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Adding corn meal into mixed elephant grass–butterfly pea legume silages improves nutritive value and dry matter recovery

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Abstract

The objective of this study was to describe and explain the effect of adding corn meal (CM) on losses, fermentation characteristics and nutritional value of silages from two elephant grass [*Cenchrus purpureus* (Schumach.) Morrone] genotypes (Taiwan A-146 2.37 and IRI-381) mixed with butterfly pea (*Clitoria ternatea* L.) legume. The forage was harvested at 75 days of regrowth from elephant grass plots intercropped with butterfly pea legume and ensiled with or without CM at 5% of dry matter (DM) content. Greater gas losses (12 g/kg) and pH (4.2) were observed in the Taiwan A-146 2.37 + butterfly pea silages. The greatest crude protein content was observed in the 'Taiwan A-146 2.37' + butterfly pea silage added with CM (116 g/kg). Silages with additive and those containing IRI-381 had a greater acid detergent fibre content (367 and 366 g/kg, respectively). CM increased the silage DM (221 g/kg), remaining water-soluble carbohydrates contents (26 g/kg) and *in vitro* digestibility of DM. The aerobic stability was maintained until 45 h after opening the silos. All silages presented a good fermentative profile and were not affected by the relatively large proportion of butterfly pea (>34%) in the ensiled mass as indicated by the reduced contents of butyric acid and ammonia nitrogen. CM reduces total losses, increases DM recovery and improves the nutritional value of silages from mixed elephant grass–butterfly pea legume.

Introduction

Irregular rainfall distribution and unfavourable conditions for forage production require alternatives to support animal production (Furtado *et al*., 2019; Lemos *et al*., 2021). Ensiling is one of the most used forage conservation methods (Santos *et al*., 2018*a*) adopted by many producers worldwide, and is considered key to intensifying ruminant production in the tropics (Santos *et al*., 2020).

Elephant grass [*Cenchrus purpureus* (Schumach.) Morrone] can be considered a good option for silage production, mainly in warm climate regions (Gusmão *et al*., 2018; Furtado *et al*., 2019) due to its greater productivity and acceptability by the animals, as well as easy cultivation and handling (Ribeiro *et al*., 2020). It can be considered an alternative to more traditional silage crops like corn (*Zea mays* L.) and sorghum [*Sorghum bicolour* (L.) Moench] (Bernardes *et al*., 2018). When well-managed, it may present a good nutritive value (Monção *et al*., 2020). However, the production potential of this perennial grass depends on nutrient replenishment, especially in cut-and-carry/silage production systems.

Despite elephant grass potential for silage production, it has limitations such as low dry matter (DM) and water-soluble carbohydrates (WSCs) (Kung Junior *et al*., 2018), and a higher buffer capacity (BC) (Bezerra *et al*., 2019; Silva *et al*., 2020). These characteristics can hinder the rapid decline in pH and compromise the fermentation process (Ferreira *et al*., 2013), which may favour the predominance of undesirable bacteria (*Clostridium* sp.) responsible for butyric fermentation (Ribas *et al*., 2021), and contribute to reducing silage nutritional value.

Nutritive additives and moisture absorbents have been used to improve the nutritive value and fermentation processes of silages for decades (Muck *et al*., 2018). The fermentation process can be improved by increasing the DM content, WSC concentration and the population of lactic acid bacteria (LAB) present in the forage (Kung Junior *et al*., 2018) or added at the ensiling time through bacterial inoculants that are a source of LAB (Rabelo *et al*., 2018).

The inclusion of other forage species, such as forage legumes, may contribute to increasing DM content, besides the crude protein (CP) content of silage (Ribeiro *et al*., 2020; Lemos *et al*., 2021). Among possible options is butterfly pea (*Clitoria ternatea* L.), a legume with forage potential that stands out for its tolerance to prolonged drought periods (Abreu *et al*., 2014; Santos *et al*., 2017, Pereira *et al*., 2020). Additionally, the presence of legumes may reduce the need for fertilizers due to the potential of atmospheric nitrogen fixation through symbiosis with bacteria and can improve diet and animal performance (Silva *et al*., 2010; Boddey *et al*., 2020).

In general, forage legumes usually present high BC, which impedes the rapid decline of pH in the ensiled mass and may reduce the fermentation capacity (Liu *et al*., 2018). Heinritz *et al*. (2012) evaluated the silage quality of different forage legumes, including butterfly pea. They reported that butterfly pea showed the best fermentation capacity (68) and the most favourable WSC/BC ratio (1.7), which contributed to greater silage quality. It is known that increasing the WSC and DM contents may improve fermentation and reduce DM losses (Borreani *et al*., 2018). Among possible additives that may be used to increase DM content and improve fermentation capacity, corn meal (CM) presents wide availability and can be successfully used as an absorbent, potentially minimizing losses during fermentation (Andrade *et al*., 2012; Muck *et al*., 2018).

Based on this context, the objective of this study was to describe and explain the effect of CM additive on gas losses (GL) and effluent losses (EL), nutritional value, fermentation characteristics and aerobic stability of mixed elephant grass–butterfly pea silages.

Materials and methods

Local, treatments and experimental design

The experiment was conducted at the Animal Science Department of the Federal Rural University of Pernambuco (UFRPE), and the forage was collected at the Carpina Sugarcane Experimental Station (EECAC), located in Carpina (07°51′03″S, 35°15′17″W, and 180 m altitude), Pernambuco, Brazil. The soil of the region is classified as Distrocohesive Yellow Argisol (Santos *et al*., 2018*b*). The climate at the location is classified as dry tropical (Alvares *et al*., 2013), and the average temperature and annual precipitation are around 24°C and 1082 mm, respectively. During the experimental forage growth year (2020), the accumulated rainfall was 735 mm, and average temperatures were 28.9°C, according to the APAC (2020) and data from EECAC.

Elephant grass genotypes (IRI-381 and Taiwan A-146 2.37) were established in 2014, initially managed in monoculture and established in furrows spaced 1.0 m apart, forming plots of 25 m^2 (5 m × 5 m) of total area, with 9 m^2 (3 m × 3 m) of useful area, managed under cutting until 2018. In August 2018, butterfly pea (*C. ternatea* L.) was introduced between the rows of elephant grass to reduce the need for nitrogen application and maintain elephant grass productivity. The elephant grass was harvested at the ground level and the butterfly pea at 20 cm stubble height. During the rainy season, the intercropped plots were fertilized with 60 kg/ha of K_2O and 70 kg/ha of P_2O_5 according to Cavalcanti *et al*. (2008) and considering the following soil chemical properties: 0.05 $cmol_c/dm^3$ of K; 0.06 $cmol_c/dm^3$ of Na; 1.60 $cmol_c/dm^3$ of Ca; 0.80 $cmol_c/dm^3$ of Mg; 0.00 Al^{3+} and 2.40 $cmol_c/dm^3$ of H + Al; besides 5 mg/dm^3 P and pH 5.5.

The elephant grass genotype IRI-381 is considered a tall type, and Taiwan A-146 2.37 is a dwarf type and both were intercropped by butterfly pea, an adapted forage legume. These two genotypes were chosen based on results obtained from a previous study conducted in the same area (Lemos *et al*., 2021).

The completely randomized design was used, with a 2 × 2 factorial arrangement corresponding to the two elephant grass genotypes plus butterfly pea, with and without CM as additive, and four replicates. The botanical composition was determined (Mannetje and Jones, 2000) by collecting 1 kg of forage (fresh matter) from each plot and quantifying the proportions of grass and legume based on the DM forage mass (FM). The Taiwan A-146 2.37 + butterfly pea intercropping had 38.6% of legume, and IRI-381 + butterfly pea 34.1% of legume in the mixtures.

Silage production

The ensiling process occurred in July 2020, after 75 days regrowth. The FM was chopped into a stationary machine to obtain particles sized from 2 to 3 cm. In the treatment with additive, 5% of CM were added based on the DM content of the ingredients (95% of forage and 5% of CM). The DM content of forage was estimated immediately before harvesting.

During the ensiling process, the forage was stored in cylindrical polyvinyl chloride (PVC) experimental silos (15 cm in diameter and 76 cm in height), containing washed and dried sand (3.5 kg) placed in a cotton bag at the bottom of the silos for the capture of effluents. The forage was compacted in silos with the aid of a wooden stake until reaching a density of 600 kg/m^3 of fresh matter. After compaction, the silos were sealed with PVC lids fitted with Bunsen valves, sealed with adhesive tape, weighed and stored at room temperature for 60 days.

Sampling procedures

FM sampling was conducted before ensiling and the CM at the time of ensiling for further analysis and at the opening of the silos, where silage samples were taken from the median portion of the silo, after disposal of approximately 10 cm from the top layer.

The fresh samples were stored in a freezer for further analysis, while the other part was dried in a forced-air oven at 55°C for 72 h, ground in a Willey mill to pass a 1 mm screen, and stored in plastic containers for determination of chemical–bromatological composition.

BC, pH, WSCs, ammonia nitrogen and organic acids analysis

BC was determined in fresh samples collected before ensiling (Playne and McDonald, 1966). The concentrations of WSCs and remaining water-soluble carbohydrates (WSCr) were determined in samples collected before and after ensiling and dried in a forced-draft oven at 55°C for 72 h, according to the methodology described by Bezerra Neto and Barreto (2011).

To determine the pH, 9 g of fresh silage sample from each treatment was weighed in a 250 ml beaker, adding 60 ml of distilled water, homogenized and read after 30 min using a pH meter (Silva and Queiroz, 2002). Ammonia nitrogen ($N\text{-}NH_3$) was determined according to the methodology described by Bolsen *et al*. (1992). For the determination of $N\text{-}NH_3$, 25 g of fresh silage sample were weighed in a 250 ml beaker, added 200 ml of 0.2 N sulphuric acid (H_2SO_4), then the containers were sealed with plastic film, and refrigerated for 48 h. After this period, the samples were filtered on filter paper, and sub-samples were collected and then distilled using the micro-Kjeldahl method to determine total nitrogen.

To determine the concentration of organic acids (acetic, butyric, propionic and lactic acids) and ethanol, 25 g of fresh silage were weighed, diluted in 225 ml of distilled water, and homogenized in an industrial blender for 1 min. The resulting water extract was filtered through filter paper, and 100 ml of the extract was acidified with H_2SO_4 at 50%, and then filtered through fast filter paper. After filtration, 2 ml of the extract received 1 ml of 20% metaphosphoric acid solution and 0.2 ml of 1% carbolic acid solution, used as an internal standard. Samples were centrifuged for 10 min at 15 000 rpm, and the supernatant was collected in Eppendorf and frozen until analysis. The organic acids and ethanol were detected by high performance liquid chromatograph (HPLC, Ciola and Gregory, Master CG model) (Kung Junior and Ranjit, 2001).

Evaluation of chemical composition and in vitro *digestibility of DM*

Dry samples were used to determine the DM, mineral matter (MM), organic matter (OM), CP contents (determined using a fixed conversion factor of 6.25), ether extract (AOAC, 2006), neutral detergent fibre (NDF), acid detergent fibre (ADF) (Van Soest *et al*., 1991) and lignin (LIG) (Van Soest, 1994), neutral detergent insoluble protein and acid detergent insoluble protein (Detmann *et al*., 2012). Hemicellulose (HEM) and cellulose (CEL) values were estimated, obtained by the difference between the percentage of FDN-FDA and FDA-LIG, respectively (Detmann *et al*., 2012). The *in vitro* digestibility of DM (DIVMS) was determined using the artificial incubator 'ANKOM Daisy Incubator' (ANKOM® Technology Corporation, Fairport, NY) and bovine rumen fluid, under the methodology proposed by Holden (1999). The chemical compositions of the mixtures before ensiling are shown in Table 1.

Losses and dry matter recovery

GL, EL and dry matter recovery rate (DMR) were estimated at the time of opening the silos (Jobim *et al*., 2007). The GL values were obtained by subtracting the weights of the silos before opening (at the end of the ensiling process) and after closing, expressed as the percentage of dry mass added to the silo according to the following equation:

$$GL = (WCSi - WCSf)/(FMi \times DMi) \times 100 \qquad (1)$$

where GL = gas losses (%MS); WCSi = weight of silo filled at closure (kg); WCSf = weight of silo filled at opening (kg); FMi = fresh forage mass at closing (kg) and DMi = dry matter content of forage at closing.

EL were estimated after the removal of the entire silage, by subtracting the weight of the sand after the removal of the silage and the original weight of the sand, quantified before filling the silos according to the following equation:

$$EL = [(WVf - ST) - (WVi - ST)]/FMi \times 100 \qquad (2)$$

where E = effluent losses (kg/t of silage); WVf = weight of empty silo with sand in the opening (kg); ST = silo tare; WVi = weight of empty silo with sand at closing (kg); ST = silo tare and FMi = fresh forage mass at closing (kg).

The DMR rate was obtained by the quotient between the amount of DM recovered from the silos at the opening and the amount of DM initially packed, and expressed as a percentage according to the following equation:

$$DMR = [(FMf \times DMf)/(FMi \times DMi)] \times 100 \qquad (3)$$

where DMR = dry matter recovery rate (%); FMf = fresh forage mass at opening (kg); DMf = dry matter content at opening

Table 1. Chemical composition, BC and *in vitro* digestibility of DM of CM and elephant grass [*C. purpureus* (Schumach.) Morrone]–butterfly pea (*C. ternatea* L.) forage mixtures before ensiling

	Taiwan A-146 2.37 + butterfly pea		IRI-381 + butterfly pea		
Variable	Without additive	With additive	Without additive	With additive	CM
WSC (g/kg DM)	160	168	151	165	206
BC (N eq.mg/100 g)	51	44	49	38	18
DM (g/kg DM)	205	211	182	219	801
EE (g/kg DM)	21	26	20	24	48
Ash (g/kg DM)	106	104	100	102	52
OM (g/kg DM)	894	896	900	898	948
CP (g/kg DM)	118	122	115	119	101
NDF (g/kg DM)	669	650	688	656	188
ADF (g/kg DM)	370	345	389	362	42
CEL (g/kg DM)	321	296	333	315	–
HEM (g/kg DM)	296	311	298	295	138
LIG (g/kg DM)	52	48	53	47	–
NDIP (g/kg DM)	95	94	109	103	194
ADIP (g/kg DM)	32	23	33	36	–
IVDDM (g/kg DM)	454	430	418	457	795

WSC, water soluble carbohydrates; BC, buffer capacity; DM, dry matter; OM, organic matter; EE, ether extract; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; CEL, cellulose; HEM, hemicellulose; LIG, lignin; NDIP, neutral detergent insoluble protein; ADIP, acid detergent insoluble protein; NFC, non-fibrous carbohydrates; IVDDM, *in vitro* digestibility of dry matter.

Table 2. GL and EL and DMR mixed elephant grass [*C. purpureus* (Schumach.) Morrone]–butterfly pea (*C. ternatea* L.) silages, with and without CM as additive

	Genotype (G)		Additive (A)			*P* value		
Variable	Taiwan A-146 2.37	IRI-381	Without	With	SD	G	A	G × A
GL (g/kg DM)	12.0[A]	10.0[B]	11.5	10.7	0.40	0.042	0.325	0.637
EL (kg/t)[a]	79.5	83.6	89.3	73.5	4.51	0.622	0.086	0.495
DMR (g/kg DM)	794	819	779[B]	835[A]	1.10	0.130	0.006	0.537

DM, dry matter; SD, standard deviation of mean; G, genotype effect; A, additive effect; G × A, interaction effect.
Means followed by different letters in the lines (for the same effect – genotype or additive) differ from each other by the Tukey's test at 5% probability.
[a]Results based on fresh matter.

(%); FMi = fresh forage mass at closing (kg) and DMi = dry matter content of forage at closing (%).

Aerobic stability test

The aerobic stability test was performed by collecting a subsample of 3 kg of silage from each treatment when the silos were opened, placing the forage in plastic buckets without compaction, and kept in an acclimatized room with a temperature of approximately 22 ± 1.5°C. Silage and ambient temperatures were recorded daily at three times a day for 4 days to assess the time (in h) for a 2°C rise in temperature in relation to ambient temperature. An alcohol thermometer with a scale from −10 to 150°C, inserted at 10 cm in the middle of the silage mass, was used to record the temperature of the silage. In addition, the room temperature was measured using digital thermometers located at different points in the room. The loss of aerobic stability was defined as the time in hours for the temperature to rise by 2°C compared to the room temperature (Kung Junior *et al*., 2000).

Statistical methods

Data were submitted to the Shapiro–Wilk residuals normality test ($P \leq 0.05$) and analysed using the GLM procedure of the SAS® OnDemand for Academic Students, through analysis of variance. In the case of the significant *F* test, the means were compared using the Tukey's test at 5% probability, according to the below statistical model:

$$Y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ij} \quad (4)$$

where Y_{ij} = value observed in the repetition (silos); α_i = elephant grass genotype effect (1–2); β_j = effect of CM additive (1–2); $(\alpha\beta)_{ij}$ = effect of the interaction between the elephant grass genotype and the additive (CM) and ε_{ij} = residual error.

Results

Losses, DMR and fermentation profile

No genotype × additive interaction ($P > 0.050$) was observed for GL, EL and DMR (Table 2). There was genotype effect for GL ($P = 0.042$), with higher values for Taiwan A-146 2.37, as well as additive effect for DMR ($P = 0.056$), with higher values for silages with CM.

There was no genotype × additive interaction effect ($P > 0.050$) for the fermentation profile (Table 3). Taiwan A-146 2.37 presented lower ($P = 0.028$) ethanol value than IRI-381 but higher ($P = 0.009$) pH value, as well as an additive effect for the values of WSCr ($P = 0.006$), with higher concentration in silages containing CM.

Nutritive value

Genotype × additive interaction effect ($P = 0.038$) was observed for CP (Table 4). There was a genotype effect ($P < 0.050$) for MM, OM and ADF contents. The Taiwan A-146 2.37 genotype had higher ADF and MM content and, consequently, lower OM content.

Regarding the additive, there was an effect ($P < 0.050$) for DM, CP, NDF, ADF, CEL, HEM and IVDDM contents. The additive silages had higher contents of DM, CP and IVDDM and lower

Table 3. Fermentation profile of silages of mixed elephant grass [*C. purpureus* (Schumach.) Morrone]–butterfly pea (*C. ternatea* L.) with and without CM

	Genotype (G)		Additive (A)			*P* value		
Variable	Taiwan A-146 2.37	IRI-381	Without	With	SD	G	A	G × A
pH	4.2[A]	4.1[B]	4.13	4.15	0.01	0.009	0.221	0.608
N-NH_3 (g/kg N-total)	9.0	9.6	8.6	10.0	1.10	0.622	0.254	0.488
WSCr (g/kg DM)	19.2	20.4	13.8[B]	25.8[A]	1.50	0.547	0.0002	0.717
Ethanol (g/kg DM)	5.8	7.01	5.9	7.0	0.95	0.028	0.234	0.281
Acetic acid (g/kg DM) (AA)	12.0	18.0	15.0	15.0	1.80	0.214	0.878	0.760
Propionic acid (g/kg DM)	2.0	2.0	2.0	1.0	0.20	0.776	0.121	0.806
Butyric acid (g/kg DM)	0.3	0.4	0.3	0.2	0.03	0.479	0.207	0.743
Lactic acid (g/kg DM) (LA)	105.0	101.0	96.0	110.0	5.01	0.734	0.212	0.301
LA/AA ratio	9.7	6.4	6.8	7.0	0.90	0.050	0.118	0.081

pH, hydrogen potential; N-NH_3, ammonia nitrogen, expressed in g per kg of total nitrogen; WSCr, water-soluble carbohydrates remaining in the silages; SD, standard deviation of mean.
Means followed by different letters in the lines (for the same effect – genotype or additive) differ from each other by the Tukey's test at 5% probability.

Table 4. Nutritive value of mixed elephant grass [*C. purpureus* (Schumach.) Morrone]–butterfly pea (*C. ternatea* L.) silages, with or without CM as additive

	Genotype (G)		Additive (A)			*P* value		
Variable (g kg DM)	Taiwan A-146 2.37	IRI-381	Without	With	SD	G	A	G × A
DM[a]	218	213	210[B]	221[A]	2.7	0.195	0.018	0.429
MM	98[A]	93[B]	97	94	1.1	0.005	0.083	0.090
OM	902[B]	908[A]	903	906	2.1	0.009	0.565	0.464
EE	33	31	30	33.0	1.1	0.400	0.221	0.558
NDF	592	601	609[A]	584[B]	4.1	0.089	0.001	0.955
ADF	358[B]	366[A]	367[A]	357[B]	2.4	0.027	0.007	0.901
CEL	309	310	314[A]	305[B]	2.1	0.637	0.043	0.788
HEM	234	236	241[A]	229[B]	2.3	0.499	0.002	0.536
LIG	49	55	53	51	1.6	0.070	0.550	0.774
NDIP	52	54	52	54	0.6	0.263	0.273	0.359
ADIP	38	39	39	37	1.0	0.709	0.369	0.905
IVDDM	420	413	412[B]	421[A]	2.0	0.091	0.033	0.995

WSC, water soluble carbohydrates; BC, buffer capacity; DM, dry matter; OM, organic matter; MM, mineral matter; EE, ether extract; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; CEL, cellulose; HEM, hemicellulose; LIG, lignin; NDIP, neutral detergent insoluble protein; ADIP, acid detergent insoluble protein; NFC, non-fibrous carbohydrates; IVDDM, *in vitro* digestibility of dry matter; SD, standard deviation of mean.
Means followed by different letters in the lines (for the same effect – genotype or additive) differ from each other by the Tukey's test at 5% probability.
[a]Results based on fresh matter.

NDF, ADF, CEL and HEM levels. The Taiwan A-146 2.37 silage with additive had higher CP content than without additive, but it did not differ from the IRI-381 silage with additive.

Aerobic stability

The results of the aerobic stability assessment showed that the silages remained stable during a 45 h period of exposure to oxygen (Fig. 1), indicated by the increase in the silage temperature of 2°C compared to room temperature.

Discussion

Losses, DMR and fermentation profile

Greater GL values observed in Taiwan A-146 2.37 silages may reflect the higher pH value of this silage (Table 2). The botanical composition of ensiled FM revealed a slightly greater proportion of the butterfly pea at the time of forage harvest at the Taiwan A-146 2.37 plots compared to the IRI-381 plots, which may have reflected in a greater buffering capacity of the ensiled mass and, consequently, greater resistance to lowering the pH. However, the presence of the forage legume did not contribute to limiting pH values since all the silages presented a satisfactory fermentative profile (Table 3).

Although the GL was higher in Taiwan A-146 2.37 silages without CM, the GL values observed in both silages (Table 2) can be considered reduced compared to the consulted literature. Andrade *et al.* (2010), when evaluating elephant grass silages with or without cassava meal, coffee husk or cocoa meal at levels of 0, 10, 20 and 30% of fresh matter, observed GL values around 61 g/kg DM in non-additive silages. Rigueira *et al.* (2018) also observed GL values around 66 g/kg DM in elephant grass silage

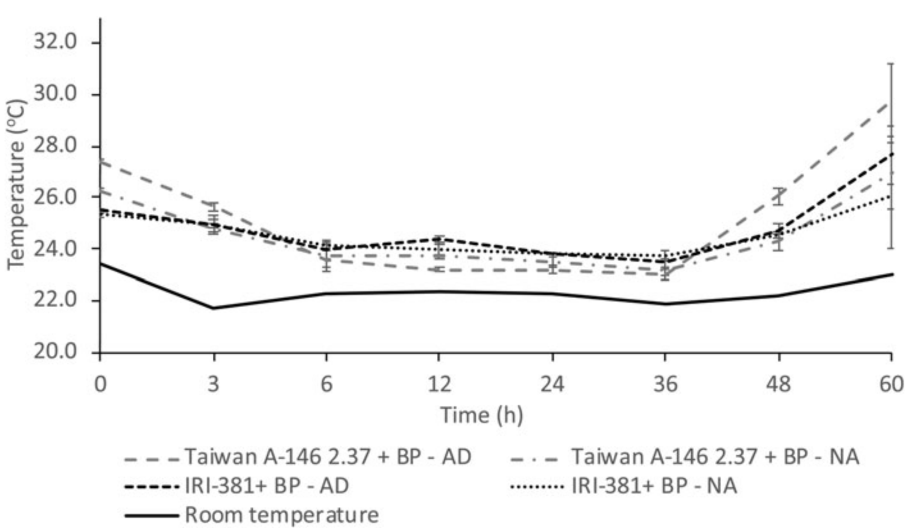

Fig. 1. Temperature variation of mixed elephant grass [*C. purpureus* (Schumach.) Morrone] genotypes–butterfly pea (*C. ternatea* L.) (BP) silages with (AD) and without (NA) additive (CM) after opening the silos. Vertical bars are the standard error of the mean.

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without additives. The low GL values indicate the low occurrence of gas-producing microorganisms such as clostridia (McDonald, 1981) and the possible absence of secondary fermentations, confirming the adequate fermentation profile due to the reduced N-NH_3 and butyric acid concentrations (Table 3).

The EL values can be considered high (average of 82 kg/t fresh FM) (Table 2), especially when compared to other studies with elephant grass silage, such as those by Zanine *et al.* (2010) (42 kg/t FM), Santos *et al.* (2013) (25 kg/t fresh mass) observed for the Taiwan A-146 2.37 genotype and Rigueira *et al.* (2018) (51 kg/t FM). Andrade *et al.* (2010), on the other hand, observed EL values above (145 kg/t FM) when evaluating elephant grass silages without additive at DM contents around 187 g/kg DM, close to the DM value observed for the genotype IRI-381 (182 g/kg DM) in the present study. The great EL was probably related to the low DM contents of the forage before ensiling (Table 1), which is a feature in elephant grass silages that do not receive a moisture reduction technique (Ribas *et al.*, 2021). The relatively high moisture content in the ensiled mass is one of the main factors that contribute to increased EL and the type of silo, degree of compaction and physical processing of the forage (Rigueira *et al.*, 2018; Lemos *et al.*, 2020).

The inclusion of 5% CM tended to reduce EL values ($P = 0.0864$), with variations between 89 kg/t FM, in non-additive silages and values of 74 kg/t FM, in silages containing CM, contributing to the greatest DMR in additive silages. The greater DMR can be used as one of the indicators of the fermentative efficiency of the silage, as well as the reduced loss of the nutritive value of the silage. The losses generally reduce the nutrients in the silage and proportionally increase the fibrous constituents, resulting in lower DM digestibility and, consequently, lower animal consumption and performance.

These results may indicate the potential of CM used to reduce the moisture content of the ensiled mass, reflecting its high DM content (Table 1). Although a trend towards EL reduction was observed for silages with CM, the absence of significance at 5% probability may be a result of the small amount of CM used (5%). Increasing the proportion of CM as additive could provide better results when minimizing total DM losses resulting mainly from lower EL, which in large proportions promote a reduction in the nutritive value of the silage in terms of vitamins, minerals, carbohydrates and soluble proteins (Kung Junior *et al.*, 2018; Muck *et al.*, 2018). However, the cost may be a limiting factor.

Despite the difference between the genotypes, with Taiwan A-146 2.37 silages presenting greater pH, the pH values found in the present study can be considered satisfactory even with the presence of a relatively large proportion of the forage legume in the ensiled mass (>35%). It is important to mention that there was no effect of additive on pH values and ammonia nitrogen contents, indicating the absence of undesirable microorganisms, as indicated in the fermentation profile (Table 3).

In general, silages produced from legumes tend to present greater pH values when compared to grass silages, given the higher BC, which makes it difficult to reduce the pH during the fermentation process (Ribeiro *et al.*, 2020). However, the presence of the butterfly pea did not compromise the fermentation capacity of the ensiled mass, and all silages had pH values within the range considered ideal (3.8–4.2) for adequate forage conservation (McDonald, 1982; Bernardes *et al.*, 2018). The pH should be low enough to inhibit the growth of bacteria responsible for secondary fermentation, maintaining conservation and reducing losses in the nutritional value of the silage (Muck *et al.*, 2018). Lemos *et al.* (2021), evaluating silages of IRI-381 and Taiwan A-146 2.37 elephant grass genotypes intercropped with butterfly pea, did not report high pH values (3.97). Santos *et al.* (2013) reported a pH of 4.2 in Taiwan A-146 2.37 silage without forage legume. Thus, the pH values obtained in the present study revealed an adequate fermentation process.

The N-NH_3 values of all silages (Table 3) were greater than those observed by Andrade *et al.* (2010) (1.6–3.9 g/kg N total), evaluating elephant grass silage with and without the inclusion of additives. Despite this, all silages showed N-NH_3 concentrations below 100 g/kg N total, indicating reduced proteolysis. The N-NH_3 values above 120 g/kg N total are indicative of strong proteolysis since N-NH_3 comes from the degradation of the protein fraction (true protein, peptides, amino acids, amines and amides) by the action of undesirable microorganisms (Musco *et al.*, 2016; Furtado *et al.*, 2019). In addition to being a widespread moisture-absorbent additive that can contribute to reduced losses during fermentation processes, according to Monteiro *et al.* (2011), CM may present reasonable concentrations of WSC. Bezerra *et al.* (2019) reported that the WSC content in elephant grass silage increased with the addition of ground corn. According to the authors, even though corn is starch-rich, it contains reasonable amounts of other soluble sugars, increasing the concentration of WSC in the silage that can be used as a substrate for LAB. In the present study, adding CM did not result in a significant increase in WSC (Table 1), despite the silage with additive show a slightly greater WSCr (Table 3).

According to Mendieta-Araica *et al.* (2009), the WSC contents in silages with an adequate fermentative profile must be at least 2 g/kg DM, values above those observed in the present study. Other studies evaluating elephant grass silage without additive reported even lower WSCr values. Lemos *et al.* (2021) reported values of 1.85 g/kg DM in elephant grass silages mixed with butterfly pea, while elephant grass silages without the legume presented 1.34 g/kg DM. Santos *et al.* (2013) observed even lower WSCr contents in silages of genotypes Mott (0.6 g/kg DM), Elephant B (1.1 g/kg DM), Taiwan A-146 2.114 (0.6 g/kg DM), HV 241 (0.7 g/kg DM) and Taiwan A-146 2.37 (0.8 g/kg DM).

WSCs are the primary substrate for LAB, and may contribute to a rapid decline in pH and for controlling the development of other undesirable microorganisms such as clostridia during the fermentation process (Muck, 2010; Borreani *et al.*, 2018) since it is related to lactic acid accumulation in the ensiled mass. Despite its importance, WSC values above 2 g/kg may contribute to lower aerobic stability after opening the silo, since fungi and yeasts can quickly use soluble sugars, a phenomenon that increases the temperature and reduces the quality of the silage (Muck *et al.*, 2018).

The greater ethanol concentrations observed in the silages of the IRI-381 genotype may be related to the lower DM content (185 g/kg DM) of this genotype compared to Taiwan A-146 2.37 (208 g/kg DM), which may have contributed to an environment more conducive to alcoholic fermentation. Tran *et al.* (2017) evaluated the ethanol concentration in elephant grass silages with or without exogenous bacterial inoculants (*Lactobacillus paracasei* + *Lactococcus lactis*). They observed that the control silage (without inoculant) and lower DM content (156 g/kg DM) showed a greater concentration of ethanol (18 g/kg DM) compared to silage added with exogenous microorganisms (DM = 189 g/kg DM; ethanol = 10 g/kg DM). In the present study, the values of organic acids indicate a satisfactory fermentation profile in all silages.

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The concentrations of organic acids (lactic, acetic, propionic and butyric acid) and ethanol were not affected by the addition of CM (Table 3). Costa *et al.* (2020) observed an increase in lactic acid concentration in elephant grass silage added with 30% of faveira pod meal (*Parkia platycephala* Benth.) added to the ensiled mass. Although CM showed a slightly greater concentration of WSC than the ensiled FM (Table 1), the amount used was not enough to increase the production of lactic acid (Muck, 2010). Even with a relatively large proportion of butterfly pea (>35%) in the ensiled FM, butyric acid and lactic acid concentrations indicate an adequate fermentative profile (Table 3). The presence of lactic acid is an important indicator of the fermentation profile, as it suggests that fermentation occurred properly, effectively reduced pH and undesirable fermentation was restricted (Musco *et al.*, 2016; Borreani *et al.*, 2018).

Nutritional value

The use of CM as an additive increased the silage DM content (801 g/kg DM), contributing to greater recovery, indicating its potential to reduce the moisture content of the ensiled mass (Bezerra *et al.*, 2019). This same trend was observed by Andrade *et al.* (2012), who observed a reduction in moisture content of elephant grass silages containing 5% (238 g/kg DM) and 10% (289 g/kg DM) of CM compared to non-additive silages (210 g/kg DM). The increase in DM content results in a significant reduction in GL and EL and greater DM recovery. The reduced DM contents, common in tropical grasses, can favour the development of *Clostridium* bacteria, responsible for butyric acid production (Ribas *et al.*, 2021). Borreani *et al.* (2018) indicate that values of at least 250 g/kg DM are already capable of supporting a good fermentation process. In the present study, it was possible to observe that DM values around 200 g/kg DM can also result in good quality silage, with reduced losses in the nutritive value, as long as the stages of its preparation are properly followed.

Taiwan A-146 2.37 genotype silages showed greater MM contents, consequently, lower OM contents (Table 4). Santos *et al.* (2013) reported similar levels of MM for Taiwan A-146 2.37 (100 g/kg DM) silage without additive using forage harvested at 56 days of regrowth. Therefore, at the levels observed in this research, the MM contents (95 g/kg DM) of all silages are within acceptable limits for this variable. In addition, MM can also vary with several factors, including age after regrowth (Lopes *et al.*, 2021).

IRI-381 showed greater ADF content compared to Taiwan A-146 2.37 genotype. According to Silva *et al.* (2021), tall genotypes like IRI-381 generally show greater stem elongation. The greater development of plant structural tissues increases the deposition LIG and participation of sclerenchyma, considered less digestible and a proportional reduction in more digestible tissues (Sanchês *et al.*, 2018). However, despite differences in ADF, there was no difference in the IVDDM values between the genotypes.

The genotypes showed similar LIG content, with averages of 52 g/kg DM. Lemos *et al.* (2021) reported differences in LIG contents between genotypes of different types (tall *v.* dwarf), both cultivated intercropped with the butterfly pea. They reported mean LIG values higher than those found in this study [Taiwan A-146 2.37 (83 g/kg DM) and IRI-381 (109 g/kg DM)], which contributed to lower IVDDM. In addition to genotype, the adopted harvest interval may contribute to LIG and DM content changes and WSC. However, a larger harvest interval can also contribute to a linear increase in NDF, ADF and LIG, reducing CP and IVDDM contents (Lopes *et al.*, 2021) and resulting in silage with reduced nutritional value.

The addition of CM reduced the fibrous fractions (NDF, ADF, CEL and HEM) in the silages (Table 4). The lower concentration of fibrous content in the FM (Table 1) results from the greater nutritional value of CM, which contributed to greater IVDDM. Monteiro *et al.* (2011), evaluating elephant grass silage with rice (*Oryza sativa* L.) bran and CM, also observed a reduction in NDF and ADF contents in the silage and increased CP in the ensiled mass.

The lower values of the fibrous fractions observed in the additive silages (Table 4) contributed to greater IVDDM (421 g/kg DM) compared to silages without CM (412 g/kg DM). Even though the mixed silages with CM added showed greater IVDDM, the values reported in the present study were below those obtained in other studies with elephant grass silage (Costa *et al.*, 2020; Lemos *et al.*, 2021). These results may be related to the inclusion of the butterfly pea in the ensiled forage. It is known that legumes usually present leaves with greater digestibility than tropical grasses; however, they may present structural parts such as more lignified stems and branches (Castro-Montoya *et al.*, 2020), which probably contributed to reducing IVDDM.

Mixed Taiwan A-146 2.37–butterfly pea silages with CM showed greater CP concentration than without CM and did not differ from the IRI-381 silages (Table 5). In general, the silages showed values above the minimum CP required by ruminants of 70 g/kg DM (Valadares Filho *et al.*, 2016), which can be explained by the presence of the butterfly pea in all treatments. Despite the presence of the legume, the $N\text{-}NH_3$ concentration in the silage indicated the absence of proteolysis during fermentation, indicating that most of N content of butterfly pea was preserved, contributing to increase silage nutritive value.

Aerobic stability

The silages remained stable up to 36 h after exposure to air (Fig. 1). After this period, there was a gradual increase in temperature through aerobic deterioration caused by undesirable microorganisms (Guim *et al.*, 2002) until the total loss of stability after 45 h of opening the silos. Mixed Taiwan A-146 2.37–butterfly pea silage without additives showed more accentuated temperature elevation. This may be related to the higher pH value recorded in this treatment (Table 3) and a slightly greater proportion of butterfly pea in the ensiled mass combined with the absence of CM.

The increase in the temperature of the silages results from the action of microorganisms (bacteria and fungi) is inherent to the ensiled mass, which develops when exposed to oxygen and

Table 5. Interaction effect of genotype × additive (CM) on CP content (g/kg DM) of mixed elephant grass [*C. purpureus* (Schumach.) Morrone]–butterfly pea (*C. ternatea* L.) silages, with or without CM

	Additive (g/kg DM)			
Genotype	Without	With	*P* value	SD
Taiwan A-146 2.37	113^{aB}	116^{aA}	0.038	0.4
IRI-381	113^{aA}	114^{aA}		

Means followed by distinct letters, lowercase in the column and uppercase in the row, differ from each other by Tukey's test at 5% probability.

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initiates the aerobic deterioration of the nutrients present in the silage (Kung Junior *et al.*, 2018). Lemos *et al.* (2020) reported a break in the stability of elephant grass silages added with fibrolytic enzymes right after the first hour of evaluation. On the other hand, Andrade *et al.* (2012) evaluated elephant grass silages supplemented with cornmeal and soybean hulls and showed aerobic deterioration only after 48 h, close to the value observed in the present study.

High-quality silages, with high concentrations of WSC tend to be deteriorated mainly by filamentous fungi and yeasts, which can result in less aerobic stability due to the rapid degradation of soluble sugars after opening the silos, which causes an increase in temperature and reduction in the quality of the silage (Wilkinson and Davies, 2013; Muck *et al.*, 2018). In grass-based silages, which generally show low DM and WSC contents, fermentation stability is generally low at pH above 4.5 becoming more prone to spoilage by aerobic bacteria (enterobacteria) instead of mould and yeast when compared to maize or sorghum silages (Bernardes *et al.*, 2018).

Under the conditions in which these silages were produced and, given their expected deterioration, the use of mixed elephant grass–butterfly pea silages should occur before 45 h of exposure to the air, aiming for minimal loss of its nutritional value after opening the silos.

The use of nutritive additives and moisture absorbers has become important strategies for the successful ensiling of tropical grasses (Borreani *et al.*, 2018). However, the results obtained in this research showed that it is possible to obtain silages of good fermentative profile from elephant grass–butterfly pea legume mixture, even with DM around 20%.

Conclusions

The mixed IRI-381 (tall elephant grass) or Taiwan A-146 2.37 (dwarf elephant grass)–butterfly pea legume results in good quality silages, with little difference in the nutritive value. The use of forage of elephant grass intercropped with butterfly pea legume does not negatively influence the fermentation profile of the silages, even with the relatively large participation (34–39%) of the legume in the ensiled mass. The addition of 5% CM reduces losses and increases the recovery of DM and nutritive value of elephant grass silages, but the non-inclusion did not limit the fermentation characteristics of mixed elephant grass–butterfly pea legume.

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