doi: 10.17138/TGFT(10)134-142

Research Paper

Protein and carbohydrate fractionation of silages made from maize, *Urochloa* species and their mixtures

Fraccionamiento de proteínas y carbohidratos de ensilajes de maíz, especies de Urochloa *y sus mezclas*

DANIEL AUGUSTO ALVES TEIXEIRA[1], KÁTIA APARECIDA DE PINHO COSTA[2], MARIANA BORGES DE CASTRO DIAS[2], KÁTIA CYLENE GUIMARÃES[2], PATRÍCIA SOARES EPIFANIO[2] AND PATRICK BEZERRA FERNANDES[3]

[1]*Universidade Federal de Goiás, Goiânia, GO, Brazil.* ppgz.evz.ufg.br
[2]*Instituto Federal Goiano, Rio Verde, GO, Brazil.* ifgoiano.edu.br
[3]*Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, Brazil.* ufms.br

Abstract

New feed assessment systems and methodologies for ruminants are being used with the aim of maximizing the use of nutrients by animals. The Cornell Net Carbohydrate and Protein System (CNCPS) considers the dynamics of ruminal fermentation and the potential loss of nitrogen in feed evaluation. We used this system to evaluate the protein and carbohydrate fractionation of silages made from maize and *Urochloa* species alone and in combination (70:30). The experiment was carried out under a completely randomized experimental design with 4 replications. Treatments comprised silages made from the following forages: maize (*Zea mays*); Congo grass (*Urochloa ruziziensis*); Xaraes palisadegrass (*U. brizantha* 'Xaraés'); Paiaguas palisadegrass (*U. brizantha* 'BRS Paiaguás'); 70% maize + 30% Congo grass; 70% maize + 30% Xaraes palisadegrass; and 70% maize + 30% Paiaguas palisadegrass. The results showed that despite the *Urochloa* exclusive silages having higher crude protein concentration than maize and mixed silages, they have a higher proportion of unavailable fractions with slow degradation rates, which can compromise animal performance. The maize silage and mixed silages had higher percentages of protein and carbohydrates with high degradation potential than *Urochloa* exclusive silages. Therefore, mixed silages represent one more alternative to provide forage with good nutritional value for ruminant feeding in times of feed shortage. Mixing grass and maize at ensiling would increase the volume of silage produced relative to ensiling maize alone without any significant reduction in quality of the silage produced. However, further studies are needed to determine the appropriate combinations of maize and grass at ensiling to produce silage with the desired nutritional value for the particular application and class of animals being fed. Feeding studies with animals would verify production levels achieved with the various silages.

Keywords: Ruminal degradation, total carbohydrates, tropical forage, *Zea mays*.

Resumen

Se están utilizando nuevos sistemas y metodologías de evaluación de alimentos para rumiantes con el objetivo de maximizar el uso de nutrientes por parte de los animales. El Cornell Net Carbohydrate and Protein System (CNCPS) es un sistema que considera la dinámica de la fermentación ruminal y la pérdida potencial de nitrógeno en la evaluación de alimentos. Por tanto, el objetivo de este estudio fue evaluar el fraccionamiento de proteínas y carbohidratos del ensilado de maíz y de especies de *Urochloa*, solos o combinados en proporción 70:30. El estudio se llevó a cabo bajo un diseño experimental completamente al azar con cuatro repeticiones. Los tratamientos comprendían los siguientes tipos de ensilado: maíz (*Zea mays*), *U. ruziziensis*, *U. brizantha* cv. Paiaguás, *U. brizantha* 'Xaraés', 70% maíz + 30% *U. ruziziensis*, 70% maíz + 30%

Correspondence: K.A.P. Costa, Instituto Federal Goiano, Rodovia Sul Goiana - km 1 - Zona Rural, Rio Verde, GO, CEP 75901-970, Brazil. E-mail: katia.costa@ifgoiano.edu.br

U. brizantha 'BRS Paiaguás', 70% maíz + 30% *U. brizantha* 'Xaraés'. Los resultados mostraron que los ensilajes hechos de solo forrajes del género *Urochloa*, si bien tenían mayores contenidos de proteína cruda que los de maíz y mixtos, presentan una mayor proporción de fracciones no disponibles con bajas tasas de degradación, lo que puede comprometer la ganancia de peso. Los ensilajes de maíz y mixtos presentaron niveles más altos de proteína y carbohidratos con alto potencial de degradación que los ensilajes de solo pasto. Por lo tanto, los ensilajes mixtos representan una alternativa más para brindar alimentos con buen valor nutritivo para la alimentación de los rumiantes en épocas de escasez de forraje. Mezclar pasto y maíz en el ensilaje aumentaría el volumen de ensilaje producido comparado con el ensilaje de maíz solo, sin una reducción significativa en la calidad del ensilaje producido. Sin embargo, se necesitan más estudios para determinar las combinaciones apropiadas de maíz y pasto en el ensilado para producir ensilaje con el valor nutricional deseado para la aplicación particular y la clase de animales que se alimentan. Los estudios de alimentación con animales permitirían verificar los niveles de producción alcanzados con los distintos ensilajes.

Palabras clave: Carbohidratos totales, degradación ruminal, forrajes tropicales, *Zea mays*.

Introduction

Conservation of forage as silage has become a common practice in Central Brazil in order to maintain livestock production throughout the year, since growth and forage quality of tropical grasses are reduced during the dry season (Daniel et al. 2019).

Of materials recommended for silage making, maize is considered the standard, owing to its high energy concentration and favorable characteristics for silage, i.e. high dry matter yield, high soluble carbohydrate concentration and low buffering capacity (Ferraretto et al. 2018). As a result, maize silage is the primary silage used in diets for dairy cows in Brazil and USA (Grant and Adesogan 2018; Daniel et al. 2019).

Other forages such as tropical grasses, e.g. *Urochloa* spp., produce high forage yields with nutritive value adequate to support weight gains, when harvested at the appropriate time. On the other hand, low soluble carbohydrate concentration in most tropical forages may limit the proper fermentation process (Anjos et al. 2020).

In this context, the production of silages of mixtures of maize and tropical grass forage becomes an interesting option, with benefits such as increased silage mass (Souza et al. 2019; Oliveira et al. 2020) and balanced nutritional value, with possible increased crude protein concentration (Paludo et al. 2020). Maize silage is considered an energy feed, with lower crude protein concentration than tropical grass silage, depending on the stage of harvesting of the grass and soil fertility/N application rates (Souza et al. 2019).

The chemical composition of mixed silage, e.g. maize + pasture grass, may differ from that of maize silage, since 2 distinct forages are involved. Therefore, the determination of protein and carbohydrate fractions is necessary to estimate the nutritional value of these mixed silages, important in livestock feeding, to formulate balanced diets and to improve the synergism between nutrients, so as to optimize animal performance (Bumbieris Junior et al. 2011).

Owing to the advancement of research related to ruminant nutrition, new systems and methodologies for food evaluation have been developed. The Net Carbohydrate and Protein System (CNCPS) aims to: estimate degradation rates of different feed fractions in the rumen; synchronize production of protein and carbohydrate in the rumen to maximize utilization and consequently microbial production; and also minimize nitrogen losses (Sniffen et al. 1992; Higgs et al. 2015).

Currently, there is a lack of information on protein and carbohydrate fractionation in mixed silages. The hypothesis is that mixing *Urochloa* species with maize at ensiling would improve silage quality, with higher fractions of proteins and carbohydrates with rapid and intermediate degradation rates. Therefore, the objective of this study was to evaluate the protein and carbohydrate fractionation of silages made from maize and *Urochloa* species alone and in combination. Data for dry matter yields, fermentation profiles and chemical composition of the silages have been published by Teixeira et al. (2021).

Materials and Methods

Experimental site

The forages for the experiment were planted in the field (17°48' S, 50°55' W; 748 masl) in Rio Verde, Goiás state, in a soil characterized as a Dystroferric Red Latosol (Santos et al. 2018). Before the beginning of the experiment, soil samples were collected from the 0–20 cm horizon with the aid of a Dutch soil auger for soil chemical analysis. Characteristics of the soil were as follows: 450, 200 and 350 g/kg clay, silt and sand, respectively; pH in $CaCl_2$: 5.4; Ca: 2.1 cmolc/dm^3; Mg:

1.3 cmolc/dm^3; Al: 0.05 cmolc/dm^3; Al + H: 2.3 cmolc/dm^3; K: 0.24 cmolc/dm^3; cation exchange capacity (CEC): 5.94 cmolc/dm^3; base saturation (V1): 61.3%; P (Mehlich): 1.2 mg/dm^3; and OM: 27.5 g/kg.

Statistical design, treatments and crop planting

The experimental design was completely randomized with 4 replications. Treatments were: maize (Zea mays); Congo grass (*Urochloa ruziziensis*, syn. B*rachiaria ruziziensis*); Xaraes palisadegrass (*U. brizantha*, syn. *B. brizantha*, 'Xaraés'); Paiaguas palisadegrass (*U. brizantha*, syn. *B. brizantha*, 'BRS Paiaguás'); 70% maize + 30% Congo grass; 70% maize + 30% Xaraes palisadegrass; and 70% maize + 30% Paiaguas palisadegrass (Teixeira et al. 2021). Proportions of forages were calculated on a fresh matter basis.

Maize and grasses were sown with MF 510 double disc seeders; the maize hybrid used was P3779H. Simple superphosphate was applied at 1,000 kg/ha along with 80 kg KCl/ha. When the corn plants reached the development stage V4 (4 leaves) and V6 (6 leaves), 50 kg N/ha was applied as urea.

A row spacing of 50 cm was used for maize, and the pasture grass was sown down the centre of the maize inter-rows. All species were sown at a depth of 2 cm. Each plot was 14 m long and 6.5 m wide. The material used for silage production was the central 4 rows, less 0.5 m on each end.

During the experiment, chlorfenapyr insecticide was applied twice at a rate of 0.5 L/ha of the commercial product, using a sprayer. Weed control was performed manually.

Ensilage

For the ensiling process, both maize and grasses were harvested at 105 days following sowing (maize at soft dough-hard dough transition phase and grass preflowering), and the dry matter concentration of maize forage was 338 g/kg. The forages were cut separately at a height of 20 cm from the soil using a manual harvester. Subsequently, the forages were chopped separately into segments of approximately 10 mm using a stationary chopper. Then, for the mixed silages, combinations of fresh maize forage and the fresh appropriate grass on a 70:30 basis were prepared and thoroughly mixed.

The material was packed into experimental PVC silos, measuring 10 cm in diameter and 40 cm in length, and compacted with an iron pendulum throughout the process to reach an average density in the silos of 1.12 kg/dm^3. The silos were closed with PVC caps and sealed with adhesive tape to prevent the entry of air. Thereafter, the experimental silos were stored inside the laboratory at room temperature (average 25 °C).

Opening of silos and chemical analyses of silages

Fifty days after ensiling, the silos were opened and the upper and lower portions of the contents of each silo were discarded. The central portion of the silo contents was homogenized and placed in plastic trays.

Samples of the material (approximately 0.5 kg) were selected, weighed, dried in a forced-ventilation oven at 55 °C to constant weight before reweighing and then milled in a knife mill with a 1 mm sieve and stored in plastic containers prior to analysis.

Analyses of silages

Chemical analyses of the silages were carried out according to the methods described by AOAC (1990) to determine dry matter (DM) (Method 934.01), ash (Method 934.01), total N (Method 920.87) and ether extract (EE) (Method 920.85). Neutral detergent fiber (NDF) concentration was determined according to Mertens (2002), acid detergent fiber (ADF) by Method 973.18 (AOAC 1990) and lignin concentration in 13.51 M sulfuric acid (Van Soest and Robertson 1985). Total digestible nutrients (TDN) were calculated using the equation (TDN % = 105.2 – 0.68 × NDF %) proposed by Chandler (1990). These data and those for fresh forage before ensiling were published by Teixeira et al. (2021).

Non-protein nitrogen (NPN) plus nitrogen insoluble in neutral (NIND) and acid detergent (NIAD) were determined according to the methodology described by Licitra et al. (1996). Soluble nitrogen (SN) was determined according to Krishnamoorthy et al. (1983).

Protein fractionation was calculated by using the CNCPS system of Sniffen et al. (1992). Protein was analyzed and calculated in 5 fractions (A, B1, B2, B3 and C). Fraction A (non-protein N; NPN) was determined as the difference between total nitrogen (total N) and N insoluble in trichloracetic acid (TCA). Fraction B1, composed of soluble proteins that rapidly degrade in the rumen, was the difference between nitrogen soluble in borate phosphate buffer (TBF) and NPN. Fractions B2 and B3, consisting of insoluble proteins with intermediate and slow degradation rates in the rumen, were determined as the difference between the fraction insoluble in TBF and the NIND fraction (Fraction B2) and NIND minus NIAD (Fraction B3). The C fraction, consisting of insoluble and indigestible rumen proteins, was determined as the residual nitrogen in the sample after being treated with acid detergent (NIAD) and expressed as a percentage of the total N in the sample.

Total (TC) and non-fibrous (NFC) carbohydrates were determined by the expressions: TC = 100 – (% CP + % EE + % ash); and NFC = 100 – (% CP + % EE + % NDFap + mineral matter/MM), where NDFap is equivalent to the neutral detergent fiber corrected for ash and protein. The B2 fraction was calculated as NDFap – Fraction C and Fraction C by the percentage of lignin multiplied by 2.4 (Sniffen et al. 1992).

Statistical analyses

The variables were subjected to analysis of variance (R program, version R-3.1.1, with the ExpDes package) (Ferreira et al. 2015). The averages were compared using Tukey's test, with a significance level of P<0.05.

Results

Protein fractionation (Fractions A, B1, B2, B3 and C) was different (P<0.05) for the different treatments (Table 1). Fraction A (non-protein N) ranged from 39.4 to 53.3%, being highest for maize silage and lowest for pure grass silages (P<0.05), while mixed silages were intermediate (P<0.05).

On the other hand, B1 fraction ranged from 18.1 to 20.2% with no significant difference between treatments (P>0.05). For the B2 fraction (Table 1), the range was 12.7–14.3%, with maize and maize-Xaraes palisadegrass silages presenting higher values than the pure grass silages (P<0.05). Values for the B3 fraction ranged from 6.2 to 15.5% with the highest value for Congo grass silage and lowest for maize silage (P<0.05). For the C fraction (Table 1), values ranged from 7.2 to 13.4% with again highest values for the pure grass silages and lowest for maize silage (P<0.05).

Total carbohydrate and carbohydrate fractionation (fractions A + B1, B2 and C) also varied between the different silages. Total carbohydrate (TC) levels ranged from 80.1 to 88.8% (Table 2), with the highest values for the pure grass silages and the lowest for maize silage (P<0.05). For the A + B1 fraction, highest values (45.1%) were recorded for maize silage and lowest (25.9%) for Congo grass and Xaraes palisadegrass (P<0.05). In contrast, values for the B2 fraction were highest (60.9–63.9%) for pure grass silage and lowest (49.9%) for maize silage. A similar pattern emerged for Fraction C.

Table 1. Protein concentration (g/kg DM) and protein fractions (%) of silages made from maize and *Urochloa* species alone and in 70:30 mixtures.

Silage	CP[1]	Fraction				
		A	B1	B2	B3	C
Maize	64.6d[2]	53.3a	19.2a	14.2a	6.2d	7.2c
Congo grass	82.5b	39.4c	18.6a	13.2b	15.5a	13.4a
Xaraes palisadegrass	92.2b	40.1c	19.9a	13.0b	14.2b	12.9a
Paiaguas palisadegrass	101.7a	42.1c	20.2a	12.7b	13. 1b	11.8a
70% Maize + 30% Congo grass	72.5c	47.4b	18.5a	13.4ab	10.1c	10.7b
70% Maize + 30% Xaraes palisadegrass	85.1bc	48.0b	18.2a	14.3a	9.9c	9.6b
70% Maize + 30% Paiaguas palisadegrass	85.0bc	51.0b	18.1a	13.4ab	9.4cd	8.2b
CV (%)	6.51	2.0	3.8	8.5	8.4	5.2
s.e.m.	0.27	0.46	0.38	0.55	0.48	0.28

[1]Data from Teixeira et al. (2021).

[2]Means within columns followed by different letters differ by Tukey test at 5% probability.

Table 2. Total carbohydrates (%) and carbohydrate fractions (%) of silages made from maize and *Urochloa* species alone and in 70:30 mixtures.

Silage	Fraction			
	TC[1]	A + B1	B2	C
Maize	80.1c[2]	45.1a	49.9d	5.0c
Congo grass	88.1a	25.9d	63.9a	10.1a
Xaraes palisadegrass	88.8a	28.6d	61.7ab	9.6a
Paiaguas palisadegrass	86.9a	30.2c	60.9ab	8.9ab
70% Maize + 30% Congo grass	83.6b	32.6b	60.0bc	7.5b
70% Maize + 30% Xaraes palisadegrass	82.7b	36.0b	56.4c	7.6b
70% Maize + 30% Paiaguas palisadegrass	82.6b	35.3b	56.9c	7.8b
CV (%)	0.7	4.4	2.8	8.3
s.e.m.	0.31	0.73	0.83	0.34

[1]TC: total carbohydrate.

[2]Means within columns followed by different letters differ by Tukey test at 5% probability.

Discussion

This study has shown that mixing *Urochloa* grasses with maize forage at ensiling has significant impacts on both protein and carbohydrate fractionation in the resulting silages.

While Paiaguas palisadegrass silage showed the highest CP concentration and pure maize silage the lowest CP concentration with mixed silages intermediate (Teixeira et al. 2021), the proportions of the various protein fractions altered the nutritive value of the protein contained. According to Lazzarini et al. (2009), ruminants should receive a diet with at least 70 g/kg CP to not compromise the efficiency of ruminal microorganisms in using the fibrous carbohydrates present in silages. Therefore, with the exception of exclusive maize silage, the other silages presented CP concentrations that were higher than was recommended by the above-mentioned authors.

The finding of the highest values for the A fraction of the proteins in maize silage in relation to the mixed and grass silages was possibly due to the amount of starch in the maize grains, which were at the soft dough-hard dough transition phase (Souza et al. 2019), which favors better degradation inside the rumen. While inclusion of 30% *Urochloa* spp. forage with maize at ensiling provided higher A fraction than in pure grass silage, which suggests better rumen degradation than for pure grass silage, the A fraction in the mixed silage was still lower than in pure maize silage.

According to Russell et al. (1992), fraction A is essential for good ruminal functioning, since ruminal carbohydrate-fermenting microorganisms use ammonia as a nitrogen source. This fraction, formerly classified as non-protein nitrogen, was reclassified as ammonia to facilitate analysis and provide a better prediction of the metabolizable protein contribution from free amino acids and small peptides (Higgs et al. 2015). These improvements increased the ability to detect the most limiting nutrient, allowing the user to refine the diet formulation to improve the productive efficiency of cattle (Van Amburgh et al. 2015).

Proteins in the ensiled material can be converted into non-protein nitrogen due to the fermentation processes that may occur inside the silo (Dong et al. 2019). Therefore, build-up of high concentrations of non-protein nitrogen in silage can result in nitrogen losses if there is a lack of a carbon skeleton readily available for microbial protein synthesis in the rumen (Santos et al. 2020). It is worth noting that plant and microbial proteolytic processes lead to changes in nitrogen compounds in silages, which can result in soluble N increasing to between 55 and 60% of total N, while NH_3-N is usually less than 10–15% of total N (Kung et al. 2018). Therefore, the balance between non-protein nitrogen and soluble carbohydrates is very important to avoid nutrient losses and keep ruminal microorganisms active (Queiroz et al. 2011).

The silages showed protein B1 fraction values ranging from 17.1 to 20.2%, which represent true soluble proteins and tend to be extensively degraded in the rumen (Pires et al. 2009). Bacteria that ferment structural carbohydrates use this fraction as a nitrogen source (Sniffen et al. 1992). High proportions of the B1 fraction may result in nitrogen leakage to the intestines if there is lack of a carbon skeleton that is readily available for microbial protein synthesis; this confirms the importance of the appropriate carbohydrate and protein balance for ruminal microorganisms (Queiroz et al. 2011; Negrão et al. 2014).

The lower protein B2 fraction values (Table 1) observed in the *Urochloa* silages may be related to the higher concentrations of the fibrous fraction and lower digestibility of grasses (Teixeira et al. 2021). The pure grass silages showed average concentrations of NDF of 698 g/kg DM and ADF of 422 g/kg DM. Higher fiber fractions reduce the ruminal degradation of silages, since the B2 fraction represents the protein fraction with intermediate degradation rates because it is associated with potentially degradable fiber with a slower degradation rate (Sniffen et al. 1992).

The B3 fraction values correspond to cell wall-associated proteins with slow rumen degradation (Leite et al. 2021) and which are mainly digested in the intestines (Ferreira et al. 2018). Even though pure *Urochloa* spp. silages presented higher crude protein concentrations than maize and mixed silages (Teixeira et al. 2021; Table 2), there was a lower degradation rate due to the higher NDF concentrations in tropical grasses relative to maize. Although the *Urochloa* silages presented higher concentrations of the B3 fraction, mixing maize and grass when ensiling reduced the overall concentration of the B3 fraction. This would result in better utilization of the resulting silage by ruminants.

The lower protein C fraction values (considered indigestible) in the maize silage may have been related to the lower lignin concentration in maize relative to the grasses (Teixeira et al. 2021), because the C fraction corresponds to lignin-associated proteins, tannin-protein complexes and products from the Maillard reaction, which are highly resistant to microbial breakdown and enzyme activity along the gastrointestinal tract (Licitra et al. 1996).

According to Van Soest (1994), an increase in the C fraction in silages may occur due to the formation of Maillard reaction products, caused by the temperature increase inside the silo resulting from undesirable fermentations. This phenomenon is generally common in silages with a high moisture content and with a higher amount of fibrous fractions, characteristics commonly found in tropical grasses, which explains the higher concentration of this indigestible fraction in the *Urochloa* silages and the mixed silages. Low concentrations of this indigestible fraction in the maize silage are interesting from a nutritional point of view (Branco et al. 2010). Viana et al. (2012) evaluated the protein fractionation of silages from different forages and found that maize silage presented a C fraction of 14.1%, which suggests that further studies are needed to determine if the concentrations recorded for maize in the current study are abnormal.

Protein fractionation is important because rate and level of protein degradation in the rumen have marked impacts on supplying nitrogen to rumen microorganisms as well as N losses if rate of breakdown is too rapid.

The finding of the highest total carbohydrate values (TC) in the *Urochloa* silages would be a function of higher NDF, ADF and lignin levels present in those tropical grasses at ensiling (Teixeira et al. 2021), since Brandstetter et al. (2019) reported that fiber present in tropical grasses represents most of the total carbohydrates in the pasture. However, all silages contained at least 80% total carbohydrates, which is adequate to support satisfactory animal performance.

The high non-fibrous carbohydrate levels, represented by the A + B1 fraction in the maize silage would be due to the starch content of the maize grains, which were at the soft dough-hard dough transition phase (Souza et al. 2019), which gives rise to these fractions. High starch concentrations in corn silage result in high energy content and high dry matter degradation (Refat et al. 2017), which is followed by high rates of passage of ingesta and high propionate production, that is related to increased dry matter intake by animals (Oba and Allen 2000). According to the Cornell Net Carbohydrate and Protein System model, fraction A consists of sugars, and the B1 fraction consists of starch, pectin and glucans (Sniffen et al. 1992).

Feed with high concentration of the A + B1 fraction is a good source of energy for ruminal microbial growth (Carvalho et al. 2007), and the higher the A + B1 fraction, the greater the need to supply rapidly degrading proteins to synchronize carbohydrate and protein fermentation in the rumen, i.e. a simultaneous release of energy and nitrogen, which has an important effect on the final products of fermentation and animal production (Russell et al. 1992). With regard to potentially digestible fibrous carbohydrates, corresponding to the B2 fraction, the high values observed in pure grass silages can be explained by the high NDF concentrations in these tropical grasses at ensiling (Teixeira et al. 2021). It is of interest that the mixed silages had lower A fraction than maize silage but B1 and B2 fractions were not significantly different for pure maize and mixed silages.

The B2 fraction provides slow energy in the rumen and may affect efficiency of microbial synthesis and hence animal performance. In cases of high levels of this fraction, forage must be supplemented with energy sources that are readily available in the rumen, when there are no protein limitations in terms of quantity or quality (Epifanio et al. 2014). In this context, it is important to highlight that making mixed silages lowered B2 fraction levels in the silage relative to those for pure grass silage.

Concentrations of the C fraction, considered indigestible in the gastrointestinal tract, were clearly higher in the Urochloa silages, possibly reflecting the high lignin concentrations in grass at ensiling.

It is known that the B2 fraction, which is the main component of tropical forage silages, presents a slow degradation rate, which combines with the C fraction (indigestible) to impair animal consumption via rumen physical limitation, reducing animal performance (Ferreira et al. 2018). It is necessary to examine strategies to increase the quality of silage produced, possibly by mixing cereals and grasses, but appropriate levels and quality of tropical forages must be determined, so as not to compromise the quality of the silage produced.

Conclusions

Despite the *Urochloa* exclusive silages having higher crude protein concentration than maize and mixed silages, they have a higher proportion of unavailable fractions with slow degradation rates, which can compromise animal performance. The maize silage and mixed silages had higher levels of protein and carbohydrates with high degradation potential than pure grass silages. Therefore, mixed silages represent one more alternative to provide forage with good nutritional value for ruminants in times of feed shortage.

Mixing grass and maize at ensiling would increase the volume of silage produced relative to ensiling maize alone without any significant reduction in quality of the silage produced. However, further studies are needed to determine the appropriate combinations of maize and grass at ensiling to produce silage with the desired nutritional value for the particular application and class of animals being fed. Feeding studies with animals would verify production levels achieved with the various silages.

Acknowledgments

The authors thank the Instituto Federal Goiano for financially supporting this study.

References

(Note of the editors: All hyperlinks were verified 4 February 2022).

Anjos AJ; Coutinho DN; Freitas CAS; Macêdo AJS; Sena HP; Mata e Silva BC; Oliveira GM; Raimundi TAJ. 2020. Potentials and challenges in making silages using tropical forages. Scientific Electronic Archives 13:129–136. doi:10.36560/13920201205

AOAC (Association of Official Analytical Chemists). 1990. Official methods of analysis. 15th Edn. AOAC Inc., Arlington, VA, USA.

Branco RH; Rodrigues MT; Silva MMC da; Rodrigues CAF; Queiroz AC de; Araújo FL de. 2010. Effect of dietary forage fiber levels on intake, production and efficiency of utilization of nutrients of lactating goats. Revista Brasileira de Zootecnia 39:2477–2485. (In Portuguese). doi: 10.1590/S1516-35982010001100022

Brandstetter EV; Costa KAP; Santos DC; Souza WF de; Silva VC; Dias MBC. 2019. Protein and carbohydrate fractionation of Jiggs Bermuda grass in different seasons and under intermittent grazing by Holstein cows. Acta Scientiarum. Animal Sciences 41:e43363. doi: 10.4025/actascianimsci.v41i1.43363

Bumbieris Jr VH; Jobim CC; Emile JC; Rossi R; Calixto Jr M; Branco AF. 2011. Ruminal degradability and carbohydrates and proteins fractioning of triticale silages in singular culture or in mixtures with oat and/or legumes. Semina: Ciências Agrárias 32:759–770. (In Portuguese). doi: 10.5433/1679-0359.2011v32n2p759

Carvalho GGP de; Garcia R; Pires AJV; Pereira OG; Fernandes FÈP; Obeid JA; Carvalho BMA de. 2007. Carbohydrate fractioning of elephantgrass silage wilted or enriched with cocoa meal. Revista Brasileira de Zootecnia 36:1000–1005. (In Portuguese). doi: 10.1590/S1516-35982007000500003

Chandler P. 1990. Energy prediction of feeds by forage testing explorer. Feedstuffs 62(36):1–12.

Daniel JLP; Bernardes TF; Jobim CC; Schmidt P; Nussio LG. 2019. Production and utilization of silages in tropical areas with focus on Brazil. Grass and Forage Science 74:188–200. doi: 10.1111/gfs.12417

Dong Z; Chen L; Li J; Yuan X; Shao T. 2019. Characterization of nitrogen transformation dynamics in alfalfa and red clover and their mixture silages. Grassland Science 65:109–115. doi: 10.1111/grs.12230

Epifanio PS; Costa KAP; Guarnieri A; Teixeira DAA; Oliveira SS; Silva VR da. 2016. Silage quality of *Urochloa brizantha* cultivars with levels of Campo Grande Stylosanthes. Acta Scientiarum. Animal Sciences 38:135–142. doi: 10.4025/actascianimsci.v38i2.29631

Epifanio PS; Costa KAP; Severiano EC; Cruvinel WS; Bento JC; Perim RC. 2014. Fermentative and bromatological characteristics of Piata palisadegrass ensiled with levels of meals from biodiesel industry. Semina: Ciências Agrárias 35:491–504. (In Portuguese). doi: 10.5433/1679-0359.2014v35n1p491

Ferraretto LF; Shaver RD; Luck BD. 2018. Silage review: Recent advances and future technologies for whole-plant and fractionated corn silage harvesting. Journal of Dairy Science 101:3937–3951. doi: 10.3168/jds.2017-13728

Ferreira DJ; Zanine AM; Lana RP; Souza AL de; Negrão FM; Geron LJV; Parente HN; Parente MOM. 2018. Carbohydrate and protein fractioning of grass silages with added dehydrated brewery residue. Ciencia e Investigación Agraria

45:192–199. rcia.uc.cl/index.php/ijanr/article/view/1807

Ferreira EB; Cavalcanti PP; Nogueira DA. 2015. Experimental Designs package [Package 'ExpDes']. The Comprehensive R Archive Network. bit.ly/3Mh1aDK

Grant RJ; Adesogan AT. 2018. Journal of Dairy Science silage special issue: Introduction. Journal of Dairy Science 101:3935–3936. doi: 10.3168/jds.2018-14630

Higgs RJ; Chase LE; Ross DA; Van Amburgh ME. 2015. Updating the cornell net carbohydrate and protein system feed library and analyzing model sensitivity to feed inputs. Journal of Dairy Science 98:6340–6360. doi: 10.3168/jds.2015-9379

Krishnamoorthy U; Sniffen CJ; Stern MD; Van Soest PJ. 1983. Evaluation of a mathematical model of rumen digestion and an in vitro simulation of rumen proteolysis to estimate the rumen-undegraded nitrogen content of feedstuffs. British Journal of Nutrition 50:555–568. doi: 10.1079/BJN19830127

Kung L; Shaver RD; Grant RJ; Schmidt RJ. 2018. Silage review: Interpretation of chemical, microbial and organoleptic components of silages. Journal of Dairy Science 101:4020–4033. doi: 10.3168/jds.2017-13909

Lazzarini I; Detmann E; Sampaio CB; Paulino MF; Valadares Filho SC; Souza MA de; Oliveira FA. 2009. Intake and digestibility in cattle fed low-quality tropical forage and supplemented with nitrogenous compounds. Revista Brasileira de Zootecnia 38:2021–2030. doi: 10.1590/S1516-35982009001000024

Leite RG; Cardoso AS; Fonseca NVB; Silva MLC; Tedeschi LO; Delevatti LM; Ruggieri AC; Reis RA. 2021. Effects of nitrogen fertilization on protein and carbohydrate fractions of Marandu palisadegrass. Scientific Reports 11:14786.doi: 10.1038/s41598-021-94098-4

Licitra G; Hernandez TM; Van Soest PJ. 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Animal Feed Science and Technology 57:347–358. doi: 10.1016/0377-8401(95)00837-3

Mertens DR. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beaker or crucibles: collaborative study. Journal of AOAC International 85:1217–1240. pubmed.ncbi.nlm.nih.gov/12477183/

Negrão FM; Zanine AM; Cabral LS; Souza AL de; Alves AG; Ferreira DJ; Dantas CCO; Lehmkuhl A. 2014. Fractionation of carbohydrates and protein and rumen degradation kinetic parameters of *Brachiaria* grass silage enriched with rice bran. Revista Brasileira de Zootecnia 43:105–113. doi: 10.1590/S1516-35982014000300001

Oba M; Allen MS. 2000. Effects of brown midrib 3 mutation in corn silage on productivity of dairy cows fed two concentrations of dietary neutral detergent fiber: 3. Digestibility and microbial efficiency. Journal of Dairy Science 83:1350–1358. doi: 10.3168/jds.S0022-0302(00)75002-8

Oliveira SS; Costa KAP; Souza WF; Santos CB dos; Teixeira DAA; Silva VC e. 2020. Production and quality of the silage of sorghum intercropped with Paiaguas palisadegrass in different forage systems and at different maturity stages. Animal Production Science 60:694–704. doi: 10.1071/AN17082

Paludo F; Costa KAP; Dias MBC; Silva FAS e; Silva ACG; Rodrigues LG; Silva SAA; Souza WF; Bilego UO; Muniz MP. 2020. Fermentative profile and nutritive value of corn silage with Tamani guinea grass. Semina: Ciências Agrárias 41:2733–2746. doi: 10.5433/1679-0359.2020v41n6p2733

Pires AJV; Carvalho GGP de; Garcia R; Carvalho Jr JN de; Ribeiro LSO; Chagas DMT. 2009. Fractioning of carbohydrates and protein of elephant grass silages with coffee hulls, cocoa meal and cassava meal. Revista Brasileira de Zootecnia 38:422–427. (In Portuguese). doi: 10.1590/S1516-35982009000300004

Queiroz MFS; Berchielli TT; Morais JAS; Messana JD; Malheiros EB; Ruggieri AC. 2011. Digestibility and ruminal parameters in beef cattle fed palisade grass (*Brachiaria brizantha* cv. Marandu). Archivos de Zootecnia 60:997–1008. (In Portuguese). doi: 10.4321/S0004-05922011000400016

Refat B; Prates LL; Khan NA; Lei Y; Christensen DA; McKinnon JJ; Yu P. 2017. Physiochemical characteristics and molecular structures for digestible carbohydrates of silages. Journal of Agricultural and Food Chemistry 65: 8979–8991. doi: 10.1021/acs.jafc.7b01032

Russell BJ; O'Connor JD; Fox DG; Van Soest PJ; Sniffen CJ. 1992. A net carbohydrate and protein system for evaluation of cattle diets: I. Ruminal fermentation. Journal of Animal Science 70:3551–3561. doi: 10.2527/1992.70113551x

Santos HG dos; Jacomine PKT; Anjos LHC dos; Oliveira VA de; Lumbreras JF; Coelho MR; Almeida JA de; Araujo Filho JC de; Oliveira JB de; Cunha TJF. 2018. Sistema Brasileiro de Classificação de Solos. 5th Edn. Embrapa Solos, Brasília, DF, Brasil. bit.ly/3gUTs3s

Santos KC; Carvalho FFR; Carriero MM: Magalhães ALR; Batista AMV; Fagundes GM; Bueno ICS. 2020. Use of different carbohydrate sources associated with urea and implications for *in vitro* fermentation and rumen microbial populations. Animal Production Science 60:1028–1038. doi: 10.1071/AN18633

Sniffen CJ; O'Connor JD; Van Soest PJ; Fox DG; Russell JB. 1992. A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. Journal of Animal Science 70:3562–3577. doi: 10.2527/1992.70113562x

Souza WF de; Costa KAP; Guarnieri A; Severiano EC; Silva JT da; Teixeira DAA; Oliveira SS; Dias MBC. 2019. Production and quality of the silage of corn intercropped with Paiaguas palisadegrass in different forage systems and maturity stages. Revista Brasileira de Zootecnia 48:e20180222. doi: 10.1590/rbz4820180222

Teixeira DAA; Costa KAP; Souza WF de; Severiano EC; Guimarães KC; Silva JT da; Oliveira SS; Dias MBC. 2021. Fermentation profile and nutritive value of maize silage with *Brachiaria* species. Australian Journal of Crop

Science 15:695–702. doi: 10.21475/ajcs.21.15.05.p3004
Van Amburgh ME; Collao-Saenz EA; Higgs RJ; Ross DA; Recktenwald EB; Raffrenato E; Chase LE; Overton TR; Mills JK; Foskolos A. 2015. The Cornell Net Carbohydrate and Protein System: Updates to the model and evaluation of version 6.5. Journal of Dairy Science 98:6361–6380. doi: 10.3168/jds.2015-9378
Van Soest PJ. 1994. Nutritional ecology of the ruminant. 2nd Edn. Cornell University Press, Ithaca, NY, USA.
Van Soest PJ; Robertson JB. 1985. Analysis of forages and fibrous foods. A Laboratory Manual for Animal Science. Cornell University, Ithaca, NY, USA.
Viana PT; Pires AJV; Oliveira LB de; Carvalho GGP de; Ribeiro LSO; Chagas DMT; Nascimento Filho CS; Carvalho AO. 2012. Fractioning of carbohydrates and protein of silages of different forages. Revista Brasileira de Zootecnia 41:292–297. (In Portuguese). doi: 10.1590/S1516-35982012000200009

(Received for publication 23 December 2020; accepted 28 January 2022; published 31 May 2022)