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Chemical composition of *Pennisetum purpureum* silages with inclusion of dehydrated *Bixa orellana* residue

Composición química de ensilajes de *Pennisetum purpureum* con inclusión de residuo deshidratado de *Bixa orellana*

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Abstract

This study aimed to evaluate the fermentative characteristics and the chemical composition of *Pennisetum purpureum* cv. Roxo silages with addition of dehydrated *Bixa orellana*. A completely randomized design with four treatments (0, 15, 30, and 45 g kg^{-1}) and five replicates was used. The variables analyzed were pH, ammoniacal nitrogen, effluent loss and dry matter recovery, and dry matter, crude protein, neutral detergent fiber, acid detergent fiber, and hemicellulose content. The data were subjected to analysis of variance and regression at a 5 % probability level ($p < 0.05$) using the statistical package SISVAR®. A linear increase of 0.0038 and 0.3305 percentage units was observed for each 1 g kg^{-1} of dehydrated annatto residue included, on pH and dry matter recovery. Ammoniacal nitrogen and effluent loss values reduced linearly by 0.0096 and 0.2473 percentage units. Each 1 g kg^{-1} of dehydrated annatto residue included in the *Pennisetum purpureum* silage promoted a linear increase of 0.4235, 0.1333, 0.5347, and 0.6450 percentage units in dry matter, crude protein, and neutral detergent fiber and hemicellulose, respectively. There was a reduction of 0.1107 percentage units in acid detergent fiber content. It was concluded that dehydrated annatto residue has the potential to serve as a silage moisture sequestrant for use in ruminant feed.

Keywords: Additive, agro-industrial coproduct, forage conservation, moisture sequestrant, qualitative characteristics.

Resumen

El objetivo del estudio fue evaluar las características fermentativas y la composición química de ensilajes de pasto *P. purpureum* cv. Roxo con adición de residuo deshidratado de *Bixa orellana*. Se utilizó un diseño completamente al azar con cuatro tratamientos (0, 15, 30 y 45 g kg^{-1}) y cinco repeticiones. Las variables analizadas fueron pH, nitrógeno amoniacal, pérdidas de efluentes y recuperación de materia seca, contenido de materia seca, proteína bruta, fibra detergente neutra, fibra detergente ácida y contenido de hemicelulosa. Los datos fueron sometidos a análisis de varianza y regresión al 5 % de probabilidad ($p < 0.05$) utilizando el paquete estadístico SISVAR®. Se observó un aumento lineal de 0.0038 y 0.3305 unidades porcentuales en el pH y la recuperación de materia seca por cada 1 g kg^{-1} de residuo de achiote deshidratado incluido. Los valores de nitrógeno amoniacal y las pérdidas de efluentes se redujeron linealmente en 0.0096 y 0.2473 unidades porcentuales. Cada 1 g kg^{-1} de residuo de achiote deshidratado incluido en el ensilaje de pasto elefante promovió un aumento lineal de 0.4235, 0.1333, 0.5347 y 0.6450 unidades porcentuales en materia seca, proteína bruta y fibra detergente neutra y hemicelulosa, respectivamente. Hubo una reducción de 0.1107 unidades porcentuales en el contenido de fibra detergente ácida. Se concluyó que el residuo de achiote deshidratado tiene el potencial de secuestrar la humedad del ensilaje para su uso en alimentos para rumiantes.

Palabras claves: aditivo, coproducto agroindustrial, conservación de forrajes, secuestrante de humedad, características cualitativas.

Introduction

Brazil has great potential for pasture-based livestock production, mainly due to its large availability of sunlight and water, in addition to its vast territorial extension (Barbero *et al.*, 2021). These facts made it the world's largest producer and exporter of beef in 2024, according to the United States Department of Agriculture (USDA) and the Brazilian Agricultural Research Corporation (Florentino, 2024).

The practice of ensiling began in Brazil at the end of the 19th century. However, from 1920 onwards, when the import of machines and tractors gained strength in the country, the technique spread over the years, initially with corn and then with sorghum and elephant grass in the 1960s (Daniel *et al.*, 2019).

Studies are needed to develop conservation techniques that maximize surplus production during the rainy season of the year, aiming to use it during the dry period when forage is scarce. One of these conservation techniques is ensiling, which consists of preserving harvested green plants in a moist state via fermentative processes while maintaining their nutritional value (Negrão *et al.*, 2024).

One of the most commonly used grasses for silage production is *Pennisetum purpureum Schumach* (elephant grass). An important factor about *P. purpureum* is its nutritional balance point, suitable for silage production. It culminates in high moisture content, low soluble carbohydrate content, and high buffering power, which can negatively influence fermentation.

Because of its low production costs, elephant grass silage has been an alternative for animal supplementation, since it has high productivity and good palatability. However, due to its ideal cutting time, it has a high moisture content, which can interfere in the fermentation process, causing loss of nutrients. Therefore, the use of different additives in this silage is one of the alternatives that aims to minimize nutritional losses and enhance production quality.

According to McDonald (1981), plants ensiled with high moisture produce a large number of effluents, which carry highly digestible nutrients, sugars, and organic acids, reducing the nutritional value of the silage. One way to reduce these losses is the addition of a co-product with high hygroscopic powder that benefits fermentation during the forage conservation process, such as annatto (*Bixa Orellana* L.) residue (Rêgo *et al.*, 2010), bean (*Phaseolus vulgaris* L.) residue (Negrão *et al.*, 2020), or rice bran (*Oryza sativa*) (Santos *et al.*, 2024).

It was hypothesized that including dehydrated annatto residue would increase the dry matter of the ensiled forage and, consequently, the crude protein content of the silage produced. Considering the availability of annatto and its residues, the objective was to evaluate the chemical composition and losses of *P. purpureum* cv. Roxo silages with the inclusion of dehydrated annatto residue.

Materials and methods

The experiment was conducted at the Federal Institute of Science and Technology – IFRO, on the Colorado do Oeste campus, which is located in the municipality of Colorado do Oeste/RO, at coordinates 13°07'39''S 60°29'68''W, at 410 m of altitude, climate type Aw, according to Koopen's classification, hot and humid tropical, characterized by two well-defined seasons: dry and hot from April to September and rainy and hot from October to March.

The *P. purpureum* cv. Roxo used in the research was already established without fertilization. Before its use, a standardization cut was made close to the ground. The grass was cut for the ensiling process in January 2018, when there was high rainfall, with an average height of 1.80 m.

The experiment was carried out in a completely randomized design with four treatments and five replications. Based on the natural matter of the grass, the treatments consisted of *P. purpureum* cv. Roxo silage without the inclusion of dehydrated annatto residue, *P. purpureum* cv. Roxo silage + 15 g kg^{-1} of dehydrated annatto residue, *P. purpureum* cv. Roxo silage + 30 g kg^{-1} of dehydrated annatto residue, and *P. purpureum* cv. Roxo silage + 45 g kg^{-1} of dehydrated annatto residue.

The annatto residue was obtained in partnership with an annatto processing company in the municipality of Cabixi-RO. Due to its high moisture content, it was necessary to dry it in a forced circulation oven at 65 °C for 72 hours. After drying, the residue was crushed using a Thomas Willey knife mill equipped with a 1 mm mesh sieve.

The dehydrated annatto residue and the *P. purpureum* cv. Roxo forage mass were homogenized on plastic canvas according to each treatment, then placed into the experimental bags and manually compacted using an iron socket, whose lower part was shaped to ensure proper compaction inside the bag.

The silages were prepared in PVC bags of 0.1 m in diameter and 0.40 m in height, with a volume of 3.14×10^{-3} m^3, provided with "Bunsen" type valves to allow the free escape of fermentation gases. Approximately 2 kg of the material was weighed and compacted using a wooden stick, reaching a standardized degree of compaction of 700 to 800 kg/m^3 for all samples. Correct compaction is important to exclude as much oxygen as possible and ensure anaerobic conditions favouring the preservation of nutrients. 1 kg of fine sand was placed, separated from the forage by a layer of fine screen (shading), to capture effluent. After weighing each bag, they were sealed with hot glue to prevent effluent leakage and stored in a cool, ventilated place.

The experimental silos were stored in a covered area at room temperature and opened 40 days after sealing. A completely randomized design was used with four replicates per treatment. Dehydrated annatto was added at the time of ensiling, according to the treatment, taking care to ensure good homogenization of the material, using 0, 15, 30 and 45 g kg^{-1}, based on the natural material and the chemical composition determined in the Bromatology and Animal Feeding Laboratory of the Federal Institute of Rondônia, Colorado do Oeste campus. The mixture of the additive with the chopped grass was prepared on a plastic tarp to prevent contact of the material with the floor surface, thus avoiding possible contamination. The chemical composition of *P. purpureum* cv. Roxo and dehydrated annatto residue used for ensiling is shown in Table 1.

Losses due to effluents, gases and total dry matter (DM) were quantified according to equations proposed by Jobim *et al.* (2007). Effluent production was measured by calculating the difference in weights of the bag and TNT bag with sand before and after ensiling, in relation to the amount of natural material in the ensiled sample. After opening (40 days) the material in the experimental bag, the set (silo + lid + TNT with wet sand + screen) was weighed and the production of effluent drained to the bottom of the bag was estimated by subtracting the weight of the same set before ensiling, according to Equation 1:

$$E\ (kg/ton\ GF) = [(Psvaa - Ts)] - (Psa - Ts)] / GFfi \times 100 \quad \textbf{Eq.1}$$

Where:

E (kg/ton NM): Effluent losses;

Psvaa: Weight of the whole bag (kg) when closing the silage;

Ts: Bag tare;

Psa: Weight of the bag with sand (kg) before adding the chopped green forage;

GFfi: Green forage mass (kg) used to make the silage.

Table 1. Chemical composition (average values) of *P. purpureum* cv. Roxo and dehydrated annatto residue (*B. orellana*) before the ensiling procedure

	DM (%)	CP (%)	NDF (%)	ADF (%)	HEM (%)
Pennisetum purpureum cv. Roxo	14.60	5.96	43.62	30.75	12.86
Bixa orellana residue	81.24	12.65	75.43	24.01	51.40

DM: dry matter, CP: crude protein, NDF: neutral detergent fiber, ADF: acid detergent fiber, HEM: hemicellulose.

The loss of dry matter resulting from gas production was determined by the difference between the gross weight of dry matter in the ensilage and at the opening, in relation to the quantity of dry matter ensiled, subtracting the weight of the ensiled set at both stages from the total ensiled weight, according to Equation 2:

$$G\ (\%\ DM) = [(Pschf - Pscha)/(NMfi \times DNfi)] \times 1000 \quad \textbf{Eq.2}$$

Where:

G (% DM): Gas losses;

Pschf: Weight of the full bag (kg) at the closing of the silage;

Pscha: Weight of the full bag (kg) at the opening;

NMfi: Natural mass (kg) of ensiled forage;

DMfi: Dry matter (%) of ensiled forage.

Total dry matter recovery was determined by the difference between the gross weight of dry matter at ensiling and at opening, in relation to the amount of dry matter ensiled, according to Equation 3:

$$RDM\ (\%) = (GFfo \times DMfo) / (DMi \times DMsi) \times 100 \quad \textbf{Eq.3}$$

Where:

RDM (%): Dry matter recovery;

GFfo: Forage green mass (kg) at the time of ensiling;

DMfo: Forage dry matter (%) at the time of ensiling;

DMi: Silage mass (kg) at the opening of the bags;

DMsi: Silage dry matter (%) at the opening of the bags.

Upon opening the bag, a surface layer of 5 cm was discarded, and only the central portion of each experimental bag was considered. A sample of approximately 550 g of silage was collected, packed in plastic bags, and stored in a freezer.

Subsequently, a 50 g subsample was taken, to which 100 mL of distilled water was added. Then, the mixture was blended for 1 minute. The resulting sample was filtered through filter paper, and its pH was measured using a bench potentiometer (Detmann *et al.*, 2012). The analysis of ammonia nitrogen was carried out according to the methodology described by Detmann *et al.* (2012).

Afterward, the silage samples were packed in paper bags and pre-dried in an oven with forced air circulation regulated at 65 °C for 72 hours. Subsequently, the samples were ground using a stationary Thomas-Willey knife mill equipped with a 1 mm sieve and stored in polyethylene containers to evaluate the chemical composition.

Laboratory analyses were carried out to determine the contents of dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and hemicellulose (HEM), according to methodologies described by Detmann *et al.* (2012).

The data obtained regarding pH, ammonia nitrogen, loss through effluents, recovery of dry matter, and chemical constituents of *P. purpureum* cv. Roxo silages with the inclusion of dehydrated annatto residue were statistically analysed using analysis of variance (*t*-test). In cases of statistical significance, regression analysis was performed, testing first- and second-degree polynomial models at a 5 % level of significance, using the SISVAR® statistical package.

Results and discussion

A linear increase ($p < 0.05$) of 0.0038 percentage units was observed on pH values with the inclusion of dehydrated annatto residue (Table 2). The increases in pH values were 3.78, 3.91, 3.94, and 3.96 with the addition of 0, 15, 30, and 45 g kg^{-1} of dehydrated annatto residue in grass silage, respectively. Even with this increase, which is somewhat undesirable, the pH remained at values considered adequate and characteristic of proper fermentation (between 3.8 and 4.2), which prevents proteolysis and the consequent production of butyric acid (McDonald, 1981).

Yeasts are present in all phases of ensiling; however, when present during the anaerobic phase, they compete with lactic acid-producing bacteria for the sugars available in the medium, reducing the production of lactic acid and, consequently, increasing the pH. Another factor that may have contributed to the inhibition of lactic acid-producing bacteria is the presence of organic solvents used in the bixin extraction process, which, due to their nature, may have caused a toxic effect on fermentative lactic acid bacteria (Muck, 2010).

Garcez *et al.* (2023) evaluated Tifton 85 silages in natura with and without the addition of corn, soybean, and rice bran, in order to determine if there was an improvement in the conservation and nutritional value of the ensiled product. The authors did not observe significant differences in the pH values of the silages obtained and throughout the times of aerobic exposure. However, after 144 hours of air exposure, a loss of aerobic stability was observed in the silages added with soybean and rice bran, leading to an increase in the pH values by more than 0.2 units in relation to the pH at the time the bags were opened.

For ammonia nitrogen ($N\text{-}NH_3$) values, a reduction ($p < 0.05$) of 0.0096 percentage units was observed for each 1 g kg^{-1} of dehydrated annatto residue included in *P. purpureum* cv. Roxo silage (Table 2). For all addition levels, the silages presented satisfactory $N\text{-}NH_3$ levels, remaining below 12 g kg^{-1}, that, according to McDonald (1981), is the limit value below which good quality silages are classified.

Araújo *et al.* (2021) evaluated the fermentation characteristics, sensory analysis and aerobic stability of Piata grass (*Urochloa brizantha*) silages with addition of 0, 2.5, 5 and 7.5 % of curauá fiber residue and observed a linear reduction in gas production, an increase in the $N\text{-}NH_3$/N-total content in the treatments with 0 %, 2.5 % and 5 % addition, and a reduction in the N-NH3/N-total content in the treatment with 7.5 %. There was also an increase in pH values. Only the treatment with 2.5 % of curauá (*Ananas erectifolius*) residue showed a break in stability. The analyzed silages showed little aerobic deterioration. The authors concluded that the inclusion of curauá fiber residue in the Piata grass silage contributed to the increase in the temperature and pH of the silages and the loss of $N\text{-}NH_3$.

With the addition of 15, 30 and 45 % of dehydrated annatto residue, the effluent loss was reduced in relation to the control to 4.40, 2.82 and 1.80 kg t^{-1} DM, respectively (Table 2). The dehydrated annatto residue was an excellent sequester of *P. purpureum* cv. Roxo moisture, as it has a high amount of dry matter, generating less leaching of nutrients via silage effluent.

The high rates of loss through effluents in silages with lower levels of dehydrated annatto residue are due to the silage's high moisture content, which favours the metabolic activity of yeast and nutrient losses through leaching.

Table 2. Average values of pH, ammonia-N, effluent loss, and dry matter recovery in *P. purpureum* cv. Roxo silages with the inclusion of dehydrated annatto residue

	Levels				Regression equation	CV %	R^2
Variables	0 %	15 %	30 %	45 %			
pH	3.78	3.91	3.94	3.96	Y = 3.82 + 0.0038x*	1.67	0.82
$N\text{-}NH_3$ [2]	3.09	2.58	2.37	2.68	Y = 2.896 – 0.0096x*	5.27	0.37
EL[3]	13.64	4.40	2.82	1.80	Y = 11.23 – 0.2473x*	32.79	0.78
RDM[1]	74.75	89.53	90.12	91.08	Y = 78.93 + 0.3305x*	5.08	0.67

*Significant at 5 % probability. 1: Percentage. 2: Percentage in dry matter. 3: kg/ton. CV = coefficient of variation (%).

$N\text{-}NH_3$: ammonia-N, EL: effluent loss, RDM: dry matter recovery.

For every 1 g kg^{-1} of dehydrated annatto residue included in the *P. purpureum* cv. Roxo silage, there was an increase ($p < 0.05$) of 0.3305 percentage units in dry matter recovery (Table 2). The high dry matter content of the dehydrated annatto residue (81.24 g kg^{-1}, Table 1) efficiently reduced the moisture content of the silage by diluting the amount of water in it.

The dehydrated annatto residue proved to be effective in absorbing moisture from the silage, as it provided a linear increase ($p < 0.05$) in the dry matter (DM) content of *P. purpureum* cv. Roxo silages, estimated at 0.4235 g kg^{-1} for every 1 g kg^{-1} included (Table 3).

Furthermore, the use of additives contributes to reducing nutritional deficiencies, increasing the growth of rumen microorganisms, and enhancing microbial fermentation. This leads to greater extraction of forage carbohydrates, increased production of volatile fatty acids, and improved efficiency in the use of metabolizable energy from forage (Santos *et al.*, 2019).

The crude protein (CP) content of *P. purpureum* cv. Roxo silages increased linearly ($p < 0.05$) with the inclusion of dehydrated annatto residue, estimating that for every 1 g kg^{-1} included, there was an increase in the content of crude protein by 0.1333 percentage units (Table 3).

Regarding the crude protein content, Mertens (1994) point out that 7 g kg^{-1} of crude protein is sufficient for maintaining the rumen microbiota when there is satisfactory forage consumption due to the protein supply to rumen microorganisms. Considering this, it was possible to reach the 7 % level with the inclusion of 9.37 g kg^{-1} of dehydrated annatto residue.

The annatto residue presented a value of 12.65 % (Table 1) of crude protein, demonstrating its potential for inclusion as a protein source in animal feed. When used together with *P. purpureum* cv. Roxo (5.96 % of CP), it provides silage with good nutritional values (Table 3).

The addition of dehydrated annatto residue promoted a linear increase ($p < 0.05$) in the neutral detergent fiber (NDF) content, estimated at 0.5347 percentage units (Table 3). The dehydrated annatto residue has a higher NDF content than *P. purpureum* cv. Roxo (75 and 43 g kg^{-1}, respectively, Table 1), justifying the high NDF content in treatments with greater inclusion of the residue. Thus, the progressive increase in NDF content may imply a reduction in dry matter intake due to the physical effect of filling the rumen with excessively fibrous material, reducing the rate of food passage through the digestive tract (Mertens, 1994).

The acid detergent fiber (ADF) content was linearly reduced ($p < 0.05$) by 0.1107 percentage units (Table 3) with the inclusion of dehydrated annatto residue. The data revealed a dilution effect as *P. purpureum* cv. Roxo has an ADF value (30.7 g kg^{-1}) higher than that found in dehydrated annatto residue (24 g kg^{-1}).

The reduction in ADF levels is extremely important as it indicates an increase in the nutritional value of silages since this reduction increases the digestibility of dry matter (Mertens, 1994). Therefore, it can be inferred that the addition of dehydrated annatto residue increases the ruminant's use of some constituents of the silage cell wall.

There was a linear increase ($p < 0.05$) in hemicellulose (HEM) levels of 0.6450 percentage units for each 1 g kg^{-1} of dehydrated annatto residue added to *P. purpureum* cv. Roxo silage over hemicellulose values (Table 3).

Santos *et al.* (2019) observed an increasing linear behaviour of 0.18 and 0.04 in the NDF and ADF contents, respectively, as the inclusion levels of *Theobroma grandiflorum* (Willd. Ex Spreng.) K Schum. seed cake increased. However, they did not observe a significant effect for the hemicellulose values, that is, there was no influence on the constitution of the cell wall.

Table 3. Chemical composition (average values) of *P. purpureum* cv. Roxo silages with the inclusion of dehydrated annatto residue

	Levels				Regression equation	CV %	R^2
Variables	0 %	15 %	30 %	45 %			
DM[1]	16.74	23.60	31.11	35.45	Y = 17.18 + 0.4235x*	5.24	0.98
CP[2]	5.12	8.48	10.23	11.20	Y = 5.75 + 0.1333x*	10.21	0.93
NDF[2]	40.55	60.47	62.44	66.62	Y = 45.48 + 0.5347x*	5.71	0.79
ADF[2]	28.03	23.44	23.37	22.52	Y = 26.83 - 0.1107x*	5.13	0.73
HEM[2]	12.51	37.03	39.07	44.10	Y = 18.65 + 0.6450x *	10.13	0.78

*Significant at 5 % probability.1: Percentage. 2: Percentage in dry matter. CV = coefficient of variation (%).
DM: dry matter, CP: crude protein, NDF: neutral detergent fiber, ADF: acid detergent fiber, HEM: hemicellulose.

Conclusion

The inclusion of dehydrated annatto residues in elephant grass silage improves the fermentative and nutritional quality by reducing effluent losses and increasing the dry matter and crude protein content of the forage.

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