

## Article

# Qualitative Production of Mixture Silage within a Sustainable Concept

Karina Batista \*  and Campos, F.P. 

Instituto de Zootecnia—IZ, Agência Paulista de Tecnologia dos Agronegócios—APTA, 56 Heitor Penteado St. Centro, Nova Odessa 13380-011, SP, Brazil

\* Correspondence: batistakarim@gmail.com

**Abstract:** Climate change and seasonality in forage production have caused alterations in animal feed. Thus, this study evaluated the composition of silages from soybean (*Glycine max* (L.) Merrill) mixed with tropical grasses. The experimental design was randomized blocks with four replications. Treatments were silage from soybeans, silage from soybeans with Aruana Guinea grass (*Megathyrsus maximus* cv. Aruana), and silage from soybeans with Congo grass (*Urochloa ruziziensis* cv. Comum). Silos were stored for 60 days in the laboratory at room temperature. The silage from soybeans with Aruana Guinea grass showed the highest contents of dry matter, crude fiber, neutral detergent fiber, insoluble nitrogen in neutral detergent fiber, and insoluble nitrogen in acid detergent fiber but the lowest levels of crude protein and ether extract. The highest content of mineral material and hemicellulose was observed in the silage from soybeans with Congo grass. The silages from soybeans and soybeans with Congo grass showed no significant differences for acid detergent fiber and lignin. In conclusion, the use of tropical grasses as a component to improve the quality of silage from soybeans is an alternative for forage conservation in ruminant production systems, especially at the dry season.

**Keywords:** climate change; forage conservation; mixed silage; legume-grass silage; sustainable livestock



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## 1. Introduction

Animal production and its byproducts play an important role in providing protein food for the ever-growing global population. Making production more efficient has been the key to solving environmental, social, and economic problems, leading us to sustainability. A permanent challenge, therefore, is to identify opportunities to maximize the efficiency of ruminant feed sustainably [1]. In general, animal feed in countries with tropical and subtropical climates is based on forage production, which is abundant and of good quality during the rainy season but which decreases rapidly during the dry season. Thus, seasonality in forage production causes producers to use conservation practices, such as ensiling, to store the forage [2]. Silage is a product of forage fermentation under acidic and anaerobic conditions and can be produced from most crops [3,4].

Soybean (*Glycine max* (L.) Merrill) is one of the major crops in the world, and the very high quality of its protein makes it an important component of animal feed for both milk and meat production. Despite this, the soybean crop is rarely used for ensiling since its silage has an unpleasant odor and relatively high levels of butyric acid, which makes it unattractive for consumption by animals [3]. However, its mixture with tropical grasses can improve the silage fermentation process due to the higher dry matter (DM) content of tropical grasses, eliminating the unpleasant odor and reducing butyric acid production [5]. Thus, factors that hinder the silage process of the isolated soybean could be canceled with an appropriate combination in the silage of the forage plants, such as tropical grasses, since the unfavorable characteristics of both could be improved [6].

Mixed silage with two or more species, such as grasses and legumes, could be an agricultural practice for animal feed in the dry season [6]. In this way, the intercropping of

legumes with forage species of the Poaceae family (cereals or grasses) could be proposed as a suitable alternative to improve the forage nutritional composition for animal feed [4]. One of the main objectives of intercropping systems for forage production to ensiling is the complementary effect of desirable nutritional features of two or more crops [7].

Intercropping between grain-producing plants and tropical grasses has been widely used in agriculture. However, the quality of mixed material harvested in intercropped systems is uncertain. Grasses are nitrogen-consuming C4 plants, while soybeans are nitrogen-fixing C3 plants, and, as both can be sown at the same time, their intercropping and mechanized harvesting become feasible. In addition, soybeans intercropped with tropical grasses allow different soil horizons to be explored due to their different root lengths. Thus, a better-quality forage can be produced given the greater amount of nitrogen absorbed by grasses and soybean crops [8].

According to the findings of [4], the forage produced in the intercropping system between soybean and maize had the highest crude protein (CP) and the lowest acid detergent fiber (ADF) and neutral detergent fiber (NDF). Moreover, total digestible nutrients (TDN) and ashes were higher in the forage produced in the intercropping system than in the maize in monoculture. Adding to that, [5] reported that the CP in the silage of soybean intercropped with maize was higher than in the silage of maize in monoculture. The silage of maize in monoculture and maize intercropped with soybean was similar to the *in vitro* digestibility of dry matter (IVDDM) and losses of gases. In addition, [5] emphasized that the ensiling of soybeans with low DM is not recommended due to the significant potential for unfavorable fermentation and loss of nutrients. The authors of [6] evaluated the composition of silage from several soybean genotypes and pointed out that the differences in CP and IVDDM among the silage were due to the fermentation problems during the ensiling process. The authors of [6] also emphasized that the low DM content of the silage from various soybean genotypes led to the highest production of butyric acid, exceeding 1%.

The quality of the forage produced within the intercropping systems with soybeans has been the subject of much research. However, there are few studies about the quality of the preserved forage as silage. This lack of information for the silage of mixtures of soybeans with tropical grasses is due to the fact that soybean-specialized farmers direct the crop to grain production only, in addition to the fact that many ranchers do not see soybeans as an economically viable forage. Thus, starting from the hypothesis that the mixture of soybeans with tropical grasses can be a sustainable alternative for silage production, this study aimed to evaluate the chemical composition, fermentative parameters, and *in vitro* digestibility of DM and organic matter (OM) of silages from soybeans intercropped with tropical grasses.

## 2. Materials and Methods

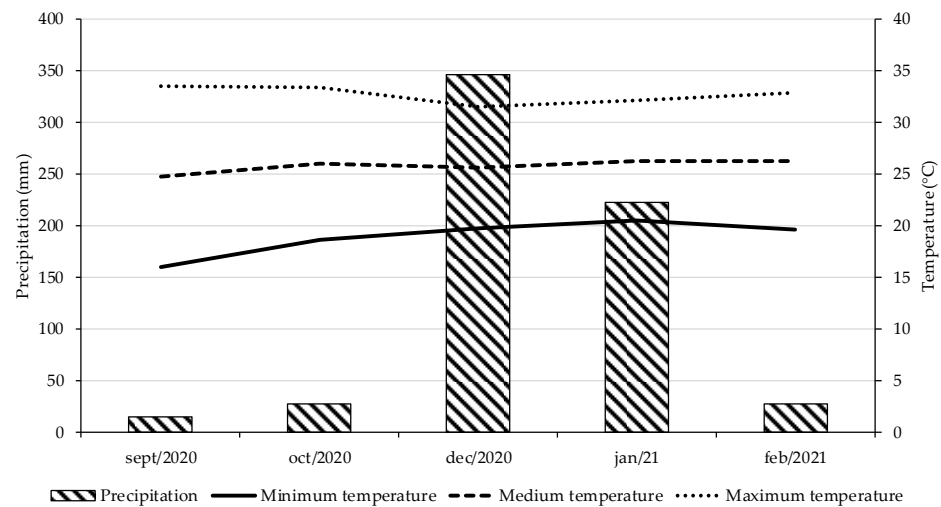
### 2.1. Experiment Location, Treatments, and Plant Cultivation

An experiment was carried out in southeastern Brazil (22°42' S, 47°18' W, and 570 m altitude) on a Red-Yellow Argisol (Ultisol) [9]. According to Köppen's classification, the local climate is Aw type, which stands for a rainy tropical forest with rains in the summer and drought in the winter [10]. Figure 1 shows the data regarding the climate. During the experimental period, soybeans intercropped with tropical grasses were grown in the summer season.

The experimental design was a randomized block with four replications. Treatments consisted of (1) silage from soybeans in monoculture (*Glycine max* (L.) Merrill) (Soy), (2) silage from soybeans intercropped with Aruana Guinea grass (*Megathyrsus maximus* cv. Aruana) (S + AGG), and (3) silage from soybeans intercropped with Congo grass (*Urochloa ruziziensis* cv. Comum) (S + CG).

The cultivation plots had 72 m<sup>2</sup> (3.6 × 20.0 m). In the soybean monoculture, the rows were spaced 0.45 m apart. In the intercropping system between soybeans and tropical grasses, the rows were spaced 0.225 m apart. Soybean sowing density was 300 thousand

plants per hectare. Grass seeds with 60% cultural value were sown at a density of about  $6 \text{ kg ha}^{-1}$ .



**Figure 1.** Temperature and precipitation during the soybean and tropical grass cultivation.

The soybean cultivar used was M6410IPRO (INTACTA RR2 PRO<sup>®</sup>, Agro Bayer, São Paulo, Brazil). At sowing time, soybean seeds were inoculated with *Bradyrhizobium japonicum*, and only soybean rows were fertilized with  $17 \text{ kg ha}^{-1}$  of N,  $59 \text{ kg ha}^{-1}$  of  $\text{P}_2\text{O}_5$ , and  $34 \text{ kg ha}^{-1}$  of  $\text{K}_2\text{O}$  [11]. The fertilizer hose from the grass rows were closed at sowing time. Thus, the grass rows were not fertilized.

## 2.2. Harvesting and Ensiling

At the beginning of soybean maturity, when pods on the main stem showed a mature color (R7 stage), the plants were harvested manually at about 25 cm high. When there were rows of soybeans and grass in the plot, one meter of the green mass of soybeans and one parallel meter of the green mass of grass were harvested at three different points in the central row of each plot. When there were only soybeans in the plot, the soybeans were harvested at three different points in the central row, and at each point, one meter of green mass was harvested.

The proportions of soybeans and grasses were determined in the mixture of the treatments. Hence, in the mass of silage from soybeans intercropped with Aruana Guinea grass, the means values were soybeans 3.83% and Aruana Guinea grass 96.17%, and in the mass of silage from soybeans intercropped with Congo grass, the means values were soybeans 16.03 and Congo grass 83.97. After harvesting, the samples were weighed and subsequently chopped (Forage harvester JF 50 maxxium, Itapira, Brazil) and ensiled. A sample of each material was separated at the time of harvest to determine the chemical composition and in vitro digestibility parameters of the materials before ensiling as an initial quality reference (Table 1).

The chopped material was stored in experimental silos made of PVC, measuring 0.3 m in length and 0.1 m in diameter. This material was compacted with a wooden tamper inside the silo and coated with a transparent and resistant plastic bag ( $30 \text{ cm} \times 40 \text{ cm} \times 0.20 \text{ mm}$ ). After filling, the silos were closed with PVC covers equipped with a Bunsen-type valve for gas to escape. The silos were stored at room temperature in the laboratory for 60 days in randomized blocks with four replications, following the same experimental design as the experimental field. After 60 days, the silos were weighed and opened, homogenizing the material inside each silo. At that time, liquid effluents were not observed in the silos. After homogenization, a 200 g sample was collected to determine the DM, chemical composition, and in vitro digestibility, and a 20 g sample was collected to determine the fermentative parameters of the silage produced.

**Table 1.** Means values of DM, CP, CF, NDF, ADF, EE, MM, NDIN, ADIN, lignin, cellulose, hemicellulose, IVDDM, IVDOM, TDN, and NFC in the materials before being ensiled.

Parameters	Soy	AGG	CG	S + AGG	S + CG
DM (g kg <sup>-1</sup> )	200.00	255.90	196.58	259.90	199.70
CP (g kg <sup>-1</sup> )	174.40	133.40	131.25	135.01	149.70
CF (g kg <sup>-1</sup> )	351.80	434.70	369.60	463.50	359.20
NDF (g kg <sup>-1</sup> )	719.50	765.00	724.15	820.20	709.10
ADF (g kg <sup>-1</sup> )	622.20	735.30	675.40	765.10	653.20
EE (g kg <sup>-1</sup> )	7.10	14.00	12.20	6.10	8.90
MM (g kg <sup>-1</sup> )	78.20	91.10	83.80	75.30	73.60
NDIN (g kg <sup>-1</sup> )	234.80	411.40	196.80	319.90	192.70
ADIN (g kg <sup>-1</sup> )	-	93.80	58.70	185.00	45.00
Lignin (g kg <sup>-1</sup> )	193.60	181.40	115.89	221.30	123.10
Cellulose (g kg <sup>-1</sup> )	428.60	553.90	559.50	543.80	530.00
Hemicellulose (g kg <sup>-1</sup> )	97.30	29.70	48.70	55.20	56.00
IVDDM (g g <sup>-1</sup> )	0.580	0.516	0.502	0.515	0.509
IVDOM (g g <sup>-1</sup> )	0.592	0.523	0.507	0.524	0.519
TDN (g kg <sup>-1</sup> )	538.50	427.30	421.10	407.60	443.30
NFC (g kg <sup>-1</sup> )	20.90	-	48.61	-	58.80

Soy: only soybeans; AGG: only Aruana Guinea grass; CG: only Congo grass; S + AGG: soybeans with Aruana Guinea grass; and S + CG: soybeans with Congo grass. DM: dry matter; CP: crude protein; CF: crude fiber; NDF: neutral detergent fiber; ADF: acid detergent fiber; EE: ether extract; MM: mineral material; NDIN: neutral detergent insoluble nitrogen; ADIN: acid detergent insoluble nitrogen; IVDDM: in vitro digestibility of dry matter; IVDOM: in vitro digestibility of organic matter; TDN: total digestible nutrients; and NFC non-fibrous carbohydrates.

### 2.3. Chemical Analyses

For the determination of the chemical composition and digestibility, the samples were dried in a forced-air circulation oven at 55 °C for 72 h and then ground in a Wiley mill to a particle size of 1.0 mm (Micro Mill Type Wiley, Piracicaba, Brazil). For the DM content, the samples were dried in a forced-air circulation oven at 105 °C for 72 h. Finally, for fermentation parameters, the samples were frozen until the analysis. In addition, for chemical composition correction of the data, the DM subsamples were dried in an oven at 105 °C for 3 h (method 934.01) [12].

The parameters evaluated were (a) crude protein (CP) [13], (b) crude fiber (CF) [14], (c) neutral detergent fiber (NDF) [15], (d) acid detergent fiber (ADF) [15], (e) ether extract (EE) (method 920.39) [12], (f) mineral material (MM) (method 942.05) [12], (g) non-nitrogen extract (NNE) [14], (h) neutral detergent insoluble nitrogen (NDIN) [16], (i) vacid detergent insoluble nitrogen (ADIN) [16], (j) lignin [17], (k) cellulose [14], (l) hemicellulose [14], (m) in vitro digestibility of dry matter (IVDDM) and organic matter (IVDOM) [16], (n) total digestible nutrients (TDN) [18], (o) non-fibrous carbohydrates (NFC) [19], (p) pH [14], (q) lactic acid [20,21], (r) acetic acid [22,23], (s) propionic acid [22,23], (t) butyric acid [23], (1998), and (u) N-ammonium from silage [15].

The true in vitro digestibility of the samples (DM, NDF, and organic matter (OM)) was determined by the in vitro System—Ankom Daisy Incubator. In the incubation, 1.0 g of sample (ground to 1 mm) was weighed in Ankom-type TNT bags (Ankom F-57) and transferred to glass bottles with 1600 mL of nutrient solution (NS, buffer and macro- and micromineral solutions, with a pH of around 7.0) and 400 mL of rumen fluid. Before incubation, pH adjustments were performed by adding NaOH or HCl. Then, the flasks were closed with CO<sub>2</sub> saturation and incubated in an oven with forced ventilation at 39.5 °C for 48 h, following procedures according to [16]. Ruminant liquid was collected from two crossbred Holstein cows' cannulated grass-grazing system (ad libitum). The ruminal liquid was filtered through double-folded gauze in thermos bottles and again filtered through gauze and 2 cm thick glass wool under continuous injection of CO<sub>2</sub> stored in a hermetically sealed thermos bottle. Duplicate samples were used for each treatment, and the samples were incubated separately with rumen fluid plus NS. An Ankom-type

TNT bag was used without the addition of a sample and with buffered ruminal liquid (the control sample) to remove the effect of possible sample residues in ruminal liquids. After 48 h of incubation, the undigested residues from each treatment were washed with a neutral detergent solution, hot distilled water, and acetone and dried in an oven at 105 °C for 12 h, and the NDF residues were recovered in the bags. The digestibility of the DM, NDF, and OM was estimated sequentially based on the solid residue remaining in each TNT bag, according to [16].

#### 2.4. Calculations of the Contribution and Proportion of Each Forage in the DM, CP, CF, NDF, ADF, EE, MM, Lignin, NDIN, ADIN, Cellulose, and Hemicellulose in the Silages

Equations (1) and (2) were used for the calculation of the means values of the proportion of soybeans and grasses in the DM, CP, CF, NDF, ADF, EE, MM, lignin, NDIN, ADIN, cellulose, and hemicellulose in the silages.

$$PSb = \frac{(PSsm \times bpS)}{100} \quad (1)$$

where PSbp is means value of the proportion of soybeans in the bromatological parameter in the silage, PSsm is the proportion of soybeans in the silage mass, and bps is the bromatological parameter of soybeans.

$$PGbp = \frac{(PGsm \times bpG)}{100} \quad (2)$$

where PGbp is the proportion of the grass in the bromatological parameter in the silage, PGsm is the proportion of grass in the silage mass, and bpG is the bromatological parameter of grass.

Equations (3)–(6) were used for the calculation of the means values of the contribution of soybeans and grasses in the DM, CP, CF, NDF, ADF, EE, MM, lignin, NDID, ADIN, cellulose, and hemicellulose in the silages.

$$PCSsm = \left( \frac{CSsm}{(CGsm + CSsm)} \right) \times 100 \quad (3)$$

where PCSsm is the percentage contribution of the soybeans in the silage mass, CSsm is the contribution of the soybeans in the silage mass, CGsm is the contribution of the grass in the silage mass, and CSsm is the contribution of the soybeans in the silage mass.

$$PCGsm = \left( \frac{CGsm}{(CGsm + CSsm)} \right) \times 100 \quad (4)$$

where PCGsm is the percentage contribution of the grass in the silage mass, CGsm is the contribution of the grass in the silage mass, CGsm is the contribution of the grass in the silage mass, and CSsm is the contribution of the soybeans in the silage mass.

$$PCSbp = \left( \frac{PPS \times pbMS}{100} \right) \quad (5)$$

where PCSbp is the percentage contribution of the soybeans in the bromatological parameters of the mixture silage, PPS is the percentage proportion of the soybeans, and pbMS is the bromatological parameter of the mixture silage.

$$PCGbp = \left( \frac{PPG \times pbMS}{100} \right) \quad (6)$$

where PCGbp is the percentage contribution of the grass in the bromatological parameters of the mixture silage, PPG is the percentage proportion of the grass, and pbMS is the bromatological parameter of the mixture silage.

### 2.5. Statistical Analysis

The experimental design was a randomized block with four replications. The results were subjected to variance analysis using the ANOVA procedure of the SAS software (Statistical Analysis System—SAS/STAT®9.2) [24], and the means of the treatment were compared by Tukey's test at 5% probability according to the model written in Equation (7). When necessary, outliers were removed.

$$Y_{ij} = \mu + \tau_i + \xi_{ij} \quad (7)$$

where  $Y_{ij}$  is the  $ij$ -th observation,  $\mu$  is a constant for all observations (general means),  $\tau_i$  is the effect of the  $i$ -th treatment, and  $\xi_{ij}$  is the random error (measurement errors, uncontrollable factors, differences between experimental units, etc.).

### 3. Results

The chemical composition varied among the silages (Table 2). Silage from soybeans intercropped with Aruana Guinea grass had the highest contents of DM, CF, NDF, NDIN, and ADIN, as well as the lowest contents of CP and EE (Table 2). The highest ADF content was observed in the silage from soybeans intercropped with Aruana Guinea grass, but it did not differ from the silage of soybeans. The highest contents of MM and hemicellulose, as well as the lowest of cellulose, were observed in the silage from soybeans intercropped with Congo grass (Table 2). The highest lignin content was observed in the silage from soybeans intercropped with Aruana Guinea grass, but it did not differ from the silage from soybeans intercropped with Congo grass (Table 2).

**Table 2.** Means values of DM, CP, CF, NDF, ADF, EE, MM, NDIN, ADIN, lignin, cellulose, hemicellulose, IVDDM, IVDOM, TDN, and NFC in the silages.

Parameters	Means			Standard Error			p-Value
	Soy	S + AGG	S + CG	Soy	S + AGG	S + CG	
DM (g kg <sup>-1</sup> )	179.40 b	257.80 a	191.20 b	10.00	12.30	10.00	0.0013
CP (g kg <sup>-1</sup> )	139.00 a	116.30 b	154.30 a	5.30	4.30 b	4.30	0.0051
CF (g kg <sup>-1</sup> )	364.30 b	449.30 a	369.60 b	11.30	13.80	11.30	0.0016
NDF (g kg <sup>-1</sup> )	662.90 b	711.90 a	648.80 b	8.50	8.50	8.50	0.0002
ADF (g kg <sup>-1</sup> )	630.30 ab	681.20 a	604.60 b	13.60	13.60	13.60	0.0140
EE (g kg <sup>-1</sup> )	26.40 a	17.10 b	25.30 a	0.80	1.00	8.30	0.0005
MM (g kg <sup>-1</sup> )	81.40 b	82.90 b	119.80 a	4.10	4.10	5.00	0.0029
NDIN (g kg <sup>-1</sup> )	84.50 b	209.80 a	93.30 b	3.30	5.30	3.30	0.0001
ADIN (g kg <sup>-1</sup> )	69.90 b	119.70 a	68.30 b	4.70	5.80	4.70	0.0003
Lignin (g kg <sup>-1</sup> )	96.50 b	162.40 a	148.10 ab	10.80	10.80	10.80	0.0123
Cellulose (g kg <sup>-1</sup> )	531.40 a	534.90 a	446.50 b	59.00	59.00	59.00	0.0035
Hemicellulose (g kg <sup>-1</sup> )	416.00 b	223.00 c	518.00 a	35.00	43.00	35.00	0.0366
IVDDM (g g <sup>-1</sup> )	0.543	0.541	0.543	0.0004	0.0006	0.0004	0.9957
IVDOM (g g <sup>-1</sup> )	0.551	0.547	0.555	0.0024	0.0037	0.0024	0.8634
TDN (g kg <sup>-1</sup> )	504.60	467.30	515.20	14.10	14.10	14.10	0.0731
NFC (g kg <sup>-1</sup> )	85.10	69.50	62.80	12.60	12.60	12.60	0.4040

Means followed by different lowercase letters in the rows for each variable differed from each other by the F test ( $p \leq 0.05$ ). SE: standard error.

The contents of DM, CP, CF, NDF, ADF, EE, NDIN, and ADIN showed no significant differences between the silages from soybeans and soybeans intercropped with Congo grass (Table 2). The MM and cellulose contents of the silages from soybeans and soybeans intercropped with Aruana Guinea grass did not show significant differences (Table 2), while the significant differences between silages from soybeans intercropped with Aruana Guinea grass and soybeans intercropped with Congo grass for cellulose content can be due to differences in grass proportions in each ensiled mass. The ADF content showed no statistical difference among the silages (Table 2). The lignin content of silage from

soybeans intercropped with Congo grass showed no statistical difference from the silage from soybeans (Table 2).

The IVDDM and IVDOM, TDN, and NFC of the silages did not show significant differences. Therefore, the presence of grasses in the silages did not interfere with these parameters (Table 2). The means values of the digestibility of DM and OM, TDN, and NFC silages were  $0.542 \text{ g g}^{-1}$ ,  $0.551 \text{ g g}^{-1}$ ,  $495.7 \text{ g kg}^{-1}$ , and  $72.47 \text{ g kg}^{-1}$ , respectively.

The largest DM loss was observed in the silage from soybeans intercropped with Aruana Guinea grass, which also had the lowest ensiled material density (Table 3). The silages from soybeans and soybeans intercropped with Congo grass showed no significant differences for DM loss. The values of pH, propionic acid, and ammonia-N in silages showed significant changes (Table 3). Silage from soybeans had a higher pH value, differing from silages from soybeans intercropped with tropical grasses that showed no significant differences between them. The highest levels of propionic acid and ammonia-N were observed in the silage from soybeans, which did not differ statistically from the silage from soybeans intercropped with Aruana Guinea grass. Thus, Congo grass in the silage promoted a reduction in the production of these compounds.

**Table 3.** Means values of DM losses, density, pH value, lactic acid, acetic acid, propionic acid, butyric acid, and N-ammonia in the silages.

Parameters	Means			Standard Error			p-Value
	Soy	S + AGG	S + CG	Soy	S + AGG	S + CG	
DM losses (%)	2.24 b	6.52 a	1.15 b	0.26	0.32	0.26	0.0001
Density ( $\text{kg m}^{-3}$ )	591.85 b	554.14 b	661.36 a	10.88	13.33	10.88	0.0002
pH value	5.52 a	4.97 b	4.63 b	0.08	0.08	0.08	0.0001
Lactic acid ( $\text{mg mL}^{-1}$ )	0.889	1.099	0.922	0.08	0.08	0.08	0.2091
Acetic acid (mM)	21.41	21.09	17.42	1.93	1.93	1.93	0.2624
Propionic acid (mM)	0.014 a	0.011 ab	0.010 b	0.00	0.00	0.00	0.007
Butyric acid (mM)	1.218	1.154	1.170	0.10	0.10	0.10	0.8617
Ammonia-N (% of total N)	4.88 a	4.08 ab	3.262 b	0.22	0.22	0.22	0.0008

Means followed by different lowercase letters in the rows for each variable differed from each other by the F test ( $p \leq 0.05$ ).

The silages showed no significant differences for lactic acid, acetic acid, and butyric acid contents (Table 3). The mean contents of lactic, acetic, and butyric acids in silages were  $0.97 \text{ mg mL}^{-1}$ ,  $19.97 \text{ mM}$ , and  $1.18 \text{ mM}$ , respectively. In general, silage from soybean plants intercropped with Aruana Guinea and silage from soybeans plant intercropped with Congo grasses had better characteristics than silage soybeans. Grasses ensiled with soybean promoted a balanced fermentation, improving the pH value and production of volatile fatty acids (Table 3), while the highest pH value in the silage from soybeans also corresponded to the highest acetic acid and butyric acid contents. In addition, silage from soybeans had a higher ammonia-N content (Table 3).

The proportion of Aruana Guinea grass to silage from soybeans intercropped with Aruana Guinea grass were significantly higher for DM, CP, CF, NDF, ADF, EE, MM, lignin, NDIN, ADIN, cellulose and hemicellulose contents than the proportion of Congo grass to silage from soybeans intercropped with Congo grass (Table 4). In addition, soybean proportions to silage from soybeans intercropped with Aruana Guinea grass were significantly less for these contents than soybean proportions to silage from soybeans intercropped with Congo grass, While for the grasses' contribution, no significant differences were observed for the MM and cellulose contents (Table 5). The Aruana Guinea grass had a higher contribution to the contents of DM, CF, NDF, ADF, lignin, NDIN, ADIN in the silage from soybeans intercropped with Aruana Guinea grass than Congo grass in the silage from soybeans intercropped with Congo grass (Table 5).

**Table 4.** Means values of the proportion of soybeans and grasses in DM, CP, CF, NDF, ADF, EE, MM, lignin, NDIN, ADIN, cellulose, and hemicellulose in the silages.

Parameters	Soybeans					Grasses				
	Means		Standard Error		<i>p</i> -Value	Means		Standard Error		<i>p</i> -Value
	S + AGG	S + CG	S + AGG	S + CG		S + AGG	S + CG	S + AGG	S + CG	
DM (%)	3.19 b	12.75 a	0.07	0.07	0.0001	96.81 a	87.25 b	0.07	0.07	0.0001
CP (%)	5.00 b	15.39 a	0.10	0.10	0.0001	94.99 a	84.62 b	0.10	0.10	0.0001
CF (%)	3.58 b	12.80 a	0.06	0.06	0.0001	96.42 a	87.20 b	0.06	0.06	0.0001
NDF (%)	3.25 b	10.91 a	0.16	0.16	0.0001	96.72 a	89.27 b	0.06	0.06	0.0001
ADF (%)	3.07 b	10.65 a	0.13	0.13	0.0001	96.93 a	89.35 b	0.13	0.13	0.0001
EE (%)	5.77 b	19.18 a	0.72	0.72	0.0001	94.23 a	80.82 b	0.72	0.72	0.0001
MM (%)	5.56 b	18.08 a	0.13	0.13	0.0001	94.44 a	81.92 b	0.13	0.13	0.0001
Lignin (%)	3.91 b	16.97 a	0.28	0.28	0.0001	96.09 a	83.03 b	0.78	0.78	0.0001
NDIN (%)	0.81 b	5.25 a	0.20	0.20	0.0001	99.19 a	94.75 b	0.20	0.20	0.0001
ADIN (%)	2.61 b	13.77 a	0.78	0.78	0.0001	97.39 a	86.23 b	0.17	0.17	0.0001
Cellulose (%)	2.81 b	8.95 a	0.08	0.08	0.0001	97.19 a	91.05 b	0.08	0.08	0.0001
Hemicellulose (%)	8.01 b	14.56 a	0.59	0.59	0.0015	91.99 a	85.44 b	0.59	0.59	0.0015

Means followed by different lowercase letters in the rows for each variable differed from each other by the F test ( $p \leq 0.05$ ).

**Table 5.** Means values of the contribution of soybeans and grasses in DM, CP, CF, NDF, ADF, EE, MM, lignin, NDID, ADIN, cellulose, and hemicellulose in the silages.

Parameters	Soybeans					Grasses				
	Means		Standard Error		<i>p</i> -Value	Means		Standard Error		<i>p</i> -Value
	S + AGG	S + CG	S + AGG	S + CG		S + AGG	S + CG	S + AGG	S + CG	
DM (%)	0.83 b	2.32 a	0.04	0.04	0.0001	25.45 a	15.97 b	0.07	0.07	0.0001
CP (%)	0.49 b	2.24 a	0.09	0.09	0.0001	9.27 b	12.30 a	0.56	0.56	0.0028
CF (%)	1.67 b	4.54 a	0.09	0.09	0.0001	44.91 a	30.93 b	1.15	1.15	0.0001
NDF (%)	2.43 b	7.15 a	0.10	0.10	0.0001	72.53 a	58.36 b	1.50	1.50	0.0001
ADF (%)	2.22 b	6.57 a	0.09	0.09	0.0001	70.16 a	55.20 b	1.66	1.66	0.0001
EE (%)	0.10 b	0.53 a	0.02	0.02	0.0001	1.57 b	2.11 a	0.11	0.11	0.0036
MM (%)	0.46 b	1.47 a	0.13	0.13	0.0001	7.79	7.38	0.71	0.71	0.6541
Lignin (%)	0.75 b	1.66 a	0.11	0.14	0.0006	20.30 a	8.61 b	1.25	0.97	0.0001
NDIN (%)	0.16 b	0.46 a	0.01	0.01	0.0001	18.62 a	8.69 b	0.28	0.28	0.0012
ADIN (%)	0.32 b	0.86 a	0.01	0.01	0.0001	11.97 a	5.86 b	0.58	0.58	0.0002
Cellulose (%)	1.50 b	4.59 a	0.19	0.19	0.0001	51.76	46.68	2.25	2.25	0.1072
Hemicellulose (%)	0.20 b	0.55 a	0.01	0.01	0.0001	2.38 b	4.21 a	0.22	0.22	0.0017

Means followed by different lowercase letters in the rows for each variable differed from each other by the F test ( $p \leq 0.05$ ).

In the silages from soybeans intercropped with tropical grasses, Aruana Guinea grass (96.81%) had a higher proportion of CP content in the silage from soybeans intercropped with Aruana Guinea grass than Congo grass (87.25%) in the silage from soybeans intercropped with Congo grass (Table 4). However, the contribution of Aruana Guinea grass in the silage from soybeans intercropped with Aruana Guinea grass to CP content was 3.03% lower than Congo grass in the silage from soybeans intercropped with Congo grass (Table 5). Similar responses were observed for EE and hemicellulose contents (Tables 4 and 5). For EE content, the proportion of Aruana Guinea grass (94.23%) in the silage from soybeans intercropped with Aruana Guinea grass was higher than Congo grass (80.82%) in the silage from soybeans intercropped with Congo grass. Nevertheless, the contribution of Congo grass in the silage from soybeans intercropped with Congo grass to EE content was 0.54% higher than Aruana Guinea grass in the silage from soybeans intercropped with Aruana Guinea grass. For hemicellulose, the proportion of the Aruana Guinea grass (91.99%) in the silage from soybeans intercropped with Aruana Guinea grass was higher than Congo grass (85.44%) in the silage from soybeans intercropped with Congo grass, but the contribution of Congo grass in the hemicellulose content of the silage from soybeans intercropped with Congo grass was 1.8% higher than Aruana Guinea grass in the silage from soybeans intercropped with Aruana Guinea grass (Table 5).

Silage mass in soybeans intercropped with Congo grass had 10.39% more CP from the soybeans compared to that of the silage mass in soybeans intercropped with Aruana Guinea grass (Table 4). We observed that an inclusion of 5.0% soybeans in the silage from soybeans intercropped with Aruana Guinea grass contributed only 0.49% in CP (Table 5). In short, silage from this treatment had a higher grass proportion and hence lower CP content in the silage. Likewise, other nutrients, such as DM, NDF, and ADF, showed the same behavior, in which the silage from soybeans intercropped with Congo grass had 9.56, 7.66, and 7.58% more of the referred nutrients than in the silage from soybeans intercropped with Aruana Guinea grass (Table 4). Still, the soybean contribution to the ensiled mass was low, about 0.83% for DM, 2.4% for NDF, and 2.2% for ADF. As for the other nutrients, percentages were even lower (Tables 4 and 5).

#### 4. Discussion

The DM content in the silage from soybeans intercropped with Aruana Guinea grass revealed that this grass can be a good option to increase the quality of ensiled materials compared to others [4]. In addition, soybeans harvested with Aruana Guinea grass at the same time eliminate the mixing step required for the ensiling of secondary crops grown in isolation [5].

The CP content of silages varied between 11.6 and 15.4%. According to [2], the protein content in silage must exceed 7% for the proper growth of ruminal bacteria, and levels below 7% may impair silage consumption and nutrient digestibility due to ruminal nitrogen deficiency. The high CP content in the silage from soybeans intercropped with Congo grass reflected lower nitrogen losses since lower levels of ammonia-N were observed. This was consistent with [25], who reported that adequate DM levels provide lower effluent losses and, consequently, lower proteolysis. Silage from soybeans had higher N-ammonia content. It reflected nitrogen loss in the fermentation process, probably butyric acid, which is undesirable as the growth of bacteria of the genus *Clostridium* is promoted. The authors of [5] observed that the silage from soybeans had a higher CP content associated with higher ammonia-N content (A1 fraction) and non-digestible (high-C fraction) content than silage from soybeans intercropped with corn. The authors also pointed out that these values indicate the lower protein quality of silage from soybeans.

Differences in CP and CF contents between silage from soybeans intercropped with Aruana Guinea grass and the other silages can be attributed to the diluting effect of forages that make up the mixtures of the silage as the proportion of ensiled materials varied between silages, with a lower proportion of soybeans in the CP (5.0% soybeans and 95.0% Aruana Guinea grass) (Table 4) and CF (3.58% soybeans and 96.42% of Aruana Guinea grass) of the silage from soybeans intercropped with Aruana Guinea grass. The authors of [25] also observed a reduction in the CP content of silage with a reduction in the proportion of legumes in relation to the grass in the silage masses.

Fibrous fractions in forages must be quantified and qualified for characterization of their nutritive value. These fractions have a strong relationship with intake regulation, digestibility, passage rate, and chewing activity in ruminant feeding. High-fiber diets often tend to have less energy density and limited intake due to the filling of the rumen, although low-fiber ruminant diets may increase metabolic disturbances [26]. NDF and ADF contents in silage from soybeans intercropped with Aruana Guinea grass were, on means, 1.1 times higher than other silages (Table 2). However, the 63.10 g kg<sup>-1</sup> difference in NDF between silage from soybeans intercropped with Aruana Guinea grass and silage from soybeans intercropped with Congo grass may be related to the proportion of grass in each silage (Tables 2 and 4). The authors of [27] studied the chemical composition of forages from grasses intercropped with soybeans and observed differences in ADF and NDF contents between forage from soybeans intercropped with Congo grass and soybeans intercropped with *Panicum maximum* cv. Mombaça. The authors emphasized that the higher the ADF content found, the lower the digestibility reached. We observed the same, with

silage from soybeans intercropped with Aruana Guinea grass having the highest ADF and lowest digestibility.

The highest EE content was observed in the silage from soybeans and the silage of soybeans intercropped with Congo grass, that is, silage masses with a higher proportion of soybean in the ensiled material (Table 4). According to [28], EE content is the fat-soluble component in silages and has a positive correlation with silage quality since, in general, silages with high EE content promote high energy.

Regarding differences in MM content, the material of the silage from soybeans intercropped with Congo grass in pre-silage (Table 1) may have favored higher content of MM (12.0%) in the silage (Table 2), as already reported by [6].

The contents of NDIN and ADIN (Table 2) demonstrate that silage from soybeans intercropped with Aruana Guinea grass has a higher fraction of nitrogen bound to the cell walls (NDIN and ADIN); similar results were observed by [29]. However, according to [25], differences between NDIN and ADIN represent the slowly degradable true protein and are considered as the B3 fraction, in which silage from soybeans intercropped with Aruana Guinea grass would present 9.01%, making it less attractive than silage from soybeans intercropped with Congo grass, which would present 2.50%.

Lignin is the main limiting factor for the cell-wall degradation of feed in the rumen. However, rumen degradation of soybeans is less problematic in terms of resistance than grasses, which depend on their stem–leaf relationship [25]. Normally, C3 plants (legumes) have fibrous structures in their leaves that are more digestible than C4 plants (grasses) due to the carbon bonds. The lower lignin content in silage from soybeans and silage from soybeans intercropped with Congo grass may be related to the grass maturity stage at harvesting for ensiling. Therefore, maturity changes cellulose, hemicellulose, and lignin contents, which are constituents of the cell wall [4]. This fact can be observed in the results in Table 2, which reveals higher levels of CF, ADF, and NDF and a lower hemicellulose content in silage from soybeans intercropped with Aruana Guinea grass.

Hemicellulose is the most digestible fiber fraction and is where cell wall-forming monosaccharides are incorporated [30]. The authors of [31,32] reported that when plants enter advanced growth stages and senescence, lignin-forming compounds are formed. According to [27], it is interesting that silage has a low cellulose content and high hemicellulose content, as ruminants transform these components, through the action of their bacterial flora into short-chain fatty acids (SCFA). In the present study, we noted that silage from soybeans intercropped with Congo grass had low cellulose content and high hemicellulose content compared to the other silages. Although higher hemicellulose contents in silage are desirable to increase volatile fatty acids (VFAs) in the rumen, tropical forages should have higher proportions of cellulose in their fibrous fractions, and ruminants are specialized in using them in the rumen environment efficiently when compared to monogastric animals. During forage conservation, soluble sugars and highly degradable fibers, such as hemicelluloses, are the first to be used within the first 12 h of fermentation. Thus, during the fermentation of sugars and highly degradable fibers, anaerobic microorganisms quickly form lactic acids, which are responsible for reducing the silage pH value, thus improving the stability of the ensiled mass [30]. This fact was confirmed by the numerical contents of lactic acid (1.1 vs. 0.92,  $p > 0.05$ ) for treatments that had lower soybean proportions in the silage (S + AGG, 3.19% versus S + CG, 12.75% in DM). Legumes are known for having greater buffering power for pH value reduction in preserved silages [32]. This was also observed for the silage from soybeans (soy, 5.5 pH value vs. 4.95 for S + AGG and 4.63 for S + CG).

The highest ADF values corresponded to the lowest TDN contents (Table 2). TDN refers to nutrients that animals can use, and they are negatively correlated with ADF content in forage. In other words, as the ADF content increases in forage, TDN decreases; therefore, the nutrients will be less used by animals [4]. Table 2 reveals that the lowest NFC values corresponded to the highest fibrous components (NDF, ADF, and lignin contents).

NFC make up the fraction of foods with high energy-production capacity, especially starch, sugars, and pectin [33].

DM losses in the present study (1.15 to 6.52%) are considered low regardless of the silage, and the presence of liquid effluents was not observed (Table 3). Silages' density varied from 554.14 to 661.36 kg m<sup>-3</sup> (Table 3). These high densities provided lower DM losses and corroborate the results of [34], who mentioned that DM losses decrease as silage density increases. DM losses associated with fermentation in silos are primarily a function of carbon dioxide production but depend on dominant microbial species and fermented substrates [34].

The pH value of an ensiled sample is a measure of its acidity [35]. The silage from soybeans had the highest pH value, differing from the other silages tested (Table 3). According to [36], increases in the pH value of silage from soybeans occurs mainly due to an increase in its buffering power, which is caused by proteolysis. This reaction occurs by releasing ammonia during fermentation, which makes it difficult for the pH value to decrease as this silage has a basic pH value character. The lower pH value in silage from soybeans intercropped with grasses reveals that the presence of grasses in ensiled material may have reduced the buffering effect of soybeans, evidencing the technical feasibility of intercropped silages [7]. On the other hand, the highest pH value in the silage from soybeans also corresponded to the highest acetic acid and butyric acid contents (Table 3).

Ammonia-N content in silage is indicative of protein degradation during ensiling [36]. Ammonia-N content in silage from soybeans intercropped with Aruana Guinea grass was intermediate compared to the other silages. The highest ammonia-N content was observed in the silage from soybeans (Table 3); therefore, protein degradation was higher in this silage. However, [37] highlighted that ammonia-N content below 10%, as observed in the present study, indicates a low degradation of protein compounds by proteolytic enzymes, which are secreted especially by bacteria of the genus *Clostridium*; the author posited that relatively low pH values may have helped to prevent the development of these bacteria (genus *Clostridium*), which are among the main spoilage microorganisms in silages. In the present study, the lowest pH value was also correlated to the lowest ammonia-N content (Table 3). The highest content of ammonia-N in silage from soybeans may have resulted from a greater proteolytic activity in this silage. Proteolysis can promote the accumulation of ammonia-N, while reducing the true protein content in silages [5]. Briefly, fermentation increases soluble N (between 55 and 60% of total N) and ammonia-N contents (generally less than 10–15% of total N) [35].

Lactic acid is a product of silage fermentation and can rapidly reduce the silage pH value, thus preventing the development of undesirable microorganisms [38]. Table 3 shows that the content of lactic acid in silages ranged from 0.89 to 1.10 mg ml<sup>-1</sup>. The authors of [35] mentioned that, in general, lactic acid content (pKa of 3.86) in silages ranges from 2 to 4% of DM and that this acid is stronger than the other main acids found in silages, such as acetic acid (pKa of 4.75) and propionic acid (pKa of 4.87). However, [39] emphasized that lactic acid acts directly on the fermentation process, decreasing the pH value of the ensiled mass and thus keeping it within acceptable values of acetic and propionic acids.

In the current study, the acetic acid content in silages ranged from 17.42 mM to 24.40 mM, with no significant difference among silages (Table 3). In general, the acetic acid content in silages ranges from 1 to 3% of the DM, and when consumed by ruminants, it can be absorbed by the rumen and used for energy or incorporated into milk or body fat. Moderate content of acetic acid in silage may be beneficial because it inhibits yeasts, improving silage stability when exposed to the air [35].

Propionic acid inhibits the development of undesirable fungi in silage [38]. In good quality silages, propionic acid is usually found in very low quantity (<0.1%), and, when consumed, it is absorbed by the rumen and converted into glucose by the ruminant liver [35]. The low propionic acid content in silage from soybeans intercropped with Aruana Guinea grass and silage from soybeans intercropped with Congo grass highlighted better fermentation conditions for these silages than silage from soybeans.

Butyric acid is generally produced by bacteria of the genus *Clostridium*, which causes poor fermentation and an unpleasant odor in silages [38]. Therefore, it should not be detectable in well-fermented silages [35]. In this study, the butyric acid content in the silages ranged from 1.15 mM to 1.22 mM, with no significant differences among them (Table 3). *Clostridium* bacteria are capable of fermenting sugars into butyric acid (*Saccharolytic* bacteria) and converting lactic acid into butyric acid. Paradoxically, silages with butyric acid tend to be stable when exposed to the air, as it has strong antifungal characteristics [35].

Upon comparing the silages, silage from soybeans with Aruana Guinea grass had a higher proportion of grass than did the silage from soybeans with Congo grass, which had a higher proportion of soybeans in the DM (12.75% vs. 3.19%) (Table 4). This difference may have been due to an imbalance in soybean crops during the development phase. In this sense, competition and poor availability of environmental resources, such as water and light, are among the factors that can promote changes in the development of plants [32]. Shading effects on soybeans can reduce the yield when intercropped, reducing the photosynthetic rate [32], soybean leaf proportion or size, and the leaf area index in the silage mass [5]. Thus, soybeans have lower participation in the silage from soybeans with Aruana Guinea grass (0.83% of DM).

According to [40], the mean dry biomass production of the soybeans in monoculture was 84.13 kg ha<sup>-1</sup>; of the soybeans intercropped with Aruana Guinea grass, 53.87 kg ha<sup>-1</sup>; and of the soybeans intercropped with Congo grass, 88.38 kg ha<sup>-1</sup>. In terms of grasses, the mean of dry biomass production in Aruana Guinea grass intercropped with soybeans was 5527.28 kg ha<sup>-1</sup>, and the mean of dry biomass production in Congo grass intercropped with soybeans was 5189.61 kg ha<sup>-1</sup>.

## 5. Conclusions

The silage from soybeans intercropped with Aruana Guinea grass and silage from soybeans intercropped with Congo grass had better nutritional and fermentative characteristics than silage soybeans. Then, the use of the tropical grasses as a component to improve the quality of silages from soybeans is an alternative for forage conservation in ruminant production systems, especially during the dry season. The growth stage of the grass at the time of cutting for ensilage can cause changes in the fiber content of the intercropped silage.

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