect on the decrease of CF content of corn stover silage ($p < 0.05$). The decrease was caused by the high addition of molasses that caused a high growth rate of LAB; hence more CF could be broken down, which finally decreased the silage CF content. Putra et al. **[22]** stated that the decrease in the crude fiber of silage is caused by the hemicellulose hydrolysis process that takes place during the ensilage process.

Analysis of variance showed that the interaction of leucaena proportion and dose of molasses significantly affected ($p < 0.05$), but the dose of molasses partially was not significantly affecting the hemicellulose content of corn stover silage. The hemicellulose content decreased in line with the increase of leucaena proportion ($p < 0.05$). The low hemicellulose content of L45 treatment compared to other treatments was presumably caused by hydrolysis of hemicellulose during ensilage processes. Close to half of hemicellulose content was degraded during the ensilage process **[22,23]**. Widyastuti et al. **[24]** stated that three possibilities caused the degradation of hemicellulose, i.e. 1) was degraded by the hemicellulose enzyme of the plant itself, 2) degraded by the hemicellulose enzyme bacteria, and 3) was hydrolyzed by organic acids during fermentation processes.

The addition of leucaena in corn stover silage showed a significant effect ($p < 0.05$) on the NDF and ADF content of the silage. The higher proportion of leucaena in corn stover silage indicated lower ADF and NDF content. A similar effect also showed by molasses, the higher molasses added the lower ADF and NDF content of corn stover silage ($p < 0.05$). Acid detergent fiber and Neutral detergent fiber content in this experiment is closely related to the decrease of crude fiber of corn stover silage after ensilage processes.

Silage fermentability quality

The result showed that the pH of corn stover silage was significantly affected by the addition of leucaena proportion ($p < 0.05$). The supplementation of legumes in haymaking caused the increase in pH value reported by **[25]**. The experiment showed that the increase of leucaena proportion was followed by the increase of pH value (3.58 *vs* 3.65 *vs* 3.89 *vs* 4.07; for each treatment, $p < 0.05$). This condition clearly showed the high buffer capacity of protein components of silage pH. Piltz & Burns **[26]** stated that buffer capacity is how much acid is needed to succeed in silage convertion; the high value of buffer capacity of particular feed material will require much more acid as an agent of conversion and *vice versa*.

The addition of molasses significantly affects the pH of silage ($p < 0.05$). The pH value obtained in this experiment differs from to result reported by **[21]**, who obtained a pH value of 4.5 for the silage mixture between corn husk and leucaena (ratio 75:25) and showed the buffer capacity of Protein caused by leucaena addition. The addition of molasses dose affected the increase of glycolytic activities, so LAB could use the availability of hydrogen ion (H^+) as an electron acceptor in producing lactic acid as a fermentation product. Nevertheless, Ohshima et al. **[27]** stated that fermentation with the addition of several LAB strains is more critical than diluted carbohydrates that reduce alfalfa silage's buffer capacity. The low pH value in silage indicated the high production of lactic acid during fermentation **[28,29]**. However, in our study, no interaction effect was recorded between leucaena and molasses on the pH value of corn stover silage.

Ammonia silage concentration in each treatment of L0, L15, L30, and L45 showed an increase of NH_3 concentration (5.53, 6.24, 6.90, and 8.27 mg/100 ml (p < 0.05). This result is similar to the result reported by **[28]**. Th was associated with the high buffer capacity of legumes that supported the production of other organic acids except for lactic acid. The decrease in pH affects the formation of ammonia because there was no hydrolysis of protein which means the lower the NH_3-N concentration in the silage, the better the silage quality. Ammonium concentration of silage was significantly affected by molasses (p < 0.05); as described earlier, molasses added to silage possess a vital role as an energy source for epiphytic LAB, which was formed from growth modulation processes. Cazzato et al. **[30]** reported that the inoculation of *L. plantarum* in silage-making significantly suppressed the NH_3 formation. Lactic Acid Bacteria, which were formed, provide advantages in decreasing pH, ammonium, and butyric acid production and increasing lactic acid concentration **[31,32]**. The concentration of ammonium in the silage was associated with protein degradation levels caused by plant enzymes or the activity of microbes, particularly microbial enzyme activities.

In vitro dry matter and organic matter digestibility

Digestibility is the number of feed ingredients that can be digested by the digestive tract of livestock in the rumen and then absorbed by livestock in the small intestine. The results of our study showed that the DMD and OMD of silage increased linearly with the increasing portion of the addition of leucaena (p < 0.05). This increased digestibility of corn stover silage is caused by a decrease in the portion of crude fiber in the silage due to an increase in the portion of leucaena. These results confirmed by **[22]** showed the same thing, namely an increase in the digestibility of native grass silage that received leucaena supplementation up to a level of 30% under in vitro conditions. Other researchers, Barros-Rodríguez et al. **[33]**, also reported that under in sacco condition, the addition of 20% to 40% of legume in silage significantly increased the digestibility of silage.

Likewise, the effect of the dose of molasses treatment was an increase in the digestibility value of corn stover silage (p <0.05). Thus, increasing portions of leucaena and molasses onto corn straw silage will impact the DMD and OMD of corn stover silage. The high DMD in feed indicates the quality of this feed. The results of our study also showed a significant interaction of both treatment factors on the increased digestibility of OMD and OMD of corn stover silage (p <0.05). The increased digestibility of corn stover silage in our study was due to the availability of sufficient N sources of protein origin, which was positively correlated with a decrease in crude fiber content as well as the availability of soluble carbohydrates, which supported faster rumen microbial proliferation which in turn improved overall rumen performance. Qu et al. **[34]** suggest that higher CP content and lower fiber content in legumes than in grass may affect digestion.

CONCLUSION

This study shows that the inclusion of leucaena in 30% to 45% is very effective in increasing and improving the chemical composition of corn stover silage because this proportion significantly suppresses the content of crude fiber and its fractions and increases the CP content of the silage. Likewise, the inclusion of molasses at a dose of 4% also positively contributed to the quality of the resulting silage, especially its effect in suppressing the buffer capacity of proteins resulting in low pH values and NH_3-N concentrations in silage. Overall, there was a synergistic interaction between the addition of the proportion of Leucaena and the dose of molasses in increasing the chemical composition, quality of silage fermentation, and increasing the digestibility of silage in the rumen with the best combination obtained at the proportion of leucaena of 45% with a dose of molasses of 4%. Further, an in vivo study should be carried out to investigate the direct effect of increasing the proportion of leucaena and the dose of molasses in corn stover-based silage on live livestock, especially the effect on overall production performance.

CONFLICT OF INTERESTS

The authors report no conflict of interest.

AUTHORS CONTRIBUTION

All authors developed the theory and supervised the research. Yusuf Akhyar Sutaryono, Dahlanuddin, Ryan Aryadin Putra, Mardiansyah, Enny Yuliani, Harjono, Mastur, and Sukarne contributed to the sample collection and analysis calculations. Yusuf Akhyar Sutaryono, Ryan Aryadin Putra, Mardiansyah, Luh Sri Ernawati and Dahlanuddin contributed to the writing and final version of the manuscript.

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Table 1. Chemical composition of *Leucaena leucocephala* and corn stover

Chemical Composition	Corn stover	Leucaena
Dry matter, % DM	89.92	87.53
Organic matter, %	94.12	91.53
Crude Protein, %	5.48	23.47
Crude Fiber, %	30.56	20.16

Table 2. Nutrient composition, characteristics fermentative and in vitro digestibility of mixture silage corn stover with different inclusion of leucaena and doses of molasses.

Variable	L0			L15			L30			L45			*s.e.m*	*P-value*		
	M2	M4	M6	M2	M4	M6	M2	M4	M6	M2	M4	M6		L	M	L x M
Nutrient composition (%)																
Dry matter	92.50±0.24^{a}	92.93±0.77^{a}	94.13±0.51^{c}	93.94±0.48^{bc}	93.17±0.56^{ab}	95.74±0.49^{d}	95.96±0.45^{d}	95.60±0.61^{d}	95.11±0.62^{d}	95.97±0.65^{d}	95.36±0.54^{d}	95.96±0.17^{d}	0.309	<0.001	0.001	<0.001
Organic matter	91.68±0.65^{c}	91.53±0.56^{bc}	90.19±1.11^{a}	91.48±0.22^{bc}	90.61±0.23^{ab}	90.88±0.50^{abc}	91.72±0.25^{c}	91.65±0.40^{c}	91.23±0.15^{bc}	91.66±0.30^{c}	90.96±0.23^{abc}	91.72±0.51^{c}	0.288	0.093	0.014	0.032
Crude protein	5.21±0.35^{a}	6.52±0.17^{b}	6.60±0.13^{b}	6.94±0.12^{c}	8.20±0.34^{d}	8.78±0.19^{f}	8.31±0.13^{de}	8.60±0.16^{ef}	9.64±0.29^{g}	10.42±0.17^{h}	10.95±0.18^{i}	11.89±0.16^{j}	0.114	<0.001	<0.001	<0.001
Crude fiber	31.49±0.65^{g}	30.59±0.17^{f}	29.41±0.50^{e}	29.75±0.34^{e}	28.00±0.35^{d}	27.79±0.21^{cd}	27.64±0.34^{cd}	26.09±0.77^{b}	25.55±0.32^{b}	27.17±0.47^{c}	25.67±0.32^{b}	23.33±0.32^{a}	0.251	<0.001	<0.001	0.002
Hemmicelulose	24.09±0.36^{g}	22.30±0.91^{def}	22.20±1.37^{cde}	22.59±1.12^{defg}	23.21±0.40^{efg}	23.94±0.49^{fg}	21.17±0.53^{cd}	22.91±0.52^{efg}	21.60±1.69^{cde}	18.52±0.61^{a}	20.58±0.73^{bc}	19.46±0.84^{ab}	0.515	<0.001	0.204	0.008
Acid Detergent Fiber	44.37±0.46	43.01±0.78	41.10±0.93	43.17±0.54	41.23±0.36	39.59±0.16	42.47±0.64	39.84±0.57	38.65±0.88	41.73±0.14	38.56±0.25	36.46±0.14	0.325	<0.001	<0.001	0.052
Neutral detergent Fiber	68.47±0.42^{h}	65.32±0.15^{fg}	63.30±0.64^{d}	65.76±0.81^{g}	64.44±0.46^{ef}	63.53±0.48^{de}	63.63±0.56^{de}	62.76±0.55^{d}	60.25±0.81^{c}	60.25±0.61^{c}	59.15±0.59^{b}	55.92±0.88^{a}	0.341	<0.001	<0.001	0.001
Silage fermentation characteristic																
pH value	4.11±0.12	4.12±0.14	3.98±0.17	4.02±0.19	3.90±0.18	3.77±0.17	3.73±0.21	3.70±0.10	3.51±0.16	3.60±0.19	3.59±0.16	3.55±0.13	0.075	<0.001	0.008	0.835
N-NH_3 (mg N/ml)	6.45±1.66	4.89±1.00	5.22±1.36	6.95±0.86	6.76±1.06	4.99±0.79	8.49±1,20	6.93±1.03	5.28±1.47	9.45±1.66	7.84±1.53	7.52±1.22	0.570	<0.001	<0.001	0.365
In vitro rumen digestibility (%)																
Dry Matter Digestibility	38.10±0.45^{a}	38.65±0.44^{ab}	39.56±0.28^{b}	41.82±0.62^{c}	43.90±0.52^{d}	46.15±0.38^{e}	43.67±0.72^{d}	45.82±0.12^{e}	47.34±0.56^{f}	46.95±0.75^{ef}	49.11±0.88^{g}	51.24±0.54^{h}	0.324	<0.001	<0.001	0.001
Organic Matter Digestibility	40.12±0.64^{a}	39.03±0.60^{a}	41.56±0.28^{b}	42.31±0.51^{b}	45.01±0.78^{c}	46.93±0.76^{de}	45.24±0.40^{c}	46.78±0.59^{d}	48.12±0.98^{ef}	48.54±1.06^{f}	50.77±1.09^{g}	53.30±0.37^{h}	0.431	<0.001	<0.001	0.001

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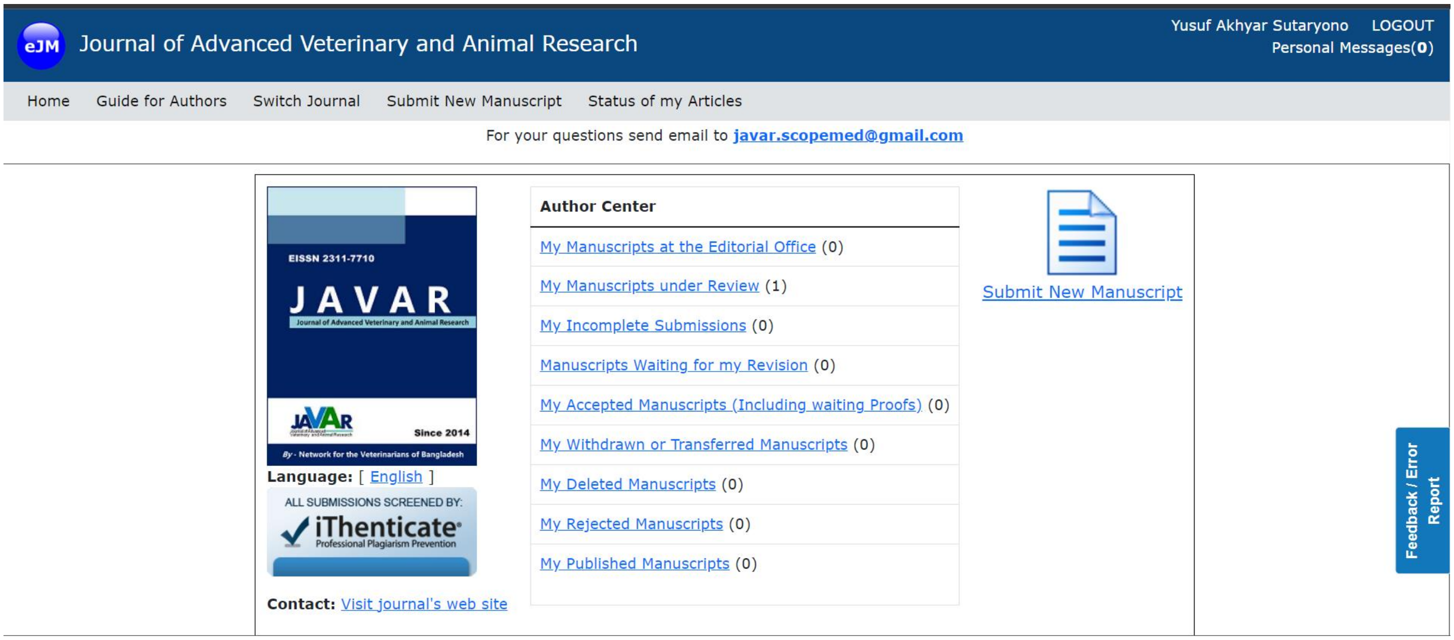

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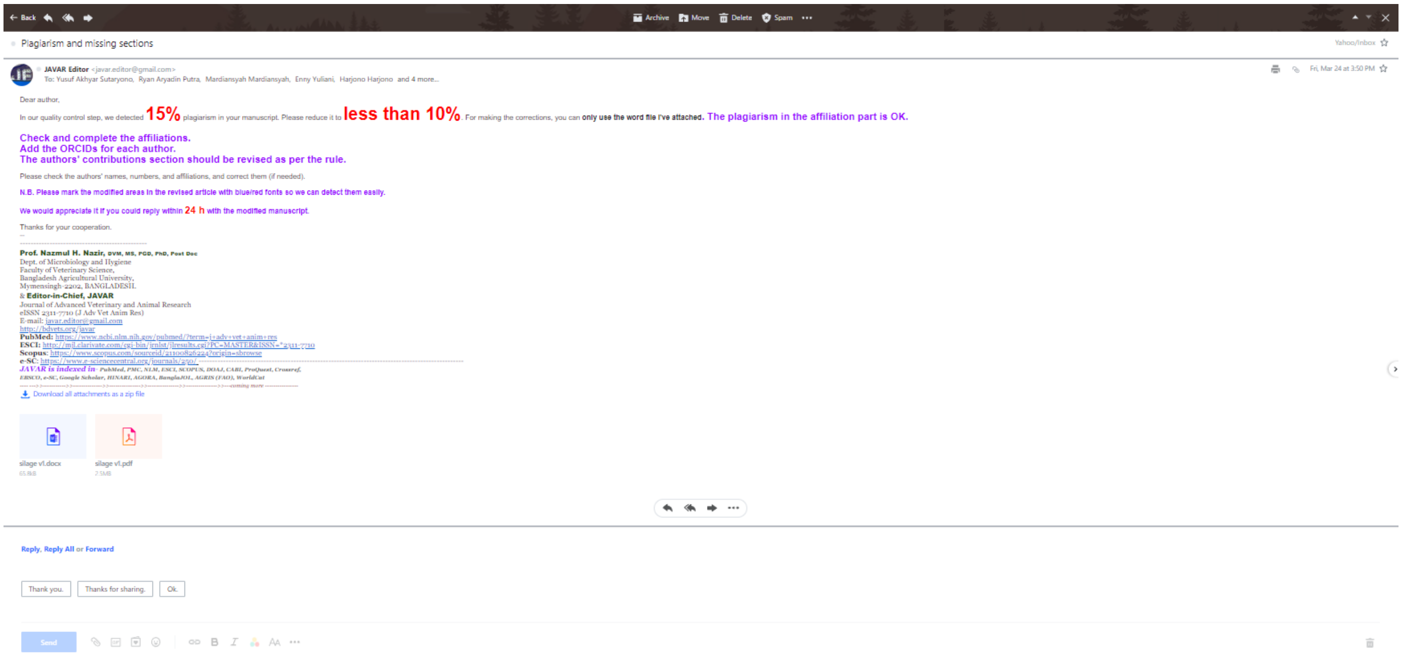
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Letter

Article Title: Mixed leucaena and molasses can increase the nutritional quality and rumen degradation of corn stover silage

Letter Subject: Article Revision Letter for Authors - (JAVAR-2023-01-06)

Letter:
Dear Yusuf Akhyar Sutaryono,

Your manuscript entitled "Mixed leucaena and molasses can increase the nutritional quality and rumen degradation of corn stover silage" (Ms.Nr. JAVAR-2023-01-06) was reviewed by expert reviewers of the Journal of Advanced Veterinary and Animal Research. As an initial decision, your manuscript was found interesting, but some revisions must be made before it can reach a publishable value.
Please answer all the comments below point-by-point in an accompanying response letter to your revised submission.

You should send your revised manuscript via the online system of ScopeMed on my.ejmanager.com.

Sincerely yours,

Nazmul H. Nazir, PhD
Editor-in-Chief
Journal of Advanced Veterinary and Animal Research

COMMENTS for Authors:

=> Reviewer # 1

The purpose of this study was to determine the effect of leucaena at different proportions and doses of molasses on the Nutrient Quality, silage fermentation characteristic, and In vitro Digestibility of corn stover silage.

I have no objection to giving my positive comments on it, as there is a novelty.

=> Reviewer # 2

The authors have described novelty. From this point of view, the article has merit to be accepted.
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File name: silage_v1.docx (65.83K)
Word count: 6015
Character count: 34644

Original Article

Mixed leucaena and molasses can increase the nutritional quality and rumen degradation of corn stover silage

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ABSTRACT

Objective: The study was conducted to determine the effect of leucaena at different proportions and doses of molasses on the nutritional quality, silage fermentation characteristic, and in vitro Digestibility of corn stover silage.
Materials and Methods: The study was designed in a completely randomized factorial design 3*3 pattern. The first factor was the proportion addition of leucaena i.e., L0 (0%), L15 (15%), L30 (30%), and L45 (45%) of inclusion of leucaena on the DM basis of corn stover. The second factor was the dose of inclusion of molasses, i.e., M2 (2%), M4 (4%), and M6 (6%) on the fed basis of silage. Each treatment had five replications. The variables observed included chemical composition (DM, OM, CP, CF, hemicellulose, ADF, and NDF), silage fermentation characteristics (pH and NH_3-N), dry matter digestibility (DMD), and organic matter digestibility (OMD) under in vitro conditions.
Results: The result shows that the inclusion of leucaena in the proportion of 30% to 45% is very effective in increasing and improving the chemical composition of corn stover silage, significantly suppresses the content of crude fiber, and increases the CP content of the silage. Likewise, the inclusion of molasses at a dose of 4% also positively contributed to the quality of the resulting silage, especially its effect in suppressing the buffer capacity of proteins resulting in low pH values and NH_3-N concentrations in silage.
Conclusions: It was concluded that the inclusion of leucaena in 30% to 45% and the inclusion of molasses at a dose of 4% is very effective in increasing and improving the chemical composition, silage fermentability characteristics, and digestibility of corn stover silage.

Keywords: corn stover, silage, leucaena, water-soluble carbohydrate.

INTRODUCTION

Feed availability for cattle in West Nusa Tenggara Province fluctuates between the rainy and dry seasons. Due to the erratic seasonal rainfall in this area, it causes forage throughout the year to fluctuate in quantity and quality **[1],** and those fluctuations than the growth and body weight of Bali cattle raised by farmers in this province. With high feed availability in the rainy season, the body weight growth is very high, but during the dry season, the body weight of cattle will decrease rapidly due to less quantity and quality of feed availability **[2]**.

The high fluctuation of feed availability in this region needs to be addressed with the use of feed that is available in large numbers, easy to be accessed, and cheap. The most widely known in this region is corn. The availability of corn stover in this region is extensive due to the large amount of land planted with corn. Until recently, most of this corn stover was left wasted, returned to the soil, and burned **[3]**. Burning this biomass destroys organic matter potential for cattle feed **[4]**, and causes massive environmental pollution due to the high carbon released into the atmosphere **[5]**.

The use of corn stover as feed has drawbacks; approximately 50–70% of corn stover is composed of cellulose, hemicellulose, and lignin, affecting the utilization efficiency **[6]**. Especially its low protein content, so it needs to be mixed with other high protein feeds, one of which is widely adopted and used as cattle feed in this area is *Leucaena leucocephala cv. Tarramba* **[7,8]**. Adding protein is expected to respond positively **[9]**. However, adding high-protein materials in the silage has its problems. The problem is the low DM content, WSC concentration, and high buffering capacity, mainly when harvesting **[10]**. Hence, it is necessary to consider adding water-soluble carbohydrates to overcome them. One of the most widely available WSC at low prices is molasses. The addition of molasses can provide a fast carbohydrate substrate for lactic acid bacteria in producing lactic acid **[11]**, and silage fermentation efficiency can be achieved **[12]**, and finally can improve livestock performance **[13,14]**. The study aimed to test the effect of several inclusions of leucaena and the doses of molasses in increasing and improving the chemical composition, silage fermentability characteristics, and digestibility of corn stover silage.

MATERIALS AND METHODS

Silage preparation process

The material used in this experiment is corn stover, leucaena, and molasses. Corn stover was collected randomly from corn stover fields in the Central Lombok district, West Nusa Tenggara Province, Indonesia, while molasses was obtained from a molasses trader in Mataram city. Corn stover and leucaena leaves were then chopped to 3-6 cm in size. Before chopping the corn stover, leucaena leaves were let dry under the shade for 6 hours to achieve a water content of approximately 65%. The experiment was conducted on a laboratory scale. Silage was made from mixed corn stover and leucaena in a 5kg mixture, with a leucaena proportion of 0, 15, 30 and 45% of the total mix. Molasses were applied in 2, 4, and 6% doses into the corn stover and leucaena. All materials were mixed well, placed into a plastic container, pressed and vacuumed to reduce oxygen in the silo, and then sealed. Finally, all silos (plastic containers) were placed in a sterile room and left for fermentation for 21 days before being harvested.

Sample analysis procedure

Before the ensiling process, the procedure of **[15]** was applied for calculation the dry matter (DM), organic matter (OM), crude protein (CP), and crude fiber (CF) content. Hemicellulose, neutral detergent fiber (NDF), and acid detergent fiber (ADF) content were analyzed according to the method described by Van Soest et al **[16]** in Table 1. Fermentation characteristics were analyzed based on pH and NH_3-N. Analysis of pH silage followed the procedure **[17]** using a pH meter (Metrohm 691 pH electrode). Analysis of NH_3-N concentration follows the procedure of **[18]** using a spectrophotometer with a reading wavelength of 640 nm. In vitro digestibility was analyzed using the methods developed in **[19]**. In vitro tubes were filled with samples consisting of rumen fluid and artificial saliva solution (McDougal solution) with 1:4 ratios. The CO_2 was provided simultaneously to enable the anaerobic condition in tubes that will be incubated. The incubation was conducted in the water bath at 39-40°C for 48 hours.

Experimental design

The study was designed in a completely randomized factorial design 3*3. The first factor was the proportion addition of leucaena, as follows: L0 (0%), L15 (15%), L30 (30%), and L45 (45%) of inclusion of leucaena on the DM basis of corn stover. The second factor was the dose of inclusion of molasses, as follows: M2 (2%), M4 (4%), and M6 (6%) on the fed basis of silage. Each treatment had five replications. Hence there were 60 experimental units.

Data analysis

The variables observed included chemical composition (DM, OM, CP, CF, hemicellulose, ADF and NDF), silage fermentation characteristics (pH and NH_3-N), dry matter digestibility (DMD), and organic matter digestibility (OMD) under In vitro conditions. All data obtained were then processed with Statistical Product and Service Solutions (SPSS) software version 20 based on the design used. If there are differences between treatments, Duncan New Multiple Range Test (DNMRT) was applied.

RESULTS

Effect on the chemical composition of silage

The results showed that the increase of leucaena proportion substantially affects the value of DM, CP, CF, hemicellulose, ADF, NDF of corn stover silage ($p < 0.05$; Table 2). Specifically, the increase of leucaena increased the CP content and decreased CF and its fractions. The CP content in L0, L15, L30 and L45 were 6.11%, 7.98%, 8.85% and 11.09 respectively ($p < 0.05$).

A similar result is shown by adding a dose of molasses, where increasing the dose significant affected corn stover silage nutrient, except for hemicellulose content. The most potent effect of molasses was indicated by the NDF value increase of 64.54%, 62.92%, and 60.76% for M2, M4, and M6, respectively ($p < 0.05$; Table 2). A significant effect of interaction between leucaena and molasses was also shown by DM, OM, CP, CF, hemicellulose, and NDF ($p < 0.05$).

Effect on silage fermentability quality

The experiment showed no effect of the interaction of leucaena and molasses on the silage fermentation quality. Although the addition of leucaena and molasses significantly affected the pH and NH_3-N of the silage ($p < 0.05$; Table 2). Based on the partially effect, the pH value increased substantially in line with the increase of leucaena proportion in the silage (3.58 to 4.07 on average; $p < 0.05$), but there was a significant decrease when molasses was added (3.87 to 3.70 on average; $p < 0.05$) with the lowest value is in L0 treatment.

The increase of leucaena proportion increased the NH_3-N concentration of silage ($p < 0.05$), the value of NH_3-N concentration caused by the increase of leucaena proportion were 5.2 mg/100 ml (L0), 6.24 mg/100 ml (L15), 6.90 mg/100 ml (L30), and 8.27 mg/100 ml (L45) (Table 2). On the other hand, the NH_3-N concentration decreased with the increase of molasses dose ($p < 0.05$) with pH values of 7.87 mg/100 ml, 6.61 mg/100 ml, and 5.76 mg/100 ml for M2, M4, and M6 respectively.

Effect on dry matter and organic matter digestibility

Dry matter and organic matter digestibility of the silage increased concomitantly with the increase of the proportion of leucaena and molasses dose ($p < 0.05$). Moreover, a significant interaction between leucaena and molasses ($p < 0.05$) affected the DMD and OMD of silage. Dry and organic matter digestibility of silage increased significantly with the leucaena proportion of 45% of total silage (49.10% and 50.87%, respectively). The DMD and OMD values with the addition dose of molasses at 2% were 42.63% and 44.37%, while 4% were 44.06% and 45.40% ($p < 0.05$).

DISCUSSION

Chemical composition of silage

The result showed the significant effect of leucaena and molasses addition on the silage quality ($p < 0.05$). The increase in DM content was the direct effect of leucaena addition on the silage. The increasing DM content obtained in this study is clearly due to increased CP content silage (Table 2). In this regard, mixing several materials with high DM content in silage or feed-making could increase the DM content compared to the DM content used as single feed material. Another researcher also reported a similar result in increased DM content in silage when the proportion of legume (Cowpea) is added **[20]**. The increase in the content of DM silage due to the increased dose of molasses is thought to be caused by the high contribution of single-cell protein from LAB (as indicated by increasing CP silage, Table 2), which may have an overall impact on the content of DM silage when chemical composition analysis is carried out. However, in our study, no investigation was carried out on the population and epiphytic diversity of LAB that grew during the ensiling process. The results study by Rambau et al **[21]** also showed an increase in DM silage due to the combined effect of bio slurry-digester with molasses. Silage with high dry matter content shows that their nutrient contained also increases; for example, this study shows the increase in CP and energy silage. Silage with high protein and energy content is identified as a quality feed that can optimize cattle growth. By increasing silage quality, the amount of silage consumed by cattle will increase as the quality of silage increases and vice versa; when there is a decrease in quality, the amount consumed will also decrease.

Table 2 shows that the OM content decreased slightly in line with an increased dose of molasses ($p < 0.05$), contrary to the effect of molasses dose that increases the DM content of silage. The decrease of OM affected by the increase of molasses dose was presumed caused by using several nutrients by lactic acid bacteria (LAB) into a soluble product during the ensiling process. The rate of LAB population increase during the ensilage process might be very high; hence there was a need for high energy that caused the high use of OM. The increased number of lactic acid bacteria causes their nutritional needs to increase **[22]**. Microorganisms need essential nutrients, especially energy sources, to support cell multiplication **[23]**. In the anaerobic fermentation process, fermented sugar is used in high amounts during intensive fermentation phase at the aerobic respiration period, but when the fermentation process enters the stable phase, the demand for the substrate is reduced **[24]**. There was a significant interaction between leucaena and molasses on DM and OM content of silage. The best interaction was shown at the treatment of 45% leucaena and 6% molasses with DM value of 95.97% (p < 0,05).

In all treatments, increasing CP content was observed. ($p < 0.05$; Table 2). The CP content increased with the increase of leucaena proportion in silage can be explained that the increase in CP content in silage is a direct effect of increasing the proportion of leucaena which is added. As is known, leucaena has a high CP content of 23.47% compared to the CP content of corn stover, which is 5.48% (See Table 1). Thus, in this case, the mixed feed materials have an associative effect where increasing the proportion of leucaena addition leads to linearly increasing the CP content. This result was confirmed by **[25]**, which reported that adding leucaena to 37% in prepared cactus silage produced the highest nitrogen.

An increase in silage protein content also occurs caused by the increased number of additive molasses applied. The higher the molasses concomitant with the higher crude protein content of silage. These results were associated with the LAB growth as a direct amount of molasses source for WSC in the silage. An increased dose of molasses means more energy is available for LAB to grow and proliferate. With high proliferation rate of LAB would contribute to the increase in the crude protein content of silage through the contribution of their single-cell protein. The dead bacteria will also be considered CP when analyzing the crude protein content of silage. The current results contrary with found by Rambau et al. **[21]**, who reported a substantial numerical reduction in CP silage in their study by adding fermentable carbohydrate additives. The highest CP content at 11.89% found in this experiment is enough to fulfill cattle needs for maintaining their life. Furthermore, Putra et al. **[26]** stated that to meet the CP requirement of cattle, and it requires a minimum of 12% crude protein content in its ration.

The low CF content of silage on leucaena proportion of 45% was caused by the increase of leucaena proportion in the corn stover silage. Leucaena has lower CF content than corn stover (20.16 *vs*. 30.56, Table 1); hence, the higher the leucaena added, the lower the crude fiber. The CF content of corn stover-only silage was 30.50%, while the silage with the proportion of leucaena 15%, 30% and 45% was 28.52%, 2[illegible]43% and 25.39%, respectively ($p < 0.05$). This result agreed with Bureenok et al **[27]**, who reported a decrease in the content of the fiber fraction in silage when mixed with legume *Stylosanthes guianensis* compared to silage prepared from guinea grass only. Furthermore, the inclusion leucaena of 30% to the base material of native grasses silage decreased the crude fiber content of the silage **[26]**. Silage with low CF can increase their value by increasing degradation in the rumen and leading to more benefits for consumed cattle.

Moreover, the increase of molasses as silage additive showed a significant effect on the decrease of CF content of corn stover silage (p < 0.05). The decline was caused by the high addition of molasses that caused a high growth rate of LAB; hence more CF could be broken down, which finally decreased the silage CF content. Putra et al. **[28]** stated that the decrease in the crude fiber of silage is caused by the hemicellulose hydrolysis process that takes place during the ensilage process. However, unfortunately, due to limitations in our research, there was no testing on the growth rate of LAB during the ensiling process.

Analysis of variance showed that the interaction of leucaena proportion and dose of molasses significantly affected ($p < 0.05$), but the dose of molasses partially was not significantly affecting the hemicellulose content of corn stover silage. The hemicellulose content values for each treatment can be seen in Table 2. The hemicellulose content decreased with increased leucaena proportion ($p < 0.05$). Compared to other treatment interactions, it was partially caused by a decrease in the contribution of hemicellulose due to the addition of leucaena but also caused by the hydrolysis of hemicellulose during ensiling processes. The hydrolysis of hemicellulose during the ensiling process is intended to make it more soluble, which can then be used as needed to meet fermentation requirements. As Widyastuti et al. **[29]** stated that three possibilities caused the degradation of hemicellulose, i.e., 1) was degraded by the hemicellulose enzyme of the plant itself, 2) degraded by the hemicellulose enzyme bacteria, and 3) was hydrolyzed by organic acids during fermentation processes.

The higher proportion of leucaena in corn stover silage indicated lower ADF and NDF content. A similar effect also showed by molasses; the higher molasses added, the lower the ADF and NDF content of corn stover silage. Their combination also significantly affected NDF content ($p < 0.05$). Although, somehow, the combined effect has no significance on the NDF content of the corn stover silage. Acid detergent fiber and neutral detergent fiber content in this experiment are closely related to the decrease of crude fiber of corn stover silage after ensilage processes.

The decreased content of ADF and NDF obtained in this study can be illustrated by the disruption of the complex's lignin-carbohydrate during ensilage. Microbes degrade the released soluble carbohydrates to meet their needs, as explained in the crude fiber section in this paper. Cellulose, hemicellulose, lignin, and silica are the constituent components of ADF. Dilaga et al **[23]** explained that the low content of the ADF fraction obtained in their study was due to the ability of microbes to separate hemicellulose-lignin linking to making up the cell walls, and part of the hemicellulose was also degraded, causing the low content of the ADF fraction. However, the discussion regarding reducing the CF fraction in silage is still incomplete and needs further study.

Silage fermentability quality

The result showed that the pH of corn stover silage was significantly affected by the addition of leucaena proportion and the dose of molasses ($p < 0.05$), but there was no recorded interaction effect between treatment factors. The increase of leucaena proportion has followed the increase in pH value (3.58 *vs* 3.65 *vs* 3.89 *vs* 4.07; for each treatment, $p < 0.05$). This condition clearly showed the buffer capacity of protein components of silage. Other researchers also showed that there was an increase in the pH value in corn stover silage with a mixture of Common Veth and Alfafa legumes **[30]**. The high buffer capacity of particular feed material will require much more acid as an agent of conversion and *vice* versa. The critical pH for silage is about <4.5. The pH range produced in our study met these criteria.

The low pH of silage can prevent undesired microorganisms competing for the use of fermented sugar, pursuing other fermentation pathways, and producing a variety of metabolic products. The materials that have been ensiled have an almost neutral pH, and the substrate for fermentation is made of raw materials **[24]**, as well as from outside inputs like silage additives.

Due to its low price and constant availability, molasses was considered the most excellent sugar substrate for silage preservation. Adding molasses in silage affected the increase of glycolytic activities, to produce lactic acid as a fermentation product, LAB could use the hydrogen ion's (H^+) availability as an electron acceptor. The low pH value in silage indicated the dynamics of the fermentation during the ensiling process **[31]** one of which can determine the production of lactic acid and may have prevented protein degradation during the ensiling process. However, in our study, no interaction effect was observed between leucaena and molasses on the pH value of corn stover silage.

The good indication of silage preservation during fermentation is indicated by ammonia nitrogen, a component of the non-protein in silage **[32,33]**. The concentration of NH_3-N in the silage was associated with protein degradation caused by plant enzymes or the activity of microbes, particularly microbial enzyme activities. Ammonia nitrogen silage concentration in each treatment of L0, L15, L30, and L45 showed an increase of NH_3-N concentration (5.53, 6.24, 6.90, and 8.27 mg/100 ml ($p < 0.05$). This result is similar to the effect reported by Bureenok et al **[27]**. There was associated with the high buffer capacity of legumes that supported the production of other organic acids except for lactic acid **[34]**. The decrease in pH affects the formation of NH_3-N because there was no hydrolysis of protein which means the lower NH_3-N concentration in the silage. Ammonium concentration of silage was significantly affected by molasses ($p < 0.05$); as described earlier, molasses added to silage possess a vital role as an energy source for epiphytic LAB, which was formed from growth modulation processes. Cazzato et al. **[35]** reported that the inoculation of *L. plantarum* in silage significantly suppressed the NH_3-N formation. Lactic Acid Bacteria, which were formed, provide advantages in decreasing pH, ammonium, and butyric acid production, and increasing lactic acid concentration **[36,37]**.

In vitro dry matter and organic matter digestibility

Digestibility is the number of feed ingredients that can be digested by the digestive tract of livestock in the rumen and then absorbed by livestock in the small intestine. The results of our study showed that the DMD and OMD of silage increased linearly with the increasing portion of the addition of leucaena ($p < 0.05$). This increased digestibility is caused by a decrease in CF due to an increase in the portion of leucaena. The presence of legumes in the feed provides a source of nitrogen for rumen microbes. The available nitrogen source can promote cell multiplication for them with the availability of carbon and ATP. Kariyani et al **[7]** described mixed leucaena and cassava chips with a maximum level inclusion of 47.5% and cassava pulp with maximum level inclusion of 28% achieved high live weight gain in Bali cattle. Leucaena base diet without mixed with maize stover produced digestibility of 60.6%, while combining leucaena with maize stover had a digestibility of 58.8% with a mixture ratio of 75:25 **[38]**. In the context of silage, our research results were confirmed by **[28,39]** also reported that adding 20% to 40% of legume in silage significantly increased the digestibility of silage.

Likewise, the effect of the dose of molasses treatment was an increase in the digestibility value of corn stover silage (p <0.05). Thus, increasing portions of leucaena and molasses in corn straw silage will impact the DMD and OMD of corn stover silage. The high DMD in the feed indicates the quality of this feed. The results of our study also showed a significant interaction of both treatment factors on the increased digestibility of OMD and OMD of corn stover silage (p <0.05). The increased digestibility of corn stover silage in our study was due to the availability of sufficient N sources of protein origin, which was positively correlated with a decrease in crude fiber content as well as the availability of soluble carbohydrates, which supported faster rumen microbial proliferation which in turn improved overall rumen performance. Qu et al. **[40]** suggest that higher CP content and lower fiber content in legumes than in grass may affect digestion.

Overall, the interaction effect between leucaena and molasses addition used in this study needs to be looked at as directly as it might have a significant influence on animal nutrition. Therefore, predicting the subsequent association effect on cattle output is extremely challenging. As a result, the findings of this association effect merit future animal research to establish how these changes in mixed silage impact on cattle production.

CONCLUSION

This study shows that the inclusion of leucaena in 30% to 45% is very effective in increasing and improving the chemical composition of corn stover silage because this proportion significantly suppresses the content of CF and its fractions and increases the CP content of the silage. Likewise, the inclusion of molasses at a dose of 4% also positively contributed to the quality of the resulting silage, especially its effect in suppressing the buffer capacity of proteins resulting in low pH values and NH_3-N concentrations in silage. Overall, there was a synergistic interaction between leucaena and molasses in increasing the chemical composition, silage fermentation quality and improving the rumen digestibility with the best combination obtained at the proportion of leucaena of 45% with a dose of molasses of 4%. Further, an in vivo study should be carried out to investigate the direct effect of increasing the proportion of leucaena and the dose of molasses in corn stover-based silage, especially the effect on overall production performance.

List of abbreviations

Missing this section. Add this section. Check our style carefully.

Acknowledgment

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Conflict of interests

The author has declared that no competing interests during the research and writing of the manuscript

Authors' contributions

All authors developed the theory and supervised the research. Yusuf Akhyar Sutaryono, Ryan Aryadin Putra, Dahlanuddin, Mardiansyah, Enny Yuliani, Harjono, Mastur, and sukarne contributed to the sample collection and analysis calculations. Yusuf Akhyar Sutaryono, Ryan Aryadin Putra, Dahlanuddin, Mardiansyah, and Luh Sri Ernawati contributed to the writing and final version of the manuscript.

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Table 1. Chemical composition of *Leucaena leucocephala cv. Tarramba* and corn stover

Chemical Composition	**Corn stover**	**Leucaena**
Dry matter, % DM	89.92	87.53
Organic matter, %	94.12	91.53
Crude Protein, %	5.48	23.47
Crude Fiber, %	30.56	20.16

Table 2. Nutrient composition, characteristics fermentative and in vitro digestibility of mixture silage corn stover with different inclusion of leucaena and doses of molasses.

Variable	L0			L15			L30			L45			*s.e.m*	*P-value*		
	M2	M4	M6	M2	M4	M6	M2	M4	M6	M2	M4	M6		L	M	L x M
Nutrient composition (%)																
Dry matter	92.50±0.24[a]	92.93±0.77[a]	94.13±0.51[c]	93.94±0.48[bc]	93.17±0.56[ab]	95.74±0.49[d]	95.96±0.45[d]	95.60±0.61[d]	95.11±0.62[d]	95.97±0.65[d]	95.36±0.54[d]	95.96±0.17[d]	0.309	<0.001	0.001	<0.001
Organic matter	91.68±0.65[c]	91.53±0.56[bc]	90.19±1.11[a]	91.48±0.22[bc]	90.61±0.23[ab]	90.88±0.50[abc]	91.72±0.25[c]	91.65±0.40[c]	91.23±0.15[bc]	91.66±0.30[c]	90.96±0.23[abc]	91.72±0.51[c]	0.288	0.093	0.014	0.032
Crude protein	5.21±0.35[a]	6.52±0.17[b]	6.60±0.13[b]	6.94±0.12[c]	8.20±0.34[d]	8.78±0.19[f]	8.31±0.13[de]	8.60±0.16[ef]	9.64±0.29[g]	10.42±0.17[h]	10.95±0.18[i]	11.89±0.16[j]	0.114	<0.001	<0.001	<0.001
Crude fiber	31.49±0.65[g]	30.59±0.17[f]	29.41±0.50[e]	29.75±0.34[e]	28.00±0.35[d]	27.79±0.21[cd]	27.64±0.34[cd]	26.09±0.77[b]	25.55±0.32[b]	27.17±0.47[c]	25.67±0.32[b]	23.33±0.32[a]	0.251	<0.001	<0.001	0.002
Hemmicellulose	24.09±0.36[g]	22.30±0.91[def]	22.20±1.37[cde]	22.59±1.12[defg]	23.21±0.40[efg]	23.94±0.49[fg]	21.17±0.53[cd]	22.91±0.52[efg]	21.60±1.69[cde]	18.52±0.61[a]	20.58±0.73[bc]	19.46±0.84[ab]	0.515	<0.001	0.204	0.008
Acid Detergent Fiber	44.37±0.46	43.01±0.78	41.10±0.93	43.17±0.54	41.23±0.36	39.59±0.16	42.47±0.64	39.84±0.57	38.65±0.88	41.73±0.14	38.56±0.25	36.46±0.14	0.325	<0.001	<0.001	0.052
Neutral detergent Fiber	68.47±0.42[h]	65.32±0.15[fg]	63.30±0.64[d]	65.76±0.81[g]	64.44±0.46[ef]	63.53±0.48[de]	63.63±0.56[de]	62.76±0.55[d]	60.25±0.81[c]	60.25±0.61[c]	59.15±0.59[b]	55.92±0.88[a]	0.341	<0.001	<0.001	0.001
Silage fermentation characteristic																
pH value	4.11±0.12	4.12±0.14	3.98±0.17	4.02±0.19	3.90±0.18	3.77±0.17	3.73±0.21	3.70±0.10	3.51±0.16	3.60±0.19	3.59±0.16	3.55±0.13	0.075	<0.001	0.008	0.835
NH_3-N (mg N/ml)	6.45±1.66	4.89±1.00	5.22±1.36	6.95±0.86	6.76±1.06	4.99±0.79	8.49±1,20	6.93±1.03	5.28±1.47	9.45±1.66	7.84±1.53	7.52±1.22	0.570	<0.001	<0.001	0.365
In vitro rumen digestibility (%)																
Dry Matter Digestibility	38.10±0.45[a]	38.65±0.44[ab]	39.56±0.28[b]	41.82±0.62[c]	43.90±0.52[d]	46.15±0.38[e]	43.67±0.72[d]	45.82±0.12[e]	47.34±0.56[f]	46.95±0.75[ef]	49.11±0.88[g]	51.24±0.54[h]	0.324	<0.001	<0.001	0.001
Organic Matter Digestibility	40.12±0.64[a]	39.03±0.60[a]	41.56±0.28[b]	42.31±0.51[b]	45.01±0.78[c]	46.93±0.76[de]	45.24±0.40[c]	46.78±0.59[d]	48.12±0.98[ef]	48.54±1.06[f]	50.77±1.09[g]	53.30±0.37[h]	0.431	<0.001	<0.001	0.001

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Best regards,

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Thank you for the advice that has been given. We present several statements that we have discussed and followed according to the reviewer's comments:

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2. In the discussion section and several other sections, we have added a more extensive discussion (you can see from the sections highlighted in **blue**) in our manuscript.
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Mixed leucaena and molasses can increase the nutritional quality and rumen degradation of corn stover silage

Manuscript ID :	JAVAR-2023-01-06
Manuscript Type :	Original Article
Submission Date :	06-Jan-2023

Abstract : Objective: The study was conducted to determine the effect of leucaena at different proportion and doses of molasses on the nutritional quality, silage fermentation characteristic, and In vitro digestibility of corn stover silage. Materials and Methods: The study was designed in a completely randomized factorial design 3 3 pattern. The first factor was the proportion addition of leucaena i.e. L0 (0%), L15 (15%), L30 (30%), and L45 (45%) of inclusion of leucaena on the DM basis of corn stover. The second factor was the dose of inclusion of molasses, i.e. M2 (2%), M4 (4%), and M6 (6%) on the fed basis of silage. Each treatment had five replications. Variables: The variables observed included chemical composition (DM, OM, CP, CF, hemicellulose, ADF, and NDF), silage fermentation characteristics (pH and NH3-N), dry matter digestibility (DMD), and organic matter digestibility (OMD) under in vitro conditions. Results: The result shows that the inclusion of leucaena in the proportion of 30% to 45% is very effective in increasing and improving the chemical composition of corn stover silage, significantly suppresses the content of crude fiber, and increases the CP content of the silage. Likewise, the inclusion of molasses at a dose of 4% also positively contributed to the quality of the resulting silage, especially its effect in suppressing the buffer capacity of proteins resulting in low pH values and NH3-N concentrations in silage. Conclusions: It was concluded that the inclusion of leucaena in 30% to 45% and the inclusion of molasses at a dose of 4% is very effective in increasing and improving the chemical composition, silage fermentability characteristics, and digestibility of corn stover silage.

Keywords : corn stover, silage, leucaena, water-soluble carbohydrate.

For your questions please send message to javar.scopemed@gmail.com

ORIGINAL ARTICLE,

Mixed leucaena and molasses can increase the nutritional quality and rumen degradation of corn stover silage

Statement of novelty: Revealed a strong interaction between various additions of leucaena and various doses of molasses. The addition of leucaena up to 45% and molasses at a dose of 4% has significantly improve the quality of corn stover silage compared to the addition of 15% Leucaena at all doses of molasses inclusion.

Mixed leucaena and molasses can increase the nutritional quality and rumen degradation of corn stover silage

ABSTRACT

Objective: The study was conducted to determine the effect of leucaena at different proportions and doses of molasses on the nutritional quality, silage fermentation characteristic, and in vitro Digestibility of corn stover silage. **Materials and Methods**: The study was designed in a completely randomized factorial design 3*3 pattern. The first factor was the proportion addition of leucaena i.e., L0 (0%), L15 (15%), L30 (30%), and L45 (45%) of inclusion of leucaena on the DM basis of corn stover. The second factor was the dose of inclusion of molasses, i.e., M2 (2%), M4 (4%), and M6 (6%) on the fed basis of silage. Each treatment had five replications. **Variables:** The variables observed included chemical composition (DM, OM, CP, CF, hemicellulose, ADF, and NDF), silage fermentation characteristics (pH and NH_3-N), dry matter digestibility (DMD), and organic matter digestibility (OMD) under in vitro conditions. **Results:** The result shows that the inclusion of leucaena in the proportion of 30% to 45% is very effective in increasing and improving the chemical composition of corn stover silage, significantly suppresses the content of crude fiber, and increases the CP content of the silage. Likewise, the inclusion of molasses at a dose of 4% also positively contributed to the quality of the resulting silage, especially its effect in suppressing the buffer capacity of proteins resulting in low pH values and NH_3-N concentrations in silage. **Conclusions:** It was concluded that the inclusion of leucaena in 30% to 45% and the inclusion of molasses at a dose of 4% is very effective in increasing and improving the chemical composition, silage fermentability characteristics, and digestibility of corn stover silage.

Keywords: corn stover, silage, leucaena, water-soluble carbohydrate.

INTRODUCTION

Feed availability for cattle in West Nusa Tenggara Province fluctuates between the rainy and dry seasons. Due to the erratic seasonal rainfall in this area, it causes forage throughout the year to fluctuate in quantity and quality **[1],** and those fluctuations than the growth and body weight of Bali cattle raised by farmers in this province. With high feed availability in the rainy season, the body weight growth is very high, but during the dry season, the body weight of cattle will decrease rapidly due to less quantity and quality of feed availability **[2]**.

The high fluctuation of feed availability in this region needs to be addressed with the use of feed that is available in large numbers, easy to be accessed, and cheap. The most widely known in this region is corn. The availability of corn stover in this region is extensive due to the large amount of land planted with corn. Until recently, most of this corn stover was left wasted, returned to the soil, and burned **[3]**. Burning this biomass destroys organic matter potential for cattle feed **[4]**, and causes massive environmental pollution due to the high carbon released into the atmosphere **[5]**.

The use of corn stover as feed has drawbacks; approximately 50–70% of corn stover is composed of cellulose, hemicellulose, and lignin, affecting the utilization efficiency **[6]**. Especially its low protein content, so it needs to be mixed with other high protein feeds, one of which is widely adopted and used as cattle feed in this area is *Leucaena leucocephala cv. Tarramba* **[7,8]**. Adding protein is expected to respond positively **[9]**. However, adding high-protein materials in the silage has its problems. The problem is the low DM content, WSC concentration, and high buffering capacity, mainly when harvesting **[10]**. Hence, it is necessary to consider adding water-soluble carbohydrates to overcome them. One of the most widely available WSC at low prices is molasses. The addition of molasses can provide a fast carbohydrate substrate for lactic acid bacteria in producing lactic acid **[11]**, and silage fermentation efficiency can be achieved **[12]**, and finally can improve livestock performance **[13,14]**. The study aimed to test the effect of several inclusions of leucaena and the doses of molasses in increasing and improving the chemical composition, silage fermentability characteristics, and digestibility of corn stover silage.

MATERIALS AND METHODS

Silage Preparation Process

The material used in this experiment is corn stover, leucaena, and molasses. Corn stover was collected randomly from corn stover fields in the Central Lombok district, West Nusa Tenggara Province, Indonesia, while molasses was obtained from a molasses trader in Mataram city. Corn stover and leucaena leaves were then chopped to 3-6 cm in size. Before chopping the corn stover, leucaena leaves were let dry under the shade for 6 hours to achieve a water content of approximately 65%. The experiment was conducted on a laboratory scale. Silage was made from mixed corn stover and leucaena in a 5kg mixture, with a leucaena proportion of 0, 15, 30 and 45% of the total mix. Molasses were applied in 2, 4, and 6% doses into the corn stover and leucaena. All materials were mixed well, placed into a plastic container, pressed and vacuumed to reduce oxygen in the silo, and then sealed. Finally, all silos (plastic containers) were placed in a sterile room and left for fermentation for 21 days before being harvested.

Sample analysis procedure

Before the ensiling process, the procedure of **[15]** was applied for calculation the dry matter (DM), organic matter (OM), crude protein (CP), and crude fiber (CF) content. Hemicellulose, neutral detergent fiber (NDF), and acid detergent fiber (ADF) content were analyzed according to the method described by Van Soest et al **[16]** in Table 1. Fermentation characteristics were analyzed based on pH and NH_3-N. Analysis of pH silage followed the procedure **[17]** using a pH meter (Metrohm 691 pH electrode). Analysis of NH_3-N concentration follows the procedure of **[18]** using a spectrophotometer with a reading wavelength of 640 nm. In vitro digestibility was analyzed using the methods developed in **[19]**. In vitro tubes were filled with samples consisting of rumen fluid and artificial saliva solution (McDougal solution) with 1:4 ratios. The CO_2 was provided simultaneously to enable the anaerobic condition in tubes that will be incubated. The incubation was conducted in the water bath at 39-40°C for 48 hours.

Experimental Design

The study was designed in a completely randomized factorial design 3*3. The first factor was the proportion addition of leucaena, as follows: L0 (0%), L15 (15%), L30 (30%), and L45 (45%)

of inclusion of leucaena on the DM basis of corn stover. The second factor was the dose of inclusion of molasses, as follows: M2 (2%), M4 (4%), and M6 (6%) on the fed basis of silage. Each treatment had five replications. Hence there were 60 experimental units.

Data Analysis

The variables observed included chemical composition (DM, OM, CP, CF, hemicellulose, ADF and NDF), silage fermentation characteristics (pH and NH_3-N), dry matter digestibility (DMD), and organic matter digestibility (OMD) under In vitro conditions. All data obtained were then processed with Statistical Product and Service Solutions (SPSS) software version 20 based on the design used. If there are differences between treatments, Duncan New Multiple Range Test (DNMRT) was applied.

RESULTS

Effect on the chemical composition of silage

The results showed that the increase of leucaena proportion substantially affects the value of DM, CP, CF, hemicellulose, ADF, NDF of corn stover silage ($p < 0.05$; Table 2). Specifically, the increase of leucaena increased the CP content and decreased CF and its fractions. The CP content in L0, L15, L30 and L45 were 6.11%, 7.98%, 8.85% and 11.09 respectively ($p < 0.05$).

A similar result is shown by adding a dose of molasses, where increasing the dose significant affected corn stover silage nutrient, except for hemicellulose content. The most potent effect of molasses was indicated by the NDF value increase of 64.54%, 62.92%, and 60.76% for M2, M4, and M6, respectively ($p < 0.05$; Table 2). A significant effect of interaction between leucaena and molasses was also shown by DM, OM, CP, CF, hemicellulose, and NDF ($p < 0.05$).

Effect on silage fermentability quality

The experiment showed no effect of the interaction of leucaena and molasses on the silage fermentation quality. Although the addition of leucaena and molasses significantly affected the pH and NH_3-N of the silage ($p < 0.05$; Table 2). Based on the partially effect, the pH value increased substantially in line with the increase of leucaena proportion in the silage (3.58 to 4.07 on average;

$p < 0.05$), but there was a significant decrease when molasses was added (3.87 to 3.70 on average; $p < 0.05$) with the lowest value is in L0 treatment.

The increase of leucaena proportion increased the NH_3-N concentration of silage ($p < 0.05$), the value of NH_3-N concentration caused by the increase of leucaena proportion were 5.2 mg/100 ml (L0), 6.24 mg/100 ml (L15), 6.90 mg/100 ml (L30), and 8.27 mg/100 ml (L45) (Table 2). On the other hand, the NH_3-N concentration decreased with the increase of molasses dose ($p < 0.05$) with pH values of 7.87 mg/100 ml, 6.61 mg/100 ml, and 5.76 mg/100 ml for M2, M4, and M6 respectively.

Effect on dry matter and organic matter digestibility

Dry matter and organic matter digestibility of the silage increased concomitantly with the increase of the proportion of leucaena and molasses dose ($p < 0.05$). Moreover, a significant interaction between leucaena and molasses (p < 0.05) affected the DMD and OMD of silage. Dry and organic matter digestibility of silage increased significantly with the leucaena proportion of 45% of total silage (49.10% and 50.87%, respectively). The DMD and OMD values with the addition dose of molasses at 2% were 42.63% and 44.37%, while 4% were 44.06% and 45.40% ($p < 0.05$).

DISCUSSION

Chemical Composition of silage

The result showed the significant effect of leucaena and molasses addition on the silage quality ($p < 0.05$). The increase in DM content was the direct effect of leucaena addition on the silage. The increasing DM content obtained in this study is clearly due to increased CP content silage (Table 2). In this regard, mixing several materials with high DM content in silage or feed-making could increase the DM content compared to the DM content used as single feed material. Another researcher also reported a similar result in increased DM content in silage when the proportion of legume (Cowpea) is added **[20]**. The increase in the content of DM silage due to the increased dose of molasses is thought to be caused by the high contribution of single-cell protein from LAB (as indicated by increasing CP silage, Table 2), which may have an overall impact on the content of DM silage when chemical composition analysis is carried out. However, in our study, no investigation was carried out on the population and epiphytic diversity of LAB that grew during the ensiling process. The results study by Rambau et al **[21]** also showed an increase in DM silage due to the combined effect of bio slurry-digester with molasses. Silage with high dry matter content shows that their nutrient contained also increases; for example, this study shows the increase in CP and energy silage. Silage with high protein and energy content is identified as a quality feed that can optimize cattle growth. By increasing silage quality, the amount of silage consumed by cattle will increase as the quality of silage increases and vice versa; when there is a decrease in quality, the amount consumed will also decrease.

Table 2 shows that the OM content decreased slightly in line with an increased dose of molasses ($p < 0.05$), contrary to the effect of molasses dose that increases the DM content of silage. The decrease of OM affected by the increase of molasses dose was presumed caused by using several nutrients by lactic acid bacteria (LAB) into a soluble product during the ensiling process. The rate of LAB population increase during the ensilage process might be very high; hence there was a need for high energy that caused the high use of OM. The increased number of lactic acid bacteria causes their nutritional needs to increase **[22]**. Microorganisms need essential nutrients, especially energy sources, to support cell multiplication **[23]**. In the anaerobic fermentation process, fermented sugar is used in high amounts during intensive fermentation phase at the aerobic respiration period, but when the fermentation process enters the stable phase, the demand for the substrate is reduced **[24]**. There was a significant interaction between leucaena and molasses on DM and OM content of silage. The best interaction was shown at the treatment of 45% leucaena and 6% molasses with DM value of 95.97% ($p < 0,05$).

In all treatments, increasing CP content was observed. ($p < 0.05$; Table 2). The CP content increased with the increase of leucaena proportion in silage can be explained that the increase in CP content in silage is a direct effect of increasing the proportion of leucaena which is added. As is known, leucaena has a high CP content of 23.47% compared to the CP content of corn stover, which is 5.48% (See Table 1). Thus, in this case, the mixed feed materials have an associative effect where increasing the proportion of leucaena addition leads to linearly increasing the CP content. This result was confirmed by **[25]**, which reported that adding leucaena to 37% in prepared cactus silage produced the highest nitrogen.

An increase in silage protein content also occurs caused by the increased number of additive molasses applied. The higher the molasses concomitant with the higher crude protein content of silage. These results were associated with the LAB growth as a direct amount of molasses source for WSC in the silage. An increased dose of molasses means more energy is available for LAB to grow and proliferate. With high proliferation rate of LAB would contribute to the increase in the crude protein content of silage through the contribution of their single-cell protein. The dead bacteria will also be considered CP when analyzing the crude protein content of silage. The current results contrary with found by Rambau et al. **[21]**, who reported a substantial numerical reduction in CP silage in their study by adding fermentable carbohydrate additives. The highest CP content at 11.89% found in this experiment is enough to fulfill cattle needs for maintaining their life. Furthermore, Putra et al. **[26]** stated that to meet the CP requirement of cattle, and it requires a minimum of 12% crude protein content in its ration.

The low CF content of silage on leucaena proportion of 45% was caused by the increase of leucaena proportion in the corn stover silage. Leucaena has lower CF content than corn stover (20.16 *vs.* 30.56, Table 1); hence, the higher the leucaena added, the lower the crude fiber. The CF content of corn stover-only silage was 30.50%, while the silage with the proportion of leucaena 15%, 30% and 45% was 28.52%, 26,43% and 25.39%, respectively ($p < 0.05$). This result agreed with Bureenok et al **[27]**, who reported a decrease in the content of the fiber fraction in silage when mixed with legume *Stylosanthes guianensis* compared to silage prepared from guinea grass only. Furthermore, the inclusion leucaena of 30% to the base material of native grasses silage decreased the crude fiber content of the silage **[26]**. Silage with low CF can increase their value by increasing degradation in the rumen and leading to more benefits for consumed cattle.

Moreover, the increase of molasses as silage additive showed a significant effect on the decrease of CF content of corn stover silage ($p < 0.05$). The decline was caused by the high addition of molasses that caused a high growth rate of LAB; hence more CF could be broken down, which finally decreased the silage CF content. Putra et al. **[28]** stated that the decrease in the crude fiber of silage is caused by the hemicellulose hydrolysis process that takes place during the ensilage process. However, unfortunately, due to limitations in our research, there was no testing on the growth rate of LAB during the ensiling process.

Analysis of variance showed that the interaction of leucaena proportion and dose of molasses significantly affected ($p < 0.05$), but the dose of molasses partially was not significantly affecting the hemicellulose content of corn stover silage. The hemicellulose content values for each treatment can be seen in Table 2. The hemicellulose content decreased with increased leucaena proportion ($p < 0.05$). Compared to other treatment interactions, it was partially caused by a decrease in the contribution of hemicellulose due to the addition of leucaena but also caused by the hydrolysis of hemicellulose during ensiling processes. The hydrolysis of hemicellulose during the ensiling process is intended to make it more soluble, which can then be used as needed to meet fermentation requirements. As Widyastuti et al. **[29]** stated that three possibilities caused the degradation of hemicellulose, i.e., 1) was degraded by the hemicellulose enzyme of the plant itself, 2) degraded by the hemicellulose enzyme bacteria, and 3) was hydrolyzed by organic acids during fermentation processes.

The higher proportion of leucaena in corn stover silage indicated lower ADF and NDF content. A similar effect also showed by molasses; the higher molasses added, the lower the ADF and NDF content of corn stover silage. Their combination also significantly affected NDF content ($p < 0.05$). Although, somehow, the combined effect has no significance on the NDF content of the corn stover silage. Acid detergent fiber and neutral detergent fiber content in this experiment are closely related to the decrease of crude fiber of corn stover silage after ensilage processes.

The decreased content of ADF and NDF obtained in this study can be illustrated by the disruption of the complex's lignin-carbohydrate during ensilage. Microbes degrade the released soluble carbohydrates to meet their needs, as explained in the crude fiber section in this paper. Cellulose, hemicellulose, lignin, and silica are the constituent components of ADF. Dilaga et al **[23]** explained that the low content of the ADF fraction obtained in their study was due to the ability of microbes to separate hemicellulose-lignin linking to making up the cell walls, and part of the hemicellulose was also degraded, causing the low content of the ADF fraction. However, the discussion regarding reducing the CF fraction in silage is still incomplete and needs further study.

Silage fermentability quality

The result showed that the pH of corn stover silage was significantly affected by the addition of leucaena proportion and the dose of molasses ($p < 0.05$), but there was no recorded interaction effect between treatment factors. The increase of leucaena proportion has followed the increase in pH value (3.58 *vs* 3.65 *vs* 3.89 *vs* 4.07; for each treatment, $p < 0.05$). This condition clearly showed the buffer capacity of protein components of silage. Other researchers also showed that there was an increase in the pH value in corn stover silage with a mixture of Common Veth and Alfafa legumes **[30]**. The high buffer capacity of particular feed material will require much more acid as an agent of conversion and *vice* versa. The critical pH for silage is about <4.5. The pH range produced in our study met these criteria. The low pH of silage can prevent undesired microorganisms competing for the use of fermented sugar, pursuing other fermentation pathways, and producing a variety of metabolic products. The materials that have been ensiled have an almost neutral pH, and the substrate for fermentation is made of raw materials **[24]**, as well as from outside inputs like silage additives.

Due to its low price and constant availability, molasses was considered the most excellent sugar substrate for silage preservation. Adding molasses in silage affected the increase of glycolytic activities, to produce lactic acid as a fermentation product, LAB could use the hydrogen ion's (H^+) availability as an electron acceptor. The low pH value in silage indicated the dynamics of the fermentation during the ensiling process **[31]** one of which can determine the production of lactic acid and may have prevented protein degradation during the ensiling process. However, in our study, no interaction effect was observed between leucaena and molasses on the pH value of corn stover silage.

The good indication of silage preservation during fermentation is indicated by ammonia nitrogen, a component of the non-protein in silage **[32,33]**. The concentration of NH_3-N in the silage was associated with protein degradation caused by plant enzymes or the activity of microbes, particularly microbial enzyme activities. Ammonia nitrogen silage concentration in each treatment of L0, L15, L30, and L45 showed an increase of NH_3-N concentration (5.53, 6.24, 6.90, and 8.27 mg/100 ml ($p < 0.05$). This result is similar to the effect reported by Bureenok et al **[27]**. There was associated with the high buffer capacity of legumes that supported the production of other organic acids except for lactic acid **[34]**. The decrease in pH affects the formation of NH_3-N because there was no hydrolysis of protein which means the lower NH_3-N concentration in the silage. Ammonium concentration of silage was significantly affected by molasses ($p < 0.05$); as described earlier, molasses added to silage possess a vital role as an energy source for epiphytic LAB, which was formed from growth modulation processes. Cazzato et al. **[35]** reported that the inoculation of *L. plantarum* in silage significantly suppressed the NH_3-N formation. Lactic Acid Bacteria, which were formed, provide advantages in decreasing pH, ammonium, and butyric acid production, and increasing lactic acid concentration **[36,37]**.

In vitro dry matter and organic matter digestibility

Digestibility is the number of feed ingredients that can be digested by the digestive tract of livestock in the rumen and then absorbed by livestock in the small intestine. The results of our study showed that the DMD and OMD of silage increased linearly with the increasing portion of the addition of leucaena ($p < 0.05$). This increased digestibility is caused by a decrease in CF due to an increase in the portion of leucaena. The presence of legumes in the feed provides a source of nitrogen for rumen microbes. The available nitrogen source can promote cell multiplication for them with the availability of carbon and ATP. Kariyani et al **[7]** described mixed leucaena and cassava chips with a maximum level inclusion of 47.5% and cassava pulp with maximum level inclusion of 28% achieved high live weight gain in Bali cattle. Leucaena base diet without mixed with maize stover produced digestibility of 60.6%, while combining leucaena with maize stover had a digestibility of 58.8% with a mixture ratio of 75:25 **[38]**. In the context of silage, our research results were confirmed by **[28,39]** also reported that adding 20% to 40% of legume in silage significantly increased the digestibility of silage.

Likewise, the effect of the dose of molasses treatment was an increase in the digestibility value of corn stover silage (p <0.05). Thus, increasing portions of leucaena and molasses in corn straw silage will impact the DMD and OMD of corn stover silage. The high DMD in the feed indicates the quality of this feed. The results of our study also showed a significant interaction of both treatment factors on the increased digestibility of OMD and OMD of corn stover silage (p <0.05). The increased digestibility of corn stover silage in our study was due to the availability of sufficient N sources of protein origin, which was positively correlated with a decrease in crude fiber content as well as the availability of soluble carbohydrates, which supported faster rumen microbial proliferation which in turn improved overall rumen performance. Qu et al. **[40]** suggest that higher CP content and lower fiber content in legumes than in grass may affect digestion.

Overall, the interaction effect between leucaena and molasses addition used in this study needs to be looked at as directly as it might have a significant influence on animal nutrition. Therefore, predicting the subsequent association effect on cattle output is extremely challenging. As a result, the findings of this association effect merit future animal research to establish how these changes in mixed silage impact on cattle production.

CONCLUSION

This study shows that the inclusion of leucaena in 30% to 45% is very effective in increasing and improving the chemical composition of corn stover silage because this proportion significantly suppresses the content of CF and its fractions and increases the CP content of the silage. Likewise, the inclusion of molasses at a dose of 4% also positively contributed to the quality of the resulting silage, especially its effect in suppressing the buffer capacity of proteins resulting in low pH values and NH_3-N concentrations in silage. Overall, there was a synergistic interaction between leucaena and molasses in increasing the chemical composition, silage fermentation quality and improving the rumen digestibility with the best combination obtained at the proportion of leucaena of 45% with a dose of molasses of 4%. Further, an in vivo study should be carried out to investigate the direct effect of increasing the proportion of leucaena and the dose of molasses in corn stover-based silage, especially the effect on overall production performance.

CONFLICT OF INTERESTS

The author has declared that no competing interests during the research and writing of the manuscript

AUTHORS CONTRIBUTION

All authors developed the theory and supervised the research. Yusuf Akhyar Sutaryono, Ryan Aryadin Putra, Dahlanuddin, Mardiansyah, Enny Yuliani, Harjono, Mastur, and sukarne contributed to the sample collection and analysis calculations. Yusuf Akhyar Sutaryono, Ryan Aryadin Putra, Dahlanuddin, Mardiansyah, and Luh Sri Ernawati contributed to the writing and final version of the manuscript.

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Table 1. Chemical composition of *Leucaena leucocephala cv. Tarramba* and corn stover

Chemical Composition	Corn stover	Leucaena
Dry matter, % DM	89.92	87.53
Organic matter, %	94.12	91.53
Crude Protein, %	5.48	23.47
Crude Fiber, %	30.56	20.16

Table 2. Nutrient composition, characteristics fermentative and in vitro digestibility of mixture silage corn stover with different inclusion of leucaena and doses of molasses.

Variable	L0			L15			L30			L45			*s.e.m*	*P-value*		
	M2	M4	M6	M2	M4	M6	M2	M4	M6	M2	M4	M6		L	M	L x M
Nutrient composition (%)																
Dry matter	92.50±0.24[a]	92.93±0.77[a]	94.13±0.51[c]	93.94±0.48[bc]	93.17±0.56[ab]	95.74±0.49[d]	95.96±0.45[d]	95.60±0.61[d]	95.11±0.62[d]	95.97±0.65[d]	95.36±0.54[d]	95.96±0.17[d]	0.309	<0.001	0.001	<0.001
Organic matter	91.68±0.65[c]	91.53±0.56[bc]	90.19±1.11[a]	91.48±0.22[bc]	90.61±0.23[ab]	90.88±0.50[abc]	91.72±0.25[c]	91.65±0.40[c]	91.23±0.15[bc]	91.66±0.30[c]	90.96±0.23[abc]	91.72±0.51[c]	0.288	0.093	0.014	0.032
Crude protein	5.21±0.35[a]	6.52±0.17[b]	6.60±0.13[b]	6.94±0.12[c]	8.20±0.34[d]	8.78±0.19[f]	8.31±0.13[de]	8.60±0.16[ef]	9.64±0.29[g]	10.42±0.17[h]	10.95±0.18[i]	11.89±0.16[j]	0.114	<0.001	<0.001	<0.001
Crude fiber	31.49±0.65[g]	30.59±0.17[f]	29.41±0.50[e]	29.75±0.34[e]	28.00±0.35[d]	27.79±0.21[cd]	27.64±0.34[cd]	26.09±0.77[b]	25.55±0.32[b]	27.17±0.47[c]	25.67±0.32[b]	23.33±0.32[a]	0.251	<0.001	<0.001	0.002
Hemmicelulose	24.09±0.36[g]	22.30±0.91[def]	22.20±1.37[cde]	22.59±1.12[defg]	23.21±0.40[efg]	23.94±0.49[fg]	21.17±0.53[cd]	22.91±0.52[efg]	21.60±1.69[cde]	18.52±0.61[a]	20.58±0.73[bc]	19.46±0.84[ab]	0.515	<0.001	0.204	0.008
Acid Detergent Fiber	44.37±0.46	43.01±0.78	41.10±0.93	43.17±0.54	41.23±0.36	39.59±0.16	42.47±0.64	39.84±0.57	38.65±0.88	41.73±0.14	38.56±0.25	36.46±0.14	0.325	<0.001	<0.001	0.052
Neutral detergent Fiber	68.47±0.42[h]	65.32±0.15[fg]	63.30±0.64[d]	65.76±0.81[g]	64.44±0.46[ef]	63.53±0.48[de]	63.63±0.56[de]	62.76±0.55[d]	60.25±0.81[c]	60.25±0.61[c]	59.15±0.59[b]	55.92±0.88[a]	0.341	<0.001	<0.001	0.001
Silage fermentation characteristic																
pH value	4.11±0.12	4.12±0.14	3.98±0.17	4.02±0.19	3.90±0.18	3.77±0.17	3.73±0.21	3.70±0.10	3.51±0.16	3.60±0.19	3.59±0.16	3.55±0.13	0.075	<0.001	0.008	0.835
NH_3-N (mg N/ml)	6.45±1.66	4.89±1.00	5.22±1.36	6.95±0.86	6.76±1.06	4.99±0.79	8.49±1,20	6.93±1.03	5.28±1.47	9.45±1.66	7.84±1.53	7.52±1.22	0.570	<0.001	<0.001	0.365
In vitro rumen digestibility (%)																
Dry Matter Digestibility	38.10±0.45[a]	38.65±0.44[ab]	39.56±0.28[b]	41.82±0.62[c]	43.90±0.52[d]	46.15±0.38[e]	43.67±0.72[d]	45.82±0.12[e]	47.34±0.56[f]	46.95±0.75[ef]	49.11±0.88[g]	51.24±0.54[h]	0.324	<0.001	<0.001	0.001
Organic Matter Digestibility	40.12±0.64[a]	39.03±0.60[a]	41.56±0.28[b]	42.31±0.51[b]	45.01±0.78[c]	46.93±0.76[de]	45.24±0.40[c]	46.78±0.59[d]	48.12±0.98[ef]	48.54±1.06[f]	50.77±1.09[g]	53.30±0.37[h]	0.431	<0.001	<0.001	0.001

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JOURNAL OF ADVANCED VETERINARY AND ANIMAL RESEARCH
ISSN 2311-7710 (Electronic)
http://doi.org/10.5455/javar.2021.h479
A periodical of the Network for the Veterinarians of Bangladesh (BDvetNET)

March 2023
VOL 8, NO. 4, PAGES XX–XX

ORIGINAL ARTICLE

Mixed *Leucaena* and molasses can increase the nutritional quality and rumen degradation of corn stover silage

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ABSTRACT

Objective: The study was conducted to determine the effect of *Leucaena* at different proportions and doses of molasses on the nutritional quality, silage fermentation characteristic, and *in vitro* digestibility of corn stover silage.

Materials and Methods: The study was designed in a completely randomized factorial design 3*3 pattern. The first factor was the proportion addition of *Leucaena*, i.e., L0 (0%), L15 (15%), L30 (30%), and L45 (45%) of inclusion of *Leucaena* on the dry matter (DM) basis of corn stover. The second factor was the dose of inclusion of molasses, i.e., M2 (2%), M4 (4%), and M6 (6%) on the fed basis of silage. Each treatment had five replications. The variables observed included chemical composition [DM, organic matter (OM), crude protein (CP), crude fiber (CF), hemicellulose, acid detergent fiber, and neutral detergent fiber], silage fermentation characteristics (pH and NH_3-N), DM digestibility (DMD), and OM digestibility under *in vitro* conditions.

Results: The result shows that the inclusion of *Leucaena* in the proportion of 30%–45% is very effective in increasing and improving the chemical composition of corn stover silage, significantly suppresses the content of CF, and increases the CP content of the silage. Likewise, the inclusion of molasses at a dose of 4% also positively contributed to the quality of the resulting silage, especially its effect in suppressing the buffer capacity of proteins resulting in low pH values and NH_3-N concentrations in silage.

Conclusions: It was concluded that the inclusion of *Leucaena* in 30%–45% and the inclusion of molasses at a dose of 4% is very effective in increasing and improving the chemical composition, silage fermentability characteristics, and rumen degradation of corn stover silage.

ARTICLE HISTORY

Received January 06, 2023
Revised XXXX
Accepted March 05, 2023
Published XXXX

KEYWORDS

Corn stover; silage; *Leucaena*; water-soluble carbohydrate

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Introduction

Feed availability for cattle in West Nusa Tenggara Province fluctuates between the rainy and dry seasons. Due to the erratic seasonal rainfall in this area, it causes forage throughout the year to fluctuate in quantity and quality [1], and those fluctuations than the growth and body weight of Bali cattle raised by farmers in this province. With high feed availability in the rainy season, the body weight growth is very high, but during the dry season, the body weight of cattle will decrease rapidly due to less quantity and quality of feed availability [2].

The high fluctuation of feed availability in this region needs to be addressed with the use of feed that is available in large numbers, easy to be accessed, and cheap. The most widely known in this region is corn. The availability of corn stover in this region is extensive due to the large amount of land planted with corn. Until recently, most of this corn stover was left wasted, returned to the soil, and burned [3]. Burning this biomass destroys organic matter (OM) potential for cattle feed [4], and causes massive environmental pollution due to the high carbon released into the atmosphere [5].

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How to cite: Sutaryono YA, Putra RA, Mardiansyah M, Yuliani E, Harjono H, Mastur M, et al. Mixed *Leucaena* and molasses can increase the nutritional quality and rumen degradation of corn stover silage. J Adv Vet Anim Res XXXX; XX(XX):XX–XX.

The use of corn stover as feed has drawbacks; approximately 50%–70% of corn stover is composed of cellulose, hemicellulose, and lignin, affecting the utilization efficiency [6]. Especially its low protein content, so it needs to be mixed with other high protein feeds, one of which is widely adopted and used as cattle feed in this area is *Leucaena leucocephala cv. Tarramba* [7,8]. Adding protein is expected to respond positively [9]. However, adding high-protein materials in the silage has its problems. The problem is low dry matter (DM) content, water-soluble carbohydrate (WSC) concentration, and high buffering capacity, mainly when harvesting [10]. Hence, it is necessary to consider adding WSC to overcome them. The most widely available WSC at low prices is molasses. The addition of molasses can provide a fast carbohydrate substrate for lactic acid bacteria (LAB) in producing lactic acid [11], and silage fermentation efficiency can be achieved [12], and finally can improve livestock performance [13,14]. The study aimed to test the effect of several additional levels of *Leucaena* and the doses of molasses in increasing and improving the chemical composition, silage fermentability characteristics, and digestibility of corn stover silage.

Materials and Methods

Silage preparation process

The material used in this experiment is corn stover, *Leucaena*, and molasses. Corn stover was collected randomly from corn stover fields in the Central Lombok district, West Nusa Tenggara Province, Indonesia, while molasses was obtained from a molasses trader in Mataram city. Corn stover and *Leucaena* leaves were then chopped to 3–6 cm in size. Before chopping the corn stover, *Leucaena* leaves were let dry under the shade for 6 h to achieve a water content of approximately 65%. The experiment was conducted on a laboratory scale. Silage was prepared from mixed corn stover and *Leucaena* in a 5 kg mixture, with a *Leucaena* proportion of 0%, 15%, 30%, and 45% of the total mix. Molasses were applied in 2%, 4%, and 6% doses into the corn stover and *Leucaena*. All materials were mixed well, placed into a plastic container, pressed and vacuumed to reduce oxygen in the silo, and then sealed. Finally, all silos (plastic containers) were placed in a sterile room and left for fermentation for 21 days before being harvested.

Sample analysis procedure

Before the ensiling process, the procedure of [15] was applied to calculate the DM, OM, crude protein (CP), and crude fiber (CF) content. The method described by Van Soest et al. [16] has been applied to analyze the content of hemicellulose, neutral detergent fiber (NDF), and acid detergent fiber (ADF). Fermentation characteristics were analyzed based on pH and NH_3-N. Analysis of pH silage followed the procedure [17] using a pH meter (Metrohm 691 with pH electrode). Analysis of NH_3-N concentration follows the procedure of [18] using a spectrophotometer with a reading wavelength of 640 nm. *In vitro* digestibility was analyzed using the methods developed in [19]. *In vitro* tubes were filled with samples consisting of rumen fluid and artificial saliva solution (McDougal solution) with 1:4 ratios. Carbon dioxide gas (CO_2) was provided simultaneously to enable the anaerobic condition in tubes that will be incubated. Incubation was conducted in the water bath at 39°C–40°C for 48 h.

Experimental design

The study was designed in a completely randomized design with 3*3 factorial pattern. The first factor was the proportion addition of *Leucaena*, as follows: L0 (0%), L15 (15%), L30 (30%), and L45 (45%) of inclusion of *Leucaena* on the DM basis of corn stover. The second factor was the dose of inclusion of molasses, as follows: M2 (2%), M4 (4%), and M6 (6%) on the fed basis of silage. Each treatment had five replications. Hence, there were 60 experimental units.

Data analysis

The variables observed included the chemical composition of silage (DM, OM, CP, CF, hemicellulose, ADF, and NDF), silage fermentation characteristics (pH and NH_3-N), DM digestibility (DMD), and organic matter digestibility (OMD) under *In vitro* conditions. All data obtained were then processed with Statistical Product and Service Solutions software version 20 based on the design used. If there are differences between treatments, Duncan New Multiple Range Test was applied.

Results

Effect on the chemical composition of silage

The results showed that the increase of *Leucaena* proportion substantially affects the value of DM, CP, CF, hemicellulose, ADF, and NDF of corn stover silage ($p < 0.05$; Table 2). Specifically, the increase of *Leucaena* increased the CP content and decreased CF and its fractions. The CP content in L0, L15, L30 and L45 were 6.11%, 7.98%, 8.85% and 11.09% respectively ($p < 0.05$).

A similar result is shown by adding a dose of molasses, where increasing the dose significantly affected corn stover silage nutrient, except for hemicellulose content. The most potent effect of molasses was indicated by the NDF value increase of 64.54%, 62.92%, and 60.76% for M2, M4, and M6, respectively ($p < 0.05$; Table 2). A significant effect of interaction between *Leucaena* and molasses was also shown by DM, OM, CP, CF, hemicellulose, and NDF ($p < 0.05$).

Effect on silage fermentability quality

The experiment showed no effect of the interaction of *Leucaena* and molasses on the fermentation quality of silage. Although the addition of *Leucaena* and molasses significantly affected the pH value and NH_3-N concentration of silage ($p < 0.05$; Table 2). Based on the partial effect, the pH value increased substantially in line with the increase of *Leucaena* proportion in the silage (3.58–4.07 on average; $p < 0.05$), but there was a significant decrease when molasses was added (3.87–3.70 on average; $p < 0.05$) with the lowest value is in L0 treatment.

The increase of *Leucaena* proportion increased the NH_3-N concentration of silage ($p < 0.05$), the value of NH_3-N concentration caused by the increase of *Leucaena* proportion was 5.2 (L0), 6.24 (L15), 6.90 (L30), and 8.27 mg/100 ml (L45) (Table 2). On the other hand, the NH_3-N concentration decreased with the increase of molasses dose ($p < 0.05$) with pH values of 7.87, 6.61, and 5.76 mg/100 ml for M2, M4, and M6 respectively.

Effect on DMD and OMD

DMD and OMD of the silage increased concomitantly with the increased proportion of *Leucaena* and molasses dose ($p < 0.05$). Moreover, a significant interaction between *Leucaena* and molasses ($p < 0.05$) affected the DMD and OMD of silage. DMD and OMD of silage increased significantly with the *Leucaena* proportion of 45% of total silage (49.10% and 50.87%, respectively). The DMD and OMD values with the additional dose of molasses at 2% were 42.63% and 44.37%, while 4% were 44.06% and 45.40% ($p < 0.05$).

Discussion

Chemical composition of silage

The result showed the significant effect of *Leucaena* and molasses addition on the silage quality ($p < 0.05$). The increase in DM content was the direct effect of the *Leucaena* addition on the silage. The increasing DM content obtained in this study is clearly due to increased CP content silage (Table 2). In this regard, mixing several materials with high DM content in silage or feed-making could increase the DM content compared to the DM content used as single feed material. Another researcher also reported a similar result in increased DM content in silage when the proportion of legume (Cowpea) is added [20]. The increase in DM silage content due to increased molasses dosage is thought to be due to the high contribution of single-cell protein from LAB (indicated by increased silage CP, Table 2), which may have an overall impact on DM silage content when chemical composition analysis is carried out. However, in our study, no investigation was carried out on the population and epiphytic diversity of LAB that grew during the ensiling process. The results study by Rambau et al. [21] also showed an increase in DM silage due to the combined effect of bio slurry-digester with molasses. Silage with a high DM content shows that the nutrient contained also increases; for example, this study shows an increase in CP and energy silage. Silage with high protein and energy content is identified as a quality feed that can optimize cattle growth. By increasing silage quality, the amount of silage consumed by cattle will increase as the quality of silage increases and vice versa; when there is a decrease in quality, the amount consumed will also decrease.

Table 2 shows that the OM content decreased slightly in line with an increased dose of molasses ($p < 0.05$), contrary to the effect of molasses dose that increases the DM content of the silage. The decrease of OM is affected by the increase of molasses dose possibility caused by using several nutrients by LAB into a soluble product during the ensiling process. The rate of the LAB population increase during the ensilage process might be very high; hence there was a need for high energy that caused the high use of OM. The increased number of LAB causes their nutritional needs to increase [22]. Microorganisms need essential nutrients, especially energy sources, to support cell multiplication [23]. In the anaerobic fermentation process, fermented sugar is used in high amounts during the intensive fermentation phase during the aerobic respiration period, but when the fermentation process enters the stable phase, the demand for the substrate is reduced [24]. The DM and OM content of corn stover silage showed significant differences due to the interaction effect between *Leucaena* and molasses. The best interaction was shown at the treatment of 45% *Leucaena* and 6% molasses with a DM value of 95.97% ($p < 0.05$).

In all treatments, increasing CP content was observed ($p < 0.05$; Table 2). The increase in protein content with increasing *Leucaena* content in silage can be explained by the fact that the increase in CP content in silage is a direct effect of increasing the proportion of *Leucaena*, which is known to have a high CP content of 23.47% compared to the CP content of corn stover, which is 5.48% (Table 1). Thus, in this case, mixed feed materials have an associative effect, where increasing the proportion of *Leucaena* leads to a linear increase in CP content. This result was confirmed by [25], who reported that adding *Leucaena* to

Table 1. Chemical composition of *L. leucocephala cv. Tarramba* and corn stover.

Chemical Composition	Corn stover	*Leucaena*
DM, %	89.92	87.53
OM, %	94.12	91.53
CP, %	5.48	23.47
CF, %	30.56	20.16

Table 2. Nutrient composition, characteristics fermentative and *in vitro* digestibility of mixture silage corn stover with different inclusion of *Leucaena* and doses of molasses.

Variable	L0			L15			L30			L45			SEM	*p*-value		
	M2	M4	M6	M2	M4	M6	M2	M4	M6	M2	M4	M6		L	M	L × M
Nutrient composition (%)																
DM	92.50 ± 0.24[a]	92.93 ± 0.77[a]	94.13 ± 0.51[c]	93.94 ± 0.48[bc]	93.17 ± 0.56[ab]	95.74 ± 0.49[d]	95.96 ± 0.45[d]	95.60 ± 0.61[d]	95.11 ± 0.62[d]	95.97 ± 0.65[d]	95.36 ± 0.54[d]	95.96 ± 0.17[d]	0.309	<0.001	0.001	<0.001
OM	91.68 ± 0.65[c]	91.53 ± 0.56[bc]	90.19 ± 1.11[a]	91.48 ± 0.22[bc]	90.61 ± 0.23[ab]	90.88 ± 0.50[abc]	91.72 ± 0.25[c]	91.65 ± 0.40[c]	91.23 ± 0.15[bc]	91.66 ± 0.30[c]	90.96 ± 0.23[abc]	91.72 ± 0.51[c]	0.288	0.093	0.014	0.032
CP	5.21 ± 0.35[a]	6.52 ± 0.17[b]	6.60 ± 0.13[b]	6.94 ± 0.12[c]	8.20 ± 0.34[d]	8.78 ± 0.19[f]	8.31 ± 0.13[de]	8.60 ± 0.16[ef]	9.64 ± 0.29[g]	10.42 ± 0.17[h]	10.95 ± 0.18[i]	11.89 ± 0.16[j]	0.114	<0.001	<0.001	<0.001
Crude fiber	31.49 ± 0.65[g]	30.59 ± 0.17[f]	29.41 ± 0.50[e]	29.75 ± 0.34[e]	28.00 ± 0.35[d]	27.79 ± 0.21[cd]	27.64 ± 0.34[cd]	26.09 ± 0.77[b]	25.55 ± 0.32[b]	27.17 ± 0.47[c]	25.67 ± 0.32[b]	23.33 ± 0.32[a]	0.251	<0.001	<0.001	0.002
Hemmicelulose	24.09 ± 0.36[g]	22.30 ± 0.91[def]	22.20 ± 1.37[cde]	22.59 ± 1.12[defg]	23.21 ± 0.40[efg]	23.94 ± 0.49[fg]	21.17 ± 0.53[cd]	22.91 ± 0.52[efg]	21.60 ± 1.69[cde]	18.52 ± 0.61[a]	20.58 ± 0.73[bc]	19.46 ± 0.84[ab]	0.515	<0.001	0.204	0.008
ADF	44.37 ± 0.46	43.01 ± 0.78	41.10 ± 0.93	43.17 ± 0.54	41.23 ± 0.36	39.59 ± 0.16	42.47 ± 0.64	39.84 ± 0.57	38.65 ± 0.88	41.73 ± 0.14	38.56 ± 0.25	36.46 ± 0.14	0.325	<0.001	<0.001	0.052
NDF	68.47 ± 0.42[h]	65.32 ± 0.15[fg]	63.30 ± 0.64[d]	65.76 ± 0.81[g]	64.44 ± 0.46[ef]	63.53 ± 0.48[de]	63.63 ± 0.56[de]	62.76 ± 0.55[d]	60.25 ± 0.81[c]	60.25 ± 0.61[c]	59.15 ± 0.59[b]	55.92 ± 0.88[a]	0.341	<0.001	<0.001	0.001
Silage fermentation characteristic																
pH value	4.11 ± 0.12	4.12 ± 0.14	3.98 ± 0.17	4.02 ± 0.19	3.90 ± 0.18	3.77 ± 0.17	3.73 ± 0.21	3.70 ± 0.10	3.51 ± 0.16	3.60 ± 0.19	3.59 ± 0.16	3.55 ± 0.13	0.075	<0.001	0.008	0.835
NH_3-N (mg N/ml)	6.45 ± 1.66	4.89 ± 1.00	5.22 ± 1.36	6.95 ± 0.86	6.76 ± 1.06	4.99 ± 0.79	8.49 ± 1.20	6.93 ± 1.03	5.28 ± 1.47	9.45 ± 1.66	7.84 ± 1.53	7.52 ± 1.22	0.570	<0.001	<0.001	0.365
In vitro rumen digestibility (%)																
DMD	38.10 ± 0.45[a]	38.65 ± 0.44[ab]	39.56 ± 0.28[b]	41.82 ± 0.62[c]	43.90 ± 0.52[d]	46.15 ± 0.38[e]	43.67 ± 0.72[d]	45.82 ± 0.12[e]	47.34 ± 0.56[f]	46.95 ± 0.75[ef]	49.11 ± 0.88[g]	51.24 ± 0.54[h]	0.324	<0.001	<0.001	0.001
OMD	40.12 ± 0.64[a]	39.03 ± 0.60[a]	41.56 ± 0.28[b]	42.31 ± 0.51[b]	45.01 ± 0.78[c]	46.93 ± 0.76[de]	45.24 ± 0.40[c]	46.78 ± 0.59[d]	48.12 ± 0.98[ef]	48.54 ± 1.06[f]	50.77 ± 1.09[g]	53.30 ± 0.37[h]	0.431	<0.001	<0.001	0.001

37% of the prepared cactus silage produced the highest nitrogen.

The increasing protein content of silage also occurs cause of the increased number of additive molasses applied. The higher the molasses, the higher the CP content of the silage. These results were associated with LAB growth as a direct amount of molasses source for WSC in the silage. An increased dose of molasses means more energy is available for LAB to grow and proliferate. The high proliferation rate of LAB would contribute to the increase in the CP content of silage through the contribution of their single-cell protein. The dead bacteria will also be considered CP when analyzing the CP content of the silage. The current results are in contrast to those found by Rambau et al. [21], who reported a substantial numerical reduction in CP silage in their study by adding fermentable carbohydrate additives. The highest CP content at 11.89% found in this experiment is enough to fulfill cattle needs for maintaining their life. Furthermore, Putra et al. [26] stated that to meet the CP requirement of cattle, it requires a minimum of 12% CP content in its ration.

The low CF content of silage with *Leucaena* proportion of 45% is probably by the increase of *Leucaena* proportion in the corn stover silage. *Leucaena* has lower CF content than corn stover (20.16 *vs.* 30.56, Table 1); hence, the higher the *Leucaena* added, the lower the CF. The CF content of corn stover-only silage was 30.50%, while the silage with the proportion of *Leucaena* 15%, 30%, and 45% was 28.52%, 26,43%, and 25.39%, respectively ($p < 0.05$). This result agreed with Bureenok et al. [27], who reported a decrease in the content of the fiber fraction on silage when mixed with legume *Stylosanthes guianensis* compared to silage prepared from Guinea grass only. Furthermore, the inclusion *Leucaena* of 30% to the base material of native grasses silage decreased the CF content of the silage [26]. Silage with low CF can increase their value by increasing degradation in the rumen and leading to more benefits for consumed cattle.

In addition, the increase in molasses as a silage additive significantly decreased the CF content of corn stover silage ($p < 0.05$). The decline trough by the high addition of molasses leads to a high growth rate of LAB; hence more CF could break down, which finally decreased the silage CF content. Putra et al. [28] stated that the decrease in the CF of silage is due to the hemicellulose hydrolysis process that takes place during the ensiling process. However, unfortunately, due to limitations in our research, there was no testing on the growth rate of LAB during the ensiling process.

The current result showed that the interaction of *Leucaena* proportion and a dose of molasses significantly affected ($p < 0.05$), but the dose of molasses partially was not significantly affecting the hemicellulose content of corn stover silage. The hemicellulose content values for each treatment are presented in Table 2. The hemicellulose content decreased with increased *Leucaena* proportion ($p < 0.05$). Compared to other treatment interactions, it was partly owing to a decrease in the contribution of hemicellulose due to the addition of *Leucaena* but also caused by the hydrolysis of hemicellulose during ensiling processes. The hydrolysis of hemicellulose during the ensiling process is intended to make it more soluble, which can then be used as needed to meet fermentation requirements. As Widiyastuti et al. [29], stated that three possibilities caused the degradation of hemicellulose, i.e., 1) was degraded by the hemicellulose enzyme of the plant itself, 2) degraded by the hemicellulose enzyme bacteria, and 3) was hydrolyzed by organic acids during fermentation processes.

The higher proportion of *Leucaena* in corn stover silage indicated lower ADF and NDF content. A similar effect also showed by molasses; the higher molasses added, the lower the ADF and NDF content of corn stover silage. Their combination also significantly affected NDF content ($p < 0.05$). Although, somehow, the combined effect has no significance on the NDF content of the corn stover silage. The ADF and NDF contents in this experiment are closely related to the decrease of CF of corn stover silage after ensilage processes.

The decreased content of ADF and NDF obtained in this study can be illustrated by the disruption of the complex lignin-carbohydrate during ensilage. Microbes degrade the released soluble carbohydrates to meet their needs, as explained in the CF section in this paper. Cellulose, hemicellulose, lignin, and silica are the constituent components of ADF. Dilaga et al. [23] explained that the low content of the ADF fraction obtained in their study was due to the ability of microbes to separate hemicellulose-lignin linking to making up the cell walls, and part of the hemicellulose was also degraded, causing the low content of the ADF fraction. However, the discussion regarding reducing the CF fraction in silage is still incomplete and needs further study.

Silage fermentability quality

The result showed that the pH of corn stover silage was significantly affected by the addition of *Leucaena* proportion and the dose of molasses ($p < 0.05$), but there was no recorded interaction effect between treatment factors. The increase of *Leucaena* proportion has followed the increase in pH value (3.58 *vs.* 3.65 *vs.* 3.89 *vs.* 4.07; for each treatment, $p < 0.05$). This condition clearly showed the buffer capacity of protein components of silage. Other researchers also showed that there was an increase in the pH value in corn stover silage with a mixture of Common Veth and Alfafa legumes [30]. The high buffer capacity of particular feed material will require much more acid as an agent of conversion and vice versa. The critical pH for silage is about <4.5. The pH range produced in our study met these criteria. The low pH of silage can prevent undesired microorganisms from competing for the use of fermented sugar, pursuing other fermentation pathways, and producing a variety of metabolic products. The materials that have been ensiled have an almost neutral pH, and the substrate for fermentation is made of raw materials [24], as well as from outside inputs like silage additives.

Due to its low price and constant availability, molasses was considered the most excellent sugar substrate for silage preservation. Adding molasses in silage affected the increase of glycolytic activities, to produce lactic acid as a fermentation product, LAB could use the hydrogen ions (H^+) availability as an electron acceptor. The low pH value in silage indicated the dynamics of the fermentation during the ensiling process [31] one of which can determine the production of lactic acid and may have prevented protein degradation during the ensiling process. However, in our study, no interaction effect was observed between *Leucaena* and molasses on the pH value of corn stover silage.

The good indication of silage preservation during fermentation is indicated by ammonia nitrogen, a component of the non-protein in silage [32,33]. The concentration of NH_3-N in the silage relates to protein degradation caused by plant enzymes or the activity of microbes, particularly microbial enzyme activities. The Ammonia nitrogen silage concentration in each treatment of L0, L15, L30, and L45 showed an increase in NH_3-N concentration (5.53, 6.24, 6.90, and 8.27 mg/100 ml ($p < 0.05$). This result is similar to the effect reported by Bureenok et al. [27]. There was associated with the high buffer capacity of legumes that supported the production of other organic acids except for lactic acid [34]. The decrease in pH affects the formation of NH_3-N because there was no hydrolysis of protein which means the lower NH_3-N concentration in silage. Ammonium concentration of silage was significantly affected by molasses ($p < 0.05$); as described earlier, molasses added to silage possess a vital role as an energy source for epiphytic LAB, that form from growth modulation processes. Cazzato et al. [35] reported that the inoculation of *L. plantarum* in silage significantly suppressed the NH_3-N formation. LAB, which was formed, provides advantages in decreasing pH, ammonium, and butyric acid production, and increasing lactic acid concentration [36,37].

In vitro DMD and OMD

Digestibility is the number of feed ingredients that can be digested by the digestive tract of livestock in the rumen and then absorbed by livestock in the small intestine. The results of our study showed that the DMD and OMD of silage increased linearly with the increasing portion of the addition of *Leucaena* ($p < 0.05$). This increased digestibility is owing to a decrease in CF due to an increase in the portion of *Leucaena*. The presence of legumes in the feed provides a source of nitrogen for rumen microbes. The available nitrogen source can promote cell multiplication for them with the availability of carbon and ATP. Kariyani et al. [7] described mixed *Leucaena* and cassava chips with a maximum level inclusion of 47.5% and cassava pulp with a maximum level inclusion of 28% achieved high live weight gain in Bali cattle. *Leucaena* base diet without mixed with maize stover produced a digestibility of 60.6% while combining *Leucaena* with maize stover had a digestibility of 58.8% with a mixture ratio of 75:25 [38]. In the context of silage, our research results were confirmed by [28,39] also reported that adding 20%–40% of legume in silage significantly increased the digestibility of silage.

Likewise, the effect of the dose of molasses treatment was an increase in the digestibility value of corn stover silage ($p < 0.05$). Thus, increasing portions of *Leucaena* and molasses in corn straw silage will impact the DMD and OMD of corn stover silage. The high DMD in the feed indicates the quality of this feed. The current results of our study also showed a significant interaction of both treatment factors on the increased digestibility of OMD and OMD of corn stover silage ($p < 0.05$). The increased digestibility of corn stover silage in our study was due to the availability of sufficient N sources of protein origin, which was positively correlated with a decrease in CF content as well as the availability of soluble carbohydrates, which supported a faster rumen microbial proliferation which in turn improved overall rumen performance. Qu et al. [40] suggest that higher CP content and lower fiber content in legumes than in grass may affect digestion.

Overall, the interaction effect between *Leucaena* and molasses addition used in this study needs to be looked at as directly as it might have a significant influence on animal nutrition. Therefore, predicting the subsequent association effect on cattle output is extremely challenging. As a result, the finding of this association effect provides an opportunity for future research to establish how these changes in mixed silage impact cattle production.

Conclusion

This study shows that the inclusion of *Leucaena* in 30%–45% is very effective in increasing and improving the chemical composition of corn stover silage because this proportion significantly suppresses the content of CF and its fractions and increases the CP content of the silage. Likewise, the inclusion of molasses at a dose of 4% also positively contributed to the quality of the resulting silage, especially its effect in suppressing the buffer capacity of proteins resulting in low pH values and NH_3-N concentrations in silage. Overall, there was a synergistic interaction between *Leucaena* and molasses in increasing the chemical composition, silage fermentation quality, and improving the rumen digestibility with the best combination obtained at the proportion of *Leucaena* of 45% with a dose of molasses of 4%. Further, an *in vivo* study should be carried out to investigate the direct effect of increasing the proportion of *Leucaena* and the dose of molasses in corn stover-based silage, especially the effect on overall production performance.

List of abbreviations

L, *Leucaena*; M, Molasses; LAB, lactic acid bacteria; DM, Dry matter; OM, organic matter; CF, Crude fiber; CP, Crude protein; ADF, Acid detergent fiber; NDF, Neutral detergent fiber; WSC, water-soluble carbohydrate; NH3-N, ammonia; DMD, Dry matter digestibility; OMD, Organic matter digestibility.

Acknowledgments

The authors thank the University of Mataram Board of Research and Community Service (LPPM) for fully funding this research through the university PNBP Scheme (Contract Number: 2840/UN18.L1/PP/2021).

Conflict of interest

The author has declared that no competing interests during the research and writing of the manuscript

Authors' contribution

All authors contribute to developing the theory and supervised the research. Yusuf Akhyar Sutaryono, Ryan Aryadin Putra, Dahlanuddin, Mardiansyah, Enny Yuliani, Harjono, Mastur, and Sukarne contributed to the sample collection and analysis calculations. Yusuf Akhyar Sutaryono, Ryan Aryadin Putra, Dahlanuddin, Mardiansyah, and Ni Luh Sri Ernawati contributed to the writing and final version of the manuscript.

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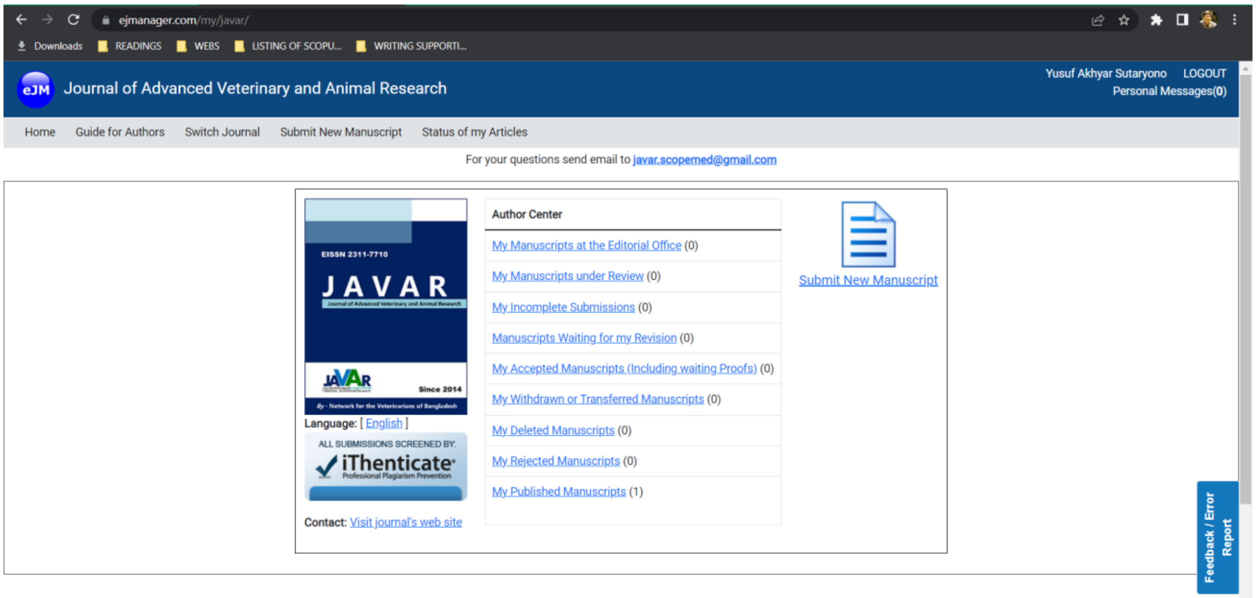

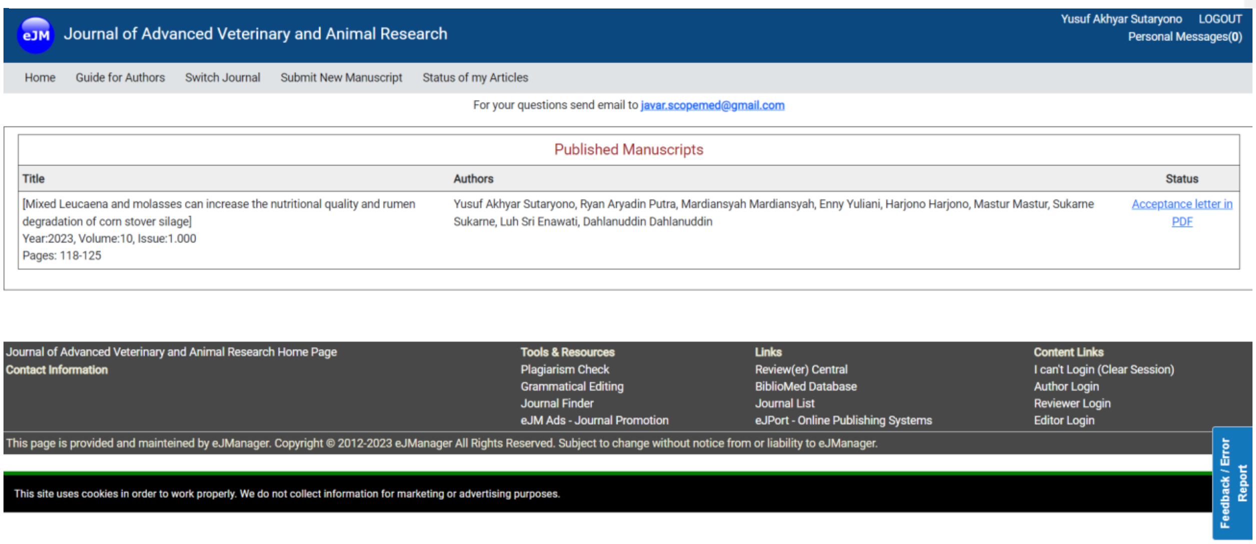

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JOURNAL OF ADVANCED VETERINARY AND ANIMAL RESEARCH
ISSN 2311-7710 (Electronic)
http://doi.org/10.5455/javar.2023.j660
A periodical of the Network for the Veterinarians of Bangladesh (BDvetNET)

March 2023
VOL 10, NO. 1, PAGES 118–125

ORIGINAL ARTICLE

Mixed *Leucaena* and molasses can increase the nutritional quality and rumen degradation of corn stover silage

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ABSTRACT

Objective: The study was conducted to determine the effect of *Leucaena* at different proportions and doses of molasses on the nutritional quality, silage fermentation characteristic, and *in vitro* digestibility of corn stover silage.

Materials and Methods: The study was designed in a completely randomized factorial design 3*3 pattern. The first factor was the proportion addition of *Leucaena*, i.e., L0 (0%), L15 (15%), L30 (30%), and L45 (45%) of inclusion of *Leucaena* on the dry matter (DM) basis of corn stover. The second factor was the dose of inclusion of molasses, i.e., M2 (2%), M4 (4%), and M6 (6%) on the fed basis of silage. Each treatment had five replications. The variables observed included chemical composition [DM, organic matter (OM), crude protein (CP), crude fiber (CF), hemicellulose, acid detergent fiber, and neutral detergent fiber], silage fermentation characteristics (pH and NH_3-N), DM digestibility (DMD), and OM digestibility (OMD) under *in vitro* conditions.

Results: The result shows that the inclusion of *Leucaena* in the proportion of 30%–45% is very effective in increasing and improving the chemical composition of corn stover silage, significantly suppresses the content of CF, and increases the CP content of the silage. Likewise, the inclusion of molasses at a dose of 4% also positively contributed to the quality of the resulting silage, especially its effect in suppressing the buffer capacity of proteins resulting in low pH values and NH_3-N concentrations in silage.

Conclusions: It was concluded that the inclusion of *Leucaena* in 30%–45% and the inclusion of molasses at a dose of 4% is very effective in increasing and improving the chemical composition, silage fermentability characteristics, and rumen degradation of corn stover silage.

ARTICLE HISTORY

Received January 06, 2023
Revised March 05, 2023
Accepted March 05, 2023
Published March 31, 2023

KEYWORDS

Corn stover; silage; *Leucaena*; water-soluble carbohydrate

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Introduction

Feed availability for cattle in West Nusa Tenggara Province fluctuates between the rainy and dry seasons. Due to the erratic seasonal rainfall in this area, it causes forage throughout the year to fluctuate in quantity and quality [1], and those fluctuations of the growth and body weight of Bali cattle raised by farmers in this province. With high feed availability in the rainy season, the body weight growth is very high, but during the dry season, the body weight of cattle will decrease rapidly due to less quantity and quality of feed availability [2].

The high fluctuation of feed availability in this region needs to be addressed with the use of feed that is available in large numbers, easy to be accessed, and cheap. The most widely known in this region is corn. The availability of corn stover in this region is extensive due to the large amount of land planted with corn. Until recently, most of this corn stover was left wasted, returned to the soil, and burned [3]. Burning this biomass destroys organic matter (OM) potential for cattle feed [4], and causes massive environmental pollution due to the high carbon released into the atmosphere [5].

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How to cite: Sutaryono YA, Putra RA, Mardiansyah M, Yuliani E, Harjono H, Mastur M, et al. Mixed *Leucaena* and molasses can increase the nutritional quality and rumen degradation of corn stover silage. J Adv Vet Anim Res 2023; 10(1):118–125.

The use of corn stover as feed has drawbacks; approximately 50%–70% of corn stover is composed of cellulose, hemicellulose, and lignin, affecting the utilization efficiency [6]. Because of its low protein content, so it needs to be mixed with other high protein feeds, one of which is widely adopted and used as cattle feed in this area is *Leucaena leucocephala cv. Tarramba* [7,8]. Adding protein is expected to respond positively [9]. However, adding high-protein materials in the silage has its problems. The problem is low dry matter (DM) content, water-soluble carbohydrate (WSC) concentration, and high buffering capacity, mainly when harvesting [10]. Hence, it is necessary to consider adding WSC to overcome them. The most widely available WSC at low prices is molasses. The addition of molasses can provide a fast carbohydrate substrate for lactic acid bacteria (LAB) in producing lactic acid [11], and silage fermentation efficiency can be achieved [12], and finally can improve livestock performance [13,14]. The study aimed to test the effect of several additional levels of *Leucaena* and the doses of molasses in increasing and improving the chemical composition, silage fermentability characteristics, and digestibility of corn stover silage.

Materials and Methods

Silage preparation process

The material used in this experiment is corn stover, *Leucaena*, and molasses. Corn stover was collected randomly from corn stover fields in the Central Lombok district, West Nusa Tenggara Province, Indonesia, while molasses was obtained from a molasses trader in Mataram city. Corn stover and *Leucaena* leaves were then chopped to 3–6 cm in size. Before chopping the corn stover, *Leucaena* leaves were let dry under the shade for 6 h to achieve a water content of approximately 65%. The experiment was conducted on a laboratory scale. Silage was prepared from mixed corn stover and *Leucaena* in a 5 kg mixture, with a *Leucaena* proportion of 0%, 15%, 30%, and 45% of the total mix. Molasses were applied in 2%, 4%, and 6% doses into the corn stover and *Leucaena*. All materials were mixed well, placed into a plastic container, pressed and vacuumed to reduce oxygen in the silo, and then sealed. Finally, all silos (plastic containers) were placed in a sterile room and left for fermentation for 21 days before being harvested.

Sample analysis procedure

Before the ensiling process, the procedure of [15] was applied to calculate the DM, OM, CP, and CF content. The method described by Van Soest et al. [16] has been applied to analyze the content of hemicellulose, neutral detergent fiber (NDF), and acid detergent fiber (ADF). Fermentation characteristics were analyzed based on pH and NH_3-N. Analysis of pH silage followed the procedure [17] using a pH meter (Metrohm 691 with pH electrode). Analysis of NH_3-N concentration follows the procedure of [18] using a spectrophotometer with a reading wavelength of 640 nm. *In vitro* digestibility was analyzed using the methods developed in [19]. *In vitro* tubes were filled with samples consisting of rumen fluid and artificial saliva solution (McDougal solution) with 1:4 ratios. Carbon dioxide (CO_2) gas was provided simultaneously to enable the anaerobic condition in tubes that will be incubated. Incubation was conducted in the water bath at 39°C–40°C for 48 h.

Experimental design

The study was designed in a completely randomized design with 3*3 factorial pattern. The first factor was the proportion addition of *Leucaena*, as follows: L0 (0%), L15 (15%), L30 (30%), and L45 (45%) of inclusion of *Leucaena* on the DM basis of corn stover. The second factor was the dose of inclusion of molasses, as follows: M2 (2%), M4 (4%), and M6 (6%) on the fed basis of silage. Each treatment had five replications. Hence, there were 60 experimental units.

Data analysis

The variables observed included the chemical composition of silage (DM, OM, CP, CF, hemicellulose, ADF, and NDF), silage fermentation characteristics (pH and NH_3-N), dry matter digestibility (DMD), and organic matter digestibility (OMD) under *In vitro* conditions. All data obtained were then processed with Statistical Product and Service Solutions software version 20 based on the design used. If there are differences between treatments, Duncan's New Multiple Range Test was applied.

Results

Effect on the chemical composition of silage

The results showed that the increase of *Leucaena* proportion substantially affects the value of DM, CP, CF, hemicellulose, ADF, and NDF of corn stover silage ($p < 0.05$; Table 2). Specifically, the increase of *Leucaena* increased the CP content and decreased CF and its fractions. The CP content in L0, L15, L30 and L45 were 6.11%, 7.98%, 8.85% and 11.09% respectively ($p < 0.05$).

A similar result is shown by adding a dose of molasses, where increasing the dose significantly affected corn stover silage nutrient, except for hemicellulose content. The most potent effect of molasses was indicated by the NDF value increase of 64.54%, 62.92%, and 60.76% for M2, M4, and M6, respectively ($p < 0.05$; Table 2). A significant effect of interaction between *Leucaena* and molasses was also shown by DM, OM, CP, CF, hemicellulose, and NDF ($p < 0.05$).

Effect on silage fermentability quality

The experiment showed no effect of the interaction of *Leucaena* and molasses on the fermentation quality of silage. Although the addition of *Leucaena* and molasses significantly affected the pH value and NH_3-N concentration of silage ($p < 0.05$; Table 2). Based on the partial effect, the pH value increased substantially in line with the increase of *Leucaena* proportion in the silage (3.58–4.07 on average; $p < 0.05$), but there was a significant decrease when molasses was added (3.87–3.70 on average; $p < 0.05$) with the lowest value is in L0 treatment.

The increase of *Leucaena* proportion increased the NH_3-N concentration of silage ($p < 0.05$), the value of NH_3-N concentration caused by the increase of *Leucaena* proportion was 5.2 (L0), 6.24 (L15), 6.90 (L30), and 8.27 mg/100 ml (L45) (Table 2). On the other hand, the NH_3-N concentration decreased with the increase of molasses dose ($p < 0.05$) with pH values of 7.87, 6.61, and 5.76 mg/100 ml for M2, M4, and M6 respectively.

Effect on DMD and OMD

DMD and OMD of the silage increased concomitantly with the increased proportion of *Leucaena* and molasses dose ($p < 0.05$). Moreover, a significant interaction between *Leucaena* and molasses ($p < 0.05$) affected the DMD and OMD of silage. DMD and OMD of silage increased significantly with the *Leucaena* proportion of 45% of total silage (49.10% and 50.87%, respectively). The DMD and OMD values with the additional dose of molasses at 2% were 42.63% and 44.37%, while 4% were 44.06% and 45.40% ($p < 0.05$).

Discussion

Chemical composition of silage

The result showed the significant effect of *Leucaena* and molasses addition on the silage quality ($p < 0.05$). The increase in DM content was the direct effect of the *Leucaena* addition on the silage. The increasing DM content obtained in this study is clearly due to increased CP content silage (Table 2). In this regard, mixing several materials with high DM content in silage or feed-making could increase the DM content compared to the DM content used as single feed material. Another researcher also reported a similar result in increased DM content in silage when the proportion of legume (Cowpea) is added [20]. The increase in DM silage content due to increased molasses dosage is thought to be due to the high contribution of single-cell protein from LAB (indicated by increased silage CP, Table 2), which may have an overall impact on DM silage content when chemical composition analysis is carried out. However, in our study, no investigation was carried out on the population and epiphytic diversity of LAB that grew during the ensiling process. The results study by Rambau et al. [21] also showed an increase in DM silage due to the combined effect of bio slurry-digester with molasses. Silage with a high DM content shows that the nutrient contained also increases; for example, this study shows an increase in CP and energy silage. Silage with high protein and energy content is identified as a quality feed that can optimize cattle growth. By increasing silage quality, the amount of silage consumed by cattle will increase as the quality of silage increases and vice versa; when there is a decrease in quality, the amount consumed will also decrease.

Table 2 shows that the OM content decreased slightly in line with an increased dose of molasses ($p < 0.05$), contrary to the effect of molasses dose that increases the DM content of the silage. The decrease of OM is affected by the increase of molasses dose possibility caused by using several nutrients by LAB into a soluble product during the ensiling process. The rate of the LAB population increase during the ensilage process might be very high; hence there was a need for high energy that caused the high use of OM. The increased number of LAB causes their nutritional needs to increase [22]. Microorganisms need essential nutrients, especially energy sources, to support cell multiplication [23]. In the anaerobic fermentation process, fermented sugar is used in high amounts during the intensive fermentation phase during the aerobic respiration period, but when the fermentation process enters the stable phase, the demand for the substrate is reduced [24]. The DM and OM content of corn stover silage showed significant differences due to the interaction effect between *Leucaena* and molasses. The best interaction was shown at the treatment of 45% *Leucaena* and 6% molasses with a DM value of 95.97% ($p < 0.05$).

In all treatments, increasing CP content was observed ($p < 0.05$; Table 2). The increase in protein content with increasing *Leucaena* content in silage can be explained by the fact that the increase in CP content in silage is a direct effect of increasing the proportion of *Leucaena*, which is known to have a high CP content of 23.47% compared to the CP content of corn stover, which is 5.48% (Table 1). Thus, in this case, mixed feed materials have an associative effect, where increasing the proportion of *Leucaena* leads to a linear increase in CP content. This result was confirmed by [25], who reported that adding *Leucaena* to

Table 1. Chemical composition of *L. leucocephala cv. Tarramba* and corn stover.

Chemical Composition	Corn stover	*Leucaena*
DM, %	89.92	87.53
OM, %	94.12	91.53
CP, %	5.48	23.47
CF, %	30.56	20.16

Table 2. Nutrient composition, characteristics fermentative and *in vitro* digestibility of mixture silage corn stover with different inclusion of *Leucaena* and doses of molasses.

Variable	L0			L15			L30			L45			SEM	*p*-value		
	M2	M4	M6	M2	M4	M6	M2	M4	M6	M2	M4	M6		L	M	L × M
Nutrient composition (%)																
DM	92.50 ± 0.24^{a}	92.93 ± 0.77^{a}	94.13 ± 0.51^{c}	93.94 ± 0.48^{bc}	93.17 ± 0.56^{ab}	95.74 ± 0.49^{d}	95.96 ± 0.45^{d}	95.60 ± 0.61^{d}	95.11 ± 0.62^{d}	95.97 ± 0.65^{d}	95.36 ± 0.54^{d}	95.96 ± 0.17^{d}	0.309	<0.001	0.001	<0.001
OM	91.68 ± 0.65^{c}	91.53 ± 0.56^{bc}	90.19 ± 1.11^{a}	91.48 ± 0.22^{bc}	90.61 ± 0.23^{ab}	90.88 ± 0.50^{abc}	91.72 ± 0.25^{c}	91.65 ± 0.40^{c}	91.23 ± 0.15^{bc}	91.66 ± 0.30^{c}	90.96 ± 0.23^{abc}	91.72 ± 0.51^{c}	0.288	0.093	0.014	0.032
CP	5.21 ± 0.35^{a}	6.52 ± 0.17^{b}	6.60 ± 0.13^{b}	6.94 ± 0.12^{c}	8.20 ± 0.34^{d}	8.78 ± 0.19^{f}	8.31 ± 0.13^{de}	8.60 ± 0.16^{ef}	9.64 ± 0.29^{g}	10.42 ± 0.17^{h}	10.95 ± 0.18^{i}	11.89 ± 0.16^{j}	0.114	<0.001	<0.001	<0.001
CF	31.49 ± 0.65^{g}	30.59 ± 0.17^{f}	29.41 ± 0.50^{e}	29.75 ± 0.34^{e}	28.00 ± 0.35^{d}	27.79 ± 0.21^{cd}	27.64 ± 0.34^{cd}	26.09 ± 0.77^{b}	25.55 ± 0.32^{b}	27.17 ± 0.47^{c}	25.67 ± 0.32^{b}	23.33 ± 0.32^{a}	0.251	<0.001	<0.001	0.002
Hemmicelulose	24.09 ± 0.36^{g}	22.30 ± 0.91^{def}	22.20 ± 1.37^{cde}	22.59 ± 1.12^{defg}	23.21 ± 0.40^{efg}	23.94 ± 0.49^{fg}	21.17 ± 0.53^{cd}	22.91 ± 0.52^{efg}	21.60 ± 1.69^{cde}	18.52 ± 0.61^{a}	20.58 ± 0.73^{bc}	19.46 ± 0.84^{ab}	0.515	<0.001	0.204	0.008
ADF	44.37 ± 0.46	43.01 ± 0.78	41.10 ± 0.93	43.17 ± 0.54	41.23 ± 0.36	39.59 ± 0.16	42.47 ± 0.64	39.84 ± 0.57	38.65 ± 0.88	41.73 ± 0.14	38.56 ± 0.25	36.46 ± 0.14	0.325	<0.001	<0.001	0.052
NDF	68.47 ± 0.42^{h}	65.32 ± 0.15^{fg}	63.30 ± 0.64^{d}	65.76 ± 0.81^{g}	64.44 ± 0.46^{ef}	63.53 ± 0.48^{de}	63.63 ± 0.56^{de}	62.76 ± 0.55^{d}	60.25 ± 0.81^{c}	60.25 ± 0.61^{c}	59.15 ± 0.59^{b}	55.92 ± 0.88^{a}	0.341	<0.001	<0.001	0.001
Silage fermentation characteristic																
pH value	4.11 ± 0.12	4.12 ± 0.14	3.98 ± 0.17	4.02 ± 0.19	3.90 ± 0.18	3.77 ± 0.17	3.73 ± 0.21	3.70 ± 0.10	3.51 ± 0.16	3.60 ± 0.19	3.59 ± 0.16	3.55 ± 0.13	0.075	<0.001	0.008	0.835
NH_3-N (mg N/ml)	6.45 ± 1.66	4.89 ± 1.00	5.22 ± 1.36	6.95 ± 0.86	6.76 ± 1.06	4.99 ± 0.79	8.49 ± 1.20	6.93 ± 1.03	5.28 ± 1.47	9.45 ± 1.66	7.84 ± 1.53	7.52 ± 1.22	0.570	<0.001	<0.001	0.365
In vitro rumen digestibility (%)																
DMD	38.10 ± 0.45^{a}	38.65 ± 0.44^{ab}	39.56 ± 0.28^{b}	41.82 ± 0.62^{c}	43.90 ± 0.52^{d}	46.15 ± 0.38^{e}	43.67 ± 0.72^{d}	45.82 ± 0.12^{e}	47.34 ± 0.56^{f}	46.95 ± 0.75^{ef}	49.11 ± 0.88^{g}	51.24 ± 0.54^{h}	0.324	<0.001	<0.001	0.001
OMD	40.12 ± 0.64^{a}	39.03 ± 0.60^{a}	41.56 ± 0.28^{b}	42.31 ± 0.51^{b}	45.01 ± 0.78^{c}	46.93 ± 0.76^{de}	45.24 ± 0.40^{c}	46.78 ± 0.59^{d}	48.12 ± 0.98^{ef}	48.54 ± 1.06^{f}	50.77 ± 1.09^{g}	53.30 ± 0.37^{h}	0.431	<0.001	<0.001	0.001

37% of the prepared cactus silage produced the highest nitrogen.

The increasing protein content of silage also occurs caused by the increased number of additive molasses applied. The higher the molasses, the higher the CP content of the silage. These results were associated with LAB growth as a direct amount of molasses source for WSC in the silage. An increased dose of molasses means more energy is available for LAB to grow and proliferate. The high proliferation rate of LAB would contribute to the increase in the CP content of silage through the contribution of their single-cell protein. The dead bacteria will also be considered CP when analyzing the CP content of the silage. The current results are in contrast to those found by Rambau et al. [21], who reported a substantial numerical reduction in CP silage in their study by adding fermentable carbohydrate additives. The highest CP content at 11.89% found in this experiment is enough to fulfill cattle needs for maintaining their life. Furthermore, Putra et al. [26] stated that to meet the CP requirement of cattle, it requires a minimum of 12% CP content in its ration.

The low CF content of silage with *Leucaena* proportion of 45% is probably by the increase of *Leucaena* proportion in the corn stover silage. *Leucaena* has lower CF content than corn stover (20.16 *vs.* 30.56, Table 1); hence, the higher the *Leucaena* added, the lower the CF. The CF content of corn stover-only silage was 30.50%, while the silage with the proportion of *Leucaena* 15%, 30%, and 45% was 28.52%, 26,43%, and 25.39%, respectively ($p < 0.05$). This result agreed with Bureenok et al. [27], who reported a decrease in the content of the fiber fraction on silage when mixed with legume *Stylosanthes guianensis* compared to silage prepared from Guinea grass only. Furthermore, the inclusion *Leucaena* of 30% to the base material of native grasses silage decreased the CF content of the silage [26]. Silage with low CF can increase their value by increasing degradation in the rumen and leading to more benefits for consumed cattle.

In addition, the increase in molasses as a silage additive significantly decreased the CF content of corn stover silage ($p < 0.05$). The decline trough by the high addition of molasses leads to a high growth rate of LAB; hence more CF could break down, which finally decreased the silage CF content. Putra et al. [28] stated that the decrease in the CF of silage is due to the hemicellulose hydrolysis process that takes place during the ensiling process. However, unfortunately, due to limitations in our research, there was no testing on the growth rate of LAB during the ensiling process.

The current result showed that the interaction of *Leucaena* proportion and a dose of molasses significantly affected ($p < 0.05$), but the dose of molasses partially was not significantly affecting the hemicellulose content of corn stover silage. The hemicellulose content values for each treatment are presented in Table 2. The hemicellulose content decreased with increased *Leucaena* proportion ($p < 0.05$). Compared to other treatment interactions, it was partly owing to a decrease in the contribution of hemicellulose due to the addition of *Leucaena* but also caused by the hydrolysis of hemicellulose during ensiling processes. The hydrolysis of hemicellulose during the ensiling process is intended to make it more soluble, which can then be used as needed to meet fermentation requirements. As Widiyastuti et al. [29], stated that three possibilities caused the degradation of hemicellulose, i.e., 1) degraded by the hemicellulose enzyme of the plant itself, 2) degraded by the hemicellulose enzyme bacteria, and 3) hydrolyzed by organic acids during fermentation processes.

The higher proportion of *Leucaena* in corn stover silage indicated lower ADF and NDF content. A similar effect also showed by molasses; the higher molasses added, the lower the ADF and NDF content of corn stover silage. Their combination also significantly affected NDF content ($p < 0.05$). Although, somehow, the combined effect has no significance on the NDF content of the corn stover silage. The ADF and NDF contents in this experiment are closely related to the decrease of CF of corn stover silage after ensilage processes.

The decreased content of ADF and NDF obtained in this study can be illustrated by the disruption of the complex lignin-carbohydrate during ensilage. Microbes degrade the released soluble carbohydrates to meet their needs, as explained in the CF section in this paper. Cellulose, hemicellulose, lignin, and silica are the constituent components of ADF. Dilaga et al. [23] explained that the low content of the ADF fraction obtained in their study was due to the ability of microbes to separate hemicellulose-lignin linking to making up the cell walls, and part of the hemicellulose was also degraded, causing the low content of the ADF fraction. However, the discussion regarding reducing the CF fraction in silage is still incomplete and needs further study.

Silage fermentability quality

The result showed that the pH of corn stover silage was significantly affected by the addition of *Leucaena* proportion and the dose of molasses ($p < 0.05$), but there was no recorded interaction effect between treatment factors. The increase of *Leucaena* proportion has followed the increase in pH value (3.58 *vs.* 3.65 *vs.* 3.89 *vs.* 4.07; for each treatment, $p < 0.05$). This condition clearly showed the buffer capacity of protein components of silage. Other researchers also showed that there was an increase in the pH value in corn stover silage with a mixture of Common Veth and Alfafa legumes [30]. The high buffer capacity of particular feed material will require much more acid as an agent of conversion and vice versa. The critical pH for silage is about <4.5. The pH range produced in our study met these criteria. The low pH of silage can prevent undesired microorganisms from competing for the use of fermented sugar, pursuing other fermentation pathways, and producing a variety of metabolic products. The materials that have been ensiled have an almost neutral pH, and the substrate for fermentation is made of raw materials [24], as well as from outside inputs like silage additives.

Due to its low price and constant availability, molasses was considered the most excellent sugar substrate for silage preservation. Adding molasses in silage affected the increase of glycolytic activities, to produce lactic acid as a fermentation product, LAB could use the hydrogen ions (H^+) availability as an electron acceptor. The low pH value in silage indicated the dynamics of the fermentation during the ensiling process [31] one of which can determine the production of lactic acid and may have prevented protein degradation during the ensiling process. However, in our study, no interaction effect was observed between *Leucaena* and molasses on the pH value of corn stover silage.

The good indication of silage preservation during fermentation is indicated by ammonia nitrogen, a component of the non-protein in silage [32,33]. The concentration of NH_3-N in the silage relates to protein degradation caused by plant enzymes or the activity of microbes, particularly microbial enzyme activities. The Ammonia nitrogen silage concentration in each treatment of L0, L15, L30, and L45 showed an increase in NH_3-N concentration (5.53, 6.24, 6.90, and 8.27 mg/100 ml ($p < 0.05$). This result is similar to the effect reported by Bureenok et al. [27]. There was associated with the high buffer capacity of legumes that supported the production of other organic acids except for lactic acid [34]. The decrease in pH affects the formation of NH_3-N because there was no hydrolysis of protein which means the lower NH_3-N concentration in silage. Ammonium concentration of silage was significantly affected by molasses ($p < 0.05$); as described earlier, molasses added to silage possess a vital role as an energy source for epiphytic LAB, that form from growth modulation processes. Cazzato et al. [35] reported that the inoculation of *L. plantarum* in silage significantly suppressed the NH_3-N formation. LAB, which was formed, provides advantages in decreasing pH, ammonium, and butyric acid production, and increasing lactic acid concentration [36,37].

In vitro DMD and OMD

Digestibility is the number of feed ingredients that can be digested by the digestive tract of livestock in the rumen and then absorbed by livestock in the small intestine. The results of our study showed that the DMD and OMD of silage increased linearly with the increasing portion of the addition of *Leucaena* ($p < 0.05$). This increased digestibility is owing to a decrease in CF due to an increase in the portion of *Leucaena*. The presence of legumes in the feed provides a source of nitrogen for rumen microbes. The available nitrogen source can promote cell multiplication for them with the availability of carbon and ATP. Kariyani et al. [7] described mixed *Leucaena* and cassava chips with a maximum level inclusion of 47.5% and cassava pulp with a maximum level inclusion of 28% achieved high live weight gain in Bali cattle. *Leucaena* base diet without mixed with maize stover produced a digestibility of 60.6% while combining *Leucaena* with maize stover had a digestibility of 58.8% with a mixture ratio of 75:25 [38]. In the context of silage, our research results were confirmed by [28,39] also reported that adding 20%–40% of legume in silage significantly increased the digestibility of silage.

Likewise, the effect of the dose of molasses treatment was an increase in the digestibility value of corn stover silage ($p < 0.05$). Thus, increasing portions of *Leucaena* and molasses in corn straw silage will impact the DMD and OMD of corn stover silage. The high DMD in the feed indicates the quality of this feed. The current results of our study also showed a significant interaction of both treatment factors on the increased digestibility of OMD and OMD of corn stover silage ($p < 0.05$). The increased digestibility of corn stover silage in our study was due to the availability of sufficient N sources of protein origin, which was positively correlated with a decrease in CF content as well as the availability of soluble carbohydrates, which supported a faster rumen microbial proliferation which in turn improved overall rumen performance. Qu et al. [40] suggest that higher protein content and lower fiber content in legumes than in grass may affect digestion.

Overall, the interaction effect between *Leucaena* and molasses addition used in this study needs to be looked at as directly as it might have a significant influence on animal nutrition. Therefore, predicting the subsequent association effect on cattle output is extremely challenging. As a result, the finding of this association effect provides an opportunity for future research to establish how these changes in mixed silage impact cattle production.

Conclusion

This study shows that the inclusion of *Leucaena* in 30%–45% is very effective in increasing and improving the chemical composition of corn stover silage because this proportion significantly suppresses the content of CF and its fractions and increases the CP content of the silage. Likewise, the inclusion of molasses at a dose of 4% also positively contributed to the quality of the resulting silage, especially its effect in suppressing the buffer capacity of proteins resulting in low pH values and NH_3-N concentrations in silage. Overall, there was a synergistic interaction between *Leucaena* and molasses in increasing the chemical composition, silage fermentation quality, and improving the rumen digestibility with the best combination obtained at the proportion of *Leucaena* of 45% with a dose of molasses of 4%. Further, an *in vivo* study should be carried out to investigate the direct effect of increasing the proportion of *Leucaena* and the dose of molasses in corn stover-based silage, especially the effect on overall production performance.

List of abbreviations

L, *Leucaena*; M, Molasses; LAB, Lactic acid bacteria; DM, Dry matter; OM, Organic matter; CF, Crude fiber; CP, Crude protein; ADF, Acid detergent fiber; NDF, Neutral detergent fiber; WSC, Water-soluble carbohydrate; NH3-N, Ammonia; DMD, Dry matter digestibility; OMD, Organic matter digestibility.

Acknowledgments

The authors thank the University of Mataram Board of Research and Community Service (LPPM) for fully funding this research through the university PNBP Scheme (Contract Number: 2840/UN18.L1/PP/2021).

Conflict of interest

The author has declared that no competing interests during the research and writing of the manuscript

Authors' contribution

All authors contribute to developing the theory and supervised the research. Yusuf Akhyar Sutaryono, Ryan Aryadin Putra, Dahlanuddin, Mardiansyah, Enny Yuliani, Harjono, Mastur, and Sukarne contributed to the sample collection and analysis calculations. Yusuf Akhyar Sutaryono, Ryan Aryadin Putra, Dahlanuddin, Mardiansyah, and Luh Sri Ernawati contributed to the writing and final version of the manuscript.

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