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Chemical composition and fermentation characteristics of elephant grass silage with biodiesel industry co-products

Composição química e características fermentativas de silagens de capim elefante contendo coprodutos da indústria do biodiesel

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ABSTRACT

*The objective of this research was to evaluate the effect of three concentrations (3, 6, and 9%) of forage turnip (**Raphanus sativus**) and physic nut (**Jatropha curcas**) cakes on dry matter, crude protein, ether extract, neutral detergent fiber, acid detergent fiber, lignin, acid detergent insoluble nitrogen neutral detergent insoluble nitrogen contents, in vitro dry matter digestibility, pH values and concentrations of* $N\text{-}NH_3$ *in elephant grass silages. It was used an entirely randomized design in factorial arrangement [(2×3)+1]. Experimental PVC silos were used and ensiled material was kept for 62 days. The addition of cakes increased the dry matter contents (P<0.05). The fibrous fractions were reduced (P<0.05) with the inclusion of cakes during the grass ensilage and the CP contents increased (P<0.05). The forage turnip cake provided the same pH and* $N\text{-}NH_3$ *values in ideal levels and the physic nut, added to 9%, increased those values (P<0.05). IVDMD was reduced (P<0.05) when the cakes were added. These co-products can be used in small amounts for elephant grass ensilage in order to provide improvement in chemical and fermentation characteristics of the silages. Nevertheless, physic nut cake shows limitations for its use in animal feeding due to the presence of toxic compounds, making necessary studies for their identification and elimination.*

Key words: *ensilage,* in vitro *digestibility,* ***Jatropha curcas****,* ***Pennisetum purpureum****,* ***Raphanus sativus.***

RESUMO

*Objetivou-se avaliar o efeito da adição de três concentrações (3, 6, e 9%) das tortas de nabo forrageiro (**Raphanus sativus**) e de pinhão manso (**Jatropha curcas**) sobre os teores de matéria seca, proteína bruta, extrato etéreo, fibra em detergente neutro, fibra em detergente ácido, lignina, nitrogênio insolúvel em detergente ácido, nitrogênio insolúvel em detergente neutro, digestibilidade in vitro da matéria seca, valores de pH e concentrações de* $N\text{-}NH_3$ *em silagens de capim-elefante. Utilizou-se delineamento inteiramente casualizado em arranjo fatorial [(2×3)+1]. Foram utilizados silos experimentais de PVC e o material ensilado permaneceu por 62 dias. A adição das tortas proporcionou aumento nos teores de matéria seca (P<0,05). As frações fibrosas foram diminuídas (P<0,05) com a inclusão das tortas no momento da ensilagem do capim e os teores de PB aumentados (P<0,05). A torta de nabo forrageiro proporcionou a manutenção dos valores de pH e de* $N\text{-}NH_3$ *em níveis ideais e a de pinhão manso, adicionada a 9%, elevou esses valores (P<0,05). A DIVMS foi diminuída (P<0,05) quando as tortas foram adicionadas. As tortas de nabo forrageiro e pinhão manso podem ser utilizadas em pequenas quantidades na ensilagem do capim-elefante por melhorarem as características químicas e fermentativas das silagens. Contudo, a torta de pinhão manso apresenta limitações para alimentação animal, pois apresenta compostos tóxicos, sendo necessários estudos para sua identificação e eliminação.*

Palavras-chave: *digestibilidade* in vitro*, ensilagem,* ***Jatropha curcas****,* ***Pennisetum purpureum****,* ***Raphanus sativus.***

INTRODUCTION

The use of agroindustrial co-products as source of nutrients in animal diets has been practiced

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for decades, since millions of tons of these materials are produced each year. In animal production, food represents the biggest part of production costs, making it indispensable to efficiently use the available resources to maximize the animal performance.

Elephant grass (***Pennisetum purpureum***, Schum.) is very well adapted to climate and soil conditions of almost all Brazilian territory. Despite species from ***Pennisetum*** gender show a high dry matter per hectare production, they are also characterized by having high moisture and low concentration of soluble carbohydrates, limiting its use in silage production. However, the use of additives as agroindustrial co-products during ensilage process can minimize these problems (NEIVA et al., 2006).

Biodiesel brought a large number of new co-products, once cakes and meals that are being produced, existing then, necessity to study the viability of inclusion of these new alternative food sources in animal diets. Inclusion of these alternative food sources could increase dry matter in the silage content, ensuring a good standard of anaerobic fermentation. High moisture contents maintain low osmotic pressure, providing the development of ***Clostridium*** bacteria, which change sugars into butyric acid, acetic acid, ammonia and carbon dioxide, compromising the silage quality (MCDONALD et al., 1991). The objective of this research was to evaluate the effect of including forage turnip and physic nut cakes, derived from oleaginous grains cold pressing for biodiesel production, on chemical composition and fermentation characteristics of elephant grass silage.

MATERIAL AND METHODS

Three concentrations (3; 6; and 9%) of forage turnip and physic nut cakes were evaluated, and added during the ensilage of elephant grass. The treatments were composed of: 100% elephant grass; 97% elephant grass + 3% forage turnip cake; 94% elephant grass + 6% forage turnip cake; 91% elephant grass + 9% forage turnip cake; 97% elephant grass + 3% physic nut cake; 94% elephant grass + 6% physic nut cake and; 91% elephant grass + 9% physic nut cake.

A randomized design, in a factorial arrangement [(2×3)+1] (two types of cake: forage turnip and physic nut; and three concentrations of the referred cakes: 3; 6; and 9%) was used, with an additional treatment (elephant grass pure ensiled), with three replicates per each treatment.

Elephant grass was cut to 10cm of soil with 70 days of age, and chopped in two centimeters particles. Cakes were obtained from a biodiesel production plant. Oil extraction was realized using a mini mechanical press, model MPE-40 Ecirtec LTDA. Samples of elephant grass and cakes were, initially, dried (55^{o}C for 72h) and grinded in a Wiley type mill (1mm) for further chemical analysis.

Cakes were homogenized before ensilage. Forage and cake were mixed and ensiled in PVC silos with diameter of 10cm and 60cm of height, adapted with Bunsen valves. Approximately four kilograms of material were put in each silo, in a way to reach a density of 700kg m^{-3}. Packing was manual, and after thorough packing, the silos were closed and sealed. Sixty two days after, the silos were opened and the sensorial features (texture and smell, besides the presence of fungi) were observed. Silage samples for analysis were obtained from the middle of PVC silo. These samples were weighed and dried in forced ventilation air oven at 55°C for 72h and the rest was allocated in plastic bags properly identified and kept in freezer (-18^{o}C). After that, pre-drying samples were ground in a Wiley type mill, using sieve with perforation of 1mm, stored in plastic pots properly identified.

An additional sample of 250g of material was collected from each silo and its juice was extracted to determine pH values and ammonia nitrogen content.

Dry matter (DM), mineral matter (MM), ether extract (EE) and crude protein (CP) contents were determined (AOAC, 1998). Neutral detergent fiber (NDF), acid detergent fiber (ADF), cellulose (CEL), lignin (LIG), neutral detergent insoluble nitrogen insoluble nitrogen in neutral detergent (NDIN) and acid detergent insoluble nitrogen (ADIN) contents were determined using VAN SOEST et al. (1991) method. A potentiometer was used to determine pH and buffering capacity (BC) was measured according to PLAYNE & MCDONALD (1966); ammonia nitrogen (N-NH_3) as percentage of the total nitrogen, according to CÂNDIDO (2000), and two stages techniques were used to determine *in vitro* dry matter digestibility (IVDMD).

Data were tested for normality and homokedasticity assumptions, and so the analysis of variance was realized using "F" test at 5% probability level. Once the significance of "F" test was verified, the average of the treatments were compared using the Tukey test ($P<0.05$). For statistics analyses, the procedure PROC GLM of the statistical pack SAS (SAS, 1999) was used.

RESULTS AND DISCUSSION

Silage added with forage turnip cake showed good sensorial characteristics, in opposition to physic nut cake silage that showed problems, such as pronounced odor and dark color, indication of protein degradation, which was demonstrated in the amount of ammonia nitrogen observed in these silages (Table 1).

Increasing levels of forage turnip cake promoted reductions (P<0.05) in NDF content silages (Table 1), due to the fact that forage turnip cake presented lower NDF content than Elephant Grass (Table 2), while physic nut cake addition had not effect on NDF content (P>0.05).

Similar results, in relation to the reduction of NDF contents of silages with addition of by-products, were found by NEIVA et al. (2006), when added increasing levels of by-product of passion fruit industry (56.4% of NDF) to elephant grass, and CARVALHO et al. (2008) that added different levels of cocoa meal (43.6% of NDF) to elephant grass ensilage.

ADF values (Table 1) of silages containing physic nut cake were higher (P<0.05) than the ones with forage turnip cake only in the concentrations of 6 and 9%. This fact is because of the higher concentration of ADF in physic nut cake (37.77%). Reductions in this fiber fraction were also observed by BATISTA et al. (2006), when added growing concentrations of mesquite pod (11.90% of ADF) in elephant grass silage (38.09% of ADF) and by PEREIRA et al. (1999), that added citrus pulp pellets (24.00% of ADF) also in elephant grass silage (47.40% of ADF). There was no effect (P>0.05) of physic nut cake addition on ADF contents, due to the higher ADF values (Table 1).

Addition of forage turnip cake to elephant grass, during the ensilage process, provided more dry matter silage digestibility (P<0.05) than in silages added with physic nut cake (Table 1). This fact is mainly due to the higher digestibility of forage turnip cake in relation to the physic nut cake (66.66 and 51.07%, respectively). Another possibility would be higher presence of toxins (phytates, tripsin inhibitors, curcin and forbol esters) present in physic nut cake (MAKKAR et al., 1997; MARTINEZ-HERRERA et al., 2006).

The addition of 9% of forage turnip cake caused reduction in the IVDMD (Table 1) of silages (P<0.05), that was not observed in 3 or 6% level (P>0.05). The lowest value obtained (41.1% of IVDMD) could start dropping dry matter intake of animals ingesting this kind of silage (Van SOEST, 1994).

Silage pH value (Table 1), except the silages containing physic nut cake in the concentrations 3 and 9% (4.7 and 5.2 units, respectively), indicated appropriate conditions for the development of a good fermentation process, according to the values indicated by MCDONALD et al. (1991), between 3.8 and 4.2. The addition of forage turnip cake did not vary (P>0.05) pH values. According to MCDONALD et al. (1991), in

Table 1 - Neutral detergent fiber and acid detergent fiber contents, *in vitro* dry matter digestibility, ammonia nitrogen concentrations and pH values of elephant grass due to different concentrations and types of added cake

Silage	Concentration of cake (%)[4]			Additional treatment[8]
	3	6	9	
	NDF (%DM) CV[1] = 1.5%			
EG[5]+FTC[6]	62.30^{Ba}	59.31^{Bb}	52.18^{Bc}	71.8*
EG+PNC[7]	65.71^{Aa}	63.67^{Aa}	60.17^{Aa}	
	ADF (%DM) CV[1] = 3.4%			
EG+FTC	42.02^{Aa}	38.68^{Ba}	33.74^{Bb}	42.4*
EG+PNC	43.54^{Aa}	42.75^{Aa}	40.1^{Aa}	
	IVDMD[2] (%DM) CV[1] = 1.7%			
EG+FTC	45.2^{Aa}	45.0^{Aa}	41.1^{Ab}	49.4*
EG+PNC	41.7^{Ba}	39.2^{Bb}	30.6^{Bc}	
	pH CV[1] = 1.8%			
EG+FTC	3.9^{Ba}	3.9^{Ba}	3.8^{Ba}	4.2*
EG+PNC	4.7^{Ab}	4.1^{Ac}	5.2^{Aa}	
	$N\text{-}NH_3$[3] (%total N) CV[1] = 3.2%			
EG+FTC	4.2^{Bb}	5.4^{Aa}	5.5^{Ba}	5.9*
EG+PNC	9.8^{Ab}	5.0^{Bc}	15.2^{Aa}	

Averages followed by the same lower case in the line and by the same capital letter in the column, do not differ among them by the Tukey test (5%). [1]Coefficient of variation. [2]*in vitro* dry matter digestibility. [3]Ammonia nitrogen as the percentage of total nitrogen. [5]Elephant grass. [6]Forage turnip cake. [7]Physic nut cake. [8]Elephant grass pure ensilaged. *Significant (P<0.05) (additional treatment × factorial).

Table 2 - Averages of chemical composition, *in vitro* digestibility and buffering capacity of elephant grass and co-products used in the ensilage.

Variable	Elephant grass (EG)	------Co-products------	
		Forage turnip cake (FTC)	Physic nut cake (PNC)
DM (%)*[1]	21.27	92.83	92.96
OM (%DM)[2]	86.13	91.82	94.27
MM (%DM)[3]	13.87	8.18	5.73
CP (%DM)[4]	6.74	31.62	17.44
EE (%DM)[5]	4.76	26.02	27.54
NDF (%DM)[6]	77.83	21.71	47.62
ADF (%DM)[7]	49.30	13.71	37.77
CEL (%DM)[8]	46.58	12.05	37.32
LIG (%DM)[9]	9.73	3.74	3.64
NDIN (%Total N)[10]	36.48	32.44	50.22
ADIN (%Total N)[11]	17.83	24.36	32.85
IVDMD (%)[12]	55.17	66.66	51.07
BC[13]	34.33	16.70	18.42

[*]Fresh matter basis; [1]Dry matter; [2]Organic matter; [3]Mineral matter; [4]Crude protein; [5]Ether extract; [6]Neutral detergent fiber; [7]Acid detergent fiber; [8]Cellulose; [9]Lignin; [10]Neutral detergent insoluble nitrogen; [11]Acid detergent insoluble nitrogen; [12]*In vitro* dry matter digeslibility; [13]Buffering capacity (eq.mg NaOH/100g DM).

conventionally conserved silages, high pH values promote a higher production of acetic and butyric acid, characteristic of undesirable fermentation processes.

Evaluating silages of elephant grass with growing levels of citrus pulp (0; 2.5; 5; 7.5; 10; 12.5 and 15%), RODRIGUES et al. (2005) found pH values (3.87; 3.86; 3.89; 3.74; 3.84; 3.80; 3.89; respectively) that were similar to the ones found in this study.

Excluding the average value of $N\text{-}NH_3$ in the level 9% (Table 1) of physic nut cake (15.2%), other values were below 10%, which is considered the maximum acceptable value to classify silage as unspoilt according to MCDONALD et al. (1991). Higher values indicate intense proteolysis in the silages that cause darkening and reduction in their nutritive value. However, the levels of $N\text{-}NH_3$ in physic nut cake did not affect its chemical composition.

An interaction concentrations × cakes was not verified in relation to DM, CP, EE, NDIN, ADIN, CEL, and LIG contents (Table 3). Silage DM contents increased only with higher concentrations of forage turnip cake (P<0.05). Silages containing physic nut cake, in all concentrations, presented a similar DM contents (P>0.05) to the ones found only in the silages of elephant grass (22.01%).

The addition of forage turnip cake in the concentrations of 6 and 9% caused significant increase (P<0.05) in DM contents. None of the experimental silage treatments reached the minimum level of 28% of DM assumed by MCCULLOUGH (1977) as being necessary for predominance of lactic fermentation and inhibition of clostridium fermentation. Similar effects were found by GONÇALVES et al. (2004), when included increasing levels of by-products for the processing of West Indian cherry and guava with DM contents superior to 87%.

In relation to CP contents, it was showed that higher contents of CP with the addition of physic nut cake when compared to elephant grass silages, were only found (P<0.05) in bigger concentrations (6 and 9%). On the other hand, after adding forage turnip cake, it was observed that even in the lowest evaluated concentration, higher contents of CP were obtained (P<0.05) than in pure elephant grass silages.

The cakes used in this research caused significant elevations (P<0.05) in EE contents (Table 3). This could be due to the high content of EE present in the forage turnip cakes. This high content of EE is, mainly, a consequence of low efficiency of oil extraction cold process. This characteristic could limit the use of products from this process, once high contents of EE (superior to 7%) in ruminant diets can cause decrease in dry matter intake, besides decreasing the rate of fiber degradation (PALMQUIST, 1994).

A decrease (P<0.05) in NDIN content of silages was observed when the cakes of forage turnip and physic nut cakes were added at the concentration

Table 3 - Dry matter, crude protein, ether extract, neutral detergent insoluble nitrogen, acid detergent insoluble nitrogen, cellulose and lignin contents of pure elephant grass silages and silages added to different concentrations of forage turnip and physic nut cakes

Variable	EGS[4]	---Silage---						CV[1] (%)
		---EG[5] + (%) FTC[6]---			---EG + (%) PNC[7]---			
		3	6	9	3	6	9	
DM	22.1^{b}	22.0^{b}	25.7^{a}	27.2^{a}	22.3^{b}	25.1^{ab}	24.7^{ab}	4.8
CP[2]	7.0^{d}	10.1^{b}	10.5^{b}	12.1^{a}	8.3^{cd}	8.6^{c}	9.2^{bc}	5.1
EE[2]	4.2^{e}	7.1^{d}	8.8^{bc}	11.4^{a}	6.4^{d}	7.3^{cd}	10.1^{ab}	7.3
NDIN[3]	31.4^{a}	28.3^{ab}	27.6^{ab}	22.1^{b}	25.8^{ab}	25.6^{ab}	22.4^{b}	9.7
ADIN[3]	10.7^{abc}	8.7^{cd}	9.1^{bcd}	6.9^{d}	12.9^{a}	11.6^{ab}	10.3^{abc}	9.7
CEL[2]	43.4^{a}	37.4^{bc}	36.0^{c}	31.0^{d}	42.7^{a}	41.3^{ab}	41.0^{abc}	4.5
LIG[2]	9.3^{a}	8.8^{a}	8.2^{a}	8.0^{a}	7.1^{a}	7.1^{a}	6.9^{a}	12.5

Averages followed by the same case in the line do not differ among them by the Tukey test (P>0.05).[1]Coefficient of variation; [2]Dry matter basis; [3]% of total nitrogen; [4]Elepahnt grass silage; [5]Elephant grass; [6]Forrage turnip cake; [7]Physic nut cake. DM=Dry matter, CP=Crude protein, EE=Ether extract, NDIN=Neutral detergent insoluble nitrogen, ADIN=Acid detergent insoluble nitrogen, CEL=Cellulose, LIG=lignin.

of 9%. However, for the contents of ADIN, there were reductions (P<0.05) only at the addition of 9% of forage turnip cake. These results were not expected due to the superiority in NDIN and ADIN contents of both cakes having the same contents found in elephant grass. All average values of ADIN found in this study were lower than 20%, a limiting value for using nitrogen, which according to VAN SOEST & MANSON (1991), is due to the reduction in its availability and in the decrease of dry matter digestibility.

All silage with addition of forage turnip cake had cellulose contents lower (P<0.05) than the ones verified in silages with elephant grass only. Considering the different concentrations of evaluated forage turnip cake, the lower contents of cellulose were obtained with the higher addition level (9%), which reached 12.4% less of cellulose than silages which had only elephant grass. On the other hand, silages with the addition of physic nut cake did not differ among them (P>0.05) in relation to the contents of cellulose, showing average contents of 67% that were similar (P>0.05) to the ones found just with elephant grass (43.4%). These results were expected due to the fact of forage turnip cake presents 34.53% less cellulose than elephant grass and physic nut cake have a very similar concentration to elephant grass (Table 1). Lignin content did not differ between treatments (P>0.05) due to the lower contents of phenolic compound in the cakes, the mean value were 7.91%.

CONCLUSION

The inclusion of up to 9% of forage turnip cake improves chemical composition and provides good fermentation characteristics of elephant grass silage. In contrast, the inclusion of physic nut cake should be limited to 3%, because despite improving chemical composition of elephant grass silage, this co-product, added in highest levels, depreciates the silage fermentation parameters. Nevertheless, it should be considered that physic nut cake shows limitations to be used in animal feeding due to the presence of antinutritional and toxic compounds, being necessary studies for a better identification and elimination of them.

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