



# **Effects of Dehydrated Cupuaçu Almond Bran Inclusion on Fermentation Profile and Bromatological Composition of *Pennisetum purpureum* Grass Silage**

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## **Authors' contributions**

*This work was carried out in collaboration among all authors. Authors JGDR and FDMN designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors ESM, FHBC, ADPC and AAM managed the analyzes of the study. Authors JGDR and FDMN managed the literature searches. All authors read and approved the final manuscript.*

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## ABSTRACT

This study aimed to evaluate the fermentative characteristics and the bromatological composition of elephant grass silages by adding dehydrated almonds of cupuaçu. A completely randomized design was used with four treatments (0, 15, 30, and 45%) and five replications. The variables analyzed were pH, effluent losses and dry matter recovery, dry matter, crude protein, neutral detergent fiber, acid detergent fiber, and hemicellulose. The data were submitted for analysis of variance and regression to the level of 5% probability using the statistical package SISVAR®. With the inclusion of dehydrated cupuaçu almonds was observed a linear increase ( $P < 0.05$ ) of 0.05, 1.14, 0.63, 2.43, and 2.52 percentage units for each percentage on pH, dry matter recovery, dry matter, neutral detergent fiber, and hemicellulose. On the other hand, for each 1% of the additive added was observed reduction of 0.20, 1.15, 0.035, 0.8, 0.38, and 0.36 for N-NH<sub>3</sub>, buffering power, effluents, gas loss, crude protein, and acid detergent fiber, respectively. The addition of dehydrated cupuaçu almonds to elephant grass silage promotes improvements in the silage composition; however, the amount to be added must be moderated as this additive increases the pH and the amount of fiber in the silage.

*Keywords: Forage conservation; Theobroma grandiflorum; nutrition.*

## 1. INTRODUCTION

Although it is relatively easy to obtain good-quality silage from corn and sorghum, it is also possible to produce medium- to good-quality silage using grasses, with grasses from the elephant group (*Pennisetum purpureum* Schum) being most recommended. After corn and sorghum, this is one of the forages with the best characteristics for ensiling, given its high productivity, great adaptability, ease of cultivation, good animal acceptability, and, when new, good nutritional value.

Considering that a large part of the annual forage production is concentrated in the rainy season and that accumulated growth reduces nutritional value, elephant grass when used as silage, is another alternative in animal feed in the dry season, in addition to enabling more efficient use of production from the cultivated area. Elephant grass, a perennial plant with high production potential and good chemical composition, is an economically more attractive alternative than other annual crops for silage production [1]. For this purpose, cutting the forage when new has been recommended aims for better nutritional value; however, it is necessary to adopt ways to eliminate excess moisture from the plant [2].

In ensiling processes under laboratory conditions, where care is taken regarding the size of the forage cut and compaction of the mass, good fermentation patterns have been observed, even when plants with a low dry matter content are used. Even in these

processes, overheating of the mass has not occurred, which, according to Abdalla et al [3], would cause an increase in the percentage of nitrogen insoluble in acid detergent, which is poorly digested in the rumen.

Through knowledge about the production characteristics of forage grasses, which are seasonal (high productivity in the rainy season and low productivity in the dry season), studies are necessary to develop conservation techniques to take advantage of surplus production during the rainy season of the year, aiming to use it in the period in which there is a forage deficit that coincides precisely with the dry period of the year. Another justification for the development and execution of the project is based on sustainable food production, highlighting that cupuaçu residue is improperly discarded in the environment, and experiments that aim to give these surpluses a noble destination fulfill their social role.

The primary purpose of contributing to human nutrition is also highlighted by transforming coarse food that is not consumable by humans into animal protein of high biological value through animal consumption. This research will contribute to the qualification of human resources capable of being absorbed into public teaching and research bodies, federal and state, and for use in the private sector in its different areas of activity. The aim is to bring the results to producers who want to reduce production costs by inserting low-cost waste that is easily accessible and available to animals in times of low forage supply.

However, research with this residue is incipient for the conditions of the State of Rondônia and Brazil. Thus, the evaluation of this agro-industry residue, in the form proposed in this research project, will result in scientific information necessary to define rational strategies for using optimal levels of cupuaçu almond bran residue in the production of elephant grass silage. In an attempt to minimize these problems, the use of by-products would be an alternative, since due to the rapid growth in the use of cupuaçu in the North region, large quantities of waste are available, including cupuaçu (*Theobroma grandiflorum*), which many They are often left in the yards of agribusinesses, which can cause serious environmental problems.

Considering the availability of cupuaçu almonds, there are still no records of research of this nature in the State of Rondônia, even with the abundance of residues from this crop. Therefore, the objective of this research will be to evaluate levels of cupuaçu almond meal as a hygroscopic additive in elephant grass silage.

## 2. MATERIALS AND METHODS

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

The experiment was carried out in a completely randomized design with four treatments and five replications. The treatments evaluated were: pure grass silage, elephant grass silage + 10 g/kg of dehydrated cupuaçu almond meal, elephant grass silage + 20 g/kg of dehydrated cupuaçu almond meal, and grass silage elephant + 30 g/kg of dehydrated cupuaçu almond bran, based on the natural matter of the grass.

The institution supplied the cupuaçu almond. When obtained, the almonds had high humidity, making it necessary to dry them in a forced circulation oven at 65°C for 72 hours. After drying, the almonds were crushed in a Thomas Willey knife mill equipped with a mesh sieve with 1 mm screens. As for elephant grass, we used a plantation already established in the Campus

area. It was only necessary to make one cut to standardize the grass before use.

The cupuaçu bran residue and the elephant grass forage mass, corresponding to each treatment, were homogenized on plastic canvas and then placed in experimental PVC pipe silos measuring 50 cm in length and 100 mm in diameter and equipped with a valve made of thin hoses adapted to its lid, in order to allow the escape of gases from the fermentation process. At the bottom of each experimental silo, 2 kg of sand was placed, separated from the forage by a layer of plastic mesh, aiming to measure the amount of effluents produced.

At the time of ensiling, a sample of chopped green forage and forage containing cupuaçu almond bran residue was collected, placed in paper bags, and pre-dried in a forced air ventilation oven at 55°C for 48 hours. The pre-dried samples were weighed and ground in a Thomas-Willey knife mill equipped with a mesh sieve with 1 mm diameter screens.

At 40 days after ensiling, the silos were opened, where the filled silos, the deteriorated silage, and the silage suitable for consumption by the animals were weighed for the quantitative determination of gas and effluent losses, dry matter recovery based on gravimetric differences, using equations adapted from Jobim et al [4].

The quantification of dry matter losses in silages in the form of gases was calculated due to the difference in Weight using the following equation:  $G(\% \text{ DM}) = [(P_{schf} - P_{scha}) / (MV_{fi} \times MS_{fi})] \times 1000$ , in which  $G(\% \text{ DM})$ = Gas losses,  $P_{schf}$  = WeightWeight of the full silo (kg) when closing the silage,  $P_{scha}$ = Weight of the full silo (kg) at the opening,  $MV_{fi}$ = Green mass (kg) of ensiled forage and  $MS_{fi}$ = Matter Dryness (%) of ensiled forage.

As for effluent losses, these are calculated due to the difference in sand weight and related to the mass of fresh forage at closure. To this end, the equation  $E [(kg/ton \text{ MV}) = (P_{svaa} - T_s)] - (P_{sa} - T_s) / MV_{fi} \times 100$  was used, where  $E [(kg/ton \text{ MV})$ = Effluent losses,  $P_{svaa}$  = WeightWeight of the empty silo with sand (kg) when closing the silage,  $T_s$ = Silo tare;  $P_{sa}$ = Weight of the silo with sand (kg) before adding the chopped green forage and  $MV_{fi}$ = Green mass of forage (kg) used to make the silage.

To determine dry matter recovery, the following equation was used:  $RMS(\%) = [(MVfo \times MSfo)/(MSi \times MSSi)] \times 100$ , where: RMS(%)= Dry Matter Recovery in percentage, MVfo= Green mass of forage (kg) at the time of ensiling, MSfo= Dry matter of forage (%) at the time of ensiling, MSi= Mass of silage (kg) at the opening of the silos and MSSi= Dry matter of silage (%) when opening the bags.

Another point that should be observed about elephant grass silage with the addition of dehydrated cupuaçu almond bran is that it has a high concentration of lactic acid, making it more susceptible to deterioration after opening [5]. It is also known that higher protein levels interfere with the silage fermentation process, increasing the buffering power, impairing the rapid drop in the pH of the ensiled material, and may increase the storage time for this drop and pH stabilization to occur [6].

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 Silva and Queiroz [7].

The data obtained regarding losses due to gases and effluents, dry matter recovery, fermentation pattern, and bromatological constituents of elephant grass silage were analyzed statistically using an analysis of variance. In cases of

significance, proceeded regression analysis will be carried out, testing first—and second-degree polynomial models at a 5% significance level using the SAEG program (1999) version 8.1. The choice of the regression model will be made considering the values of P and R2, because if it is significant, the value of R2 will be disregarded.

### 3. RESULTS AND DISCUSSION

The addition linear increase ( $P < 0.05$ ) of 0.05 percentage units for each percentage unit of inclusion of dehydrated cupuaçu almonds on pH values (Fig 1). The pH ranged from 3.57 g/kg to 5.05 g/kg, including 0 g/kg and 30 g/kg of dehydrated cupuaçu almonds, respectively. Mahanna et al [8] points out that the increase in the pH of silages occurs mainly due to the presence of yeast, which, by consuming the lactic acid present during the fermentation period, generates undesirable imbalances and causes an increase in pH. Suitable pH values for silages are 3.8 to 4.2, according to McDonald [9]. Thus, using this additive raises the pH of the silage to very high levels.

The inclusion of dehydrated cupuaçu almonds reduced the N-NH3 content of the silages by 0.20 g/kg for each 1% of the additive added. These values are considered very high, as according to McDonald [9], the N-NH3 values considered desirable for silages are below 12%.

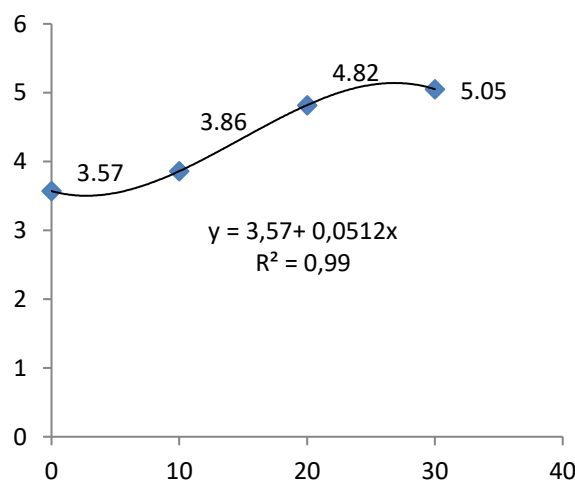
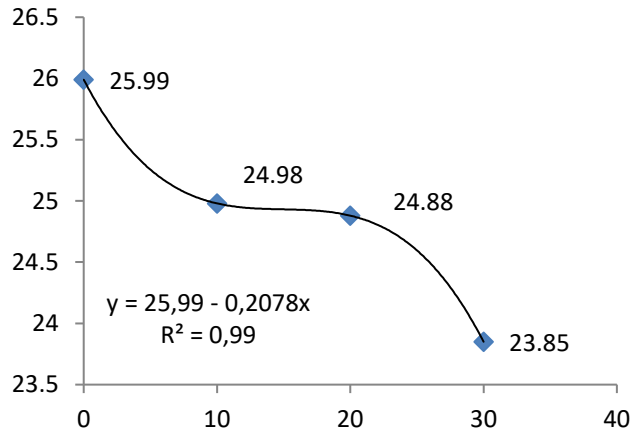


Fig. 1. Average pH values of elephant grass silage with the addition of dehydrated cupuaçu almonds

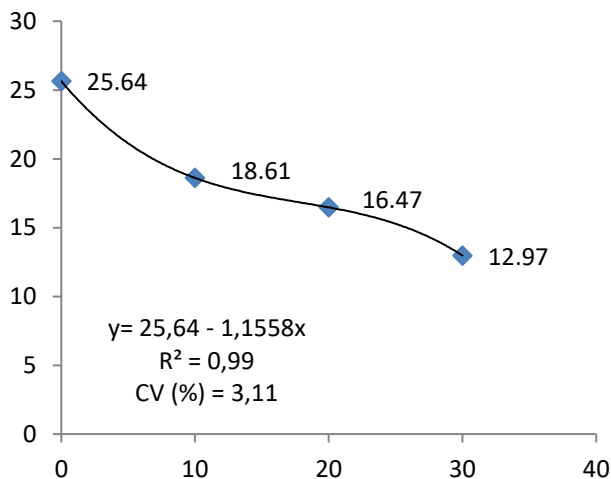


**Fig. 2. Average ammonia-N values of elephant grass silage with the addition of dehydrated cupuaçu almonds**

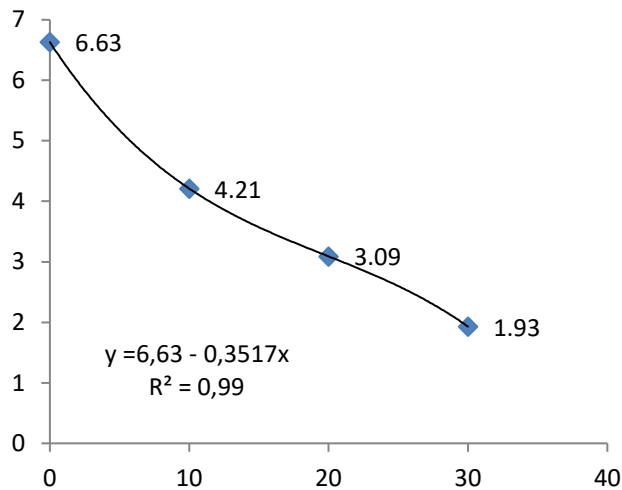
There was a reduction of 1.15 g/kg in the value of buffering power for each 1% addition of dehydrated cupuaçu almonds. The reduction in buffering power is desirable, as it indicates the intensity to which the silage resists the change in pH. The forage, when ensiled at an early vegetative development stage, has high nutritional quality but has a low dry matter content and high buffering power, preventing the rapid reduction of pH and enabling the development of undesirable fermentations [10].

Therefore, the inclusion of dehydrated cupuaçu almonds is efficient in solving this problem of increasing buffering power.

Including dehydrated cupuaçu almonds caused a reduction of 0.035 g/kg for each 1% addition of dehydrated almonds (Fig 4). Dehydrated cupuaçu almonds efficiently reduce silage moisture, which reduces loss through effluents, which is undesirable as it causes the leaching of nutrients via silage effluent.



**Fig. 3. Average values of the buffering power of elephant grass silage with the addition of dehydrated cupuaçu almonds**

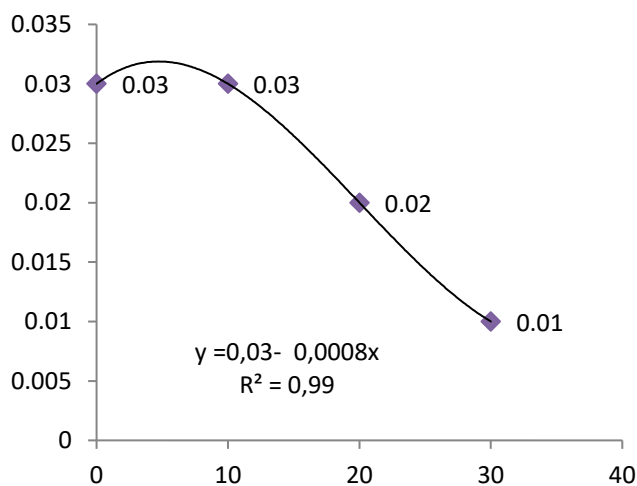


**Fig. 4. Average loss values due to effluents of elephant grass silage with the addition of dehydrated cupuaçu almonds**

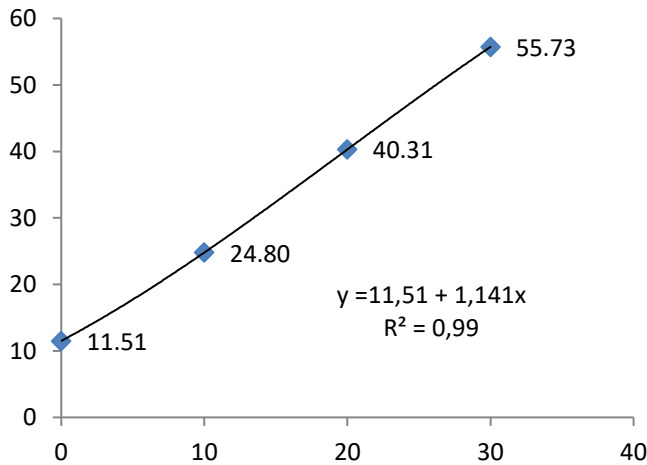
The inclusion of dehydrated cupuaçu almonds reduced gas loss by 0.8 g/ton (Fig 5). The reduction in gas loss was greater as the level of inclusion of dehydrated cupuaçu almonds was increased, demonstrating the additive's moisture absorption capacity when mixed with chopped elephant grass forage. The reduction in gas losses is probably due to the reduction of gas-producing microorganisms, such as

enterobacteria and clostridial bacteria, which develop in poorly preserved silage [11].

Silage dry matter recovery values increased linearly ( $P < 0.05$ ) with the inclusion of dehydrated cupuaçu almonds. A 1.14 g/kg increase in dry matter recovery was observed for each 1g/kg additive inclusion (Fig 6).



**Fig. 5. Average values of gas loss of elephant grass silage with the addition of dehydrated cupuaçu almonds**

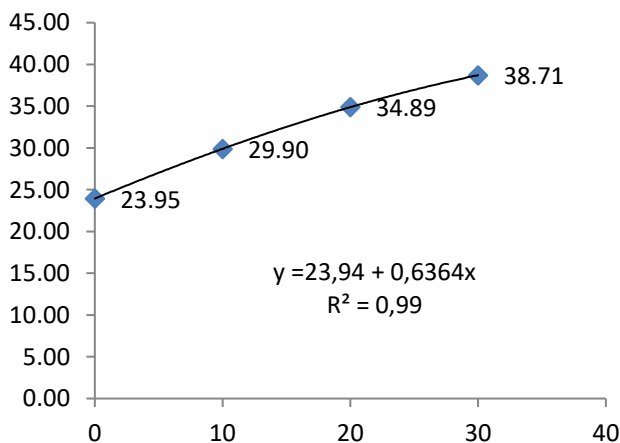


**Fig. 6. Average values of dry matter recovery from elephant grass silage with the addition of dehydrated cupuaçu almonds**

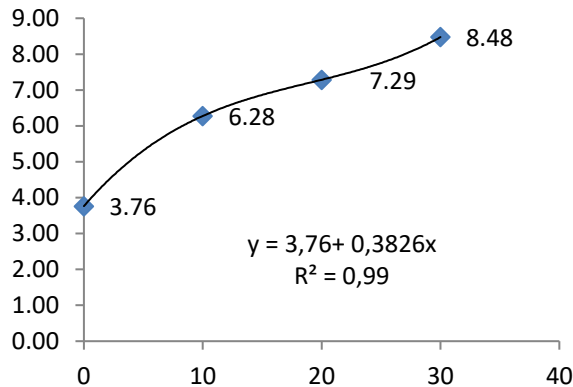
Dehydrated cupuaçu almonds proved to be effective in absorbing moisture from silage, as it provided a linear increase ( $P < 0.05$ ) in the dry matter (DM) content of silages, estimated at 0.63g/kg every 1% of inclusion (Fig 7). For the fermentation process to occur satisfactorily, the dry matter content of the forage must be between 30% and 35%. Dry matter levels below 30% allow the proliferation of bacteria of the genus *Clostridium*, which are responsible for undesirable fermentations and, consequently, for losses observed in silages [9]. The appropriate

level of DM was achieved by including 20 g/kg of dehydrated cupuaçu almonds.

There was an increase of 0.38 g/kg in crude protein content for each 1 g/kg addition of dehydrated cupuaçu almonds (Fig 8). The adequate crude protein content for maintaining the rumen microbiota, according to McDonald et al [12], is 7% when there is satisfactory forage consumption due to the protein supply for rumen microorganisms. Adding 20 g/kg of dehydrated annatto almonds already provides the recommended value of 7% crude protein.



**Fig. 7. Average dry matter values of elephant grass silage with the addition of dehydrated cupuaçu almonds**



**Fig. 8. Average crude protein values of elephant grass silage with the addition of dehydrated cupuaçu almonds**

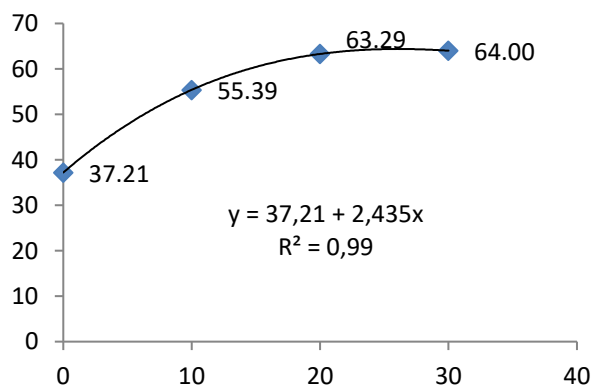
The addition of dehydrated cupuaçu almonds promoted a linear increase ( $P < 0.05$ ) in the neutral detergent fiber (NDF) content, estimating an increase of 2.43 g/kg (Fig 9). The value of 64 g/kg in NDF content was reached by including 30 g/kg of dehydrated cupuaçu almonds in the elephant grass silage.

According to Resende et al [13], the progressive increase in NDF content causes a reduction in DM intake due to the physical effect of filling the rumen with excessively fibrous material, reducing the rate of food passage through the digestive tract.

There was a 0.36 g/kg reduction in the acid detergent fiber content (Fig 10) for each 1 g/kg of dehydrated cupuaçu almonds. According to Cruz et al [14], the ADF content indicates the

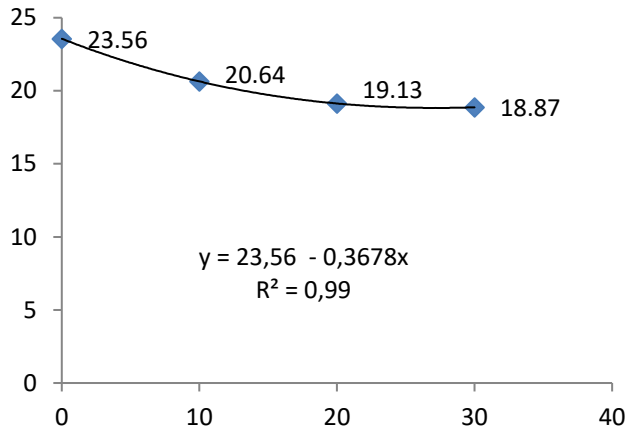
digestibility of the silage, as it contains the highest proportion of lignin, an indigestible fiber fraction, demonstrating the amount of undigestible fiber. The inclusion of dehydrated cupuaçu almonds increased the use of dry matter digestibility by reducing the ADF content.

The hemicellulose (HEM) content increased by 2.52 g/kg for every 1 g/kg dehydrated cupuaçu almonds (Fig 11). The higher concentration of hemicellulose in the treatment with greater inclusion of dehydrated cupuaçu almonds can be justified by the higher concentration of lignin in this treatment, which leads to the hypothesis of protection of hemicellulose by lignin, which hinders the action of specific enzymes in their degradation throughout the process—day of silage fermentation [15].

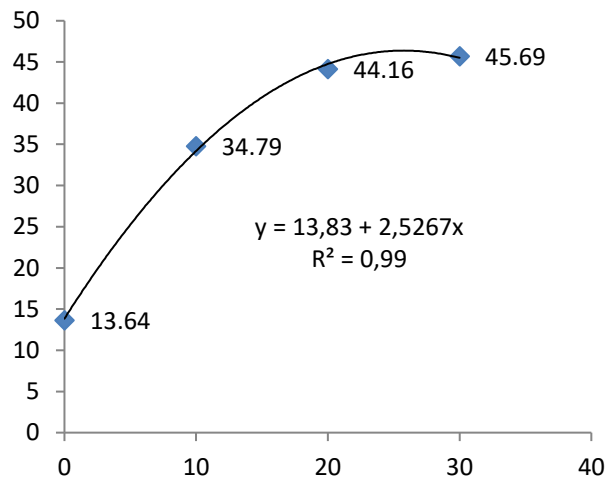


**Fig. 9. Average neutral detergent fiber values of elephant grass silage with the addition of dehydrated cupuaçu almonds**





**Fig. 10. Average values of acid detergent fiber from elephant grass silage with the addition of dehydrated cupuaçu almonds**



**Fig. 11. Hemicellulose contents of elephant grass silage with the addition of dehydrated cupuaçu almond bran**

#### 4. CONCLUSION

The addition of dehydrated cupuaçu almonds to elephant grass silage promotes improvements in the silage composition; however, the amount to be added must be moderated as this additive increases the ph and the amount of fiber in the silage.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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